Fractionation of Milk Fat Globule Membranes in butter processing

Annamari Jukkola

Fractionation of Milk Fat Globule Membranes in butter processing

Annamari Jukkola

A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Chemical Engineering, at a public examination held at the lecture hall Ke2 of the school on 31st May 2019 at 12 noon.

Aalto University
School of Chemical Engineering
Department of Bioproducts and Biosystems
Bio-Based Colloids and Materials (BiCMat)

Supervising professor

Professor Orlando J. Rojas, Aalto University, Finland

Thesis advisors

Doctor Riitta Partanen, Valio Ltd., Finland

Doctor Antti Heino, Valio Ltd., Finland

Preliminary examiners

Associate Professor Lars Wiking, Aarhus University, Sweden

Doctor Marie-Caroline Michalski, INSA Lyon, France

Opponent

Doctor José Sanchez-Marcano, The European Institute of Membranes (IEM), France

Aalto University publication series

DOCTORAL DISSERTATIONS 90/2019

© 2019 Annamari Jukkola

ISBN 978-952-60-8549-4 (printed)

ISBN 978-952-60-8550-0 (pdf)

ISSN 1799-4934 (printed)

ISSN 1799-4942 (pdf)

<http://urn.fi/URN:ISBN:978-952-60-8550-0>

Unigrafia Oy

Helsinki 2019

Finland

Printed matter
4041-0619

Author

Annamari Jukkola

Name of the doctoral dissertation

Fractionation of Milk Fat Globule Membranes in butter processing

Publisher School of Chemical Engineering

Unit Department of Bioproducts and Biosystems

Series Aalto University publication series DOCTORAL DISSERTATIONS 90/2019

Field of research Bioproduct Technology

Manuscript submitted 1 March 2019

Date of the defence 31 May 2019

Permission for public defence granted (date) 25 April 2019

Language English

☐ **Monograph**

☒ **Article dissertation**

☐ **Essay dissertation**

Abstract

Bovine milk contains components that are significant to human nutrition and most beneficial if present in infant formulas. Such components are mainly associated with the biological membrane surrounding the milk fat globules, the Milk Fat Globule Membrane (MFGM). MFGM, one of the most complex fractions in milk, contains proteins and polar lipids that form a trilayer structure. Compared to its native form, MFGM structure is disrupted during milk processing, leading to the release of valuable components and affecting associated structural and functional properties. Therefore, novel methods are demanded for the isolation, fractionation and deployment of MFGM.

In this thesis, we apply concepts relevant to colloid and surface chemistry to establish new processing methods aimed to maximizing the nutritional and technological value of MFGM components. We propose cream microfiltration prior to the traditional buttermaking to separate fat globules from other dairy constituents, such as proteins, lactose and minerals. Filtered cream fractions were churned, upon which phase inversion facilitated the production of butter (termed here as "ideal butter") and buttermilk, the latter of which was enriched with MFGM fragments but depleted of other dairy components.

The microfiltration resulted in a protein separation efficiency of 93% and the filtered cream was found suitable for buttermaking, without affecting negatively the process. MFGM damage was observed under intense processing conditions, leading to losses in polar lipids (up to 20%). Moreover, the pH drastically effected filtration performance given its roles in modulating the electrostatic interactions and in fouling the filtration membrane. The optimisation of the process in relation to shearing forces and other factors, was found critical in defining the structure and possible losses of fat globules and MFGM. MFGM-rich ideal buttermilk powder was surface-active and capable of forming relatively stable food emulsions. Such material is proposed for the formulation of nutraceutical and infant formulas.

Keywords Milk fat, dairy, MFGM, microfiltration, separation

ISBN (printed) 978-952-60-8549-4

ISBN (pdf) 978-952-60-8550-0

ISSN (printed) 1799-4934

ISSN (pdf) 1799-4942

Location of publisher Helsinki

Location of printing Helsinki **Year** 2019

Pages 97

urn <http://urn.fi/URN:ISBN:978-952-60-8550-0>

Tekijä

Annamari Jukkola

Väitöskirjan nimi

Maitorasvamembraanien erotus voiprosessista

Julkaisija Kemian tekniikan korkeakoulu**Yksikkö** Biotuotteiden ja biotekniikan laitos**Sarja** Aalto University publication series DOCTORAL DISSERTATIONS 90/2019**Tutkimusala** Biotuotetekniikka**Käsikirjoituksen pvm** 01.03.2019**Väitöspäivä** 31.05.2019**Väittelyluvan myöntämispäivä** 25.04.2019**Kieli** Englanti☐ **Monografia**☒ **Artikkeliväitöskirja**☐ **Esseeväitöskirja****Tiivistelmä**

