UNIVERSITEIT GENT

FACULTY OF BIOSCIENCE ENGINEERING

Academic year 2011-2012

Fat crystallization and partial coalescence in dairy creams: Role of monoacylglycerols

by

ir. Eveline Fredrick

Promotor: Prof. dr. ir. Koen Dewettinck

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in the Applied Biological Science: Chemistry

Voor Bart en mijn lieve ouders

References pictures frontpage:
CSLM-image: Royal FrieslandCampina
SEM-image: Quorum Technologies (www.quorumtech.com)
Whipped cream: (www.wasabimon.com)

Promotor: Prof. dr. ir. Koen Dewettinck

Laboratory of Food Technology and Engineering
Department of Food Safety and Food Quality

Dean: Prof. dr. ir Guido Van Huylenbroeck

Rector: Prof. dr. Paul Van Cauwenberghe

UNIVERSITEIT
GENT

FACULTY OF BIOSCIENCE ENGINEERING

Academic year 2011 - 2012

Fat crystallization and partial coalescence in dairy creams: Role of monoacylglycerols

by

ir. Eveline Fredrick

Promotor: Prof. dr. ir. Koen Dewettinck

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in the Applied Biological Science: Chemistry

Dutch translation of the title:

Vetkristallisatie en partiële coalescentie in zuivelromen: Rol van monoacylglycerolen

For citation:
FREDRICK, E. (2011). Fat crystallization and partial coalescence in dairy creams: Role of monoacylglycerols. PhD thesis, Ghent University, Belgium, 203p.

ISBN-number: 978-90-5989-469-3

The author and the promoter give the authorization to consult and to copy parts of this work for personal use only. Every other use is subject to the copyright laws.
Permission to reproduce any material contained in this work should be obtained from the author.

Woordje vooraf

-Oef! Eindelijk! Woehoe!- waren zowat de eerste kreten die in mijn hoofd opkwamen na indiening van dit proefschrift. Dat dit werk er gekomen is zonder slag of stoot of een éénmanszaakje is, zal je me niet horen zeggen. Ik kon rekenen op velen die op één of andere manier hun steentje bijdroegen. Hun bijdrage varieerde van wetenschappelijke ondersteuning, motiverende woorden, vriendschap, ontspanning,... Sommigen slaagden er zelfs in al deze te combineren. Dit is dan ook de ideale gelegenheid om even terug te blikken en om met welverdiende complimentjes te gooien.

Vooreerst wil ik mijn promotor Prof. dr. ir Koen Dewettinck bedanken voor de kansen die ik de afgelopen 5,5 jaar gekregen heb en voor zijn geloof in mijn kunnen. Het resultaat van telkens weerkerende brainstorms over ‘harde’ en ‘zachte’ bollen in room heeft nu eindelijk vorm gekregen en is zowat de rode draad doorheen dit proefschrift. Daarnaast heb ik als academisch assistent ook van jou de mogelijkheid gekregen om mijn vaardigheden op andere vlakken te ontplooien door het uitwerken van projectaanvragen, het coördineren van het intensief trainingsprogramma zuiveltechnologie, adviesverlening bij enkele dienstverleningsprojecten, het leiden van de onderzoeksgroep zuivel, ...

De overige leden van de examencommissie, Prof. dr. Els Van Damme, Prof. dr. ir. Paul Van der Meeren, Prof. dr. ir. Tiny van Boekel, dr. Jan De Block, dr. ir. Veerle De Graef en Mevr. Hanneke Zijtveld – van der Wiel, wens ik te bedanken voor het vrijmaken van hun kostbare tijd om dit proefschrift vakkundig te beoordelen en suggesties te formuleren.

Het onderwerp van dit doctoraatsonderzoek werd aangebracht en financieel ondersteund door Royal FrieslandCampina. Gedurende de afgelopen jaren heb ik met velen rond de tafel gezeten om dit complexe verhaal tot een goed einde te brengen. Bij deze, in het bijzonder mijn dank aan: Hanneke Zijtveld – Van der Wiel, Sabine Fischer, Marc Custinne, Marcel Paques, Anno Koning, Erix Schokker en Werner Clauwaert. Het was aangenaam samenwerken!

Voor dit onderzoek had ik de eer en het genoegen om samen te werken met de wereldwijd gerenomeerde zuivelspecialist Em. Prof. dr. ir. Pieter Walstra. Hij was mijn mentor van bij het begin tot het einde van mijn onderzoek. Zijn kennis tilde niet enkel dit onderzoek op een hoger niveau maar droeg ook in grote mate bij tot mijn persoonlijke vorming als wetenschappelijk onderzoeker. Pieter en Paulien, ik wens jullie het allerbeste toe en ik hoop bij jullie nog eens op de koffie te komen in Wageningen!

Bij aanvang van dit onderzoek kon ik rekenen op inzichten van Prof. dr. ir. Imogen Foubert. Helaas werden onze wegen na een kleine twee jaar gescheiden. Toch heb ik in deze korte periode veel van jouw kritische blik op onderzoeksresultaten opgestoken. Imogen, bedankt en hopelijk kruisen onze wegen elkaar nog eens.

During my PhD research, I supervised several master thesis students. Greenfield, Justice, Filip and Puji, thanks for your contribution and critical look to this work. I hope you are still enjoying scientific research! Good luck, with your future careers!

Niet alle analyses werden uitgevoerd aan het FTE-labo. Ik kon rekenen op vele helpende handen van andere labo's binnen en buiten de UGent. Daarom een woord van dank aan de collega's van het PaInt-labo (UGent) o.l.v Prof. dr. ir. Paul Van der Meeren voor het gebruik van hun infrastructuur (Mastersizer, droptensiometer, densitometer) en hun technische hulp, aan Prof. dr. ir. Paula Moldenaers en ir. Tom Verwijlen van de afdeling Toegepaste Reologie en Kunstofverwerking (KULeuven) voor hun deskundige hulp bij de uiterst complexe grensvlakrheologische analyses en aan Kristof Debeuf (BIOSTAT, UGent) voor zijn advies bij de statistische dataverwerking.

Aan mijn lieftallige FTE-collega's. Op 5,5 jaar zag ik er velen komen en gaan. Ik zou zonder veel moeite enkele pagina's door kunnen gaan om iedereen een persoonlijk dankwoordje toe te kennen maar vrees dat ik er toch maar enkele kan uitnemen. Bart en Davy, bij jullie vond ik een mooi evenwicht tussen leerrijke wetenschappelijke discussies en grappen, gieren en grollen tussen het werk door. Man, wat heb ik wat afgelachen! Benny, ze zeggen wel eens dat iedereen vervangbaar is, maar jij bewijst voor mij dat uitzonderingen de regel bevestigen. Geen vraag of babbel was jou teveel ookal zat je steeds tot over je oren in het werk. Ik ben blij dat ik jou goed heb leren kennen en hopelijk kunnen we in de toekomst nog enkele fietstochtjes samen rijden. Claudia de chocolate-lady van den bureau. Ik sta versteld van wat jij allemaal kan combineren. Bedankt om mij van tijd tot tijd van een 'chocoladeshot' te voorzien en mij het Westvlaams machtig te maken. Ik kan het nu goed gebruiken! Veerle, jou heb ik zien komen en gaan op den bureau. Bedankt om me op de hoogte te houden van alle nieuwtjes ;-). Met Frédéric, de bolleboos van de groep, heb ik enkele kleinere projectjes opgestart. Vooral de aangename SEM-zoektocht is me bijgebleven en heeft me een heel mooi plaatje opgeleverd! Nathalie, als collega-assistent hadden we veel raakvlakken. In onze samenwerking vulden we elkaar aan waar nodig en in de gelijklopende PhD-eindfase was je als lotgenote een grote steun. In de laatste maanden kon ik ook genieten van de kritische blik van Bart en Maarten en hun vakkundig advies op mijn geschreven tekst. Bedankt voor jullie tijd! FTE-collega's, het was me een waar genoegen om met jullie mijn universiteitsloopbaan af te ronden. Nu geef ik de fakkel door aan Kim, een jonge dynamische toffe meid, die de RG-dairy ongetwijfeld nieuw leven in zal blazen. Veel succes!

Beste vriendjes en vriendinnetjes van Gent, Lovendegem en omstreken. Jullie zijn de beste ontspanning! We hebben stuk voor stuk een drukke agenda maar we vergeten nooit tijd voor elkaar te maken. Dat blijkt uit onze vaste uitstapjes (spaghetti op maandag en/of pintjes op vrijdag) en onze jaarlijkse reisjes (weekendjes, skireis, surfreis en/of meidentrip). Een ideale formule om energie op te doen en de dag erna er opnieuw goed in te vliegen. Aan de meiden Annelies, Lieselot, Sara en Sofie nog een extra bedankje om me in één van de laatste weken van het drukke schrijven te verrassen met een ontbijtje aan huis. Pieter en Gijs, jullie hebben voor mij het pad geëffend en me getoond hoe een PhD-traject af te leggen. Ik heb veel van jullie ongelofelijk ‘knappe’ koppen opgestoken! Een dubbele dankjewel aan Pieter die zijn professioneel oog liet vallen op een aantal van mijn figuren.

Bij mijn vriend Bart kreeg ik er als kers op de taart een tweede familie bij. Linda, Rik, Sofie, Arno en -niet te vergeten mijn oogappeltje- Neal, jullie zijn fantastisch en maken het plaatje compleet.

Lieve broer, Steven, jij bent altijd mijn grote voorbeeld geweest en wat heb ik een bewondering voor jouw werklust! Bij jou en Lies voel ik me altijd superwelkom en kan ik ongegeneerd mijn hart luchten. Bedankt om er voor mij te zijn!

Mama en papa, onbeschrijflijk wat jullie voor mij al gedaan hebben! Jullie hebben een groot vertrouwen in mijn kunnen, hebben mij de nodige kansen gegeven en zijn dan ook de reden waarom ik met succes dit traject heb afgelegd. Mama, jij was diegene met de bemoedigende woorden als het eens tegenzat en papa, jij wist me op de juiste manier te triggeren om het onderste uit de kan te halen. Op beiden konden Bart en ik ook dagelijks rekenen tijdens onze verbouwingen. Een titanenwerk dat door jullie helpende handen een haalbare kaart is geworden!

Bartje, waar moet ik beginnen... Jij maakt van mij een perfect gelukkige vrouw! Van bij het begin waren we een ideale match en werken we samen aan onze dromen: mooie reizen, genieten van fietsen, het zeetje, ons huisje, ons tuintje en binnenkort ook ons kindje. Door jou kan ik zaken makkelijker een plaats geven en ga ik relaxer door het leven. De afgelopen maanden stond je er voor veel zaken alleen voor. Nu de tijden vol stress, deadlines en weekendwerk voorbij zijn, is het nu mijn beurt om vele taakjes van jou over te nemen en kunnen we samen verder onze vele dromen waarmaken.

TABLE OF CONTENT

TABLE OF CONTENT .... **I**

LIST OF SYMBOLS AND ABBREVIATIONS .... **IX**

SUMMARY .... **XV**

SAMENVATTING .... **XXI**

OUTLINE OF THE RESEARCH **1**

PART I: MILK FAT CRYSTALLIZATION **7**

1. LITERATURE REVIEW 9
   - *1.1. Introduction 9*
   - *1.2. Chemical composition of milk fat 9*
     - 1.2.1. Major components 10
     - 1.2.2. Minor components 11
     - 1.2.3. Factors governing the fat composition 11
   - *1.3. Fat crystallization 12*
     - 1.3.1. General principles of primary crystallization 13
       - 1.3.1.1. Thermodynamic driving force 14
       - 1.3.1.2. Nucleation 14
       - 1.3.1.3. Crystal growth 16
       - 1.3.1.4. Polymorphism 17
         - A. Polymorphism based on subcell packing 17
         - B. Polymorphism based on longitudinal stacking 18
         - C. Polymorphic evolutions 19
       - 1.3.1.5. Compound crystals 20
       - 1.3.1.6. Factors affecting primary crystallization 21
     - 1.3.2. Bulk versus emulsified fat 22
     - 1.3.3. Effect of small-molecule surfactants 24
     - 1.3.4. Recent research on milk fat crystallization 26
2. DESCRIPTION OF MATERIALS AND METHODS 29
   - *2.1. Materials 29*
     - 2.1.1. Natural cream 29
     - 2.1.2. Recombined cream 30
       - 2.1.2.1. Chemical characterization of ingredients 30
         - A. Anhydrous milk fat 30
         - B. Sweet cream buttermilk powder 30
         - C. Monoacylglycerols 30
       - 2.1.2.2. Production on pilot plant 31
   - *2.2. Methods 31*
     - 2.2.1. Differential scanning calorimetry 31
     - 2.2.2. Time-resolved synchrotron X-ray diffraction 32
     - 2.2.3. Static laser light scattering 33
     - 2.2.4. Nuclear magnetic resonance 33
     - 2.2.5. Interfacial tension analyses 35

2.2.6. Statistical analyses ... 36

3. MILK FAT CRYSTALLIZATION IN BULK AND EMULSIFIED STATE: NATURAL AND RECOMBINED CREAM ... 37

3.1. Introduction ... 37

3.2. Research strategy ... 38

3.3. Results and discussion ... 39

3.3.1. Characterization of the natural and recombined cream ... 39

3.3.2. DSC-Analyses ... 39

3.3.3. XRD-measurements ... 41

3.3.4. NMR-measurements ... 45

3.3.5. Discussion ... 46

3.4. Concluding remarks ... 49

4. EFFECT OF MONOACYLGLYCEROLS ON MILK FAT CRYSTALLIZATION IN RECOMBINED CREAM ... 51

4.1. Introduction ... 51

4.2. Research strategy ... 52

4.3. Results and discussion ... 53

4.3.1. Particle size distribution ... 53

4.3.2. DSC-analyses ... 54

4.3.2.1. Crystallization temperatures ... 54

A. Monoacylglycerols ... 54

B. Cream with monoacylglycerols ... 55

4.3.2.2. Isothermal crystallization behavior of cream ... 55

4.3.3. NMR-analyses ... 57

4.3.4. Interfacial tension analyses ... 57

4.3.5. Discussion ... 60

4.4. Concluding remarks ... 62

PART II: PARTIAL COALESCENCE ... 65

5. LITERATURE REVIEW ... 67

5.1. Cream ... 67

5.1.1. Definitions ... 67

5.1.2. Physical properties ... 67

5.1.2.1. Particle size distribution ... 68

5.1.2.2. Viscosity ... 68

5.1.2.3. Physical stability ... 69

5.1.3. Mechanism of partial coalescence in oil-in-water emulsions ... 72

5.1.4. Factors governing partial coalescence rate ... 75

5.1.4.1. Process parameters ... 76

A. Flow conditions ... 76

B. Temperature ... 77

5.1.4.2. Composition and formulation ... 86

A. Fat volume fraction ... 86

B. Particle size of globules and clumps ... 87

C. Oil-water interfacial layer composition ... 88

D. Continuous aqueous phase composition ... 96

E. Dispersed oil phase compostition ... 97

5.2. Whipped cream ... 97

5.2.1. Definitions ... 97

5.2.2. Physical properties ... 100

5.2.3. Mechanism of structure build-up ... 100

5.2.4. Factors governing the whipping properties ... 103

5.2.4.1. Process parameters ... 104

A. Flow condition ... 104

B. Temperature ... 104

5.2.4.2. Composition and formulation of the cream ... 105

A. Fat volume fraction ... 105

B. Particle size ... 105

C. Surface-active components ... 106

D. Continuous aqueous phase composition ... 108

E. Dispersed oil phase composition ... 108

5.3. Concluding remarks ... 109

6. DESCRIPTION OF MATERIALS AND METHODS ... 111

6.1. Materials ... 111

6.2. Methods ... 111

6.2.1. Rheology ... 111

6.2.1.1. Rotational viscosity analyses ... 111

6.2.1.2. Oscillatory interfacial shear rheology ... 112

6.2.2. Static laser light scattering ... 113

6.2.3. Nuclear magnetic resonance analyses ... 113

6.2.4. Interfacial tension analyses ... 113

6.2.5. Microscopy ... 113

6.2.5.1. Bright-field light microscopy ... 113

6.2.5.2. Confocal scanning laser light microscopy ... 114

6.2.6. Protein load analysis ... 114

6.2.7. Whipping properties ... 116

6.2.7.1. Whipping process ... 116

6.2.7.2. Overrun ... 116

6.2.7.3. Firmness ... 117

6.2.7.4. Serum loss ... 117

6.2.8. Statistical analyses ... 117

7. POTENTIALS OF A RHEOLOGICAL METHOD TO STUDY PARTIAL COALESCENCE IN DAIRY WHIPPING CREAMS ... 119

7.1. Introduction ... 119

7.2. Research strategy ... 120

7.3. Results and discussions ... 120

7.3.1. Mechanism of shear-induced partial coalescence ... 120

7.3.2. Effect of temperature ... 123

7.3.2.1. Solid fat content ... 124

7.3.2.2. Shear-induced partial coalescence ... 124

7.3.2.3. Whipping properties ... 126
7.3.2.4. Discussion ... 127
7.3.3. Effect of Shear rate ... 129
7.3.3.1. Shear-induced partial coalescence ... 129
7.3.3.2. Whipping properties ... 129
7.3.3.3. Discussion ... 130
7.3.4. Effect of fat content ... 130
7.3.4.1. Shear-induced partial coalescence ... 131
7.3.4.2. Whipping properties ... 131
7.3.4.3. Discussion ... 133
7.4. Concluding remarks ... 134
8. EFFECT OF MONOACYLGLYCEROLS ON PARTIAL COALESCENCE IN RECOMBINED CREAM ... 135
8.1. Introduction ... 135
8.2. Research strategy ... 136
8.3. Results and discussion ... 137
8.3.1. Shear-induced partial coalescence ... 137
8.3.2. Whipping properties ... 140
8.3.3. Physicochemical properties of the recombined creams and of their oil-water interface ... 143
8.3.3.1. Protein displacement ... 144
8.3.3.2. Interfacial tension ... 145
8.3.3.3. Interfacial viscoelasticity ... 146
8.3.3.4. Fat crystallization ... 149
8.3.4. Discussion ... 150
8.4. Concluding remarks ... 154
9. POTENTIALS OF MONOACYLGLYCEROLS TO IMPROVE WHIPPING PROPERTIES OF RECOMBINED CREAM ... 155
9.1. Introduction ... 155
9.2. Research hypotheses ... 156
9.3. Results and discussions ... 156
9.3.1. Binary mixture of monoacylglycerols ... 156
9.3.1.1. Shear-induced partial coalescence ... 156
9.3.1.2. Whipping properties ... 157
9.3.1.3. Physicochemical properties of the recombined creams and of their oil-water interface ... 158
A. Interfacial tension ... 158
B. Interfacial viscoelasticity ... 159
C. Fat crystallization ... 160
9.3.1.4. Discussion ... 161
9.3.2. Fat reduction by using monoacylglycerols ... 162
9.3.2.1. Shear-induced partial coalescence ... 162
9.3.2.2. Whipping properties ... 163
9.3.2.3. Discussion ... 163
9.4. Concluding remarks ... 165

GENERAL CONCLUSIONS ... 167

REFERENCE LIST **173**

ANNEXES **195**

ANNEX I 197
ANNEX II 197
ANNEX III 197
ANNEX IV 197
ANNEX V 198
ANNEX VI 198
ANNEX VII 198

CURRICULUM VITAE **199**

LIST OF SYMBOLS AND ABBREVIATIONS

α	Capture efficiency
γ	Interfacial tension without surface-active components or strain
$\dot{\gamma}$	Shear rate
γ_{OW}	Oil-water interfacial tension
γ_{SO}	Solid-oil interfacial tension
γ_{WS}	Water-solid interfacial tension
σ	Shear stress
Γ	Protein load
ΔH_m	Melting enthalpy
$\Delta\mu$	Difference in chemical potential
ΔP	Laplace pressure
η	Viscosity
$[\eta]$	Intrinsic viscosity
η_s	Viscosity of the solvent
θ	Scattering angle (Part I) or contact angle (Part II)
θ_w	Contact angle, as measured in the aqueous phase
θ_O	Contact angle, as measured in the oil phase
Λ	Stress concentration factor
λ	Wavelength
Π	Surface pressure
Π_{cr}	Critical surface pressure
ρ_c	Density of the continuous phase
ρ_d	Density of the dispersed phase
ρ_h	Density of the heavy phase
ρ_l	Density of the light phase
φ	Fat volume fraction
φ_{eff}	Effective volume fraction
φ_{max}	Maximum volume fraction attainable
ψ	Crystal volume fraction
AMF	Anhydrous milk fat
AFM	Atomic force microscopy
CB	Cocoa butter
C	Concentration of the solute
C_s	Saturation concentration of the solute
CLA	Conjugated linoleic acid

CLSM	Confocal laser scanning microscopy
CNO	Coconut oil
DAG	Diacylglycerol
d	Distance
D	Fractal dimensionality or globule diameter
$D_{3,2}$	Volume-surface average or Sauter diameter
DSC	Differential scanning calorimetry
ESRF	European synchrotron radiation facility
FA	Fatty acid
FFA	Free fatty acid
FID	Free induction decay
g	Gravitational acceleration
G'	Storage/elastic modulus (rheological parameter)
G’’	Loss/viscous modulus
HDPE	High density polyethylene
HLB	Hydrophilic-lipophilic balance
HMF	High-melting fraction of milk fat
HSFO	Hydrogenated sunflower oil
L_{aq}	Liquid FID-signal of the aqueous phase of cream
L_{cr}	Liquid FID-signal of cream
$\mathrm{L'}_{\mathrm{cr}}$	Liquid FID-signal of milk fat in cream corrected for the aqueous phase
L_{MF}	Liquid FID-signal of milk fat
$\mathrm{L}_{\mathrm{oil}}$	Liquid FID-signal of an oil
LLL	Trilaurin
LMF	Low-melting fraction of milk fat
$m_{pr,\ interface}$	Mass proteins at the interface
m_{serum}	Mass serum phase
$m_{unwhipped}$	Mass unwhipped cream
$m_{whipped}$	Mass whipped cream
MAG	Monoacylglycerol
MAG-L	Monoacylglycerols rich in lauric acid
MAG-O	Monoacylglycerols rich in oleic acid
MAG-S	Monoacylglycerols rich in stearic acid
MFGM	Milk fat globule membrane
MF-NC	Milk fat extracted from natural cream
MF-RC	Milk fat used for recombined cream
MMF	Middle-melting fraction of milk fat

NC	Natural cream
NMR	Nuclear magnetic resonance
P_{inside}	Pressure inside the droplet/bubble
$P_{outside}$	Pressure outside the droplet/bubble
PL	Phospholipid
PO	Palm oil
POl	Palm olein
Polysorbate 20	Polyoxyethylene sorbitan monolaurate
r	Radius
r_1 and r_2	Radii of the curvature
$r_{crystal}$	Radius of the protruding crystal
R_g	Universal gas constant
$r_{globule}$	Radius of the globule
RC	Recombined cream
RSO	Rapeseed oil
$S_{cr,T_{5°C}}$	Solid fat content of cream at 5℃
$S_{MF,T_{5°C}}$	Solid fat content of milk fat at 5℃
SAXS	Small-angle X-ray scattering
SCBMP	Sweet cream buttermilk powder
SFC	Solid fat content
SFO	Sunflower oil
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SMP	Skimmed milk powder
SMUF	Simulated milk ultrafiltrate
SOE	Sucrose oligoesters
SSS	tristearin
T_α	Final melting point or clear point of the α-polymorph
T_β	Final melting point or clear point of the β-polymorph
$T_{\beta'}$	Final melting point or clear point of the β’-polymorph
T_{cr}	Crystallization temperature
$T_{cr,chain}$	Chain crystallization temperature
T_m	Final melting temperature
T_{max}	Temperature a few degrees below final melting point
T_{ch}	Churning time
t_{max}	Time at T_{max}
T_{wh}	Whipping temperature

t_{wh}	Whipping time
t,T-program	Time-temperature program
TAG	Triacylglycerol
TEM	Transmission electron microscopy
UHT	Ultra-high temperature
WAXD	Wide-angle X-ray diffraction
WP	Whey protein
WPC	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate
x_{aq}	Mass fraction of the aqueous phase
y	Diameter of the aggregating crystals

SUMMARY

Dairy cream is a fat-rich fluid milk product that, from a microstructural point of view, can be described as an oil-in-water emulsion. Highly fat-rich creams or whipping creams can be processed into whipped cream which is a rigid foam that can be defined as an aerated partially destabilized oil-in-water emulsion. During the whipping process, air is introduced and fat globules start to interact by means of a partial coalescence mechanism. As a result, in the ultimate whipped cream a network of partially coalesced fat globules envelops the air bubbles and immobilizes the serum phase. The presence of fat crystals and a substantial fat content are thereby indispensable. Moreover, the amount and rate of partial coalescence control to a large extent the final microstructure and, hence, the sensory attributes and physicochemical properties (overrun, stability, firmness, creaminess, fattiness, etc.) of whipped cream. In particular factors governing the globule size, the oil-water interfacial properties and the fat crystallization behavior of cream control the partial coalescence kinetics and may, hence, be usefull to improve the whipping behavior of dairy creams

Nowadays, besides natural dairy cream, derived directly from fresh cow's milk, often recombined dairy cream is used. The latter is made by recombining milk products, mostly milk fat and a low-fat milk powder, and water. The benefits of using these creams are that the composition and formulation can easily be modified for product development goals and that the raw materials can efficiently be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce. Nevertheless, recombined whipped cream seems to have unfavorable divergent physicochemical and sensory properties as compared to whipped cream derived from natural cream. This may partly be attributed to a variation in susceptibility of the fat globules towards partial coalescence between both creams during processing. Given that monoacylglycerols (MAGs), as oil-soluble small-molecule surfactants, are able to modify the fat globule size, the oil-water interfacial properties and the fat crystallization in recombined creams, MAGs can play a key role in governing the partial coalescence rate and, hence, probably in improving the structure build-up of the recombined whipped creams.

Generally, this manuscript aimed at more understanding of a few of the underlying phenomena important for whipping of dairy creams. More specifically, in Part I (Chapter 1-4) milk fat crystallization in cream was addressed, which is an essential process preceding partial coalescence, and in Part II (Chapter 5-9) the partial coalescence in cream with and without air inclusion was discussed. Throughout both parts, the effects of MAGs were extensively surveyed with the intention of, firstly, getting more fundamental insight in the mechanism clarifying the effect of different MAGs on partial coalescence of recombined cream and on their whipping properties and, secondly, exploring the potentials of MAGs to improve the physicochemical properties of dairy recombined whipped creams.

Part I: Milk fat crystallization

In Chapter 1 an overview was given of (1) the chemical composition of milk fat, of (2) the general basic principles on fat crystallization in bulk and in emulsions, of (3) the effect of small-molecule surfactants on the crystallization behavior and of (4) recent studies on milk fat crystallization. Besides, more specific brief literature reviews on the 'state of the art' are provided as an introduction to each experimental chapter.

The description of the materials and methods was collected in Chapter 2 as a lot of the experimental work in the research chapters of Part I was based on the same spectrum of analytical procedures. It covered, firstly, the substrates used and, secondly, a description of common techniques applied to study fat crystallization. Thereby, special attention was drawn to adapting, if necessary, the well-known conventional methods in order to investigate fat crystallization in cream in a proper way. Thirdly, the techniques used to measure oil-water interfacial tensions and fat globule sizes of cream were reported.

In Chapter 3, the first experimental chapter of Part I, a comparison is made between the mechanism and kinetics of milk fat crystallization in bulk and emulsified state and between recombined and natural cream. Milk fat in bulk or emulsified state showed a similar crystallization mechanism at temperature conditions closely related to the ones applied during the industrial production of whipping cream (fast cooling to 5℃). First, α-crystals were formed and, second, β'-crystals grew at the expense of the α-crystals. However, some α-crystals still remained and additional crystallization during further cooled storage took place. The observed mechanism was explained by the formation of compound crystals. Although these similarities between the milk crystallization mechanism in bulk and in cream, the overall crystallization rate was found to be lower in both natural and recombined cream. This difference was to a large extent explained by the widely accepted theory of nucleation in the dispersed phase. To achieve full crystallization, nucleation has to take place in each individual globule. The time required to induce nucleation is thereby related to the volume of the globules and if the globules have the same size, nucleation is a random process. The crystallization behavior of natural cream deviated from that of recombined cream since the triacylglycerol composition of the individual globules varied in natural cream. In the considered natural cream this was manifested in an earlier start and a more scattered α-β'-polymorphic transition and in a lower amount of solid fat after 5 days storage at 5℃ while this was not observed for recombined cream.

In Chapter 4, the effect of MAGs which differ in chain length and degree of saturation on the primary crystallization behavior of milk fat in recombined cream was elucidated at the same experimental conditions as in Chapter 2. The long-chain unsaturated MAGs did not show significant effects on the primary crystallization behavior and on the solid fat content. A similar crystallization mechanism compared to the reference recombined cream, as studied in Chapter 3, was observed. In contrast, recombined creams containing long-chain saturated MAGs revealed a different nucleation mechanism. Interfacial heterogeneous nucleation took place during cooling. As a consequence, in the presence of long-chain saturated MAGs the crystallization started at a higher temperature and the crystal growth and the α-β' polymorphic transition was accelerated. Intermediate behavior was observed with saturated MAGs with a mid-chain length. These MAGs accelerated the α-β' polymorphic transition while they did not show any effect on the nucleation and the crystal growth. It was postulated that the intermediate behavior is ascribed to co-crystallization of the MAGs once crystallization has started. Furthermore, regardless of the type and concentration of MAGs, the mechanism apart from the nucleation was similar to the reference cream and the solid fat content after prolonged storage at 5℃ was unaffected.

Part II: Partial coalescence

A detailed critical literature review on partial coalescence was given in Chapter 5. In the first section, after a brief description of the general physical properties of cream, the mechanism of partial coalescence was extensively discussed. Next, the factors controlling the kinetics of partial coalescence were addressed. The second section dealt with the microstructure of whipped cream and the role of partial coalescence in the whipping process. Finally, factors govering the whipping properties were summarized. Moreover, via an introductory section the 'state of the art' was reported in each research chapter of Part II.

The materials and methods were collected in one chapter (Chapter 6) since they all are relevant to the following experimental chapters. In addition, in Chapter 7, a rheological method to survey shear-induced partial coalescence kinetics was first introduced as a qualitative predictive tool for the whipping properties of dairy creams. This technique was validated by linking the partial coalescence rate and the whipping properties of natural cream by varying the flow condition, the temperature and the fat content. Moreover, in combination with microscopic and particle size analyses it was possible to get more insight in the mechanism of the ongoing shear-induced partial coalescence in the rheometer.

The rheological method introduced in Chapter 7, is used in Chapter 8 to unravel the effect of two long-chain MAGs which differ in saturation degree on partial coalescence in recombined cream and on their whipping properties. The same MAGs as in Chapter 4 were used. The unsaturated MAGs showed an increased partial coalescence rate in recombined cream resulting in a lower whipping time and overrun and a higher stability and firmness of whipped recombined creams while the saturated MAGs demonstrated the exact opposite effects. The

decreased partial coalescence rate in recombined cream with the saturated MAGs provoked a higher whipping time and overrun and a lower stability and firmness of the whipped recombined creams. The divergent behavior of the MAGs on the susceptibility of fat globules towards partial coalescence was mechanistically explained by the different behavior of MAGs at the oil-water interface, by using the results obtained in both Chapter 4 and Chapter 8. If the MAGs were able to crystallize at the oil-water interface and if they thereby induced heterogeneous interfacial nucleation of the milk fat during cream production, the protrusion of the obtained smaller crystals was suggested to be limited resulting in a decreased partial coalescence rate. On the contrary, MAGs that behave as a liquid at the oil-water interface enhanced the deformability of the interface which presumably enhanced the protrusion of the fat crystals through the oil-water interface causing ultimately an increased partial coalescence rate. In this study, the long-chain saturated MAGs behaved as a solid while its unsaturated counterpart appeared to be in the liquid state at the oil-water interface.

Following the fundamental research on the effect of MAGs on milk fat crystallization (Chapter 4) and partial coalescence (Chapter 8) it was explored in Chapter 9 whether the long-chain MAGs which differ in saturation degree can be used in practice to improve whipping properties of recombined cream. The separate use of one type of MAG in a full-fat cream showed rather poor whipping behavior: some beneficial whipping properties were attained but this occurred at the expense of other desired whipping properties. Alternatively, when both the MAGs are introduced in one full-fat cream the negative effects of one MAG can be counterbalanced by the other causing ultimately improved whipping properties of recombined cream. On the contrary, when also the fat content of recombined cream is varied, the separate use of the long-chain unsaturated MAGs in one cream became beneficial. In fact, whipped recombined cream with a reduced fat content containing unsaturated MAGs demonstrated comparable whipping properties to the full-fat variant without MAGs.

SAMENVATTING

Zuivelroom is een vetrijk vloeibaar melkproduct dat vanuit microstructureel oogpunt beschreven kan worden als een olie-in-water emulsie. Een zeer vetrijke room of slagroom kan verwerkt worden tot opgeklopte room of geslagen room. Dit is een stevige schuim dat kan gedefinieerd worden als een beluchte gedeeltelijk gedestabiliseerde olie-in-water emulsie. Tijdens het opklopproces wordt er lucht in de room geslagen en wordt er een netwerk gevormd van partieel gecoalesceerde vetglobulen die enerzijds de luchtbellen omgeven en anderzijds de serumfase gevangen houden. De aanwezigheid van vetkristallen in de vetglobulen en een aanzienlijk vetgehalte van de room is daarbij essentieel. Evenals bepaalt de mate en de snelheid van partiële coalescentie grotendeels de uiteindelijke microstructuur en derhalve de sensorische eigenschappen en fysicochemische eigenschappen (luchtinslag, stabiliteit, stevigheid, romigheid, vettigheid,...) van opgeklopte room. Vooral factoren die de melkvetkristallisatie, de olie-water grensvlakeigenschappen en de partikelgrootte van slagroom beïnvloeden, controleren de snelheid van partiële coalescentie.

In de praktijk wordt er naast natuurlijke room, dat rechtstreeks wordt afgeleid van verse koemelk, vaak gebruik gemaakt van gerecombineerde room. Dit wordt geproduceerd door het hersamenstellen van melkafgeleiden, doorgaans melkvet en melkpoeder met een laag vetgehalte, en door toevoeging van water. De voordelen van deze romen zijn enerzijds de flexibiliteit waarmee de samenstelling en formulatie kan gewijzigd worden naargelang de gewenste producteigenschappen en anderzijds de daling in kostprijs om de hoofdingrediënten te bewaren en te transporteren naar regio’s waar verse melk schaars is en/of waar geschikte opslagplaatsen voor verse zuivelproducten ontbreken. Desondanks blijken gerecombineerde opgeklopte romen ongewenste afwijkende fysicochemische en sensorische eigenschappen te vertonen in vergelijking met natuurlijke romen. Mogelijks is dit te wijten aan een verschil in vatbaarheid voor partiële coalescentie van de vetglobulen in beide romen. In de wetenschap dat monoacylglycerolen, als vetoplosbare oppervlakteactieve stof, het vermogen bezitten om de grootte van de vetglobulen, de olie-water grensvlakeigenschappen en het vetkristallisatiegedrag te beïnvloeden, kunnen monoacylglycerolen een rol spelen in het beheersen van partiële coalescentie en derhalve mogelijkerwijs in het verbeteren van de microstructurele opbouw van gerecombineerde opgeklopte room.

In dit manuscript staat doorgaans het begrijpen van de onderliggende fenomenen die van primordiaal belang zijn bij het opkloppen van room centraal. Meer specifiek werd in Deel I (Hoofdstuk 1-4) melkvetkristallisatie, het essentieel proces dat partiële coalescentie voorafgaat, behandeld en in Deel II (Hoofdstuk 5-9) werd er dieper ingegaan op de partiële coalescentie in room met en zonder luchtinslag. Doorheen beide delen werden de invloeden van monoacylglycerolen in kaart gebracht met als doel enerzijds inzicht te verwerven in het mechanisme die de gevolgen van het gebruik van monoacylglycerolen in room verklaren en anderzijds het vermogen na te gaan van deze oppervlakteactieve stoffen om de opklopeigenschappen van gerecombineerde romen te verbeteren.

Deel 1: Melkvetkristallisatie

Nadat in Hoofdstuk 1 een overzicht werd gegeven van de chemische samenstelling van melkvet, werden de algemene basisprincipes van vetkristallisatie in bulk en emulsies toegelicht. Vervolgens werden de invloeden van oppervlakteactieve stoffen op de algemene vetkristallisatie en de recente studies aangaande melkvetkristallisatie behandeld. Bijkomend werd een meer specifieke duiding betreffende de stand van zaken van de wetenschappelijke literatuur bij elk onderzoekshoofdstuk gegeven.

Daar veel experimenteel werk van Deel I gebaseerd is op eenzelfde gamma aan analytische procedures werd de beschrijving van de aangewende materialen en methoden gebundeld in een afzonderlijk hoofdstuk (Hoofdstuk 2). Het omvat (1) de gebruikte substraten, (2) een omschrijving van de technieken om de olie-water grensvlakspanning en de vetglobulegrootte te bepalen en (3) een beschrijving van de veelvoorkomende technieken aangewend in vetkristallisatieonderzoek. Hierbij werd voornamelijk aandacht besteed aan essentiële aanpassingen van de conventionele procedures die doorgevoerd werden om vetkristallisatie in room op een correcte manier te bestuderen.

In Hoofdstuk 3, het eerste experimentele hoofdstuk van Deel I, werd het mechanisme en de kinetiek van melkvetkristallisatie in bulk en in room in het algemeen en ook in natuurlijke en in gerecombineerde room vergeleken. Melkvet in bulk en in room vertoonden een vergelijkbaar kristallisatiemechanisme bij temperatuurscondities conform met de industrieel toegepaste condities bij de productie van slagroom (snelle koeling naar ± 5°C). Tijdens de koeling werden α-kristallen gevormd en groeiden vervolgens β’-kristallen ten koste van de α-kristallen. Een aantal α-kristallen hielden daarbij stand en postkristallisatie tijdens aanhoudende bewaring trad op. Het geobserveerde mechanisme werd verklaard door de vorming van mengkristallen. Niettegenstaande de gelijkenissen in het kristallisatiemechanisme was de algemene kristallisatiesnelheid van melkvet in beide romen vertraagd ten opzichte van bulk melkvet. Dit verschil werd verklaard door de algemeen aanvaarde nucleatietheorie in emulsies. Tevens week het kristallisatiegedrag van natuurlijke room af van dat van gerecombineerde room doordat de lipidensamenstelling van de individuele melkvetglobulen in natuurlijke room varieert. In deze studie resulteerde dit in een gewijzigde start en meer gespreide α-β’ polymorfe overgang en een lagere hoeveelheid vast vetgehalte in vergelijking met bulk melkvet. Bij gerecombineerde room werd dit niet vastgesteld.

In Hoofdstuk 4 werd de invloed van monoacylglycerolen die verschillen in ketenlengte en verzadigingsgraad van de veresterde vetzuren op de primaire kristallisatie van melkvet in gerecombineerde room ontrafeld. Dezelfde experimentele condities als in Hoofdstuk 3 werden aangewend. De langketen onverzadigde monoacylglycerolen vertoonden geen significante effecten op de primaire kristallisatie en het vast vetgehalte. Eenzelfde kristallisatiemechanisme als in de referentieroom (zonder monoacylglycerolen), zoals onderzocht in Hoofdstuk 3, werd vastgesteld. Gerecombineerde romen die langketen verzadigde monoacylglycerolen bevatten, vertoonden daarentegen een verschillend nucleatiemechanisme. Grensvlak gemedieerde heterogene nucleatie van melkvet grijpt plaats tijdens de koeling. Bijgevolg in aanwezigheid van de langketen verzadigde monoacylglycerolen startte de kristallisatie bij hogere temperaturen en werd de kristalgroei en de α-β’ polymorfe overgang versneld. Intermediair gedrag werd geobserveerd bij gebruik van monoacylglycerolen met een middellange ketenlengte. Zij versnelden de α-β’ polymorfe overgang terwijl geen significante invloed op de nucleatie en kristalgroei van melkvet vastgesteld werd. Dit intermediaire gedrag werd toegeschreven aan co-kristallisatie van de monoacylglycerolen eens de melkvetkristallisatie is geïnitieerd. Tevens werd er vastgesteld dat onafhankelijk van het type en de concentratie aan monoacylglycerolen het kristallisatiemechanisme na nucleatie en het finale vast vetgehalte na aanhoudende bewaring bij 5℃ voor vijf dagen niet werd beïnvloed.

Deel II: Partiële coalescentie

Een gedetailleerd kritisch literatuuroverzicht aangaande partiële coalescentie werd gegeven in Hoofdstuk 5. In het eerste gedeelte, na een korte beschrijving van de algemene fysische eigenschappen van room, werd het mechanisme van partiële coalescentie zonder luchtinslag beschreven. Vervolgens werden de factoren die de kinetiek van partiële coalescentie beheren uitvoerig besproken. In het tweede gedeelte werd de microstructuur van geslagen room en de rol van partiële coalescentie in de structuuropbouw tijdens opkloppen behandeld. Alsook werden de factoren die de opklopeigenschappen van slagroom beïnvloeden onder de loep genomen. Net zoals in Deel I werd elk onderzoekshoofdstuk in Deel II ingeleid met een meer specifiek overzicht van de stand van zaken aangaande de wetenschappelijke literatuur van toepassing.

De beschrijving van de materialen en methoden werd verzameld in één hoofdstuk (Hoofdstuk 6) daar al het experimenteel onderzoek in Deel II stoelt op dezelfde aangewende technieken en substraten. Aansluitend werd in Hoofdstuk 7 de toepasbaarheid van een reologische methode uiteengezet om de snelheid van partiële coalescentie bij een constante afschuifsnelheid in kaart te brengen. In combinatie met microscopische evaluaties en partikelgrootteanalyzes werd het bovendien mogelijk om meer inzicht te verwerven in het mechanisme van partiële coalescentie in de reometer. Een vergelijkende studie van de reologische techniek en de opklopeigenschappen van zuivelromen bij verschillende temperaturen, afschuifsnelheden en vetgehaltes toonde bovendien aan dat de reologische methode aangewend kan worden als een kwalitatieve voorspellende techniek voor de opklopeigenschappen.

De reologische methode, geïntroduceerd in Hoofdstuk 7, werd vervolgens gebruikt om de invloed van twee langketen monoacylglycerolen die verschillen in verzadigingsgraad op de partiële coalescentie in gerecombineerde room en op hun opklopgedrag te onderzoeken. Dezelfde monoacylglycerolen als in Hoofdstuk 4 werden gehanteerd. De onverzadigde monoacylglycerolen veroorzaakten een verhoging in snelheid van partiële coalescentie. Na opkloppen resulteerde dit enerzijds in een verlaagde opkloptijd en luchtinslag en anderzijds in een verhoogde stabiliteit en stevigheid van opgeklopte gerecombineerde room. De verzadigde tegenhanger ontketende daarentegen het omgekeerde effect. De vertraagde partiële coalescentie in gerecombineerde room met de langketen verzadigde monoacylglycerolen lokte een verhoogde opkloptijd en luchtinslag en een verlaagde stabiliteit en stevigheid uit. Deze uiteenlopende invloeden van de monoacylglycerolen op de gevoeligheid voor partiële coalescentie en op de opklopeigenschappen van gerecombineerde romen werden toegeschreven aan het verschil in gedrag van de monoacylglycerolen aan het olie-water grensvlak. Indien monoacylglycerolen in staat zijn te kristalliseren aan het grensvlak en daarbij heterogene nucleatie in melkvet berokkenen (tijdens de productie van gerecombineerde room) wordt het uitpuilen van kristallen vanuit de vetglobule in de waterige fase verhinderd. Dit doet bijgevolg de gevoeligheid van de vetglobulen voor de partiële coalescentie dalen. Monoacylglycerolen die zich gedragen als een vloeistof aan het olie-water grensvlak verhogen de vervormbaarheid van het grensvlak dat vermoedelijk het uitpuilen van de vetkristallen door het olie-water grensvlak bevordert. Dit veroorzaakte uiteindelijk de toegenomen gevoeligheid van de vetglobulen voor partiële coalescentie. In de beschouwde condities induceerden de gekristalliseerde langketen verzadigde monoacylglycerolen heterogene nucleatie terwijl de onverzadigde tegenhanger de vervormbaarheid van het grensvlak bevorderden.

Aansluitend op de fundamentele bevindingen aangaande de invloed van monoacylglycerolen op de melkvetkristallisatie (Hoofdstuk 4) en op de partiële coalescentie (Hoofdstuk 8) in gerecombineerde room, werd in Hoofdstuk 9 het potentieel van het gebruik van monoacylglycerolen om de opklopeigenschappen van gerecombineerde romen te verbeteren aangetoond. In een eerste luik werd proefondervindelijk bewezen dat een binair mengsel van elkaar tegenwerkende monoacylglycerolen verbeterde opklopeigenschappen bewerkstelligen ten opzichte van romen waarbij één van deze twee monoacylglycerolen afzonderlijk werd aangewend. Het tweede luik van Hoofdstuk 9 illustreerde hoe monoacylglycerolen die het vermogen bezitten om partiële coalescentie te versnellen de opklopeigenschappen kunnen verbeteren van slagroom met een verlaagd vetgehalte.

OUTLINE OF THE RESEARCH

Dairy cream is a fat-rich fluid milk product and can, from a microstructural point of view, be described as an oil-in-water emulsion. The so-called milk fat globules in natural cream, derived from fresh milk, contain the milk lipids and are dispersed in a continuous aqueous skimmed milk phase. Cream can be further processed into whipped cream which is a rigid dairy foam that is often used as a topping for desserts, cakes and ice creams. During whipping air is introduced in the cream and the milk fat globules destabilize by means of a partial coalescence mechanism due to the presence of fat crystals in the milk fat globules and due to the applied mechanical agitation. As a result, whipped cream consists of a network of partially coalesced fat globules which immobilizes the air bubbles and the cream serum phase. In order to obtain a high-quality whipped cream with desired physicochemical and sensory properties (overrun, stability, firmness, creaminess, etc.), the partial coalescence rate needs thus to be controlled and a substantial amount of fat seems to be indispensible. However, it is assumed that a substantial fat reduction is feasible when an efficient arrangement of the fat globules in whipped cream can be achieved by controlling the partial coalescence rate.

From literature, it is clear that monoacylglycerols (MAGs), being small-molecule surfactants, may govern partial coalescence rate and, hence, the whipping properties of cream. MAGs are known to change the fat globule size, the oil-water interfacial properties and the fat crystallization behavior of oil-in-water emulsions which all influence the partial coalescence rate (Figure 1). Note that these factors strongly interact with each other. However, an elaborate study that surveys the effect of different types of MAGs and a well-funded mechanistic explanation of their effects in dairy whipping cream is still lacking.

Hence, MAGs are extensively examined in this manuscript. The obtained fundamental insights aim at providing knowledge on how the MAGs can be used in a proper way for custom made whipped creams. In this study, recombined cream was chosen as a substrate to elucidate the effects of MAGs since it provides more flexibility to control the physical properties of creams. Recombined creams are made by recombining milk products, mostly milk fat and a low-fat milk powder, with water. An additional benefit of using recombined creams is that their starter materials can easily be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce. Nevertheless, whipped recombined cream compared with whipped natural cream seems to have unfavorable divergent physicochemical properties (i.e. lower stability and overrun). Therefore, the differences between natural and recombined cream are also addressed in this research.

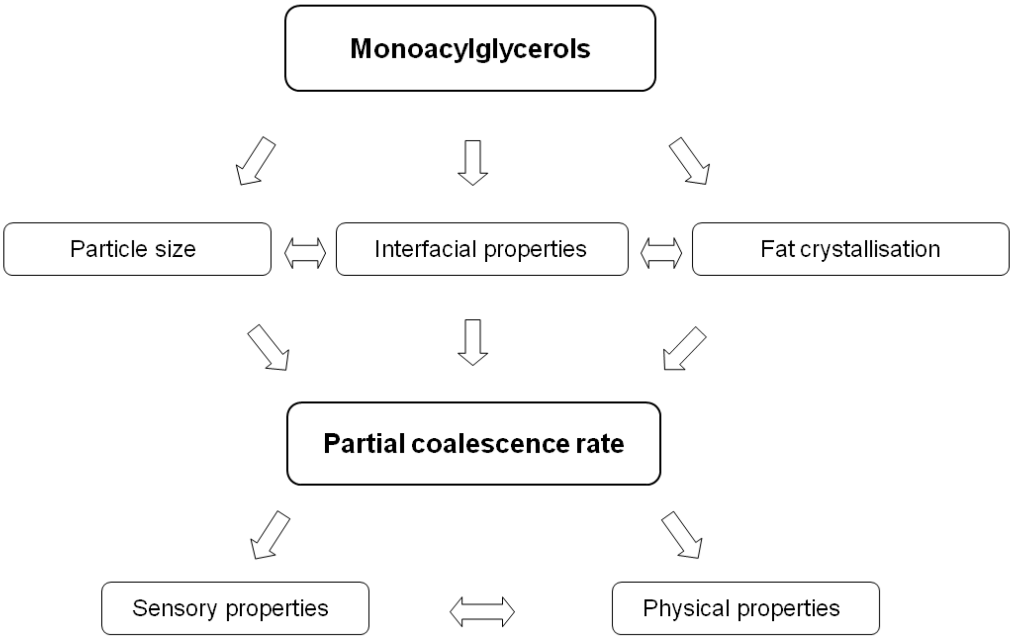


Figure 1 Schematic presentation of how monoacylglycerols may affect partial coalescence rate and, hence, the sensory and physical properties of whipped cream.

This manuscript consists of two parts. Part I deals with the milk fat crystallization which is indispensable for partial coalescence to occur. In Part II partial coalescence with and without air inclusion is extensively addressed. Furthermore, with respect to using MAGs in cream, the effects of MAGs on the milk fat crystallization behavior (Part I) is linked with the effects on partial coalescence rate and, hence, on the whipping properties (Part II). Figure 2 summarizes schematically the structure of this research.

Part I: Milk fat crystallization

Chapter 1 provides a literature review which summarizes the chemical composition of milk fat and the general principles of fat crystallization in bulk and in emulsified state. In addition, a critical review of researches dealing with the general effects of small-molecule surfactants on fat crystallization and of recent studies on milk fat crystallization are included in this literature review. After the description of the materials and methods applied (Chapter 2) in Part I, the obtained research results are handled. In Chapter 3, the milk fat crystallization behavior in bulk and emulsified state are investigated. Furthermore, attention is drawn to the differences between milk fat crystallization in natural and recombined cream. Chapter 4 uses the knowledge acquired in Chapter 3 to study the effect of three types of MAGs which differ in chain length and degree of saturation on the milk fat crystallization in recombined cream.

Part II: Partial coalescence

Chapter 5 outlines a literature review on partial coalescence in oil-in-water emulsions and in whipped cream and critically summarizes the factors governing the rate of partial coalescence with and without air inclusion. Chapter 6 lists the materials and methods used in part II and Chapter 7 introduces a rheological method to study the partial coalescence rate in dairy whipping cream. The applicability of the introduced rheological method is valorized by linking the influence of temperature, shear rate and fat content on the partial coalescence rate in natural cream with their effect on the whipping properties of natural cream. Chapter 8 addresses the effects of MAGs on the partial coalescence rate and the whipping properties. Based on the findings in Chapter 4 and the examined physicochemical properties of the recombined creams and of their oil-water interfaces a mechanistic explanation is put forward explaining the effects of MAGs on partial coalescence and, hence, on the whipping properties. Chapter 9 uses the research results attained in Chapter 7 and Chapter 8 to explore the potentials of MAGs to improve the whipping properties of recombined cream. Two case studies are addressed. Firstly, the applicability of a binary mixture of MAGs to improve whipping properties is examined and, secondly, the use of MAGs to achieve a substantial fat reduction in whipped cream products with an acceptable overrun and stability are probed.

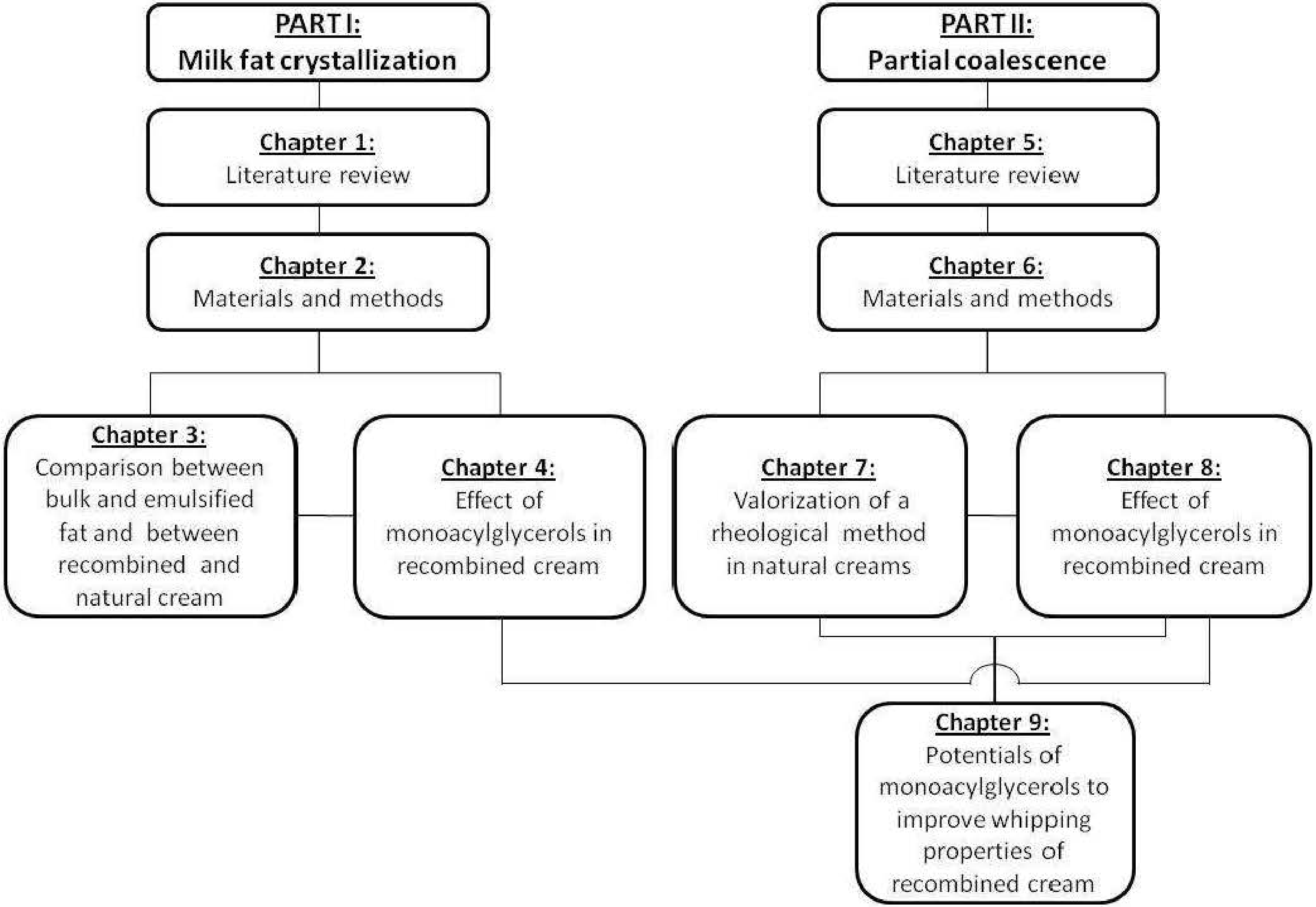

PART I:
Milk fat crystallization
Chapter 1:
Literature review
Chapter 2:
Materials and methods
Chapter 3:
Comparison between bulk and emulsified fat and between recombined and natural cream
Chapter 4:
Effect of monoacylglycerols in recombined cream
PART II:
Partial coalescence
Chapter 5:
Literature review
Chapter 6:
Materials and methods
Chapter 7:
Valorization of a rheological method in natural creams
Chapter 8:
Effect of monoacylglycerols in recombined cream
Chapter 9:
Potentials of monoacylglycerols to improve whipping properties of recombined cream

Figure 2 Outline of the research.

PART I: MILK FAT CRYSTALLIZATION

1. LITERATURE REVIEW

1.1. Introduction

Cream is a fat-rich fluid milk product derived from physical separation of milk into skimmed milk and cream. From a microstructural point of view, cream can, similar to milk, be described as an oil-in-water emulsion. The so-called milk fat globules, which contain the milk lipids, are dispersed in a continuous aqueous skimmed milk phase. This general description refers to cream that is made directly from fresh cow's milk and is indicated as *natural cream* in this manuscript. Nowadays, besides natural cream often *recombined* cream is used which is prepared by recombining milk products, mostly milk fat and a low-fat milk powder, with water. The benefits of using recombined cream are that the composition and formulation can easily be modified for product development goals and that the raw materials can efficiently be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce.

Milk fat is one of the main constituents of milk, cream and their derivatives. It mainly consists of a complex mixture of triacylglycerols (TAGs) which may solidify in well-arranged crystalline structures. These shaped milk fat crystals determine to a large extent the physicochemical and sensory properties of primarily the fat-rich dairy products.

The following literature review is intended to summarize the general aspects of the chemical composition of milk fat and the common principles of fat crystallization in bulk and in emulsified state. In addition, the latter includes a critical review of researches dealing with the common effects of small-molecule surfactants on fat crystallization and of recent studies on milk fat crystallization.

1.2. Chemical composition of milk fat

As for most other food fats, the main components of bovine anhydrous milk fat are triacylglycerols (TAGs) (95 - 98 wt%). The minor components of the milk lipids include mainly diacylglycerols (DAGs) (0.3 - 1.6 wt%), monoacylglycerols (MAGs) (0.016 - 0.038 wt%), phospholipids (PLs) (0.8 - 1.0 wt%), sterols (0.22 - 0.41 wt%) and free fatty acids (FFA) (0.1 - 0.44 wt%) [1-3].

Below the general aspects of the major and minor components and the influencing factors governing their content are briefly summarized. For more detailed information on these chemical aspects of milk fat the reader might consult the extended review by Jensen [4].

1.2.1. Major components

TAGs consist of three fatty acids (FAs) esterified on a glycerol backbone. Milk fat TAGs can contain over 250 [1] to 400 [4, 5] different FAs. The broad range of FAs that mainly differ in chain length and degree of saturation contributes to the wide melting range of milk fat. Table 1.1 gives an overview of the major FAs present in milk fat expressed in wt%. The FAs have primarily an even carbon number and are generally linear and saturated. The most abundant FA is palmitic acid (C16:0) followed by oleic acid (C18:1). Characteristic for milk fat is the significant proportion of short-chain FAs (C4 to C6), *trans* FAs, like vaccenic acid (C18:1 *t*11) [6] and conjugated linoleic acid (CLA) (C18:2 *c*9*t*11) [7]. The short-chain FAs are synthesized in the mammary gland of the cow and, with respect to the physical properties, they determine to a large extent the distinctive crystallization and melting behavior of milk fat. The presence and the amount of CLA and *trans* FAs is determined by both the microbial isomerization and biohydrogenation process of dietary unsaturated FAs in the rumen and the activity of Δ^9-desaturase in the mammary gland [7, 8]. Note that the concentrations of branched [9], hydroxyl [5], keto [10] and odd chain length FAs are higher in dairy fats compared to vegetable fats.

Table 1.1 Nomenclature and composition of the major FAs in milk fat, adapted from [4, 11].

Short notation (Cx:y)[1]	Triavial name	Average range (wt%)
C4:0	Butyric	2 - 5
C6:0	Caproic	1 - 5
C8:0	Caprylic	1 - 3
C10:0	Capric	2 - 4
C12:0	Lauric	2 - 5
C14:0	Myristic	8 - 14
C15:0	Pentadecanoic	1 - 2
C16:0	Palmitic	22 - 35
C16:1	Palmitoleic	1 - 3
C17:0	Margaric	0.5 - 1.5
C18:0	Stearic	9 - 14
C18:1[2]	Oleic	20 - 30
C18:2	Linoleic	1 - 3
C18:3	Linolenic	0.5 - 2

[1] where x is the number of carbon atoms in the FA and y is the number of double bonds in the FA

[2] Includes about 3% *trans* isomers [12]

On the glycerol backbone the FAs are not randomly distributed. The short-chain FAs are, for instance, primarily esterified on the sn-3 position [4]. Although this somewhat stereospecific distribution of the FAs, an identification of all milk fat TAGs is not feasible due to the highly complex FA-pattern. Milk fat may contain several thousands of TAGs, most in traces [4]. Therefore the chemical composition of different milk fats is generally evaluated using the FA-profile.

1.2.2. *Minor components*

All non-TAG components present in milk fat can be defined as minor components. The concentrations of these components are dependent on the definition of milk fat [2]. When milk fat refers to the apolar phase of milk, the concentration of the sterols and PLs will be higher than when milk fat is defined as, the industrial product, anhydrous milk fat (AMF). The latter is the fat phase gained from butter after several centrifugal separation steps. PLs and sterols which are mainly originally located at the milk fat globule membrane (MFGM) of milk can, though at lower concentrations, be found in AMF due to its incomplete exclusion as a result of their solubility in the TAG-mixture.

In bovine milk, the PLs amount to about 1 wt% of the total milk lipids. The main PLs are phosphatidylcholine (25.4%), phosphatidylethanolamine (37.2%), phosphatidylserine (2.8%) and sphingomyelin (23.6%) [4, 13]. Their FA-composition differs from that of the TAGs in milk fat. More long-chain unsaturated FAs and no short-chain FAs are detected [14].

FFAs, MAGs and DAGs are present because of the unfinished synthesis of TAGs or due to the action of lipases. An elevated FFA-content, in particular when the short-chain FFA-content is increased, contributes to a rancid off-flavour of butter [15].

Furthermore, the unsaponifiable fraction of milk fat includes sterols, tocopherols, hydrocarbons, carotenoids, aliphatic alcohols, squalene and fat soluble vitamins (A, D, E and K). Most of the sterols are cholesterol (>95%), although traces of lanosterol, dihydrolanosterol and β-sitosterol are also present [14].

1.2.3. *Factors governing the fat composition*

The chemical composition of milk fat is primarily influenced by the feed, the breed (genetics), the stage of lactation (physiological condition) and possible udder infections (mastitis) of the cow [16]. When all these factors are kept constant differences in milk fat composition between the different individuals can still be noticed [1]. In the dairy industry milk is pooled before further processing to limit variation in milk fat composition.

The most important aspect causing the chemical FA-variability is the feed factor which can be easily illustrated by the differences observed between summer and winter milk fat [17]. The switch from stall feed (silaged feed) in the winter to fresh grass during the summer months causes an increase in the amount of unsaturated FAs in milk fat and, hence, a change in physical properties of mainly the fat-rich dairy products, like butter. For instance, summer butter is softer than winter butter. Also *trans* FAs are strongly subjected to seasonal variation of the feeding [4]. Besides the seasonal variation in the feeding, many studies demonstrate intentional manipulations of the FA-composition by feeding the cow with supplements of various fats and oils, as reviewed by Ashes et al. [18]. Next to the chemical composition of the supplements, its physical appearance may also contribute to a higher proportion of unmodified dietary FAs available for milk fat synthesis in the udder [19-21]. Dietary fats present in a specific matrix may escape biohydrogenation in the rumen of the cow. Moreover, several minor components, such as carotene and fat-soluble vitamins, are strongly affected by their content in the feed.

The breed of the cow is mainly selected by man according to the intended use and the local conditions, such as climate, feed, terrain and customs resulting in variability in milk composition. Regarding the lactation period, the proportion of short-chain FAs is, for instance, low at the start and increases during the first weeks of the lactation period [4, 16].

1.3. Fat crystallization

Crystallization of milk fat is a complicated phenomenon given that it consists of a mixture of a very broad range of TAGs and small quantities of other components, as has been shown in Section 1.2. During processing and subsequent storage of dairy products, the shaped fat crystals create a fat crystal network which defines the characteristic physical and sensory properties of predominantly the fat-rich dairy products [1, 19]. For instance, in butter the fat crystal network properties determine greatly its consistency. It has to be high enough to withstand collapse under its own weight and low enough to have a spreadable butter and to avoid a waxy mouth feel. Furthermore, the fat crystal network in butter holds the liquid oil in the matrix, preventing oiling-off, and provides stability to the water droplets against coalescence. When milk fat is dispersed in fat globules, a space-spanning network is formed inside the fat globule which is of importance for its stability towards partial coalescence and, hence, for the manufacturing of butter, whipped cream and ice cream. Partial coalescence is extensively addressed in Chapter 5.

The general processes involved in the development of a fat crystal network are schematically presented in Figure 1.1. TAGs may nucleate upon supercooling (cooled below their melting point) and, subsequently, primary crystals grow and develop a specific morphology and size. Upon further processing, when a certain solid fraction is reached, fat crystals may start to aggregate due to van der Waals attractions [20, 21]. This aggregation process proceeds until a continuous three dimensional network is formed. Note that in fats, once nucleation is achieved, these events are usually not chronological [22, 23]. Nucleation and crystal growth may still go on and new crystal aggregates will be shaped which tend to fill the pores in the existing network. Moreover, parts of the crystallizing molecules are deposited onto the existing crystals and can cause sintering, i.e. the formation of solid bridges between of the aggregated crystals and aggregates [1]. Eventually, a firm network may be formed with small pores. Upon storage the fat crystal network properties can be largely affected due recrystallization [20].

In this section some general concepts of bulk fat primary crystallization and its controlling factors are first introduced. Secondly, the differences in crystallization behavior between bulk and emulsified fat are touched. Thirdly, the potentials of small-molecule surfactants as crystal structure modifiers are discussed and, finally, recent researches on milk fat crystallization in bulk and cream systems are briefly summarized.

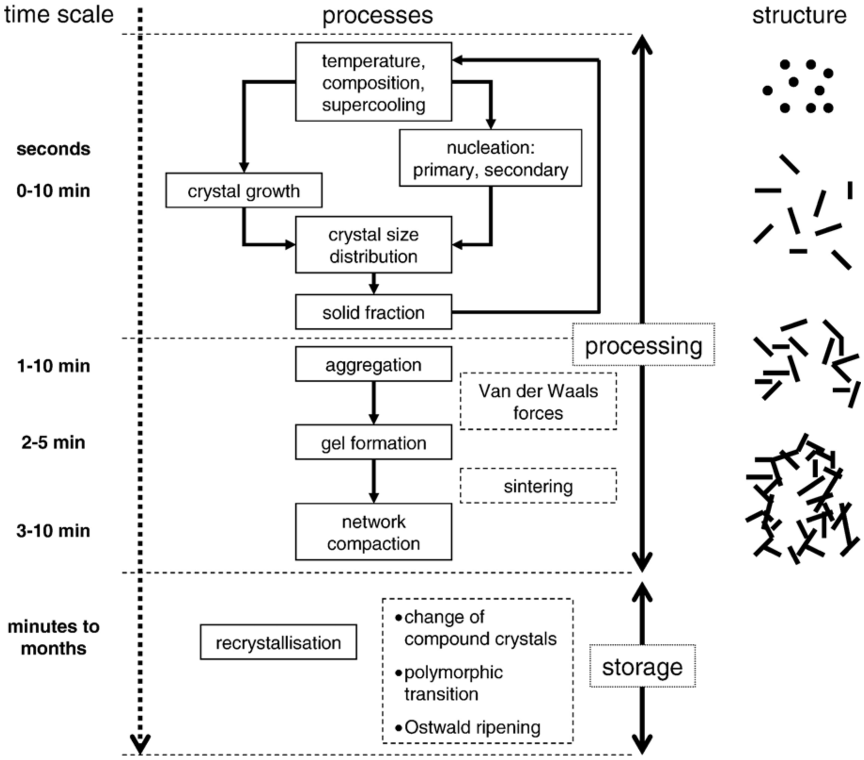

time scale
processes
structure
temperature, composition, supercooling
nucleation: primary, secondary
crystal growth
crystal size distribution
solid fraction
seconds
0-10 min
1-10 min
2-5 min
3-10 min
minutes to months
aggregation
Van der Waals forces
gel formation
sintering
network compaction
processing
recrystallisation
•change of compound crystals
•polymorphic transition
•Ostwald ripening
storage

Figure 1.1 Schematic presentation of the processes involved in fat crystallization [20].