Lehmän maito sisältää monia ravitsemuksellisesti tärkeitä ainesosia, jotka ovat erityisen hyödyllisiä äidinmaidon korvikkeiden rikastuksessa. Nämä ainesosat sijoittuvat maidossa rasvapisaroita ympäröivään maitorasvamembraaniin (eng. Milk Fat Globule Membrane, MFGM), joka on yksi maidon monimuotoisimmista fraktioista. MFGM sisältää useita eri proteiineja ja polaarisia lipidejä kolmikerroksisessa rakenteessa. Tämä biologinen rakenne vaurioituu usein maitoa prosessoitaessa, mikä johtaa MFGM:n komponenttien irtoamiseen ja funktionaalisuuden menetykseen. Tästä syystä tarvitaan uusia menetelmiä, joiden avulla MFGM voidaan erottaa maidosta hallitusti.

Tämän tutkielman tarkoituksena oli selvittää mikrosuodatuksen soveltuvuutta MFGM:n erotukseen sekä arvioida erotusprosessin kriittisiä parametreja hyödyntäen kolloidi- ja pintakemian käsitteitä. Työn menetelmässä mikrosuodatus lisättiin perinteiseen voinvalmistusprosessiin ennen kirnuamista, jotta kerman sisältämät rasvapallot saatiin erotettua muista maidon komponenteista kuten proteiineista, laktoosista ja mineraaleista. Suodatettu kerma kirnuttiin, jolloin faasit erottuivat voiksi ("ideaali voiksi") ja MFGM-rikastetuksi kirnumaidoksi.

Mikrosuodatuksessa saavutettiin 93% erotustehokkuus proteiinien suhteen. Suodatettu kerma soveltui hyvin voinvalmistukseen ilman muutostarpeita myöhempiin prosessivaiheisiin. Voimakkaan prosessoinnin havaittiin kuitenkin vahingoittavan MFGM rakennetta johtaen jopa 20% polaaristen lipidien menetykseen suodatuksessa. Lisäksi pH:lla havaittiin olevan merkittävä vaikutus suodatustehokkuuteen johtuen sen vaikutuksesta makromolekyylien sähköisiin vuorovaikutuksiin ja siten suodatuskalvon tukkeutumiseen. Mikrosuodatusprosessi tulee optimoida leikkauvoimien ja muiden muuttujien suhteen, jotta rasvapisaroiden pilkkoutuminen ja MFGM-komponenttien permeaatio suodatuksessa voidaan minimoida. Työssä osoitettiin myös MFGM-rikastetun ideaalikirnumaidon pinta-aktiivisuus ja sen soveltuvuus emulgaattoriksi. Työssä kehitetty raaka-aine tarjoaa luontaisen ja ravitsemuksellisesti arvokkaan emulgaattorivaihtoehdon elintarvikesovelluksiin, kuten äidinmaidonkorvikkeisiin.

Avainsanat Maitorasva, maito, MFGM, mikrosuodatus, separointi**ISBN (painettu)** 978-952-60-8549-4**ISBN (pdf)** 978-952-60-8550-0**ISSN (painettu)** 1799-4934**ISSN (pdf)** 1799-4942**Julkaisupaikka** Helsinki**Painopaikka** Helsinki**Vuosi** 2019**Sivumäärä** 97**urn** <http://urn.fi/URN:ISBN:978-952-60-8550-0>

Preface

I feel extremely emotional to see the four-year journey of working as PhD student coming to its end. During this time, I have understood that being a part of the scientific world, especially in the form of a PhD student, is truly a unique experience. Not only have I learned myriad interesting and useful scientific practices, but I have learned so much about myself and got the opportunity to question everything, develop myself, learn and influence. This experience would not have been possible without the funding from Valio Ltd. Support was also received from Agronomiliiton tiede ja –tutkimussäätiö (Science and research foundation of the Association of Academic Agronomists). I am very grateful for their financial support.

Words cannot express how grateful I am to my supervising professor Orlando Rojas. You have truly inspired me with your positive - yet extremely professional approach and made me feel so warmly welcome to the group, even though the connection between colloids and surfaces and milk may have seemed foreign to me at the time started. I feel privileged to learn from you.