1.3.1. General principles of primary crystallization

Primary crystallization includes the generation of sufficient thermodynamic driving force, the nucleation and the crystal growth. Furthermore, the TAGs may crystallize in different polymorphic forms and compound crystals may be created during primary crystallization.

This section introduces some general principles and vocabulary and includes a brief discussion about process parameters controlling the rate of the various involved crystallization processes. More information on the fundamental thermodynamical aspects of lipid crystallization can be found in elaborated high-quality reviews by Boistelle [24], Garside [25], Aquilano and Sgualdino [26], Sato [27], Himawan et al. [28] and Marangoni [29].

1.3.1.1. Thermodynamic driving force

For all crystallization processes, the driving force is the difference in chemical potential $\Delta\mu$ ($J.mol^{-1}$) (or partial molar Gibbs free energy) between the liquid and the solid phase. In lipids, the crystallization can be considered as from solution. The lower melting TAGs, thereby, act as a solvent for the higher melting ones, the solutes. Hence, crystallization can be achieved when the system is supersaturated: the concentration of the solute C exceeds the saturation concentration C_s. At a given temperature (T) the difference in chemical potential can be written as:

$$\Delta\mu = R_{\mathrm{g}} T \ln \frac{C}{C_s} \tag{1.1}$$

with R_g the universal gas constant ($8.314\ J.K^{-1}.mol^{-1}$) and T the absolute temperature (K).

In addition, fat crystallization can also be considered from the melt. In the molten state above the melting temperature of the highest melting TAGs, the interaction between individual TAGs is too low to counteract the thermal Brownian motion. However, upon cooling the thermal motion is decreased and the interaction between the TAGs, i.e. van der Waals forces, may exceed the kinetic energy of the TAGs. The thermodynamic driving force for nucleation from the melt is, therefore, proportional to the temperature difference between the actual temperature and the melting temperature (T_m). This temperature difference is called supercooling and, occasionally, undercooling. The difference in chemical potential ($\Delta\mu$) can be written as:

$$\Delta\mu = \Delta H_{\mathrm{m}} \frac{T_m - T}{T_m} \tag{1.2}$$

with ΔH_m the melting enthalpy.

1.3.1.2. Nucleation

When the driving force is large enough, nucleation may occur via bimolecular interactions which lead to the formation of ordered domains [21]. Three types can be distinguished: primary nucleation either homogeneous or heterogeneous and secondary nucleation.

TAGs are flexible molecules that have to assume a specific conformation to be stable in a nucleus. This is a relatively slow process giving rise to the existence of a metastable region [29]. Figure 1.2 presents the thermodynamical limits at which each type of nucleation will occur with respect to the solubility curve.

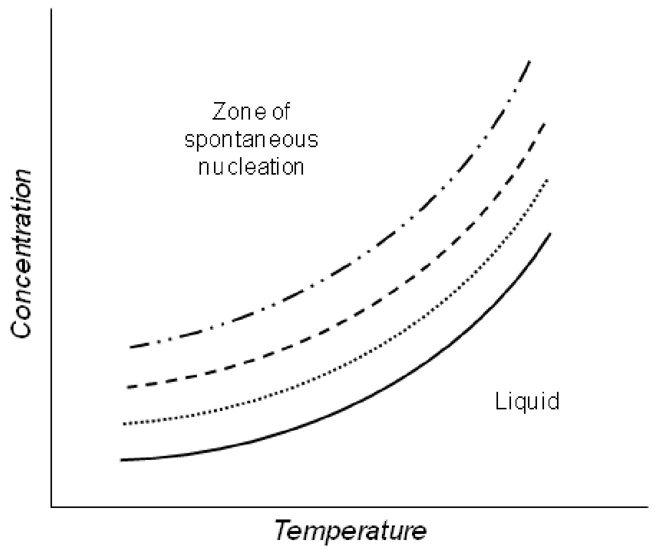


Figure 1.2 Summary of the thermodynamical limits for the different types of nucleation with respect to the solubility curve (_______): primary homogeneous (▬ · · ▬), primary heterogeneous (- - - - -) and secondary nucleation (……….), after [21, 30].

Primary homogeneous nucleation occurs without catalysis through a foreign surface. Therefore, severe supersaturation or supercooling conditions are required. Kloek [21] reported that a supercooling of 30K is essential for homogeneous nucleation. However, a spontaneous occurrence of homogeneous nucleation rarely occurs in natural bulk fat systems. Before proper supercooling for homogeneous nucleation is achieved, foreign surfaces may induce *primary heterogeneous nucleation* at a supercooling of 1-3K [25]. These foreign surfaces, often referred to as impurities, may act as catalytic nucleation sites by lowering the activation free energy. The effectiveness of the foreign surface largely depends on the match between the structure of the nucleating site and the crystal lattice. Examples of catalytic impurities are crystallizer walls, blades of impellers, mono- and diacylglycerols, other minor polar lipids or surfactants and even dust particles [23, 31].

Once primary nucleation, whether homogeneous or heterogeneous, has occurred it is easy to imagine a catalyzing effect of the already present fat crystals to create new nuclei. This phenomenon is called *secondary nucleation*. Small pieces of growing crystals are removed from the surface facilitating the new crystal formation elsewhere. Secondary nucleation occurs mainly if the TAG-composition is broad [32, 33] and is highly relevant for industrial applications [22, 24, 31]. For instance, it is the underlying mechanism of crystal seeding. Kloek [21] and Garside [25] distinguished three types of secondary nucleation: true, apparent and contact. True secondary nucleation occurs when nucleation takes place in the vicinity of growing crystals (not on their surface) while apparent secondary nucleation occurs at fragments of growing crystals that are washed off by flow along the crystals and act as nuclei. Contact secondary nucleation results from the collision of crystals, crystallizer walls, impellers, etc.

1.3.1.3. Crystal growth

Fat crystal growth involves both the diffusion of TAGs from the bulk solution across a boundary layer and the incorporation of TAGs into the crystal lattice of an existing nucleus or crystal [29]. However, TAGs can also become detached. The growth rate is therefore determined by the resultant of attachment and detachment of TAGs to the crystal surface [33].

The shape of the crystal surface plays a critical role in the crystal growth rate [24, 28]. Generally, three distinct surfaces in order of growth rate are described: flat, corrugated and kinked surfaces. However, in the last decades, growth rate is mainly described based on two types of surfaces: smooth and rough surfaces [23, 28]. Smooth surfaces are characterized by an atomically immediate change in the degree of crystalline order across the solid-liquid boundary, while rough surfaces are structurally diffuse, with the crystalline degree varying continuously over the scale of a few atomic planes across the solid-liquid boundary. The growth rate at the rough surface is faster because the attachment involves contact with more molecules than in smooth surfaces which involves contact with only one molecule. The more contact sites, the higher the ’sticking probability’. Crystal growth at a smooth surface occurs via a 2D-nucleation mechanism [33].

Next to the internal factors of the crystals (structure, bonds and defects) also external factors such as viscosity, temperature and composition of the liquid phase play a role [24, 26]. The composition and the temperature determine the supersaturation and/or supercooling of the TAGs and, thus, the difference in chemical potential between the crystal and the solution or the melt which is positively related with the crystal growth rate [33]. An increased viscosity (i.e. decreasing temperature) can decrease the transport of TAGs to the crystal surface and the crystal growth rate will, thus, be diffusion limited. Diffusion limited crystal growth rate can be easily shown by stirring the fat system. If the growth rate is then enhanced, it is diffusion limited in quiescent conditions. In addition, higher viscosity implies the slower dissipation of the crystallization heat away from the growing crystal [29]. Viscosity is, hence, inversely related to the crystal growth rate.

Crystal growth rate is usually a very slow process in natural fats [33]. For each large and flexible TAG-molecule it takes a long time before the right position and conformation are obtained to fit into the crystal lattice of a growing crystal. Moreover, fat is a multicomponent system in which several TAGs may be supersaturated and go in competition with the same nucleating site [29].

Compared to nucleation, the driving force required for crystal growth is lower than for nucleation [29]. Therefore, at higher supersaturation or supercooling, nucleation will dominate resulting in a large number of nuclei and, hence, crystals [34].

1.3.1.4. Polymorphism

Polymorphism is the phenomenon whereby the molecules with the same chemical composition can crystallize in different unit cell structures. Upon melting the same identical liquid phases are obtained. However, the different polymorphic modifications have different physical properties such as melting point, density, melting/crystallization heat,…

A. Polymorphism based on subcell packing

Larsson [35] classified the basic polymorphs of TAGs into three different types according to their hydrocarbon subcell packing: α, β’ and β. A subcell is the smallest unit of repetition along the FA-chain axis and their packing is characterized by their interchain distances. These distances or ‘short spacings’ are independent of the chain length of the TAGs and can be measured by wide-angle X-ray diffraction ($8.5 < \theta < 13°$) [36-38]. Figure 1.3 shows a schematic representation of a cross-sectional view of the subcell of the three basic polymorphs.

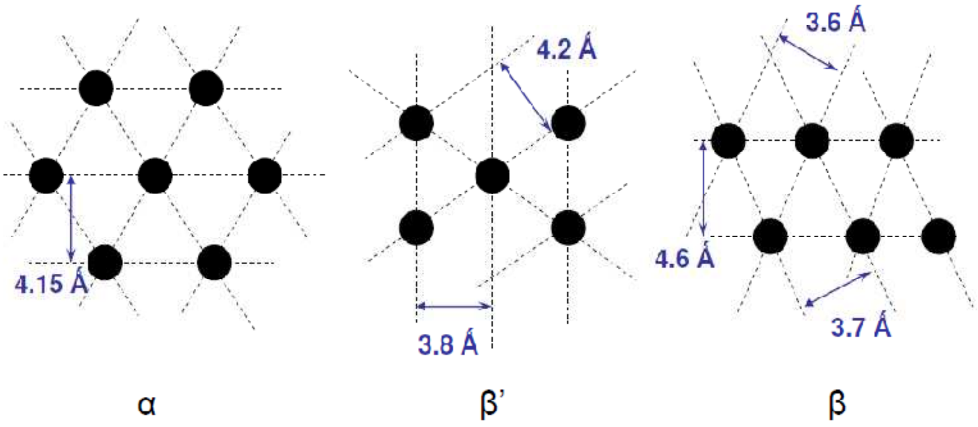


Figure 1.3 Cross-sectional view of the subcell packing of different polymorphs in which the lengths of short spacings are indicated [30].

Furthermore, the orientation and degree of freedom of the FA-chains differ between the three basic polymorphs. In the crystal lattice of an α-polymorph the hydrocarbon chains are positioned perpendicular to the methyl end group plane and are assumed to oscillate and rotate with a high degree of molecular freedom while in the β’- and β-crystals they are tilted with respect to the methyl end group plane (angle between 50-70°) and exhibit less oscillatory freedom. In the β'-polymorph the hydrocarbon chain of the adjacent zigzag FA-chains are in different planes whereas in the β-polymorph all zigzag FA-chains are positioned in the same plane.

The three different basic polymorphs differ in stability, melting point, melting enthalpy and density. As a direct result of the more rigid packing, the β-polymorph has the lowest Gibbs free energy and is, thus, the most stable one and shows the highest melting point, melting enthalpy and density. The α-polymorph has the highest Gibbs free energy and is, hence, the least stable polymorph and reveals the lowest melting point, melting enthalpy and density. The β'-polymorph shows intermediate behavior [31]. Table 1.2 summarizes the subcell packing, the main short spacings and physical properties like melting point and enthalpy of fusion of the three basic polymorphs of tristearin.

Table 1.2 Some properties of the three basic polymorphic forms in tristearin, adapted from [1, 33].

Properties	**Polymorphic form**		
	α	**β'**	**β**
Subcell packing	Hexagonal	Orthorhombic	Triclinic
Main short spacing(s) (Å)	4.15	3.8 and 4.2	4.6
Melting point (°C)	55	63	73
Enthalpy of fusion (kJ.mol^{-1})	110	150	546

B. *Polymorphism based on longitudinal stacking*

TAG-crystals are made by the stacking of TAG-layers. The thickness of one crystal layer strongly depends on the number of FA-sublayers. There can be 2 (2L) or 3 (3L) sublayers which correspond with a layer thickness or 'long spacing' of 40-50 Å and 55-75 Å, respectively, as shown in Figure 1.4. Long spacings can be measured by small-angle X-ray diffraction ($0° < \theta < 5°$). Roughly, 2L-structures are generated mostly by long-chain, high-melting, trisaturated TAGs or TAGs with a very similar FA-moiety, whereas 3L-forms are usually related to low-melting, long-chain monounsaturated and/or mixed long- and short-chain TAGs because of chain sorting. The differing chains may segregate into a specific layer [27, 28, 39]. Moreover, the layer thickness in TAG-crystals depends on the chain length and the degree of saturation of the FA-chains and the angle of tilt of the TAG-molecules with respect to the methyl end group plane.

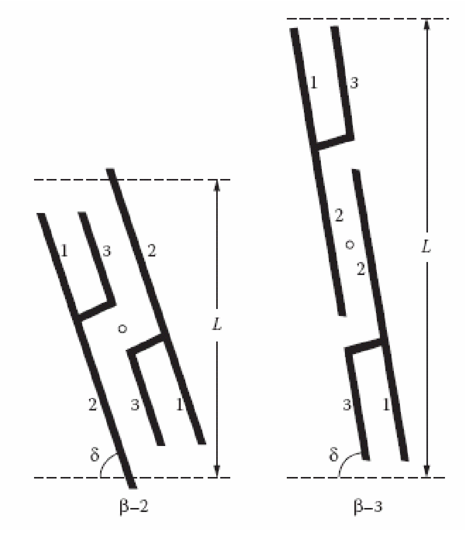


Figure 1.4 Double (2L) (at the left side) and triple chair (3L) (at the right side) arrangement of TAGs in the β-polymorph with L the layer thickness (or long spacings) and δ the angle of tilt [31].

C. *Polymorphic evolutions*

The polymorphs in natural fat are monotropic modifications [40]. Regardless of the conditions, the β-polymorph is more stable than the other basic polymorphs and transformations will only take place in the direction of the more stable form.

When TAGs are cooled to a temperature at which several polymorphs may exist, the crystallization will start in the least stable polymorph [41]. For instance, below the melting point of the α-polymorph, the crystallization will start in the α-polymorph although the supercooling to create the β and β'-crystals is higher because of their higher melting point. The more rapid formation of the least stable polymorph is caused by the differences in activation energy between the different polymorphs. The α-polymorph has a lower activation energy. Due to the high flexibility and loose packing of the FAs in the α-crystal the thermal activation processes involved in the rearrangement are minimized. The more stable polymorphic modifications have a more rigid packing [42]. Hence, these forms show higher activation energies and more difficulties to nucleate. Upon storage or heating the more stable forms may arise by means of a polymorphic transition, as shown in Figure 1.5. Note that these transformations can occur within the solid phase or by melting and recrystallization.

At proper conditions, all three basic polymorphs can be crystallized directly from the melt. However, at temperatures just below the melting points of the stable crystals, they do not readily form. The crystallization is delayed (increased induction time) because there is more time required to organize the molecules in the more rigid packing.

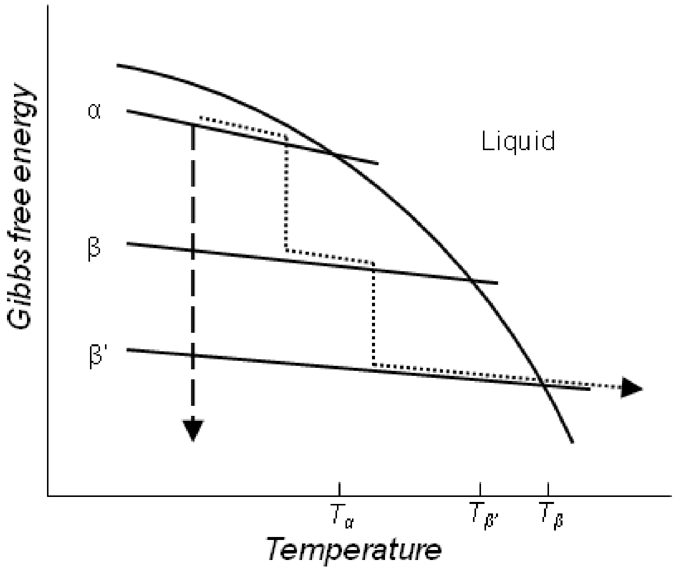


Figure 1.5 The Gibbs free energy - temperature relation of the three basic polymorphs (α, β and β’) and their possible evolution during storage (– – – – –) and heating (................), after [43]. With T_α, T_β and $T_{\beta'}$ the final melting temperature or clear point of the α, β and β’-polymorph, respectively.

1.3.1.5. Compound crystals

Compound (or mixed) crystals contain two or more different TAG-molecules that are not identical, but have a very similar shape and configuration [33]. The driving force for compound crystallization is that the total supersaturation of molecules that can form a compound crystal is much higher than that of each of the individual TAG-molecule separately [1, 33].

TAGs may readily form compound crystals in the α-polymorph. In this modification the FA-chains appear to oscillate and rotate with a considerable molecular freedom. The formation of mixed crystals will, therefore, hardly disturb the α-crystal packing. In the β’ polymorph, and even more in the β-form, the compound crystal formation is restricted to small groups of similar TAGs due to their denser crystal packing [33, 44].

The compound crystal formation depends greatly on the degree of supercooling. At a greater degree of supercooling more low-melting TAGs can be incorporated in the crystal lattice of the high-melting TAGs.

Extensive compound crystal formation occurs in milk fat and may result in [1, 33]:

- A narrowed melting range
- Higher solid fat content. Even cooling to a lower temperature before bringing to the final temperature gives higher solid fat contents than direct cooling to the latter.
- Longer persistence of metastable polymorphs

1.3.1.6. Factors affecting primary crystallization

In a bulk fat system, without changing its composition, the fat crystallization mechanism and kinetics will mainly be defined by the applied time-temperature (*t,T)* program, including the actual temperature, cooling rate and storage time, and whether or not agitation is carried out.

Form equation 1.1 and 1.2, it can be deduced that the actual temperature stipulates the degree of supersaturation and supercooling. As a consequence, the lower the temperature, the higher the driving force for nucleation to take place. Compared to nucleation, the driving force required for crystal growth is lower than for nucleation. Therefore, at higher supersaturation or supercooling, nucleation will dominate resulting in a large number of nuclei and, hence, smaller crystals. At lower supercooling or supersaturation crystal growth is favored resulting in fewer large crystals [34]. In addition, since natural fats are multicomponent TAG-systems, more TAGs will be supersaturated or supercooled with decreasing temperature [33]. This implies above all an increased solid fat fraction but also an increased compound crystal formation.

At a fast cooling rate, nucleation is forced to take place in a short timescale [19]. Hence, nucleation only happens at severe decreased induction times (i.e. increased nucleation rate) and, thus, at higher supercooling. Consequently, the right TAG-conformation is more readily attained and compound crystals are favored. The latter involves an increased solid fat content as has been shown by Herrera and Hartel [34]. Furthermore, the cooling rate also sets the polymorphic modification of the shaped TAG-crystal. If the time spent between the clear/melting points of the stable and the metastable polymorph is shorter than the induction time of the stable polymorph, the TAG-crystals will solidify below the clear point of the metastable polymorph in this less stable conformation [41]. The latter has a lower induction time because of its lower activation energy, although it has a lower degree of supercooling [42]. As a result, at a high cooling rate crystallization starts at severe supercooling resulting in a large number of small compound crystals in a metastable polymorphic modification. The reverse is true for low cooling rates.

Upon storage, as shown in Figure 1.1, aggregation of fat crystals and sintering between crystals and aggregates takes place. Moreover, recrystallization involving Ostwald ripening, changes in polymorphism, composition and morphology of the crystals may occur [20]. Recrystallization is in particular pronounced when compound crystals are shaped.

For given *t,T*-conditions, the mass transfer and heat transfer will be highly dependent on whether or not mechanical agitation is applied. If agitation is applied the primary crystallization is mainly accelerated and the crystal sizes and the final fat crystal network properties may be affected [45-49]. At low shear rates agglomeration can take place whereas at higher shear rates smaller crystals may be formed. Smaller crystals are formed due to an enhanced nucleation and/or breakdown of the crystals and their aggregates by the impeller [20]. In addition, crystals may also show orientation at severe agitation conditions [45].

1.3.2. Bulk versus emulsified fat

In oil-in-water emulsions the fat is subdivided into numerous small particles. As a result, new phenomena arise, causing the fat crystallization to be more complex than in bulk fat systems. In particular the nucleation mechanism may strongly deviate. Regarding heterogeneous nucleation, three types can be distinguished (Figure 1.6) [50]:

- **Volume heterogeneous nucleation**: In bulk fat systems heterogeneous nucleation is common due to the presence of guest molecules or foreign surfaces (Section 1.3.1.2). During emulsification these catalytic impurities are, like the fat, partitioned [33, 51, 52]. The number of impurities per droplet may thereby vary between many per droplet to approximately zero depending on particle size. Consequently, an increased supercooling is generally observed and the kinetics will initially be proportional to the volume of the droplets, and, thus, to its cube diameter [52]. Note that when the number of droplets exceed the number of impurities or when the bulk mass is impurity free, the fat crystallizes by a homogeneous nucleation mechanism [51, 53, 54].

- **Interfacial heterogeneous nucleation**: In emulsions an enormous interfacial area is created which can serve as nucleating sites if the hydrophobic portion of the surface-active components have a similar molecular structure as the crystallizing components [53, 55]. Hence, the nucleation rate is proportional to the interfacial area of the droplets and, thus, to its square diameter [51, 52].

- **Interdroplet heterogeneous nucleation**: Droplets rapidly move because of Brownian diffusion and, thereby, frequently collide. If a crystallized globule collides with a supercooled (liquid) droplet, nucleation may be induced in the latter, provided that the crystal phase of the solid droplet comes into contact with the supercooled liquid [33, 52, 56-58]. Note that this is a secondary nucleation process since it happens only if primary, either homogeneous or heterogeneous, has taken place [52].

As in bulk fat systems, the nucleation type and rate in emulsions will be affected by the entire *T*-history program and flow conditions. In addition, in emulsions the droplet/particle size distribution will also have a pronounced effect [54, 59]. The above-mentioned effects of dispersing the oil into droplets on nucleation will have an influence on the crystal growth and polymorphic evolutions during subsequent crystallization and, consequently, on the final amount, size, morphology and composition of the fat crystals and its network in the fat globules.

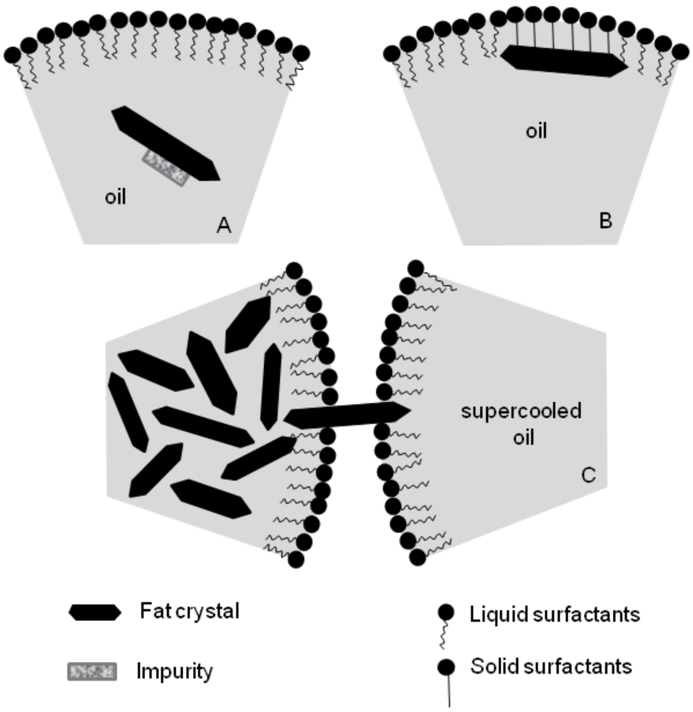


Figure 1.6 Schematic presentation of the different heterogeneous nucleation mechanisms in oil-in-water emulsions: (A) Volume heterogeneous nucleation, (B) interfacial heterogeneous nucleation and (C) interdroplet heterogeneous nucleation.

Another important difference between fat in bulk and in an emulsion is that the heat is transported to or from the globules by the aqueous phase. Because of the very small oil compartments, temperature changes happen in a few seconds throughout the whole volume of the droplet. When oil is cooled in a bulk system, the temperature gradient is more pronounced. At the lower temperatures (i.e. near the cooling medium) crystals may rapidly grow, aggregate and sinter which prevents a rapid heat transfer in the highly viscous mass. Hence, an uneven distribution of the *t,T*-history in bulk occurs which can cause problems with the interpretation of experimental research results. The latter should be taken into account when drawing conclusions for emulsified system from bulk crystallization studies. Moreover, the very short timescale of the various stages that can be realized in emulsions allows studying the crystallization at constant temperature.

An excellent review of studies on fat crystallization in emulsions is provided by Coupland [51] and the fundamentals of the primary crystallization are clearly summarized by Povey [52].

1.3.3. Effect of small-molecule surfactants

Apart from the TAG-composition, the physical properties of fats can be influenced by minor components such as small-molecule surfactants or emulsifiers which are present by nature or added on purpose. Small-molecule surfactants are amphiphilic molecules containing a hydrophobic and a hydrophilic moiety. The hydrophobic moiety, mostly composed of FAs, attracts the TAGs in the fat system depending on the chain length and the degree of saturation homogeneity between them. The hydrophilic moiety performs rather a repulsive power. Depending on the miscibility of the surfactant and the fat, the surfactant can either retard or accelerate the nucleation, crystal growth and/or polymorphic transition [43]. The surfactant may act as impurities and, thereby, perform a catalytic action resulting in an acceleration of the heterogeneous nucleation. The rate of crystal growth alters as a result of adsorption of the surfactant on the fat crystal surface or inclusion in the fat crystal lattice. Considering the interaction of surfactants with the polymorphic transition, surfactants were initially used to retard polymorphic transitions. However, studies with tristearin later showed that surfactants can accelerate the polymorphic transition as well [60]. Distinction is made between liquid and solid surfactants. The liquid surfactants accelerated the polymorphic transition in a tristearin system. They provide a higher mobility in the tristearin crystals thus promoting the polymorphic transition. Solid surfactants exhibit an ambiguous effect; they can both retard or accelerate polymorphic transitions. The different effects are attributed to the capacity of surfactants to create hydrogen bonds between the hydroxyl group of the hydrophilic head of the surfactant and the carbonyl group of the neighboring TAGs. Surfactants that are capable to form hydrogen bonds retard and the others accelerate the polymorphic transition due to the increasing mobility of the TAGs caused by the absent FAs in some surfactants compared with TAGs [60].

Table 1.3 gives an overview of studies that handle the effects of various food grade small-molecule surfactants on the different levels of primary fat crystallization in bulk systems. The type of fat considered and whether the rate of nucleation, of crystal growth and/or of the polymorphic transition is investigated are also indicated in the table. Despite the numerous studies in bulk fat systems, it was not possible to report a straightforward accelerating or retarding influence on the primary crystallization kinetics. The rate of nucleation, crystal growth and polymorphic transition is greatly defined by (1) the chain length and degree of saturation of FAs in both the surfactant and the crystallizing TAG-system, (2) the number of FAs present in the surfactants, (3) the type of hydrophilic moiety, (4) the concentration of the surfactants and (5) the applied *t,T*-program. As a result of the influence of small-molecule surfactants on the primary crystallization kinetics, the surfactants may also show a change in amount, shape (morphology) and size of the crystals and its fat crystal network properties (including sintering) [61-72].

Table 1.3 Overview of studies on the effect of small-molecule surfactants on the rate of nucleation, of crystal growth and/or of polymorphic transition in different bulk fat systems.

Type of surfactant	Type of fat[1]	Nucleation[2]	Crystal growth[2]	Polymorphic transitions[2]	Ref.
Phospholipids	HSFO	X	-	-	[73]
	PO	XX	X	-	[61, 73]
	CNO	X	-	-	[73]
	CB	X	X	X	[74-76]
	MF-CB	X	X	X	[77]
	MF	X	X	-	[78-80]
Sucrose esters	HMF-SFO	X	X	X	[63, 64, 81]
	SSS	-	-	X	[82]
	HSFO	X	-	X	[83]
	PKO	X	X	-	[84]
	PO	X	-	-	[85]
Polyglycerol ester	PO	X	X	X	[67]
	HMF-SFO	X	-	X	[63]
Polyglycerol polyricinoleate	HCSO	-	-	X	[86]
Sorbitan polyesters	PO	-	-	X	[87]
	RSO	-	-	X	[88]
	CB	X	-	X	[68, 89, 90]
	SSS	-	-	X	[82, 91, 92]
	RSO	-	-	X	[93]
Monoacylglycerols	PO	X	X	X	[71, 94-96]
	HSFO	X	-	-	[94, 95]
	CNO	X	-	-	[94, 95]
	MF	X	X	-	[97]
	LLL	-	X	-	[69, 70]
Diacylglycerols	PO	X	X		[98, 99]
	POl	X	X	-	[30, 100]
	MF	X	X	-	[97, 101]
	LLL	-	X	-	[69, 70]
	SSS	-	-	X	[92]
	HRSO	-	-	X	[102]
	CB	-	-	X	[103]
other acylglycerols	SSS	-	-	X	[91]

[1] HSFO = Hydrogenated sunflower oil; PO = Palm oil; CNO = Coconut oil; CB = Cocoa butter; MF-CB = Blend of milk fat and cocoa butter; MF = Milk fat; HMF-SFO = Blend of high-melting milk fat and sunflower oil; HCSO = Hydrogenated cottonseed oil; SSS = tristearin; RSO = Rapeseed oil; LLL = Trilaurin; POl = Palm olein.

[2] 'X' indicates that this effect is discussed while '-' indicates that the effect is not considered.

The number of studies on the effect of surfactants on emulsified TAG-crystallization is quite limited. However, recently the group of Sato studied extensively the effect of hydrophobic sucrose and polyglycerol esters in oil-in-water emulsions in which the oil phase is composed of palm oil [85], a palm mid fraction [104-107], palm stearin [108] or palm kernel oil [84, 104]. Generally, it was observed that the nucleation is mainly accelerated in the presence of these surfactants. Moreover, crystallization of palm kernel oil is initiated in a more stable polymorph in the presence of sucrose esters [84]. The effect on the nucleation rate of both the sucrose and the polyglycerol esters increases with the chain length of the saturated FAs and with the degree of FA interesterification on the hydrophilic moiety of the surfactants. Furthermore, it is postulated that the higher nucleation rate is attributed to interfacial heterogeneous nucleation rather than to volume heterogeneous nucleation. For hydrophobic sucrose esters in a palm mid fraction oil-in-water emulsion, interfacial heterogeneous nucleation was demonstrated by using a microbeam small-angle X-ray diffraction technique [109]. The lamellar planes of the fat crystals are found to be parallel oriented to the oil-water interface in the presence of the sucrose esters while otherwise a rather random orientation is observed. Sucrose esters oriented the FA-chains of the TAG-molecules by hydrophobic interactions causing interfacial heterogeneous nucleation.

1.3.4. Recent research on milk fat crystallization

During the last decade the group of Ollivon has put considerable efforts into investigating the crystallization behavior of milk fat in bulk (AMF) [39, 110-114] and natural cream [59, 111, 115-117]. In their research, they focus mainly on elucidating the thermal and structural behavior of milk fat crystals in terms of polymorphism (sub-α, α, β’ and β) and longitudinal stacking (2L and 3L) by coupling simultaneously differential scanning calorimetry (DSC) and X-ray diffraction (XRD) recordings in both isothermal and non-isothermal conditions. Contradictory to the complex TAG-composition of milk fat only a few crystalline structures are formed in both bulk fat and cream systems. Whatever the cooling rate mainly α and β’-crystals are formed which are longitudinally packed in 2L- and/or 3L-stackings. Traces of the β-polymorph are only found in bulk fat systems at low cooling rates and at isothermal conditioning at 4℃ after quench cooling. This clearly shows that mixed crystals in milk fat are omnipresent. The structural compatibility, required for the formation of compound crystals, within the heterogeneous group of TAGs in milk fat can be explained by the specific positional distribution of the FAs on the glycerol backbone in milk fat [118].

The main differences observed between natural cream and bulk milk fat systems are the favored formation of unstable crystals and the delayed transition to more stable species, which appear in particular at low cooling rates (< 0,15℃.min^{-1}) [113, 115]. Bulk milk fat nucleates as β’2L-crystals whereas milk fat in cream nucleates in a α3L-polymorph at higher supercooling then in bulk milk fat. The cooling was not slow enough to allow stable varieties to be formed in cream due to the lack of nucleation centers in most of the globules. Moreover, by thoroughly investigating the X-ray diffraction peaks the group of Ollivon postulated that in fat globules the crystals are more disordered and/or are smaller. This was attributed to the higher supercooling required to induce nucleation and the crystal packing constraints because of the presence a curved interface. This packing constraint is positively related to the curvature of the droplets and, hence, increases with decreasing particle sizes [59]. Furthermore, the structural evolutions in natural cream were recorded for both the small globules (1-3 µm) and the large fat globules (> 5 µm) separately [116]. Similar crystalline forms appeared but at different degrees of supercooling. In addition, the long spacings slightly varied depending on the globules size which was mainly explained by the differences in FA-composition between the individual milk fat globules [119].

Some recent studies dealing with the effect of small-molecule surfactants on bulk milk fat crystallization can be found, as is indicated in Table 1.3. Foubert et al. [97] investigated the temperature and concentration dependent effect of MAGs of oleic and stearic acid on milk fat crystallization. MAGs of stearic acid interact with the nucleation and crystal growth rate dependent on the degree of supersaturation, which is determined both by temperature and concentration. For MAGs of oleic acid a more straightforward relation was detected: the crystal growth rate is enhanced while the induction time is less affected. In contrast to MAGs, adding PLs and DAGs unambiguously retard nucleation and crystal growth of milk fat [78-80, 97]. Regarding the effect of small-molecule surfactants on the milk fat crystallization in cream are the available studies rather scarce. In recombined dairy cream, Miura [120] investigated the effect of bovine and soybean PLs on the milk fat crystallization and concluded that soybean PLs decreased the crystallization temperature during cooling and consequently lowered the solid fat content compared to recombined creams containing bovine PLs.

Besides the above-mentioned researches, other recent studies deal primarily with bulk milk fat and focus on investigating milk fat crystallization upon shearing [45], on the crystallization of milk fat fractions [39, 114, 121] and on the milk fat network formation [122-124]. However, these topics are beyond the scope of this manuscript.

2. DESCRIPTION OF MATERIALS AND METHODS

In this chapter the materials and basic principles of the methods used in Chapter 3 and 4 are discussed. Moreover, the chemical characterization of the applied materials is included in this chapter.

2.1. Materials

2.1.1. Natural cream

The commercially available natural cream (NC) (Debic, FrieslandCampina Professionals, Lummen, Belgium) was produced via industrial skimming of fresh raw milk. The fat content amounts to 35 wt% and the cream was UHT-treated to prolong the microbiological shelf life and carrageenan (0.01 wt%) (Lactarin®, FMC biopolymers, Brussels, Belgium) was added to lengthen the physical stability. The milk fat from NC (MF-NC) was isolated by successive churning of the cream, melting and centrifugation of the obtained butter and separation and drying of the milk fat. The fatty acid (FA) profile of MF-NC obtained was characterized by using the AOCS Official Method Ce 1b-89. The GC-analyses were carried out on an Interscience Thermofocus GC with RTX-2330 column (cyanopropyl polysiloxane, 60 m length, 0.25 mm internal diameter, 0.2 µm layer thickness). The FA-analyses were performed in triplicate (Table 2.1).

Table 2.1 FA-profile in wt% of milk fat extracted from natural cream (MF-NC) and milk fat used for recombined cream (MF-RC).

FA (%)	MF-NC	MF-RC
C4:0	3,14 ± 0,03	3,41 ± 0,04
C6:0	1,76 ± 0,01	1,87 ± 0,04
C8:0	1,59 ± 0,01	1,59 ± 0,03
C10:0	3,92 ± 0,02	3,67 ± 0,01
C12:0	5,11 ± 0,07	4,77 ± 0,04
C14:0	13,86 ± 0,00	13,38 ± 0,08
C14:1	1,69 ± 0,04	1,72 ± 0,01
C15:0	1,57 ± 0,31	1,29 ± 0,00
C16:0	33,86 ± 0,02	31,24 ± 0,29
C16:1	2,29 ± 0,03	2,51 ± 0,01
C17:0	0,77 ± 0,02	0,40 ± 0,05
C17:1	0,29 ± 0,00	0,31 ± 0,01
C18:0	8,58 ± 0,01	8,82 ± 0,06
C18:1t	0,95 ± 0,01	1,56 ± 0,11
C18:1c	17,42 ± 0,02	19,42 ± 0,23
C18:2	1,04 ± 0,01	1,05 ± 0,03
C20:0	0,08 ± 0,00	0,07 ± 0,00
C18:3	0,37 ± 0,01	0,43 ± 0,00
C20:1	0,26 ± 0,02	0,53 ± 0,02

2.1.2. Recombined cream

Recombined creams (RCs) with and without monoacylglycerols (MAGs) were made by recombining anhydrous milk fat (AMF) (FrieslandCampina cheese & butter, Noordwijk, The Netherlands), sweet cream butter milk powder (SCBMP) (FrieslandCampina, The Netherlands), carrageenan (Lactarin®, FMC Biopolymers) and potable water.

2.1.2.1. Chemical characterization of ingredients

A. Anhydrous milk fat

The FA-profile of AMF used for the manufacturing of RCs (MF-RC) (Table 2.1) is somewhat different from MF-NC. MF-RC contained an increased amount of unsaturated FAs at the expense of saturated FAs compared to MF-NC.

B. Sweet cream buttermilk powder

By using SCBMP all the components of the milk serum and the milk fat globule membrane of NC, including milk phospholipids, were incorporated in the RCs. The powder is made by drying a mixture of skimmed milk (70 wt%) and buttermilk (30 wt%) and contains lactose (45.5 wt%), proteins (33.1 wt%), fat (10.4 wt%) including polar lipids (3.4 wt%) and ash (7.7 wt%).

C. Monoacylglycerols

Three types of distilled MAGs were selected in which the main FA-component (>75 wt%) differs in chain length and/or saturation degree: monoacylglycerols rich in oleic (MAG-O) (Palsgaard, Juelsminde, Denmark), lauric (MAG-L) (Palsgaard) and stearic acid (MAG-S) (Puratos Group, Grootbijgaarden, Belgium). Table 2.2 gives the FA-profile determined by the method described in Section 2.1.1.

Table 2.2 FA-profile in wt% of monoacylglycerols rich in oleic acid (MAG-O), lauric acid (MAG-L) and stearic acid (MAG-S).

FA (%)	MAG-O	MAG-L	MAG-S
C10:0	-	0,18 ± 0,00	-
C12:0	-	**99,30** ± 0,04	0,15 ± 0,01
C14:0	0,07 ± 0,00	0,45 ± 0,03	0,36 ± 0,02
C16:0	4,92 ± 0,04	-	19,00 ± 0,19
C18:0	3,28 ± 0,03	-	**78,89** ± 0,31
C18:1	**82,08** ± 0,06	-	0,56 ± 0,07
C18:2	8,25 ± 0,01	-	0,10 ± 0,03
C18:3	0,46 ± 0,00	-	-
C20:0	-	-	0,48 ± 0,01

2.1.2.2. Production on pilot plant

SCBMP (7,2 wt%) and carrageenan (0,01 wt%) were dispersed in water and preheated to 50℃ and the MF-RC (35 wt%) was melted and preheated to 50℃. Subsequently, the pre-emulsion was prepared by thoroughly mixing of the aqueous phase and fat phase with an ultra turrax (10000 rpm) for 10 min at 50℃. The pre-emulsion was then transferred to a continuous pilot installation (APV plate heat exchangers) in which the cream was successively homogenized (0-10 bar at 75℃), UHT-treated (6 s at 140℃), rapidly cooled (until 5℃) and aseptically packaged in HDPE-bottles of 1L. RCs with MAGs were prepared with the same procedure and the concentrations used vary between 0.035 wt% and 0.5 wt% calculated on cream-basis.

2.2. Methods

2.2.1. Differential scanning calorimetry

An indirect differential scanning calorimetry (DSC) method was applied to study the isothermal crystallization behavior of milk fat in bulk and in cream at 5℃. The direct/conventional DSC-method was not appropriate as a result of, first, milk fat crystallization during cooling and, second, the rather low resolution of the crystallization peak of milk fat in cream during the isothermal period. The low peak resolution in cream is partly caused by the decreased fat content in cream (35 wt%) compared to the bulk samples (dilution effect). Foubert et al. [125] described this indirect method, referred to as the stop-and-return method, in great detail and Figure 2.1 gives a schematic representation of the *t,T*-program. Distinct from the direct method is that the isothermal period in the indirect method is interrupted by applying a heating step. During heating, the crystals formed, both during the cooling and the considered isothermal period, completely melt. The melting heat calculated from the melting peak recorded by the DSC is thereby a measure for the amount of crystallization heat released during both the cooling and the considered isothermal period. By using this *T*-cycle for several defined isothermal periods, an isothermal crystallization profile can be constructed by plotting the calculated melting heats as a function of time. The prerequisite to use this indirect method for emulsions is that the emulsions remain stable during the repetitive *T*-cycling [125]. Destabilization can be tested by monitoring the variation of the crystallization temperature during the repetitive *T*-cycles. The crystallization temperature (T_{cr}) can be defined as the temperature at which the crystallization starts during cooling (the onset temperature of the crystallization). When destabilization occurs, the T_{cr} will increase due to an increased particle size, possibly because of partial coalescence. An unaffected crystallization temperature is, thus, required when applying the indirect DSC-method for oil-in-water emulsions like cream.

The analyses were performed in triplicate with a TA Q1000 DSC (TA Instruments, New Castle, Delaware, USA). The DSC was calibrated with indium (TA Instruments), azobenzene (Sigma-Aldrich, Bornem, Belgium) and undecane (Acros Organics, Geel, Belgium) prior to analysis. Nitrogen was used to purge the system. Milk fat and cream samples (5-15mg) were sealed in hermetic alodined aluminium pans (TA Instruments) and an empty pan was used as a reference. The applied *t,T*-program was as follows: (a) holding at 65℃ for 10 min, (b) cooling at 25℃.min^{-1} to 5℃, (c) holding at 5℃ for *x* min (*x* may vary from 0 to 120 min) and (d) heating at 20℃.min^{-1} until 65℃. This *t,T*-program or *T*-cycle is now repeated for several defined isothermal periods. The melting curves recorded were integrated using a horizontal linear baseline with a fixed end point at 55℃. The obtained average melting heat (J.g^{-1}) was plotted as a function of the holding time at 5℃. The plotted Y- error bars represent the standard deviations (with n = 3).

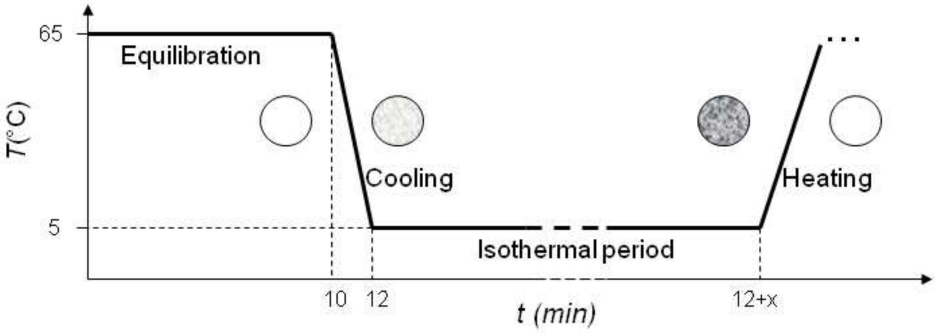


Figure 2.1 Schematic presentation of the *t,T*-program of the indirect DSC-method to study isothermal crystallization of cream at 5℃.

The T_{cr} and the final melting temperature (T_m) of the three MAGs addressed in Chapter 4 were determined in triplicate by applying the same *T,t*-program with x = 120 min. T_m is defined as the temperature at which the MAG-crystals created during cooling and isothermal crystallization at 5℃ are completely melted.

2.2.2. Time-resolved synchrotron X-ray diffraction

Real-time X-ray diffraction (XRD) measurements were performed on the Dutch-Flemish (DUBBLE) beamline BM26B at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). The experiments were conducted at a fixed wavelength λ of 1,24 Å. A curved 1D-microstrip gas chamber detector was used for wide-angle X-ray diffraction (WAXD) while a 2D-multiwire gas-filled detector was applied for small-angle X-ray scattering (SAXS). The samples were enclosed into a perforated aluminum DSC-cup and the gaps were covered by thin mica. The temperature was controlled by a Linkam hot stage (PE 94, Tadworth, UK). The following *t,T*-program was applied: heating to 65℃, holding at that temperature for 10 minutes, cooling at 25 °C.min $^{-1}$ to 5°C, holding at 5°C for 120 min and heating to 65°C at 5 °C.min $^{-1}$. Scattering patterns were taken every 30 s from the start of the isothermal period. The known reflections of silverbehenate and silicon powder were respectively used to calibrate the SAXS- and WAXD-scattering angles, 2θ. In the case of SAXS, intensities are shown as a function of s, with $s = 1/d = 2\sin\theta/\lambda$ and d the distance. All scattering patterns were corrected for the detector response and normalized to the intensity of the primary beam which is measured by an ionisation chamber placed after the sample. The 2D SAXS-powder patterns were radially averaged to yield 1D-patterns and, finally, a melt pattern was subtracted as a background. For WAXD, the intensities are plotted as a function of the short spacings d (Å).

2.2.3. Static laser light scattering

The size distribution of fat globules was determined using a Mastersizer S (Malvern Instruments, Malvern, U.K.) equipped with a 300RF lens. The refractive index values for milk fat and water were fixed at 1.4920 and 1.3300, respectively, whereas the imaginary refractive index of the dispersed phase was set at 0.01. Polydisperse analysis was selected. The creams were successively heated (50°C), diluted (1: 100) in a sodium dodecyl sulphate (SDS) solution (1 wt%) and then diluted (about 1:10000) with deionized water in the MSX-17 (Malvern) sample unit at 20°C under moderate stirring and pumping conditions. The heating of the cream and the subsequent rapid measurement at room temperature was performed to avoid scattering of fat crystals present in the milk fat globules [126]. SDS was used since this anionic surfactant is known to deflocculate non-covalently bound aggregates of fat droplets [127-129] and to dissociate casein micelles [127]. It is important to heat before the dilution in SDS, since freshly created partially coalesced fat globules can be easily split due to the ‘Lanza’ effect [130]. The volume-surface weighted or Sauter diameter $D_{3,2}$ was used to characterize and compare the particle size of the creams. Particle size analyses were performed in triplicate.

2.2.4. Nuclear magnetic resonance

Solid fat content (SFC) was measured with a Maran Ultra 23 MHz pulsed-field gradient nuclear magnetic resonance (NMR) (Oxford Instruments, Abingdon, UK). For bulk milk fat, SFC was measured using the indirect SFC-method. In this method the SFC is calculated after determining the liquid FID-signals (free induction decay), at 70 µs, of milk fat (L_{MF}) and rapeseed oil (L_{oil}) per gram both at crystallization or holding temperature ($T_{5°C}$) and at a temperature above the final melting point of milk fat (i.e.. 45°C; $T_{45°C}$). The following equation was used to calculate the SFC at $T_{5°C}$ of bulk milk fat ($S_{MF,T_{5°C}}$):

$$S_{MF,T_{5°C}} = 100 - 100 \frac{L_{oil,T_{45°C}}}{L_{oil,T_{5°C}}} \frac{L_{MF,T_{5°C}}}{L_{MF,T_{45°C}}} \tag{2.1}$$

The above-described SFC-method is inappropriate for cream samples since the aqueous phase of cream contributes to the FID-signal. Therefore, the measured liquid FID-signal (at 70 µs) of cream (L_{cr}) per gram was corrected for the aqueous phase. This by measuring the liquid FID-signal of the aqueous phase (L_{aq}) separately and subtracting it from the liquid FID-signal of cream taking into account the mass fraction of the aqueous phase (x_{aq}):

$$L'_{cr} = L_{cr} - x_{aq}L_{aq} \tag{2.2}$$

L'_{cr} is the corrected FID-liquid signal per gram of cream. This correction was performed at both $T_{5°C}$ and $T_{45°C}$. For NC, the aqueous phase was obtained by churning the cream with a Hobart mixer and separating the buttermilk from the milk fat by using a Büchner funnel provided with a filter paper. The aqueous phase of RC was simulated by dispersing 11,1 wt% SCBMP in water. As a control for the corrections made for the aqueous phase, the following condition should be fulfilled:

$$L_{cr,T_{45°C}} = x_{aq}L_{aq,T_{45°C}} + (1 - x_{aq})L_{MF,T_{45°C}} \tag{2.3}$$

This was performed for every sample and no deviations were observed. Finally, the SFC of cream at $T_{5°C}$ ($S_{cr,T_{5°C}}$) is then calculated using the following equation:

$$S_{cr,T_{5°C}} = 100 - 100 \frac{L_{oil,T_{45°C}}}{L_{oil,T_{5°C}}} . \frac{L'_{cr,T_{5°C}}}{L'_{cr,T_{45°C}}} \tag{2.4}$$

This indirect NMR-method for cream was optimized in FrieslandCampina Corporate Research (Deventer, The Netherlands).

The NMR-tubes were filled with ±0,5 mL sample and the mass was determined on an analytical balance (Sartorius CP225D, Germany). All samples were first placed in a water bath at 45°C. After 10 min the FID-signal was recorded at 45°C. Subsequently, the samples were transferred in a bath at 5°C and the FID-signal was recorded after holding the samples for 2h, 1 day and 3 and/or 5 days at 5°C. In order to avoid interruption of the isothermal period the NMR-probe was thermally controlled at 5°C. NMR-analyses were performed in triplicate and the plotted Y-error bars represent the standard deviations (with n=5).

2.2.5. Interfacial tension analyses

To measure the oil-water interfacial tension (γ_{OW}) in a convenient way, the drop tensiometer (Tracker, IT concept, Longessaigne, France) was used. By means of an axisymmetric drop shape analysis, this instrument is able to study the γ_{OW} as a function of *t* and *T*. The *T* is controlled by means of an external water bath (Julabo, Seelbach, Germany) which manages the temperature of the cuvette in which the droplet is shaped.

Two equations are used by the Tracker software to obtain the interfacial tension:

1. The Laplace-Young equation:

$$\Delta P = \gamma\left(\frac{1}{r_1} + \frac{1}{r_2}\right) \tag{2.5}$$

   With ΔP the Laplace pressure, γ the interfacial tension and r_1 and r_2 the radii of the curvature at a point on the interface

2. Equation for hydrostatic equilibrium at a plane passing through a point at the interface:

$$2\pi x\gamma \sin\theta = V(\rho_{h-}\,\rho_l)g + \pi x^2 \Delta P \tag{2.6}$$

   With *g* the gravitation constant, *x* the radius of the plane, *V* the volume beneath the plane and ρ_h and ρ_L the density of the heavy and the light phase, respectively.

In order to not deviate too much from the RC-systems in this research, the similar concentrations of MAGs and SCBMP were used. The applied concentrations to prepare the aqueous and oil phase were therefore recalculated from emulsion-based concentrations to water-based and oil-based concentrations.

The oil phase was purified sunflower oil. To remove the polar lipids, the sunflower oil was treated with silica. The γ_{OW} was measured of an aqueous droplet in a continuous oil phase. The reverse system was not applicable because the aqueous phase, containing SCBMP, was not translucent. The γ_{OW} measured for a water droplet in the purified oil is 29,5 ± 1,2 mN.m^{-1} which corresponds well with values found in literature (28-30 mN.m^{-1}).

A droplet was shaped at *T* = 40℃. After conditioning for 10 min at 40℃ a coo ling rate of ± 0.3 ℃.min^{-1} is applied and γ_{OW} was measured every 10s. Measurements were performed in triplicate. In the graphs the average γ_{OW} are plotted as a function of *T* and the error bars represent the standard deviations (n=3).

During the interfacial tension measurements the density of the different phases will vary as a function of *T*. Therefore, the density as a function of *T* of the different aqueous and oil phases was measured by using an Anton Paar density meter (Graz, Austria). After fitting a 3rd order polynomial equation to the curve the different coefficients were programmed in the software of the IT concept Tracker drop tensiometer.

2.2.6. Statistical analyses

SPSS software (version 15, SPSS Inc., Chicago, Illinois, USA) was used for statistical comparison of the T_{cr}, $D_{3,2}$ and SFCs. All the reported values are the average of at least three replicates. After checking the prerequisites, analyses of variance (one-way ANOVA) were carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. All tests were performed at a 95% significance level.

3. MILK FAT CRYSTALLIZATION IN BULK AND EMULSIFIED STATE: NATURAL AND RECOMBINED CREAM

3.1. Introduction

Milk fat crystallization determines to a large extent the physicochemical and sensory properties of a wide variety of fat-rich dairy products. In products like whipped cream, ice cream and traditional butter, cream is used as an essential ingredient. During processing of these products the milk fat crystallization occurs predominantly in cream and, thus, in the oil-in-water emulsified state. Cream is first subjected to a specific time-temperature (*t,T*-) program to obtain partially crystallized fat globules and subsequently the cream is exposed to a severe mechanical agitation in which the fat globules destabilize through a mechanism known as partial coalescence for which fat crystals are indispensable. The generated partially coalesced fat globules give thereby the desired structure to the products. The extent and rate of partial coalescence defines largely the final macroscopic product properties (stability, firmness, creaminess, overrun, etc.) which are primarily governed by the *T*-history of the cream [130-134]. *T*-history, including both the applied *t,T*-program during processing and the storage conditions of the cream, defines mainly the milk fat crystallization mechanism and kinetics and, as a consequence, also the solid fat content, the amount, morphology, polymorphism, composition and size of the crystals and the final arrangement of the crystals in the fat globules. However, it need be remarked that partial coalescence is also affected by the flow conditions, particle size distribution and composition of the aqueous phase, the fat phase and the oil-water interfacial layer as will extensively be discussed in Chapter 5.

Two main important aspects determine the complexity of investigating milk fat crystallization in cream: the composition of milk fat is significantly different from other fats in foods and the crystallization occurs in small droplets. Both these complicating factors are extensively discussed in Chapter 1.

During the last decade, following the work of Walstra and van Beresteyn [54], the group of Ollivon has put considerable efforts into investigating the crystallization behavior of milk fat in bulk (anhydrous milk fat) [39, 110-114] and natural cream [59, 111, 115-117]. In their research, they focus mainly on elucidating the thermal and structural behavior of milk fat crystals in terms of polymorphism (sub-α, α, β' and β) and longitudinal stacking (2L and 3L) by coupling simultaneously differential scanning calorimetry (DSC) and X-ray diffraction (XRD) recordings in both isothermal and non-isothermal conditions. The main difference observed during cooling is that in natural cream the formation of unstable crystals is favored because of the lack of catalytic impurities and that the transition to a more stable species is delayed, especially when slow cooling is applied (< 0,15 ℃.min^{-1}) [113, 115]. At higher cooling rates these differences are less pronounced. Moreover, independent of the *t,T*-program the crystals in natural cream have compared to bulk milk fat crystals a smaller size and/or have a less organized structure [59, 111, 115].

In practice, besides natural cream, derived from milk by means of centrifugation, often recombined cream is used. It is obtained by recombining milk products, mostly milk fat and a low-fat milk powder, with water. The benefits of using recombined cream are that the composition and formulation can easily be modified for product development goals and that the raw materials can easily be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce. Nevertheless, the recombined dairy products like ice cream and whipped cream seem to have unfavorable divergent physicochemical and sensory properties as compared to products made from natural cream. This can probably be partly attributed to a variation in susceptibility to partial coalescence of the fat globules of natural and recombined cream during processing. The differences in composition of the interfacial layer [135] and/or fat crystallization behavior [54] between the two types of cream are potential explanations (see Chapter 5). Hence, understanding of the crystallization behavior of milk fat in natural cream and recombined cream is desirable.

3.2. Research strategy

This study applies a combination of advanced techniques (differential scanning calorimetry, time-resolved X-ray diffraction and nuclear magnetic resonance) allowing a thorough understanding of the effect of recombination on the isothermal crystallization mechanism and kinetics of milk fat. Therefore, a comparison is made between the milk fat crystallization in recombined cream, natural cream, the bulk milk fat used for the recombined cream and the bulk milk fat extracted from the natural cream.

The fatty acid composition and, thus, also the triacylglycerol (TAG) composition of milk fat can vary depending on numerous factors such as feeding, climatic conditions, stage of lactation and breed of the cow (Section 1.2.3). These differences in chemical composition can have an important influence on the crystallization behavior of milk fat [136, 137]. In this contribution, the effect of the chemical composition of lipids on the crystallization behavior is circumvented by pairwise comparing, at one hand, the crystallization of natural cream (NC) with the milk fat extracted from the natural cream (MF-NC) and, on the other hand, the crystallization of recombined cream (RC) with the milk fat used for its production (MF-RC). The fatty acid composition of MF-NC and MF-RC is given in Table 2.1 (Chapter 2).

3.3. Results and discussion

3.3.1. Characterization of the natural and recombined cream

In emulsions the crystallization behavior can be affected to a large extent by the particle size distribution. The smaller the droplet, the higher the supercooling needed to induce crystallization [54, 59]. The latter counts until the supercooling for homogeneous nucleation is reached. In order to eliminate this influencing factor, a RC with a comparable order of magnitude of particle size distribution to NC was produced by adapting the homogenization pressure. Figure 3.1 gives the particle size distribution of NC and RC with a $D_{3,2}$ of 3,14 ± 0,19 µm and 3,46 ± 0,18 µm, respectively.

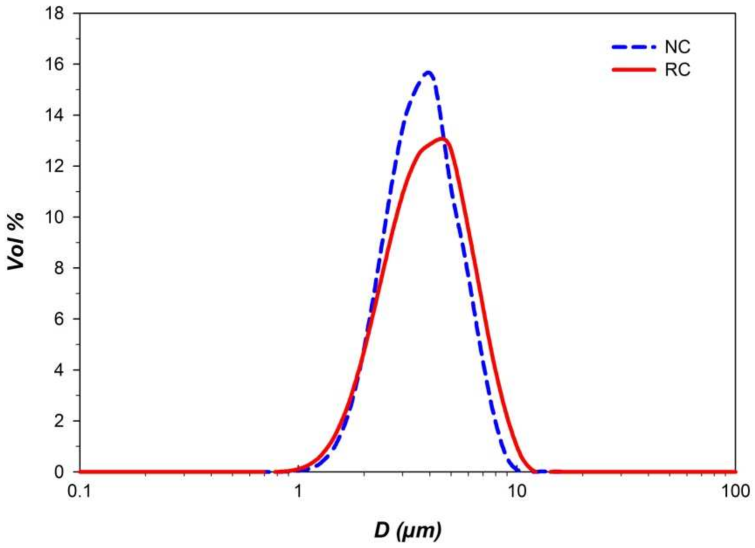


Figure 3.1 Volume-weighted particle size distribution of NC and RC.

3.3.2. DSC-analyses

The isothermal crystallization at 5℃ was studied by means of the indirect DSC-method for NC, MF-NC, RC and MF-RC. Upon *T*-cycling the crystallization temperature (T_{cr}) of NC and RC was unaffected (data not shown), which makes the cream appropriate for this indirect DSC-method [125]. Figure 3.2 A and B show the crystallization profiles of NC versus MF-NC and RC versus MF-RC, respectively. To facilitate quantitative assessment between bulk and emulsified milk fat, the measured values of melting heat in the cream samples were recalculated as melting heat per g fat.

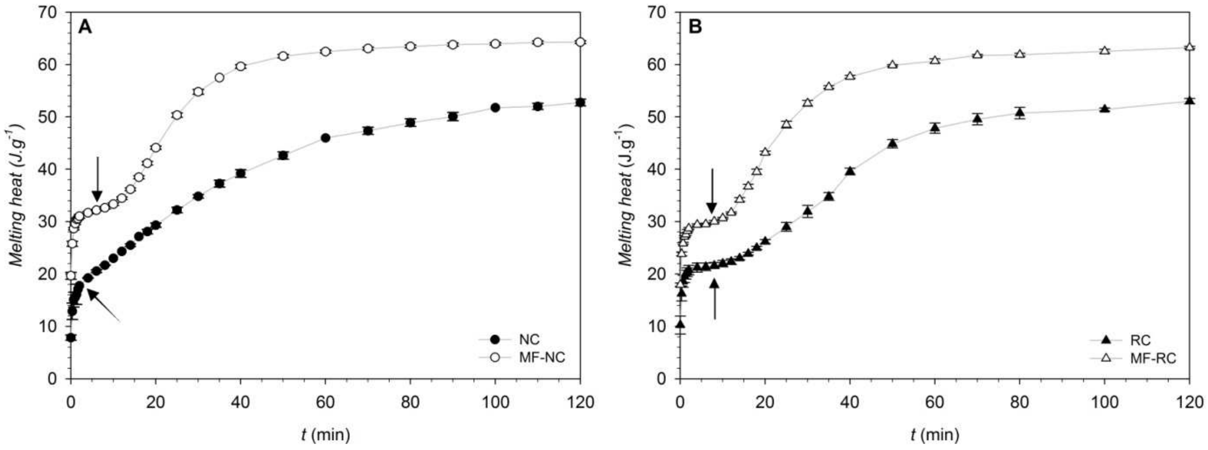


Figure 3.2 Isothermal crystallization profiles of (A) NC versus MF-NC and (B) RC versus MF-RC at 5℃ constructed by plotting the melting heat per g fat as a function of the holding time.

For all these samples melting heat can already be detected from the instant the temperature reaches 5℃ (*t* = 0 min) indicating that milk fat both in bulk and in cream started to crystallize during the preceded cooling. The crystallization temperatures (T_{cr}) of the different samples are given in Table 3.1. Generally, it is expected that due to emulsification the supercooling needed to induce crystallization will be increased [53, 54]. Nevertheless, in this case the crystallization temperature and, thus, the supercooling of MF-NC and NC are not significantly different and the differences between MF-RC and RC are rather small. This can probably be explained by the high cooling rate (25 ℃.min^{-1}) applied. This cooling rate was chosen as a compromise between the industrially applied high cooling rates and the potential of the DSC to cool linearly in the considered *T*-range. Table 3.2 shows the crystallization temperature of NC and MF-NC as a function of cooling rate. It can be observed that T_{cr} increases more in MF-NC than in NC with decreasing cooling rate. The temperature at which the milk fat in bulk starts to crystallize decreased thus strongly with increasing cooling rate which is in agreement with ten Grotenhuis [41]. In cream, this effect is less pronounced because of lack of nucleation at higher temperature due to a shortage of catalytic impurities in the fat globules.

Table 3.1 The T_{cr} of MF-RC, RC, MF-NC and NC.

Sample	T_{cr} (℃)
MF-RC	13.29 ± 0.28^a
RC	11.10 ± 0.57^b
MF-NC	14.61 ± 0.33^c
NC	14.44 ± 0.18^c

$^{a-d}$different letters indicate significant differences.

Another similarity in Figure 3.2 is that the crystallization occurs in two steps. This two-step crystallization can be a result of a crystallization of different fractions, a polymorphic transition with or without additional crystallization or a combination thereof [138, 139]. Next to these overall similarities, differences between bulk and cream can be identified counting for both NC and RC. The rate of the second step (as measured by the slope) is higher for bulk milk fat samples than for the emulsified ones. Moreover, a significantly higher amount of melting heat is observed during the first two hours of crystallization. This difference can be attributed to a difference in SFC, in crystal composition, polymorphic form or a combination of these factors.

Table 3.2 The T_{cr} as a function of cooling rate for NC and MF-NC.

Cooling rate **(°C.min^{-1})**	$T_{cr,\ NC}$ **(°C)**	$T_{cr,\ MF\text{-}NC}$ **(°C)**
0.5	$17.74 \pm 0.55^{a,x}$	$23.27 \pm 0.63^{a,y}$
1	$16.65 \pm 0.45^{a,b,x}$	$17.73 \pm 0.50^{b,x}$
2	$16.11 \pm 0.67^{a,b,c,x}$	$16.58 \pm 0.12^{b,c,x}$
10	$15.24 \pm 0.23^{b,c,x}$	$15.63 \pm 0.21^{c,d,x}$
20	$14.39 \pm 0.56^{c,x}$	$14.89 \pm 0.51^{d,x}$
25	$14.44 \pm 0.18^{c,x}$	$14.61 \pm 0.33^{d,x}$

$^{a\text{-}d}$different letters indicate significant differences between different cooling rates.
^{x,y}different letters indicate significant differences between NC and MF-NC.

Furthermore, regarding the onset of the second step (as indicated by an arrow in Figure 3.2) a difference between bulk and cream can be identified which only counts for NC. The onset of the second step takes place earlier for NC than for MF-NC while in RC and MF-RC a fairly simultaneous onset is observed. Besides, it is remarkable that the slope of the second step in RC is steeper than in NC.

In order to further explain the differences observed in the above-mentioned DSC-experiments, the crystallization mechanism behind the two-step crystallization had to be elucidated (Section 3.3.3) and the SFC (Section 3.3.4) had to be measured for both samples.

3.3.3. XRD-measurements

The crystallization mechanism of milk fat in bulk and in emulsified state was elucidated using small-angle (SAXS) and wide-angle (WAXD) XRD-experiments. These analyses were performed for NC, MF-NC, RC and MF-RC. Figure 3.3 and Figure 3.4 show the evolution of SAXS- and WAXD-profiles, respectively, of both MF-NC and NC.

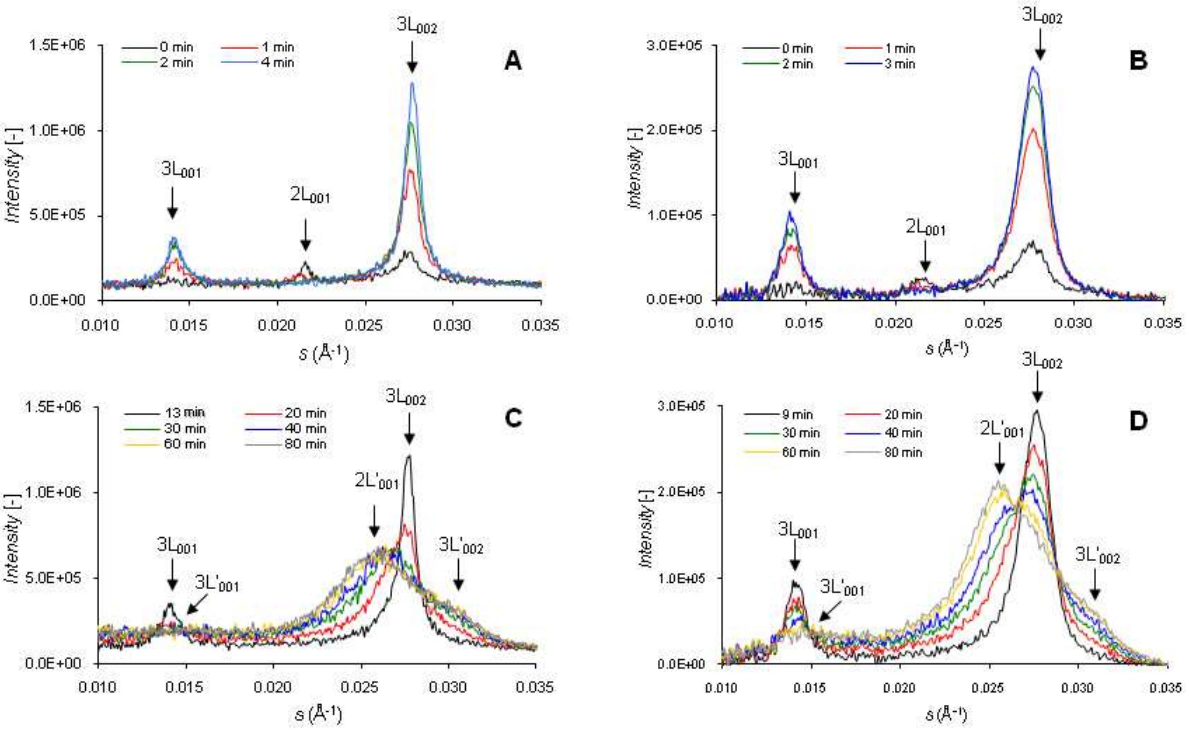


Figure 3.3 Evolution of the long spacings (SAXS-patterns) at 5°C of MF- NC during (A) the first crystallization step and (C) the second crystallization step and NC during (B) the first crystallization step and (D) the second crystallization step.

For clarity in Figure 3.3, the evolution of the long spacings for both MF-NC and NC are plotted into two graphs showing separately the evolution of the first and the second step. During the first step three individual peaks can be identified in both MF-NC and NC. A small peak at s = 0,022 Å^{-1} (d = 46,5 Å) is present at time 0 min and vanishes during the first minutes of isothermal crystallization. A longitudinal organization of TAG with a thickness of 40-50 Å typically corresponds with a double chain length structure. The peak can thus be associated with an unstable 2L-stacking and is indicated as $2L_{001}$ in Figure 3.3. The two other peaks at s = 0,014 Å^{-1} (d = 71,4 Å) and s = 0,028 Å^{-1} (d = 35,7 Å) appear simultaneously at the beginning of the isothermal period and grow until a maximum intensity is reached. For MF-NC this maximum intensity is reached later (t = 4 min) than in NC (t = 3 min). The concurrent appearance of the peaks and the position of the peaks indicate that the peak at 35,7 Å is a second order peak of the peak at 71,4 Å. The latter first order peak representing the actual layer thickness has a typical value of a 3L-stacking (between 55 and 75 Å). The first order and the second order peak are therefore labeled as $3L_{001}$ and $3L_{002}$ in Figure 3.3. Despite the complexity in the SAXS-patterns, only one peak at 4,15 Å appears, superimposed on the liquid signal (broad peak at 4.5 Å) [140], in the WAXD-patterns of both MF-NC and NC during the first step (Figure 3.4), which unequivocally corresponds with an α-polymorph [141, 142].

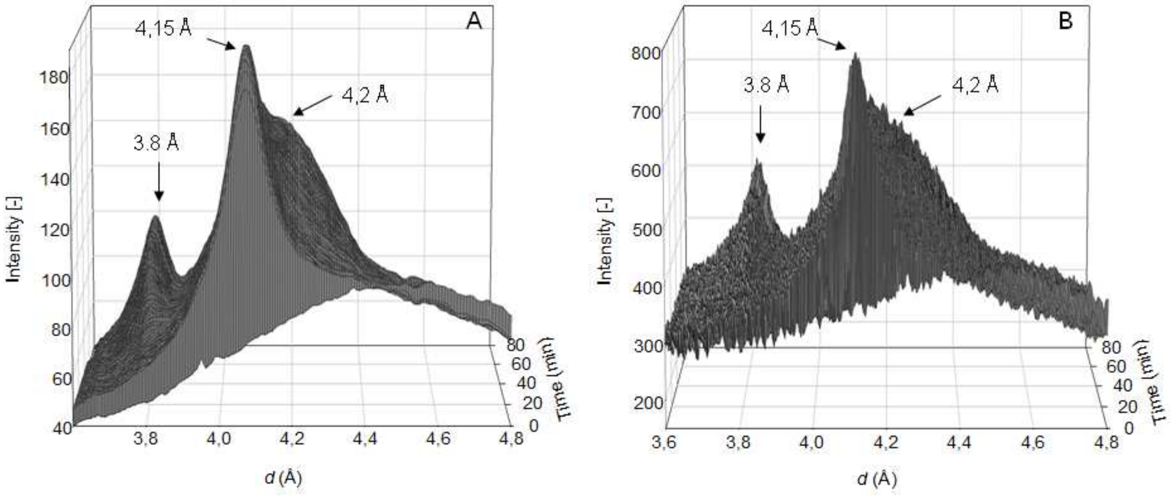


Figure 3.4. Evolution of the short spacings (WAXD-patterns) at 5℃ of (A) MF- NC and (B) NC.

At the beginning of the second step (after 13 min in MF-NC and after 9 min in NC at 5℃), the $3L_{001}$- and $3L_{002}$-peak start to decline, a broad peak appears at s = 0,026 Å^{-1} (d = 38,46 Å) with a shoulder at around s = 0,030 Å^{-1} (d = 33,3 Å) and the $3L_{001}$-peak shifts to a higher s-value or a lower long spacing (s = 0,015 Å^{-1} or d = 66,7 Å). Similar to the $3L_{001}$- and $3L_{002}$-peak appearing during the first step, the peak at s = 0,015 Å^{-1} (d = 66,7 Å) and the one at s = 0,030 Å^{-1} (d = 33,3 Å) can be linked to the first order and the second order of the 3L-packing, respectively. The peaks are therefore indicated as $3L'_{001}$ and $3L'_{002}$ in Figure 3.3. The peak at s = 0,026 Å^{-1} (d = 38,5 Å) falls within the range of the 2L-packing and is labeled as $2L'_{001}$. In the WAXD-patterns of both MF-NC and NC a new sharp peak appears at d = 3,8 Å and simultaneously the sharp α-peak at d = 4,15 Å declines, broadens and shifts slightly to higher values during the second step. The appearance of a short spacing at 3,8 Å is characteristic for the orthorhombic subcell stacking of a β'-polymorph [141, 142]. The two-step crystallization can, thus, undoubtedly be related to an α-β'-polymorphic transition.

Considering the XRD-data during the isothermal crystallization at 5℃ two questions regarding the structure of the crystals still remain unanswered. First, the presence of residual α-crystals at the end of the applied isothermal period is questionable. The broad peak in between 4,1 – 4,3 Å in Figure 3.4 A and B, which includes one of the typical short spacing of the β'-polymorph at 4,2 Å, makes it unfeasible to identify the characteristic α short spacing at 4,15 Å. Second, if α-crystals are present, the univocal assignment of the long spacings (the 3L'-peak and 2L'-peak) in the second step to a polymorphic form is uncertain. The 3L'- and the 2L'-peak grow simultaneously at the expense of the α3L-peak during the second step. To answer these questions XRD-patterns were further recorded during heating at 5°C.min^{-1} after 120 min isothermal crystallization at 5℃ of MF -NC and NC (Figure 3.5 and Figure 3.6). The rate was chosen high enough to block further polymorphic evolution and low enough to allow recording sufficient XRD-patterns.

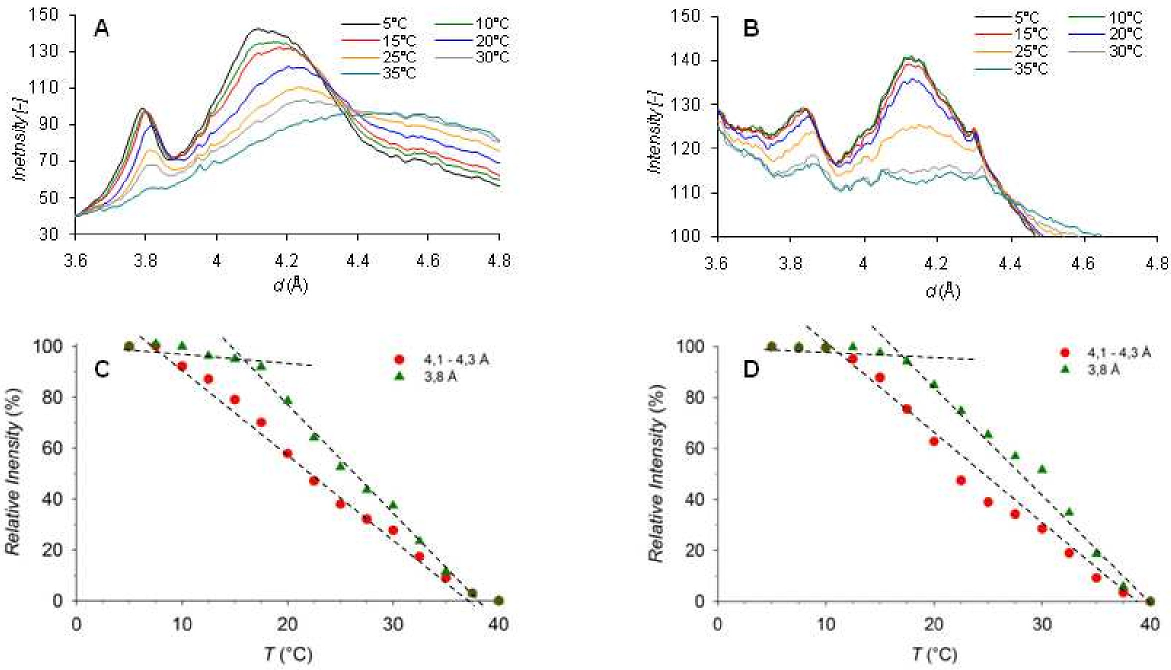


Figure 3.5 Evolution of the short spacings (WAXD-patterns) during heating (5 ℃.min^{-1}) after an isothermal crystallization period of 120 min at 5℃ of (A) MF -NC and (B) NC and the corresponding relative evolution of the peak maximum at 3,8 Å and peak maximum at 4,1 – 4,3 Å of (C) MF-NC and (D) NC.

Figure 3.5 A and B show the evolution of WAXD-patterns during heating of MF-NC and NC respectively. The relative intensity of the broad peak between 4,1 - 4,3 Å possibly corresponding to α- and β’- polymorphs and the peak at 3,8 Å corresponding with the β’-polymorph are plotted as a function of temperature in Figure 3.5 C and D. These relative intensities allow us to asses the temperatures at which the peaks start to decrease. It can be observed that the broad peak between 4,1 – 4,3 Å starts to decline at *T* > 7,5℃ in MF-NC and at *T* > 10℃ in NC while the peak at 3,8 Å in both MF -NC and NC persists and starts to decline substantially if *T* > 15℃. This dissimilar evolution of the two peaks at *T* < 15℃ confirms the presence of α-crystals, given that in the presence of only β’-crystals the two peaks should decrease concurrently. The evolution of the SAXS-patterns simultaneously recorded with the WAXD-patterns during the heating of MF-NC and NC is shown in Figure 3.6 A and B, respectively. For both samples it can be concluded that at *T* = 15℃ the 3L’$_{001}$- and the 3L’$_{002}$-peaks have disappeared and only the 2L’$_{001}$-peak remains. The latter slowly disappears at *T* > 15 ℃. The melting of the α-crystals, as concluded from the WAXD-pattern, coincidences, thus, with the disappearance of the 3L’-peaks below 15℃. The α-crystals can therefore be associated with the 3L longitudinal organization (3L’) after 120 min crystallization while the β’-polymorph are crystallized in the 2L longitudinal packing (2L’).

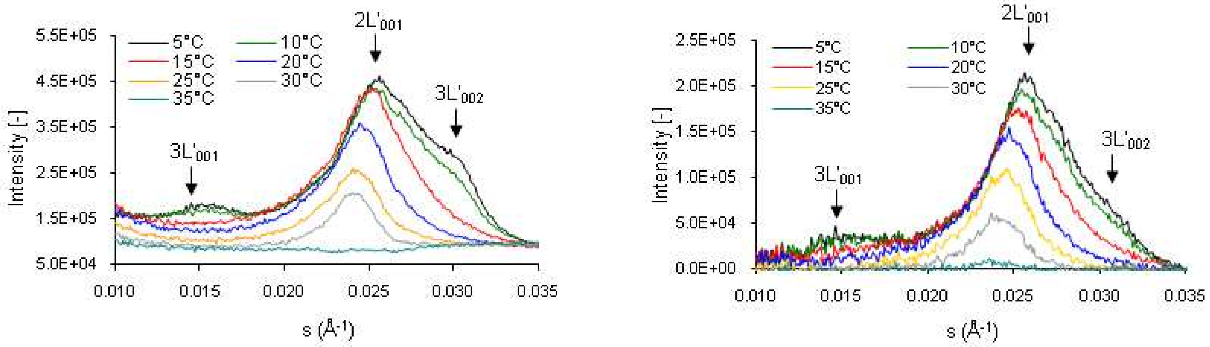


Figure 3.6 Evolution of the long spacings (SAXS-patterns) during heating (5 ℃.min $^{-1}$) after an isothermal crystallization period of 120 min at 5℃ of (A) MF- NC and (B) NC.

Analogue results were obtained for RC and MF-RC and the same conclusions regarding the crystallization mechanism were drawn (data not shown).

3.3.4. NMR-measurements

Figure 3.7 A and B show the SFC of MF-NC versus NC and MF-RC versus RC, respectively, after 2 hours, 1 day, 3 and 5 days isothermal crystallization at 5℃. After 120 min, it can be observed that the SFC of both NC and RC is lower than MF-NC and MF-RC, respectively. During the first three days the SFC still increases for all these samples while after 5 days an equilibrium seems to be reached. The differences observed between the cream samples and the bulk samples after 120 min still persist in NC; while RC has attained the same SFC as MF-RC.

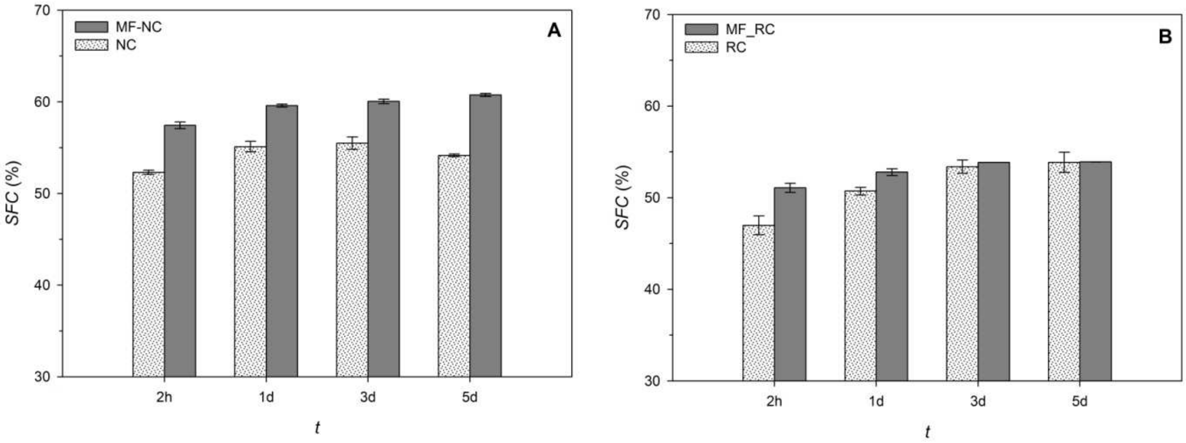


Figure 3.7 SFC (%) of (A) NC and MF-NC and of (B) RC and MF-RC at 2 hours,1 day, 3 and 5 days storage at 5℃.

3.3.5. Discussion

From the XRD-results described above the following crystallization mechanism is suggested for milk fat in bulk, NC and RC:

- During cooling: liquid → liquid +α2L
- First crystallization step at 5℃: liquid + α2L → liquid + α3L
- Second crystallization step at 5℃: liquid + α3L → liquid + β'2L' + α3L'

From the DSC-measurement in Figure 3.2, after 120 min it seems that an equilibrium in the milk fat crystallization was reached in NC, MF-NC, RC and MF-RC. However, SFC-measurements show some extra crystallization during the first 3 days. The polymorphic transition probably continues after 120 min of crystallization at 5℃ most likely combined with some additional crystallization of the TAGs which did not crystallize immediately.

This proposed mechanism is for the most part in agreement with the findings of Lopez et al. [111] who studied the isothermal crystallization at 4℃ of natural cream and its milk fat after quench cooling. The differences between their results regarding the mechanism is that they detected traces of a β-polymorph and didn't associate the α- and β'-polymorphs to the two different long spacings (2L en 3L) present after the polymorphic transition. However, Lopez et al. [111] gave some proper suggestions about the polymorphic correspondence which are confirmed by the experimental data in this research.

At 5℃ in milk fat, the high-melting fraction (HMF) and the middle-melting fraction (MMF) of milk fat will crystallize and constitute the solid phase while the low-melting fraction (LMF) will mainly be present in the liquid phase (the melt) [143]. Therefore, the solid phase will primarily consist of TAGs containing three long-chain saturated fatty acids and TAGs containing two long-chain saturated fatty acids and a short-chain saturated or a long-chain unsaturated fatty acid.

Due to the rapid cooling applied, the crystallization starts in the metastable α-polymorph [110, 139, 144, 145]. β'-crystals are not formed directly from the melt because the time spent between the β'- and α-clear point (35℃ and 20℃ respectively) is shorter than the induction time of the β'-crystals (35 min at 20℃) [41, 146, 147]. Below the α-clear point the induction time of the α-polymorph is lower than for the β'-polymorph, even though the supercooling for the α-polymorph is lower. The longer induction time of the more stable polymorph is attributed to their higher activation free energy which hinders direct nucleation at the given conditions [42].

The metastable α2L-packing appears already during cooling which readily transforms into an α3L-packing from the moment 5℃ is reached. This fast transition probably occurs because a 3L-packing appears to be a more appropriate longitudinal packing to accommodate TAGs in which one or two of the three fatty acid chain moieties are largely different from the other [27]. Both the long-chain monounsaturated TAGs and the TAGs containing a mixture of long-chain and short-chain fatty acids from the crystallizing MMF of milk fat will be incorporated together with the HMF in the α3L-packing at 5℃, despite of their differences in melting points, molecular volume and chemical structure. Hence, compound crystals of HMF and MMF are formed upon rapid cooling to 5℃. This is in agreement with several earlier studies [118, 144, 148, 149]. The structural compatibility, required for the formation of compound crystals, within the heterogeneous group of TAGs in MMF and HMF can be explained by the specific positional distribution of the fatty acids on the glycerol backbone in milk fat [118]. Moreover, in α-crystals the fatty acid chains appear to oscillate and rotate with considerable molecular freedom. The α-packing in the compound crystals will therefore hardly be disturbed when TAGs of MMF and HMF are incorporated [44].

From a thermodynamic point of view, fat crystals can irreversibly transform to other polymorphs at a given condition. In this system the polymorphic transition provokes a molecular segregation of the initially formed crystals in different crystalline structures. The 2L'β'-crystals grow at the expense of 3Lα-crystals but still some α-crystals (3L'α) persist. The higher lateral density of the chains in the β'-polymorph causes a rather limited solid miscibility in the more stable polymorph [44]. Therefore, only TAGs with fatty acids rather similar in chain length and saturation degree will fit in a 2L'β'-organization. The remaining supersatured TAGs, which are unable to crystallize in the 2L'β'-packing, will recrystallize or remain in a α3L' structural organization. The α3L'-crystals will, thus, be enriched in TAGs containing one short-chain fatty acid compared with the initial α3L-crystals. The latter explains the decrease in the long spacings of the α3L'-crystals (66,7 Å) against the α3L-crystals formed at the beginning of the isothermal period (71.4 Å). This segregation of the initially formed compound crystals suggests that the polymorphic evolution takes place via the liquid implying the simultaneous dissolution of the existing crystals and the formation of new 'purer' crystals.

Besides the similarities regarding the crystallization mechanism between NC, MF-NC, RC and MF-RC, the above-described DSC- and NMR-results show differences between cream and MF which counts for both NC and RC. The DSC-measurements demonstrate an overall decreased amount of melting heat necessary to melt the crystals formed in cream during the considered isothermal period and a slower α-β'- polymorphic transition in cream than in bulk milk fat. The NMR-measurements show that the SFC of the creams is substantially lower than in bulk milk fat after 2h storage at 5℃. The latter explains to a large extent the lower amount of melting heat measured in the cream by DSC. However, differences in composition and in the amount of α- and β'-crystals present will probably also contribute to differences in melting heat measured between the cream and the milk fat samples. Moreover, from all of these observations, it can be deduced that the crystallization kinetics is delayed if the milk fat is dispersed in numerous milk fat globules. Although only small or no differences in crystallization temperature between milk fat in cream and in bulk were observed, the explanation can mainly be found on the level of nucleation. When primary nucleation, whether homogeneous or heterogeneous, occurs in a bulk milk fat, the crystallization can readily spread throughout the whole liquid via secondary nucleation at severe supercooling [32]. Secondary nucleation will also occur if milk fat is dispersed in numerous globules but only in the globule in which primary nucleation took place. Therefore, to achieve full crystallization in cream nucleation needs to happen in every individual globule but will not simultaneously arise in each globule. Nucleation is a random process for globules of the same size [54]. Moreover, the fat globules show a polydisperse particle size distribution and the time needed for nucleation is related to the globule volume. At isothermal conditions, the nucleation rate in the small globules will be lower than in larger globules. This scattered nucleation behavior in cream counts for both the α- and β'-nucleation and as a consequence the overall crystallization in cream is more spread in time which is manifested in an overall decreased crystallization rate.

NC shows also some differences with MF-NC other than RC with MF-RC. The α-β'-polymorphic transition in NC starts before MF-NC and the final amount of SFC reached after 5 days storage at 5℃ in cream is still lower than in MF-NC. In RC no substantial differences in the start of the polymorphic transition or in SFC after 5 days at 5℃ can be observed. These additional differences in NC may be attributed to the variation in TAG-composition between the individual globules in milk as is shown in several studies [116, 119, 150, 151]. Regarding the fatty acid composition in milk, even a distinction can be made between small milk fat globules (D < 3 µm) and large milk fat globules (D > 5 µm). Independent of the season the small milk fat globules contain more lauric, myristic and palmitoleic acid and less stearic acid than large milk fat globules [116, 119]. As a result, the MF-NC is thus a blend of various milk fats with a different TAG-composition originating from the naturally constituted milk fat globules of NC. The observed earlier start of the polymorphic transition than in MF-NC is therefore probably caused by only a part of the fat globules which have a TAG-composition for which the β'-nucleation rate is accelerated at the given conditions. Moreover, the β'-nucleation will be more spread in time in NC than in RC which explains the retardation of the polymorphic transition rate compared to RC in which the TAG-composition of each globule is similar and equal to MF-RC. The lower SFC in NC compared to MF-NC can potentially be explained by a decreased SFC in a large amount of fat globules in the considered timeframe. Regarding the RC, an equal amount of SFC is observed after 5 days storage of RC and MF-RC at 5℃ indicating that the delayed crystallization due to emulsification does not interfere with the final amount of SFC reached.

3.4. Concluding remarks

Milk fat in bulk or in cream showed a similar crystallization mechanism if rapidly cooled to 5℃. First, α-crystals were formed and, second, β'-crystals grew at the expense of the α-crystals. However, some α-crystals still remained and additional crystallization during further cooled storage took place. The observed mechanism was explained by the formation of compound crystals. Initially complex compound crystals were formed which, subsequently, partly segregated via the liquid state involving a compositional and/or polymorphic change of the newly formed crystals.

Nevertheless, the overall crystallization rate was lower in both NC and RC. This difference was to a large extent explained by the widely accepted theory of nucleation in the dispersed phase. To achieve full crystallization, nucleation has to take place in each individual globule. The time required to induce nucleation is thereby related to the volume of the globules and if the globules have the same size, the nucleation is a stochastic process. The crystallization behavior of NC deviated from that of RC since the TAG-composition of the individual globules varies in NC. The latter caused further variations in the crystallization kinetics. In the considered NC this was manifested in an earlier start and a more scattered α-β'-polymorphic transition and a lower amount of solid fat after 5 days storage at 5℃.

The acquired knowledge on the crystallization behavior of milk fat in recombined cream is used in Chapter 4 to explore the effect of different monoacylglycerols on the crystallization behavior of milk fat in recombined cream at similar conditions as in this chapter.

4. EFFECT OF MONOACYLGLYCEROLS ON MILK FAT CRYSTALLIZATION IN RECOMBINED CREAM

4.1. Introduction

From the literature review (Section 1.3.3) it is clear that small-molecule surfactants, depending on their miscibility in the fat, can interact (retard or accelerate) with the nucleation, crystal growth and/or polymorphic transitions [43]. As a consequence, crystals differing in size and morphology may be formed. Moreover, the number of crystals and the solid fat content (SFC) may be affected.

The effect of monoacylglycerols (MAGs) on bulk fat crystallization is well-studied for several fat systems. In general, it is accepted that MAGs can autoassociate in inverse micelles which may act as templates for heterogeneous nucleation [54]. Niiya et al. [152] found that saturated MAGs increased the melting point and accelerated the crystal growth slightly in hydrogenated soybean and palm kernel oil while in the presence of unsaturated MAGs the melting point decreased and crystal growth was apparently accelerated. Sambuc et al. [94] concluded that the addition of a mixture of MAGs of palmitic and stearic acid decreased the induction time of vegetable fats. Smith et al. [69] and Smith and Povey [70] discussed a trilaurin model system and they concluded that crystal growth rate increased in the presence of MAGs of lauric acid, while it was hardly affected by the MAGs with a chain length deviating too much from lauric acids. Foubert et al. [97] investigated the temperature and concentration dependent effect of MAGs of oleic and stearic acid on milk fat crystallization. MAGs of stearic acid interact with the nucleation and crystal growth rate dependent on the degree of supersaturation, which is determined both by temperature and concentration. For MAGs of oleic acid a more straightforward relation was detected: the crystal growth rate is enhanced while the induction time is less affected. Fredrick et al. [71] studied the effect of 2% MAGs of hydrogenated palm oil and sunflower oil on the isothermal crystallization of palm oil at 25℃. The MAGs of sunflower oil only accelerate the polymorphic transition whereas MAGs of hydrogenated palm oil also affect the crystallization on nucleation level. The latter accelerated the nucleation rate which even caused crystallization during cooling. The higher degree of saturation of the MAGs of hydrogenated palm oil and the match in chain length with palm oil are suggested as the main factors causing the differences with the effect of MAGs of sunflower oil. Later on, Basso et al. [96] studied also the effect of MAGs on palm oil but they addressed both the isothermal (25℃) and non-isothermal crystallization and used two saturated MAGs which differ in chain length: MAGs of palmitic and behenic acid. Both MAGs led to the formation of many nuclei resulting in accelerated crystal growth and the formation of many small crystals. The effect of MAGs of behenic acid was more pronounced. At isothermal conditions the SFC was not affected. Conversely, Vereecken et al. [153] recently described that MAGs can be used as SFC-provider when they are used at higher concentrations. The effect is strongly dependent on the fatty acid (FA)-composition of the MAGs.

Opposed to the number of studies in bulk fat systems, researches dealing with the effect of MAGs on fat crystallization behavior in oil-in-water emulsions are rather limited. Skoda and Van den Tempel [53], showed an increased crystallization temperature in the presence of MAGs of stearic acid in model emulsions of which the oil phase is composed of tristearin (SSS) or paraffin oil. A more recent study of Davies et al. [154] suggested that in the presence of unsaturated MAGs more spiky SSS crystals are formed while smaller spherical crystals are created in the presence of MAGs of stearic acid. However, no straightforward evidences are given of these morphological hypotheses. Studies on real dairy cream systems are nonexistent. Hence, the main objective of this chapter is to study the effect of MAGs on the primary crystallization kinetics (nucleation, crystal growth and polymorphism) of milk fat in recombined cream (RC) and on the SFC during storage. Moreover, the effect of chain length and saturation degree is investigated.

4.2. Research strategy

Three different commercial distilled MAGs were selected which differ in chain length and/or saturation degree: MAG rich in oleic (MAG-O), stearic (MAG-S) and lauric (MAG-L) acid. The FA-composition is given in Table 2.2. The applied concentrations in the RCs varied between 0% and 0.5% (wt%). However, RC with 0.5% MAG-O was not included in this research since its stability was too low to study the crystallization behavior.

To characterize the RCs the particle size distributions were determined. The crystallization behavior was studied using differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). For both techniques the conventional methods were adapted so that they were appropriate to conduct crystallization experiments in oil-in-water emulsions like cream, as described in Chapter 2. For DSC-analyses the crystallization curves were indirectly constructed by using the melting heats [125]. Corrections for the free induction decay (FID) signal of the aqueous phase were implemented in the frequently applied indirect NMR-method to measure SFC in bulk fat systems [125]. It was chosen to study the isothermal crystallization at 5℃ after a fast cooling step (2 0 ℃.min $^{-1}$) because this resembles most the production process conditions. Additionally, interfacial tension measurements were performed with a drop tensiometer to investigate the presence and the behavior of the MAGs at the oil-water interface. To conduct these measurements, it was required to leave the real dairy cream systems. Macroscopic droplets originating from bulk phases were made.

It should be mentioned that within each set of cream pilot production the same batch of anhydrous milk fat (AMF) and sweet cream buttermilk powder (SCBMP) was used and that the RC without MAGs is used as the reference cream. A comparative study within each set between the reference RC and the RC with MAGs was applied to identify the effect of the chain length, saturation degree and concentration of the MAGs on the studied variables. This comparative approach excludes potential variation between production sets due to, for instance, different storage and processing conditions of both the RCs and the starter material or due to slightly different composition of the starter material.

4.3. Results and discussion

4.3.1. Particle size distribution

In oil-in-water emulsions the crystallization behavior can be affected to a large extent by the particle size distribution. The smaller the droplet is, the higher the supercooling needed to induce crystallization [54, 59]. The latter counts until the supercooling for homogeneous nucleation is reached. Moreover, the SFC may be increased with increasing particle size of the emulsion [53, 117]. In order to eliminate this influencing factor, the different RCs were produced with a comparable order of magnitude of particle size distribution independent of the type and concentration of MAGs. Although substantial differences in Sauter diameter or volume-surface weighted diameter ($D_{3,2}$) can be observed (Table 4.1), MAGs show a limited effect on particle size distribution at the highest applied concentrations (Figure 4.1): 0.2% MAG-O, 0.5% MAG-S and 0.5% MAG-L. Also no large variations between the reference RCs without MAGs of different production sets can be observed.

Table 4.1 $D_{3,2}$ (µm) of RCs with different concentrations of MAG-O, MAG-L and MAG-S.

[MAG] (%)	MAG-O	MAG-S	MAG-L
0	3,28 ± 0,02	3,46 ± 0,01	3,10 ± 0,02
0,035	3,32 ± 0,29	3,39 ± 0,01	3,26 ± 0,01
0,07	3,25 ± 0,01	3,26 ± 0,16	3,12 ± 0,01
0,1	3,28 ± 0,02	3,25 ± 0,18	3,01 ± 0,01
0,2	3,11 ± 0,01	3,29 ± 0,02	2,98 ± 0,05
0,5	-	3,15 ± 0,09	2,32 ± 0,01

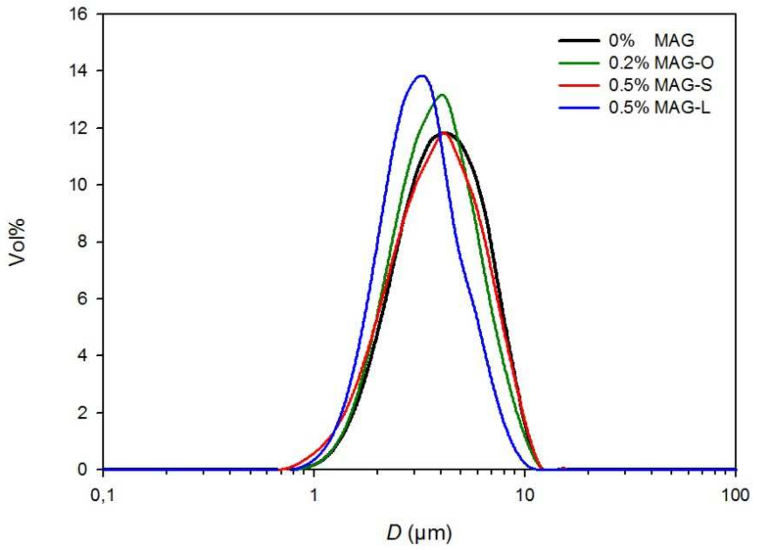


Figure 4.1 Particle size distribution of RCs with 0% MAGs, 0.2% MAG-O, 0.5% MAG-S and 0.5% MAG-L.

4.3.2. DSC-analyses

4.3.2.1. Crystallization temperatures

A. Monoacylglycerols

Because of the differences in chain length and saturation degree of the major fatty acids of the applied MAGs, the crystallization behavior of the bulk MAGs will largely be affected. Table 4.2 gives the temperature at which crystallization starts (T_{cr}) upon cooling and the final melting temperature (T_m) upon heating. Both the T_{cr} and the T_m of MAG-O, MAG-S and MAG-L are substantially different. The T_{cr} measured are in general slightly lower than found in literature [155] which can be explained by the difference in *T*-history, in FA-composition and in purity of the commercial distilled MAGs used. Impurities like diacylglycerols (DAGs) and TAGs delay the crystallization of MAGs [155].

Table 4.2 Crystallization temperature (T_{cr}) and final melting temperature (T_m) of the MAG-O, MAG-S and MAG-L measured at a cooling rate of 25 ℃.min^{-1} and heating rate of 20 ℃.min^{-1}.

	T_{cr} (℃)	T_m (℃)
MAG-O	11.51 ± 0.21	29.81 ± 0.19
MAG-S	68.02 ± 0.10	74.24 ± 0.15
MAG-L	35.89 ± 0.04	42.15 ± 0.22

B. Cream with monoacylglycerols

Table 4.3 shows the crystallization temperatures of the RCs with different types and concentrations of MAGs. In the presence of MAG-S a significant increasing trend in T_{cr} can be observed if [MAG-S] ≥ 0.07%. Because of the high T_{cr} of MAG-S, they can serve as heterogeneous nuclei to induce crystallization of the milk fat in RC at higher temperatures, if the concentration of MAG-S is high enough. This can be due to interfacial heterogeneous nucleation or volume heterogeneous nucleation depending on the presence and the behavior of the MAGs at the oil-water interface or in the dispersed oil phase, respectively [50]. The increasing trend in T_{cr} as a function of [MAG] is observed in neither the RCs with MAG-O nor with MAG-L. For MAG-O, this is not surprising, since the T_{cr} of MAG-O (11.51 ± 0.21℃) is similar to that of milk fat in RC without MAGs (11.35 ± 0.1℃). However, the T_{cr} of pure MAG-L (35.89 ± 0.04℃) is substantially higher than the T_{cr} of milk fat in RC without MAGs (11.70 ± 0.21℃).

Table 4.3 The T_{cr} (℃) of RCs at different MAG-concentrations.

[MAG] (%)	MAG-O (℃)	MAG-S (℃)	MAG-L (℃)
0	11.35 ± 0.10 ^{a,b}	11.88 ± 0.04^{a}	11.70 ± 0.21^{a}
0,035	11.21 ± 0.08^{a}	11.78 ± 0.03^{a}	11.72 ± 0.14^{a}
0,07	-	13.33 ± 0.04^{b}	11.72 ± 0.18^{a}
0,1	11.30 ± 0.11^{a,b}	14.15 ± 0.12^{c}	11.47 ± 0.18^{a}
0,2	11.59 ± 0.09^{b}	15.78 ± 0.21^{d}	11.76 ± 0.21^{a}
0,5	-	16.81 ± 0.25^{e}	11.84 ± 0.31^{a}

$^{a-e}$ Different letters indicate significant differences ($p < 0.05$) within one type of MAGs.

4.3.2.2. Isothermal crystallization behavior of cream

To study the isothermal crystallization behavior of RC at 5℃ it is required to construct the crystallization profiles in an indirect way, as described in Section 2.2.1. The prerequisite to do this is that the stability of the cream is guaranteed as a function of the applied *T*-cycli [125]. For all RCs investigated the stability was controlled and assured. No destabilization was detected.

Figure 4.2 A shows the isothermal crystallization curves of RCs with different [MAG-O] at 5℃ after fast cooling (25℃.min^{-1}). The crystallization profiles show all a two-step crystallization indicating a similar crystallization mechanism as described in Section 3.3.5. First, α-crystals grow from the melt during cooling and at the beginning of the isothermal period (first step). Second, β'-crystals are shaped at the expense of α-crystals (second step) while still some α-crystals persist. However, the latter have a different composition than the initially created α-crystals during cooling. No significant effect of MAG-O on the crystallization mechanism and kinetics can be detected.

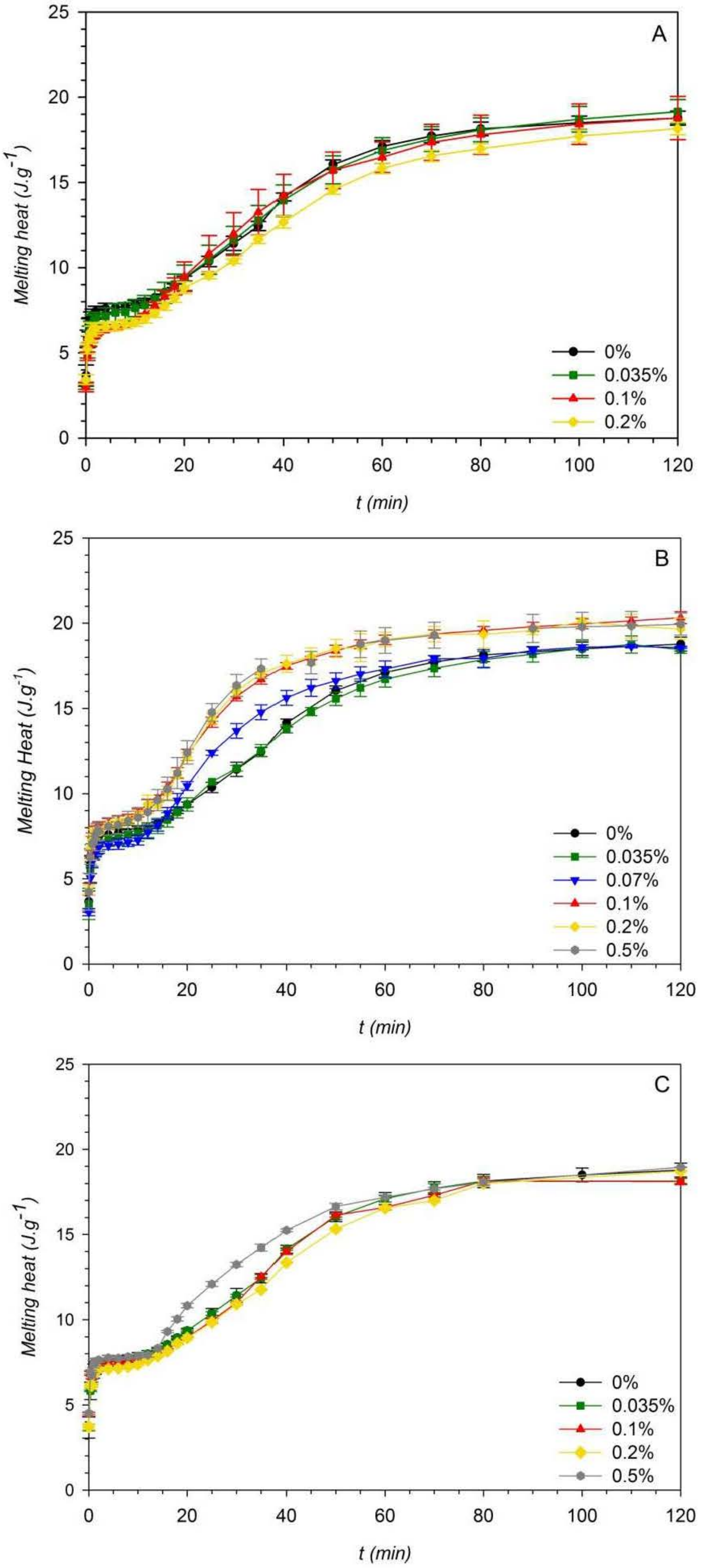


Figure 4.2 Isothermal crystallization curves at 5℃ of RCs at different concentrations of (A) MAG-O, (B) MAG-S and (C) MAG-L constructed by plotting the melting heat per g cream sample as function of the holding time.

For MAG-S the results are shown in Figure 4.2 B. Next to the similarities in the two-step crystallization mechanism between the different variants, some differences in crystallization kinetics can clearly be detected. At [MAG-S] > 0.07%, the slope of the second step is steeper and, hence, the α-β’ polymorphic transition seems faster than at lower concentrations. Furthermore, at [MAG-S] > 0.1% the melting heat required to melt the crystals, created during each applied isothermal period, is higher. The latter probably indicates a higher SFC, differences in TAG-composition of the fat crystals or a combination thereof.

Figure 4.2 C gives the results for RCs with MAG-L. The only difference observed here is the increased rate of the second step and, hence, of the α-β’ polymorphic transition at the highest concentrations of MAG-L (0.5%). No significant difference in the final melting heat can be detected.

4.3.3. *NMR-analyses*

In order to study the effect of MAGs on milk fat crystallization in cream at 5℃ during storage the SFC was measured for the highest applied concentrations of the different MAGs. SFC was measured after 2h, 1 day and 5 days storage at 5℃ and compared with the reference RC without MAGs of the same production set (Figure 4.3). The results of the statistical analyses are given in Annex I. Independent of the type and concentration of MAG, SFC increases during storage. Measurement after 5 days didn’t show further increase indicating that equilibrium has been reached (data not shown). Figure 4.3 A and C gives the results for MAG-O and MAG-L, respectively. No significant differences as a function of [MAG] can be observed. In the presence of MAG-S significant differences in SFC with increasing [MAG-S] after 2h and 1 day storage can be detected, as shown in Figure 4.3 B. However, after storage for 5 days these differences vanished.

4.3.4. *Interfacial tension analyses*

Small-molecule surfactants may create upon cooling a two-dimensional (2D) crystal at the oil-water interface below a certain temperature. This phenomenon is called *chain crystallization* and occurs at the *chain crystallization temperature* ($T_{cr,chain}$). The latter depends strongly on the concentration, the chain length and saturation degree of the hydrophobic moiety of the small-molecule surfactants. For MAGs this phenomenon was studied by Lutton et al. [156] and Krog and Larsson [157] and could be detected as a sharp decrease in the oil-water interfacial tension (γ_{OW}) upon cooling. In oil-in-water emulsions chain crystallization may have further consequences: first, the 2D-crystals at the oil-water interface may serve as heterogenic nuclei to induce crystallization of the TAGs and, second, proteins adsorbed at the oil-water interface may be displaced [157].

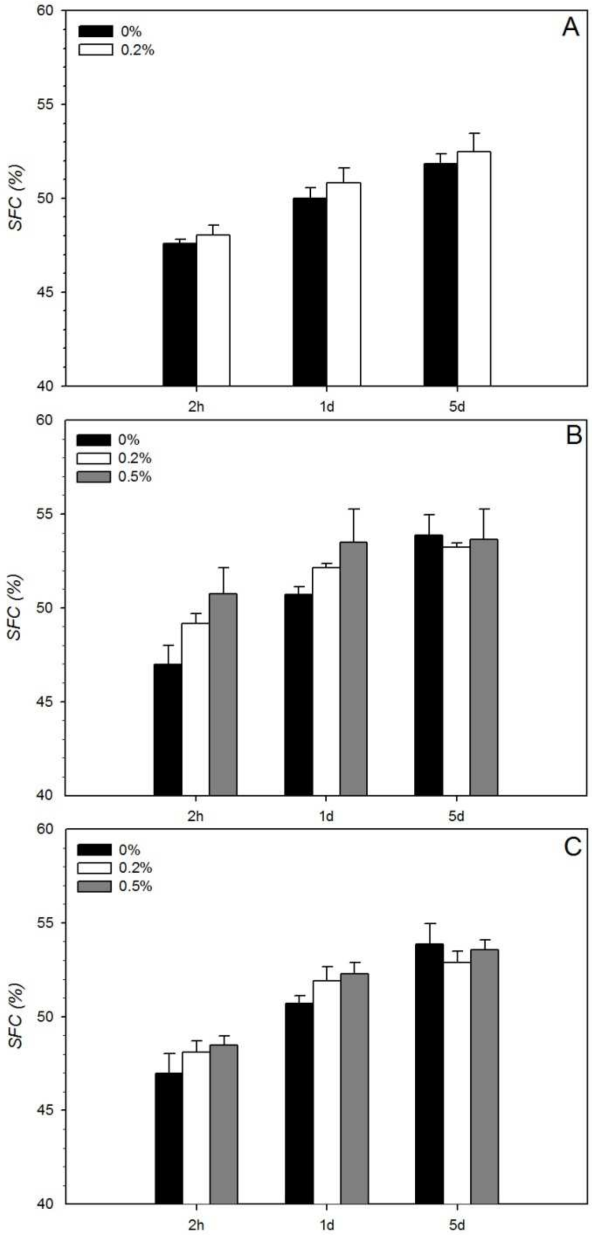


Figure 4.3 SFC (%) of RC without MAGs and (A) with 0.2% MAG-O, (B) with 0.2% and 0.5% MAG-S and (C) with 0.2% and 0.5% MAG-L after storage for 2h, 1 day and 5 days at 5℃.

To elucidate the presence and the physical state of MAGs at the interface, interfacial tension measurements were performed. Direct measurement on the oil-water interface of cream was unfeasible. Therefore, it was essential to deviate from the real cream system. In order to not diverge too far from the applied RC-systems, the same concentrations of SCBMP and MAG were used. The applied SCBMP- and MAG-concentrations to prepare the aqueous and oil phase were, thus, recalculated from emulsion-based concentrations to water-based and oil-based concentrations, respectively. For instance, a RC with 0.2% MAG-S with 35% fat has an oil phase which contains 0.6 % (= 0.2/35*100) MAG-S.

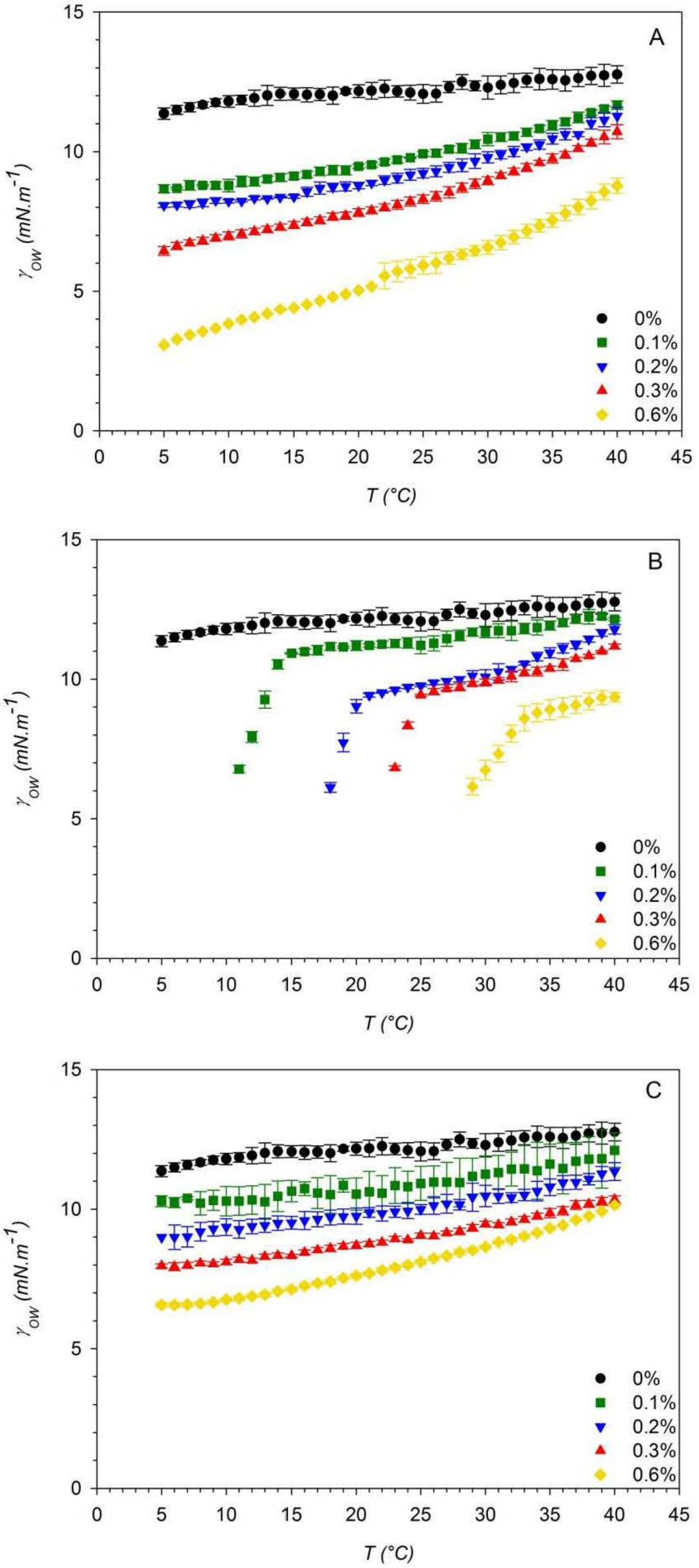


Figure 4.4 Interfacial tension γ_{OW} (mN.m^{-1}) of an interface created between buttermilk and purified sunflower oil during cooling as a function of concentration of (A) MAG-O, (B) MAG-S or (C) MAG-L (oil-based concentrations).

Figure 4.4 shows the γ_{OW} of oil-buttermilk systems in the presence of the different types and concentrations of MAGs during cooling. In the presence of MAGs, γ_{OW} is lower at all temperatures showing that MAG-molecules are present at the oil-water interface independent of the type of MAG. In addition, with increasing concentration of MAG, γ_{OW} decreases further regardless of the temperature. More MAGs are probably positioned at the oil-water interface when the concentration increases. In the presence of MAG-S, a discontinuity in the slope of the γ_{OW} - curves is observed regardless of its concentration. These discontinuities in slope or the sharp decreases in γ_{OW} clearly show that chain crystallization is taking place [156, 157]. For instance, at 0.6% MAG-S, the slope changes at a $T_{cr,chain}$ = 33℃. At T < 29℃, γ_{OW} becomes too low to keep the droplet pending on the needle of the drop tensiometer. $T_{cr,chain}$ decreases with decreasing concentrations of MAG-S which is conform with the results of Lutton et al. [156] and Krog and Larsson [157]. No discontinuities in the γ_{OW} - curves and, hence, no chain crystallization is observed in the presence of MAG-O and MAG-L irrespective of the concentration.

4.3.5. Discussion

MAG-S is the only type of the investigated MAGs that shows an effect on the nucleation since an increased T_{cr} is observed and, thus, heterogeneous nucleation takes place. This accords well with the findings of Skoda and Van den Tempel [53] who revealed an increased nucleation rate in model emulsions due to the presence of MAG-micelles in the oil droplets. However, in the current study interfacial measurements (Section 4.3.4) indicate the presence and the crystallization of MAG-S at the oil-water interface demonstrating that interfacial heterogeneous nucleation takes place rather than volume heterogeneous nucleation. MAG-S create a template at the oil-water interface inducing the crystallization of milk fat TAGs. Subsequently, crystallization proceeds throughout the whole volume of the droplets. Nevertheless, in the presence of MAG-S the measured $T_{cr,chain}$ are rather high compared to the T_{cr} of the RCs. For instance, at 0.2% MAG-S, which corresponds with 0.6% MAG-S in the oil phase, the T_{cr} and $T_{cr,chain}$ are 16.81 ± 0.25℃ and 33℃, respectively. This is probably caused by the difference in ratio of the interfacial area to the oil volume in the drop tensiometer and in the real cream system. In the drop tensiometer this ratio is very low compared with the real cream system. More MAG-molecules will, thus, be available to position at the oil-water interface. As a consequence, the measured $T_{cr,chain}$ is likely to be an overestimation of the actual $T_{cr,chain}$ and, hence, the T_{cr} in the real cream systems. Yet, chain crystallization in the cream system is believed to take place as it also occurs at lower MAG-S concentrations (Figure 4.4). In literature interfacial heterogeneous nucleation was established for other small-molecule surfactants with a long-chain saturated hydrophobic tail [53, 84, 104, 105, 107, 158]. Furthermore, it is reasonable to assume that the observed increased nucleation rate due to heterogeneous nucleation of small-molecule surfactants results in more and smaller crystals as was suggested by Basso et al. [96], Fredrick et al. [71] and Sakamoto et al. [67] in bulk fat systems and by Arima et al. [107] in model oil-in-water emulsions. In this study several attempts have been taken to visualize fat crystals in the milk fat globules with microscopic techniques. Unfortunately, due to the small sizes of the fat crystals and the numerous sample preparation steps, in particular for electron microscopic techniques, the size, the morphology and the arrangement of the fat crystals in the globules could not be observed.

In contrast to MAG-S, for both MAG-O and MAG-L no increased T_{cr} is observed. Also, chain crystallization is not observed at the applied concentrations implying that interfacial heterogeneous nucleation does not take place. These discrepancies between the effect of MAGs with different chain length and saturation degree of the hydrophobic moiety are also perceived for sucrose oligoesters (SOEs) [50, 84, 104, 105] and DAGs [158]. SOEs and DAGs of palmitic and stearic acid were found to accelerate interfacial heterogeneous nucleation in both palm kernel and n-hexadecane oil-in-water emulsions while SOEs and DAGs of lauric and oleic acid do not show any effect on the nucleation rate. It should be remarked that at higher concentrations of MAG-L chain crystallization would probably take place because of the rather large difference in T_{cr} of the reference RC (11.70 ± 0.21℃) and the bulk MAG-L (35.89 ± 0.04℃). For MAG-O it will not take place at any concentration due to similar T_{cr} of the reference RC and the bulk MAG-O: 11.35 ± 0.10℃ and 11.51 ± 0.21℃, respectively.

The SFC-data at the highest applied concentrations of MAG-S, confirm that the increase in melting heat during the whole isothermal period, as measured by DSC, can at least partly be ascribed to an increase in solid fraction. Since MAG-S act as catalytic impurities, more nucleation sites in RC with MAG-S will be present which increases the possibility that crystallization has started in more individual globules than in RC without MAGs. In addition, due to the interfacial heterogeneous nucleation mechanism in the presence of MAG-S the composition of the formed crystals will possibly be different which further contributes to the differences in the measured melting heat. At T_{cr} of RC without MAGs more milk fat TAGs will be supercooled resulting in a more mixed TAG-composition of the initially created crystals than these shaped at the elevated T_{cr} of RC with MAG-S (probably less mixed). Further follow-up of the SFC during storage at 5℃ showed that the differences vanished after 5 days. This suggests that the MAG-S show an apparent accelerating effect on the crystal growth rate due to the increase in heterogeneous nucleation until the equilibrium SFC is attained.

On the level of polymorphic transitions both MAG-L and MAG-S seem to have an accelerating effect. The effect of MAG-S is thereby more pronounced and is observed already at lower concentrations while the effect for MAG-L is only observed at the highest applied concentration. In literature acceleration of polymorphic transitions is observed for several small-molecule surfactants, including MAGs, at isothermal conditions in bulk fat systems [60, 71]. Small-molecule surfactants are thereby thought to create crystal imperfections within the growing crystals owing to their low structure affinity with the TAGs. In this case acceleration can therefore probably be explained by incorporation of MAG-S and MAG-L in the crystal lattice. The latter implies that MAG-L probably co-crystallizes with the milk fat TAGs. Although no further indication of co-crystallization is found, it is believed that this is possible because of the high T_{cr} of bulk MAG-L. For MAG-S the difference in polymorphic transition rate can also be related to the difference in TAG-composition of the initially formed crystals. In the presence of MAG-S the α-crystals will have a narrower TAG-composition which enhances the polymorphic transition rate [1].

4.4. Concluding remarks

The effect of MAGs which differ in chain length and degree of saturation on the primary crystallization behavior of milk fat at processing conditions (*t,T*-program) closely related to the ones applied during the production of whipping cream (fast cooling to 5℃) was investigated. Furthermore, SFC of milk fat during storage was followed.

The long-chain unsaturated MAGs (MAG-O) didn't show significant effects on the primary crystallization behavior and SFC. A similar crystallization mechanism compared to the reference RC was observed. Nucleation of α-crystals occured in the droplet (volume heterogeneous/homogenous nucleation). Subsequently, the growing α-crystals partly transformed into β'-crystals while still some α-crystals persisted. However, the latter had a different composition than the initially created α-crystals during cooling.

A difference in nucleation mechanism was detected in cream containing the long-chain saturated MAGs (MAG-S). Interfacial heterogeneous nucleation took place during cooling. As a consequence, the crystallization started at higher temperature and the crystal growth and the α-β' polymorphic transition was accelerated compared to the reference RC. However, the mechanism after nucleation was found to be similar to the reference RC and the SFC after prolonged storage at 5℃ was unaffected.

Intermediate behavior was observed with saturated MAGs with a mid-chain length (MAG-L). These MAGs accelerated the α-β' polymorphic transition while they did not show any effect on the nucleation and the crystal growth. Moreover, these MAGs did not affect the SFC. It was postulated that the intermediate behavior can be ascribed to co-crystallization of the MAGs once crystallization has started.

The effect of MAGs on the nucleation mechanism and crystallization kinetics may result in a different number, size, arrangement and morphology of the crystals in the fat globule. However, a proper microscopic method to visualize fat crystals in emulsion droplets is still lacking.

The differences in crystallization behavior of the fat globules with different MAGs may have consequences for the partial coalescence rate and, hence, the whipping properties of the RCs investigated in this chapter. Therefore, the effect of the MAGs on partial coalescence and on the whipping properties is further assessed in Chapter 8 and Chapter 9 of this manuscript.

PART II: PARTIAL COALESCENCE

5. LITERATURE REVIEW

5.1. Cream

5.1.1. Definitions

Cream is a fat-rich fluid milk product that can be defined as a fat-in-skimmed milk emulsion (oil-in-water type) derived by physical separation from milk. This description refers to the fat-rich top layer that rises on fresh milk when left to stand and is indicated as *natural cream* in this manuscript. Fat globules have a lower density than the milk serum phase causing the droplets to move upwards. In practice, this creaming process is accelerated by a cream separator to obtain cream and skimmed milk [1]. After standardization different types of cream can be manufactured, mainly differing in fat content (Table 5.1).

Table 5.1 Different types of cream (US Code of Federal Regulations-Title 21- Part 131).

Type	Milk fat content	uses
Half and Half cream	12% fat (range 10.5% - 18%)	Cream in coffee Cooking
Single cream	20%	Cooking
Light cream	20% (range 18% - 30%)	Coffee or table cream Cooking
Whipping cream	30%-36%	Toppings and fillings
Heavy whipping cream	>36%	Whipping
Double cream	48%	British whipping cream
Clotted cream	55% - 60 %	British served with scones

Nowadays, besides natural cream, *recombined cream* is often used. It is obtained by recombining milk products (e.g. milk fat and milk powders) with or without the addition of potable water (CODEX STAN 288-1976). The benefit of using recombined cream is the flexibility to modify the composition and formulation for product development goals as well as for avoiding seasonal variations. In addition, in regions where fresh milk is not readily available and/or where suitable storage facilities are scarce recombined cream is used to reduce the storage and transport costs.

5.1.2. Physical properties

Like other oil-in-water emulsions, creams are physically mainly characterized by their fat volume fraction φ or fat content (Table 5.1), particle/droplet size distribution (Section 5.1.2.1) and viscosity (Section 5.1.2.2). Furthermore, determination of these properties is an appropriate tool to evaluate the physical stability of the oil-in-water emulsions (Section 5.1.2.3).

5.1.2.1. Particle size distribution

The volume-surface average diameter or Sauter diameter ($D_{3.2}$) of milk is about 3.4 µm, but can vary according to breed, diet, fat yield of the milk, milking frequency and stage of lactation [1, 151, 159, 160]. During processing of milk, it can be further affected by several treatments, in particular by homogenization [161]. The average particle size of natural cream is similar to milk but can be slightly higher depending on the separation efficiency during cream separation. Homogenized creams have a $D_{3,2}$ < 1 µm [1]. For recombined cream the average particle size is dependent on the type and concentrations of surface-active material (proteins and small-molecule surfactants) [162, 163], the viscosity of the aqueous phase (stabilizers) [33, 164, 165], and the type of homogenization equipment and the applied process parameters (flow condition, time and temperature) [162, 163].

Several techniques are available to measure the particle size distribution of oil-in-water emulsions:

- Particle counters (number based) (i.e. sensing zone technique, moving sensing zone technique)
- Microscopy combined with image analyses
- Chromatographic techniques (separation)
- Gravitation/centrifugation techniques (separation techniques based on Stokes' law)
- Static and dynamic light scattering (optical technique)
- Acoustic techniques
- Pulsed field gradient nuclear magnetic resonance (NMR)

Every technique has its advantages and disadvantages and even the use of several techniques doesn't guarantee yielding exactly the same particle size distribution.

5.1.2.2. Viscosity

Viscosity (η) can be defined as the resistance of a fluid to flow and can be calculated from:

$$\sigma = \eta\dot{\gamma} \tag{5.1}$$

with σ the *shear stress* (N.m^{-2} or Pa) necessary to deform the liquid at a certain *shear rate* $\dot{\gamma}$ (s^{-1}). For *Newtonian liquids* like milk and stable cream (φ < 0.4) the viscosity is independent of the shear rate [1].

The viscosity of dispersions such as oil-in-water emulsions can be determined by the *Einstein equation,* if the dispersed volume fraction φ is smaller than 0.01 [1, 162]:

$$\eta = \eta_s(1 + 2.5\varphi) \tag{5.2}$$

with η_s the viscosity of the solvent or the continuous phase.

For higher volume fractions φ, the *Krieger-Doughtery equation* is applicable for hard spherical particles [1, 162]:

$$\eta = \eta_s(1 - \frac{\varphi}{\varphi_{max}})^{-[\eta]\varphi_{max}} \tag{5.3}$$

With φ_{max} the maximum volume fraction attainable and $[\eta]$ the intrinsic viscosity. For monodisperse and polydisperse systems φ_{max} is about 0.7 and 0.8, respectively. The value of φ in the equation has to be the effective volume fraction (φ_{eff}) which can substantially deviate from φ. This in particular in dispersions where the solvent is partly immobilized or captured because of the presence of irregularly shaped particles, particles that show interfacial roughness or aggregated particles. Moreover, it should be stressed that according to the Krieger-Doughtery equation the viscosity is independent of particle size.

For cream-like systems the Krieger-Doughtery equation applies reasonably well using the polydisperse φ_{max} (= 0.8). In cream both fat globules and casein micelles are dispersed and differ largely in size: 1-10 µm and 0.2 µm, respectively. In addition, note that the viscosity of cream is also highly dependent on the temperature, pH and the presence of clusters/aggregates [1].

5.1.2.3. Physical stability

From a thermodynamic point of view, cream-like macroemulsions are by definition unstable systems and, therefore, a better stability can only be achieved by affecting the kinetics. Various types of instability mechanisms are at the basis of changes in size, number and arrangements of droplets in oil-in-water emulsions. Figure 5.1 illustrates the types of instability and, below, the mechanisms are briefly discussed [1, 33, 162, 166, 167].

- ***Ostwald ripening*****.** This involves the transport by diffusion of molecules of the disperse phase from small to large particles, due to the differences in *Laplace pressure*[1]. Laplace stated that the pressure at the concave side of a curved interface is higher than the pressure at the convex side by an amount that is proportional to the interfacial tension times the curvature. The result is that the solubility of the disperse phase in the continuous phase is increased to a larger extent for small particles than for large ones; hence, small particles shrink (and will eventually disappear) and large particles grow. The rate at which the transport will occur is about proportional to the solubility of the dispersed phase molecules in the continuous phase. Given that the solubility of nearly all natural triacylglycerols (TAGs) in most aqueous systems is negligible, the Ostwald ripening can mainly be ignored in food oil-in-water emulsions like cream.

---

[1] In emulsion the Laplace pressure ΔP is the pressure difference between the inside and the outside of a droplet. The effect is caused by the interfacial tension γ between two liquids. For a spherical emulsion droplet with a radius *r* the Laplace pressure ΔP can be calculated as: $\Delta P = P_{inside} - P_{outside} = \frac{2\gamma}{r}$.

– ***Creaming***. This is caused by the difference in mass density between the dispersed oil and the continuous water phase. In nearly all oil-in-water emulsions the difference is negative, causing the droplets to move upwards. The rate of creaming of a separate droplet is defined by *Stokes' law*[2]. However, if the volume fraction of the oil droplets is high, creaming is also dependent on several additional variables. Anyway, it always results in the formation of a cream layer in which the droplets are in close contact. Creaming rate can be enhanced by centrifugation to up to hundreds or even thousands time that caused by gravity.

– ***Aggregation***. Droplets frequently come close to each other due to Brownian motion. It will then depend on the forces acting on the droplets whether they will stay in close contact for a substantial time or not. There are two kinds of interaction forces: internal and external. The internal forces can be attractive or repulsive. It is the balance of all these internal forces as a function of interdroplet distance that determines whether aggregation occurs or not. The main internal attractive force is the ubiquitous van der Waals attraction of which the strength depends on the composition of both phases. Besides, the presence of non-adsorbing polymers in the continuous phase can cause an attractive depletion force. The main repulsive forces are electrostatic, caused by equal electric charges on both droplets, mostly due to substances adsorbed on the interface of the droplets. The pH and the ionic strength of the continuous phase are important variables determining the extent of electric repulsion. Another important repulsive mechanism is steric repulsion, also caused by adsorbed substances. The latter may contain hydrophilic polymeric chains that protrude into the continuous phase. External forces are mainly hydrodynamic, i.e. caused by motion of the liquid (e.g. due to stirring) and are for the most part trying to disrupt the aggregates. If gentle stirring suffices to achieve disruption, the aggregation is considered to be reversible, and it is often called *flocculation* while *coagulation* refers to aggregation where strong forces are needed to disrupt the aggregates. It may be added that aggregation of particles can lead to the formation of a particle gel if the aggregation is not hindered by creaming or stirring.

---

[2] Stokes' law : $v_s = \frac{2}{9}\frac{(\rho_d-\rho_c)}{\eta} g r^2$ with v_s the creaming rate, $\rho_d - \rho_c$ the difference in density between the dispersed phase and the continuous phase; η the viscosity of the continuous phase; g the gravitational acceleration constant and *r* the radius of the globule

– ***Coalescence***. This can occur if two droplets are for some time very close with each other, be it by aggregation or in a cream layer, so that only a very thin film of the continuous phase prevents oil-oil contact. Such a film may spontaneously break, the more readily if the film diameter is larger (e.g. larger droplets), the film is thinner (e.g. the stronger the net attraction between the droplets) and the oil-water interfacial tension is lower, which depends on the type and concentration of the emulsifier used. Moreover, when proteins are used as emulsifier the interfacial viscoelastic behavior will determine to a large extent the film diameter and the subsequent film break. As soon as the film breaks, the two adjacent droplets coalesce to form one larger drop; this is due to the Laplace pressure being locally quite high, and the oil will flow to sites where the pressure is lower. The oil will then assume a spherical shape yielding the lowest Laplace pressure possible

– ***Partial coalescence***, also called *clumping*. This can only occur if the droplets contain solid particles, mainly crystals. The droplets are than called *fat globules*. In the simplest case the crystals form a solid network in the globules and a few crystals protrude from a globule into the continuous water phase. Such a crystal can, upon collision with another globule, pierce the adsorption layer of that globule, thereby making oil-oil contact, as shown in Figure 5.1. The mechanisms and factors affecting partial coalescence will be discussed in detail in Section 5.1.3 and 5.1.4.