Working on a thesis often comes across as an individual journey. I cannot take the credit of this work without expressing my gratitude to my advisors Dr. Riitta Partanen and Dr. Antti Heino and my co-authors Dr. Sanna Hokkanen, Tero Kämäräinen and Wenchao Xiang who substantially contributed to the work. Riitta and Antti, you have given me so much support in many levels. Every time I left from our meeting, I felt encouraged and motivated. Meetings with you always included many interesting scientific details, lots of ideas but also relaxed every day conversations and I never wanted them to end. Most importantly, you have given me guidance no matter the issue but also challenged me in the right way to exceed my own expectations. Sanna, I felt very happy to share the same project with you and to have somebody working with me who really understands the world of lipids. Tero, it was undoubtedly exciting to work with you and trying to solve the problems from different angles. Wenchao, your positiveness and enthusiasm made our collaboration so pleasant and productive.

I would also like to thank the whole BiCMat group. I am proud to be part of this amazing family even though I may not have been the most active member of it. I have enjoyed learning about many unfamiliar topics from you, and at the same time, sharing the knowledge from my perspective. We all share the passion for science, no matter the details. Thank you Marja Kärkkäinen, Rita Hatakka, Ritva Kivelä and Nina Heinilä with your help in the lab and conducting the experiments.

Everyone who knows me know I love coffee. Therefore, it is not surprising how much value I like to give to coffee brakes and people joining them. Besides serving much needed breaks from the lab and aid for writer's block, these moments have offered many fruitful discussions, peer support and sometimes the best least-scientific dialogues of life. So, thank you Saija, Jonna, Maija, Iina, Juuso, Naveen, Reeta, Elli, Andreas, Sara and Pegah.

A big thank you to my family, extended family and friends, especially to the boss ladies Maiju, Eve and mom, Paula. You have given me much-needed balance from the thesis and supported me to chase my dreams. I love you.

And finally, I thank Askö, the rock of my life. Thank you for being there for me, being encouraging and letting me to pour my heart out. Thank you for helping me solve the secrets of Excel many, many times and letting me to be me. It means so much to me to share my life and dreams with you.

Helsinki, February 2019

A handwritten signature in cursive script, appearing to read 'Heini Helen'.

“Always go with the choice that scares you the most, because that’s the one that is going to require the most from you”

Caroline Myss

List of Publications

This doctoral dissertation consists of a summary and of the following publications which are referred to in the text by their numerals

1. Jukkola, Annamari; Partanen, Riitta; Rojas, Orlando J; Heino, Antti. 2016. Separation of milk fat globules via microfiltration: Effect of diafiltration media and opportunities for stream valorization. *Journal of Dairy Science*, 99(11), 8644-8654. ISSN: 0022-0302. DOI: <http://dx.doi.org/10.3168/jds.2016-11422>.

2. Jukkola, Annamari; Partanen, Riitta; Rojas, Orlando J; Heino, Antti. 2018. Effect of heat treatment and pH on the efficiency of micro-diafiltration for the separation of native fat globules from cream in butter production. *Journal of Membrane Science*, 548(15), 99-107, ISSN: 0376-7388, DOI: <https://doi.org/10.1016/j.memsci.2017.11.012>.

3. Jukkola, Annamari; Hokkanen, Sanna; Kämäräinen, Tero; Partanen, Riitta; Heino, Antti; Rojas, Orlando J. 2019. Changes in milk fat globules and membrane lipids under the shear fields of microfiltration and centrifugation. *Journal of Membrane Science*, 573, 218-225, ISSN: 0376-7388, DOI: <https://doi.org/10.1016/j.memsci.2018.12.007>

4. Jukkola, Annamari; Partanen, Riitta; Xiang, Wenchao; Heino, Antti; Rojas, Orlando J. 2019. Food emulsifiers based on Milk Fat Globule Membranes and their Interactions with calcium and casein phosphoproteins. *Food Hydrocolloids*, 94, 30-37, ISSN: 0268-005X, DOI: <https://doi.org/10.1016/j.foodhyd.2019.03.005>

Author's Contribution

Publication 1: Separation of milk fat globules via microfiltration: Effect of diafiltration media and opportunities for stream valorization.

AJ was responsible for the experimental design under advisement of RP and AH. AJ performed the experimental work, including milk microfiltrations as well as chemical and physical characterization of the samples. She analysed the results, conducted the statistical analysis, performed the calculations for microfiltration performance and wrote the manuscript as principal author under supervision of OJR.

Publication 2: Effect of heat treatment and pH on the efficiency of micro-diafiltration for the separation of native fat globules from cream in butter production.

AJ was responsible for the experimental design under advisement of RP and AH. AJ performed main part of the experimental work, including cream microfiltrations, butter making, fat and dry matter analysis, particle size analysis and CLSM. Furthermore, she conducted the calculations, performed the statistical analyses, was responsible for analysing of the results and wrote the manuscript as principal author under supervision of OJR.