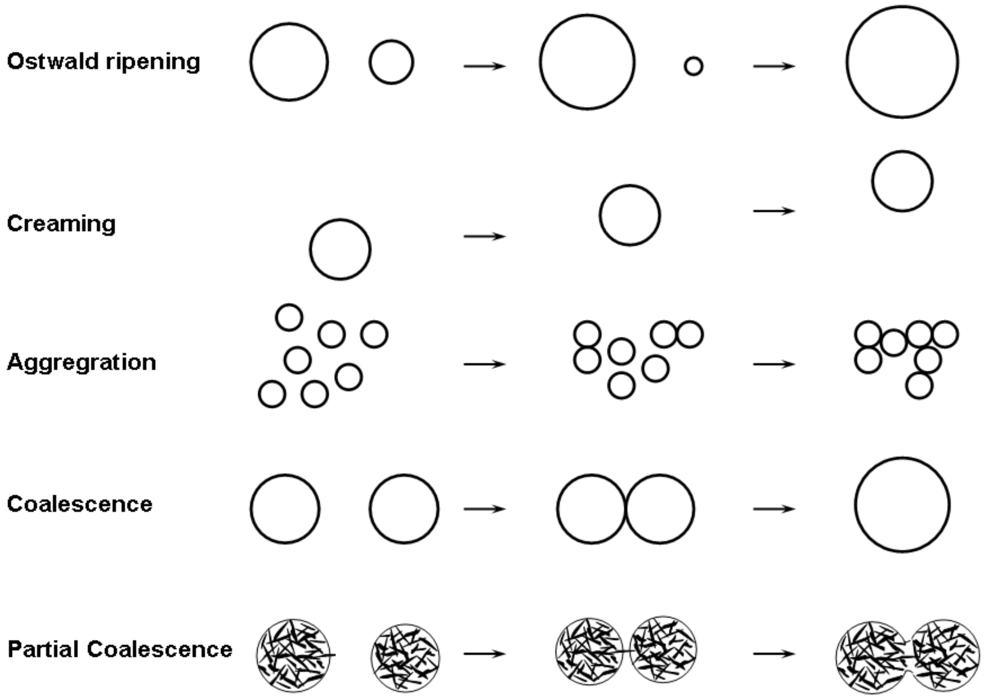


Figure 5.1 Schematic presentation of potential instability mechanisms in oil-in-water emulsions, after Walstra [1, 33, 166, 167].

These instability mechanisms may take place simultaneously in one system and, moreover, the occurrence of one instability mechanism may encourage others [162, 166, 168]. For instance, aggregation of droplets may enhance creaming, coalescence and partial coalescence depending on several conditions. ‘True’ and partial coalescence are promoted due to the extended contact time between the droplets. Partial coalescence will, in this case, especially be encouraged when the emulsion is subjected to temperature fluctuations [168].

The consequences of the instability mechanism partial coalescence show a discrepancy. On the one hand, it should be suppressed to attain an extended shelf life of commercial available food products. On the other hand, during the manufacturing of some products partial coalescence is required to achieve the desired properties in products like ice cream, butter, whipped creams, toppings, etc. [135, 169-172] It contributes to the structure formation, the physical properties (e.g. overrun, stability and firmness) and the sensory perception (e.g. fattiness and creaminess) [173] of the final food products.

Next to the pioneering work of Labuschagne [174], van Boekel [175, 176], Oortwijn and Walstra [177], Darling [171] and Boode [130, 178-181], more recent work is discussed on the mechanism of partial coalescence and on the parameters that affect the occurrence and rate of partial coalescence in Section 5.1.3 and 5.1.4, respectively. The aspects of the interaction of fat globules and/or fat clumps with air or gas bubbles and the importance of partial coalescence in the manufacture of whipped cream and other toppings are discussed in Section 5.2.3 and 5.2.4. The discussion is not limited to dairy oil-in-water emulsions.

5.1.3. Mechanism of partial coalescence in oil-in-water emulsions

Partial coalescence can only be accomplished when solid particles are present in the dispersed phase. Therefore, in an oil-in-water emulsion fat crystals are required. *Nucleation* and, hence, *fat crystallization* in the dispersed oil phase take place during cooling after reaching a certain degree of supercooling. Supercooling is the driving force for the crystallization process and can be defined as the difference between the final thermodynamic melting temperature of the crystallizing components and the actual absolute temperature. If the liquid oil is supercooled, nucleation can happen in several ways, as extensively described in Chapter 1 (Section 1.3.1.2 and Section 1.3.2). The initially formed fat crystals can move freely in the globule. Since it is thermodynamically more favorable that the fat crystals are located at the oil-water interface (as allowed by the contact angle, see Section 5.1.4.1.B.ii), crystals will have the tendency to position at that interface. Even a few of the crystals may stick out in the water phase [176]. During subsequent crystallization, as a result of van der Waals attraction, aggregation of the fat crystals will be induced and an internal fat crystal network will be formed. The fat crystals trapped in this internal network are thereby restricted in their ability to move to the interface [162, 176]. In most of the natural fats, this internal fat crystal network exists in a wide range of temperatures because of their strongly heterogeneous TAG-composition. The number of protruding crystals as well as their protruding distance are enhanced when the temperature decreases and the content of crystalline matter increases (see Section 5.1.4.1.B.i).

When two semi-crystalline globules approach, the protruding crystals may pierce the thin film between the two globules and the adsorbed layer of the second droplet (Figure 5.2), in particular when close approach of the globules is promoted [130, 176, 181]. At that time, the crystal penetrating the second globule will preferably be wetted by the oil rather than by the continuous aqueous phase. If sufficient liquid oil is available in the globules, the oil will start to flow around the crystal reinforcing the link between the two droplets [130]. An oil neck enclosing the crystal is created between the two droplets. Although this semi-solid junction becomes larger and firmer upon aging, the globules keep remnants of their original shape (as in coagulation) but there is a true molecular contact between their contents (as in 'true' coalescence). The mechanical strength of the internal fat crystal network limits the rearrangement of the crystals thus hindering the complete merging of the globules into the energetically preferred spherical shape, driven by the Laplace pressure. As a consequence, partially coalesced globules maintain an irregular shape also known as a clump when two or more globules are involved [130].

Upon heating, the fat crystal network disappears and, the globules, due to their internal contact, will merge and assume the spherical shape driven by the Laplace pressure. Finally, 'true' coalescence is established.

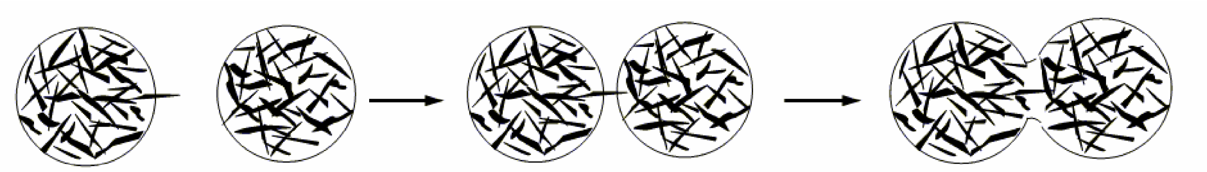


Figure 5.2 Schematic presentation of the mechanism of partial coalescence, after Boode [130].

Considering this mechanism, the following main criteria need to be fulfilled to achieve partial coalescence in an oil-in-water emulsion:

- The oil phase is semi-crystalline.
- The crystals in the oil droplets form a crystal network and at least one crystal protrudes in the aqueous continuous phase.

- The distance between two approaching globules is smaller than the size of the protruding part of the crystal.
- The remaining liquid oil is not completely immobilized/captured by the crystal network.

Partial coalescence differs from ‘true’ coalescence in the following main aspects:

– Stability against partial coalescence is usually lower than towards ‘true’ coalescence [62, 130, 166, 173, 175, 176]. The rate may differ by a factor 10^6 or even more for similar emulsions with or without crystals in the globules [33, 176]. The external stress, which forces the globules together, is locally increased at the end of the protruding fat crystals. The area over which the external stress acts, is thus much smaller than the cross-sectional area of the globule. The external stress must therefore be multiplied by a stress concentration factor Λ which is proportional to $r^2_{globule}/r^2_{crystal}$. $r_{globule}$ *and* $r_{crystal}$ refer to the radius of the globule and of the protruding crystal, respectively. As a consequence, the crystals rupture the thin aqueous film between two approaching globules and the adsorbed layer of the second globule more readily [130]. Considering ‘true’ coalescence, stress concentration is also present but it is dependent on to what extent the droplets can be deformed and create a flat film between two approaching droplets [33]. In addition, partial coalescence can be achieved at a distance where no significant repulsive interaction is sensed by the globules [171], whereas ‘true’ coalescence generally needs a much closer approach.

– Besides on the interfacial properties and the composition of the aqueous phase, the stability against partial coalescence also depends on the amount of crystalline fat (solid fat content or SFC) and on the shape, the orientation and the location of the crystals in the globules [130, 171, 176, 182].

– The formation of irregular clumps or networks of the partially coalesced globules increases the effective volume fraction φ_{eff} of the dispersed phase and thereby the viscosity [62, 130, 176]. In fact, the fat clumps can immobilize the aqueous phase when the process proceeds until a continuous space-spanning network is formed throughout the whole volume of the system [130, 135]. In that case, the emulsion is transformed in a solid-like system or solidified [130]. When two droplets completely merge into a bigger spherical one, as in ‘true’ coalescence, no large viscosity changes will be detected (cfr. equation 5.3).

– The partial coalescence rate depends greatly on the applied velocity gradient in the emulsion while in many cases the flow conditions do not show a substantial effect on the rate of ‘true’ coalescence [175, 176, 183].

5.1.4. Factors governing partial coalescence rate

Dissimilarity in the susceptibility of oil-in-water emulsions towards partial coalescence can cause variation in the final state of the emulsions. Globules that undergo fast partial coalescence will aggregate until all globules are included in a continuous network. The result is an inversion of the oil-in-water into a water-in-oil ‘emulsion’. Upon melting, a phase separation will then occur, creating an oil layer on top and an aqueous layer at the bottom. Alternatively, slow partial coalescence may lead to irregular clumps that are more prone to creaming than the original singlets. In the cream layer the clumps become more closely packed which may locally increase the rate of partial coalescence, whereas the rate in the bottom layer will be even lower than in the original emulsion. These differences in susceptibility towards partial coalescence suggest the occurrence of different types of partial coalescence. In the literature four types of partial coalescence have been experimentally established. These four types are characterized by the change in the droplet size distribution and by the presence or the absence of a fat layer on top of the emulsion both evaluated after heating and after creaming of the large clumps has occured [130, 182]. The dissimilarity in the coalescence efficiencies between the clumps and the single globules of different sizes mainly determines the changes in the particle size distribution and, thereby, the type of partial coalescence [130]. Figure 5.3 illustrates the four types of partial coalescence:

- **Type A**: All globules participate and form irregular clumps. Upon melting, an emulsion with a unimodal particle size distribution and a larger mean droplet size is obtained.
- **Type B**: Large clumps are created which cream upon melting and the remaining globules are larger than the original ones.
- **Type C**: Rapid partial coalescence between a sum of the emulsion droplets leads to large clumps which rapidly cream upon heating. The remaining droplets show an unaltered particle size distribution.
- **Type D**: Big clumps are formed, which rapidly cream upon heating and the remaining emulsion shows a size distribution of smaller droplets than in the original.

From the above, it can be assumed that the factors that significantly govern the rate of partial coalescence will affect the physical properties of the oil-in-water emulsion. The rate of aggregation or partial coalescence can be defined as the product of the encounter frequency and the capture efficiency α. The capture efficiency is the probability that two droplets stick after close approach and can be determined as the ratio of the amount of observed partially coalesced aggregates over the calculated ones in which each collision would result in the formation of an aggregate [33]. Factors affecting the collision frequency and/or the capture efficiency will consequently control the partial coalescence rate. Within the wide variety of influencing factors, distinction is made in the discussion between the process parameters and the composition and formulation of the oil-in-water emulsion.

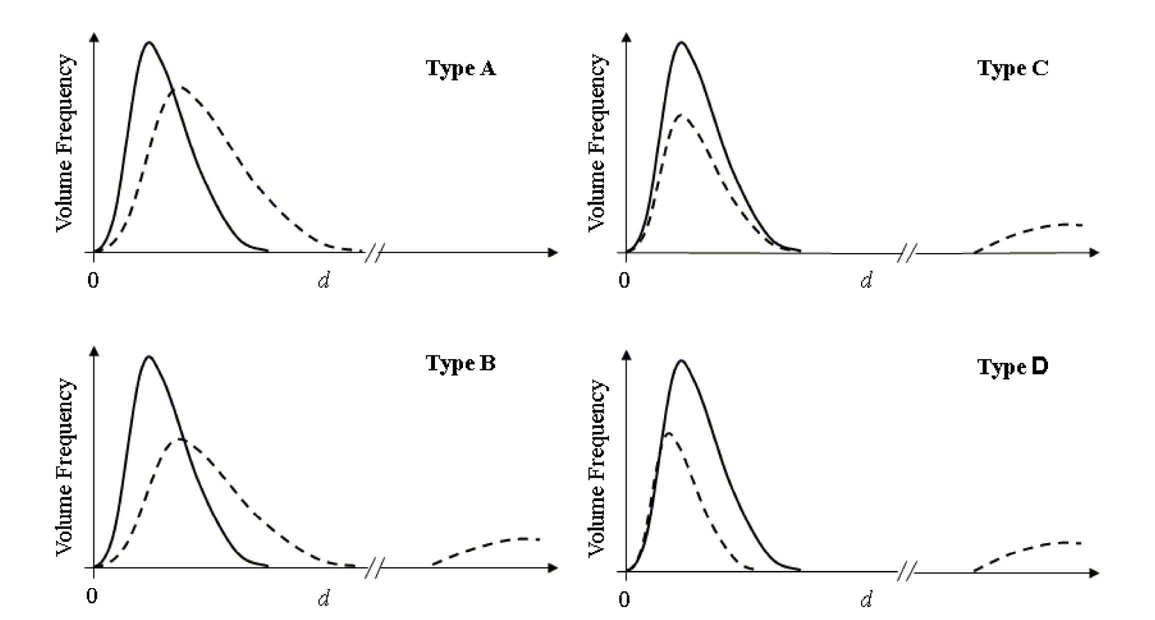


Figure 5.3 Schematic presentation of the different types of partial coalescence. The solid line represents the particle size distribution of the individual globules while the dashed lines represent the particle size distribution after partial coalescence has occurred.

5.1.4.1. Process parameters

The main process parameters affecting the occurrence and the rate of partial coalescence are the mechanical shear forces (flow conditions) and the time-temperature (*t,T*-) program applied during processing.

A. Flow conditions

The movement and, thus, the encounter frequency of droplets in an oil-in-water emulsion at rest is determined by both the Brownian motion and the creaming behavior. The encounter frequency will increase when hydrodynamic mechanical forces are present and it will be approximately proportional to the shear rate in a simple shear flow. In case of other flow types - in which Taylor vortices occur or in a turbulent flow - the encounter frequency and therefore the partial coalescence rate may even be higher [33]. These orthokinetically provoked clumping of fat globules is often referred to as *shear-induced partial coalescence* [184].

Moreover, the capture efficiency α is also increased when a velocity gradient is applied during processing as was shown by Darling [171] and van Boekel [176]. Two causes can be given for the increased capture efficiency *α* when flow is applied. First, the droplets will roll around each other resulting in a higher probability that protruding crystals will be present in the film between two approaching droplets (Figure 5.4) [176]. Second, the droplets are stronger forced together resulting in a shorter interdroplet distance than in Brownian motion [176]. A repulsion barrier may thereby be overcome and the distance of the protruding crystals may then be higher than the thickness of the film between two approaching droplets which causes the piercing of the second globule by the protruding crystal.

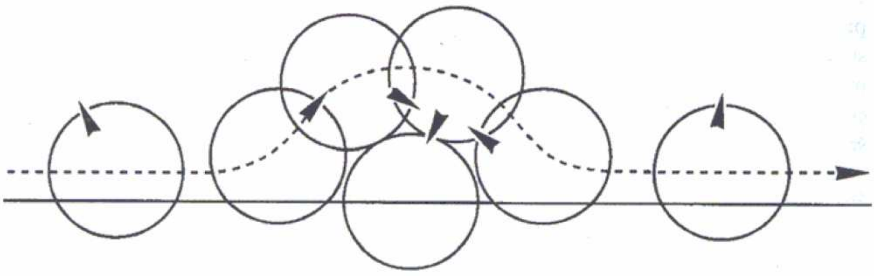


Figure 5.4 Relative movement of droplets around each other when shear flow is applied [33].

In many emulsions a shear rate threshold value can also be observed before the partial coalescence rate starts to increase with the applied shear rate [33, 185, 186]. Beneath this threshold value the globules do not clump or very slowly; presumably, the distance of approach between the globules is greater than the protrusion distance of the crystals.

On the contrary, break-up of partially coalesced clumps has to be taken into account especially when elongational flow is present in the system. The extent of partial coalescence is then dependent on both the rate of partial coalescence and the rate of clump break-up [130, 171].

B. Temperature

The actual temperature and the *T*-history define to a large extent the susceptibility of an oil-in-water emulsion towards partial coalescence as they mainly determine the physical state of the oil droplets. Crystallization of the oil droplets takes place when applying a certain degree of supercooling. Crystals nucleate and, subsequently, start to grow. The resulting solid fat content (SFC), morphology and size of the crystals and their internal arrangement will greatly affect the partial coalescence stability of the fat globules [171, 176]. Below 0℃, the water of the continuous phase can also start to freeze, which generally catalyzes partial coalescence in emulsions [187]. This part will discuss the effect of SFC, the morphology and size of the crystals and their arrangement within the globules related to the *t,T*-program applied during processing. The effect of freezing of the continuous aqueous phase is also briefly adressed.

i. *Solid fat content*

Boode [130] stated that a continuous fat crystal network is generally desired in the fat globules to induce partial coalescence. From this prerequisite, it can be deduced that the rate of partial coalescence is strongly related to the SFC of the fat globules since an adequate SFC is essential to form a continuous crystal network throughout the globules [181]. Stability against partial coalescence may, thus, be guaranteed below a certain threshold value of SFC. The crystals do not form a space-spanning network approximating the globule size and cannot protrude very far [58, 166, 181]. They may even be pushed into the globules when two approaching globules collide [33] and they may rather undergo 'true' coalescence [58, 166]. The minimum SFC needed to produce a crystal network extending throughout the whole droplet can be determined by applying the theory of fractal aggregation of the crystals. It defines a critical diameter of a fractal aggregate that permits the creation of a continuous network. This should be smaller than the globule diameter to facilitate the creation of a fat crystal network throughout the globule. Hence, the following condition needs to be fulfilled [130, 166, 181]:

$$d > y\psi^{1/(D-3)} \tag{5.4}$$

With d the globule diameter, y the (poorly defined) effective diameter of the aggregating crystals, ψ the crystal volume fraction, D the fractal dimensionality, which is less than 3, and $y\,\psi^{1/(D-3)}$ the critical diameter of the globule to form a continuous network. This implies that the minimum SFC needed depends on the globule size and the size of the crystals formed. The smaller the globule and the larger the crystals, the more SFC is needed to achieve a crystal network. The equation also involves that for an equal SFC and similar droplet size a space-spanning crystal network will more readily be formed if the crystals are smaller. Secondary nucleation is known to promote the creation of a large number of small crystals and is, thus, desired when partial coalescence is intended. It is especially favored when the oil has a very heterogeneous TAG-composition, like in milk fat [32]. If no secondary nucleation takes place, it is likely that only one or two crystals will be formed in each globule. Even though these crystals will be bigger, the globules may be less susceptible towards partial coalescence due to the absence of a crystal network in most of the small globules.

Besides the creation of a continuous fat crystal network the amount of protruding crystals and their protrusion distance are also determined by the SFC [176, 181]. Due to ongoing crystallization after the network has been formed, protrusion will especially arise from the shrinking of the globules. Globules will become short of liquid oil so that crystals can no longer be completely wetted by the oil [33, 181].

Nevertheless, as mentioned in Section 5.1.3, not only fat crystals are necessary to induce partial coalescence but the presence of liquid oil is also indispensable. The fat crystal must, after all, penetrate into the liquid region of another globule. If no oil is available, no wetting of the crystals piercing the second globule occurs and a permanent junction will not be formed [33, 180, 181]. Therefore, almost completely solidified droplets do not show partial coalescence but rather undergo aggregation [162]. Even when the oil is completely trapped in the pores of a crystal network at a lower percentage of SFC, the globules will not create clumps after collision. In addition, assuming that a crystal of one globule penetrates an adjacent globule and an initial small oil neck is created (Figure 5.5), the extent and the rate at which the globules merge is dependent on the amount of the oil that can be released from the fat crystal network during collision. The main driving force for merging is the Laplace pressure created at the rim of the initially created neck (Figure 5.5, at the right side). However, the merging will probably be counteracted by the Laplace pressure created at the oil-water interface. Due to local removal of the oil, boundary capillary pores may be created at the edge of the fat crystal network if the network does not subside under the sensed pressure (Figure 5.5, at the left side). Hence, the pore size in the fat crystal network, strongly dependent on the crystal size, defines to a large extent the availability of the oil [33] and the rate and extent of globule merge. The smaller the pores, the more the oil resists to flow out of the network since the counteracting Laplace pressure readily appears.

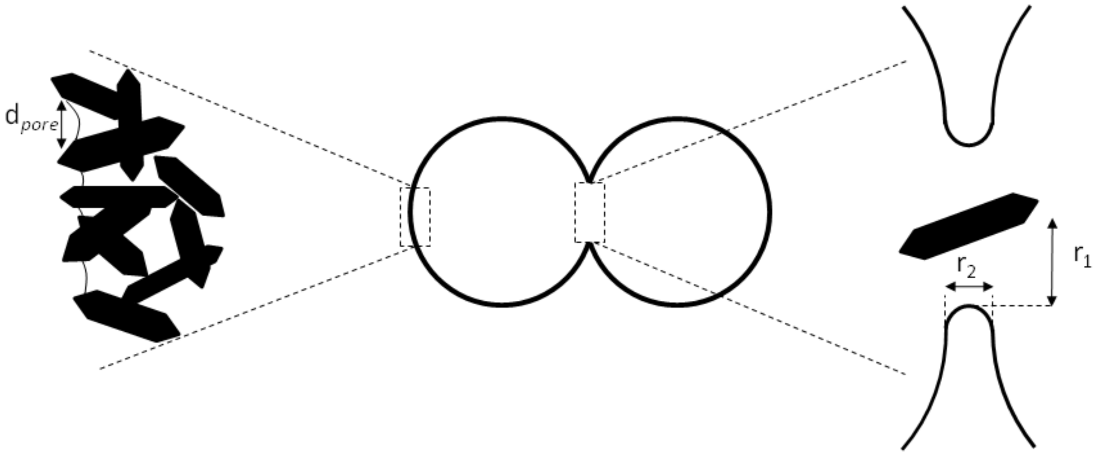


Figure 5.5 Schematic presentation of (at the right side) the geometry of an oil neck between two partially coalesced fat globules creating the driving force for globule merging and (at the left side) the formation of capillary pores at the globule interface when oil is sucked out of the globules causing counteracting capillary forces limiting the merging. With r_1 and r_2 the radii of the curvature at the oil neck and d_{pore} the pore diameter.

From the discussion above it can be assumed that an optimum SFC for partial coalescence can be established in oil-in-water emulsions [33, 162, 185, 186, 188, 189], as illustrated for milk fat in Figure 5.6. This optimum SFC is highly dependent on the composition of the fat globules and the size, the morphology and the location of the crystals. Davies et al. [189] suggested that partial coalescence is maximized in a SFC-range between 10 - 50% in model cream. Hinrichs and Kessler [186] and Hinrichs [185] stated that for natural cream the partial coalescence is maximized if the SFC is 25% (more or less at 20℃) while according to Thivilliers et al. [190] a maximum partial coalescence rate is achieved at a SFC of 10 - 15% in a recombined dairy cream during shearing. The optimum SFC can, in addition, shift when varying the flow field. At rest globules may still partially coalesce at a higher SFC than in a flow field because disruption may take place before the junction between the globules is shaped when flow is applied, especially if the flow is elongational [130, 162, 181].

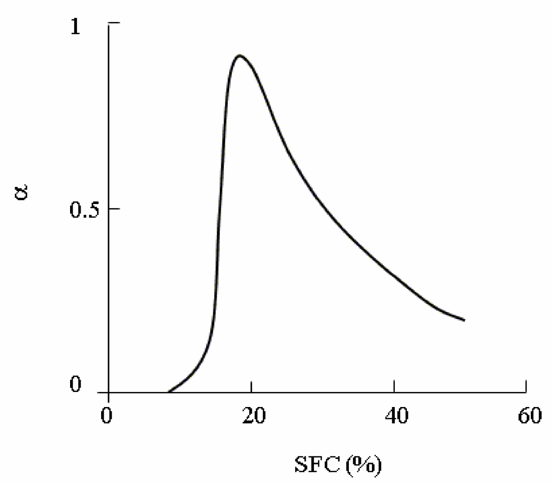


Figure 5.6 Example of the evolution of the capture efficiency α, for partial coalescence to occur, as a function of solid fat content (SFC) of milk fat [2, 19].

Furthermore, it needs to be remarked that partial coalescence also can occur when in an emulsion both solid globules and supercooled droplets are present [191]. Crystallization is induced by penetration of a protruding crystal in the supercooled liquid. The inflowing crystal serves as a site for crystal growth (secondary nucleation) [55, 56] and only partial fusion is possible [191].

In general, it can be concluded that SFC mainly affects the capture efficiency *α*. The encounter frequency is hardly affected.

ii. Arrangement of the crystals

If solids, in this case crystals, have the freedom to move to an equilibrium position at the oil-water interface, three interfacial tensions will determine the contact angle of the crystal in the aqueous phase θ_w or the oil phase θ_o. Figure 5.7 gives a schematic 2D-presentation of the contact angle of a spherical solid particle at the oil-water interface. Furthermore, it should be realized that the presence, concentration and type of surfactants in the oil or the aqueous phase can strongly alter the interfacial tensions and, hence, the contact angle [175].

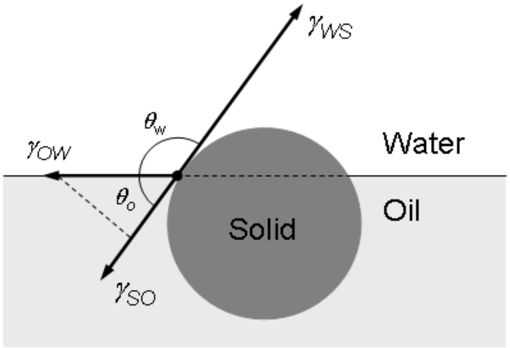


Figure 5.7 Schematic 2D-presentation of the contact angle of a spherical solid particle at the oil-water interface.

For most emulsions, the contact angle θ_w of a crystal, as measured in the aqueous phase, is between 120° and 160°. This implies that the interfacial free energy of the system is the lowest when the crystals are situated at the interface and preferentially wetted by the liquid oil phase [33]. The free energy gain, as compared with the state of complete wetting of the crystals by the oil is larger for smaller θ_w. Boode and Walstra [179] stated thereby that, irrespective of the wetting circumstances, measured by the contact angle, the free moving crystals will be tangentially oriented, as illustrated in Figure 5.8. On the other hand, because of van der Waals attraction forces, fat crystals in oil globules are considered to be aggregated in a network [179, 181, 192]. The network formation then hinders the free diffusion of the crystals to the oil-water interface. Only crystals that become detached from this network have the opportunity to reach the interface.

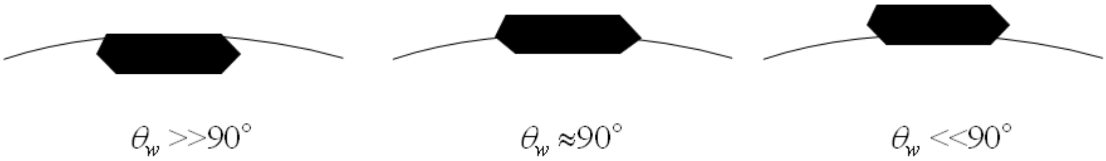


Figure 5.8 Orientation of free movable crystals at the oil-water interface after [130].

Walstra [193] was the first to classify milk fat globules according to the fat crystal arrangement in the globule. Later on, as shown in Figure 5.9 , Boode [130] extended the classification and discussed the types of fat globules in terms of capture efficiency α towards partial coalescence. The capture efficiency α of the layered (L-) type globules is related to the contact angle of the crystals. When $\theta_w >> 90°$, the crystals become preferentially wetted by the oil phase (Figure 5.8) but edges of the crystals may protrude by a few times ten nanometres because of the curved interface and the geometry and size of the crystals. The capture efficiency for partial coalescence is then for example 10^{-6}. This situation will also take place at $\theta_w \approx 90°$ when the crystals are not forced into either phase (Figure 5.8). When $\theta_w << 90°$, crystals are preferentially wetted by the aqueous phase and located at the outside of the oil-water interface. This may cause aggregation of globules by bridging, but the occurrence of partial coalescence is probably quite rare.

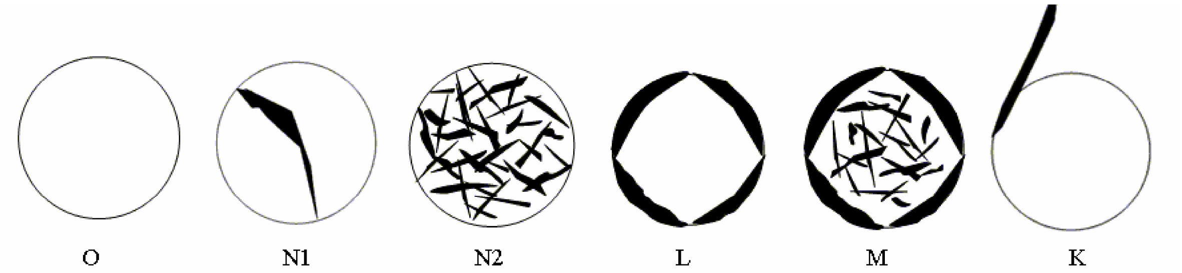


Figure 5.9 Schematic presentation of types of semi-crystalline globules as observed with the polarized microscope, after Walstra and Boode [130, 193].

If crystals are kept in a crystal network, such as in needle (N-) type globules (Figure 5.9), radial orientation of some boundary crystals can be observed [181]. In the N-type globules, the protrusion distance may presumably depend on (1) θ_w at the moment when the crystal becomes oriented at the interface (before network formation), (2) the size and shape of the crystals and (3) the extent of shrinking of the liquid perimeter of the globule due to ongoing crystallization of its content. The crystals may even stick out further from the N-type globules than from the L- and mixed (M-) type. Therefore, the N-type globules may be more susceptible towards partial coalescence leading to a wide range of capture efficiencies α depending on the amount of crystals present at the interface. In the exceptional N1-type case in which only a few crystals are present in the globule the capture efficiency α would be approximately 10^{-6}. N2-type crystallized globules, on the other hand, contain numerous protruding crystals and partial coalescence will become more efficient, $10^{-6} < \alpha < 1$.

In K-type crystallized globules, crystals are too thick to follow the curvature and can grow out of the oil globule if $\theta_w \leq 90°$. The large protrusion takes place especially when some water-soluble small-molecule surfactants are present at the interface. Nearly all encounters will lead to partial coalescence, α approaches 1.

The type of globule depends not only on the properties of the fat [181] but also on the actual temperature [194] and the *T*-history [59, 178].

iii. Crystal size, polymorphism and morphology

Emulsion stability decreases with increasing crystal size at rest and in couette flow [180]. The larger the crystals, the larger the protrusion distance of the crystals can be. As a consequence, the emulsions will become more prone to partial coalescence. The size of the crystals is initially determined by the cooling rate. In general, the higher the cooling rate, the larger the undercooling before nucleation occurs and the smaller the crystals within the globule [59]. The subsequent *t,T*-program and storage temperature will then determine the final size of the crystals.

Moreover, the *t,T*-program may define the crystal polymorphism. Johansson [62] maintained that α- and β'-crystals are more polar than β-crystals which are almost completely wetted by the oil. Polymorphic transitions may, therefore, modify the position of the crystals in the globule and the crystal network formation and, subsequently, may alter the capture efficiency α. Besides this hypothesis, so far, no relationship between polymorphism and susceptibility towards partial coalescence has been established.

Davies et al. [154] showed that in sodium caseinate stabilized emulsions with a 40% groundnut-tristearin (5%) oil phase the morphology of the crystals influence the rate of partial coalescence. They postulated that, when flow is applied, needle-like spherulites make the globules more susceptible towards partial coalescence than small rounded spherulites. The latter crystals were not able to pierce the interface of the globule. Golemanov et al. [191] observed similar effects of the morphology of paraffin crystals on the stability.

iv. Tempering

The stability of an oil-in-water emulsion is markedly affected by applying a tempering step at a temperature (T_{max}) where only little solid fat is left [130, 177, 178, 188, 195-199]. In concentrated emulsions 'body' formation, 'gelling' or 'solidification' of the emulsion may even be accomplished. These transformed emulsions do not flow anymore under their own weight as a consequence of partial coalescence. In less concentrated emulsions, the tempering results only in a higher susceptibility towards partial coalescence when the emulsion is subjected to a velocity gradient [178].

The pioneering work of Boode et al. [178] describes a potential mechanism elucidating the susceptibility of an oil-in-water emulsion towards partial coalescence after applying a tempering period. The suggested internal changes of the fat globule are given in Figure 5.10. Before the tempering period, at low temperature, the fat crystals are trapped in a continuous fat crystal network. These crystals cannot move to the thermodynamically favored interfacial position. Upon heating, the fat melts and as a result some crystals disappear and others become smaller. The remaining small crystals are then no longer kept in a continuous network so they can move freely to the energetically favored oil-water interface. This occurs only if the tempering period at T_{max} is long enough. Approximately 1 in 1000 collisions of the crystals with the interfacial region overcome the boundary activation free energy. Upon slow recooling, recrystallization starts immediately without deep supercooling since the remaining crystals serve as catalytic impurities. As a consequence, the number of crystals does not increase greatly but the crystal size increases significantly. Moreover, the crystals stick out farther in the aqueous phase and even more crystals will protrude, since they presumably started to grow in the boundary region. These larger and increased amount of protruding crystals make the emulsion more susceptible towards partial coalescence at rest (especially for concentrated emulsions) or in flow. Recently, Thivilliers et al. [188, 198] observed during tempering at T_{max} of milk fat- and cocoa butter-in-water emulsions an increase in particle size and in *G'* (storage modulus) which shows that clusters of partially coalesced globules are already formed at T_{max}. During subsequent recooling, a sharp increase of *G'* is observed. They attributed the sharp increase rather to oil crystallization than to ongoing partial coalescence, although no experimental evidence of this is given. Moreover, the same research group also showed that by applying a tempering period '*jamming*' can occur in stead of partial coalescence [198, 200, 201]. 'Jamming' refers to gelling caused by flow hindering due to surface roughness in the presence of crystals near the interface, i.e. the crystals are too small to pierce the neighbouring globules. The occurrence of 'jamming' is favored when the globule size is small, takes place in both paraffin and TAG-emulsions and depends on the tempering conditions and composition of the interfacial layer.

An alternative explanation for the decreased stability of an oil-in-water emulsion after tempering is given by Mutoh et al. [195] and Sugimoto et al. [197]. They maintained that the large crystals formed as a result of tempering may affect the conformation and the adsorption behavior of the proteins which causes globules to aggregate. The created aggregates are described as the main cause for the enhanced solidification in the oil-in-water emulsions. Partial coalescence, as such, is not mentioned in these papers.

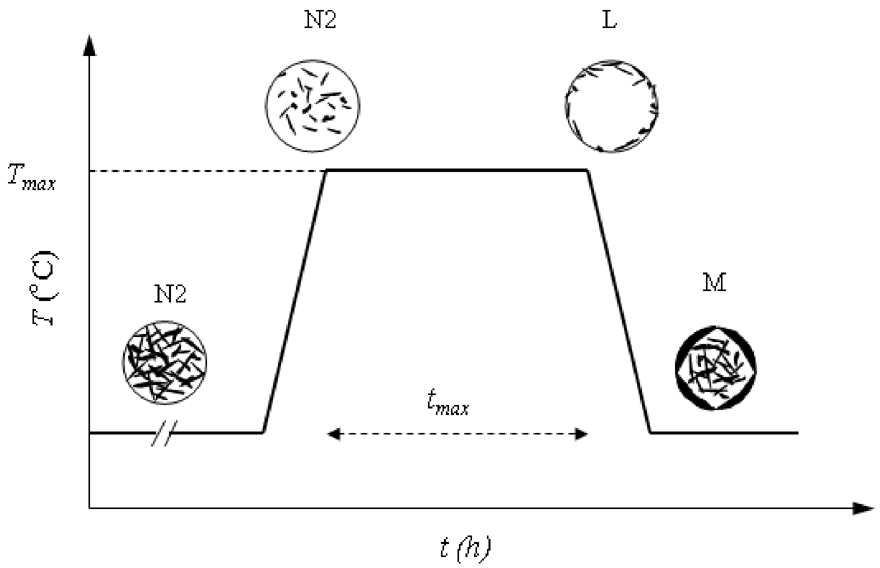


Figure 5.10 Schematic presentation of changes in type of semi-crystalline fat globule during tempering of oil-in-water emulsions, after Boode [178].

The time at T_{max} (t_{max}) and the cooling rate after the tempering period is paramount for the effectiveness to increase the extent of partial coalescence [178, 198]. T_{max} defines the SFC and the amount of free movable crystals present during the tempering period while t_{max} defines the final arrangement of the fat crystals in the globule and the initial extent of partial coalescence before recooling. When cooling rapidly, supercooling can occur which implies the formation of small crystals, especially when secondary nucleation takes place, and no enhanced destabilization will occur [178]. The slower the cooling rate, the larger the crystals and their protrusion distance which increase the partial coalescence rate [168, 178]. Moreover, large crystals will create larger pores and, thus, increase the permeability of the fat crystal network. More oil will become available for the fast creation of a strong persistent junction when a crystal penetrates the globule. In addition, when slow cooling is applied, more time is spent in the semi-solid region where the partial coalescence rate is maximized [168].

From the above, it can be concluded that tempering mainly affects the capture efficiency α. Further, it should be noted that tempering may also have an effect on the morphology and polymorphism of the crystals, the final SFC in the globules and the sintering of the internal crystal network. But so far no research confirmed these effects.

v. Ice crystal formation

Thanasukarn et al. [187, 202] investigated the destabilization of palm oil-in-water emulsions (40%) by temperature cycling. The emulsions were repeatedly heated above the final melting point of palm oil and cooled to temperatures where only oil crystallizes or where both the aqueous phase and the oil phase crystallize. The observed extent of partial coalescence was markedly higher when both phases contain a solid fraction. Below the freezing point of the aqueous phase ice crystals primarily force the droplets together. Presumably, the ice crystals can damage the adsorption layer and also induce concentration of the mineral salts in the aqueous phase, implying a reduction in electrostatic repulsion [187, 203]. All these effects clearly increase both the capture efficiency α and the encounter frequency of the globules and are especially important in the manufacturing of ice cream. In ice cream, it is the combination of ice crystallization and shear forces that mainly determines the fat destabilization. Neither the shear forces nor the ice crystallization alone results in a similar magnitude of fat destabilization [203]. In the latter research, air is also included which enhances destabilization as will be discussed for whipped cream systems in Section 5.2.

5.1.4.2. Composition and formulation

A food emulsion consists of two immiscible liquids, oil and water. In order to extend the kinetic stability emulsifiers or surface-active components, like proteins or small-molecule surfactants, are added which provide the emulsion droplets with a protective oil-water interfacial layer. Also thickening or gelling agents can be added to decrease the mobility of the emulsion droplets. During emulsification/homogenization, all these components will be distributed in the dispersed oil, the continuous aqueous phase and the oil-water interfacial layer. Moreover, the concentration of components and the process parameters during emulsification will affect the distribution and the structure of the emulsion, especially the particle size distribution. In this section, the effects of the composition and formulation on the encounter frequency and the capture efficiency α towards partial coalescence will be discussed.

A. Fat volume fraction

Hinrichs and Kessler [186] showed that emulsions with a high fat volume fraction (φ) are more susceptible to partial coalescence than those with a low fat content. A lower shear rate was necessary to destabilize the emulsion. Moreover, the ‘gel’ strength after applying a tempering was also found to be larger when φ was increased due to a larger extent of partial coalescence, as shown by Thivilliers et al. [188].

Generally, the rate of partial coalescence is approximately proportional to φ^2 if only the volume fraction and thus only the increasing encounter frequency is concerned [130, 166]. For $\varphi > 0.2$ an even stronger coalescence rate is expected [33]. Furthermore, for cream-like emulsions a minimum volume fraction of the oil phase should be present for partial coalescence to occur both at rest [198, 200] or when mechanical forces are applied [204].

B. Particle size of globules and clumps

In general, the partial coalescence rate increases with the globule size as was first established by van Boekel [175]. Later on, Boode and Walstra [180] suggested from model calculations that the capture efficiency α increases with globule size. The calculated capture efficiencies of a TAG and a paraffin emulsion are given in Figure 5.11. Solidification when applying a tempering cycle occurs at a lower fat content when TAG or paraffin emulsion contains larger globules [188, 200].

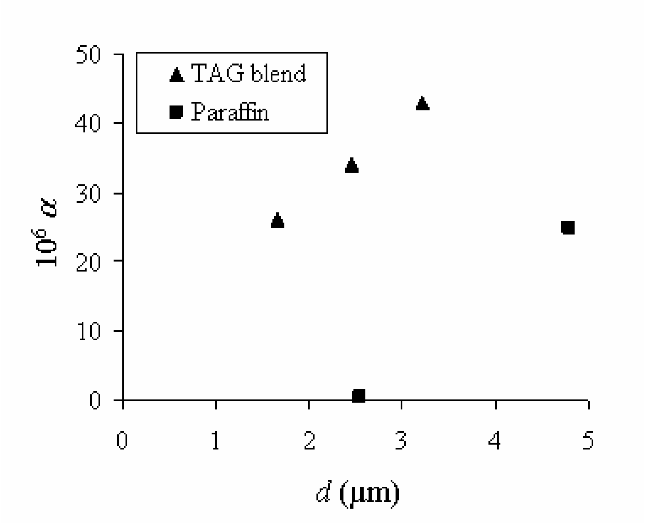


Figure 5.11 Calculated capture efficiency α as a function of globule size (μm) of a 20 % TAG- and paraffin-in-water emulsion, after Boode [130].

The effect of globule size can be attributed to:

– In a given flow field, the external force pressing the globules together is larger for larger globules and also, when similar crystal sizes are assumed, the stress concentration factor Λ will be higher since this is proportional to $r^2_{globule}$. In contrast, the internal stress (or the Laplace pressure) decreases with increased particle size. The external stress will thus be more readily higher than the internal pressure of the globules. As a consequence, two large globules will deform more strongly than smaller ones resulting in a larger area of the thin film formed between two globules and the smaller the distance between the two approaching globules [33]. The probability that a protruding crystal is located in that film and that the protruding distance is large enough to reach the second globule is therefore higher. It needs to be remarked that the presence of a network (when enough SFC) in the globules will markedly decrease the deformability of the globule.

– Since the creaming rate is proportional to $r^2_{globule}$ (Stokes' law), larger globules will cream faster forming a dense top layer. The encounter frequency will thereby be increased in the cream layer yielding higher partial coalescence rate.

– Larger crystals can be formed in larger globules [59, 205] which will, firstly, lead to greater protrusion distances [166, 200] and, secondly, to a larger pore size in the network. The greater protrusion distance involves a higher partial coalescence rate and the larger pore size makes the liquid oil more readily available as a lubricating agent for the creation of a permanent junction [33].

Further, it should be mentioned that despite the enhanced partial coalescence rate, the functionality of the globules to form a space-spanning network is reduced. With increasing particle size, a larger number of droplets are required to have the same network volume.

When partial coalescence has started, partially coalesced globules or clumps will be present. As a consequence, the collision frequency will be largely reduced. However, how the different clump sizes will affect the capture efficiency α is ambiguous. Considering the amount and size of the crystals two possibilities are realistic [130]. When the clumps are created from singlets with a few large crystals per singlet, the crystals will most likely become more engulfed by the liquid oil decreasing the reactivity of the clumps compared to the globules. Clumps originating from singlets containing a continuous network presumably become more reactive. Some oil is squeezed out to form a neck between two adjacent globules. Less oil remains to fill the network; most likely resulting in a larger protrusion distance of the crystals located at the interface.

When only the sizes of clumps are considered without variation of other parameters (crystal arrangement, amount of protruding crystals, SFC), the partial coalescence rate will increase with the size, similar to singlet globules. However, in a flow field it needs to be considered that clumps, especially the larger ones, can break up.

C. *Oil-water interfacial layer composition*

The assessment of the stability of an emulsion in which the interfacial composition is changed is a complex matter. When the amount and/or type of surface-active agent are changed before emulsification, not only the composition but also the particle size distribution, the surface load, the thickness and the viscoelasticity of the oil-water interfacial layer and the fat crystallization behavior may be altered. These varying factors make it hard to univocally compare experimental results. Even though having this in mind, some common trends can be detected in the literature and are discussed in this section.

Generally, colloidal repulsion will strongly stabilize emulsions against partial coalescence, especially in quiescent emulsions, since it defines the minimal distance between two approaching globules and thus the capture efficiency α. A large viscous stress in a given flow field may, however, overcome the repulsion as discussed in Section 5.1.4.1.A. The extent and type of colloidal repulsion is mainly dependent on the composition of the interfacial layer enclosing the fat droplets. Both steric and electrostatic repulsion may be abundant in oil-in-water emulsions stabilized by proteins and/or small-molecule surfactants. Mostly, the rate of partial coalescence is less for globules stabilized by proteins than for those stabilized by small-molecule surfactants because proteins primarily exhibit stronger steric repulsion compared to small-molecule surfactants [176, 206]. Secondly, proteins tend to form a thicker and often a more viscoelastic membrane at the oil-in-water interface [130, 176, 178, 192, 194]. The latter leads to a lower conformational flexibility at the interface and, in addition, crystals may not stick out in the aqueous phase or are not able to rupture the thick membrane of the approaching globule. Next to the interfacial composition, the composition of the continuous phase will determine to a large extent the repulsive interactions (ionic strength, pH, solvent quality,…) of the globules [180], as will be discussed in 5.1.4.2.D.

i. Proteins

In general, an increasing protein surface load decreases the partial coalescence rate [135] until a maximum load is reached [166]. This is a result of the positive correlation of the surface load with the extent of colloidal repulsion and the thickness and viscoelasticity of the interfacial layer which counts until all adsorption areas at the interface are occupied.

In literature agreement exists that emulsions stabilized with caseins are more stable against partial coalescence than those stabilized with whey proteins (WPs) [55, 177, 202, 207-209]. Figure 5.12 illustrates this with some results of Pelan et al. [207]. Among emulsions prepared with commercial available milk proteins containing both caseins and WPs, skimmed milk stabilized emulsions appear to be most stable [177, 207, 210], if the concentration of the proteins is high enough (Figure 5.12). At a low protein:fat ratio not enough proteins are available per unit area oil-water interface and clusters may be formed during emulsification [177]. The long lasting contact between globules in these clusters will encourage the occurrence of partial coalescence [162, 211].

For hydrogenated palm kernel oil (9%) emulsions stabilized by a mixture of caseins and WPs, Sourdet et al. [212] and Relkin et al. [209, 213] concluded the preferential adsorption of casein micelles over WPs and thereby a higher surface load and stability against partial coalescence of the casein containing emulsions over the 100% whey stabilized emulsions. Next to the effect on the interfacial composition, also differences in crystallization behavior between the casein containing and the 100% whey stabilized emulsions, were observed by Relkin et al. [209, 213]. The latter may also potentially explain some changes in partial coalescence rate (as discussed in Section 5.1.4.1.B).

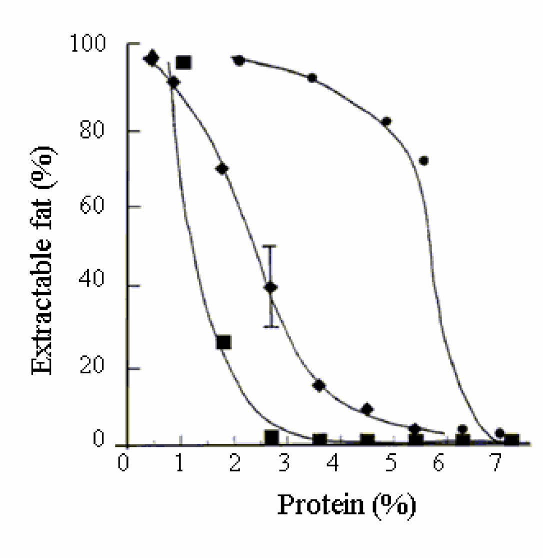


Figure 5.12 Extractable fat (%), as a measure of the amount of partial coalescence, as a function of WPC(●), caseinate (♦) and skimmed milk (■) concentration (%) in emulsions containing 20% oil. The oil phase consists of a 50:50 mixture of palm kernel oil and coconut oil [207].

Segall and Goff [210] discussed the stability of butter oil (25%) emulsions stabilized by whey protein hydrolysates (WPHs) and whey protein isolates (WPIs). To obtain a quiescently stable emulsion stabilized with WPHs, a higher protein concentration was needed compared with WPI stabilized emulsions because of the limited steric stabilization induced by WPHs. Above a certain protein concentration, on the other hand, WPHs stabilized emulsions do not show partial coalescence during shearing. The WPH-layer was considered too dense for the crystals to protrude.

Since heat treatment before and after emulsification can induce changes in protein hydrophobicity and flexibility, it may affect both aggregation properties and the protein behavior at the oil-water interface which, consequently, will affect the stability of the emulsions to a large extent [214]. The stability of casein stabilized emulsions will be far less sensitive to heat treatment than emulsions based on globular WPs. Caseins are known as heat-stable proteins while globular WPs denature quite readily [215]. Upon heating, hydrophobic amino acid side chains buried in the core of globular proteins become more exposed to the aqueous phase. The unfolding mechanism may even be followed by a covalent interaction between the WPs or WPs and caseins, if present [215].

Segall and Goff [216] showed that butter oil (24%) emulsions stabilized with pre-heated WPs are more prone to partial coalescence than those with native WPs, whereas the susceptibility of post-heated emulsions was similar. Kiokias and Bot [217, 218] and Kiokias et al. [219] investigated the tempering stability (Section 5.1.4.1.B.iv) of pre- and post-heat treated skimmed milk powder, caseinate and WPs stabilized vegetable fat (30%) emulsions. The clearest instability was observed in pre-treated WP stabilized emulsions, whereas the post-treated WP, skim milk powder and caseinate stabilized emulsions were much more stable. Furthermore, the authors maintained that emulsions remain stable as long as sufficient native WPs are available to provide a surface coverage equal to that of comparable emulsions prepared with non-heated WPs [218].

ii. Small-molecule surfactants

Small-molecule surfactants are usually more surface-active than proteins because they can pack more efficiently at the interface. Therefore, when both proteins and small-molecule surfactants are present, competitive adsorption of both oil-soluble and water-soluble small-molecule surfactants with the proteins (or protein displacement) may occur at the oil-water interface. This has been extensively studied the last decade for emulsions stabilized by purified milk proteins [220-228], WPIs [229, 230], caseinates [230, 231] and milk protein mixture [232-234]. Generally, it was found that caseins are more readily displaced than WPs [220] and water-soluble surfactants are more effective in protein displacement than oil-soluble surfactants [223, 228, 229] while the latter reduce the protein surface concentration substantially in the presence of water-soluble surfactants [222]. Mackie et al. [235] described a new ‘orogenic’ mechanism of protein displacement by small-molecule surfactants. They suggested a three stage mechanism by using AFM (atomic force microscopy) to visualize the molecular structure of the interfaces. First, the surfactant molecules nucleate at defects in the protein interface. Second, a compression of the protein network occurs due to the ongoing growing surfactant region at the interface and, finally, the interfacial network of proteins collapses and proteins are released into the aqueous phase. This mechanism, originally developed by studying air-water interfaces, seems to be applicable to oil-water interfaces and is accepted as generic [227, 236, 237]. Mackie and Wilde [238] further demonstrated, in a review, the importance of the intermolecular interactions of both ionic and non-ionic surfactants with proteins on the structure and physical properties of a mixed interface.

The displacement of proteins from the oil-water interface affects the overall stability, as summarized by Dickinson [206], including partial coalescence stability. The protein desorption reduces the thickness and changes the viscoelasticity and the repulsive interaction of the interface [236, 239, 240]. Moreover, it has been shown that when a protein-stabilized ice cream mix or dairy cream is aged in the presence of small-molecule surfactants, prior to cooling and/or whipping, the whipped products can have improved physicochemical properties [135, 169, 241-244].

Besides their effect on the protein load, small-molecule surfactants can also modify the contact angle θ_w of the fat crystals [166, 179]. In this case, they can possibly enhance the wettability of the crystals by the aqueous phase and as such increase the protrusion distance of the crystals and the emulsions will become more prone to partial coalescence [171, 176, 179].

In addition, several small-molecule surfactants can partly dissolve in the oil and may then affect the crystallization behavior in the globules [84, 85, 104, 105, 120, 241, 245]. Small-molecule surfactants can affect nucleation, crystal growth and/or the polymorphic transitions, dependent on their miscibility in the oil [43]. As a consequence the amount, the size, the morphology and the sintering of the crystals, the arrangement of the crystals in the globule, the fat crystal network and the SFC can depend on the type and the concentration of the surfactants and can, thereby, affect the partial coalescence stability of the emulsions [107, 120, 154, 241, 246].

From the above it can be deduced that the capture efficiency α of the globules for partial coalescence can be controlled when small-molecule surfactants are added. Below, the effects of monoacylglycerols (MAGs), phospholipids (PLs) and other small-molecule surfactants are described separately.

Monoacylglycerols

The displacement of the milk proteins by different types of MAG is discussed by several authors [154, 241, 247]. Barfod et al. [241] maintained that unsaturated MAGs displace the proteins to a greater extent than saturated ones, whereas Davies et al. [154] maintained the reverse conclusion. Heertje [247] postulated that the displacement of the proteins by saturated and unsaturated MAGs is concentration dependent, which can explain the disagreement. Above 0.25% saturated MAGs are more effective and below 0.25% more proteins will be displaced by the unsaturated MAGs. Barfod et al. [241] also maintained that the desorption of proteins into the aqueous phase is higher when the MAGs are able to crystallize and during the reheating readsorption of the proteins can occur. The temperature at which MAGs crystallize and protein displacement occurs, depends on the saturation degree and the chain-length of the fatty acids and the concentration of the MAGs [157]. Along with their adsorption capacity on the oil-water interface, MAGs are, thus, believed to control the destabilization of emulsions during processing.

Pelan et al. [207] studied the stability of butter oil ice cream emulsions stabilized by skimmed milk powder in the presence of saturated and unsaturated MAGs. They suggested that unsaturated MAGs are more effective in destabilizing emulsions than saturated ones, especially when the concentration is higher. The emulsions became more stable with an increasing concentration of saturated MAGs, although the protein coverage decreased slightly. The stabilizing effect of the saturated MAGs at higher concentrations is assumed to be due the occurrence of a Pickering stabilization of MAG-crystals formed at the oil-water interface.

In a more recent study, it was also found that addition of monoolein destabilizes a sodium caseinate stabilized model emulsion (φ = 40% and the oil phase consists of a mixture of groundnut oil and tristearin) stronger than the addition of monostearin or monopalmitin [154, 189]. Moreover, it appeared that the amount of tristearin needed to induce partial coalescence decreased with an increasing monoolein concentration in the emulsions [189]. Although monoolein showed the lowest level of protein displacement, these emulsions were more susceptible towards partial coalescence due to its influence on the morphology of the crystals. The fat crystals formed in monoolein containing emulsions were spiky spherulites. These crystals can easily pierce the interface during agitation. In a quiescent emulsion the crystals are not forced through the interface which implies a quiescently stable emulsion that readily destabilizes during shear flow. The monostearin containing emulsions stayed orthokinetically stable since small tristearin crystals are formed during crystallization which may be not sufficiently needle-like to force into the interfacial layer. Monopalmitin containing emulsions showed less resistance against partial coalescence than monostearin containing emulsions. Larger crystals were formed in monopalmitin containing emulsions compared to emulsions containing monostearin. Both Pelan et al. [207] and Davies et al. [154, 189] deduced thus that the amount of protein displacement would not always be the restricting factor to determine the rate of partial coalescence.

However, Miura et al. [248] observed that saturated MAGs (monopalmitin) destabilize palm oil emulsions (40% oil phase stabilized by sodium caseinate) more readily than unsaturated MAGs (monoolein) when the emulsions were repeatedly tempered. The higher susceptibility of monopalmitin containing emulsions was ascribed to the agglomeration of the higher melting TAGs of palm oil near the interface and the dislocation of the lower melting TAGs to the ‘center’ of the globules during *T*-cycling. Such a rearrangement of palm oil TAGs then would not occur in monoolein containing emulsions. In a second study Miura et al. [249] studied the effect of saturated MAGs with different chain lengths and concluded that the effectiveness for destabilizing the emulsion is dependent on the match between the chain length of the MAGs and those of the TAGs of the dispersed oil. It was shown that emulsions were destabilized if the MAGs have a chain length within a range of two carbons of the most abundant fatty acid in the oil phase (for palm oil this is palmitic acid).

The above-mentioned studies show that the stability in emulsions containing MAGs varies with the applied treatments. Pelan et al. [207] and Davies et al. [154, 189] studied the orthokinetic stability during and after shearing whereas Miura et al. [248, 249] considered the perikinetical stability during repeated tempering.

Phospholipids

Oortwijn and Walstra [177] and Boode [130] observed, after tempering, no ‘rebodying’ or solidification of cream (as discussed in Section 5.1.4.1.B.iv) produced by emulsifying milk fat in skimmed milk, unless soy lecithins were added to the fat. Recently, Miura [120, 250] studied the solidification behavior of reconstituted cream (40% butter oil) after storage at 0℃ upon addition of soy or bovine milk PLs. Dispersion of bovine milk PLs in the oil phase before homogenization stabilized the cream whereas soy PLs did not. The presence of bovine milk PLs in the aqueous phase before homogenization destabilizes the emulsion since PL-protein aggregates are formed which prevents the PL-molecules from organizing at the oil-water interface. The discrepancy between the effect of soy and bovine milk PLs was ascribed to their different effect on the crystallization behavior of the butter oil. Melsen [182] showed that the orthokinetic stability was similar for both recombined creams obtained by emulsifying milk fat in skimmed milk or in buttermilk, although the buttermilk had a much higher PL-content than the skimmed milk. The recombined cream made from skimmed milk can be made more susceptible towards partial coalescence when soy lecithin is added to the milk fat prior to emulsification. A minimum concentration of PLs has to be exceeded before a remarkable change in stability appears upon shearing. Fang and Dalgleish [251] stated, in addition, that the amount of protein displacement is different for different types of PLs dependent on their relative solubility in the oil phase and that it is in good agreement with the occurrence of ‘true’ coalescence. Partial coalescence was not considered in the latter study since no solid fat was present in the oil droplets. However, the above described studies already suggest that the PL-effect will be dependent on the composition of the PLs.

Other small-molecule surfactants

McClements et al. [55] and Dickinson et al. [56] investigated the effect of polyoxyethylene sorbitan monolaurate (polysorbate 20) and sodium dodecyl sulphate (SDS) on the interdroplet nucleation in emulsions containing a mixture of solid and supercooled liquid droplets. In the presence of proteins, McClements et al. [55] concluded an enhanced crystallization in the liquid droplets when polysorbate 20 or SDS were added. As a result of the protein displacement by polysorbate 20 and SDS, the interfacial layer becomes thinner which increases the probability of the solid globules to penetrate the supercooled liquid droplet and act thereby as nucleating site for crystal growth. Dickinson et al. [56], for systems without proteins, concluded an enhanced crystallization rate when the concentration of polysorbate 20 is increased.

Mutoh et al. [195] studied thirteen oil-soluble emulsifiers in order to retard the ‘rebodying’ or solidification of a vegetable fat (40%) emulsion after tempering. They concluded that due to addition of citric acid esters of monostearate and low HLB sucrose esters of stearic acids the emulsions stay in the liquid state after tempering. The enhanced stability of the emulsions was ascribed to two different causes. The incorporation of citric acid esters of monostearate enhanced the electrostatic repulsion, while the incorporation of low HLB sucrose esters of stearic acids changed the crystallization behavior presumably by the formation of smaller crystals, also after tempering.

The effect of the fatty acid chain of polysorbate surfactants in paraffin emulsions are thoroughly discussed by Golemanov et al. [191]. They maintained that the saturated fatty acids with a long chain (C16 and C18) are more effective in stabilizing the emulsion than the ones with a shorter chain (C12), because of the formation of a compact adsorbed interfacial layer, which can act as nucleation sites (interfacial heterogeneous nucleation) for the paraffin in the globules. The presence of a double bound in polysorbate, despite a long chain-length contributes to a lower stability due to geometrical constraints which preclude the formation of a compact adsorption monolayer. Thivilliers et al. [198] showed, in addition, that a saturated polysorbate with a mid-chainlength (C12) in casein stabilized milk fat emulsions enhances the fluidity of the interfacial layer and promote the transport of crystals through the interface which involves longer protrusion distances of the fat crystals contributing, hence, to an increase in partial coalescence rate.

Similar to the findings of Golemanov et al. [191], Arima et al. [107] concluded that after addition of sucrose oligoesters containing palmitic acid moieties an enhanced stability of palm-mid-fraction emulsion due to an enhanced heterogeneous nucleation is obtained. In the considered system, partial coalescence only occurred after an α-β’ polymorphic transition which results in a morphology change into long protruding needle-shaped crystals. Sucrose oligoesters that can retard this polymorphic transition combined with the sucrose oligoesters that enhance the interfacial nucleation decrease the partial coalescence rate more effectively.

D. Continuous aqueous phase composition

As already mentioned, the pH and the ionic strength of the continuous phase affect the repulsive interactions and thereby the distance of approach between the fat globules which changes the partial coalescence probability [130]. Thanasukarn et al. [187] studied the impact of salt addition (NaCl) on the stability of hydrogenated palm oil emulsions (20%) stabilized by WPIs during *T*-cycling. Their measurements suggest that NaCl promotes partial coalescence, attributing to the closer approach of the globules by screening the electrostatic repulsion. The destabilization was even more enhanced at temperatures where part of the aqueous phase was crystallized (Section5.1.4.2.D). Kiokias and Bot [217] and Kiokias et al. [219] showed that acidification of a vegetable fat (20%) emulsion stabilized by WPCs made it less stable during *T*-cycling.

Besides the pH and the ionic strength, also effects of other components such as sugar, carrageenan and other polysaccharides have been evaluated. Sugar can modify the functionality of the emulsifiers, increase the aqueous phase viscosity and change the freezing behavior of water. Thanasukarn et al. [187] observed an improved stability by adding sugar to a palm oil emulsion stabilized with WPIs. The stability increase was attributed to the ability of sugar to decrease the amount of frozen water, to modify the ice crystal structure, to alter the protein structure and/or to reduce the collision frequency of the droplets. For carrageenan, often used as a stabilizer in cream products, in relation to partial coalescence it is found that the adsorption of serum proteins (added after homogenization) is reduced in the presence of carrageenan when extra proteins are added [216]. Carrageenan may interact with charged proteins and thereby acts as a barrier for protein movement preventing the serum proteins from reaching the interface. The decreased surface load enlarges the probability of partial coalescence to occur. Furthermore, Hinrichs [185] showed that addition of carrageenan to the continuous aqueous phase did not affect the partial coalescence susceptibility of the fat globules when flow is applied. Hayati et al. [252] studied the effect of several other polysaccharides on the stability of a mayonnaise-like emulsion of which the oil phase has an SFC of 15% and in which 1.25% polysaccharides are dispersed in the continuous water phase. In this study, all applied polysaccharides were able to increase the stability against partial coalescence by providing a sufficiently thick continuous phase and/or by supplying a protective coating around the globule. However, carboxymethylcellulose, guar gum and locust bean gum showed the ability to reduce partial coalescence to a larger extent than xanthan gum and gum Arabic. The decreased capacity of xanthan gum and gum Arabic was attributed to an insufficient polysaccharide concentration. It was believed that the applied concentration of gum Arabic was not enough to saturate all surfaces of the droplets favoring the emulsion destabilization whereas for xanthan gum an inadequate thick continuous water phase was formed which didn’t sufficiently reduce the collision frequency of the globules.

Furthermore, Boode [130] maintained that addition of SDS to the aqueous phase shortly after partial coalescence can disperse the created aggregates into the original globules. Partial coalescence can thus be reversed by changing the composition of the aqueous phase. This is explained by the ‘Lanza effect’. Adsorption of SDS on the crystal interface gives the crystals more affinity for the aqueous phase which involves the destruction of the oil neck. After a longer time, addition of SDS will not reverse partial coalescence presumably because of sintering of the crystals located in the oil-neck between the globules will occur.

E. Dispersed oil phase compostition

The composition of the oil phase, together with the composition of the interface and the *t,T*-program defines mainly the crystallization behavior (Section 5.1.4.1.B) and is thus one of the main factors that will determine the rate of partial coalescence. In addition, it is observed that not only surface-active molecules, i.e. small-molecule surfactants and proteins, but also the fat used in the emulsion formulation participates in the development of the interfacial characteristics [232, 233].

5.2. Whipped cream

5.2.1. Definitions

Whipped cream is a rigid dairy foam which is widely used as a topping for desserts, pastries, cakes and ice cream. From a microstructural point of view, whipped cream can be described as an aerated partially destabilized oil-in-water emulsion. The air bubbles dispersed in the serum phase of whipped cream are mainly immobilized by partially coalesced fat globules which create a semi-continuous network throughout the whole product. Though, some parts of the air bubble surface are stabilized by a protein layer [208, 253, 254]. This microstructure is widely accepted and demonstrated by applying several microscopic techniques [135, 170, 254-259]. Figure 5.13, Figure 5.14 and Figure 5.15 show confocal laser scanning microscopic (CLSM), transmission electron microscopic (TEM) and scanning electron microscopic (SEM) images of dairy whipped cream, respectively.

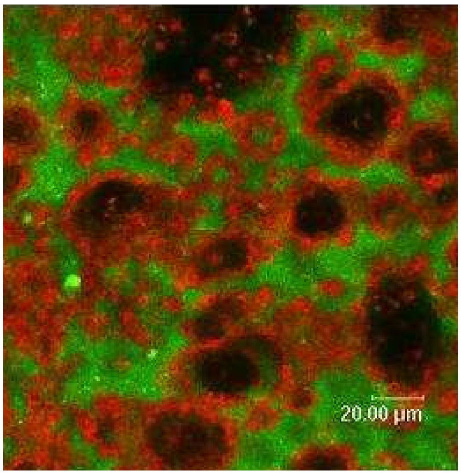


Figure 5.13 Confocal laser light scanning micrograph of whipped cream: in red the fat globules, in green the protein-rich serum phase and in black the air bubbles (Royal FrieslandCampina).