Publication 3: Changes in milk fat globules and membrane lipids under the shear fields of microfiltration and centrifugation

AJ was responsible for the experimental design under advisement of RP and AH. AJ performed main part of the experimental work, including cream microfiltrations and centrifugal separations, zeta potential and particle size measurements as well as SDS-PAGE analyses. SH was responsible for the polar lipid analysis and TK conducted the flow and shear estimations. AJ analysed the results and wrote the manuscript as principal author under supervision of OJR.

Publication 4: Food emulsifiers based on Milk Fat Globule Membranes and their Interactions with calcium and casein phosphoproteins

AJ was responsible for the experimental design under advisement of RP and AH. AJ prepared MFGM material and supervised the experimental work related to emulsion preparations and characterization. WX performed the surface tension measurements. AJ analysed the results and wrote the manuscript as principal author under supervision of OJR.

List of Abbreviations and Symbols

BTN	Butyrophilin
CD36	Cluster of differentiation 36
CLSM	Confocal laser scanning microscopy
CN	Casein
DF	Diafiltration
FFA	Free fatty acids
MF	Microfiltration
MFG	Milk fat globule
MFGM	Milk fat globule membrane
Muc1	Mucin 1
PAS6/7	Periodic acid and Schiff 6/7
PBS	Phosphate buffered saline
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PL	Polar lipid
PP3	Proteose peptone 3
PS	Phosphatidylserine
SDS-PAGE	Sodium dodecyl sulphate Polyacrylamide gel electrophoresis
SM	Sphingomyelin
SMUF	Skim milk ultrafiltration permeate
TMP	Transmembrane pressure
UTMP	Uniform transmembrane pressure
WGA	Wheat germ agglutinin
XO	Xanthine oxidase

<i>α-La</i>	<i>α-lactalbumin</i>
<i>β-Lg</i>	<i>betalactoglobulin</i>
ζ	Zeta potential

Contents

1.	Introduction	1
2.	Background	3
2.1	Milk fat globules and their surrounding membrane.....	3
2.2	Effect of processing on milk fat globules.....	5
2.3	Microfiltration	7
2.4	Ideal butter process	8
3.	Experimental.....	10
3.1	Materials	10
3.1.1	Milk based components.....	10
3.1.2	Other materials	10
3.2	Methods	11
3.2.1	Microfiltration	11
3.2.2	Centrifugal separation	12
3.2.3	Butter making	12
3.2.4	Emulsion preparation.....	13
3.2.5	Analysis.....	13
4.	Results and Discussion	15
4.1	Factors affecting whole milk and cream microfiltration	15
4.1.1	Diafiltration media	15
4.1.2	Heat treatment	16
4.1.3	pH	17
4.1.4	Protein permeation.....	18
4.2	Changes in the physicochemical properties of the milk... ..	20
4.2.1	Particle size	20
4.2.2	Zeta potential.....	22
4.3	Evaluation of MFGM functionality	23
4.3.1	Microstructural observations	23
4.3.2	Partitioning of MFGM components	25
4.3.3	Surface activity and emulsifying properties.....	27
4.4	Ideal butter process	28
5.	Concluding Remarks.....	31

1. Introduction

Milk constitutes the primary nutrition for any mammalian neonate. It contains many bioactive components that are critical for the health and development of infants (Dewettinck et al., 2008). Such bioactive components are mostly located at the surface layer of the milk fat globules, called Milk Fat Globule Membrane (MFGM). Infant formulas derived from bovine milk can be supplemented with MFGM to resemble the composition of human milk and to meet the nutrition requirements of infants. Hence, recent studies show an increasing interest in MFGM-enriched infant formulas.

MFGM is not only remarkable because of its nutritional significance, but for its technological functionalities. For instance, MFGM provides natural emulsifying and stabilizing properties in milk (Phan et al., 2016, 2014; Corredig and Dalgleish 1997, 1998), increases water holding capability in dairy products (Le et al., 2011), affects sensory characteristics (Michalski et al., 2007) and provides delivery systems for bioactive components (Liu et al., 2013). However, MFGM isolation from milk has been economically challenging, leading to the ever present need to design new separation methods.

Hence, the aim of the thesis was to evaluate a filtration process for separation of MFGM from milk. In particular, cream microfiltration was adopted prior to the traditional buttermaking process in order to separate fat globules from other dairy constituents, such as proteins, lactose and minerals. Filtered creams were churned, upon which the phases were inverted, resulting in butter and butter-milk containing MFGM fragments but depleted of other dairy components. The term “*Ideal butter*” is applied herein for such process.