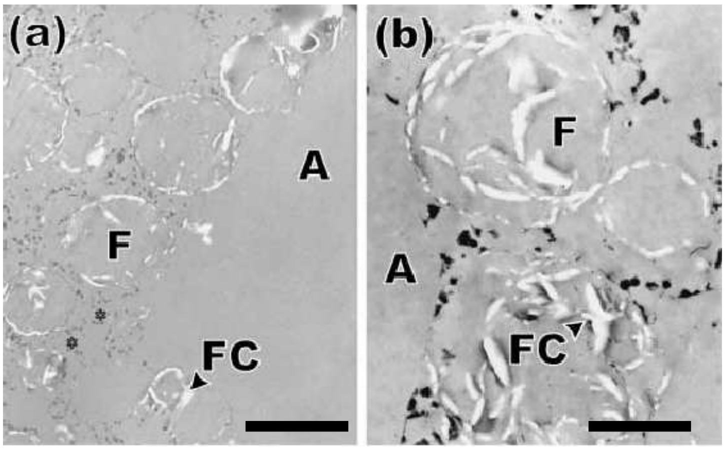


Figure 5.14 Transmission electron micrographs (TEM) of freeze-substituted and low-temperature embedded whipped cream at different magnifications: (a) length of scale bar is 4 µm and (b) length of scale bar is 1µm. Both images show at the air-bubble (A) surface fat globules (F) containing fat crystals (FC), which appear brighter, [257].

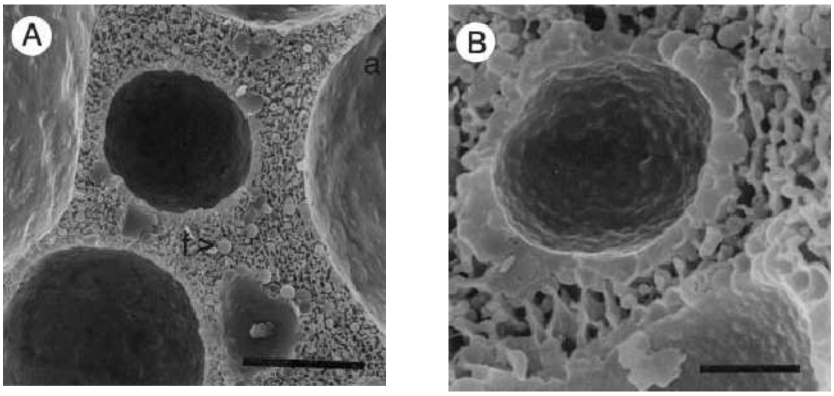


Figure 5.15: Cryo-scanning electron micrographs (cryo-SEM) of whipped cream at different magnifications: (a) Overview showing the relative size and prevalence of air bubbles and fat globules (length of scale bar is 30 µm) and (b) the internal structure of the air bubble, showing the layer of partially coalesced fat stabilizing the bubble (length of scale bar is 5 µm) [135].

In commercially available whipping cream (i.e. cream before whipping) the occurrence of the destabilization mechanism partial coalescence shows a discrepancy. On the one hand, it should be suppressed to attain an extended shelf life before whipping. On the other hand, to produce whipped cream partial coalescence is required for the structure build-up [135, 169-171] and to achieve the desired physical properties (i.e. overrun, stability, firmness) and sensory perception (i.e. fattiness and creaminess) [173] of the final product. Hence, whipping cream should be perikinetically stable and orthokinetically unstable.

Graf and Muller [260] defined an ideal foam as one that has a rigid structure, a high overrun (100-120%) and is stable against deformation. This can be achieved if the fat volume fraction φ of whipping cream is above 0.3 [17, 261]. Table 5.2 shows a typical composition of a conventional dairy whipping cream.

Table 5.2 Gross composition and some physical properties of natural whipping cream [1].

Content (%)	**Whipping cream**
Fat	35
Casein	1.7
Other protein	1.15
Lactose	3.1
Salts	0.57
Water	58.4
Physical properties	
pH	6.7
Density (kg.m^{-3})	990
$D_{3.2}$ (µm)	4.0

Next to the traditional dairy whipped cream, also *imitation creams* and *aerosol cream* exist on the market. Imitation cream is a recombined vegetable cream composed usually of a hardened lauric fat like palm kernel or coconut oil. Hardened lauric fats are appropriate for imitation creams because of its high SFC at ambient temperatures providing acceptable stability and its steep decrease in SFC at higher temperatures (close to 37℃) avoiding a waxy mouth feel [170, 262]. Compared with dairy whipped cream, the air bubbles are rather stabilized by a monolayer of individual fat globules than by a network of partially coalesced fat globules [170, 258] which detrimentally affects the storage stability of imitation creams after whipping. Aerosol cream is a whipped cream that emerges from an aerosol or spray can [1] containing dairy or vegetable cream. The overrun of these products is quite high (200%-300%) and it is mostly a close packing of the numerous bubbles that contributes to the firmness of these aerosol whipped cream [1]. Moreover, aerosol whipped cream is quite unstable. During the very fast process of bubble formation and expansion is the fat globule adsorption and clumping limited [1, 170]. Therefore, thickening agents are often inevitable to enhance the stability.

5.2.2. *Physical properties*

To assess the initial whipping properties of a cream the overrun, the air cell distribution and the firmness are often measured. Overrun is a measure for the amount of air incorporated in cream after whipping and, hence, of the volume increase. For traditional dairy whipped cream an overrun between 100% and 120% is ideal [260]. The overrun does not provide information about the air cell size distributions. To determine this, microscopic techniques (conventional light microscopy, CLSM, SEM) are often used [263-265]. The air bubble sizes measured for whipped cream varies between 20-60µm [261, 263]. The firmness/hardness of the whipped cream is mainly determined with a puncture probe placed on a texture analyzer [255, 261, 266, 267]. In so doing, the resulting firmness is highly dependent on the penetration depth and rate, the geometry of the used probe and the sample dimensions. Therefore, no general target value for the firmness of an ideal whipped cream can be set. Recently, also studies are available in which dynamic oscillatory rheology is used to study the viscoelastic behavior of whipped cream [132, 264, 268] as a measure for structure rigidity.

Like emulsions, foams are also thermodynamically unstable systems and are, thus, subjected to several instability mechanisms [1, 269, 270]:

- **Leakage/serum loss/drainage** is a gravitational instability mechanism which causes simultaneously the downwards moving of the ‘heavy’ aqueous phase around the air cells and the rising of the air cells.
- **Ostwald ripening/disproportioning** is the growth of bigger air cells at the expense of the smaller ones. The rate of disproportioning is highly dependent on the solubility of the gaseous phase in the aqueous phase and, thus, on the composition and temperature of both phases. For example, N_2O used in aerosol creams is highly soluble in water causing rapid destabilization of aerosol creams.
- **Bubble coalescence** is the fusion of two adjacent air cells leading to the loss of two smaller cells and the formation of a single larger one.

The above-mentioned instability mechanisms occur simultaneously during storage and once destabilization has started the different mechanisms are self-amplifying which eventually leads to complete phase separation of the aqueous, fat and air phase.

5.2.3. *Mechanism of structure build-up*

During whipping, cream is converted from a two-phase (fat-in-water emulsion) liquid system to a three-phase solid system in which air is incorporated and trapped by a network of fat globules [259]. Two types of partial coalescence are at the basis of this structure build-up:

shear-induced partial coalescence and surface-mediated partial coalescence [184]. Shear-induced partial coalescence is the orthokinetic destabilization of fat globules in the serum phase and is extensively discussed in Section 5.1.3 and Section 5.1.4. Surface-mediated partial coalescence occurs at the surface of an air bubble and is schematically illustrated in Figure 5.16. Fat globules enter into the air bubble surface and, subsequently, oil from the fat crystal network will be spread on the surface. If this occurs between two adjacent fat globules at the bubble surface, the fat globules will easily form a junction and partial coalescence is established. [184]. Hence, the capture efficiency *α* for partial coalescence is increased in the presence of air due to the concentration and immobilization of the fat globules at the air bubble surface as well as due to the enhancement of the junction formation as a result of oil spreading at the surface.

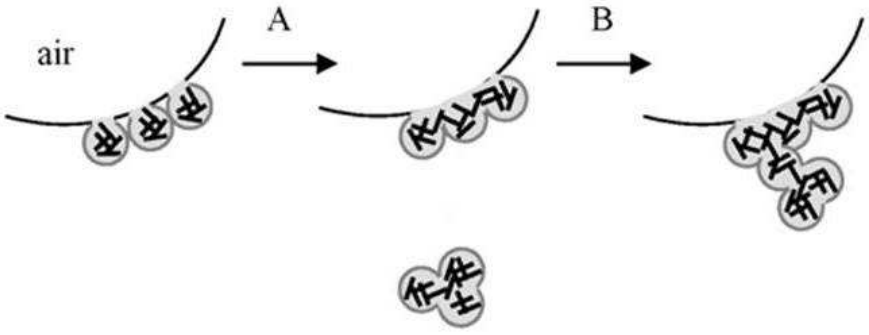


Figure 5.16 Schematic presentation of (A) surface-mediated partial coalescence combined with (B) shear-induced partial coalescence in whipped cream, adapted from Hotrum et al. [184].

The precursors for surface-mediated partial coalescence involving the entering in and the spreading of the emulsion droplets on the air bubble were extensively discussed by Hotrum et al. [184, 271-275] and Schokker et al. [276]. The entering and spreading of droplets are self-enhancing cooperative processes (Figure 5.17). The dynamic spreading of the oil, once a droplet/globule has entered, induces flow in the water phase causing additional droplet/globule transport towards the surface. Newly arrived droplets/globules will then easily coalesce or partially coalesce with the droplets or fat globules present at the air surface causing even more spreading and entering [184, 272].

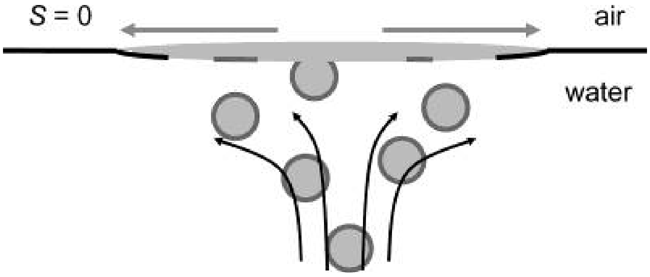


Figure 5.17 The self-enhancing cooperative process of entering and spreading of emulsion droplets at the interface [272].

In literature, the whipping process is often described as a three-stage mechanism [17, 184, 259, 261, 277] (Figure 5.18).

- **Stage 1 the protein foam formation**: In the applied turbulent flow field, large air bubbles with an initial uncovered surface (γ_{AW} = 72 mN.m^{-1}) are whisked into the cream and become incorporated as part of the foam after substantial reduction of the bubble size (120-150 µm). The system tends to lower the interfacial tensions by a diffusion-controlled adsorption of proteins (γ_{AW} = 50 mN.m^{-1}), mainly of α- and β-caseins and whey proteins. The overrun increases fast in this stage, although the viscosity and, thus, the firmness of the system are still low and the serum loss is still close to 100%. Shear-induced partial coalescence and some fat globule entering at the surface take place as well in this stage (Figure 5.18 B).

- **Stage 2 the intermediate stage**: The bubbles are further reduced in size (<60µm) but the overrun remains more or less constant. The continuous bubble break-up implies an expansion of the bubble surface area and a dynamic change in surface tension or surface pressure[3]. The newly formed interface does not contain proteins and are prone for fat globule or clump adsorption (Figure 5.18 C). If crystals stick out of the MFGM-membrane, bridging will easily occur between the fat globule and the air bubble (like in partial coalescence, Section 5.1.3). During adsorption/entering, fat globules will lose partly their MFGM [253, 259] and oil will be spread on the surface [273], both favoring surface-mediated partial coalescence. The reduction in bubble size and the adsorption of fat globules yield close packing conditions of the bubbles and the flow condition induced by the whisks is rather laminar at the end of this stage due to the increased viscosity. Bubble coalescence occurs but to a lesser extent than bubble disruption in this stage. The firmness increases gradually and the serum loss decreases significantly.

---

[3] Surface pressure can be calculated from $\Pi = \gamma_0 - \gamma$ with γ and γ_0 the actual interfacial tension and the interfacial tension without surface-active molecules and can be described as the change in interfacial tension due to the presence of surface-active components

– **Stage 3 the fat globule network formation**: A network of partially coalesced fat globules is built up which serves to keep the air bubbles in place and entrap the serum phase in the foam structure. Surface-mediated partial coalescence proceeds creating a fat-air interface (γ_{AW} = 10 mN.m^{-1}). Near the end point, the free fat globules and small clumps become scarce for the adsorption to the newly formed surface. Subsequently, bubble coalescence becomes predominant accompanied by the release of ‘shells’ of clumped fat globules due to the shrinking of the surface area (Figure 5.13). These released clumps form an extended structure throughout the serum phase (Figure 5.17 D) giving the desired firmness to whipped cream. In this final stage, the overrun is unaffected, the firmness increases exponentially and the serum loss decreases to 0%.

Further whipping promotes bubble coalescence converting the extended structure of fat globules into large butter granules which become separated from the serum phase (phase separation) and the overrun will decrease.

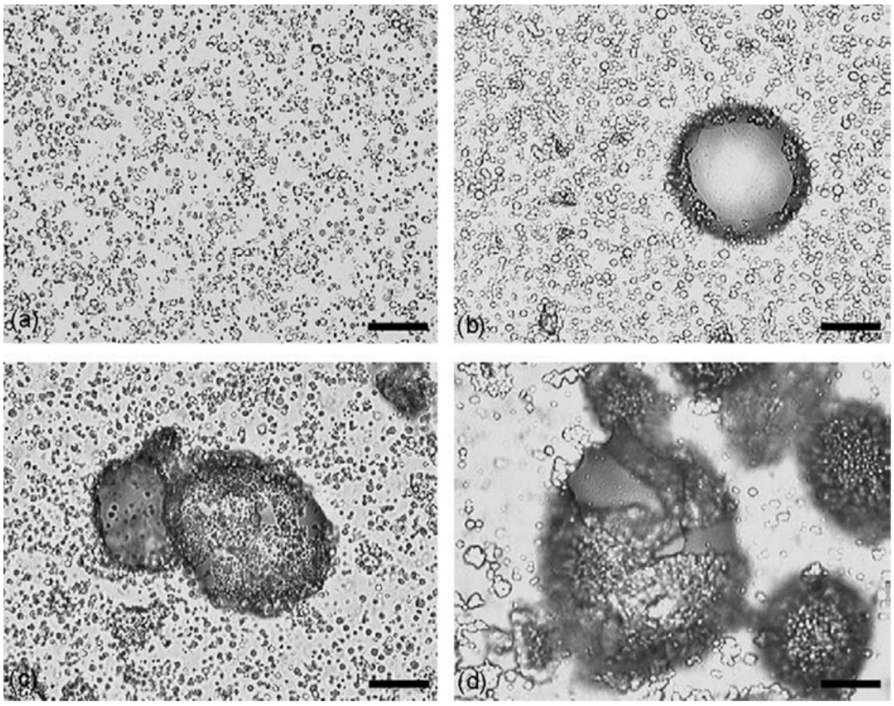


Figure 5.18 Light micrographs of cream stabilized with 1 wt% WPI of (a) before whipping, during the (b) first stage, (c) second stage and (d) third stage of whipping (length of scale bar is 100 µm) [184].

5.2.4. Factors governing the whipping properties

The whipping properties like stability, firmness, overrun and air cell size distribution and the whipping time will be highly dependent on the rate of both shear-induced and surface-mediated partial coalescence. Therefore, all factors discussed in Section 5.1.4 will have their effect on the whipping properties. This section focuses on the effect of process parameters and the effect of composition and formulation of oil-in-water emulsions on the behavior of fat globules in the presence of air bubbles and on the physical properties of whipped cream rather than on their effect on the rate and extent of partial coalescence. This discussion is not limited to dairy whipped cream.

5.2.4.1. Process parameters

A. Flow condition

Air is whisked into cream in a turbulent field in the first stage of whipping, during further whipping the flow condition becomes more and more laminar [261]. The rotational speed of the whisks decreases the whipping time [184, 261, 274]. Increased whipping speed will enhance both shear-induced (see Section 5.1.4.1.A) and surface-mediated partial coalescence. The latter is mainly attributed to the increased expansion rate of the air bubble surface causing an enhanced entering and spreading of the free globules and clumps during the second stage of whipping. A minimal rotational speed is required to reach the final stage of whipping. At lower rotational speed the expansion rate of the bubbles surface will be insufficient to cause an adequate decrease in surface pressure of the adsorbed proteins and will hinder the uptake of fat globules [261].

B. Temperature

Temperature and *T*-history are the most important factors determining the occurrence and the rate of partial coalescence. Therefore, it will obviously affect the whipping properties. However, no elaborate study on the whipping temperature (T_{wh}) is available for a wide range of T_{wh}. Ihara et al. [278] recently found that whipping time (t_{wh}), overrun and bubble diameter decreased with increasing whipping temperature for a fat dairy cream at T_{wh} between 5℃ and 15℃ due to an increased aggregation rate at higher temperatures. Moreover, SFC plays an elemental role in the stability of whipped cream. An adequate amount of SFC is required to retain the shape at the chosen storage temperature [262] and to limit excessive oil spreading out of the fat crystal network of the globules [264]. A too high amount of oil release can be detrimental for the foam stability. The oil spreading may cause squeezing out of the bordering liquid which implies local film thinning of two approaching air bubbles and will eventually result in a bubble coalescence [270]. Besides the effect of T_{wh} on fat crystal habits, it also affects the adsorption rate of proteins on the air bubbles during the first stage of whipping. At lower temperature the adsorption is reduced facilitating the fat globule entering and spreading [273].

The *T*-history of the cream before and after whipping also affects the physical properties of whipped cream. If a tempering period similar as described in Section 5.1.4.1.B.iv is applied before whipping at T_{max},which is a temperature lower than the final melting temperature of the fat (T_m), t_{wh} and serum loss are decreased and an increased firmness and elastic behavior is observed [196, 264, 277]. At T_{max} = 25°C and at low cooling rates (0.1 °C.min $^{-1}$), the highest values of *G'* (elastic modulus) were obtained while the period at T_{max} did not show significant effects [264]. This behavior was attributed to the fat crystal changes in the globule as described in 5.1.4.1.B.iv. A tempering period applied after whipping at $T_{max} < T_m$ also implied an increased elastic behavior independent of the fat content in natural cream [132] and recombined cream [268]. The optimum effect was also reached at T_{max} = 25 °C and at low cooling rates (0.1 °C.min $^{-1}$). The modifications in the foam structure during tempering involved changes in polymorphism and SFC in natural cream [132] and increased surface-mediated partial coalescence in both natural [132] and recombined cream [268]. During the period at T_{max}, *a* large fraction of the fat is melted and spread over the air bubble surface leading to the creation of a persistent junction between adjacent clusters and fat globules. Probably, sintering plays also an additional role in enhancing the elastic behavior, although not discussed in literature. Sintering of fat crystals is enhanced due to *T*-fluctuations and will thereby affect the elastic behavior of the cream.

5.2.4.2. Composition and formulation of the cream

A. Fat volume fraction

The overrun decreases and the emulsion remains more or less liquid if the fat content is lower than 20% [261]. The calculated close-packed air bubble size coated with fat globules exceeds the air bubble size produced by the whisks in the first whipping stage and can, thus, not be obtained. The achieved lower viscosity during the second stage of whipping, results in lower shear forces working on the surface and, hence, less uptake of fat globules by the bubble surfaces takes place. At 5% fat content no air bubbles can even be incorporated. At a fat content higher than 25%, a considerable stiffness is obtained [261].

B. Particle size

In recombined cream, the particle size is mainly determined by the applied homogenization pressure and the type and concentration of milk powder used [135, 163]. The particle size is generally reduced by increasing the homogenization pressure but homogenization clusters, in particular in skimmed milk stabilized creams, can easily be formed at a higher homogenization pressure [163, 279]. Generally, the t_{wh} is negatively correlated with the particle size distribution [163, 279] but for creams containing homogenization clusters the overrun and t_{wh} is lowered [163]. The clusters increase the viscosity and reduce, hence, the bubble incorporation during whipping, which is also the case in clustered homogenized natural cream [280].

The particle size distribution of natural cream can be reduced by high pressure homogenization, if properly applied [1, 161]. This homogenization step also involves a change in the interfacial composition. The decreased particles size distribution as well as the changed interfacial composition will have an effect on the whipping properties and is discussed in the next section.

C. Surface-active components

Homogenized natural creams show much longer t_{wh}, slightly higher overrun, increased firmness but similar serum loss [281]. The mechanism of air destabilization in homogenized cream seems to be different as well [170, 281]. Partial coalescence does not occur because the newly created thick fat globule membrane, which contains casein micelles, does not allow fat crystals to grow through it. Moreover, if the homogenized fat globules enter the expanding air bubble they do not lose their fat globule membrane which restricts the oil spreading. Nevertheless, casein micelles are believed to decompose at the oil-water interface which causes the release their Ca-sensitive regions. Ca-bridges between adjacent fat globules can then be formed in the last whipping stage resulting in a whipped cream in which the air bubbles are stabilized by aggregated fat globules rather than partially coalesced fat globules. The Ca-content and acidity contribute further to this aggregation mechanism of fat globules and, hence, improve the whipping properties [281, 282].

The proteins and small-molecule surfactants at the oil-water interface affect besides the shear-induced partial coalescence (Section 5.1.4.2.C.ii) also the surface-mediated partial coalescence in whipped cream. In natural cream, fat globules entering the air bubble surface easily release their MFGM which enhances the clustering at the surface. The release of the membrane in homogenized and recombined cream and, hence, also the spreadability at the air bubble surface will be highly dependent on the viscoelasticity of the oil-water interface and, therefore, on the interfacial protein load, the type of surface-active components and the applied heat treatment. For homogenized whipped natural cream the release of the membrane was not shown which causes a change in the whipping mechanism, like previously described [281]. In heat-treated WPC and SMP stabilized emulsions a decreased spreadability is observed while no effect of heating was found in Na-caseinate stabilized emulsions [276]. The cross-linking of the WPs and of WPs and caseins during heating results in an increased coherence of the oil-water protein layer. Hence, not all these interfaces will rupture, even when spreading forces are applied. In the presence of MAGs at the interface, the decreased spreadability was not observed in heat-treated WPC stabilized emulsions, in particular if unsaturated MAGs were used. MAGs, segregated in patches in between the adsorbed WPs, probably hindered the formation of a continuous protein network upon heating resulting in a lower interfacial yield stress [276].

Not only the protein load at the oil-water interface but also the amount and type of 'free' surface-active components in the aqueous phase plays an important role in the structure formation of whipped cream. Washed or deproteinated creams exhibit a lower t_{wh} [259, 277]. The fat globule adsorption/entering and spreading is fast and not hindered due to a protein barrier at the interface [163, 283]. In the presence of free proteins, t_{wh} is generally prolonged [163, 259, 281] and the entering of the droplets in the air bubble surface is dependent on the type of proteins [272, 283]. β-Lactoglobulines and soy glycinins are globular proteins that form a brittle coherent protein layer at the air bubble surface that more easily fractures than a β-casein film when exposed to shear or dilatational stresses in the second stage of whipping. A large amount of droplets enters then in the cracks between the globular protein network and an erratic spreading pattern of the droplets appears. β-caseins are random coil proteins that create a more liquid-like surface layer in which droplets enter at different 'nucleation' sites. This explains the longer t_{wh} for caseinate stabilized emulsions compared to WP stabilized emulsions [255, 274]. Moreover, in the presence of adsorbed proteins at the air bubble surface and if fat globules are entered a critical surface pressure Π_{cr} can be defined for oil spreading to occur. At a surface pressure higher than Π_{cr} no spreading takes place. For all proteins the Π_{cr} amounts to ± 15 $\mathrm{mN.m^{-1}}$ which points out that the air-water surface does not need to be completely void of adsorbed proteins in order to have oil spreading [272].

Small-molecule surfactants can enhance the spreading behavior and, thus, the surface-mediated partial coalescence also in the presence of 'free' proteins. Hotrum [283] showed that in the presence of polyoxyethylene sorbitan monolaurate and sorbitan monooleate at the oil-water interface a higher Π_{cr} could be found compared to pure protein stabilized emulsions. This, together with the frequently observed increased shear-induced partial coalescence will probably explain the often decreased t_{wh} and changed physical properties of whipped cream in the presence of small-molecule surfactants as observed in several studies [243, 244, 272, 274, 283-286].

Often an optimum concentration of small-molecule surfactants is required to meet a certain degree of destabilization of fat globules contributing to both the foam formation and stabilization. For sucrose esters this was shown by Tual et al. [243, 244]. At intermediate concentrations (ca. 0,1 wt%), sucrose esters interact with the interfacial proteins without displacing them. However, the interfacial layer is weakened facilitating the droplet disruption and spreading at the air bubble surface. At a high concentration, preferential adsorption of sucrose esters over proteins occurs and an excess of surface-active components are present in the aqueous phase promoting air inclusion. However, at these high concentrations, a readily destabilized foam is shaped due to an excess of fat globule destabilization resulting in an increased free fat content.

Furthermore, the fat:protein ratio defines the protein load at the interface and therefore the whipping properties [255, 287]. The higher the fat:protein ratio the lower the t_{wh} and the higher the firmness and the serum loss. In these studies, the authors attributed these effects on the increased sensitivity of the fat globules towards partial coalescence. Note that, even though not considered, the effect of varying the fat:protein ratio on the spreadability at the air bubble surface, on the free protein content and on the particle size distribution, as discussed above, will probably also contribute to the modified whipping properties.

D. Continuous aqueous phase composition

The effect of excess surface-active material in the aqueous phase was discussed in the previous section. In this paragraph the general effects of thickening or gelling agents (i.e. hydrocolloids), ionic strength and acidity on whipping properties are briefly discussed.

Hydrocolloids increase generally the t_{wh}, the storage stability of whipped cream and the firmness but it adversely affects the overrun, mainly by enhancing the viscosity of the aqueous phase. Nevertheless, the effect is highly dependent on the type of hydrocolloid [164, 165, 288-290].

Ca-content and acidity are known to affect the whipping properties, in particular in homogenized whipped cream [281], since they affect the electrostatic interactions between the fat globules and the thickness of the protein layer. The latter defines the crystal protrusion distance, the steric repulsion and the viscoelastic behavior of the oil-water interface. A foam can be even made of liquid droplets at a certain Ca-content and acidity [291], but has different physical properties than the conventional whipped cream.

E. Dispersed oil phase composition

A fat appropriate for whipping is partially solid at 5℃; has an adequate amount of SFC to retain the shape at ambient temperatures and shows a sharp decrease in SFC at higher temperature ($T \leq 37$℃) to avoid a waxy mouth feel [262]. The fats that fulfill these requirements are milk fats and its fractions, used in dairy whipping cream, and hardened lauric fats, used in imitation whipping creams.

The fat phase can be modified by fractionation, hydrogenation, interesterification and/or blending of the fats to improve the physical properties of whipped recombined creams mainly by changing the SFC-profile. By interesterifying a blend of hydrogenated palm kernel oil and palm stearin, an imitation whipped cream can be obtained with an enhanced stability at higher ambient temperatures (around 30℃) [262]. Attempts are also made by recombining

AMF, milk fat stearin and olein fractions in whipping cream. Whipped cream enriched with olein fractions seem to have a thicker coating of fat globules at the interface [292] but when the amount of olein fraction becomes too high the stability is largely decreased [264]. Not enough solid fat is then present to capture the oil in the globules at the oil-water interface. Alternatively, cream containing only the hard fraction of milk fat can be detrimental for the foam formation. Bubble collapse takes place due to crystals that pierce the air bubbles [293].

5.3. Concluding remarks

This literature review illustrates that profound awareness of several aspects of colloid science is essential to understand the mechanism and the kinetics of partial coalescence and its consequences regarding the whipping properties of cream-like oil-in-water emulsions. For investigating partial coalescence in food systems knowledge of colloidal interactions, fat crystallization and adsorption phenomena at the oil-water interfaces and air bubble surfaces is indispensable. Varying either the process parameters, composition or the formulation of the emulsions involves changes on both the fat crystallization kinetics in the globule (nucleation and crystal growth rate) and its final microstructure (size, morphology and polymorphisms of the crystals, the arrangement of the crystals in the globule, the fat crystal network,…). Furthermore, these factors govern the particle size, the distribution of the various components among the dispersed oil phase, the oil-water interface and the continuous aqueous phase and, as a consequence, the physicochemical properties of these phases. This all together demonstrates the complexity of researching partial coalescence in real food systems. Although keeping this in mind, some general trends upon varying certain parameters on the shear-induced and surface-mediated partial coalescence in oil-in-water emulsions can be abstracted in terms of both the encounter frequency and the capture efficiency towards partial coalescence. The flow conditions, the fat volume fraction and the physicochemical properties of the continuous aqueous phase affect both the encounter frequency and capture efficiency while the actual temperature, *T*-history and the composition of the dispersed oil and the interfacial layer affect mainly the capture efficiency towards partial coalescence. Finally, it should be stressed that whenever a parameter is varied if partial coalescence and whipping properties are investigated all other parameter should be kept constant as much as possible.

.

6. DESCRIPTION OF MATERIALS AND METHODS

In this chapter the materials and the basic principles of methods used in part II are discussed.

6.1. Materials

The same materials as in Part I were used. A detailed description of the composition of the applied creams and their starter materials can be found in Section 2.1.

6.2. Methods

6.2.1. Rheology

6.2.1.1. Rotational viscosity analyses

Rotational viscosity analyses are applied to study the kinetics of partial coalescence without the inclusion of air. Therefore, the AR2000 controlled stress rheometer (TA instruments, Brussels, Belgium) with the starch pasting cell as geometry was used. The starch pasting cell consists of a jacket, a removable cup and an impeller (diameter 32 mm and height 12 mm) (Figure 6.1). The gap between the impeller and the cup is 5500 µm, which is larger than that of the plate-plate or concentric cylinders facilitating the follow-up of partial coalescence when big aggregates are formed. The starch pasting cell is *T*-controlled. A cooling control unit is placed between a water bath (Julabo, Seelbach, Germany) and the starch pasting cell and heating is accomplished by the electrical elements present in the jacket. The actual temperature of the sample is read by a Pt 100 probe in contact with the bottom of the cup. Since the propeller produces an ill-defined flow, analytical conversion factors are not available. Therefore calibration was performed by the manufacturer to determine the conversion factor for shear rate and shear stress. For the sake of simplicity, the word 'shear rate' is used in this manuscript, although the shear rates occurring in the starch pasting cell will vary throughout the sample volume.

To follow up partial coalescence, 30 mL of cream sample was transferred in the cup and, after an equilibration period at measuring temperature, a constant shear rate was applied and the viscosity of the cream was followed as a function of time until a maximum was achieved. If no viscosity increase is observed during 8h of shearing, the measurements were stopped. The shear rate and temperature applied varied between 50 s^{-1} to 200 s^{-1} and between 5℃ to 35℃, respectively. All measurements were performed in triplicate and representative viscosity curves are plotted in the graphs. Note that in this manuscript the word viscosity actually refers to the apparent viscosity of cream.

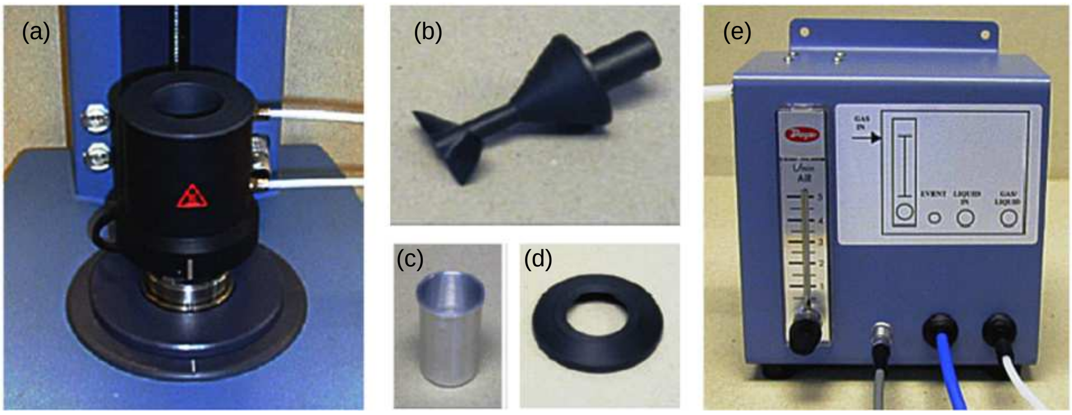


Figure 6.1 (a) Starch pasting cell with (b) removable impeller, (c) cup and (d) cap and (e) the cooling control unit (TA Instruments).

6.2.1.2. Oscillatory interfacial shear rheology

The main challenge in interfacial shear rheology is to make distinction between the rheological properties of the interface and the surrounding bulk phases. The total drag on the geometry positioned at the interface is attributed to a combined force originating from the shear stress of the interface and of the bulk phase. An appropriate geometry minimizes the ratio of the contact area between the geometry and the upper bulk phase to the perimeter of the geometry at the interface. Therefore, a double wall-ring geometry has been developed and the benefits are summarized by Vandebril et al. [294]. Figure 6.2 shows a picture of the geometry and the matching teflon cup and their dimensions. The geometry can be attached to a conventional rotational rheometer. Note that interfacial rheology is performed in two dimensions. Therefore, the rheological parameters are expressed per unit length. For instance the unit of the elastic modulus *G'* is Pa.m.

Interfacial rheological properties were determined using a double-wall ring geometry mounted to an AR-GR rotational rheometer (AR-G2, TA Instruments). The teflon cup holding the sample was filled with a sub-phase sample volume of 18,8 mL and an upper-phase sample volume of 15 mL. In this study, the sub-phase is buttermilk, made by reconstituting 11.1 wt% sweet cream buttermilk powder (SCBMP), and the upper-phase consists of purified sunflower oil with or without monoacylglycerols (MAGs). The ring of the geometry was positioned at the interface visually and the interface was allowed to equilibrate for 30 min at room temperature. At that time no differences in the measurements were observed. Subsequently, in order to define the linear viscoelastic region (LVR) strain sweeps are performed at the interface at a constant frequency of 0.1 rad.s^{-1}. Finaly, the interface was probed by means of small-amplitude frequency sweep experiments, at a frequency ranging from 0.1 to 2 rad.s^{-1} and at a constant strain or amplitude within the LVR. All measurement were performed in triplicate.

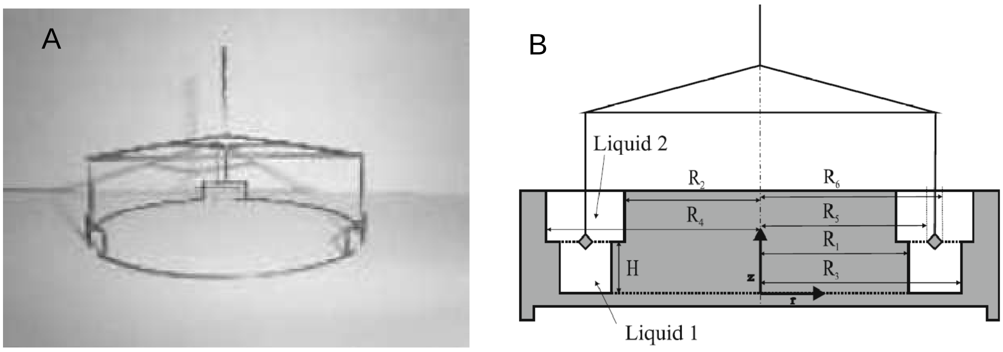


Figure 6.2 (A) The double wall-ring geometry and (B) a schematic presentation of a cross-section of the double wall-ring set-up with R_1 = 31 mm, R_2 = 30 mm, R_3 = 39.5 mm, R_4 = 40.5 mm, R_5 = 34.5 mm and R_6 = 35.5 mm, after [294].

6.2.2. Static laser light scattering

Similar procedures and equipment as described in Part I (Section 2.2.3) were applied.

6.2.3. Nuclear magnetic resonance analyses

Similar procedures and equipment as described in Part I (Section 2.2.4) were applied.

6.2.4. Interfacial tension analyses

Similar procedures and equipment as described in Part I (Section 2.2.5) were applied.

6.2.5. Microscopy

6.2.5.1. Bright-field light microscopy

To visualize the milk fat globules and the, in the rheometer shaped, partially coalesced aggregates, the cream samples were 1:10 diluted with distilled water. One drop of the diluted cream was transferred with a Pasteur pipette to a microscope slide and a cover slit was carefully placed on top of the sample to avoid air inclusion as much as possible.

Conventional bright-field microscopic analyses were conducted with a Leitz Diaplan microscope (Leitz diaplan Leica, Germany) equipped with a Linkam PE 94 temperature control system (Linkam, Surrey, United Kingdom). The images were recorded with an Olympus Color View camera and processed with cell D software (Olympus, Aartselaar, Belgium). The 100x and the 250x magnifications were mainly used.

6.2.5.2. ***Confocal scanning laser light microscopy***

To visualize the microstructure of whipped cream confocal scanning laser light microscopy (CSLM) is used. This technique is a powerful optical tool for the visualization of the structure of food products on a micrometer scale. Opposed to traditional light microscopy, the advantage of using CSLM is the imaging of a single focal plane in a sample of arbitrary thickness. The fluorescence light is collected by the objective lens and focused into a small pinhole to eliminate the out of focus light. Fluorescence is often obtained by adding a specific dye. The fluorescent dye will than spread over the samples according to local accessibility and affinity. Nowadays, CSLM-equipment offers also the possibility of multiple photo multipliers operating at different wave length intervals enabling simultaneous detection of fluorescence light from different dyes [295].

To make distinction between the three phases (aqueous, fat and air phase) in whipped cream a dual staining technique is used. Fluoroscein isothyocyanate (FITC) labels proteins non-covalently [296] and stains, thereby, the aqueous phase because of its high protein content. The fat phase is colored with nile red (NR) as was shown by van Lent et al. [297] for butter systems and by De Graef [298] for bulk palm oil systems . In this study, 0.5 mL of 1 wt% FITC dissolved in water and 0.5-1 mL of 1 wt% NR dissolved in dimethylsulfoxide were added to 500 g of cream just before whipping. After whipping, as described in Section 6.2.7.1, the stained cream samples were immediately visualized. The whipping experiments were done in duplicate and from each obtained whipped cream several samples were taken for microscopic evaluation.

A Leica inverted microscope DM IRE2 TCS SP2 (Leica microsystems, Heidelberg, Germany) equipped with a 20X objective was applied to acquire the images. Both FITC and NR were excited by a 488 nm (20mW) Argon laser. The fluorescence light emitted by the sample of the FITC and NR were separately detected using two photomultipliers (PMT's) at different wavelengths: at 500-536 nm and 595-648 nm, respectively. An overlay of the obtained images is made with ImageJ freeware.

6.2.6. *Protein load analysis*

Determination of the protein load at the oil-water interface Γ in cream was conducted to investigate the extent of protein displacement as a function of type and concentration of MAGs. To isolate the milk fat globules an adapted method based on the centrifugation method developed by Patton and Huston [299] is applied. Cream was first diluted with cooled UHT-treated skimmed milk to obtain a fat content of 5-8 wt% and 15 wt% sucrose was added. Dilution was done to limit the encounter frequency between fat globules and, hence, to avoid partial coalescence which may cause protein release during centrifugation. Sucrose was added as a density increasing agent to enhance the fat yield in the top layer after centrifugation [234]. Smaller fat globules may otherwise settle during centrifugation because of their equal or even higher density than the aqueous phase [234, 300]. The total fat content of the diluted cream was measured gravimetrically by using the Röse-Gottlieb extraction method.

After preparation of the diluted cream, it was transferred to a centrifugation tube and a washing layer consisting of simulated milk ultrafiltrate (SMUF) [301] is put on top of the cream before centrifugation (Figure 6.3). SMUF was made according to the procedure of Jenness and Koops [301] with adjustment of the pH to 6.7. Centrifugation at 3600xg for 1h was applied and a three layer system was obtained: the sediment layer containing the serum proteins of cream, the SMUF washing layer and the upper fat layer containing the intact fat globules. The centrifugation experiments were performed in triplicate for each type of cream.

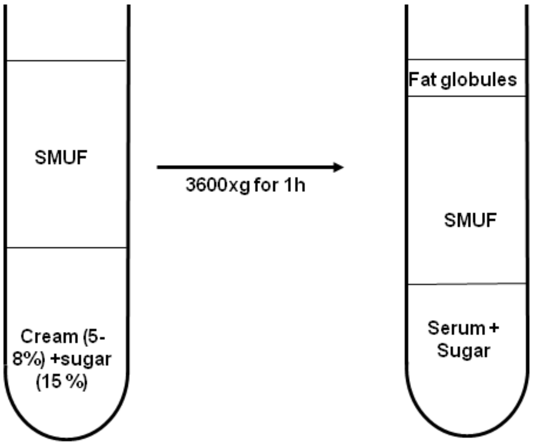


Figure 6.3 Schematic presentation of the composition of the layers before (left tube) and after (right tube) centrifugation to isolate the milk fat globules of cream.

After centrifugation, the tubes were moved to the fridge (T = 5 °C) to solidify the upper fat layer. This facilitates the isolation of the upper layer, although some fat globules may be redispersed in the liquid layer during separation. The mass, the fat content and the protein content of the fat layer were determined. The fat content was measured by means of the Weibull method [302] and the protein content was obtained using the Kjeldahl nitrogen determination method [303], using 6.38 as the protein conversion factor.

From the fat content of the upper layer and the fat content determined of the diluted cream, the fat recovery in the top layer, which was > 90%, was calculated. Taking into account this value a correction is made to calculate the total protein content ($m_{pr,\ interface}$) at the oil-water interface in cream. Using the $D_{3,2}$-values measured by laser light diffraction (Section 6.2.2), the protein load Γ can be calculated by the following equation:

$$\Gamma = \frac{m_{pr,interface}\, D_{3,2}}{6\varphi} \tag{6.1}$$

with φ the fat volume fraction.

6.2.7. Whipping properties

6.2.7.1. *Whipping process*

Whipping was performed with a Hobart N50 mixer equipped with a D-wire whip designed for maximum air inclusion in a low viscous product. Three fixed rotational speeds (120 rpm, 240 rpm or 480 rpm) can be applied and lead to consistent and thorough mixing. Unless stated otherwise, the medium speed (position 2: 240 rpm) is applied at 5℃. All creams were whipped until a defined observable end point. The optimum whipping time is defined as the time at which a cream was obtained that broke away from the wires and the bowl. Moreover, when the whipping process is stopped at this optimum whipping time (t_{wh}), the cream should not immediately start to flow around the wires of the impeller. Beyond the optimal whipping time the structure became chalkier/grainier in appearance. Whipping experiments were repeated minimally two times for each cream (i.e. two whipping batches). For each whipping batch the overrun, serum loss and firmness was determined. The whipping properties plotted in the graphs give the average values and the error bars represent the standard deviations between the two whipping batches of the same cream.

6.2.7.2. *Overrun*

Overrun is a common measure for the amount of air introduced in whipped cream and can be calculated from the comparison of the weight of equal volumes of unwhipped and whipped cream [17, 267, 304-306]:

$$Overrun\,(\%) = \frac{m_{unwhipped} - m_{whipped}}{m_{unwhipped}} * 100 \tag{6.2}$$

with $m_{unwhipped}$ and $m_{whipped}$ the mass of the unwhipped and whipped cream, respectively. After whipping the overrun was measured six times. The mass of whipped and unwhipped cream was determined in plastic containers of 200 mL. The overrun may alternatively be determined by measuring the volume increase after whipping [262].

6.2.7.3. Firmness

A measure for the firmness of whipped cream is mainly determined by a puncture test at constant speed and until the probe has reached the defined depth. The puncture probe on texture analyzer can be a cylinder, a wire frame or a cone [255, 261, 266].

Large deformation puncture measurements were conducted with a Texture Analyzer TA500 (Lloyd Instruments, UK) equipped with an acrylic cylindrical probe (diameter 25mm and height 35mm). The 200 mL containers filled with whipped cream for the overrun measurements were used as sample holder. Puncture tests were performed at a rate of 1 $mm.s^{-1}$ over a distance of 20 mm in the sample. The trigger value for the measurement started was set at 0.01 N. The force (N) required to reach this depth is defined as the firmness of the whipped cream. Puncture tests were carried out in triplicate after 1h and 24h storage at 5℃

6.2.7.4. Serum loss

Many studies use serum loss as a measure for the stability of whipped cream. This is often done by putting a defined mass of whipped cream on a sieve or filter, storing the cream for several hours at a constant temperature and measuring the amount of serum that is poured through the sieve or filter [261, 304]. Alternatively, the time at which a fixed amount of the serum was drained from the whipped cream [255] or the height of the drained serum in a bottle is measured [262, 266].

In our study, 30g of whipped cream was transferred in a funnel and placed on top of an Erlenmeyer. After storage at 20℃ for 1h and at 5℃ for 24h, the amount of serum drained from the whipped cream was gravimetrically determined in triplicate and the serum loss was calculated by:

$$serum\ loss\ (\%) = \frac{m_{serum}}{m_{whipped}} * 100 \tag{6.3}$$

with m_{serum} the mass of cream pored through the funnel and $m_{whipped}$ the initial mass of whipped cream transferred to the funnel.

6.2.8. Statistical analyses

SPSS software (version 15, SPSS Inc., Chicago, Illinois, USA) was used for statistical comparison of the Γ, the churning times (t_{ch},) and the whipping time (t_{wh}). All the reported values are the average of at least three replicates. After checking the prerequisites, analyses of variance (one-way ANOVA) were carried out to determine significant differences between the results, followed by Tukey’s post hoc test for pairwise comparison. All tests were performed at a 95% significance level.

Since for each cream sample two whipped cream batches were produced and subsequently the whipping properties (overrun, serum loss and firmness) were separately determined for each batch in triplicate, it was first verified whether a two-way ANOVA taking into account the batch effect was more powerful. If no batch effect was present a one-way ANOVA was appropriate. To decide which model can be used a likelihood ratio test was performed to compare the fit of two models. As a result one of the two models was chosen and performed, followed by a Tukey's post hoc test for pairwise comparison. All statistical analyses for the whipping properties were performed at a 95% significance level in the R 2.12.2 freeware.

7. POTENTIALS OF A RHEOLOGICAL METHOD TO STUDY PARTIAL COALESCENCE IN DAIRY WHIPPING CREAMS

7.1. Introduction

Factors controlling the rate of partial coalescence define to a large extent the whipping properties of oil-in-water emulsions, as shown in Chapter 5. Hence, assessment of these factors with a proper method is essential to adapt or develop whipped products with a desired whipping time, overrun, firmness and stability.

Shear-induced destabilization of partially crystalline fat globules in oil-in-water emulsions is addressed in several studies. Mainly rotational viscosity analyses are carried out at a constant as well as at a varying shear rate or shear stress without aerating the emulsion [154, 185, 186, 189, 192, 307]. An increase in apparent viscosity as a function of time, shear rate or shear stress is thereby ascribed to clump formation. The latter may be evidenced by direct microscopic imaging [307]. Furthermore, an oscillatory rheological approach by using the parallel plate geometry is recently reported by Thivilliers-Arvis et al. [190]. A sinusoidal maximum strain, corresponding with the strain at the periphery of the plate, out of the linear viscoelastic region is applied during a well-defined period. As a result of using the parallel plate geometry the oil-in-water emulsion is locally subjected to a variable strain proportional to its radial position. After the shearing is stopped, a threshold strain for clumping is visually determined by inspecting the radius at which macroscopic clumps appeared. This threshold strain was defined as a measure for the rate of shear-induced partial coalescence. Besides evaluating the above-mentioned rheological parameters, other studies estimate shear-induced partial coalescence rate by evaluating the particle sizes upon shearing [130, 175, 176, 182].

The precursors for surface-mediated partial coalescence (i.e. the entering in and the spreading of the emulsion droplets on air surface) on a macroscopic air surface were extensively discussed by Hotrum et al. [184, 271-275] and Schokker et al. [276]. Moreover, Hotrum et al. [274] proposed a correlation between the whipping time of vegetable recombined cream with the spreading and entering of fat globules at the surface. However, in the latter study the rate of surface-mediate partial coalescence was not considered during whipping of the vegetable creams.

None of the above-mentioned studies related the whipping properties (whipping time, overrun, firmness and stability) of dairy creams to the shear-induced partial coalescence rate as was measured by the viscosity analyses. Therefore, the aim of this study is to establish a rheological technique to survey shear-induced partial coalescence and to explore the potentials of this method to qualitatively predict the whipping properties.

7.2. Research strategy

In this chapter a rotational viscometric technique is introduced to study shear-induced partial coalescence. To gain more insight in the mechanism of partial coalescence, microscopic and particle size distribution analyses were performed at multiple marked points of the viscosity profile. To examine the potentials of this technique as a predictive tool for the whipping properties the effect of temperature, shear rate and fat content on both shear-induced partial coalescence and the whipping properties were investigated. All these experiments were performed with natural cream (NC).

7.3. Results and discussions

7.3.1. Mechanism of shear-induced partial coalescence

Figure 7.1 shows the viscosity profile of NC subjected to a constant shear rate of 150 s^{-1} at 20℃. It can be observed that after an initial lag phase (I), the viscosity (i.e. apparent viscosity) starts to increase until a local maximum (II) is attained. Subsequently, the viscosity gradually drops (III) until the viscosity abruptly increases reaching the upper limit in viscosity (IV). Further shearing resulted in a completely churned cream. Phase separation of the milk fat and the serum phase has occurred. Therefore, the time required to achieve the final maximum viscosity is called the *churning time (t_{ch})* and is, in this case, 26 min.

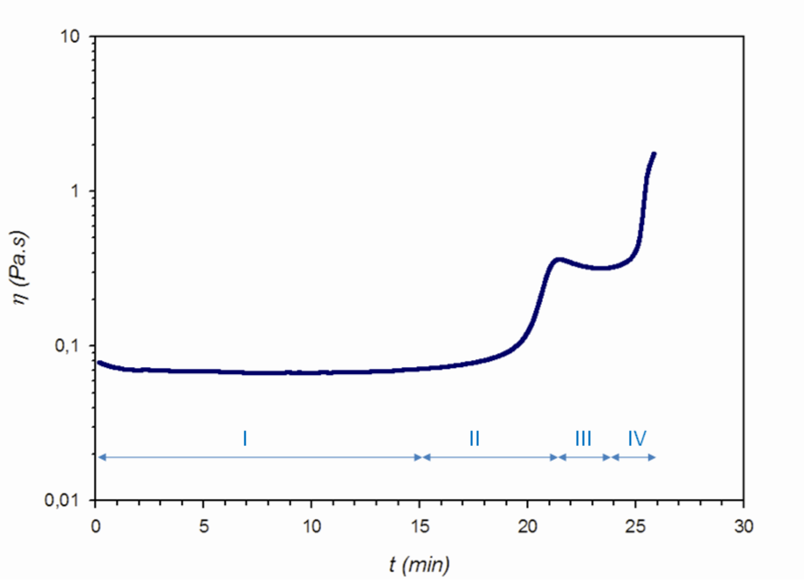


Figure 7.1 Viscosity profile of NC (T = 20℃; $\dot{\gamma}$ = 150 s^{-1}).

To elucidate what happens in the cream during shearing in the rheometer, the viscosity analyses were interrupted and bright-field microscopic evaluations of the obtained cream samples were performed. Figure 7.2 shows representative micrographs of samples taken during the different phases. During phase I individual milk fat globules together with a minority of very small aggregates are present. In phase II, large erratic or irregular aggregates were shaped whereas during phase III the aggregates became more spherical and individual droplets got scarce. Hence, the steep viscosity increase in phase II can be explained by an increase of the effective fat volume fraction due to the formation of large erratic aggregates. For the more spherical aggregates the increased effective fat volume fraction will be lower than in phase II which probably explains the gradual viscosity decrease in phase III. During phase IV, the aggregates appear to create a space-spanning network with some smaller aggregates and individual globules in between which clarifies the final steep viscosity increase just before churning.

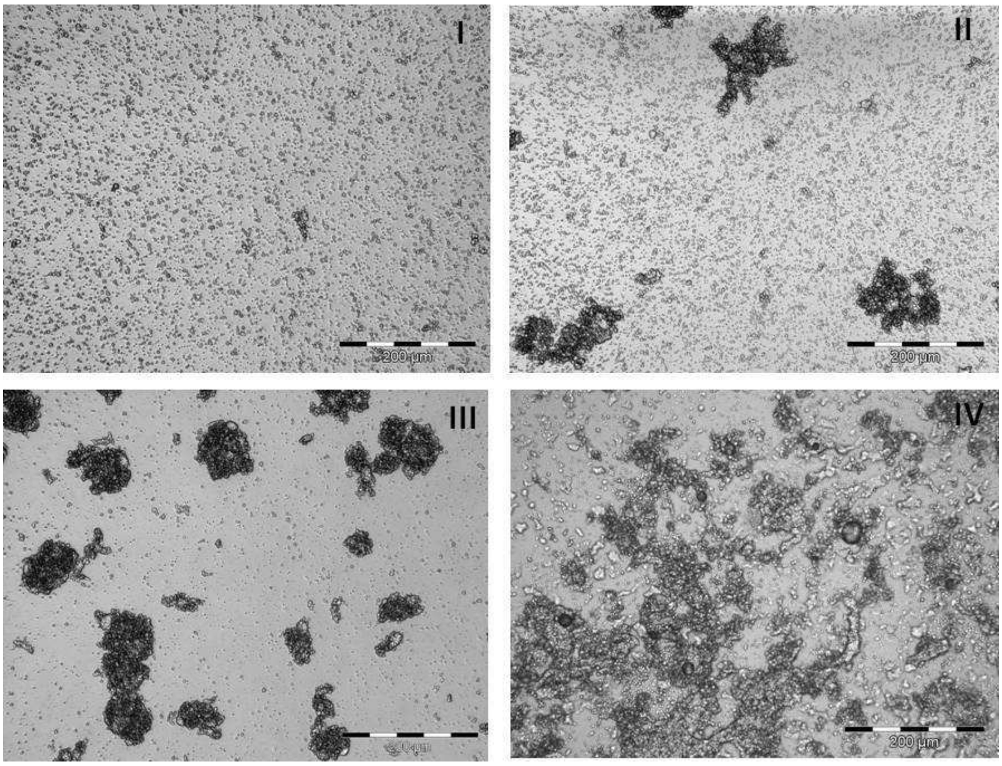


Figure 7.2 Representative micrographs of NC after interruption of the viscosity analysis (T = 20°C and $\dot{\gamma}$ = 150 s^{-1}) during phase I, II, III and IV (length of scale bar is 200 µm).

To make distinction between aggregation and partial coalescence, a cream sample taken during phase II was heated. Figure 7.3 shows the micrographs of the same cream sample but at different temperatures. Reaching 35°C the aggregates are contracted and the individual globules in the aggregates cannot be distinguished anymore. Probably, at that temperature the fat crystal network in the globules has been completely vanished. At 40°C milk fat is completely melted yielding the complete merge of the associated droplets into big spherical droplets. This clearly shows that the globules are internally connected with each other and, hence, that predominantly partial coalescence takes place during shearing in the rheometer.

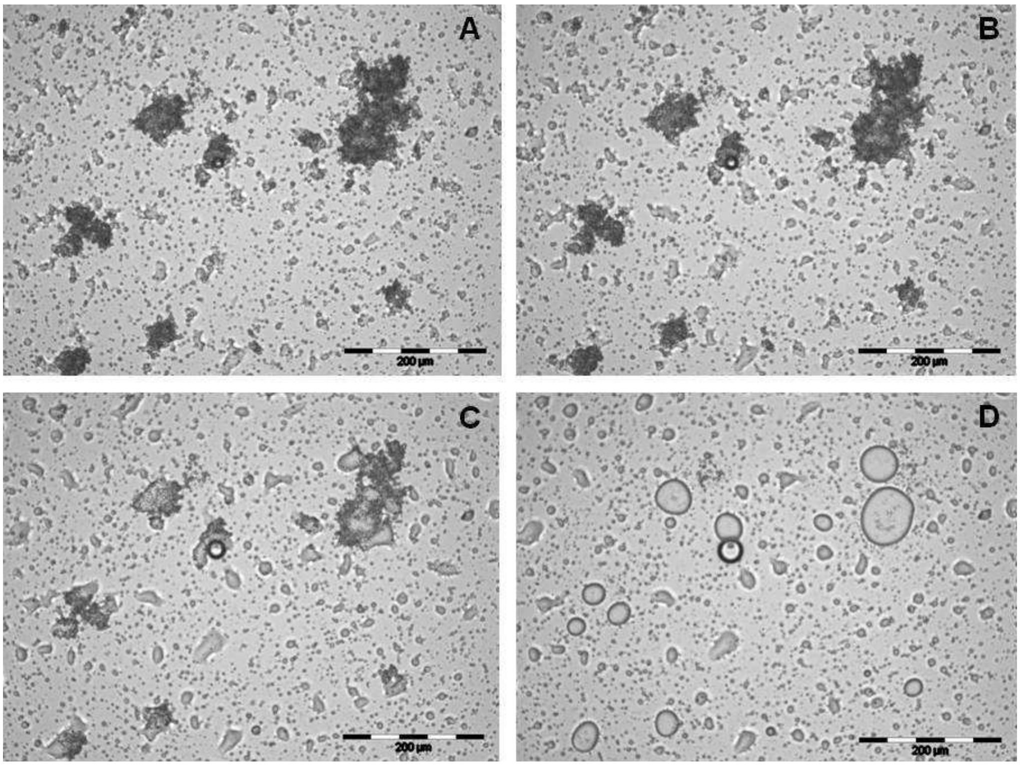


Figure 7.3 Micrographs of NC in phase II of the viscosity analysis (T = 20℃; $\dot{\gamma}$ = 150 s^{-1}) during heating at (A) 25℃, (B) 30℃, (C) 35℃ and (D) 40℃ (length of scale bar is 200 µm).

Particle size measurements (Figure 7.4) were also performed after interruption of the viscosity analyses to have a more quantitative evaluation. Prior to measuring the particle size distribution, the cream was, first, melted at 50℃ to avoid overestimation of the volume of the clumps (effective volume versus actual volume) and, second, diluted with a 1% SDS-solution to exclude analyzing flocculated droplets. The curve at 0 min in Figure 7.4 represents the particle size distribution of the individual milk fat globules in natural cream. During phase I (curve at 10 min) the distribution shows a shoulder between 6 and 10 µm which confirms the presence of small clumps whereas in phase II (curve at 20 min) two separated peaks appear. The first peak represents both the distribution of the remaining individual milk fat globules and small clumps and the second peak, at around 150 µm, characterizes the distribution of the melted large erratic clumps (Figure 7.2 B). Subsequently, in phase III (curves at 21.5 to 23.5 min) the area of the second peak grows further at the expense of the first one and concurrently the second peak maximum shifts to smaller sizes. The latter shows that the clumps shaped during phase II are disrupted while still more individual globules become incorporated in the larger clumps during phase III. The viscosity decrease in phase III (Figure 7.1) may, thus, be explained by clump break-up combined with changed clump shapes as shown by the particle size analyses (Figure 7.4) and microscopic evaluations (Figure 7.2), respectively. During phase IV (curve at 25.8 min) the second peak shifts to larger sizes. Note that the particle size analyses of cream obtained during phase IV of the viscosity profile can only be qualitatively interpreted due to the fast creaming of the largest droplets in the sample unit of the laser light scattering instrument.

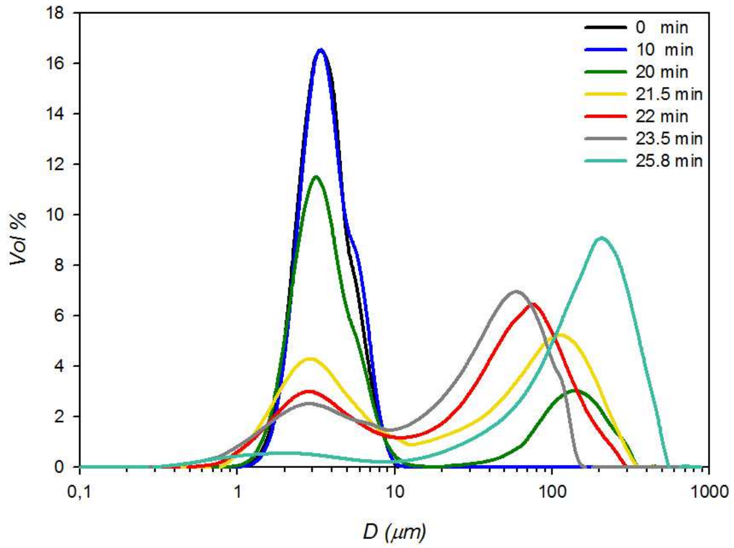


Figure 7.4 Particle size distribution of NC during the viscosity analyses (*T* = 20℃; $\dot{\gamma}$ = 150 s^{-1}) after 0, 10, 20, 21.5, 22, 23.5 and 25.8 min shearing.

7.3.2. Effect of temperature

From Section 5.1.4.1.B and Section 5.2.4.1.B of the literature review, it is clear that temperature plays a crucial role in the susceptibility of fat globules towards partial coalescence and to the extent to which globules merge if partial coalescence is accomplished. The actual temperature and *T*-history control to a large extent the fat crystal network properties of the milk fat globule, such as solid fat content (SFC), the arrangement, morphology, polymorphism, amount and size of the crystals (whether or not protruding) and the pore size between the crystals.

The creams used for the viscosity analyses and the whipping experiments were subjected to a similar *T*-history, which allows a straightforward comparative study of the effect of actual temperature. Prior to the viscosity analyses and the whipping experiments, the creams were, firstly, stored at 5℃ to ensure the presence of fat crystals and were, secondly, heated and equilibrated at the desired temperature.

7.3.2.1. Solid fat content

One of the major measurable effect of temperature on the physical state of the milk fat globules will be on the solid fat content (SFC) of the milk fat. Therefore, SFC in cream was measured after equilibration at 5℃ and after step-wise increase of the temperature (ΔT = 5℃). For these analyses an adapted SFC-method is used in which correction factors are included for the NMR-signal of the aqueous phase in cream. In Part I Section 2.2.4 a detailed description of the method is given. Figure 7.5 shows the SFC-profile. In the studied *T*-region (5℃ ≤ T ≤ 30℃) in the viscosity and whipping analyses, the SFC decreases gradually from 46.0 ± 1.3% to 5.7 ± 1.4%. At 5℃ and 10℃, a rather small difference in SFC is detected and the largest decrease in SFC is observed if the cream was heated from 15℃ to 20℃.

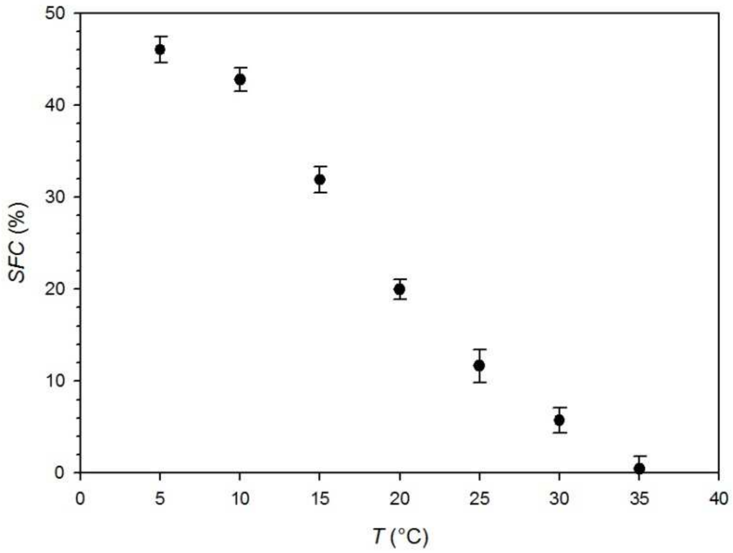


Figure 7.5 SFC-profile of NC.

7.3.2.2. Shear-induced partial coalescence

Viscosity profiles were recorded at different temperatures between 5℃ and 30℃ at a constant shear rate ($\dot{\gamma}$ = 150 s^{-1}). In Figure 7.6 only the curves from 15℃ to 30℃ are shown. At 5℃ and 10℃, the churning time (t_{ch}) was too long to include the viscosity profiles in the figure: more than 8h and 5h, respectively. At 15℃ < T < 30℃, clear differences in t_{ch} can also be observed. The t_{ch} decreases with increasing temperature until a minimum is reached around 25℃. At T > 25℃, the t_{ch} rises again. Besides the variation in t_{ch}, the viscosity profiles in Figure 7.6 vary in shape and in viscosities reached both at the first peak maximum (at the end of phase II) and at the t_{ch}.

The initial appearance of the first viscosity increase (phase II) and the lowest t_{ch} at 25℃ demonstrates that the capture efficiency and, therefore, the partial coalescence rate of NC will be the highest around 25°C and, hence, at a SFC of 11.6 ± 1.8% (Figure 7.5). At lower and higher temperatures and SFC the t_{ch} increases. This is consistent with the results of Thivilliers et al. [188, 190] who reached a maximum partial coalescence rate at a SFC of 10 - 15%. However, According to Hinrichs [186], the optimum SFC is approximately 25% corresponding to 20°C for summer cream and to 22-25 °C for winter cream. In the latter research the experimental results at higher temperatures and, thus, at lower SFC were not conducted which explains the differences with the current findings of optimum SFC. Moreover, it should be stressed that slight variations in *T*-history and composition of the creams in the considered studies may have an influence on the optimum SFC. Besides, the size, number, morphology, polymorphism and arrangement of the fat crystals can vary which may also contribute to the variation in optimum SFC-value for shear-induced partial coalescence.

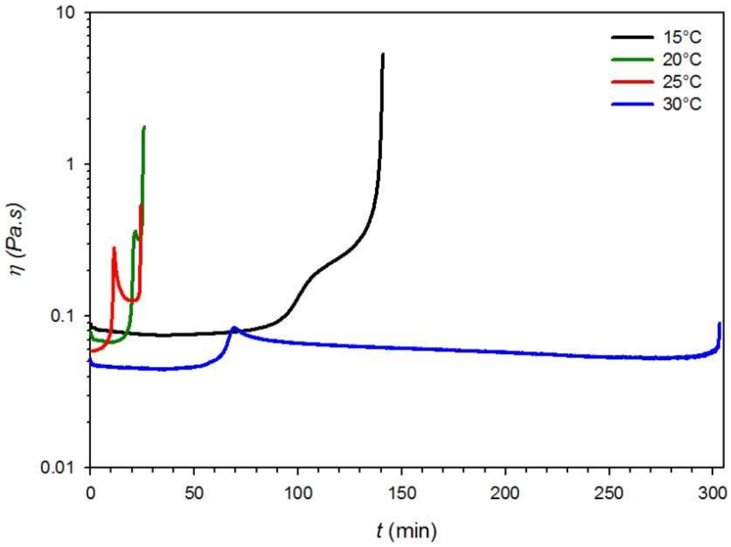


Figure 7.6 Viscosity profiles of NC at different temperatures and constant $\dot{\gamma}$ = 150 s^{-1}.

Next to the large differences in t_{ch}, Figure 7.6 reveals substantial variation in shape of the curves as a function of temperature. At 15°C, phase III (the viscosity decrease) of the viscosity profile cannot be distinguished. Probably, clump break-up did not take place to a large extent at 15°C. At *T* ≥ 20°C, phase III (the viscosity decrease) is more pronounced than at lower *T*. The erratic clumps created during phase II most likely break up more easily at higher temperatures due to their substantially lowered SFC implying a lower compression modulus of the fat crystal network and, thus, an easier collapse of the fat crystal network upon shearing. The collapse of the fat crystal network may be demonstrated by the amount of liquid oil that is collected at the outer layer of the clumps. In Figure 7.7 it can be seen that the spherical clumps shaped during phase II become more surrounded by liquid oil as the temperature increases. As a consequence, fat crystals become possibly more engulfed by the liquid oil leading to a decrease of available fat crystals to induce partial coalescence at higher temperatures. The latter together with the increased susceptibility to clump break-up explain the more pronounced phase III as temperature increases.

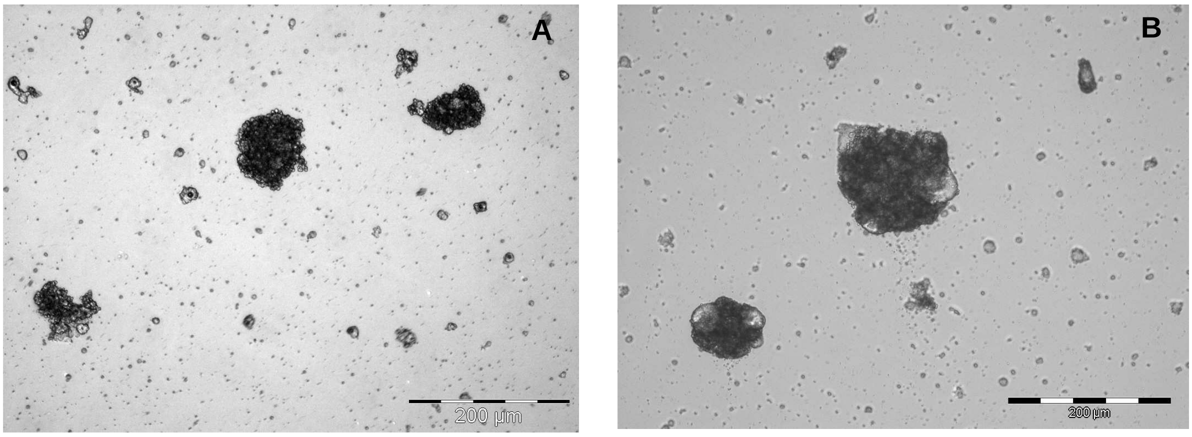


Figure 7.7 Micrographs of NC after interruption of the viscosity analyses during phase III of viscosity analyses ($\dot{\gamma} = 150\ s^{-1}$) performed at (A) 20℃ and (B) 25℃ (length of the scale bar is 200µm).

7.3.2.3. Whipping properties

Natural cream was whipped at different T_{wh} until a visible acceptable whipped cream was produced as described in Section 6.2.7.1. At T > 25℃, acceptable whipped creams could not be produced and therefore they were not considered for further research. Whipping time (t_{wh}) was recorded and the overrun (%) was measured (Figure 7.8 A). Annex II gives the results of the statistical analyses. Both the t_{wh} and overrun decrease with an increasing T_{wh}, except for 20℃ and 25℃. No significant differences in overrun between creams whipped at 20℃ and 25℃ can be observed. Ihara [278] observed similar results, although for a more limited T_{wh}-range (5℃ < T_{wh} < 15℃).

Figure 7.8 B shows the firmness of the whipped creams as a function of T_{wh} and annex II shows the results of the statistical analyses. To make comparison possible, all the puncture tests were performed at 5℃ to limit the effect of solid fat on the measured firmnesses of the whipped creams. A clear trend can be observed: the firmness increases as a function of T_{wh}, in particular after storage for 24h at 5℃. After 24h storage at 5℃ the firmness substantially increases at $T \geq$ 15℃. Probably, during storage the SFC still increases and even sintering of the fat crystal network may occur in the clumps. The latter is in particular increased due to the applied T-fluctuation.

Stability analyses by means of serum loss evaluations were also carried out. Regardless of the T_{wh}, substantial serum loss was not detected in the studied T_{wh}-range.

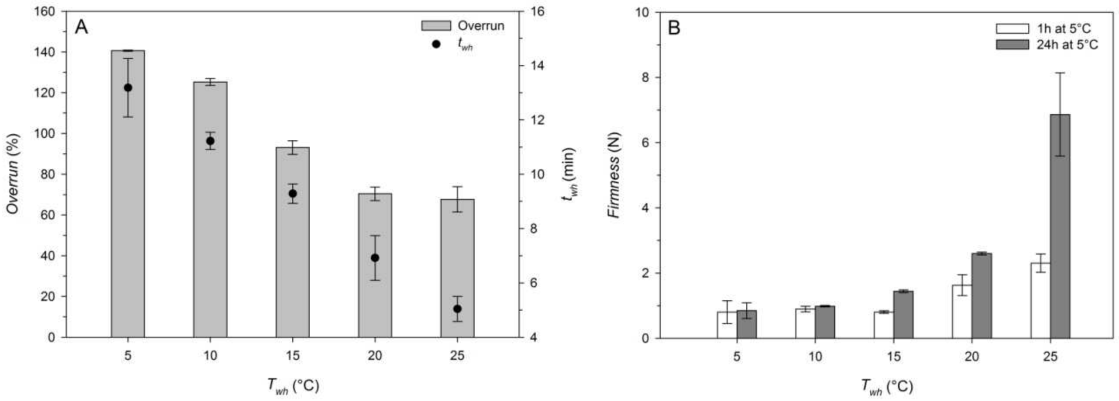
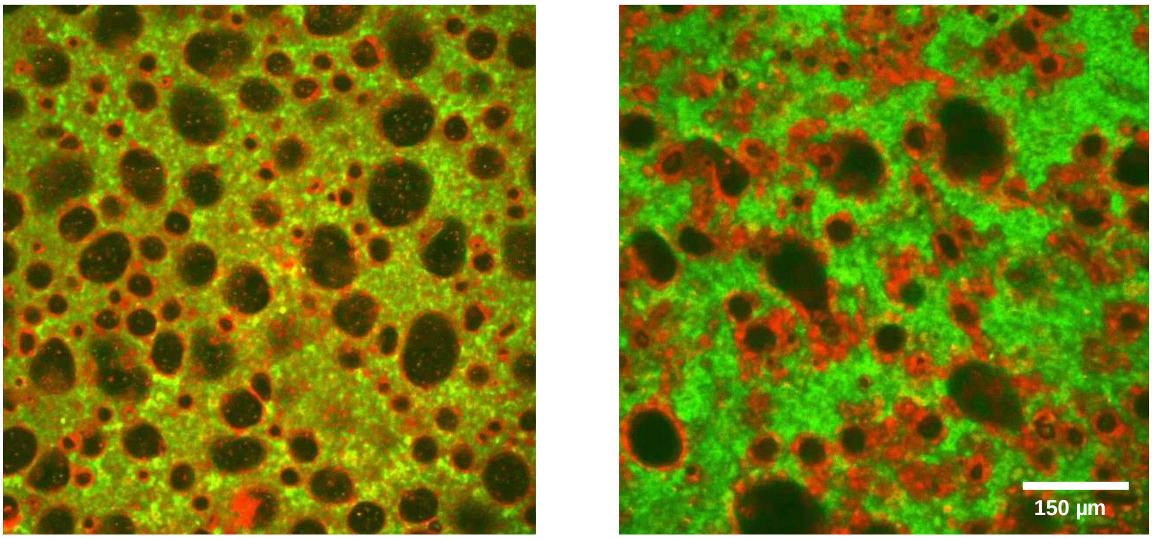


Figure 7.8 Whipping properties of NC at different T_{wh}: (A) overrun (%) and t_{wh} (min) and (B) firmness (N) after 1h and 24h storage at 5°C.

Figure 7.9 shows confocal scanning laser light microscopic (CSLM) images of NC whipped at 5°C and at 25°C. At T_{wh} = 5°C, the air bubbles are partly surrounded by a rather thin layer of partially coalesced fat globules while at T_{wh} = 25°C a higher degree of air bubble surface coverage seems to be achieved. Moreover, larger partially coalesced clumps appear in the serum phase at a T_{wh} = 25°C compared to 5°C. Hence, the overall extent of partial coalescence appears to be increased with increasing T_{wh}.

7.3.2.4. Discussion

At $T \leq 25$°C, the decreased t_{wh} as a function of T_{wh} accords well with the decreasing trend in t_{ch} as a function of the applied temperature (Section 7.3.2.2). Shear-induced and, presumably, also surface-mediated partial coalescence rate will be enhanced during whipping with increasing T_{wh}. Upon whipping, partial coalescence may thus prevail more quickly at an increased T_{wh} and the firm structure taken as the end point for whipping will then readily be obtained. As a consequence, the duration of the different stages of the whipping process, as described in Section 5.2.3, may be shorter. In particular, the changes in stage 1 of the whipping process will probably be at the basis of the altered whipping properties. In this first stage, air is incorporated and temporarily stabilized by proteins originating from the serum phase of NC. A shorter first stage will, thus, result in a lower overrun. Moreover in stage 1, shear-induced partial coalescence may be increased at higher T_{wh}. As a consequence, less fat globules will be available to efficiently envelop and immobilize the air bubbles during stage 2 of the whipping process. The thicker layer of partially coalesced clumps at the air bubbles in the CSLM-micrograph of whipped cream at T_{wh} = 25°C confirms the inefficient use of the fat globules to stabilize air bubble surfaces compared with whipped cream obtained at t_{wh} = 5°C. As a consequence, this also contributes to a final lower amount of air stabilized with an increased T_{wh}. In studies where globules are already aggregated due to the formation of homogenization clusters before whipping similar effects on overrun can be observed [163, 280].



T_{wh} = 5°C T_{wh} = 25°C

Figure 7.9 CSLM-micrographs of NCs whipped at 5°C and 25°C (length of scale bar = 150 µm). In red the fat phase and in green the protein-rich serum phase.

The increased firmness as a function of T_{wh} can mainly be explained by both the decreased overrun and the increased partial coalescence rate at a higher T_{wh}. The volume that the space-spanning partially coalesced fat globule network has to immobilize is decreased implying a denser and, hence, a firmer network.

In the discussion above, it is assumed that the surface-mediated partial coalescence, which mainly takes place during stage 2 of the whipping process, is enhanced. However, in this study surface-mediated partial coalescence rate is not separately addressed. Nevertheless, it is believed that an increased temperature and, in particular the lower SFC at a higher temperature, leads to a fast creation of complete occupied air bubble surface. Once globules or clumps enter the air bubble surface more oil will be available enhancing the spreading of the fat globules and, as a consequence, the surface-mediated partial coalescence. Figure 7.9 qualitatively indicates the differences in extent of surface-mediated partial coalescence as a function of T_{wh} by the increased degree of air bubble coverage with partially coalesced fat globules in creams whipped at 25°C compared to cream whipped at 5°C.

A stable whipped cream with an overrun of more than 100% is desired in many applications. Therefore, it can be concluded that whipping is ideally performed between 5°C and 10°C. To achieve the preferred overrun the degree of partial coalescence should be limited in the first stage of whipping and, hence, an intermediate t_{wh} is advisable.

7.3.3. Effect of Shear rate

7.3.3.1. Shear-induced partial coalescence

The applied shear rate on NC was varied at a constant T in the rheometer. Figure 7.10 shows the effect at 20℃ for shear rates ($\dot{\gamma}$) between 75 s^{-1} and 200 s^{-1}. All the four phases of the viscosity profiles can be distinguished irrespective of the applied $\dot{\gamma}$. However, the duration of the different phases of the viscosity profiles and the t_{ch} are inversely related to $\dot{\gamma}$. Since no perfect laminar shear flow is created by the rotor in the starch pasting geometry, not only the encounter frequency but also the capture efficiency for shear-induced partial coalescence will be increased (Section 5.1.4.1.A). At lower shear rates no viscosity increase was detected during 8h which indicates the existence of a threshold shear rate for partial coalescence to occur in the considered t-frame. Moreover, shear rate was also varied at different T (5℃ < T < 35℃). Similar trends were observed independent o n the temperature (data not shown).

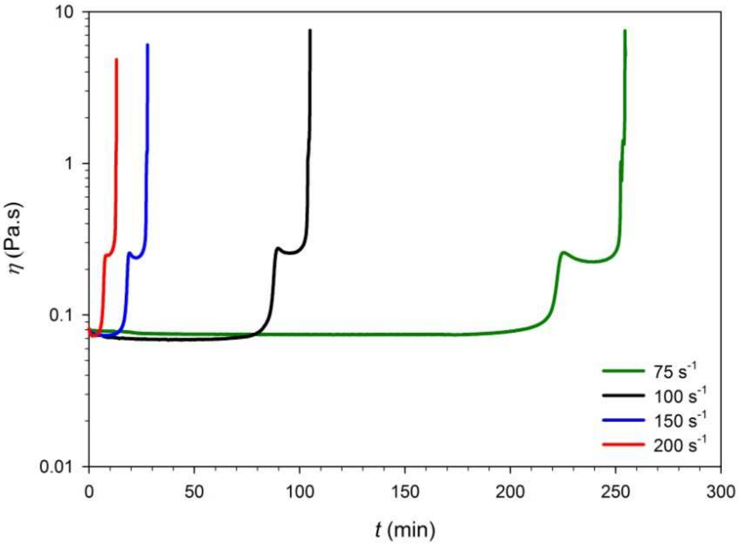


Figure 7.10 Viscosity profiles of NC at different shear rates (T = 20℃).

7.3.3.2. Whipping properties

Whipping experiments were performed at three different constant rotational speeds (120 rpm, 240 rpm and 480 rpm) and at 5℃, which was concluded as an advisable T_{wh} in the previous section. At the lowest speed no visually acceptable whipped cream was obtained. As was suggested by Van Aken [261], a minimal rotational speed is required to reach the final stage of whipping. At lower rotational speed the expansion rate of the bubble surface will be insufficient to cause an adequate decrease in surface pressure of the adsorbed proteins and will hinder the uptake and the spreading of the fat globules and, hence, the surface-mediated partial coealescence. The whipping properties at the other applied whipping speeds are given in Table 7.1. Overrun, t_{wh} and serum loss significantly decrease, while firmness significantly increases with the whipping speed.

Table 7.1 Whipping properties of NC at two different whipping speeds.

Whipping properties	**Whipping speed (rpm)**	
	240	**480**
t_{wh}	8,08 ± 0,12^{a}	3,05 ± 0,02^{b}
Overrun (%)	127,76 ± 1,46^{a}	60,46 ± 1,20^{b}
Serum loss (1h at 20℃) (%)	-	-
Serum loss (24h at 5℃) (%)	0,66 ± 0,09^{a}	-^{b}
Firmness (1h at 5℃) (N)	0,98 ± 0,04^{a}	2,18 ± 0,03^{b}
Firmness (24h at 5℃) (N)	1,27 ± 0,08^{a}	1,99 ± 0,08^{b}

$^{a-b}$ different letters indicate significant differences ($p < 0.05$) between different whipping speeds.

7.3.3.3. Discussion

Like in Section 7.3.4.3, the whipping properties can be explained by the increased shear-induced partial coalescence rate as function of shear rate (Section 7.3.3.1). At the highest whipping speed partial coalescence is possibly too fast to include and stabilize a high amount of air which leads to a dense partially coalesced network and, thus, an increased firmness and stability. A moderate whipping speed is therefore advisable. At higher whipping speeds, the overrun was too low (<100%).

It should be remarked that the flow conditions applied in the rheometer differ form the ones in the whipping experiment. Nevertheless, it seems to be possible to elucidate the same trends between the t_{ch} in viscosity analyses and t_{wh} in the whipping experiments if the temperature and the shear rate are altered. Moreover, the t_{ch} can be related to the overrun, firmness and serum loss. Although surface-mediated partial coalescence is not addressed separately, it is conceivable and accepted that, according to the correlation between the t_{ch} and t_{wh}, surface-mediated partial coalescence rate evolves concurrent with the changes in shear-induced partial coalescence rate.

7.3.4. Effect of fat content

The effect of fat content was studied by diluting the cream with cooled UHT-treated skimmed milk. As a result, the whipping creams contain a lower amount of fat but a higher amount of proteins.

7.3.4.1. Shear-induced partial coalescence

Figure 7.11 shows the viscosity profiles of NC at a fat content of 35%, 30% and 25%. The four phases of the viscosity profiles can be distinguished regardless of the fat content. However, with decreasing fat content, the duration of the different phases is prolonged and, hence, the t_{ch} is increased. The shear-induced partial coalescence rate is, thus, reduced when the cream is diluted with skimmed milk. This effect is ascribed only to the lowered collision frequency of the fat globules rather than changes in the capture efficiency upon collision. By making use of cooled skimmed milk to decrease the fat content of the whipping creams, the composition of the aqueous phase, the fat crystal network properties of the fat globules and the interfacial properties of the milk fat globule membrane are unaltered.

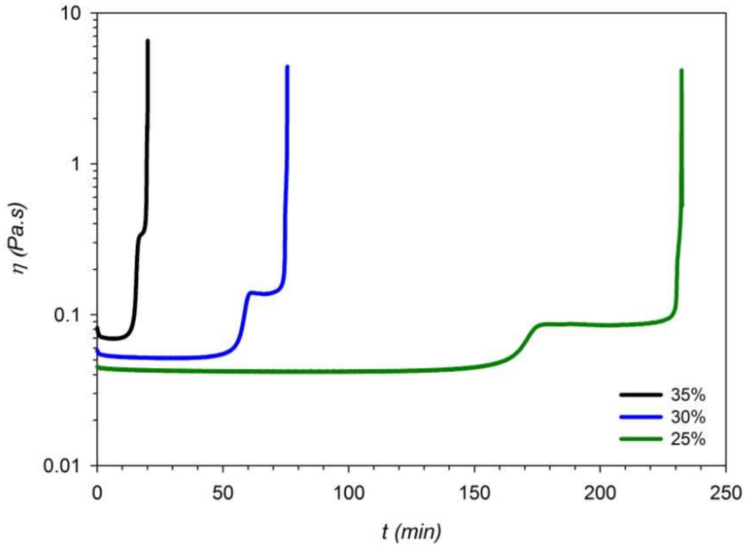


Figure 7.11 Viscosity profiles of NC with a fat content of 35%, 30% and 25% at $\dot{\gamma}$ = 150 s^{-1} and *T* = 20°C.

7.3.4.2. Whipping properties

The creams with the varying fat content were whipped at 5°C and the whipping properties are shown in Figure 7.12. Annex III shows the results of the statistical analyses. The t_{wh} and the serum loss are clearly inversely related with the fat content. The effect on overrun and firmness becomes only significant if the fat content is decreased to 25%. The overrun is increased while the firmness is substantially decreased at a fat content of 25% compared to the whipped creams with a higher fat content.

Figure 7.13 shows the CSLM-micrographs of whipped NCs with varying fat content. At a fat content of 35% and 30%, the air bubbles are covered by a thin layer of milk fat globules and in the serum phase a network of partially coalesced fat globules can be noticed. This network appears to be denser at a fat content of 35% compared with 30%. At a fat content of 25%, only locally, a rather sparse fat globule network in the serum phase and larger air bubbles appeared to be shaped. In between the larger air bubbles that are only partly covered with milk fat globules, smaller completely covered and, hence, more stabilized air bubbles can be found.

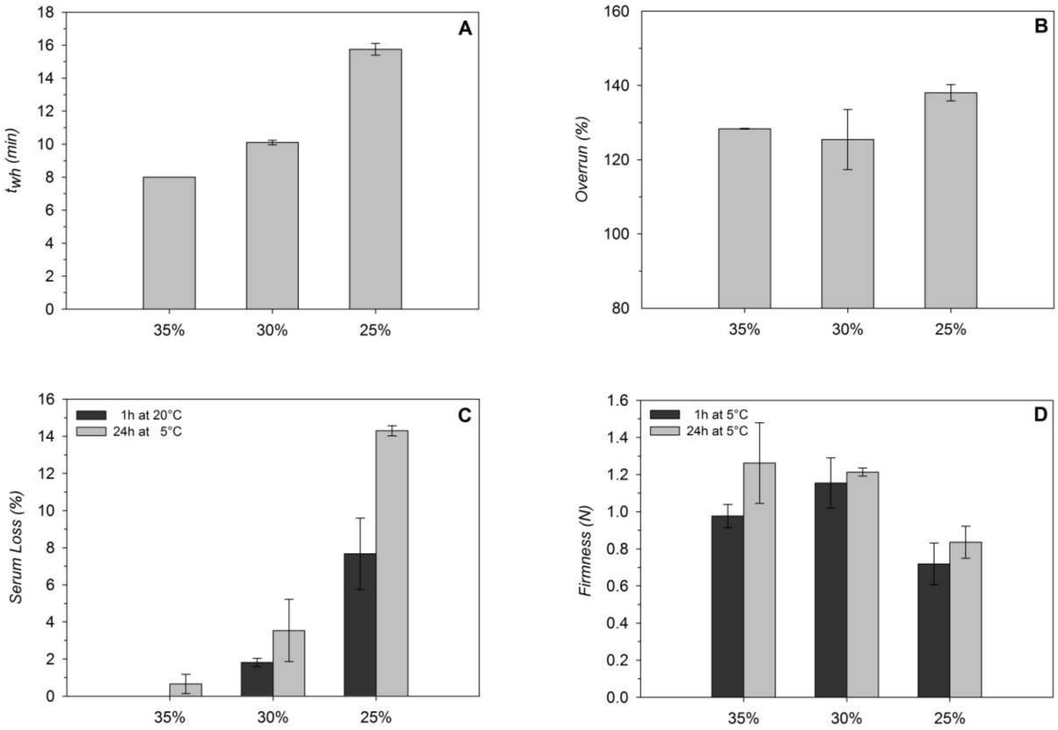
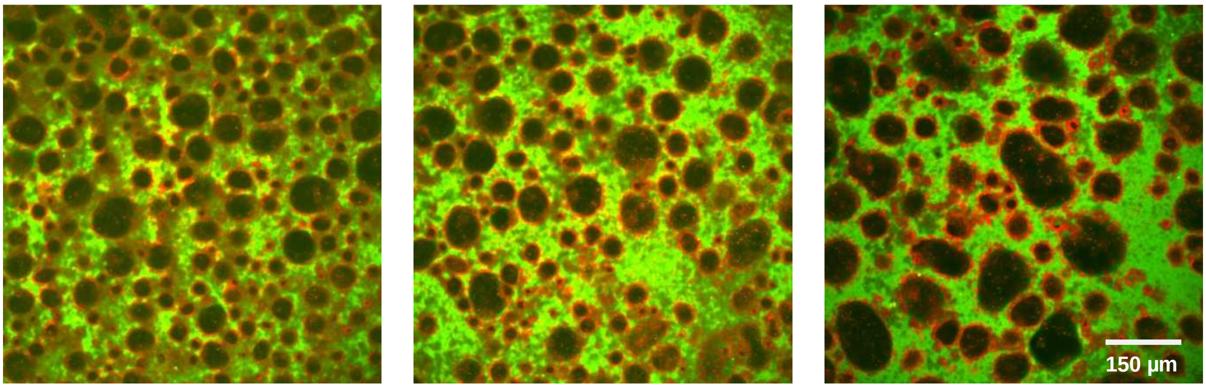


Figure 7.12 Whipping properties of NCs with a fat content of 35%, 30% and 25%: (A) t_{wh} (min); (B) *overrun* (%); (C) *serum loss* (%) after storage for 1h at 20℃ and 24h at 5℃ and (D) *firmness* (N) after 1h and 24h at 5℃.



35% 30% 25%

Figure 7.13 CLSM-micrographs of whipped NC with a different fat content (length of scale bar = 150 µm). In red the fat phase and in green the protein-rich serum phase.

7.3.4.3. Discussion

From the microstructure of whipped cream, as discussed in Section 5.2, it is clear that a substantial amount of fat is required to create a rigid dairy foam with an acceptable overrun, stability and firmness. Fat globules have to create a partially coalesced network throughout the serum phase and cover the air bubble surface. Van Aken [261] summarized the effect of fat volume fraction φ on whipped cream properties of several studies and found that a minimum fat volume fraction was required to incorporate air ($\varphi > 0.05$) and to achieve a considerable firmness ($\varphi > 0.25$). Furthermore, they concluded an increased overrun if φ decreases from 0.4 to 0.2. At $\varphi < 0.20$ the overrun substantially decreases. In the current results, like in the study of van Aken [261], the overrun is increased as a function of fat content.

Similar to the effect of temperature and shear rate, the decreased partial coalescence rate with a lower fat content, as observed by the viscosity analyses, mainly explain the increased t_{wh} and overrun of the whipped creams. However, in this case the increased amount of 'free' proteins in cream, by making use of skimmed milk to obtain a lower fat content, may also play an important role in the structure formation of whipped cream. The larger amount of surface-active components in the cream will increase the capacity to capture more air which may contribute to an additional increase in overrun.

Although fewer fat globules are present in whipped cream with a fat content of 25%, these globules have to stabilize more air (increased overrun). From the CSLM-micrographs it appears that rather large air bubbles are formed at a fat content of 25%. Presumably, not all the small air bubbles created during whipping will be sufficiently covered by fat globules and will, thereby, readily coalesce upon further whipping and storage. The broader air bubble size distribution, the lower fat globule load at air bubble surface and the lack of fat globule network in the serum phase compared to the high fat variants contribute to the disproportioning, the drainage and the bubble coalescence in whipped cream yielding the decreased stability and firmness of these creams. A whipped cream with a decreased fat content and improved whipping properties can probably be achieved at the expense of the overrun. If the overrun is lowered, the air bubble stabilization and the fat globule network in the serum phase will be enhanced. A decreased air inclusion can most likely be attained by shortening the duration of stage 1 of the whipping process and, hence, by increasing the partial coalescence rate.

7.4. Concluding remarks

In this chapter viscosity analyses of cream at a constant shear rate were proven to be an appropriate method to study the shear-induced partial coalescence kinetics. Furthermore, in combination with microscopic and particle size analyses it was possible to get more insight in the mechanism of the ongoing partial coalescence in the rheometer.

A comparative study of the viscosity analyses and the whipping properties with variation in temperature, flow conditions and fat content showed that the viscosity analyses can be used as a qualitative predictive tool for the whipping properties. The lower the churning time, the lower the whipping time and overrun will be. The lower overrun together with the increased partial coalescence rate implies a denser network and, as a consequence, an increase in firmness and decrease in serum loss. The reverse was true if increased churning times are observed.

Although the temperature affected partial coalescence rate, similar trends of shear rate are observed independent on the applied temperatures. Viscosity analyses performed at higher temperature give thus the same trend than at ideal whipping temperatures (5℃ < T_{wh} < 10℃) and can, thus, be used to study the difference in whipping properties at lower temperatures in dairy creams. This made the viscosity analyses more practically useful as a qualitative predictive tool for evaluating the whipping properties for further research (Chapter 8 and Chapter 9). Viscosity analyses at the ideal whipping temperatures would take too long to be practical.

8. EFFECT OF MONOACYLGLYCEROLS ON PARTIAL COALESCENCE IN RECOMBINED CREAM

8.1. Introduction

Nowadays, besides natural cream (NC), derived from milk by means of centrifugation, often recombined cream (RC) is used. It is obtained by recombining milk products, mostly milk fat and a low-fat milk powder, with water. The benefits of using RC are that the composition and formulation can easily be modified for product development goals and that the raw materials can easily be stored and transported to regions where fresh milk is not readily available and/or where suitable storage facilities are scarce. Nevertheless, the recombined dairy products like ice cream and whipped cream seem to have unfavorable divergent physicochemical and sensory properties as compared to products made from natural cream. This may be partly attributed to a different susceptibility towards partial coalescence of the fat globules of natural and recombined cream during processing.

From the literature review (Section 5.1.4.2.C.ii and Section 5.2.4.2.C), it is clear that small-molecule surfactants hold potential to control both shear-induced and surface-mediated partial coalescence. In oil-in-water emulsions they can affect the particle size distribution, the oil-water interfacial properties and/or the fat crystallization behavior of the oil phase. In particular, within the group of small-molecule surfactants, for monoacylglycerols (MAGs) the influence on partial coalescence rate has been shown in recombined model cream systems [154, 175, 189] and in ice cream systems [203, 207].

Davies et al. [154, 189] investigated the effect of different MAGs on the orthokinetic stability of sodium caseinate stabilized model creams of which the oil phase consists of a mixture of groundnut oil and tristearin (SSS). In these model systems, it is found that unsaturated MAGs of oleic acid destabilize emulsions more than the saturated MAGs of stearic and of palmitic acid upon shearing. Moreover, it appeared that the amount of SSS needed to induce shear-induced partial coalescence decreased with an increasing concentration of MAGs of oleic acid in the emulsions [189]. These effects were attributed to the morphological changes of the SSS-crystals in the presence of the different MAGs. The ones created in the creams containing MAGs of oleic acid were spiky spherulites which may easily pierce the oil-water interface upon shearing. With MAGs of stearic acid smaller rounded SSS-crystals were shaped which may not be able to force through the oil-water interfacial layer. The latter keeps the emulsions orthokinetically stable. Creams with MAGs of palmitic acid show less resistance against shear-induced partial coalescence than those with MAGs of stearic acid.

Larger crystals were formed in the presence of MAGs of palmitic acid. Although these are conceivable hypotheses of the effect of MAGs on the crystal morphology in emulsion systems, it has to be stated that no clear micrographs of the crystals were shown to prove these hypotheses.

Pelan et al. [207] and Goff and Jordan [203] studied the stability of dairy ice cream mixes in the presence of saturated and unsaturated MAGs. They both found that unsaturated MAGs are more effective in destabilizing ice cream mixes than their saturated counterparts. Furthermore, the emulsions became more stable with an increasing concentration of saturated MAGs [207]. Although Pelan et al. [207] and Goff and Jordan [203] agreed on the divergent effects of the saturated and the unsaturated MAGs, they put forward distinctly different explanations. Pelan et al. [207] ascribed these observed phenomena to the occurrence of Pickering stabilization in the presence of saturated MAGs while Goff and Jordan [203] attributed them to differences in oil-in-water interfacial tension. In the latter study an inverse relationship between the interfacial tension and the fat destabilization was found. Unsaturated MAGs revealed a lower oil-water interfacial tension and a higher degree of fat destabilization during whipping and freezing compared with saturated MAGs.

All of the above-mentioned studies agree on the fact that unsaturated MAGs make oil-in-water emulsions more susceptible towards partial coalescence than saturated ones but clear disagreements exist on the mechanistic explanations. In addition, no elaborate study is available on the partial coalescence during shearing and whipping of recombined dairy whipping creams containing MAGs.

Hence, the research objectives of this chapter are:

(1) To study the effect of different MAGs on both shear-induced partial coalescence in RC and on the whipping properties of RC.
(2) To elucidate the mechanism behind the effect of the MAGs on partial coalescence in RC.

8.2. Research strategy

In Chapter 4, it was shown that MAGs in recombined dairy whipping cream affect both the milk fat crystallization behavior and the oil-water interfacial tension depending on the chain length and saturation degree of the hydrophobic fatty acid (FA) chain. These effects may indicate that MAGs govern the shear-induced partial coalescence in RCs and their whipping properties in distinct ways. Hence, an initial screening of the influence of the three types of MAGs used in Chapter 4 was performed on shear-induced partial coalescence in RCs by means of viscosity analyses. The MAGs with the largest divergent effects on the churning time were selected for further research. Subsequently, the influence on the shear-induced partial coalescence and on the whipping properties was unraveled at different concentrations and protein load analyses and interfacial rheology experiments were carried out to elucidate the mechanism behind the diverging effects of these MAGs. Taking into account also the results on fat crystallization and interfacial tension analyses obtained in Chapter 4, a mechanistic explanation is formulated on how MAGs control partial coalescence and consequently the whipping properties.

Like in Chapter 4, RC was made from anhydrous milk fat (35%) and buttermilk. Within one production set of RCs, a comparative study between the reference RC and the RCs with MAGs was applied to identify the effects of MAGs on the studied variables. This comparative approach excludes potential variation between production sets due to, for instance, different storage and processing conditions of the RCs or slight differences in composition of the starter material.

8.3. Results and discussion

8.3.1. Shear-induced partial coalescence

The three types of MAGs differing in chain length and saturation degree used in Chapter 4 were considered to screen their effect on shear-induced partial coalescence. Table 2.2 gives the FA-composition of the three MAGs: MAG rich in oleic (MAG-O), stearic (MAG-S) and lauric acid (MAG-L). Within one production set for each type of MAG a RC with a concentration of 0.2% MAG and one reference RC without MAGs were produced.

Figure 8.1 shows the viscosity profiles of the RCs at a shear rate ($\dot{\gamma}$) and temperature (T) of 150 s^{-1} and 20℃, respectively. The first viscosity increase (phase II of the viscosity profile) of RCs with MAG-O and MAG-L starts nearly simultaneously and takes place earlier than in RC without MAGs. However, large differences in the duration of phase III (the viscosity decrease) of the viscosity profiles can be detected. For MAG-L the prolonged phase III causes a slightly increased churning time (t_{ch}) compared to the RC without MAGs, 21.1 min and 17.6 min, respectively. Conversely, the cream with 0.2% MAG-O showed a strongly decreased t_{ch} (6.9 min). The RC with MAG-S does not show any viscosity increase during 8h of shearing and, hence, no churning has occurred.

Since, firstly, t_{ch} has been shown to be a valuable tool to qualitatively predict the whipping properties (Chapter 7) and, secondly, MAG-O and MAG-S show the most divergent effect on the t_{ch}, these two MAGs were selected for further research. MAG-L which has a shorter chain length than both MAG-S and MAG-O and differs in saturation degree with MAG-O shows a rather intermediate effect on t_{ch}. This may be related to the fact that MAG-L, in Chapter 4, exhibited also an intermediate behavior between MAG-O and MAG-S on the milk fat crystallization. Moreover, for model creams Davies et al. [154] maintained that the saturation degree has a larger effect on the orthokinetic stability towards partial coalescence than the differences in chain length of the saturated MAGs. The shear-sensitivity towards partial coalescence is slightly increased for MAGs with a shorter saturated FA-chain compared to MAGs with a longer saturated FA-chain while unsaturated MAGs revealed an overall strongly increased destabilization effect compared to saturated MAGs.

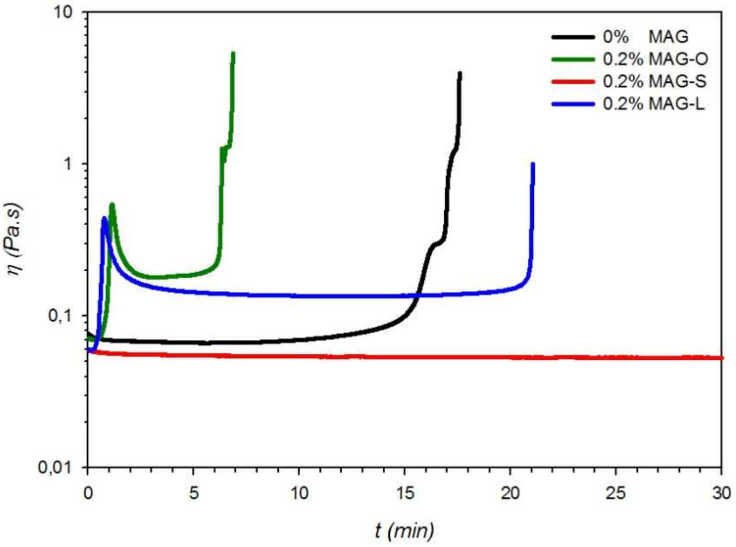


Figure 8.1 Viscosity profile of RCs without and with 0.2% MAG-O, MAG-S and MAG-L ($\dot{\gamma}$ = 150 s^{-1} and *T* = 20℃).

In order to explore the concentration effect of MAG-O and MAG-S two production sets were enclosed in this study. In the first production set variants with 0%, 0.035%, 0.07%, 0.1% and 0.2% MAG-O were produced. The same concentrations of MAG-S were applied in the second production set and, in addition, a variant with 0.5% MAGs was included. The latter concentration could not be used in RCs with MAG-O since the physical shelf life of the RCs with 0.5% MAG-O was shorter than the time required for analyses. Clumping occurred during transport and/or storage of the RCs.

Figure 8.2 A and B show the viscosity profiles of RCs at different concentrations of MAG-O and MAG-S, respectively. For creams with MAG-O, the shear-induced partial coalescence rate is clearly enhanced as a function of its concentration since the complete viscosity profiles and, thus, the t_{ch} appeared earlier with an increasing concentration of MAG-O.

Conversely, with an increasing concentration of MAG-S the viscosity profiles shift in the opposite direction which demonstrates a decrease in partial coalescence rate. Table 8.1 summarizes the t_{ch} obtained after shearing at different T. Regardless of the temperature, the same trend can be observed: MAG-O increases while MAG-S decreases the shear-induced partial coalescence rate.

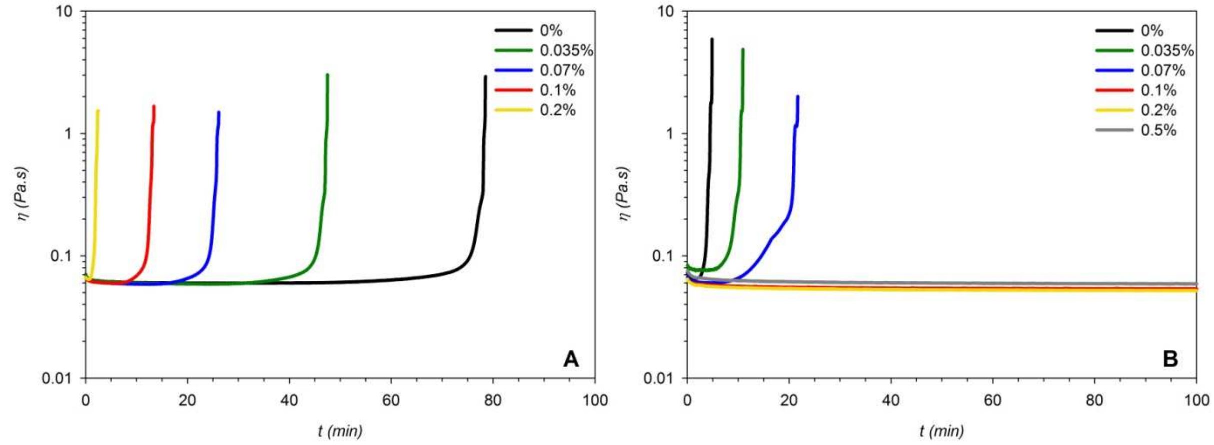


Figure 8.2 Viscosity profiles of RCs at different concentrations of (A) MAG-O and (B) MAG-S ($\dot{\gamma}$ = 150 s^{-1} and T = 20℃).

Table 8.1 The t_{ch} (min) measured by shearing ($\dot{\gamma}$ = 150 s^{-1}) at different T and concentrations of MAG-O and MAG-S.

	[MAG] **(%)**	***T*** **(℃)**		
		15	20	25
MAG-O	0	194.2 ± 0.5^{a}	78.5 ± 0.2^{a}	95.4 ± 6.7^{a}
	0.035	90.4 ± 0.1^{b}	47.9 ± 2.8^{b}	86.2 ± 6.6^{b}
	0.07	30.3 ± 0.1^{c}	26.0 ± 0.8^{c}	51.8 ± 1.7^{c}
	0.1	13.0 ± 0.3^{d}	13.4 ± 0.6^{d}	40 ± 1.0^{d}
	0.2	3.0 ± 0.2^{e}	2.5 ± 0.1^{e}	12.94 ± 0.5^{e}
MAG-S	0	10.5 ± 0.3^{a}	7.9 ± 0.0^{a}	6.9 ± 0.1^{a}
	0.035	439.5 ± 3.4^{b}	11.0 ± 0.1^{b}	8.1 ± 0.4^{b}
	0.07	>8h	23.4 ± 2.5^{c}	16.9 ± 0.6^{c}
	0.1	>8h	>8h	77.6 ± 0.8^{d}
	0.2	>8h	>8h	>8h
	0.5	>8h	>8h	>8h

$^{a-e}$ different letters indicate significant differences ($p < 0.05$) within the same temperature.

8.3.2. Whipping properties

Figure 8.3 depicts the whipping properties of the RCs at different concentrations of MAG-O and Annex IV shows the results of the statistical analyses. Generally, at *[MAG-O]* > 0.035% a significant decreasing trend in whipping time (t_{wh}), overrun and serum loss can be observed, while the firmness shows a clear increasing trend as a function of *[MAG-O]*. Between 0.1% and 0.2% no significant differences can be observed for the overrun, serum loss and firmness. In addition, similar firmnesses are measured after 1h and 24h storage at 5℃ at each concentration.

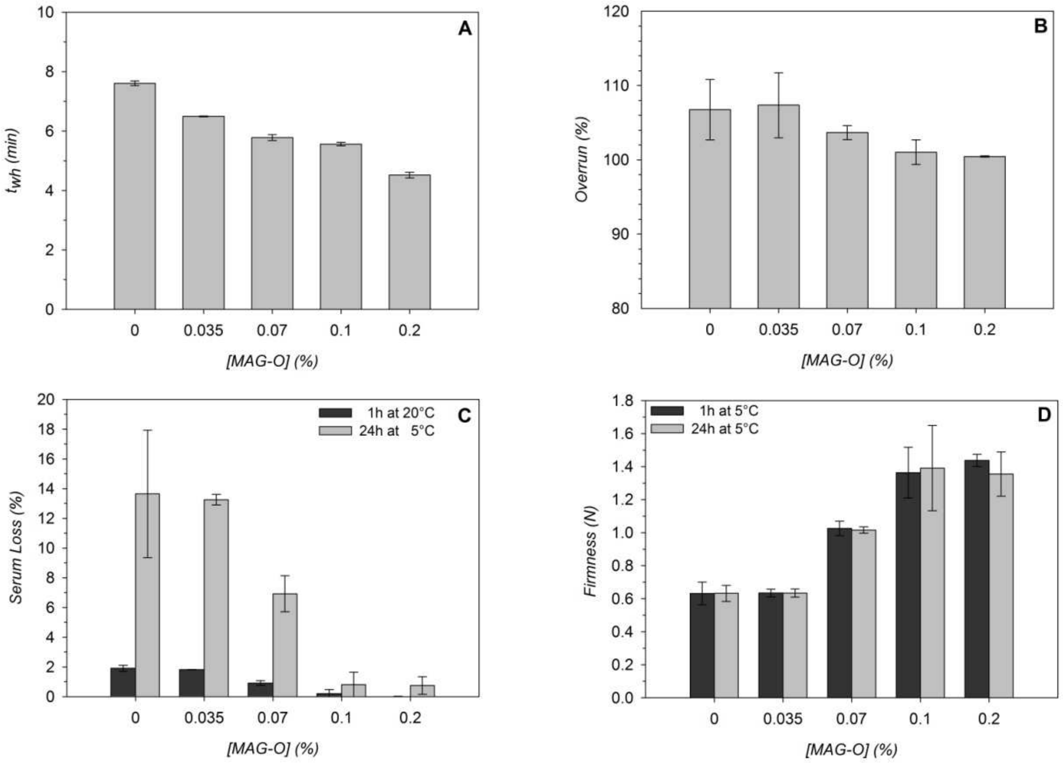


Figure 8.3 Whipping properties of RCs at different concentrations of MAG-O: (A) t_{wh} (min); (B) overrun (%); (C) serum loss (%) after storage for 1h at 20°C and 24h at 5℃ and (D) firmness (N) after 1h and 24h at 5℃.

Figure 8.4 shows the whipping properties of RCs at different concentrations of MAG-S and the statistical results are given in Annex V. Opposed to the effect of MAG-O, the t_{wh}, the overrun and the serum loss show a significant increasing trend while a decreasing trend of the firmness is observed as a function of *[MAG-S]*. Moreover, if 0.07% ≤ *[MAG-S]* ≤ 0.2%, the firmness after 24h storage at 5℃ is further decreased compared with the firmness measured after 1h. This probably indicates a substantial foam destabilization during storage. At 0.5%

MAG-S, the whipping was stopped after 20 min, although no acceptable whipped cream was obtained. This aerated 'liquid' cream reveals, as a consequence, a huge increase in serum loss after 24h at 5℃ and a low firmness just above the trigger value (0.01 N) is measured.

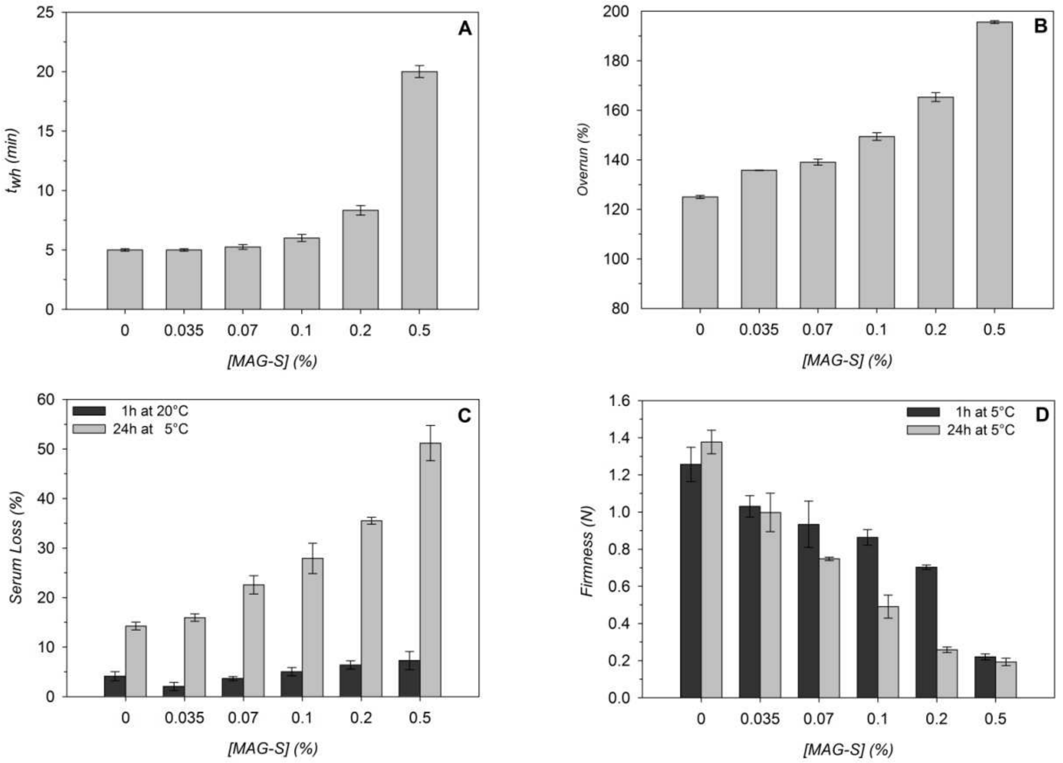
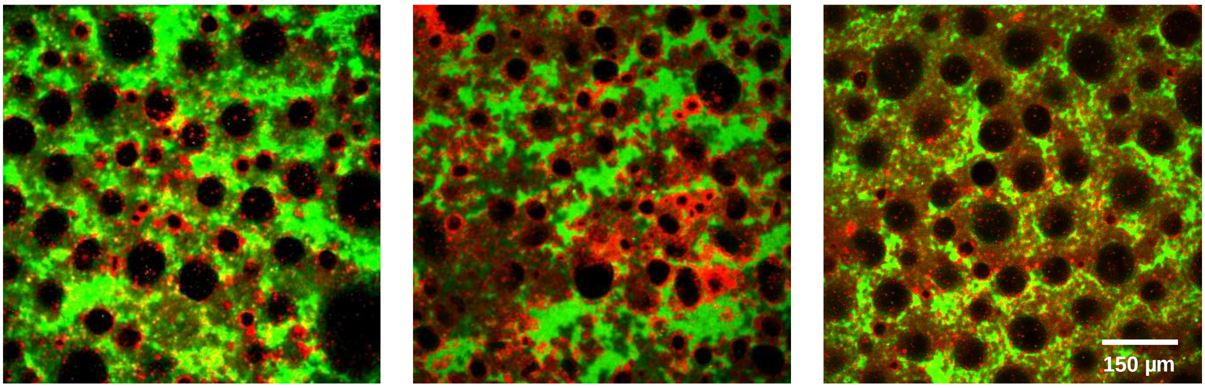


Figure 8.4 Whipping properties of RCs at different concentrations of MAG-S: (A) t_{wh} (min); (B) overrun (%); (C) serum loss (%) after storage for 1h at 20℃ and 24h at 5℃ and (D) firmness (N) after 1h and 24h at 5℃.

Figure 8.5 illustrates representative micrographs of the microstructure of the reference whipped RC and the ones with 0.2% MAG-O and MAG-S recorded with confocal scanning laser light microscopy (CSLM). In the reference RC air bubbles partly covered by fat globules can be seen. In the whipped cream containing MAG-O the air bubble surfaces are covered to a higher degree than in the reference cream. Even big clumps of fat globules can be detected both at the air bubble surfaces and in the serum phase. In the presence of MAG-S only a few small bubbles covered with fat globules can be detected. Moreover, when taking subsequent CSLM-images the fat globules in the serum phase seem to be separately moving (Brownian motion) indicating that they aren't part of a network. Hence, these images clearly show that the degree of partially coalesced fat globules both at the air bubble surface and in the serum phase is increased by using MAG-O while MAG-S decreased it.



0% MAG **0.2% MAG-O** **0.2% MAG-S**

Figure 8.5 CSLM-micrographs of whipped RC without MAGs, with 0.2% MAG-O and MAG-S (length of scale bar = 150 µm). In red the fat phase and in green the protein-rich serum phase.

Like in Chapter 7, the trends demonstrated in the whipping properties correspond well with the ones in t_{ch}, as measured by the viscosity analyses (Section 8.3.1). For MAG-O is the lower t_{ch} related to the observed decreased t_{wh}. Shear-induced and, probably, also surface-mediated partial coalescence rate is enhanced with an increasing concentration of MAG-O. As a consequence, upon whipping partial coalescence may prevail more quickly at an increased MAG-O-concentration which will limit the duration of the different stages of the whipping process. The intended rigid foam will readily be obtained. In particular the decrease of the first stage of the whipping mechanism may be at the basis of the changed whipping properties. In this first stage, air is incorporated and temporarily stabilized by proteins of the continuous aqueous phase and only shear-induced partial coalescence occurs. In the presence of MAG-O, a shorter first stage will, thus, result in a lower overrun and a substantial clump formation in the serum phase. As a consequence of the lower overrun, the partially coalesced fat globules created during the whole whipping process have to stabilize a lower volume of whipped cream compared to the reference RC. This together with the increased degree of partial coalescence causes the formation of a denser network as is confirmed by the CSLM-micrographs. The latter explains, hence, the observed higher stability and firmness of the whipped creams with MAG-O. Alternatively, in the presence of MAG-S is the first stage of the whipping process prolonged compared to the reference RC due to the decrease of shear-induced and surface-mediated partial coalescence. As a result, more air can be incorporated yielding a higher overrun. The higher overrun and the decreased degree of partial coalescence, as observed in the CSLM-micrographs, ultimately result in a decreased stability and firmness. The fat globules have to stabilize a larger volume of whipped cream and show less network formation than in the reference RC.

8.3.3. Physicochemical properties of the recombined creams and of their oil-water interface

The complexity of researching the mechanism behind the effect of MAGs on partial coalescence is that they may influence various physicochemical properties. MAGs, as a small-molecule surfactant, will affect the particle size distribution of the RCs and the oil-water interfacial properties such as the protein load, the interfacial tension and the viscoelasticity of the interfacial membrane. Furthermore, since MAGs are oil-soluble, MAGs may change the milk fat crystallization behavior in the fat globules. The opposite effects of MAG-O and MAG-S may, thus, be related to one of these effects or most likely to a combination of them. Therefore, in order to elucidate the mechanism behind the effect of MAG-O and MAG-S on partial coalescence and on whipping behavior the particle size distributions, the interfacial protein load, tension and rheology and the milk fat crystallization behavior were further investigated.

The particle size distributions can largely be affected by adding small-molecule surfactants due to their ability to decrease the interfacial tension [223, 225-227, 231]. Nevertheless, to exclude the effect of particle size of the milk fat globules on the partial coalescence rate in this study, RCs with a similar particle size distribution were produced by controlling the homogenization pressure. Figure 8.6 A and B show the particle size distribution of the two production sets of RCs at different concentrations of MAG-O and MAG-S, respectively, and confirm the similarities between the milk fat globule sizes of these RCs. This approach avoids masking the effect of MAGs on partial coalescence by a changed particle size distribution.

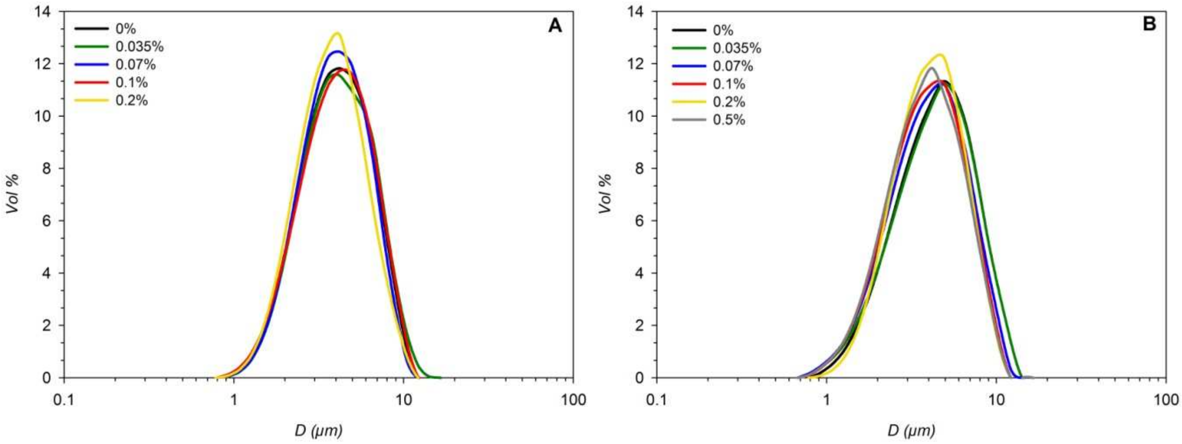


Figure 8.6 Particle size distributions of RCs at different concentrations of (A) MAG-O and (B) MAG-S.

8.3.3.1. Protein displacement

The effect of MAGs on milk protein load or the displacement of milk proteins is discussed by several authors [154, 241, 247, 308]. Barfod et al. [241] maintained that partially unsaturated MAGs displace the proteins to a greater extent than saturated ones in ice-cream mixes, whereas Davies et al. [154] maintained the reverse conclusion in model creams. Heertje [247] and Zhang and Goff [308] postulated that the protein displacement by saturated and unsaturated MAGs depends on the concentration of the MAGs and on the type of the proteins, respectively. Above 0.25% saturated MAGs are more effective and below 0.25% more proteins will be displaced by the unsaturated MAGs [247]. Unsaturated MAGs displace strongly the caseins from the interface while they are less effective in displacing whey proteins from the interface than saturated MAGs [308]. Barfod et al. [241] also maintained that the desorption of proteins into the aqueous phase is higher when the MAGs are crystallized at the interface. Upon reheating readsorption of the proteins can even occur. The temperature at which MAGs crystallize and at which, thus, protein displacement occurs, depends on the saturation degree and the chain-length of the fatty acids and the concentration of the MAGs [157].

Hence, clear disagreement exists between the effect of unsaturated and saturated MAGs on protein displacement. Therefore, in this study the protein load (Γ in mg.m^{-2}) was determined for the whipping creams considered. The method consists of a set of chemical analyses (protein and fat content) on the RCs and, on the creamed top layer obtained after centrifugation of the RCs. Subsequently, taking into account the Sauter diameter of the cream as measured by means of static laser light diffraction (see Section 2.2.3), the protein load can be calculated by equation 6.1. A more detailed description of the method can be found in Section 6.2.6.

The protein load analyses were performed only at the highest applied concentration of MAG-O and MAG-S and the results are given in Table 8.2. The protein load of the reference RC amounts to 13.53 ± 1.40 mg.m^{-2}. According to Melsen [182] the protein load varies depending on the ratios of serum proteins to the intact casein micelles and to the casein sub-micelles located at the interface. The protein load of an interface fully covered with serum proteins, intact casein micelles and casein sub-micelles amounts to 2-3 mg.m^{-2}, 40 mg.m^{-2} and 5 mg.m^{-2}, respectively. In addition, protein adsorption can be governed by the heat treatment of the protein dispersion, homogenization temperature, protein:fat ratio, fat globule size and the presence of small-molecule surfactants [309]. In this study, the milk fat globule membrane of the reference RCs will probably consist of a mixture of serum proteins, native milk fat globule proteins, intact casein micelles, sub-micelles and phospholipids. The obtained values are within the ranges suggested by Melsen [182]. Furthermore, in Table 8.2

the protein load reveals to be unchanged at the highest applied concentration of MAG-O compared to the reference RC while a decreasing trend is observed with an increasing concentration of MAG-S. MAG-S are, thus, present at the interface and capable of displacing proteins at the oil-water interface. MAG-O does not displace proteins but this does not necessarily imply the absence of MAG-O at the interface.

Table 8.2 Protein load Γ(mg.m^{-2}) at the oil-water interface of RCs with different types and concentrations of MAG.

[MAG]	**Γ (mg.m^{-2})**
0% MAG	13.53 ± 1.40^{a}
0.2% MAG-O	13.62 ± 1.02^{a}
0.2% MAG-S	10.00 ± 1.61^{b}
0.5% MAG-S	6.74 ± 0.11^{c}

$^{a-c}$ different letters indicate significant differences ($p<0.05$).

8.3.3.2. Interfacial tension

In Chapter 4 interfacial tension analyses were performed with a drop tensiometer to investigate the presence and the physical state of MAGs at the oil-water interface. The obtained results in Chapter 4 are summarized in Figure 8.7 for oil-water interfaces created between buttermilk and an oil phase containing 0.6% MAG-S or 0.6% MAG-O with respect to the reference oil-water interface without MAGs. The 0.6% oil-based concentration of the MAGs corresponds to a cream-based concentration of 0.2%. The latter is the highest possible concentration to obtain a perikinetically stable RC with either MAG-O or MAG-S.

In the presence of MAG-O or MAG-S the oil-water interfacial tension (γ_{OW}) decreases compared to the reference oil-water interface. The latter is only constituted of the sweet cream buttermilk powder (SCBMP) components (proteins and phospholipids). The lowered γ_{OW} suggests that MAGs are (at least partly) positioned at the oil-water interface. Along with their adsorption capacity on the oil-water interface, the MAGs are, thus, believed to be able to go in competition with the adsorption of the proteins and, therefore, displace the proteins. However, in the previous section, RCs containing MAG-O showed no protein displacement. Probably, the MAG-O occupy small holes or defects in the interfacial protein network where the larger proteins cannot adsorb [236]. The MAG-O may thereby deform the protein network without disintegrating it. Alternatively, MAG-O may associate with proteins through hydrophobic interactions at the interface [310-312]. Note that at a higher MAG-O content they may eventually displace the proteins.

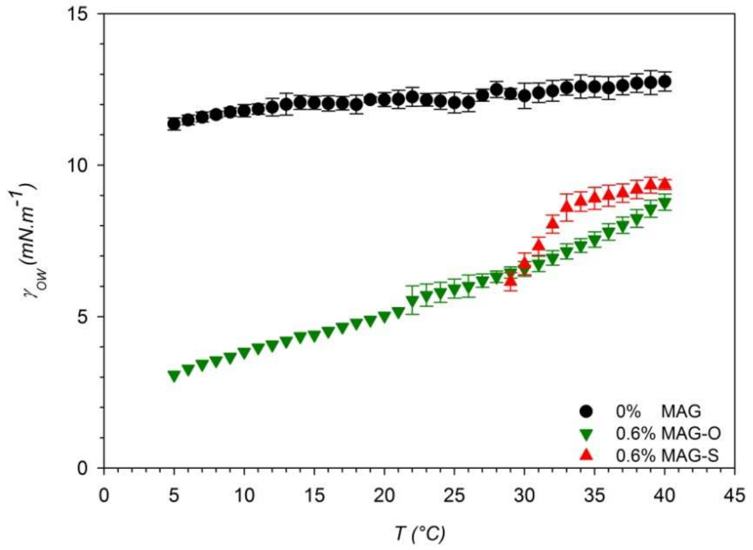


Figure 8.7 Interfacial tension γ_{OW} (mN.m^{-1}) between buttermilk and purified sunflower oil during cooling without MAGs (●), with 0.6% MAG-O (▼) and with 0.6% MAG-S (▲) (oil-based concentrations).

Moreover, upon cooling chain crystallization of MAG-S can be observed (Figure 8.7) as can be seen by the sharp decrease in γ_{OW} at 33℃ while this discontinuity in the slope of the γ_{OW} -curves is not detected with MAG-O. MAG-O stayed in the liquid state independent of the temperature. Regardless of the concentration, chain crystallization of MAG-S was observed in Chapter 4 but the chain crystallization temperature ($T_{cr,chain}$) decreased by reducing the concentration of MAG-S. Krog and Larson [157] suggested that protein displacement occurs when chain crystallization of the MAGs takes place. This is in agreement with the current results. MAG-S exhibits chain crystallization and protein displacement while MAG-O does not.

8.3.3.3. Interfacial viscoelasticity

Neither the protein load analyses (Section 8.3.3.1) nor the interfacial tension analyses (Section 8.3.3.2) provide knowledge on the viscoelasticity of the oil-water interfacial membrane. The interfacial viscoelasticity may be affected by both MAGs because of their presence at the oil-water interface, as has been demonstrated by oil-water interfacial tension measurements. Moreover, the crystallized state of the MAG-S compared to the liquid behavior of the MAG-O at the interface would probably imply a distinct viscoelastic behavior.

In literature, many interfacial rheological studies in the presence of both proteins and small-molecule surfactants are available and summarized by Murray and Dickinson [313] and Bos and van Vliet [314]. Most of these studies focus on air-water [315-319] or hydrocarbon-water [315, 317] interfaces, although significant differences in adsorption behavior on triacylglycerol

(TAG) - water interfaces can be observed [314, 315]. This is in particular the case when oil-soluble small-molecule surfactants are considered. The solubility of these surfactants in the oil phase strongly defines the interfacial adsorption. Interfacial rheological studies that address the effect of MAGs at TAG-water interface are limited [312]. Moreover, studies in the presence of a complex milk powder like SCBMP were not performed. The effect of MAGs is mainly analyzed in the presence of pure caseins [318], caseinates [316], whey protein isolates [319] and skimmed milk proteins [317]. When using SCBMP, the interface will consist of a mixture of serum proteins, caseins, milk fat globule proteins and phospholipids.

Two types of interfacial rheological techniques are mainly applied: *interfacial dilatational rheology* and *interfacial shear rheology*. Both these techniques are applied for studying the 'true' coalescence stability because both shear and dilatational deformations take place during coalescence [314]. Although there is still uncertainty about the specific implications, it is well-established that shear deformation takes place during drainage of the continuous phase between two approaching droplets (film formation) and that a dilatational deformation will occur during the film rupture. However, regarding partial coalescence the film rupture is supposed to be initiated by crystals piercing through the film and the oil-water interface which indicates that shear interfacial measurements may be more appropriate than interfacial dilatational measurements to link interfacial rheological properties to partial coalescence.

As for the interfacial tension measurements, it was required to deviate from the real emulsion system. The interfacial rheology measurements were performed at an interface between a bulk water and oil phase. In addition, because of the intrinsic difficulty of interfacial rheological experiments to make distinction between the rheological properties of the interface and the upper bulk phase, the oil phase cannot contain a fat crystal network. The presence of a fat network in the oil phase would mask the contributions of the oil-water interface to the elastic modulus (*G'*). Therefore, similar to the interfacial tension measurements, purified sunflower oil with and without MAGs and reconstituted SCBMP was used for the interfacial shear measurements. Figure 8.8 shows the interfacial elastic modulus (*G'*) and the interfacial viscous modulus (*G"*) of the reference interface as a function of the applied strain (amplitude sweep) for a fixed frequency of 0.1 rad.s^{-1}. A dominant viscous response can be observed. However, this originates mainly from the viscous upper oil phase. The geometry is also in contact with the bulk oil phase and the viscosity of the oil phase has a considerable value of 62,72 ± 0.39 mPa.s at 20℃ (i.e. viscosity measured by a conventional rheological method). This makes a reliable determination of the interfacial shear viscosity impossible. Hence, the *G"* values are merely indicative. Nevertheless, since vegetable oil exhibits a purely Newtonian viscous behavior, the upper oil layer will not contribute to *G'*. The measured *G'*-values are, therefore, unequivocally related to the interface. Figure 8.8 shows that *G'* stays unaffected if strains between 0.1 and 2% are applied. This region is called the linear viscoelastic region (LVR). The stress required to reach the applied strain is linearly related to the strain. At higher strains, the *G'* decreases indicating a structure break-down. In order to further characterize the interfacial rheological behavior, frequency sweeps were performed at a strain within the LVR. A strain of 1% appeared appropriate after performing the strain sweeps in the presence of 0.6% MAG-O and MAG-S in the oil phase (data not shown). These oil-based concentrations correspond with a cream-based concentration of 0.2% which was the highest possible concentration to obtain a perikinetically stable RC with either MAG-O or MAG-S.

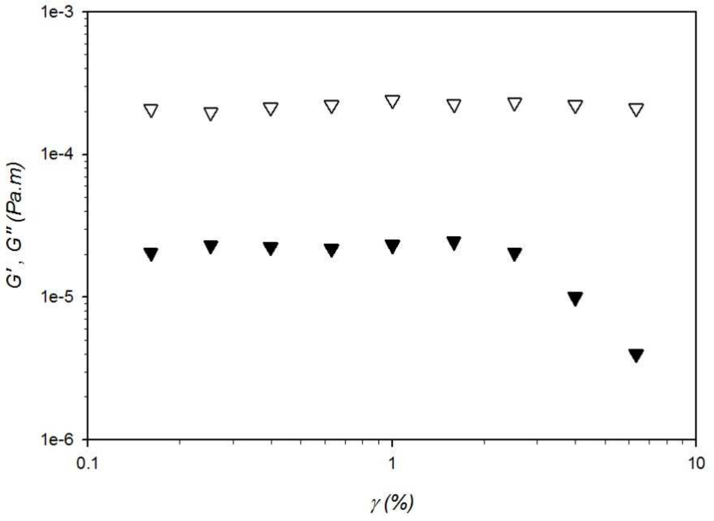


Figure 8.8 Interfacial elastic *G'* (▼) and viscous *G''* (▽) modulus (Pa.m) as a function of strain γ (%) of the interface created between buttermilk and purified sunflower oil.

In Figure 8.9, the elastic modulus (*G'*) as a function of the oscillatory frequency are plotted for an oil-water interface created in the presence an oil phase without MAGs, with 0.6% MAG-O or with 0.6% MAG-S. The clear plateaus of *G'* independent of the frequency indicate a pure elastic network at the interface. Without MAGs, the development of this elastic network can be ascribed to intermolecular covalent and non-covalent interactions of the proteins. In the presence of MAGs the *G'*-value is affected. The absolute value of *G'* is a measure for the strength of the network. A weaker interfacial elastic network is created in the presence of MAG-O (lower *G'*), while a stronger network is created in the presence of MAG-S (higher *G'*). In general, small-molecule surfactants disturb the protein-protein interactions at the interface implying a decrease in *G'* [313, 320]. This explains the effect of MAG-O on G'. However, MAG-S depicts the opposite effect. As observed in Figure 8.7, by interfacial tension analyses, at $T < 33$℃ MAG-O and MAG-S show differences in its physical state: MAG-O behaves as a liquid while MAG-S as a solid. Hence, the crystalline state of the MAG-

S contributes to the elastic behavior, even though they disorder the intermolecular protein interactions. The observed discrepancy between the elastic behavior of saturated and unsaturated MAGs is in agreement with studies of Golding and Sein [316] and Sánchez and Patino [106, 318, 319] who performed interfacial rheological experiments at the air-water interface.

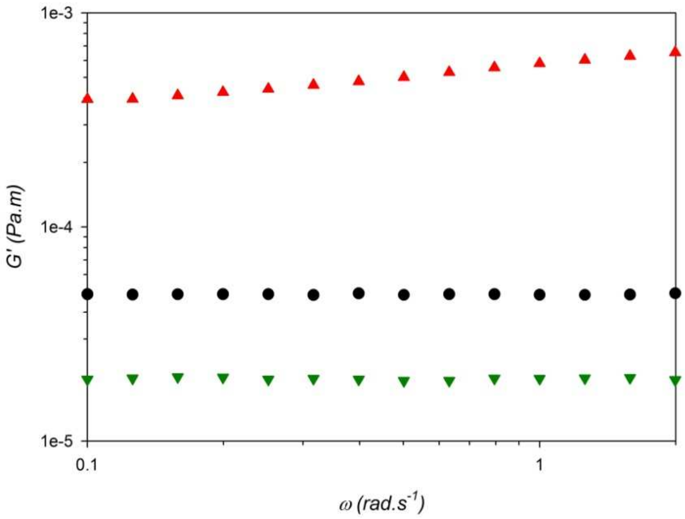


Figure 8.9 Interfacial elastic modulus *G'* (Pa.m) at different frequencies ω (rad.s^{-1}) of an interface created between buttermilk and purified sunflower oil without MAGs (●), with 0.6 % MAG-O (▼) or with 0.6% MAG-S (▲) (oil-based concentrations).

8.3.3.4. Fat crystallization

MAGs may affect the fat crystallization behavior (nucleation, crystal growth rate and polymorphic evolutions) and, therefore, the final microstructure of the milk fat globule (size, morphology and polymorphism of the crystals, the arrangement of the crystals in the globule and the fat crystal network). In Chapter 4 the primary crystallization behavior and the solid fat content (SFC) during storage of milk fat was discussed at processing conditions closely related to the ones applied during the production of whipping cream (fast cooling to 5℃).

MAG-O did not show significant effects on the primary crystallization behavior and SFC-content. A similar crystallization mechanism compared to the reference RC was observed. Nucleation of α-crystals occurs in the droplet (volume heterogeneous/homogenous nucleation) during cooling. Subsequently, the growing α-crystals partly transform into β'-crystals while still some α-crystals persist. However, the latter have a different composition than the initially created α-crystals during cooling.

A difference in nucleation mechanism is detected in cream containing MAG-S. Interfacial heterogeneous nucleation takes place during cooling. As a consequence, the crystallization starts at a higher temperature and the crystal growth and the α-β' polymorphic transition is accelerated compared to the reference RC and the RCs with MAG-O. However, the mechanism after nucleation has occurred is similar to the reference RC and the SFC after prolonged storage at 5℃ was unaffected. The difference in nucleation mechanism and crystallization kinetics may result in a different number, size, arrangement and morphology of the crystals in the fat globule. Although several attempts have been taken to visualize the milk crystals, the effect on the final crystals cannot be demonstrated.

8.3.4. Discussion

The acquired divergent effects between MAG-O and MAG-S on the interfacial protein displacement, tension and viscoelasticity and on the milk fat crystallization behavior can mainly be ascribed to whether or not chain crystallization of the MAGs has occurred. As a consequence, fat globules in the presence of MAG-O and MAG-S will develop a different microstructure at temperatures below the chain crystallization temperature ($T_{cr,chain}$). How these distinctive microstructures may develop upon cooling is schematically presented in Figure 8.10.

Regardless of the type of MAG, at temperatures well above the $T_{cr,chain}$ of MAG-S the interface may consist of a mixture of liquid MAGs and proteins, according to the interfacial tension measurements. The interfacial protein load will then probably be similar to the one obtained in the absence of MAGs. However, compared with the reference interface, proteins may have a different conformation and a lower degree of protein-protein interaction [240].

Upon cooling below the $T_{cr,chain}$ of MAG-S distinction in the nucleation mechanism of milk fat in the globule can be made between the MAGs, as was concluded in Chapter 4. In the presence of MAG-O the milk fat crystallization will start inside the droplet (primary volume heterogeneous/homogeneous nucleation) similar to the reference cream, whereas in the presence of MAG-S the crystallization will start near the interface (primary interfacial heterogeneous nucleation). The increased nucleation rate in the RCs with MAG-S was demonstrated by the increased crystallization temperature observed upon cooling as a function of the concentration of MAG-S (Table 4.3) and the interfacial tension measurement revealed that this heterogeneous nucleation took place at the interface (Figure 8.7). MAG-S crystallize in 2D-crystals which serve as a template for milk fat crystallization upon further cooling. The lamella planes of the created milk fat crystals will thereby probably be parallel oriented to the oil-water interface, as was recently observed by Arima et al. [109] in model oil-in-water emulsions. The increased nucleation rate may, furthermore, imply a higher number of nucleation sites and, hence, more primary crystals within one droplet are shaped at the interface. Besides heterogeneous nucleation, the chain crystallization of MAG-S causes protein displacement from the interface, as observed by the protein load analyses (Table 8.2).

After primary nucleation has been established during cooling, the crystallization, apart from the type of MAG, will readily spread throughout the whole volume via secondary nucleation. The latter is favored in milk fat because of their complex TAG-composition [32]. Subsequently, α-β' polymorphic transitions occur and crystals proceed to grow until an apparent equilibrium is reached (Chapter 4). However, due to differences in location of the initially created crystals the spreading behavior throughout the fat globule volume will be different. In the presence of MAG-O or in absence of MAGs the primary crystals are located inside the globule, whereas in the presence of MAG-S the crystals are positioned at the interface. The crystallization will spread from the inside of the globule to the outer layers if nucleation occurred inside the droplet. As crystallization proceeds, the larger growing crystals may start to pierce through the membrane. The latter is, especially, enhanced by a pronounced shrinkage of the fat globules with increasing solid fat content [33, 130]. Moreover, in the presence of MAG-O the piercing of crystals through the globule membrane may be more enhanced due to the reduced elasticity of the fat globule membrane, as shown with the interfacial rheological measurements. The membrane containing MAG-O will more readily be deformed and ruptured when fat crystals are growing near the interface, which is in agreement with Thivilliers et al. [198]. They observed longer protrusion distances of milk fat crystals in the presence of polysorbates at the interface which were postulated to weaken the interfacial protein-protein interactions. In addition, since MAG-O is still liquid, they may adsorb onto the crystal surface causing a change in contact angle [175]. The latter may increase the protrusion distance of the fat crystals in the aqueous phase. Conversely, in fat globules containing MAG-S, the spreading of the fat crystallization will occur in the opposite direction from the outer semi-crystalline layer/shell to the inside of the globules. The interfacial 2D MAG-S and the parallel oriented milk fat crystals will prevent, thereby, the piercing of the fat crystals through the membrane. In addition, it is reasonable to assume that the increased nucleation rate in the presence of MAG-S will cause more and smaller milk fat crystals as was also suggested by Basso et al. [96], Fredrick et al. [71] and Sakamoto et al. [67] in bulk fat systems and by Arima et al. [107] in model oil-in-water emulsions for MAGs or other small-molecule surfactants.

The eventual distinct microstructure of the fat globule dependent on the type of MAG (Figure 8.10) are at the basis of the detected differences in partial coalescence rate and, hence, in whipping behavior of the RCs. In the presence of MAG-O, the partial coalescence rate will be enhanced compared to the reference RC by (1) a higher degree and longer protrusion distances of the protruding crystals and/or (2) the weaker interfacial protein network in between the protruding crystals. The latter involves that the interfacial membranes will be more readily deformed during the film formation between two approaching globules and during their eventual merging. The enhancement of a film formation between approaching globules will increase the film area and, thus, the probability that a protruding crystal will be located in this film causing the ultimate film rupture. Moreover, during whipping, the weaker interfacial network will facilitate rupture of the membrane of the fat globules during entering at the air bubble surface. This enhances the surface spreading of fat globules and, as a consequence, also the surface-mediated partial coalescence at the air bubble surface.

In fat globules containing MAG-S the partial coalescence rate is decreased due to (1) the hindering of the piercing of fat crystals through the milk fat globule membrane, (2) the formation of smaller crystals, (3) the decreased deformability (higher elasticity) of the interfacial membrane and/or (4) the reduced probability that protruding crystals of one globule will pierce the membrane of a second globule in a ‘liquid’ region, where no crystalline MAGs are present.

It can, thus, be concluded that the occurrence of chain crystallization and subsequent interfacial heterogeneous nucleation seems to play a decisive role in the stability of RCs towards partial coalescence. This is in agreement with the findings of Arima et al. [107] in palm mid fraction (PMF) oil-in-water emulsions. They observed that hydrophobic sucrose oligoesters of palmitic acid increased the stability of the emulsions by inducing heterogeneous nucleation while the more hydrophilic sucrose monoesters did not due to the lacking of their ability to tightly pack at the interface. Moreover, it is postulated, because of the increased nucleation rate of the hydrophobic sucrose oligoesters that smaller PMF-crystals are initially created and that morphological changes of the PMF-crystals into long needle-like crystals during polymorphic transformations are prohibited. Besides, also Golemanov et al. [191] related interfacial nucleation in the presence of polysorbate surfactants to an increased stability of paraffin-in-water emulsions.

Although the consequences of protein displacement, like changes in repulsive interactions and thickness and viscoelasticity of the interfacial membrane, are often related to a decrease in stability towards partial coalescence, it revealed not to be the answer for a mechanistic clarification of the opposite effects of the MAG-O and MAG-S in RCs. This in agreement with the findings of both Pelan et al. [207] and Davies et al. [154, 189] who studied shear-induced partial coalescence in ice cream mixes and model creams, respectively. However, their proposed mechanistic explanation differs from the one suggested above. Pelan et al. [207] ascribed it to the occurrence of Pickering stabilization in the presence of saturated MAGs, while Goff and Jordan [203] attributed it to differences in oil-in-water interfacial tension.

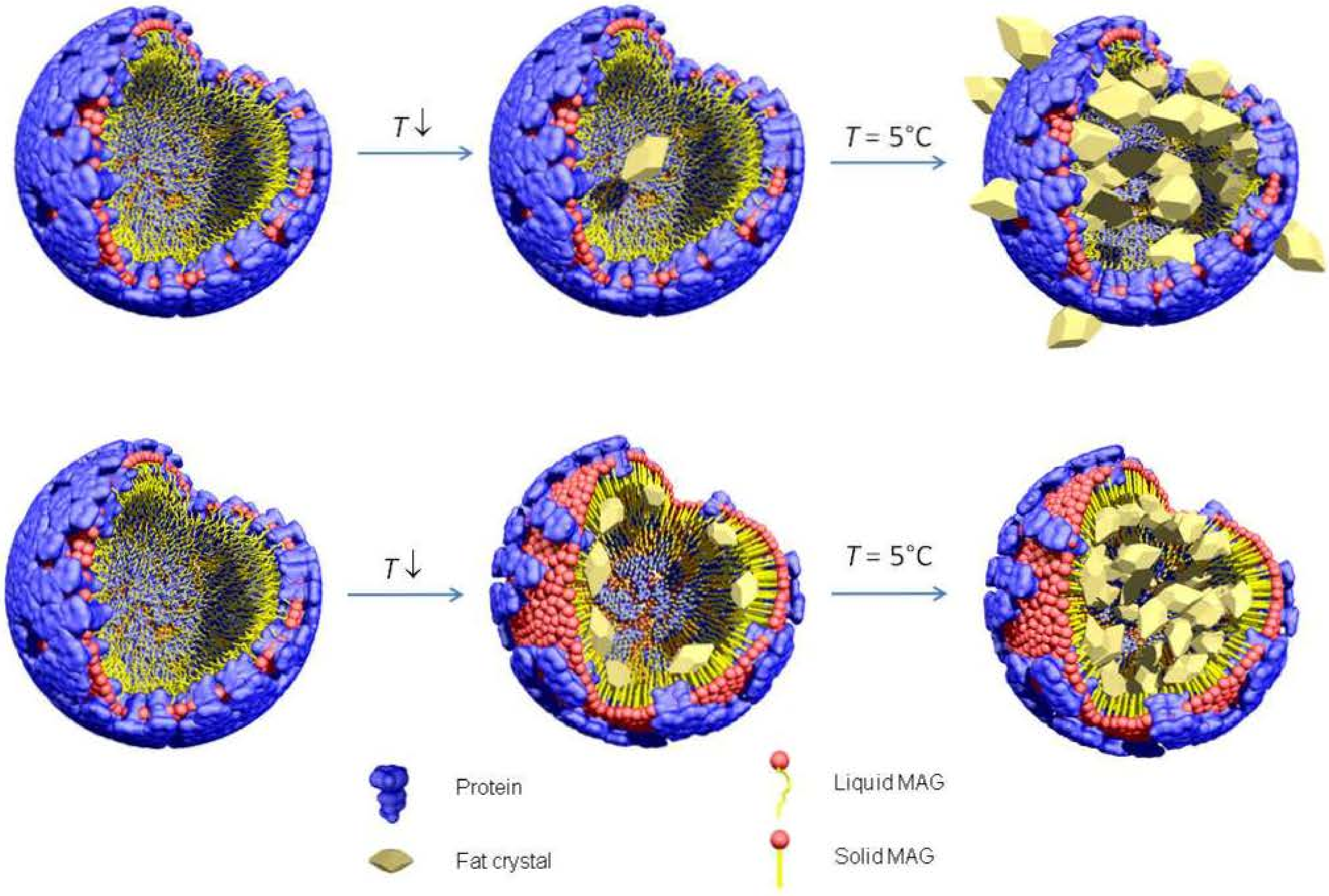

T↓
T = 5°C
T↓
T = 5°C
Protein
Fat crystal
Liquid MAG
Solid MAG

Figure 8.10 Schematic presentation of the development of the microstructure of the fat globules during cooling to 5℃ in RCs with MAG -O (at the top) and with MAG-S (at the bottom).

8.4. Concluding remarks

In this chapter the effects of two long-chain MAGs which differ in degree of saturation on shear-induced partial coalescence in RC and on the whipping behavior of RC were unraveled. The unsaturated MAGs (MAG-O) showed an increased partial coalescence rate in RC resulting in a lower t_{wh} and overrun and higher stability and firmness of the whipped RCs. The saturated MAGs (MAG-S) demonstrated the exact opposite effects. The obtained decrease in partial coalescence rate provoked a higher t_{wh} and overrun and a lower stability and firmness of the whipped RCs.

The divergent behavior of the MAGs on the susceptibility of fat globules towards partial coalescence was ascribed to the different physical state of the MAGs at the oil-water interface. If the MAGs are able to crystallize at the oil-water interface and induce thereby heterogeneous interfacial nucleation of the milk fat during cream production, the protrusion of the obtained smaller crystals is limited resulting in a decreased partial coalescence. MAGs that behave as a liquid at the oil-water interface enhance the deformability of the interface and presumably the protrusion of the fat crystals through the oil-water interface causing an increased partial coalescence rate. In this study, the long-chain saturated MAGs behaved as a solid while its unsaturated counterpart appeared to be in the liquid state at the oil-water interface.

In summary, this chapter clearly showed that MAGs can be applied to govern the whipping properties of dairy RC. Accordingly, the question rises if the MAGs considered in this study hold potentials to improve whipping properties. In Chapter 9 two cases aiming at improving whipping properties of RCs will be discussed.

9. POTENTIALS OF MONOACYLGLYCEROLS TO IMPROVE WHIPPING PROPERTIES OF RECOMBINED CREAM

9.1. Introduction

Whipped cream properties can be improved by either shortening the whipping time (t_{wh}) or increasing the overrun or the stability. The challenge is thereby to improve as many of these properties without sacrificing others.

In Chapter 8 it was found that monoacylglycerols rich in oleic (MAG-O) and rich in stearic (MAG-S) acid show clear reverse effects on the partial coalescence rate and, hence, on the whipping properties of recombined cream (RC). MAG-O in RC increases partial coalescence rate resulting in a lower t_{wh}, overrun and higher stability, while the saturated counterpart (i.e. MAG-S) demonstrates exactly the opposite effects. The decrease in partial coalescence rate in RCs with MAG-S provokes a higher t_{wh} and overrun and a lower stability in the whipped creams.

Accordingly, aiming at improving the whipping properties, MAG-O seems to be useful to shorten the t_{wh} and to increase the stability, whereas MAG-S is helpful to increase the overrun. However, when using these MAGs separately, the improved whipping properties are achieved at the expense of an unacceptable decreased overrun in RC with MAG-O and an increased t_{wh} and objectionable decreased stability in RC with MAG-S. In order to minimize these unfavorable effects of MAG-O and MAG-S, it may be beneficial to combine both the MAGs in one RC.

Besides, MAGs can perhaps be used to improve the whipping properties of creams with a reduced fat content. In Chapter 7 it was found that the t_{wh} and the overrun increases, while the stability adversely decreases as the fat content is reduced. Moreover, it was suggested that a more stable whipped cream with a reduced fat content may probably be achieved when the partial coalescence rate is increased. As a consequence, the t_{wh} and the overrun may be lowered (ideally between 100% and 120%) causing an improved air bubble stabilization and partially coalesced fat globule network in the serum phase and, hence, an increased stability. MAG-O is qualified as a surfactant that increases partial coalescence rate and, hence, reduces t_{wh} and overrun and increases stability. Therefore, MAG-O may be put forward to achieve a considerable fat reduction in whipped cream. MAG-S is not appropriate to be used for fat reduction, since the decreased partial coalescence rate increases further the t_{wh} and the overrun resulting in an additional decrease of the stability.

9.2. Research hypotheses

In this chapter, based on the findings in Chapter 7 and Chapter 8, the following research hypotheses are further explored:

(1) A binary mixture of MAG-O and MAG-S in one RC gives improved whipping properties compared to RCs in which these MAGs are used separately.
(2) MAG-O contributes to improved whipping properties of RCs with a reduced fat content.

9.3. Results and discussions

9.3.1. Binary mixture of monoacylglycerols

In order to verify whether a binary mixture of MAG-O and MAG-S gives improved whipping properties compared to using MAG-O and MAG-S separately in RC, the effect of a 50:50 mixture of MAG-O and MAG-S on the shear-induced partial coalescence rate and on the whipping properties is studied. In addition, in this section the physicochemical properties of the RCs containing both MAG-O and MAG-S and of their oil-water interface are determined to get more insight on how the mixture of MAGs behaves.

In this chapter, the 50:50 binary mixture of MAG-O and MAG-S is further encoded as MAG-OS. A comparative study within one production set is performed between the reference RC without MAGs and RCs with 0.2% MAG-O, 0.2% MAG-S and 0.2% MAG-OS.

9.3.1.1. Shear-induced partial coalescence

Viscosity analyses were performed until the RCs were completely churned into butter granules and buttermilk and

Table 9.1 depicts the obtained churning times (t_{ch}). In the presence of MAG-S and MAG-OS the t_{ch} is increased and, hence, the shear-induced partial coalescence is retarded compared to the reference RC while MAG-O enhances the shear-induced partial coalescence rate. In RCs containing MAG-OS, the effect of MAG-S seems, thus, to prevail towards shear-induced partial coalescence. However, the MAG-O in the RC with MAG-OS appears to counteract the effect of MAG-S. In Chapter 8 it was observed that a RC with only 0.1% MAG-S did not show churning upon 8h of shearing (Section 8.3.1). In RC with MAG-OS (i.e. 0.1% MAG-O and 0.1% MAG-S) churning is already achieved after 21.9 min of shearing.

Table 9.1 The t_{ch} (min) measured after shearing of RCs with 0% MAG, 0.2% MAG-O, 0.2% MAG-S or 0.2% MAG-OS ($\dot{\gamma}$ = 150 s^{-1} at T = 20℃).

[MAG]	**t_{ch} (min)**
0% MAG	8.7 ± 0.5^a
0.2% MAG-O	1.4 ± 1.02^b
0.2% MAG-S	>$8h^c$
0.2% MAG-OS	21.9 ± 0.11^d

[a-d] different letters indicate significant differences ($p < 0.05$).

9.3.1.2. ***Whipping properties***

Figure 9.1 shows the whipping properties of RCs without MAGs and with 0.2% MAG-O, MAG-S and MAG-OS and the statistical results are summarized in Annex VI. Like in Chapter 8, MAG-O and MAG-S show opposite effects on t_{wh}, overrun, stability and firmness. Considering the effect of MAG-OS, the t_{wh} of RC with MAG-OS is increased, as can be qualitatively predicted by the increase in t_{ch}, relatively to the reference RC (Section 9.3.1.1). As a result of both the hindered shear-induced partial coalescence, and, presumably, the delayed surface-mediated partial coalescence, the duration of the different stages of the whipping process may be prolonged. In the first step of the whipping process more air will be included in RC with MAG-OS, as is confirmed by the increased overrun in Figure 9.1 B. The increased volume of the whipped cream with MAG-OS and the presumable lower degree of partial coalescence both at the air bubble surface and in the serum phase may affect the firmness and the stability of the whipped cream. Indeed, in the presence of MAG-OS the serum loss after 24h at 5℃ is slightly increased and the firmness is slightly decreased compared to the reference RC. However, the serum loss after 1h at 20℃ is not significantly different with the reference cream.

Evaluating the whipping properties of RC with MAG-OS with respect to the RCs containing either MAG-O or MAG-S, it can be concluded that in general RC with MAG-OS reveals similar, though less pronounced, effects as RC with only MAG-S. MAG-S plays, thus, a dominant role during whipping in RCs containing an equal amount of MAG-O and MAG-S. However, it should be stressed that MAG-O in RC with MAG-OS appears to impede the effect of MAG-S which gains improved whipping properties compared to using only MAG-S in RC. For instance, the stability of RC with MAG-OS is not significantly affected after 1h at 20℃ and only slightly after 24h at 5℃, while in Chapter 8 (Figure 8.4) at a concentration of 0.1% MAG-S the stability of the RC is decreased to a large extent. The latter is the concentration of MAG-S present in the mixture considered in this chapter.

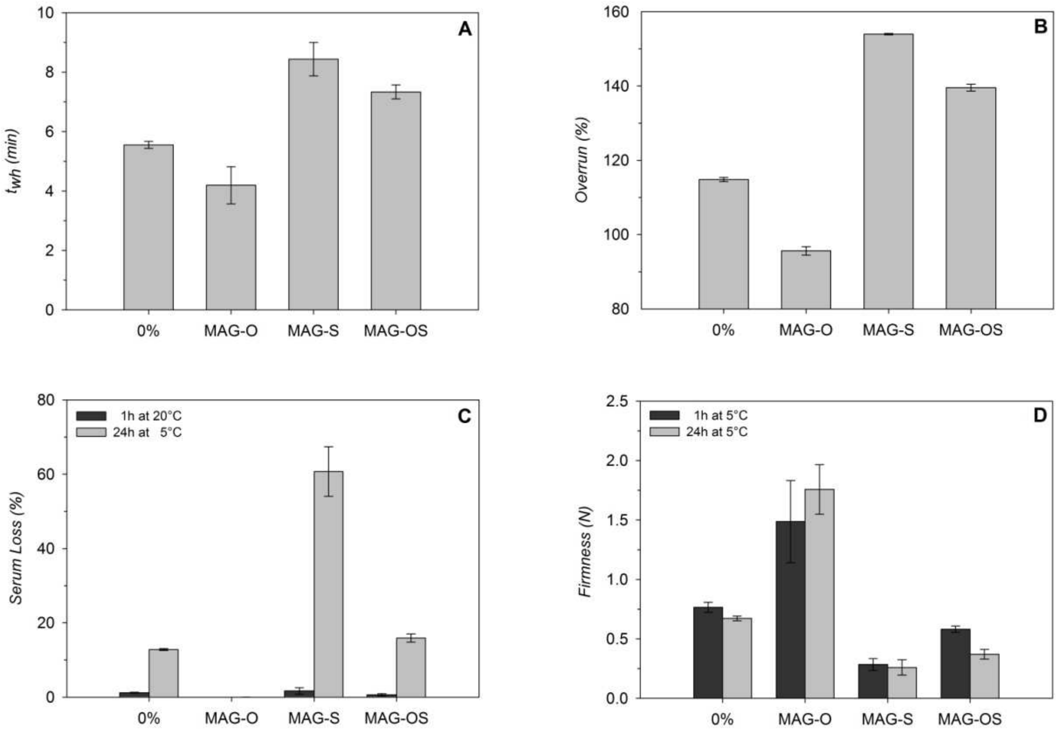


Figure 9.1 Whipping properties of RCs without MAGs, with 0.2% MAG-O, with 0.2% MAG-S or with 0.2% MAG-OS: (A) t_{wh} (min); (B) *overrun* (%); (C) *serum loss* (%) after storage for 1h at 20℃ and 24h at 5℃ and (D) *firmness* (N) after 1h and 24h at 5℃.

9.3.1.3. Physicochemical properties of the recombined creams and of their oil-water interface

In Chapter 8 the effect of MAGs on partial coalescence was linked to their ability to modify the oil-water interfacial properties and the fat crystallization behavior. In order to check how the 50:50 mixture of MAG-O and MAG-S affects these properties, the decisive effects (i.e chain crystallization, viscoelasticity of the interfacial membrane and interfacial heterogeneous nucleation) defining the final microstructure of the fat globules are addressed in this section.

A. Interfacial tension

Figure 9.2 shows the oil-water interfacial tension (γ_{OW}) curve measured during cooling of an interface created between buttermilk and purified sunflower oil with 0.6% MAG-OS with respect to γ_{OW} - curves of the interfaces created with an oil phase without MAGs, with 0.6% MAG-S or with 0.6% MAG-O. The latter were already discussed in Chapter 4 and summarized in Chapter 8. The 0.6% MAG-concentration in the oil phase corresponds with 0.2% MAGs calculated on emulsion basis. In the presence of MAGs, γ_{OW} is lower at all temperatures which shows that MAG-molecules are present at the oil-water interface. The comparable γ_{OW} at, for instance, T = 40℃ show that roughly the same amount of MAGs will be present at the interface regardless of whether the MAGs are mixed or not. In Chapter 4 (Figure 4.4) at lower MAG-concentrations substantial higher γ_{OW} were obtained.

Like in the presence of only MAG-S, a discontinuity in the slope of the γ_{OW}-curve is observed in the presence of MAG-OS which shows that chain crystallization is taking place. In the presence of both MAG-O and MAG-S, MAG-S is, hence, still able to crystallize at the interface. However, the chain crystallization occurs at different temperatures ($T_{cr,chain}$): at 33℃ and 24℃ in the presence of MAG-S and MAG-OS, respectively. This difference can be explained by the fact that the $T_{cr,chain}$ is concentration dependent, as shown in Chapter 4, and that the oil phase containing the mixture of the MAGs contains a lower amount of MAG-S (i.e. 0.3% versus 0.6%).

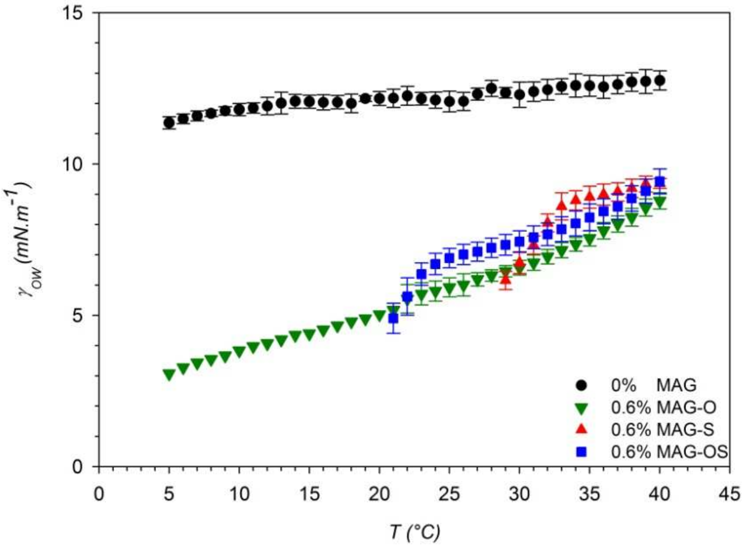


Figure 9.2 Interfacial tension γ_{OW} (mN.m^{-1}) at an oil-water interface created between buttermilk and purified sunflower oil during cooling without MAGs (●, with 0.6% MAG-O (▼), with 0.6% MAG-S (▲) or with 0.6% MAG-OS (■) (oil-based concentration).

B. *Interfacial viscoelasticity*

After defining the linear viscoelastic region (LVR) of the oil-water interface between buttermilk and an upper oil phase containing 0.6% MAG-OS a frequency sweep was performed at a strain of 1% (strain within the LVR). Figure 9.3 shows the frequency sweep of the interface between buttermilk in contact with an oil phase with 0.6% MAG-OS with respect to the data obtained in Chapter 8 (Section 8.3.3.3). For all interfaces a pure elastic behavior is observed. The *G'*-value is not affected by the applied frequency. Furthermore, in the presence of 0.6% MAG-OS, *G'* is substantially increased compared to the reference interface indicating that a stronger network is created in the presence of MAG-OS. This can be ascribed by the presence of crystalline MAG-S-crystals as shown in Section 9.3.1.3.A. However, compared to an interface created in the presence of only 0.6% MAG-S the value of *G'* is lower. This difference is attributed to probably, at the one hand, the lower amount of adsorbed MAG-S (i.e. only 0.3% MAG-S in cream with 0.6% MAG-OS) and, on the other hand, the presence of liquid MAG-O which weakens the elastic network at the interface.

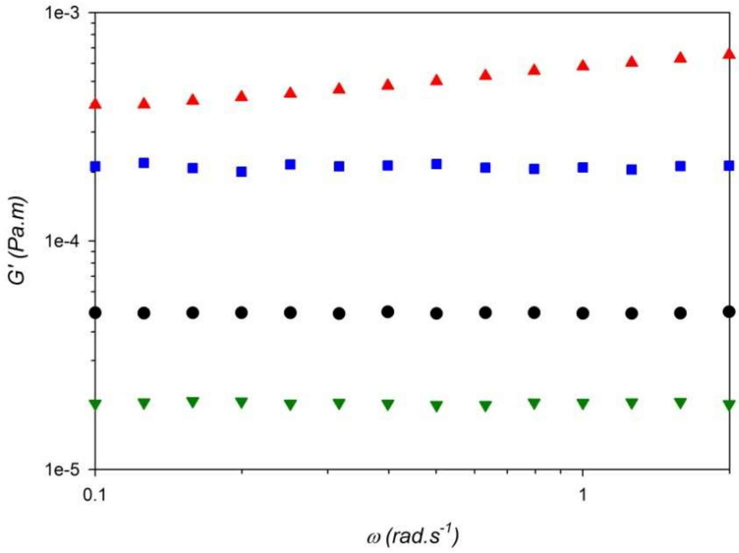


Figure 9.3 Interfacial elastic modulus *G'* (Pa.m) at different frequencies ω (rad.s^{-1}) of an interface created between buttermilk and purified sunflower oil without MAGs (●), with 0.6% MAG-O (▼), with 0.6% MAG-S (▲) or with 0.6% MAG-OS (■) (oil-based concentrations).

C. *Fat crystallization*

Since both in Chapter 8 it is postulated that only an increased nucleation rate at the oil-water interface in the presence of MAGs in RCs can be linked to differences in the partial coalescence rate and in Chapter 4 the increased nucleation rate is related to an increased crystallization temperature (T_{cr}), the T_{cr} were determined for the RCs without and with 0.2% MAG-O, MAG-S and MAG-OS (Table 9.2). The RCs with MAG-S and MAG-OS show a significant increase in T_{cr} compared to the reference RC and RC with 0.2% MAG-O. Hence, an increased nucleation rate or heterogeneous nucleation takes place in the presence of MAG-OS. The chain crystallization of the MAG-S in the presence of MAG-OS, as observed by the interfacial tension measurement, indicates the occurrence of interfacial heterogeneous nucleation in RC with MAG-OS.

However, in the presence of MAG-OS the measured $T_{cr,chain}$ are rather high compared to the T_{cr} of the RCs. For instance, at an oil-based concentration of 0.6% MAG-OS the T_{cr} of RC and $T_{cr,chain}$ amount to 11.6 ± 0.3℃ and 24℃, respectively. This is probably caused by the difference in ratio of the interfacial area to the oil volume in the drop tensiometer and in the real cream system. In the drop tensiometer this ratio is very low compared to the real cream system. More MAG-molecules will, thus, be available to position at the oil-water interface. As a consequence, the measured $T_{cr,chain}$ is likely to be an overestimation of the actual $T_{cr,chain}$ and, hence, the T_{cr} in the real cream systems.

Table 9.2 The T_{cr} of RCs without MAGs and with 0.2% MAG-O, MAG-S and MAG-OS.

[MAG]	T_{cr} (℃)		
0% MAG	10.8	±	0.2[a]
0.2% MAG-O	10.8	±	0.1[a]
0.2% MAG-S	14.5	±	0.1[b]
0.2% MAG-OS	11,6	±	0.3[c]

[a-d] different letters indicate significant differences ($p < 0.05$).

9.3.1.4. Discussion

From the above described results, it is obvious that in RC with a 50:50 binary mixture of MAG-O and MAG-S the effect of MAG-S prevails on the shear-induced partial coalescence rate, on the whipping properties and on the physicochemical properties of the RC and its interface. Hence, the microstructure of the milk fat globules may mainly resemble the one created in the presence of only MAG-S. At $T > T_{cr,\ chain}$ a mixture of MAG-S and MAG-O is present at the interface decreasing the oil-water interfacial tension with respect to the reference interface without MAGs (Figure 9.2). At $T < T_{cr,\ chain}$ MAG-S crystallize at the interface and induce, thereby, interfacial heterogeneous nucleation of milk fat in the globules resulting in the growth of many small primary milk fat crystals near the interface. Upon further cooling and storage at 5℃, crystallization spreads throughout the whole globule from the outer layer to the inside of the fat globule. Because of the presence of the rigid 'shell' of crystals at the interface milk fat crystals will be hindered to protrude. As a consequence, the partial coalescence rate is decreased compared with the reference RC and the RC with only MAG-O as is confirmed by the increased t_{ch}. In summary, like in Chapter 8, in this study the clear link between the physical behavior of the MAGs at the oil-water interface and the effect of the MAGs on partial coalescence rate is again demonstrated. Therefore, the mechanistic explanation as proposed in Section 8.3.4 is found to be also valid for mixtures of MAGs.

However, in RC with MAG-OS it should be stressed that the effect of MAG-S is substantially opposed by the action of MAG-O explaining the rather limited effect of MAG-OS compared with the effect of only MAG-S. This opposing effect of MAG-O in the mixture is in particular of interest aiming at improving the whipping properties since the use of MAGs separately seems to have a too low overrun if only MAG-O is used (i.e. < 100%) while a too low stability is reached by using only MAG-S (i.e. 60.72% serum loss with MAG-S versus 12.80% without MAGs after 24h storage at 5℃). Figure 9.1 shows that RCs with both MAG-O and MAG-S have an improved overrun at the expense of only a rather limited change in stability compared with the reference RC. In addition, the obtained whipping time and firmness of whipped RC with MAG-OS are acceptable. Hence, it can be stated that the 50:50 mixture of the MAGs improved the whipping properties to a greater extent than when used separately. Moreover, it is plausible that at different ratios of MAG-S to MAG-O and at different total concentrations of a mixture of these MAGs the whipping properties can be further optimized according to the preferred whipping properties.

9.3.2. Fat reduction by using monoacylglycerols

The same reference RC and the RC with 0.2% MAG-O as in Section 9.3.1 is used to study whether MAG-O can be used to improve the whipping properties of RC with a reduced fat content. The RCs with a fat content of 35% were diluted with cooled buttermilk to a fat content of 30% or 25%. As a result, the whipping creams contain a lower amount of fat but a higher amount of proteins.

9.3.2.1. Shear-induced partial coalescence

The creams with varying fat content were sheared until complete churning. Table 9.3 shows the t_{ch} of the reference RC and RC with 0.2% MAG-O. As a function of decreasing fat content, the t_{ch} increases regardless of the considered RC. The shear-induced partial coalescence rate is, thus, reduced when the cream is diluted with buttermilk. This effect is, like in Section 7.3.4.3, ascribed to the lowered collision frequency of the fat globules. The capture efficiency upon fat globule collision is unaffected. By making use of cooled buttermilk to decrease the fat content of the whipping cream, the composition of the aqueous phase, the milk fat crystal network properties in the globules and the interfacial properties of the milk fat globule membrane are unaltered.

Furthermore, irrespective of the fat content, the t_{ch} is lowered in the presence of MAG-O compared to the reference cream. MAG-O accelerates shear-induced partial coalescence rate as described in detail in Section 8.3.1.

Table 9.3 The t_{ch} (min) of RCs with 0% MAGs and 0.2% MAG-O at different fat contents (%)

Fat Content (%)	t_{ch} (min)					
	0% MAGs			0.2% MAG-O		
35	9.8	±	0.3[a]	1.1	±	0.0[a]
30	44.9	±	1.0[b]	4.7	±	0.2[b]
25	195.0	±	18.0[c]	16.6	±	1.4[c]

[a-c] different letters indicate significant differences ($p<0.05$)

9.3.2.2. Whipping properties

The RCs with various fat contents were whipped at 5℃ and the whipping properties are shown in Figure 9.4. Annex VII shows the results of the statistical analyses. The t_{wh}, overrun and the serum loss are inversely related with the fat content whether or not the RCs contain MAG-O. Regarding the firmness, a clear decreasing trend can be observed if the fat content is reduced in RC with MAG-O whereas for the reference RC the effect is less pronounced. Comparing the RCs with and without MAG-O, the t_{wh}, the overrun and the serum loss is significantly reduced while the firmness is significantly elevated in RCs with MAG-O regardless of the fat content. Exceptions are the serum loss measured after 1h at 20℃ and the t_{wh} of the RCs at a fat content of 30%.

9.3.2.3. Discussion

Like in natural cream (Section 7.3.4), the increased overrun with a decreasing fat content is a result of, at the one hand, the decreased partial coalescence rate and, on the other hand, the increased amount of proteins in the cream. Due to the restricted partial coalescence stage 1 of the whipping process is prolonged implying more air whisked in the cream. The increased amount of free proteins in cream with a reduced fat content enhances the ability to capture more air in the first stage of whipping. As a consequence of both the increased overrun and the lower amount of fat globules present, a more sparse partially coalesced fat globule network will be created and, hence, the stability and firmness is strongly decreased in both the RCs (i.e. with and without MAG-O) when the fat content is reduced.

The whipped RCs with MAG-O and a reduced fat content show an overall improved stability with respect to the reference RC with the same fat content. Due to the lower overrun and the increased partial coalescence rate than in the refence cream with the same fat content, a denser partially coalesced fat globule network stabilizing the air bubbles and the serum phase may be created. Moreover, for RC with 0.2% MAG-O with a fat content of 25% it can be observed that the t_{wh}, the overrun and the serum loss after 24h at 5℃ are comparable with the full-fat reference RC. This clearly demonstrates that the enhanced partial coalescence rate in the presence of MAG-O allows improving the whipping properties of RC with a reduced fat content in whipped cream.

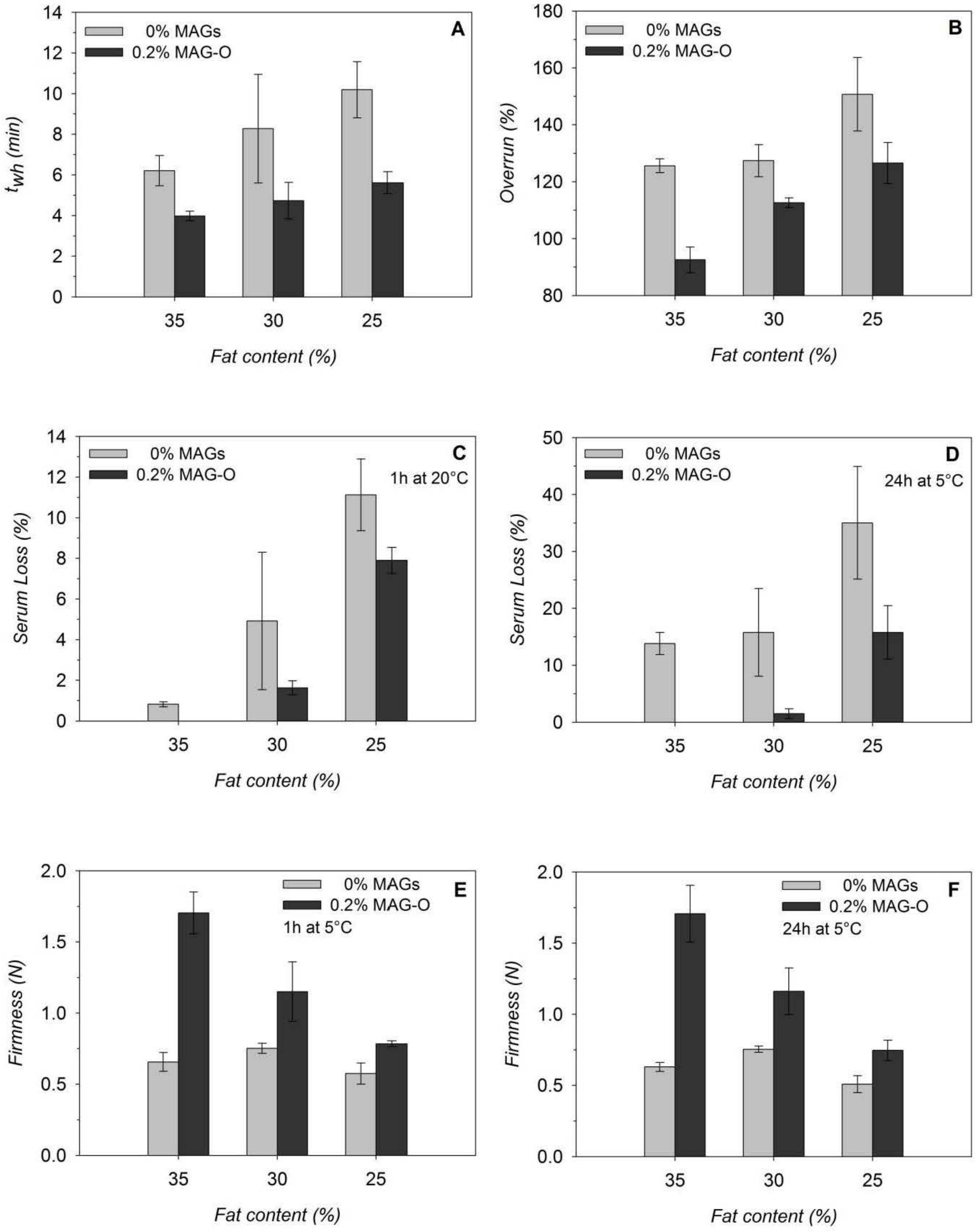


Figure 9.4 Whipping properties of RCs without MAGs, with 0.2% MAG-O as a function of fat content: (A) t_{wh} (min); (B) *overrun* (%); (C) *serum loss* (%) after storage for 1h at 20℃; (D) *serum loss* (%) after storage for 24h at 5℃; (E) *firmness* (N) after 1h at 5℃ and (F) *firmness* (N) after 24h at 5℃.

9.4. Concluding remarks

When using a 50:50 mixture of MAG-O and MAG-S in RC the effect of MAG-S prevailed. Similar, though less pronounced, effects as in RC with only MAG-S were demonstrated by studying the shear-induced partial coalescence, the whipping properties and the physicochemical properties of the RC and its interface. The rather limited effect obtained compared with the effects in RC with only MAG-S was ascribed to the opposing effect of MAG-O.

Two cases demonstrated that improved whipping properties in RC can be achieved by using MAGs. In the first case, it has been shown that a binary mixture of MAGs (i.e. MAG-O and MAG-S) gained improved whipping properties against the whipped creams that only contain one of these MAGs. The separate use of one type of MAG in a full-fat cream showed rather poor whipping behavior: some beneficial whipping properties were attained but this occurred at the expense of other desired whipping properties. In the second case, it was illustrated that MAGs which accelerate the partial coalescence (i.e. MAG-O) are appropriate to improve the physical properties of a whipped cream with a reduced fat content.

GENERAL CONCLUSIONS

Whipping cream can be processed into whipped cream which is a rigid dairy foam that can be defined as an aerated partially destabilized oil-in-water emulsion. During the whipping process air is introduced and fat globules start to interact by means of a partial coalescence destabilization mechanism. The major prerequisite for partial coalescence to occur is the presence of fat crystals.

In this research it was aimed at understanding some of the underlying phenomena important for the whipping of dairy creams. Therefore, by using a wide range of experimental techniques milk fat crystallization and partial coalescence were investigated. Moreover, throughout the whole manuscript attention is drawn to the role of monoacylglycerols in the milk fat crystallization and their consequences on partial coalescence in dairy whipping cream.

Part I: Milk fat crystallization

From the literature review, it was deduced that milk fat crystallization in recombined dairy creams is poorly investigated and that a thorough comparison between natural and recombined cream is still lacking. In addition, the effect of monoacylglycerols (MAGs) in dairy whipping creams is not addressed in literature.

By means of mainly DSC-, XRD- and NMR-techniques the milk fat crystallization behavior in bulk, natural and recombined cream was examined. To study the milk fat crystallization of creams appropriately adaptations to the conventional DSC- and NMR-techniques were implemented. As a substrate whipping creams with a 35% fat content were used and the experimental time-temperature conditions were chosen comparable to the ones industrially applied. Milk fat in bulk or in the fat globules showed a similar crystallization mechanism. First, α-crystals were formed and, second, β'-crystals grew at the expense of the α-crystals. However, some α-crystals persisted and additional crystallization during further cooled storage took place. The observed mechanism was explained by the formation of compound crystals. Although these similarities between the milk crystallization mechanism in bulk and in the creams, the overall crystallization rate was found to be lower in both natural and recombined cream. This difference was to a large extent explained by theory of nucleation in the dispersed phase. Moreover, the crystallization kinetics of natural cream deviated from that of recombined cream: the α-β' polymorphic transition started earlier and was more scattered and a lower amount of solid fat after 5 days storage at 5°C was obtained. It was argued that these effects can be ascribed to the variation in triacylglycerol composition of the individual globules in natural cream.

Following the acquired knowledge on the milk fat crystallization mechanism in cream, the effect of different MAGs on the crystallization behavior in recombined creams with a constant particle size distribution was unraveled. Long-chain unsaturated MAGs and mid-chain saturated MAGs did not affect the primary fat crystallization mechanism. Volume heterogeneous/homogeneous nucleation in the α-polymorph occurred which subsequently partly transform in β'-crystals. Moreover, after prolonged storage a similar amount of solid fat content was obtained regardless of the concentration of these MAGs. However, for the mid-chain saturated MAGs an acceleration in the α-β' polymorphic transition was observed which was suggested to be caused by a co-crystallization of these MAGs once milk fat crystallization has started. Conversely, the long-chain saturated MAGs caused a difference in nucleation mechanism. An increased nucleation rate by means of interfacial heterogeneous nucleation was demonstrated through, at the one hand, the observed elevated crystallization temperature in the recombined cream and, on the other hand, the detected chain crystallization at the oil-water interface both measured upon cooling as a function of the MAG-concentration. The presumable resulting differences in composition, number and size of the initial created α-crystals were believed to cause variations in the rate of the crystal growth and the α-β' polymorphic evolution.

Part II: Partial coalescence

The literature review that preceded the experimental work provided us, firstly, the awareness of the complexity of researching partial coalescence in oil-in-water emulsions and their whipping properties. By varying one factor of interest simultaneously different physicochemical properties of the emulsions will change. Secondly, it became clear that disagreement exists on the mechanistic clarifications of the influence of MAGs differing in saturation degree on partial coalescence in oil-in-water emulsions in general. Thirdly, it was deduced that no parallel studies of the effects of MAGs in particular on partial coalescence in dairy recombined creams and on their whipping properties could be found.

A rheological method to survey shear-induced partial coalescence was first introduced as a qualitative predictive tool for the whipping properties of dairy creams. This technique was validated by linking the partial coalescence rate and the whipping properties of natural cream by varying the flow condition, the temperature and the fat content.

By using recombined creams, equivalent to the ones in Part I, the rheological method was applied to profoundly study the effect of two long-chain MAGs which differ in saturation degree on partial coalescence. Although chemically speaking the MAGs differ only slightly (i.e. one double bond), they demonstrated opposite effects on the partial coalescence rate of the recombined cream and, hence, on their whipping properties. The unsaturated MAGs showed an increased partial coalescence rate in recombined cream resulting in a lower whipping time and overrun and a higher stability and firmness of whipped recombined creams while for the saturated MAGs the reverse is true. By means of investigating the effect of MAGs on the milk fat crystallization behavior (Part I) and on the oil-water interfacial physicochemical properties (Part I and II), a mechanism elucidating the divergent effect of the MAGs on the susceptibility of fat globules towards partial coalescence was suggested. The saturated MAGs have the ability to exhibit interfacial heterogeneous nucleation which is at the basis of the decreased partial coalescence rate due to, firstly, the hindering of the piercing of fat crystals through the milk fat globule membrane, secondly, the formation of smaller crystals, thirdly, the decreased deformability (higher elasticity) of the interfacial membrane and/or, fourthly, the reduced probability that protruding crystals of one globule will pierce the membrane of a second globule in a 'liquid' region. In contrast, the unsaturated MAGs behave as a liquid at the oil-water interface resulting in an enhanced partial coalescence rate due to (1) a higher degree and longer protrusion distance of the protruding crystals and/or (2) the weaker interfacial protein network in between the protruding crystals. The latter involves that the interfacial membranes will be more readily deformed during the film formation between two approaching globules and during their eventual merging.

Besides the fundamental research on the effect of MAGs on milk fat crystallization and partial coalescence, it was explored whether the long-chain MAGs which differ in saturation degree can be used in practice to improve whipping properties of recombined cream. The separate use of one type of MAG in a full-fat cream showed rather poor whipping behavior: some beneficial whipping properties were attained but this occurred at the expense of other desired whipping properties. Alternatively, when both the MAGs are introduced in one cream the negative effects of one MAG can be counterbalanced by the other causing ultimately improved whipping properties of recombined cream. On the contrary, when also the fat content of recombined cream is varied, the separate use of the long-chain unsaturated MAGs in one cream became beneficial. In fact, whipped recombined cream with a reduced fat content containing unsaturated MAGs demonstrated comparable whipping properties to the full-fat variant without MAGs.

Although this research dealt thoroughly with the milk fat crystallization and partial coalescence in whipping cream much is still left to be unraveled. Future research could focus on applying the optimized toolbox used throughout the whole manuscript to study the effect of other small-molecule surfactants or mixtures of these or to explore the effect of tempering of cream. Both these factors are believed to be promising to improve the whipping properties of recombined cream in particular when fat reduction is aimed. Furthermore, in order to further fundamentally survey the underlying phenomena of the controlling factors of partial coalescence development of an appropriate microscopic tool to visualize milk fat crystals in cream is advisable. Many mechanistic clarifications on factors governing partial coalescence both in this research as well as in literature rely on changes in the morphology, size and numbers of the crystals and on their arrangement in the fat globules.

REFERENCE LIST

[1] P. Walstra, J.T.M. Wouters and T.J. Geurts, *Dairy science and technology: Second edition*, CRC press, 2006, 782 pp.

[2] B. Vanhoutte, Milk fat crystallisation: fractionation and texturisation, PhD, Ghent University, Ghent, 2002, p. 364.

[3] F.E. Kurtz, The lipids of milk: Composition and properties, in: *Fundamentals of dairy chemistry: 2nd edition*, B.H. Webb, A.H. Johnson and J.A. Alford, eds, The Avi Publishing Company, Westport, connecticut, 1978, pp. 125-272.

[4] R.G. Jensen, The composition of bovine milk lipids: January 1995 to December 2000, *Journal of Dairy Science*, 85 (2002), 295-350.

[5] R.G. Jensen, Fatty acids in milk and dairy products, in: *Fatty acid in foods and their health implications*, C.K. Chow, ed, Marcel Dekker, Inc., New York, 1992, pp. 95-135.

[6] R.L. Wolff, C.C. Bayard and R.J. Fabien, Evaluation of sequential methods for the determination of butterfat fatty acid composition with emphasis on trans-18:1 acids. Application to the study of seasonal variations in French butters, *Journal of the American Oil Chemists Society*, 72 (1995), 1471-1483.

[7] M. Collomb, A. Schmid, R. Sieber, D. Wechsler and E.L. Ryhanen, Conjugated linoleic acids in milk fat: Variation and physiological effects, *International Dairy Journal*, 16 (2006), 1347-1361.

[8] J. Fritsche and H. Steinhart, Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) - a review, *Fett-Lipid*, 100 (1998), 190-210.

[9] J.K. Ha and R.C. Lindsay, Volatile branched fatty acids and phenolic compounds in aged Italian cheese flavors, *Journal of Food Science*, 56 (1991), 1241-1247.

[10] O.W. Parks, D.P. Schwartz, I. Katz and M. Keeney, Isolation and characterization of methyl ketone precursor in butter fat, *Journal of Lipid Research*, 5 (1964), 232-&.

[11] K.E. Kaylegian and R.C. Lindsay, Milk fat usage and modification, in: *Handbook of milkfat fractionation technology and application*, AOCS Press, Champaign, IL, 1995, pp. 1-18.

[12] R.G. Jensen and D.S. Newberg, Bovine milk lipids, in: *Milk composition*, R.G. Jensen, ed, Academic press, San diego, CA, 1995, pp. 543-575.

[13] W.W. Christie, R.C. Noble and G. Davies, Phospholipids in mik and dairy-products, *Journal of the Society of Dairy Technology*, 40 (1987), 10-12.

[14] R.G. Jensen, Composition of bovine milk lipids, *Journal of the American Oil Chemists Society*, 50 (1973), 186-192.

[15] G.P. McNeill, A. Odonoghue and J.F. Connolly, Quantification and identification of flavor components leading to lipolytic rancidity in stored butter, *Irish Journal of Food Science and Technology*, 10 (1986), 1-10.

[16] D.L. Palmquist, A.D. Beaulieu and D.M. Barbano, Feed and animal factors influencing milk-fat composition, *Journal of Dairy Science*, 76 (1993), 1753-1771.

[17] E. Jakubczyk and K. Niranjan, Transient development of whipped cream properties, *Journal of Food Engineering*, 77 (2006), 79-83.

[18] J.R. Ashes, S.K. Gulati and T.W. Scott, Potential to alter the content and composition of milk fat through nutrition, *Journal of Dairy Science*, 80 (1997), 2204-2212.

[19] R. Campos, S.S. Narine and A.G. Marangoni, Effect of cooling rate on the structure and mechanical properties of milk fat and lard, *Food Research International*, 35 (2002), 971-981.

[20] P. Walstra, W. Kloek and T. Van Vliet, Fat crystal network, in: *Crystallization processes in fats and lipid systems*, N. Garti and K. Sato, eds, Marcel Dekker Inc, New York, 2001, pp. 239-321.

[21] W. Kloek, Properties of fats in relation to their crystallization, PhD, Wageningen University, Wageningen, 1998, p. 241.

[22] R. Hartel, Solid-liquid equilibrium: Crystallization in foods., in: *Physical chemistry of foods*, H. Schwartzber and R. Hartel, eds, Marcel Dekker Inc., New york, 1992, pp. 47-81.

[23] B.S. Ghotra, S.D. Dyal and S.S. Narine, Lipid shortenings: a review, *Food Research International*, 35 (2002), 1015-1048.

[24] R. Boistelle, Fundamentals of nucleation and crystal growth, in: *Crystallization and polymorphism of fats and fatty acids*, N. Garti and K. Sato, eds, 31, Marcel Dekker Inc, New york, 1988, pp. 189-276.

[25] J. Garside, General principles of crystallization, in: *Food structure and behaviour*, V.M.V. Blanshard and P. Lillford, eds, Academic press, London, 1987, pp. 35-49.

[26] D. Aquilano and G. Sgualdino, Fundamental aspects of equilibrium and crystallization kinetics, in: *Crystallization processes in fats and lipid systems*, N. Garti and K. Sato, eds, Marcel Dekker Inc, New York, 2001, pp. 239-321.

[27] K. Sato, Crystallization behaviour of fats and lipids - a review, *Chemical Engineering Science*, 56 (2001), 2255-2265.

[28] C. Himawan, V.M. Starov and A.G.F. Stapley, Thermodynamic and kinetic aspects of fat crystallization, *Advances in Colloid and Interface Science*, 122 (2006), 3-33.

[29] A.G. Marangoni, Crystallization kinetics, in: *Fat crystal network*, A.G. Marangoni, ed, Marcel Dekker Inc, New York, 2005, pp. 21-82.

[30] G. Calliauw, Molecular interactions affecting the phase composition during dry fractionation of palm olein, PhD, Ghent University, Ghent, 2008, p. 275.

[31] P. Walstra, Fat crystallization, in: *Food structure and behaviour*, V.M.V. Blanshard and P. Lillford, eds, Academic Press, London, 1987, pp. 67-85.

[32] P. Walstra, Secondary nucleation in triglyceride crystallization, in: *International Conference on Colloidal Aspects of Lipids*, B. Lindman and B.W. Ninham, eds, Lund, Sweden, 1997, pp. 4-8.

[33] P. Walstra, *Physical Chemistry of Foods*, Marcel Decker, Inc, New York, 2003, 807 pp.

[34] M.L. Herrera and R.W. Hartel, Effect of processing conditions on crystallization kinetics of a milk fat model system, *Journal of the American Oil Chemists Society*, 77 (2000), 1177-1187.

[35] K. Larsson, Classification of glyceride crystal forms, *Acta Chemica Scandinavica*, 20 (1966), 2255-&.

[36] V. D'Souza, J.M. deMan and L. deMan, Short spacings and polymorphic forms of natural and commercial solid fats - a review, *Journal of the American Oil Chemists Society*, 67 (1990), 835-843.

[37] J.W. Hagemann, Thermal behavior of polymorphism of acylglycerides, in: *Crystallization and polymorphism of fats and fatty acids*, N. Garti and K. Sato, eds, Vol. 31, Marcel Dekker Inc, New York, 1988.

[38] C. Lopez, P. Lesieur, G. Keller and M. Ollivon, Thermal and structural behavior of milk fat - 1. Unstable species of cream, *Journal of Colloid and Interface Science*, 229 (2000), 62-71.

[39] C. Lopez, C. Bourgaux, P. Lesieur, A. Riaublanc and M. Ollivon, Milk fat and primary fractions obtained by dry fractionation - 1. Chemical composition and crystallisation properties, *Chemistry and Physics of Lipids*, 144 (2006), 17-33.

[40] W.W. Nawar, Lipids, in: *Food Chemistry*, O.R. Fennema, ed, Marcel Dekker, New York, 1996, pp. 239-319.

[41] E. ten Grotenhuis, G.A. van Aken, K.F. van Malssen and H. Schenk, Polymorphism of milk fat studied by differential scanning calorimetry and real-time X-ray powder diffraction, *Journal of the American Oil Chemists Society*, 76 (1999), 1031-1039.

[42] K. Sato and T. Kuroda, Kinetics of melt crystallization and transformation of tripalmitin polymorphs, *Journal of the American Oil Chemists Society*, 64 (1987), 124-127.

[43] N. Garti and J. Yano, The roles of emulsifiers in fat crystallization, in: *Crystallization processes in fat and lipid systems*, N. Garti and K. Sato, eds, Marcel Dekker Inc, New York, 2001, pp. 212-250.

[44] L.H. Wesdorp, J.A. Van Meeteren, S. De Jong, R.V.D. Giessen, P. Overbosch, P.A.M. Grootscholten, M. Struik, E. Royers and A. Don, Liquid-multiple solid phase equilibria in fats: theory and experiments, in: *Fat Crystal Networks*, A.G. Marangoni, ed, Marcel Dekker, New York, 2005, pp. 481-710.

[45] G. Mazzanti, S.E. Guthrie, E.B. Sirota, A.G. Marangoni and S.H.J. Idziak, Orientation and phase transitions of fat crystals under shear, *Crystal Growth & Design*, 3 (2003), 721-725.

[46] C. Garbolino, G.R. Ziegler and J.N. Coupland, Ultrasonic determination of the effect of shear on lipid crystallization, *Journal of the American Oil Chemists Society*, 77 (2000), 157-162.

[47] S. Sonwai and M.R. Mackley, The effect of shear on the crystallization of cocoa butter, *Journal of the American Oil Chemists Society*, 83 (2006), 583-596.

[48] M.L. Herrera and R.W. Hartel, Effect of processing conditions on physical properties of a milk fat model system: Microstructure, *Journal of the American Oil Chemists Society*, 77 (2000), 1197-1204.

[49] V. De Graef, P. van Puyvelde, B. Goderis and K. Dewettinck, Influence of shear flow on polymorphic behavior and microstructural development during palm oil crystallization, *European Journal of Lipid Science and Technology*, 111 (2009), 290-302.

[50] T.S. Awad and K. Sato, Fat crystallization in O/W emulsions controlled by hydrophobic emulsifier additives, in: *Physical properties of lipids*, A.G. Marangoni and S.S. Narine, eds, Marcel Dekker, Inc., 2002, pp. 37-62.

[51] J.N. Coupland, Crystallization in emulsions, *Current Opinion in Colloid & Interface Science*, 7 (2002), 445-450.

[52] M.J.W. Povey, Crystallization of oil-in-water emulsions, in: *Crystallization processes in fats and lipid systems*, N. Garti and K. Sato, eds, Marcel Dekker Inc, New York, 2001, pp. 251-288.

[53] W. Skoda and M. Van den Tempel, Crystallization of emulsified triglycerides, *Journal of Colloid Science*, 18 (1963), 568-584.

[54] P. Walstra and E.C.H. van Beresteyn, Crystallization of milk-fat in emulsified state, *Netherlands Milk and Dairy Journal*, 29 (1975), 35-65.

[55] D.J. McClements, E. Dickinson, S.R. Dungan, J.E. Kinsella, J.G. Ma and M.J.W. Povey, Effect of emulsifier type on the crystallization kinetics of oil-in-water emulsions containing a mixture of solid and liquid droplets, *Journal of Colloid and Interface Science*, 160 (1993), 293-297.

[56] E. Dickinson, F.J. Kruizenga, M.J.W. Povey and M. Vandermolen, Crystallization in oil-in-water emulsions containing liquid and solid droplets, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 81 (1993), 273-279.

[57] D.J. McClements and S.R. Dungan, Effect of colloidal interactions on the rate of interdroplet heterogeneous nucleation in oil-in-water emulsions, *Journal of Colloid and Interface Science*, 186 (1997), 17-28.

[58] D.J. McClements, S.W. Han and S.R. Dungan, Interdroplet heterogeneous nucleation of supercooled liquid droplets by solid droplets in oil-in-water emulsions, *Journal of the American Oil Chemists Society*, 71 (1994), 1385-1389.

[59] C. Lopez, C. Bourgaux, P. Lesieur, S. Bernadou, G. Keller and M. Ollivon, Thermal and structural behavior of milk fat - 3. Influence of cooling rate and droplet size on cream crystallization, *Journal of Colloid and Interface Science*, 254 (2002), 64-78.

[60] J.S. Aronhime, S. Sarig and N. Garti, Mechanistic considerations of polymorphic transformations of tristearin in the presence of emulsifiers, *Journal of the American Oil Chemists Society*, 64 (1987), 529-533.

[61] P.R. Smith, The effects of phospholipids on crystallisation and crystal habit in triglycerides, *European Journal of Lipid Science and Technology*, 102 (2000), 122-127.

[62] D. Johansson, Colloids in fats: The fat crystal as a functional particle, PhD, Lund University, Lund, 1994, p. 79.

[63] M. Cerdeira, V. Pastore, L.V. Vera, S. Martini, R.J. Candal and M.L. Herrera, Nucleation behavior of blended high-melting fractions of milk fat as affected by emulsifiers, *European Journal of Lipid Science and Technology*, 107 (2005), 877-885.

[64] M. Cerdeira, S. Martini, R.W. Hartel and M.L. Herrera, Effect of sucrose ester addition on nucleation and growth behavior of milk fat-sunflower oil blends, *Journal of Agricultural and Food Chemistry*, 51 (2003), 6550-6557.

[65] S. Martini, M.C. Puppo, R.W. Hartel and M.L. Herrera, Effect of sucrose esters and sunflower oil addition on crystalline microstructure of a high-melting milk fat fraction, *Journal of Food Science*, 67 (2002), 3412-3418.

[66] C. Garbolino, M. Bartoccini and E. Floter, The influence of emulsifiers on the crystallisation behaviour of a palm oil-based blend, *European Journal of Lipid Science and Technology*, 107 (2005), 616-626.

[67] M. Sakamoto, K. Maruo, J. Kuriyama, M. Kouno, S. Ueno and K. Sato, Effects of adding polyglycerols behenic acid esters on the crystallization of palm oil, *Journal of Oleo Science*, 52 (2003), 639-645.

[68] J.S. Aronhime, S. Sarig and N. Garti, Reconsideration of polymorphic transformations in cocoa butter using the DSC, *Journal of the American Oil Chemists Society*, 65 (1988), 1140-1143.

[69] P.R. Smith, D.J. Cebula and M.J.W. Povey, The effect of lauric-based molecules on trilaurin crystallization, *Journal of the American Oil Chemists Society*, 71 (1994), 1367-1372.

[70] P.R. Smith and M.J.W. Povey, The effect of partial glycerides on trilaurin crystallization, *Journal of the American Oil Chemists Society*, 74 (1997), 169-171.

[71] E. Fredrick, I. Foubert, J. Van De Sype and K. Dewettinck, Influence of monoglycerides on the crystallization behavior of palm oil, *Crystal Growth & Design*, 8 (2008), 1833-1839.

[72] D. Johansson and B. Bergenstahl, Sintering of fat crystal networks in oil during post-crystallization processes, *Journal of the American Oil Chemists Society*, 72 (1995), 911-920.

[73] E. Sambuc, Z. Dirik, G. Reymond and M. Naudet, Study on the crystallization of plastic fats .6. Influence of partial glycerides and phosphatides in absence and in presence of water .B. Case of soybean lecithins, *Revue Francaise Des Corps Gras*, 28 (1981), 13-19.

[74] J.F. Toro-Vazquez, E. Rangel-Vargas, E. Dibildox-Alvarado and M.A. Charo-Alonso, Crystallization of cocoa butter with and without polar lipids evaluated by rheometry, calorimetry and polarized light microscopy, *European Journal of Lipid Science and Technology*, 107 (2005), 641-655.

[75] C.M. Savage and P.S. Dimick, Influence of pospholipids during crystallization of hard and soft cocoa butter, *Manuf. Confect.*, 75 (1995), 127-132.

[76] D.H. Arruda and P.S. Dimick, Phospholipid-composition of lipid seed crystal isolates from ivory-coast cocoa butter, *Journal of the American Oil Chemists Society*, 68 (1991), 385-390.

[77] R.A. Tietz and R.W. Hartel, Effects of minor lipids on crystallization of milk fat-cocoa butter blends and bloom formation in chocolate, *Journal of the American Oil Chemists Society*, 77 (2000), 763-771.

[78] B. Vanhoutte, I. Foubert, F. Duplacie, A. Huyghebaert and K. Dewettinck, Effect of phospholipids on isothermal crystallisation and fractionation of milk fat, *European Journal of Lipid Science and Technology*, 104 (2002), 738-744.

[79] B. Vanhoutte, K. Dewettinck, I. Foubert, B. Vanlerberghe and A. Huyghebaert, The effect of phospholipids and water on the isothermal crystallisation of milk fat, *European Journal of Lipid Science and Technology*, 104 (2002), 490-495.

[80] L. Wiking, E. Fredrick and K. Dewettinck, Phospholipids delay crystallisation of milk fat, *Milchwissenschaft-Milk Science International*, 64 (2009), 376-379.

[81] M. Cerdeira, S. Martini, R.J. Candal and M.L. Herrera, Polymorphism and growth behavior of low-trans fat blends formulated with and without emulsifiers, *Journal of the American Oil Chemists Society*, 83 (2006), 489-496.

[82] P. Elisabettini, A. Desmedt and F. Durant, Polymorphism of stabilized and nonstabilized tristearin, pure and in the presence of food emulsifiers, *Journal of the American Oil Chemists Society*, 73 (1996), 187-192.

[83] M.L. Herrera and F.J.M. Rocha, Effects of sucrose ester on the kinetics of polymorphic transition in hydrogenated sunflower oil, *Journal of the American Oil Chemists Society*, 73 (1996), 321-326.

[84] T. Awad and K. Sato, Acceleration of crystallisation of palm kernel oil in oil-in-water emulsion by hydrophobic emulsifier additives, *Colloids and Surfaces B-Biointerfaces*, 25 (2002), 45-53.

[85] Y. Hodate, S. Ueno, J. Yano, T. Katsuragi, Y. Tezuka, T. Tagawa, N. Yoshimoto and K. Sato, Ultrasonic velocity measurement of crystallization rates of palm oil in oil-water emulsions, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 128 (1997), 217-224.

[86] D. Rousseau, S.M. Hodge, M.T. Nickerson and A.T. Paulson, Regulating the beta '->beta polymorphic transition in food fats, *Journal of the American Oil Chemists Society*, 82 (2005), 7-12.

[87] K. Kawamura, DSC thermal-analysis of crystallization behavior in palm oil .II., *Journal of the American Oil Chemists Society*, 57 (1980), 48-51.

[88] S.F. Lee and J.M. Deman, The effects of surfactants on the crystallization behavior of hydrogenated canola oil, *Canadian Institute of Food Science and Technology Journal-Journal De L Institut Canadien De Science Et Technologie Alimentaires*, 15 (1982), R17-R17.

[89] J. Schlichter, S. Sarig and N. Garti, Polymorphic transformations of cocoa butter in the presence of emulsifier, studied by the DSC, *Thermochimica Acta*, 85 (1985), 517-520.

[90] N. Garti, J. Schlichter and S. Sarig, Effect of food emulsifiers on polymorphic transitions of cocoa butter, *Journal of the American Oil Chemists Society*, 63 (1986), 230-236.

[91] J.S. Aronhime, S. Sarig and N. Garti, Dynamic control of polymorphic transformation in triglycerides by surfactants - The Button Syndrome, *Journal of the American Oil Chemists Society*, 65 (1988), 1144-1150.

[92] J. Aronhime, S. Sarig and N. Garti, Emulsifiers as additives in fats - effect on polymorphic transformations and crystal properties of fatty-acids and triglycerides, *Food Structure*, 9 (1990), 337-352.

[93] J. Hojerova, S. Schmidt and J. Krempasky, Structure of margarines made with low erucic-acid rapeseed oil, *Food Structure*, 11 (1992), 147-154.

[94] E. Sambuc, Z. Dirik, G. Reymond and M. Naudet, Study on the Crystallization of Plastic Fats .6. Influence of Partial Glycerides and Phosphatides in Absence and in Presence of Water .a. Case of Stearopalmitic Monoglycerides, *Revue Francaise Des Corps Gras*, 27 (1980), 505-512.

[95] E. Sambuc, Z. Dirik and M. Naudet, Study on the crystallization of clastic fats .6. influence of partial glycerides and phosphatides in absence and in presence of water .C. Case of equiponderal mixtures of stearopalmitic monoglycerides and soybean lecithins, *Revue Francaise Des Corps Gras*, 28 (1981), 59-65.

[96] R.C. Basso, A.P.B. Ribeiro, M.H. Masuchi, L.A. Gioielli, L.A.G. Goncalves, A.O. dos Santos, L.P. Cardoso and R. Grimaldi, Tripalmitin and monoacylglycerols as modifiers in the crystallisation of palm oil, *Food Chemistry*, 122 (2010), 1185-1192.

[97] I. Foubert, B. Vanhoutte and K. Dewettinck, Temperature concentration dependent effect of partial glycerides on milk fat crystallization, *European Journal of Lipid Science and Technology*, 106 (2004), 531-539.

[98] W.L. Siew and W.L. Ng, Influence of diglycerides on crystallisation of palm oil, *Journal of the Science of Food and Agriculture*, 79 (1999), 722-726.

[99] A.H. Saberi, O.M. Lai and J.F. Toro-Vazquez, Crystallization kinetics of palm oil in blends with palm-based diacylglycerol, *Food Research International*, 44, 425-435.

[100] W.L. Siew and W.L. Ng, Effect of diglycerides on the crystallisation of palm oleins, *Journal of the Science of Food and Agriculture*, 71 (1996), 496-500.

[101] A.J. Wright and A.G. Marangoni, Effect of DAG on milk fat TAG crystallization, *Journal of the American Oil Chemists Society*, 79 (2002), 395-402.

[102] L. Hernqvist, B. Herslof, K. Larsson and O. Podlaha, Polymorphism of rapeseed oil with a low content of erucic-acid and possibilities to stabilize the beta-prime-crystal form in fats, *Journal of the Science of Food and Agriculture*, 32 (1981), 1197-1202.

[103] S. Wahnelt, D. Meusel and M. Tulsner, Influence of isomeric diglycerides on phase-transitions of cocoa butter - investigations by isothermal DSC, *Fett Wissenschaft Technologie-Fat Science Technology*, 93 (1991), 174-178.

[104] T.S. Awad, Ultrasonic studies of the crystallization behavior of two palm fats O/W emulsions and its modification, *Food Research International*, 37 (2004), 579-586.

[105] T. Awad and K. Sato, Effects of hydrophobic emulsifier additives on crystallization behavior of palm mid fraction in oil-in-water emulsion, *Journal of the American Oil Chemists Society*, 78 (2001), 837-842.

[106] M. Sakamoto, A. Ohba, J. Kuriyama, K. Maruo, S. Ueno and K. Sato, Influences of fatty acid moiety and esterification of polyglycerol fatty acid esters on the crystallization of palm mid fraction in oil-in-water emulsion, *Colloids and Surfaces B-Biointerfaces*, 37 (2004), 27-33.

[107] S. Arima, T. Ueji, S. Ueno, A. Ogawa and K. Sato, Retardation of crystallization-induced destabilization of PMF-in-water emulsion with emulsifier additives, *Colloids and Surfaces B-Biointerfaces*, 55 (2007), 98-106.

[108] T. Sonoda, Y. Takata, S. Ueno and K. Sato, Effects of emulsifiers on crystallization behavior of lipid crystals in nanometer-size oil-in-water emulsion droplets, *Crystal Growth & Design*, 6 (2006), 306-312.

[109] S. Arima, S. Ueno, A. Ogawa and K. Sato, Scanning microbeam small-angle X-ray diffraction study of interfacial heterogeneous crystallization of fat crystals in oil-in-water emulsion droplets, *Langmuir*, 25 (2009), 9777-9784.

[110] F. Lavigne and M. Ollivon, Milk fat and its fractions, *Ocl-Oleagineux Corps Gras Lipides*, 4 (1997), 212-219.

[111] C. Lopez, C. Bourgaux, P. Lesieur and M. Ollivon, Crystalline structures formed in cream and anhydrous milk fat at 4 degrees C, *Lait*, 82 (2002), 317-335.

[112] C. Lopez, F. Lavigne, P. Lesieur, C. Bourgaux and M. Ollivon, Thermal and structural behavior of milk fat. 1. Unstable species of anhydrous milk fat, *Journal of Dairy Science*, 84 (2001), 756-766.

[113] C. Lopez, F. Lavigne, P. Lesieur, G. Keller and M. Ollivon, Thermal and structural behavior of anhydrous milk fat. 2. Crystalline forms obtained by slow cooling, *Journal of Dairy Science*, 84 (2001), 2402-2412.

[114] C. Lopez and M. Ollivon, Triglycerides obtained by dry fractionation of milk fat 2. Thermal properties and polymorphic evolutions on heating, *Chemistry and Physics of Lipids*, 159 (2009), 1-12.

[115] C. Lopez, P. Lesieur, C. Bourgaux, G. Keller and M. Ollivon, Thermal and structural behavior of milk fat 2. Crystalline forms obtained by slow cooling of cream, *Journal of Colloid and Interface Science*, 240 (2001), 150-161.

[116] M.C. Michalski, M. Ollivon, V. Briard, N. Leconte and C. Lopez, Native fat globules of different sizes selected from raw milk: thermal and structural behavior, *Chemistry and Physics of Lipids*, 132 (2004), 247-261.

[117] S. Bugeat, V. Briard-Bion, J. Pérez, P. Pradel, B. Martin, S. Lesieur, C. Bourgaux, M. Ollivon and C. Lopez, Enrichment in unsaturated fatty acids and emulsion droplet size affect the crystallization behaviour of milk triacylglycerols upon storage at 4 °C, *Food Research International*, 44 (2011), 1314-1330.

[118] A.G. Marangoni and R.W. Lencki, Ternary phase behavior of milk fat fractions, *Journal of Agricultural and Food Chemistry*, 46 (1998), 3879-3884.

[119] V. Briard, N. Leconte, F. Michel and M.C. Michalski, The fatty acid composition of small and large naturally occurring milk fat globules, *European Journal of Lipid Science and Technology*, 105 (2003), 677-682.

[120] S. Miura, T. Mutoh, Y. Shiinoki and T. Yoshioka, Emulsifying properties of phospholipids in the reconstitution of cream using butter oil, *European Journal of Lipid Science and Technology*, 108 (2006), 898-903.

[121] B. Breitschuh and E.J. Windhab, Parameters influencing cocrystallization and polymorphism in milk fat, *Journal of the American Oil Chemists Society*, 75 (1998), 897-904.

[122] L. Wiking, V. De Graef, M. Rasmussen and K. Dewettinck, Relations between crystallisation mechanisms and microstructure of milk fat, *International Dairy Journal*, 19 (2009), 424-430.

[123] G. Mazzanti, A.G. Marangoni and S.H.J. Idziak, Synchrotron study on crystallization kinetics of milk fat under shear flow, *Food Research International*, 42 (2009), 682-694.

[124] H.D. Batte and A.G. Marangoni, Fractal growth of milk fat crystals is unaffected by microstructural confinement, *Crystal Growth & Design*, 5 (2005), 1703-1705.

[125] I. Foubert, E. Fredrick, J. Vereecken, M. Sichien and K. Dewettinck, Stop-and-return DSC method to study fat crystallization, *Thermochimica Acta*, 471 (2008), 7-13.

[126] M.C. Michalski, V. Briard and F. Michel, Optical parameters of milk fat globules for laser light scattering measurements, *Lait*, 81 (2001), 787-796.

[127] T. Le Thu, J. Cocquyt, H. Nieuwenhuijse, H. Koman, K. van Lent and P. van der Meeren, Critical comparison of particle size analysis of dairy emulsions by the static laser light scattering and time-of-transition technique, *Milchwissenschaft-Milk Science International*, 61 (2006), 269-273.

[128] A. Tomas, J.L. Courthaudon, D. Paquet and D. Lorient, Effect of surfactant on some physicochemical properties of dairy oil-in-water emulsions, *Food Hydrocolloids*, 8 (1994), 543-553.

[129] A. Tomas, D. Paquet, J.L. Courthaudon and D. Lorient, Effect of fat and protein contents on droplet size and surface protein coverage in dairy emulsions, *Journal of Dairy Science*, 77 (1994), 413-417.

[130] K. Boode, Partial coalescence in oil-in-water emulsions, PhD, Landbouwuniversiteit, Wageningen, 1992, p. 159.

[131] A.S. Akalin, C. Karagozlu, G. Ender and G. Unal, Effects of aging time and storage temperature on the rheological and sensory characteristics of whole ice cream, *Milchwissenschaft-Milk Science International*, 63 (2008), 293-295.

[132] N. Drelon, E. Gravier, L. Daheron, L. Boisserie, A. Omari and F. Leal-Calderon, Influence of tempering on the mechanical properties of whipped dairy creams, *International Dairy Journal*, 16 (2006), 1454-1463.

[133] E. Frede and W. Buchheim, Buttermaking and the churning of blended fat emulsions, *Journal of the Society of Dairy Technology*, 47 (1994), 17-27.

[134] F. Ulberth, Influence of different methods of physical cream ripening on the spreadability of butter, *Milchwissenschaft-Milk Science International*, 44 (1989), 415-417.

[135] H.D. Goff, Instability and partial coalescence in whippable dairy emulsions, *Journal of Dairy Science*, 80 (1997), 2620-2630.

[136] R.G. Jensen, A.M. Ferris and C.J. Lammikeefe, Symposium - Milk-Fat Composition, Function, and Potential for Change - the Composition of Milk-Fat, *Journal of Dairy Science*, 74 (1991), 3228-3243.

[137] K. Smet, K. Coudijzer, E. Fredrick, S. De Campeneere, J. De Block, J. Wouters, K. Raes and K. Dewettinck, Crystallization behavior of milk fat obtained from linseed-fed cows, *Journal of Dairy Science*, 93 (2010), 495-505.

[138] I. Foubert, K. Dewettinck, G. Janssen and P.A. Vanrolleghem, Modelling two-step isothermal fat crystallization, *Journal of Food Engineering*, 75 (2006), 551-559.

[139] E.C.H. van Beresteyn, Polymorphism in milk-fat in relation to solid-liquid ratio, *Netherlands Milk and Dairy Journal*, 26 (1972), 117-130.

[140] K. Larsson, Molecular arrangement in glycerides, *Fett Seifen Anstrichm*, 74 (1972), 136-142.

[141] M. Kellens, Polymorphism of saturated monoacid triacylglycerols, Ph.D, Katholieke Universiteit Leuven, Leuven, Belgium, 1991.

[142] R.E. Timms, Phase behaviour of fats and their mixtures, *Progress in Lipid Research*, 23 (1984), 1-38.

[143] R.E. Timms, The phase behaviour and polymorphism of milk fat, milk fat fractions and fully hardened milk fat, *Australian Journal of Dairy Technology*, 35 (1980), 47-53.

[144] P.S. Dimick, S.Y. Reddy and G.R. Ziegler, Chemical and thermal characteristics of milk-fat fractions isolated by a melt crystallization, *Journal of the American Oil Chemists Society*, 73 (1996), 1647-1652.

[145] I.L. Woodrow and J.M. Deman, Polymorphism in milk fat shown by X-ray diffraction and infrared spectroscopy, *Journal of Dairy Science*, 51 (1968), 996-1000.

[146] G.A. van Aken, E. ten Grotenhuis, A.J. van Langevelde and H. Schenk, Composition and crystallization of milk fat fractions, *Journal of the American Oil Chemists Society*, 76 (1999), 1323-1331.

[147] G.A. van Aken and K.A. Visser, Firmness and crystallization of milk fat in relation to processing conditions, *Journal of Dairy Science*, 83 (2000), 1919-1932.

[148] J.M. Deman, Physical properties of milk fat, *Journal of Dairy Science*, 47 (1964), 1194-1200.

[149] P. Walstra and E.C.H. van Beresteyn, Additional evidence for presence of mixed crystals in milk fat, *Netherlands Milk and Dairy Journal*, 29 (1975), 238-241.

[150] H. Timmen and S. Patton, Milk fat globules- fatty acid composition, size and invivo regulation of fat Liquidity, *Lipids*, 23 (1988), 685-689.

[151] L. Wiking, J. Stagsted, B. Lennart and J.H. Nielsen, Milk fat globule size is affected by fat production in dairy cows, *International Dairy Journal*, 14 (2004), 909-913.

[152] I. Niiya, T. Maruyama, M. Imamura, M. Okada and T. Matsumoto, Effect of emulsifiers on the crystal growth of edible solid fats. III. Effects of saturated fatty acid monoglycerides, *Japanese Journal of Food Science and Technology*, 20 (1973), 182-190.

[153] J. Vereecken, I. Foubert, W. Meeussen, A. Lesaffer and K. Dewettinck, Fat structuring with partial acylglycerols: Effect on solid fat profiles, *European Journal of Lipid Science and Technology*, 111 (2009), 259-272.

[154] E. Davies, E. Dickinson and R.D. Bee, Orthokinetic destabilization of emulsions by saturated and unsaturated monoglycerides, *International Dairy Journal*, 11 (2001), 827-836.

[155] J. Vereecken, W. Meeussen, I. Foubert, A. Lesaffer, J. Wouters and K. Dewettinck, Comparing the crystallization and polymorphic behaviour of saturated and unsaturated monoglycerides, *Food Research International*, 42 (2009), 1415-1425.

[156] E.S. Lutton, C.E. Stauffer, J.B. Martin and A.J. Fehl, Solid and liquid monomolecular film at oil/H_2O Interfaces, *Journal of Colloid and Interface Science*, 30 (1969), 283-&.

[157] N. Krog and K. Larsson, Crystallization at interfaces in food emulsions - A general phenomenon, *Fett Wissenschaft Technologie-Fat Science Technology*, 94 (1992), 55-57.

[158] T. Awad, Y. Hamada and K. Sato, Effects of addition of diacylglycerols on fat crystallization in oil-in-water emulsion, *European Journal of Lipid Science and Technology*, 103 (2001), 735-741.

[159] K. Svennersten-Sjaunja, L. Wiking, A. Edvardsson, A.K. Bavius, L.B. Larsen and J.H. Nielsen, Effect of frequent milking on milk fat and protein, *Journal of Animal and Feed Sciences*, 16 (2007), 151-155.

[160] L. Wiking, J.H. Nielsen, A.K. Bavius, A. Edvardsson and K. Svennersten-Sjaunja, Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition, *Journal of Dairy Science*, 89 (2006), 1004-1009.

[161] P. Walstra, Effect of homogenization on fat globule size distribution in milk, *Netherlands Milk and Dairy Journal*, 29 (1975), 279-294.

[162] D.J. McClements, *Food Emuslions: Principles, Practice and Techniques*, CRC press LLC, Florida, 1999, 378 pp.

[163] K. van Lent, C.T. Le, B. Vanlerberghe and P. Van der Meeren, Effect of formulation on the emulsion and whipping properties of recombined dairy cream, *International Dairy Journal*, 18 (2008), 1003-1010.

[164] Q.Z. Zhao, M.M. Zhao, J.R. Li, B. Yang, G.W. Su, C. Cui and Y.M. Jiang, Effect of hydroxypropyl methylcellulose on the textural and whipping properties of whipped cream, *Food Hydrocolloids*, 23 (2009), 2168-2173.

[165] Q.Z. Zhao, M.M. Zhao, B. Yang and C. Cui, Effect of xanthan gum on the physical properties and textural characteristics of whipped cream, *Food Chemistry*, 116 (2009), 624-628.

[166] P. Walstra, Emulsion Stability, in: *Encyclopedia of Emulsion Technology*, P. Becher, ed, Vol. 4, Marcel Decker, New York, 1996, pp. 1-62.

[167] P. Walstra, Emulsions, in: *Fundamentals of interface and colloid science*, J. Lyklema, ed, Vol. V, Elsevier Academic Press, 2005, pp. 1-94.

[168] S.A. Vanapalli, J. Palanuwech and J.N. Coupland, Stability of emulsions to dispersed phase crystallization: effect of oil type, dispersed phase volume fraction, and cooling rate, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 204 (2002), 227-237.

[169] N.M. Barfod and N. Krog, Destabilization and fat crystallization of whippable emulsions (toppings) studied by pulsed NMR, *Journal of the American Oil Chemists' Society*, 64 (1987), 112-119.

[170] W. Buchheim, Microstructure of Whippable Emulsions, *Kieler Milchwirtschaftliche Forschungsberichte*, 43 (1991), 247-272.

[171] D.F. Darling, Recent advances in the destabilization of dairy emulsions, *Journal of the Society of Dairy Technology*, 35 (1982), 35-35.

[172] S.A. Vanapalli and J.N. Coupland, Emulsions under shear - the formation and properties of partially coalesced lipid structures, *Food Hydrocolloids*, 15 (2001), 507-512.

[173] J. Benjamins, M.H. Vingerhoeds, F.D. Zoet, E.H.A. de Hoog and G.A. van Aken, Partial coalescence as a tool to control sensory perception of emulsions, *Food Hydrocolloids*, 23 (2009), 102-115.

[174] J.H. Labuschagne, Churning in the absence of air, PhD, Agricultural University, Wageningen, 1962.

[175] M.A.J.S. van Boekel, Influence of fat crystals in the oil phase on stability of oil-in-water emulsions, PhD, Wageningen University, Wageningen, 1980.

[176] M.A.J.S. van Boekel and P. Walstra, Stability of oil-in-water emulsions with crystals in the disperse phase, *Colloids and Surfaces*, 3 (1981), 109-118.

[177] H. Oortwijn and P. Walstra, The membranes of recombined fat globules .4. Effects on properties of the recombined Milks, *Netherlands Milk and Dairy Journal*, 36 (1982), 279-290.

[178] K. Boode, C. Bisperink and P. Walstra, Destabilization of O/W emulsions containing fat crystals by temperature cycling, *Colloids and Surfaces*, 61 (1991), 55-74.

[179] K. Boode and P. Walstra, Partial coalescence in oil-in-water Emulsions .1. Nature of the aggregation, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 81 (1993), 121-137.

[180] K. Boode and P. Walstra, The kinetics of partial coalescence in oil-in-water emulsions, in: *Food Colloids and Polymers: Stability and Mechanical properties*, E. Dickinson and P. Walstra, eds, Royal Society of Chemistry, Camebridge, 1993, pp. 23-30.

[181] K. Boode, P. Walstra and A.E.A. Degrootmostert, Partial Coalescence in Oil-in-Water Emulsions .2. Influence of the Properties of the Fat, *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 81 (1993), 139-151.

[182] J.P. Melsen, The stability of recombined milk fat globules, PhD, Landbouwuniversiteit, Wageningen, 1987, p. 146.

[183] M.A.J.S. van Boekel and P. Walstra, Effect of couette-flow on stability of oil-in-water emulsions, *Colloids and Surfaces*, 3 (1981), 99-107.

[184] N.E. Hotrum, M.A.C. Stuart, T. van Vliet, S.F. Avino and G.A. van Aken, Elucidating the relationship between the spreading coefficient, surface-mediated partial coalescence and the whipping time of artificial cream, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 260 (2005), 71-78.

[185] J. Hinrichs, Shear stability of cream containing viscosity increasing additives, *Milchwissenschaft-Milk Science International*, 58 (2003), 130-134.

[186] J. Hinrichs and H.G. Kessler, Fat content of milk and cream and effects on fat globule stability, *Journal of Food Science*, 62 (1997), 992-995.

[187] P. Thanasukarn, R. Pongsawatmanit and D.J. McClements, Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate, *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 246 (2004), 49-59.

[188] F. Thivilliers, N. Drelon, V. Schmitt and F. Leal-Calderon, Bicontinuous emulsion gels induced by partial coalescence: Kinetics and mechanism, *Europhysics Letters*, 76 (2006), 332-338.

[189] E. Davies, E. Dickinson and R. Bee, Shear stability of sodium caseinate emulsions containing monoglyceride and triglyceride crystals, *Food Hydrocolloids*, 14 (2000), 145-153.

[190] F. Thivilliers-Arvis, E. Laurichesse, V. Schmitt and F. Leal-Calderon, Shear-induced instabilities in oil-in-water emulsions comprising partially crystallized droplets, *Langmuir*, 26 (2010), 16782-16790.

[191] K. Golemanov, S. Tcholakova, N.D. Denkov and T. Gurkov, Selection of surfactants for stable paraffin-in-water dispersions, undergoing solid-liquid transition of the dispersed particles, *Langmuir*, 22 (2006), 3560-3569.

[192] W. Xu, A. Nikolov and D.T. Wasan, Shear-induced fat particle structure variation and the stability of food emulsions: II. Effects of surfactants, protein, and fat substitutes, *Journal of Food Engineering*, 66 (2005), 107-116.

[193] P. Walstra, On crystallization habit in fat globules, *Netherlands Milk and Dairy Journal-Nederlands-Nederlands Melk En Zuiveltijdschrift*, 21 (1967), 166-&.

[194] W. Xu, A. Nikolov and D.T. Wasan, Shear-induced fat particle structure variation and the stability of food emulsions: I. Effects of shear history, shear rate and temperature, *Journal of Food Engineering*, 66 (2005), 97-105.

[195] T.A. Mutoh, H. Kubouchi, M. Noda, Y. Shiinoki and Y. Matsumura, Effect of oil-soluble emulsifiers on solidfication of thermally treated creams, *International Dairy Journal*, 17 (2007), 24-28.

[196] M. Noda and H. Yamamoto, Effects of Tempering on Physical-Properties of Whip Cream, *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi*, 41 (1994), 327-334.

[197] T. Sugimoto, T. Mori, J. Mano, T.A. Mutoh, Y. Shiinoki and Y. Matsumura, Effects of fat crystallization on the behavior of proteins and lipids at oil droplet surfaces, *Journal of the American Oil Chemists Society*, 78 (2001), 183-188.

[198] F. Thivilliers, E. Laurichesse, H. Saadaoui, F. Leal-Calderon and V. Schmitt, Thermally induced gelling of oil-in-water emulsions comprising partially crystallized droplets: The impact of interfacial crystals, *Langmuir*, 24 (2008), 13364-13375.

[199] A.L. Marquez, G.G. Palazolo and J.R. Wagner, Cream-like emulsions prepared with soy-milk 3: Effect of temperature cycling, *Grasas Y Aceites*, 56 (2005), 182-187.

[200] J. Giermanska, F. Thivilliers, R. Backov, V. Schmitt, N. Drelon and F. Leal-Calderon, Gelling of oil-in-water emulsions comprising crystallized droplets, *Langmuir*, 23 (2007), 4792-4799.

[201] F. Leal-Calderon, F. Thivilliers and V. Schmitt, Structured emulsions, *Current Opinion in Colloid & Interface Science*, 12 (2007), 206-212.

[202] P. Thanasukarn, R. Pongsawatmanit and D.J. McClements, Influence of emulsifier type on freeze-thaw stability of hydrogenated palm oil-in-water emulsions, *Food Hydrocolloids*, 18 (2004), 1033-1043.

[203] H.D. Goff and W.K. Jordan, Action of emulsifiers in promoting fat destabilization during the manufacture of ice-cream, *Journal of Dairy Science*, 72 (1989), 18-29.

[204] P.L. Marquez, G.G. Palazolo and J.R. Wagner, Cream-like emulsions prepared with soybean milk: Effect of controlled stirring on rheological behaviour., *Grasas Y Aceites*, 56 (2005), 89-95.

[205] T.A. Mutoh, S. Nakagawa, M. Noda, Y. Shiinoki and Y. Matsumura, Relationship between characteristics of oil droplets and solidification of thermally treated creams, *Journal of the American Oil Chemists Society*, 78 (2001), 177-182.

[206] E. Dickinson, Milk protein interfacial layers and the relationship to emulsion stability and rheology, *Colloids and Surfaces B-Biointerfaces*, 20 (2001), 197-210.

[207] B.M.C. Pelan, K.M. Watts, I.J. Campbell and A. Lips, The stability of aerated milk protein emulsions in the presence of small molecule surfactants, *Journal of Dairy Science*, 80 (1997), 2631-2638.

[208] Q.Z. Zhao, M.M. Zhao, J.S. Wang, C.H. Wang and B. Yang, Effects of sodium caseinate and whey proteins on whipping properties and texture characteristics of whipped cream, *Journal of Food Process Engineering*, 31 (2008), 671-683.

[209] P. Relkin, A. Ait-Taleb, S. Sourdet and P.Y. Fosseux, Thermal behavior of fat droplets as related to adsorbed milk proteins in complex food emulsions. A DSC study, *Journal of the American Oil Chemists Society*, 80 (2003), 741-746.

[210] K.I. Segall and H.D. Goff, Influence of adsorbed milk protein type and surface concentration on the quiescent and shear stability of butteroil emulsions, *International Dairy Journal*, 9 (1999), 683-691.

[211] S.A. Vanapalli, J. Palanuwech and J.N. Coupland, Influence of fat crystallization on the stability of flocculated emulsions, *Journal of Agricultural and Food Chemistry*, 50 (2002), 5224-5228.

[212] S. Sourdet, P. Relkin, P.Y. Fosseux and V. Aubry, Composition of fat protein layer in complex food emulsions at various weight ratios of casein-to-whey proteins, *Lait*, 82 (2002), 567-578.

[213] P. Relkin, S. Sourdet and P.Y. Fosseux, Fat crystallization in complex food emulsions - Effects of adsorbed milk proteins and of a whipping process, *Journal of Thermal Analysis and Calorimetry*, 71 (2003), 187-195.

[214] K.P. Das and J.E. Kinsella, Effect of heat denaturation on the adsorption of beta-lactoglobulin at the oil-water interface and on coalescence stability of emulsions, *Journal of Colloid and Interface Science*, 139 (1990), 551-560.

[215] J.E. Kinsella, Milk proteins - physicochemical and functional properties, *Crc Critical Reviews in Food Science and Nutrition*, 21 (1984), 197-262.

[216] K.I. Segall and H.D. Goff, Secondary adsorption of milk proteins from the continuous phase to the oil-water interface in dairy emulsions, *International Dairy Journal*, 12 (2002), 889-897.

[217] S. Kiokias and A. Bot, Effect of denaturation on temperature cycling stability of heated acidified protein-stabilised o/w emulsion gels, *Food Hydrocolloids*, 19 (2005), 493-501.

[218] S. Kiokias and A. Bot, Temperature cycling stability of pre-heated acidified whey protein-stabilised o/w emulsion gels in relation to the internal surface area of the emulsion, *Food Hydrocolloids*, 20 (2006), 245-252.

[219] S. Kiokias, C.K. Reiffers-Magnani and A. Bot, Stability of whey-protein-stabilized oil-in-water emulsions during chilled storage and temperature cycling, *Journal of Agricultural and Food Chemistry*, 52 (2004), 3823-3830.

[220] M. Cornec, P.J. Wilde, P.A. Gunning, A.R. Mackie, F.A. Husband, M.L. Parker and D.C. Clark, Emulsion stability as affected by competitive adsorption between an oil-soluble emulsifier and milk proteins at the interface, *Journal of Food Science*, 63 (1998), 39-43.

[221] E. Dickinson and S.T. Hong, Surface coverage of beta-lactoglobulin at the oil-water interface - influence of protein heat-treatment and various emulsifiers, *Journal of Agricultural and Food Chemistry*, 42 (1994), 1602-1606.

[222] E. Dickinson, G. Iveson and S. Tanai, Competitive adsorption in protein-stabilized emulsions containing oil-soluble and water-soluble surfactants, in: *International Symp on Food Colloids and Polymers*, E. Dickinson and P. Walstra, eds, Lunteren, Netherlands, 1992, pp. 312-322.

[223] E. Dickinson, R.K. Owusu, S. Tan and A. Williams, Oil-soluble surfactants have little effect on competitive adsorption of alpha-lactalbumin and beta-lacotglobulin in emulsions, *Journal of Food Science*, 58 (1993), 295-298.

[224] A.R. Mackie, A.P. Gunning, P.J. Wilde and V.J. Morris, Orogenic displacement of protein from the oil/water interface, *Langmuir*, 16 (2000), 2242-2247.

[225] J.L. Courthaudon, E. Dickinson and W.W. Christie, Competitive adsorption of lecithin and beta-casein in oil in water emulsions, *Journal of Agricultural and Food Chemistry*, 39 (1991), 1365-1368.

[226] J.L. Courthaudon, E. Dickinson and D.G. Dalgleish, Competitive adsorption of beta-casein and nonionic surfactants in oil-in-water emulsions, *Journal of Colloid and Interface Science*, 145 (1991), 390-395.

[227] J.L. Courthaudon, E. Dickinson, Y. Matsumura and A. Williams, Influence of emulsifiers on the competitive adsorption of whey proteins in emulsions I, *Food Structure*, 10 (1991), 109-115.

[228] J.S. Chen and E. Dickinson, Time-dependent competitive adsorption of milk-proteins and surfactants in oil-in-water emulsions, *Journal of the Science of Food and Agriculture*, 62 (1993), 283-289.

[229] J.S. Chen and E. Dickinson, Viscoelastic properties of protein-stabilized emulsions: Effect of protein-surfactant interactions, *Journal of Agricultural and Food Chemistry*, 46 (1998), 91-97.

[230] S.E. Euston, H. Singh, P.A. Munro and D.G. Dalgleish, Oil-in-water emulsions stabilized by sodium caseinate or whey protein isolate as influenced by glycerol monostearate, *Journal of Food Science*, 61 (1996), 916-920.

[231] S.E. Euston, H. Singh, P.A. Munro and D.G. Dalgleish, Competitive adsorption between sodium caseinate and oil-soluble and water-soluble surfactants in oil-in-water emulsions, *Journal of Food Science*, 60 (1995), 1124-1131.

[232] C. Granger, P. Barey, N. Combe, P. Veschambre and M. Cansell, Influence of the fat characteristics on the physicochemical behavior of oil-in-water emulsions based on milk proteins-glycerol esters mixtures, *Colloids and Surfaces B-Biointerfaces*, 32 (2003), 353-363.

[233] C. Granger, P. Barey, P. Veschambre and M. Cansell, Physicochemical behavior of oil-in-water emulsions: influence of milk protein mixtures, glycerol ester mixtures and fat characteristics, *Colloids and Surfaces B-Biointerfaces*, 42 (2005), 235-243.

[234] P. Van der Meeren, M. El-Bakry, N. Neirynck and P. Noppe, Influence of hydrolysed lecithin addition on protein adsorption and heat stability of a sterilised coffee cream simulant, *International Dairy Journal*, 15 (2005), 1235-1243.

[235] A.R. Mackie, A.P. Gunning, P.J. Wilde and V.J. Morris, Orogenic displacement of protein from the air/water interface by competitive adsorption, *Journal of Colloid and Interface Science*, 210 (1999), 157-166.

[236] V.J. Morris and A.P. Gunning, Microscopy, microstructure and displacement of proteins from interfaces: implications for food quality and digestion, *Soft Matter*, 4 (2008), 943-951.

[237] V.J. Morris, P.J. Wilde, A.R. Mackie and A.P. Gunning, Surfactant-protein interactions at air-water and oil-water interfaces observed by atomic force microscopy, in: *10th Gums and Stabilisers for the Food Industry Conference*, P.A. Williams and G.O. Phillips, eds, Wrexham, Wales, 1999, pp. 328-336.

[238] A. Mackie and P. Wilde, The role of interactions in defining the structure of mixed protein-surfactant interfaces, in: *International Workshop on Bubble and Drop Interfaces*, Genoa, ITALY, 2005, pp. 3-13.

[239] J. Palanuwech and J.N. Coupland, Effect of surfactant type on the stability of oil-in-water emulsions to dispersed phase crystallization, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 223 (2003), 251-262.

[240] P. Wilde, A. Mackie, F. Husband, P. Gunning and V. Morris, Proteins and emulsifiers at liquid interfaces, *Advances in Colloid and Interface Science*, 108-09 (2004), 63-71.

[241] N.M. Barfod, N. Krog, G. Larsen and W. Buchheim, Effects of emulsifiers on protein-fat interaction in ice-cream mix during aging .1. quantitative-analyses, *Fett Wissenschaft Technologie-Fat Science Technology*, 93 (1991), 24-29.

[242] N.M. Barfod and N. Krog, Lipid -protein interactions and fat crystallization in whippable emulsions, *Journal of the American Oil Chemists Society*, 62 (1985), 628-629.

[243] A. Tual, E. Bourles, P. Barey, A. Houdoux, M. Desprairies and J.L. Courthaudon, Effect of surfactant sucrose ester on physico-chemical properties of dairy whipped emulsions, *Sciences Des Aliments*, 25 (2005), 455-466.

[244] A. Tual, E. Bourles, P. Barey, A. Houdoux, M. Desprairies and J.L. Courthaudon, Effect of surfactant sucrose ester on physical properties of dairy whipped emulsions in relation to those of O/W interfacial layers, *Journal of Colloid and Interface Science*, 295 (2006), 495-503.

[245] H. Bunjes and M.H.J. Koch, Saturated phospholipids promote crystallization but slow down polymorphic transitions in triglyceride nanoparticles, *Journal of Controlled Release*, 107 (2005), 229-243.

[246] T. Harada and K. Yokomizo, Demulsification of oil-in-water emulsion under freezing conditions: Effect of crystal structure modifier, *Journal of the American Oil Chemists Society*, 77 (2000), 859-863.

[247] I. Heertje, Microstructural studies in fat research, *Food Structure*, 12 (1993), 77-94.

[248] S. Miura, A. Yamamoto and H. Konishi, Effect of agglomeration of triacylglycerols on the stabilization of a model cream, *European Journal of Lipid Science and Technology*, 104 (2002), 222-227.

[249] S. Miura, A. Yamamoto and K. Sato, Effect of monoacylglycerols on the stability a of model cream using palm oil, *European Journal of Lipid Science and Technology*, 104 (2002), 819-824.

[250] S. Miura, M. Tanaka, A. Suzuki and K. Sato, Application of phospholipids extracted from bovine milk to the reconstitution of cream using butter oil, *Journal of the American Oil Chemists Society*, 81 (2004), 97-100.

[251] Y. Fang and D.G. Dalgleish, Comparison of the effects of three different phosphatidylcholines on casein-stabilized oil-in-water emulsions, *Journal of the American Oil Chemists Society*, 73 (1996), 437-442.

[252] I.N. Hayati, Y.B.C. Man, C.P. Tan and I.N. Aini, Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides, *Food Hydrocolloids*, 23 (2009), 233-243.

[253] B.E. Brooker, M. Anderson and A.T. Andrews, The development of structure in whipped cream, *Food Microstructure*, 5 (1986), 277-285.

[254] M. Anderson, B.E. Brooker and E.C. Needs, The role of proteins in the stabilization/destabilization of dairy foams, in: *Food emulsions and foams*, E. Dickinson, ed, The royal society of chemistry, London, 1987, pp. 100-109.

[255] J. Van Camp, S. Van Calenberg, P. Van Oostveldt and A. Huyghebaert, Aerating properties of emulsions stabilized by sodium caseinate and whey protein concentrate, *Milchwissenschaft-Milk Science International*, 51 (1996), 310-315.

[256] S.Y. Lee and C.V. Morr, Fixation staining methods for examining microstructure in whipped cream by elektron-micoscopy, *Journal of Food Science*, 58 (1993), 124-127.

[257] A.K. Smith, H.D. Goff and B.D. Sun, Freeze-substitution and low-temperature embedding of dairy products for transmission electron microscopy, *Journal of Microscopy-Oxford*, 213 (2004), 63-69.

[258] N. Krog, N.M. Barfod and W. Buchheim, Protein-fat-surfactants interactions in whippable emulsions, in: *Food emulsions and foams*, E. Dickinson, ed, The royal society of chemistry, London, 1987, pp. 100-109.

[259] E.C. Needs and A. Huitson, The contribution of milk serum-proteins to the development of whipped cream structure, *Food Structure*, 10 (1991), 353-360.

[260] E. Graf and H.R. Muller, Fine structure and whippability of sterilized cream, *Milchwissenschaft-Milk Science International*, 20 (1965), 302-308.

[261] G.A. van Aken, Aeration of emulsions by whipping, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 190 (2001), 333-354.

[262] K. Nesaretnam, N. Robertson, Y. Basiron and C.S. Macphie, Application of hydrogenated palm kernel oil and palm stearin in whipping cream, *Journal of the Science of Food and Agriculture*, 61 (1993), 401-407.

[263] Y. Chang and R.W. Hartel, Stability of air cells in ice cream during hardening and storage, *Journal of Food Engineering*, 55 (2002), 59-70.

[264] A. Riaublanc, M. Anton, F. Mariette, C. Georges, E. Gravier, N. Drelon, A. Omari and F. Leal-Calderon, Impact of fat crystals on the foaming capacity and stability of whipped creams, *Sciences Des Aliments*, 25 (2005), 427-441.

[265] A.K. Smith, H.D. Goff and Y. Kakuda, Whipped cream structure measured by quantitative stereology, *Journal of Dairy Science*, 82 (1999), 1635-1642.

[266] K. Shamsi, Y.B.C. Man, M.S.A. Yusoff and S. Jinap, A comparative study of dairy whipping cream and palm oil-based whipping cream in terms of FA composition and foam stability, *Journal of the American Oil Chemists Society*, 79 (2002), 583-588.

[267] A.K. Smith, The stability of whipped cream structure: The effect of heat treatment and the addition of stabilizer/emulsifier, PhD, University of Guelph, Guelph, 1999, p. 166.

[268] E. Gravier, N. Drelon, L. Boisserie, A. Omari and F. Leal-Calderon, Consolidation of foams deriving from emulsions by temperature cycling ("tempering"), *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 282 (2006), 360-368.

[269] D.G. Dalgleish, Food emulsions - their structures and structure-forming properties, *Food Hydrocolloids*, 20 (2006), 415-422.

[270] A. Prins, Theory and practice of formation and stability of food foams, in: *Food emulsions and foams*, E. Dickinson, ed, The royal society of chemistry, London, 1987, pp. 100-109.

[271] N.E. Hotrum, M.A.C. Stuart, T. van Vliet and G.A. van Aken, Entering and spreading of protein-stabilized emulsion droplets at the expanding air-water interface, in: *Food Colloids, Biopolymers and Materials*, E. Dickinson and T. VanVliet, eds, 2003, pp. 192-199.

[272] N.E. Hotrum, M.A.C. Stuart, T. van Vliet and G.A. van Aken, Flow and fracture phenomena in adsorbed protein layers at the air/water interface in connection with spreading oil droplets, *Langmuir*, 19 (2003), 10210-10216.

[273] N.E. Hotrum, M.A.C. Stuart, T. van Vliet and G.A. van Aken, Spreading of partially crystallized oil droplets on an air/water interface, *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 240 (2004), 83-92.

[274] N.E. Hotrum, M.A.C. Stuart, T. van Vliet and G.A. van Aken, Proposing a relationship between the spreading coefficient and the whipping time of cream, in: *Food Colloids: Interactions, Microstructure and Processing*, E. Dickinson, ed, 2005, pp. 317-325.

[275] N.E. Hotrum, T. van Vliet, M.A.C. Stuart and G.A. van Aken, Monitoring entering and spreading of emulsion droplets at an expanding air/water interface: A novel technique, *Journal of Colloid and Interface Science*, 247 (2002), 125-131.

[276] E.P. Schokker, M.A. Bos, A.J. Kuijpers, M.E. Wijnen and P. Walstra, Spreading of oil from protein stabilised emulsions at air/water interfaces, *Colloids and Surfaces B-Biointerfaces*, 26 (2002), 315-327.

[277] H. Besner and H.G. Kessler, Interfacial interaction during the foaming of nonhomogenized cream, *Milchwissenschaft-Milk Science International*, 53 (1998), 682-686.

[278] K. Ihara, K. Habara, Y. Ozaki, K. Nakamura, H. Ochi, H. Saito, H. Asaoka, M. Uozumi, N. Ichihashi and K. Iwatsuki, Influence of whipping temperature on the whipping properties and rheological characteristics of whipped cream, *Journal of Dairy Science*, 93 (2010), 2887-2895.

[279] M. Noda and H. Yamamoto, The effect of homogenization on the physical properties of whip cream, *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi*, 43 (1996), 896-903.

[280] Z. Emam-Djome, M.E. Mousavi, A.V. Ghorbani and A. Madadlou, Effect of whey protein concentrate addition on the physical properties of homogenized sweetened dairy creams, *International Journal of Dairy Technology*, 61 (2008), 183-191.

[281] H. Besner and H.G. Kessler, Model for foam stabilization of homogenized cream by comparative examinations with non-homogenized cream, *Milchwissenschaft-Milk Science International*, 53 (1998), 609-612.

[282] F.G. Kieseker and J.G. Zadow, The whipping properties of homogenized and sterilized cream, *Australian Journal of Dairy Technology*, 28 (1973), 108-113.

[283] N.E. Hotrum, Emulsion droplet spreading at air/water interfaces: mechanisms and relevance to the whipping of cream, PhD, Wageningen University, Wageningen, 2004, p. 125.

[284] D. Kalnin, G. Garnaud, H. Amenitsch and M. Ollivon, Monitoring fat crystallization in aerated food emulsions by combined DSC and time-resolved synchrotron X-ray diffraction, *Food Research International*, 35 (2002), 927-934.

[285] J.G. Zadow and F.G. Kieseker, Manufacture of recombined whipping cream, *Australian Journal of Dairy Technology*, 30 (1975), 114-117.

[286] M.D. Eisner, S.A.K. Jeelani and E.J. Windhab, Stability of foams containing proteins, fat particles and nonionic surfactants, *Chemical Engineering Science*, 62 (2007), 1974-1987.

[287] S. Sourdet, P. Relkin and B. Cesar, Effects of milk protein type and pre-heating on physical stability of whipped and frozen emulsions, *Colloids and Surfaces B-Biointerfaces*, 31 (2003), 55-64.

[288] M.M. Camacho, N. Martinez-Navarrete and A. Chiralt, Influence of locust bean gum/lambda-carrageenan mixtures on whipping and mechanical properties and stability of dairy creams, *Food Research International*, 31 (1998), 653-658.

[289] D.W. Stanley, H.D. Goff and A.K. Smith, Texture-structure relationships in foamed dairy emulsions, *Food Research International*, 29 (1996), 1-13.

[290] K.E. Allen, B.S. Murray and E. Dickinson, Development of a model whipped cream: Effects of emulsion droplet liquid/solid character and added hydrocolloid, *Food Hydrocolloids*, 22 (2008), 690-699.

[291] K.E. Allen, B.S. Murray and E. Dickinson, Whipped cream-like textured systems based on acidified caseinate-stabilized oil-in-water emulsions, *International Dairy Journal*, 18 (2008), 1011-1021.

[292] A. Bazmi, A. Duquenoy and P. Relkin, Aeration of low fat dairy emulsions: Effects of saturated-unsaturated triglycerides, *International Dairy Journal*, 17 (2007), 1021-1027.

[293] G.M. Pilhofer, H.C. Lee, M.J. McCarthy, P.S. Tong and J.B. German, Functionality of milk-fat in foam formation and stability, *Journal of Dairy Science*, 77 (1994), 55-63.

[294] S. Vandebril, A. Franck, G.G. Fuller, P. Moldenaers and J. Vermant, A double wall-ring geometry for interfacial shear rheometry, *Rheologica Acta*, 49, 131-144.

[295] F. van de Velde, F. Weinbreck, M.W. Edelman, E. van der Linden and R.H. Tromp, Visualisation of biopolymer mixtures using confocal scanning laser microscopy (CSLM) and covalent labelling techniques, *Colloids and Surfaces B-Biointerfaces*, 31 (2003), 159-168.

[296] H. Maeda, N. Ishida, H. Kawauchi and K. Tuzimura, Reaction of fluorescein-isothiocyanate with proteins and amino acids, *Journal of Biochemistry*, 65 (1969), 777-&.

[297] K. Van Lent, B. Vanlerberghe, P. Van Oostveldt, O. Thas and P. Van der Meeren, Determination of water droplet size distribution in butter: Pulsed field gradient NMR in comparison with confocal scanning laser microscopy, *International Dairy Journal*, 18 (2008), 12-22.

[298] V. De Graef, Microstructural properties of isothermal palm oil crystallization, PhD, Ghent Univeristy, Ghent, 2009, p. 191.

[299] S. Patton and G.E. Huston, A method for isolation of milk-fat globules, *Lipids*, 21 (1986), 170-174.

[300] D.G. Dalgleish and E.W. Robson, Centrifugal fractionation of homogenized milks, *Journal of Dairy Research*, 52 (1985), 539-546.

[301] R. Jenness and J. Koops, Preparation and properties of a salt solution which simulates milk ultrafiltrate, *Nederlands Melk-En Zuiveltijdschrift*, 16 (1962), 153-164.

[302] H. Egan, R.S. Kirk and R. Sawyer, *Pearson's chemical analysis of foods* Churchill Livingstone, New York, 1981.

[303] IDF, *Milk. Determination of nitrogen content. IDF Standard 20B*, International Dairy Federation, Brussels, Belgium, 1993.

[304] K.E. Allen, E. Dickinson and B. Murray, Acidified sodium caseinate emulsion foams containing liquid fat: A comparison with whipped cream, *Lwt-Food Science and Technology*, 39 (2006), 225-234.

[305] A.K. Smith, H.D. Goff and Y. Kakuda, Microstructure and rheological properties of whipped cream as affected by heat treatment and addition of stabilizer, *International Dairy Journal*, 10 (2000), 295-301.

[306] A.K. Smith, Y. Kakuda and H.D. Goff, Changes in protein and fat structure in whipped cream caused by heat treatment and addition of stabilizer to the cream, *Food Research International*, 33 (2000), 697-706.

[307] J. Guery, E. Bertrand, C. Rouzeau, P. Levitz, D.A. Weitz and J. Bibette, Irreversible shear-activated aggregation in non-Brownian suspensions, *Physical Review Letters*, 96 (2006).

[308] Z. Zhang and H.D. Goff, On fat destabilization and composition of the air interface in ice cream containing saturated and unsaturated monoglyceride, *International Dairy Journal*, 15 (2005), 495-500.

[309] H. Oortwijn and P. Walstra, Membranes of recombined fat globules .2. Composition, *Netherlands Milk and Dairy Journal*, 33 (1979), 134-154.

[310] A. Rahman and P. Sherman, Interaction of milk-proteins with monoglycerides and diglycerides, *Colloid and Polymer Science*, 260 (1982), 1035-1041.

[311] G. Doxastakis and P. Sherman, The interaction of sodium caseinate with monoglyceride and diglyceride at the oil-water interface in corn oil-in-water emulsions and its effect on emulsion stability, *Colloid and Polymer Science*, 262 (1984), 902-905.

[312] G. Doxastakis and P. Sherman, The interaction of sodium caseinate with monoglyceride and diglyceride at the oil-water interface and its effect on interfacial rheological properties, *Colloid and Polymer Science*, 264 (1986), 254-259.

[313] B. Murray and E. Dickinson, Interfacial rheology and the dynamic properties of adsorbed films of food proteins and surfactants, *Food Science and Technology International*, 2 (1996), 131-145.

[314] M.A. Bos and T. van Vliet, Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science*, 91 (2001), 437-471.

[315] S. Roth, B.S. Murray and E. Dickinson, Interfacial shear rheology of aged and heat-treated beta-lactoglobulin films: Displacement by nonionic surfactant, *Journal of Agricultural and Food Chemistry*, 48 (2000), 1491-1497.

[316] M. Golding and A. Sein, Surface rheology of aqueous casein-monoglyceride dispersions, *Food Hydrocolloids*, 18 (2004), 451-461.

[317] S. Rouimi, C. Schorsch, C. Valentini and S. Vaslin, Foam stability and interfacial properties of milk protein-surfactant systems, *Food Hydrocolloids*, 19 (2005), 467-478.

[318] C.C. Sanchez and J.M.R. Patino, Flow-induced molecular segregation in beta-casein-monoglyceride mixed films spread at the air-water interface, *Langmuir*, 20 (2004), 6327-6334.

[319] C.C. Sanchez and J.M.R. Patino, Surface shear rheology of WPI-monoglyceride mixed films spread at the air-water interface, *Colloids and Surfaces B-Biointerfaces*, 36 (2004), 57-69.

[320] J.C. Arboleya, M.J. Ridout and P.J. Wilde, Rheological behaviour of aerated palm kernel oil/water emulsions, *Food Hydrocolloids*, 23 (2009), 1358-1365.

ANNEXES

ANNEX I

Solid fat content (SFC in %) of recombined creams with different types and concentrations of monoacylglycerols during storage at 5℃ for 2h, 1 day and 5 days.

[MAG]	2h	1d	5d
0% MAG	46,99 ± 1,03^{a}	50,73 ± 0,41^{a}	53,86 ± 1,10^{a}
0.2% MAG-S	49,15 ± 0,55^{b}	52,16 ± 0,21^{b}	53,23 ± 0,25^{a}
0.5% MAG-S	50,74 ± 1,39^{b}	53,51 ± 1,37^{b}	53,64 ± 1,61^{a}
0.2% MAG-L	48,90 ± 0,50^{a}	51,91 ± 0,75^{a}	52,90 ± 0,57^{a}
0.5% MAG-L	48,47 ± 0,51^{a}	52,30 ± 0,59^{a}	53,56 ± 0,53^{a}
0% MAG	48,05 ± 0,52^{c}	50,83 ± 0,78^{a}	52,48 ± 0,10^{a}
0.2% MAG-L	47,59 ± 0,21^{c}	49,99 ± 0,60^{a}	51,83 ± 0,55^{a}

$^{a\text{-}c}$ different letters show significant differences ($p < 0.05$) between different types and concentration of MAGs within one experimental set.

ANNEX II

Whipping properties of natural cream whipped at different whipping temperatures (T_{wh}).

T_{wh} (℃)	t_{wh} (min)	***Overrun*** (%)	***Firmness*** (N)	
			1h at 5℃	24h at 5℃
5	13.18 ± 1.67^{a}	140.63 ± 0.29^{a}	0.80 ± 0.35^{a}	0.85 ± 0.24^{a}
10	11.23 ± 0.32^{a}	125.30 ± 1.70^{b}	0.90 ± 0.08^{a}	0.98 ± 0.02^{a}
15	9.28 ± 0.35^{b}	93.09 ± 3.38^{c}	0.80 ± 0.04^{a}	1.44 ± 0.04^{b}
20	7.68 ± 1.91^{b,c}	70.45 ± 3.33^{d}	1.63 ± 0.32^{b}	2.60 ± 0.04^{c}
25	5.04 ± 0.46^{c}	67.72 ± 6.23^{d}	2.30 ± 0.28^{b}	6.86 ± 1.28^{d}

$^{a\text{-}d}$ different letters show significant differences ($p < 0.05$).

ANNEX III

Whipping properties of natural cream at different fat contents (%).

Fat content (%)	t_{wh} (min)	***Overrun*** (%)	***Serum loss*** (%)		***Firmness*** (N)	
			1h at 20℃	24h at 5℃	1h at 5℃	24h at 5℃
35%	8.0^{a}	128.3^{a}	0.0^{a}	0.7^{a}	0.98^{a}	1.26^{a}
30%	10.1^{b}	125.4^{a}	1.8^{b}	3.5^{b}	1.15^{a}	1.21^{a}
25%	15.8^{c}	138.0^{b}	7.7^{c}	14.3^{c}	0.72^{b}	0.84^{b}

$^{a\text{-}c}$different letters show significant differences ($p < 0.05$).

ANNEX IV

Whipping properties of recombined cream with different concentration of monacylglycerols rich in oleic acid (MAG-O).

[MAG-O] (%)	t_{wh} ***(min)***	***Overrun (%)***	***Serum loss (%)***		***Firmness (N)***	
			1h at 20℃	24h at 5℃	1h at 5℃	24h at 5℃
0%	7.61^{a}	106.75^{a}	1.91^{a}	13.64^{a}	0.63^{a}	0.63^{a}
0.035%	6.49^{b}	107.35^{a}	1.82^{a}	13.25^{a}	0.64^{a}	0.63^{a}
0.07%	5.78^{c}	103.67^{a,b}	0.92^{b}	6.92^{b}	1.04^{b}	1.03^{b}
0.10%	5.56^{c}	101.03^{b}	0.20^{c}	0.81^{c}	1.37^{c}	1.39^{c}
0.20%	4.52^{d}	100.44^{b}	0.01^{c}	0.75^{c}	1.45^{c}	1.36^{b,c}

$^{a\text{-}d}$ different letters show significant differences ($p < 0.05$).

ANNEX V

Whipping properties of recombined cream with different concentration of monoacylglycerols rich in stearic acid (MAG-S).

[MAG-S] (%)	t_{wh} (min)	***Overrun*** (%)	***Serum loss*** (%)		***Firmness*** (N)	
			1h at 20℃	24h at 5℃	1h at 5℃	24h at 5℃
0%	5.00^{a}	124.96^{a}	4.12^{a}	14.25^{a}	1.26^{a}	1.38^{a}
0.04%	5.10^{a}	135.78^{b}	2.05^{b}	15.96^{a}	1.03^{b}	1.00^{b}
0.07%	5.25^{b}	139.03^{c}	$3.66^{a,f}$	22.57^{b}	$0.93^{b,c}$	0.75^{c}
0.10%	6.00^{c}	149.39^{d}	$5.05^{c,f,g}$	27.92^{b}	0.86^{c}	0.49^{d}
0.20%	8.33^{d}	165.31^{e}	$6.40^{d,f,g}$	36.96^{c}	0.70^{e}	0.26^{e}
0.50%	20.00^{e}	195.61^{f}	$7.31^{e,g}$	51.18^{d}	0.22^{f}	0.19^{e}

$^{a\text{-}g}$different letters show significant differences ($p < 0.05$).

ANNEX VI

Whipping properties of recombined cream without and with 0.2% monoacylglycerols rich in oleic (MAG-O) or rich in stearic (MAG-S) acid or with 0.2% of a 50:50 mixture of both monoacylglycerols (MAG-OS).

[MAG] (%)	t_{wh} (min)	***Overrun*** (%)	***Serum loss*** (%)		***Firmness*** (N)	
			1h at 20℃	24h at 5℃	1h at 5℃	24h at 5℃
0% MAG	5.55^{a}	114.86^{a}	1.19^{a}	12.80^{a}	0.77^{a}	0.67^{a}
0.2% MAG-O	4.19^{b}	95.62^{b}	0.00^{b}	0.02^{b}	1.49^{b}	1.76^{b}
0.2% MAG-O	8.43^{c}	153.97^{c}	1.68^{c}	60.72^{c}	0.28^{c}	0.26^{c}
0.2% MAG-OS	7.33^{d}	139.56^{d}	0.62^{a}	15.90^{d}	0.58^{d}	0.37^{c}

$^{a\text{-}d}$different letters show significant differences ($p < 0.05$).

ANNEX VII

Whipping properties of recombined cream without MAGs and with MAG-O at different fat contents.

Fat content (%)	t_{wh} (min)		***Overrun (%)***	
	0% MAGs	0.2% MAG-O	0% MAGs	0.2% MAG-O
35	$6.2^{a,x}$	$4.0^{a,y}$	$125.6^{a,x}$	$92.5^{a,y}$
30	$8.3^{b,c,x}$	$4.7^{b,c,y}$	$127.4^{a,x}$	$112.6^{b,y}$
25	$10.2^{c,x}$	$5.6^{c,y}$	$150.7^{b,x}$	$126.6^{c,y}$

Fat content (%)	***Serum loss*** (%)			
	After 1h at 20℃		***After 24h at 5℃***	
	0% MAGs	0.2% MAG-O	0% MAGs	0.2% MAG-O
35	$0.80.1^{a,x}$	$-^{a,y}$	$13.8^{a,x}$	$-^{a,y}$
30	$4.9^{a,x}$	1.5^{b}	$15.8^{a,x}$	$1.6^{b,y}$
25	$11.1^{b,x}$	$7.9^{,x}$	$35.0^{b,x}$	$15.8^{c,y}$

Fat content (%)	***Firmness*** (N)			
	After 1h at 5℃		***After 24h at 5℃***	
	0% MAGs	0.2% MAG-O	0% MAGs	0.2% MAG-O
35	$0.66^{a,b,x}$	$1.70^{a,y}$	$0.63^{a,b,x}$	$1.71^{a,y}$
30	$0.75^{a,x}$	$1.15^{b,y}$	$0.75^{a,x}$	$1.16^{b,y}$
25	$0.58^{b,x}$	$0.78^{c,y}$	$0.51^{b,x}$	$0.75^{c,y}$

$^{a\text{-}c}$different letters show significant differences between creams with a different fat content ($p < 0.05$).
$^{x\text{-}y}$ different letters show significant differences between creams with and without MAG-O ($p < 0.05$).

CURRICULUM VITAE

Eveline Fredrick, geboren te Gent op 24 juni 1982, behaalde in 2000 het diploma Hoger Secundair Onderwijs, richting Wetenschappen-Wiskunde aan het Onze-Lieve-Vrouw Ten Doorn instituut te Eeklo. In 2005 studeerde zij met grote onderscheiding af als Bio-ingenieur in de Scheikunde aan de Universiteit Gent. Nadien, in Juli 2008, behaalde zij met onderscheiding het diploma van geaggregeerde voor het secundair onderwijs (AILO toegepaste biologische wetenschappen) aan de Universiteit Gent.

Vanaf december 2005 startte zij als wetenschappelijk medewerker een onderzoeksproject rond de rol van vetkristallisatie in opgeklopte zuivelproducten aan het Laboratorium voor Levensmiddelentechnologie en -proceskunde (Vakgroep Voedselveiligheid en Voedselkwaliteit, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent) in samenwerking met Royal Friesland Foods en o.l.v Prof. dr. ir Koen Dewettinck. Sinds 1 december 2007 tot op heden werkte zij als academisch assisterend personeel in de zuiveltechnologie aan datzelfde labo. Als assistent werd het reeds eerder gestarte onderzoeksproject verdergezet en dit resulteerde in meerdere publicaties in peer-reviewed wetenschappelijke tijdschriften en in presentaties op internationale wetenschappelijke conferenties. Daarnaast participeerde zij als assistent in de dienstverlenings- en onderwijsactiviteiten van de vakgroep. Zo verzorgde zij de praktische oefeningen van vakken als formulering en structurering van levensmiddelen, technologie van de zuivelproducten, van de dierlijke en van de plantaardige producten en begeleidde ze verschillende thesisstudenten. Daarnaast coördineerde zij in 2008 en 2010 het intensieve trainingsprogramma (ITP) ‘Dairy Technologie’ voor studenten uit ontwikkelingslanden.

Scientific output

A1-Publicaties

Fredrick, E., Van de Walle, D., Walstra, P., Zijtveld, H., Fischer, S., Van der Meeren, P. & Dewettinck, K. *(2011)*. Isothermal crystallization behavior of milk fat in bulk and emulsified state. *International Dairy Journal, 21, 685-695 (IF: 2,409)*.

Fredrick, E., Walstra, P. & Dewettinck, K. *(2010).* Factors governing partial coalescence in oil-in-water emulsions. *Advances in Colloid and Interface Science,* 153*(1-2)*, 30-42 *(IF: 5,675)*.

Smet, K., Coudijzer, K., Fredrick, E., De Campeneere, S., De Block, J., Wouters, J., Raes, K. & Dewettinck, K. *(2010)*. Crystallization behavior of milk fat obtained from linseed-fed cows. *Journal of Dairy Science,* 93*(2)*, 495-505 *(IF: 2,463)*.

Calliauw, G., Fredrick, E., Gibon, V., De Greyt, W., Wouters, J., Foubert, I. & Dewettinck, K. *(2010)*. On the fractional crystallization of palm olein: Solid solutions and eutectic solidification. *Food Research International,* 43*(4)*, 972-981 *(IF: 2,414)*.

Wiking, L., Fredrick, E. & Dewettinck, K. *(2009).* Phospholipids delay crystallisation of milk fat. *Milchwissenschaft-Milk Science International,* 64*(4),* 376-379 *(IF: 0,406)*.

Foubert, I., Fredrick, E., Vereecken, J., Sichien, M. & Dewettinck, K. *(2008).* Stop-and-return DSC method to study fat crystallization. *Thermochimica Acta*, 471(*1-2)*, 7-13 *(IF: 1,742)*.

Fredrick, E., Foubert, I., Van De Sype, J. & Dewettinck, K. *(2008).* Influence of monoglycerides on the crystallization behavior of palm oil. *Crystal Growth & Design,* 8*(6)*, 1833-1839 *(IF: 4,162).*

Hoofdstuk in wetenschappelijk boek:

Sichien, M.; Thienpont, N.; Fredrick, E.; Le, T.T.; Van Camp, J.; Dewettinck, K. *(2009)*. Processing means for milk fat fractionation and production of functional compounds. In: *Dairy-derived ingredients: food and nutraceutical uses* edited by Corredig, M.

Internationale lezingen

Fredrick, E., Sichien, M., Nopens, I. & Dewettinck, K. *(2010)*. Investigating the mechanism and kinetics of partial coalescence in cream by various experimental techniques. *IDF symposium on dairy products*, Troms∅, Norway, June 9-10

Fredrick, E., Walstra, P. Fischer, S., Zijtveld, H. & Dewettinck, K. *(2010).* Monoacylglycerols to control the microstructure of recombined whipped cream. *IDF symposium on dairy products*, Troms∅, Norway, June 9-10.

Dewettinck, K., De Graef, V., Fredrick, E., & Van de Walle, D. *(2010)*. Fat crystallization: from model systems to real world applications. *IDF symposium on dairy products*, Troms∅, Norway, June 9-10

Fredrick, E., Walstra, P. Fischer, S., Zijtveld, H. & Dewettinck K. *(2009).* Role of monoacylglycerols during the structure build-up of recombined whipped cream. *6th NIZO dairy conference*, The Netherlands, September 30- October 2.

Fredrick, E., Walstra, P. Fischer, S., Zijtveld, H. & Dewettinck K. *(2009)*. Factors Governing Partial Coalescence in Oil-in-Water emulsions. *IDF world Summit, Berlin*, Germany, September 21-24.

Wiking, L., De Graef, V., Fredrick, E. & Dewettinck, K *(2009)*. Crystallisation Mechanisms and Microstructure of Milk Fat - Effects of Cooling Treatments and Addition of Phospholipids. *100th AOCS Annual Meeting & Expo*, Orlando, USA, May 2-5.

Fredrick, E., Walstra, P., Zijtveld, H., Fischer, S. & Dewettinck, K. *(2009)*. Monoacylglycerols as Crystallization and Stability Modifiers of Recombined Cream. *100th AOCS Annual Meeting & Expo*, Orlando, USA, May 2-5.

Foubert, I., Fredrick, E., Vereecken, J. & Dewettinck, K *(2007)*. Applications of the stop and return indirect DSC method for fat research. *98th AOCS Annual Meeting & Expo,* Quebec, Canada, May 13-16.

Fredrick, E., Foubert, I., Hollander, F., Fischer, S., Walstra, P. & Dewettinck, K. *(2007)*. Isothermal crystallization behavior of milk fat in oil-in-water emulsions, *98th AOCS Annual Meeting & Expo*, Quebec, Canada, May 13-16.

Foubert, I., Fredrick, E., Van De Sype, J. & Dewettinck, K. *(2006)*. Influence of monoglycerides on the crystallization behavior of palm oil. *97th AOCS Annual meeting & expo*, St-Louis, USA, April 30- May 3.

Seminar lectures

Fredrick, E. (2008). Emulsion preparation: Principles and Equipment. *ILPS Short Course: Lecithin & Phospholipids in Emulsions*, Ghent, Belgium, June 5-6.

Fredrick, E. (2008). Emulsiebereiding. *Flanders' food opleiding 'Wegwijs in emulsies': Vorming, stabilisatie en karakterisering,* Affligem, België, 4 maart en 28 april.

Fredrick, E. *(2008)*. Karakterisatie van emulsies: reologie. *Flanders' food opleiding 'Wegwijs in emulsies': Vorming, stabilisatie en karakterisering,* Affligem, België, 4 maart en 28 april.

Posters

Fredrick, E., Foubert, I., Fischer, S., Walstra, P., & Dewettinck K. *(2007)*. Indirect DSC Mehod to investigate the Crystallization Behavior of Milk Fat in Bulk and Cream. *IDF World Summit*, Dublin, Ireland, October 1-4.

Fredrick, E., Foubert, I., Van de Sype, J. & Dewettinck, K. *(2006)*. Influence of Monoglycerides on the Crystallization Behavior of Palm Oil. *SyNeW-Synchrotron and Neutron Workshop*, The Hague, The Netherlands, October 12.