The thesis consists of four publications. **Publication 1** explored the feasibility of microfiltration for separating native fat globules from raw milk. Furthermore, the use of different diafiltration media was evaluated, along with their effect on filtration performance and emulsion characteristics. Moreover, the concept of *ideal butter* was examined in **Publication 2**. In particular, full fat creams of given heat treatment history were microfiltered and churned into butter. The microfiltration resulted in 78% separation efficiency (in terms of protein separation) and the filtered cream was found suitable for buttermaking with no negative consequences in the buttermaking process. The pH was observed to have a drastic effect on filtration performance. Furthermore, it was found crucial to optimize the microfiltration process in order to avoid shearing of fat globules and losses of MFGM. **Publication 3** subsequently compared microfiltration and conventional centrifugal separation. Both processes yielded a 93% separation of proteins and nearly impurity-free MFGM. However, intense processing

and flow conditions were observed to damage the MFGM. Microfiltration was proposed as a potential separation method for scaling-up. Lastly, **Publication 4** applied the MFGM material from *ideal buttermilk* as a natural component to model emulsions and investigated the interactions with protein and its emulsifying properties. MFGM proved to be surface active and was capable of producing relatively stable emulsions. Hence, MFGM is proposed as a functional ingredient for future food applications and infant formulas. An outline of the work conducted in the above publications is illustrated in Figure 1.

Figure 1. Outline of the main subjects of the thesis and their corresponding publication numbers. Schematics: yellow droplets represent milk fat globules surrounded by layer of milk fat globule membranes (purple), small blue spheres represent serum proteins, lactose and minerals.

2. Background

2.1 Milk fat globules and their surrounding membrane

Milk is an oil-in-water emulsion comprising 3-4% fat in an aqueous phase containing proteins, lactose and minerals. Nearly all of the milk fat exists in dispersed, separate fat globules with diameters between 0.1 and 15 μm (Yao et al., 2016; Lu et al., 2016). The fat globule core consists primarily of triacylglycerols, which represent more than 98% of the milk lipids, whereas diacylglycerols, monoacylglycerols, free fatty acids, polar lipids, sterols and fat-soluble vitamins are a minor part of milk lipids. It is now recognized that the minor part of milk lipids is nutritionally significant, which has been the subject of a growing research interest. For instance, a myriad of health-promoting effects has been attributed to milk polar lipids (Dewettinck et al., 2008).

The fat and aqueous phases of the milk do not mix—like water and oil (Fig. 2). Hence, the fat globules in milk are enclosed in a natural membrane, milk fat globule membrane (MFGM), which stabilizes and protects them from enzymatic degradation.

Figure 2. Emulsion oil droplets dispersed in water.

Most of the minor lipids in milk, including polar lipids, are located in MFGM. Moreover, the exclusive composition and structure of MFGM provides specific technological, nutritional and biological functions to milk fat globules. In particular, it provides natural emulsifying and stabilizing properties (Phan et al., 2016), enhances water holding capacity and acts as a delivery system for bioactive components (Liu et al., 2013; Le et al., 2011). Moreover, various important

biological functions such as antimicrobial and antiviral activities, gut maturation and development of the central nervous system in the neonate have been associated to the MFGM (Gallier et al., 2015; Contarini and Povolo, 2013; Dewettinck et al., 2008).

Figure 3. Milk fat globules and enveloping membranes: (A) Schematic illustration of milk fat globule membrane (MFGM). The core, the fat globule, is surrounded by a polar lipid trilayer (tail-and-head molecules in yellow) interspersed with various proteins and enzymes (purple, blue and red (left)). Membrane fragments can be detached from the interface (right). (B) Milk fat globule size distribution (Michalski et al., 2001). (C) Confocal microscopy image of milk fat globules surrounded by glycoproteins and lipids (Gallier et al., 2012). (D) Transmission electron microscopy of MFGM (Gallier et al., 2012). (E) Organization of polar lipids in the MFGM (Zheng et al., 2014). (F) Schematic illustration of the trilayer MFGM structure interspersed with proteins (Lopez et al., 2011). Figures B-F modified from Jukkola and Rojas (2017). All figures reproduced with permission from the respective sources.

MFGM is certainly the most diverse fraction of milk. The structure of MFGM is vastly complex and dynamic, which is characteristic of biological membranes (Jukkola and Rojas, 2017). In its native form, MFGM is highly structured with main components comprising a complex mixture of proteins, glycoproteins, enzymes, neutral lipids, polar lipids and cholesterol. Furthermore, the structure of the membrane encompasses a trilayer phospholipid structure: first and closest to the core, is a monolayer surrounded by a layer of proteins and, atop, a phospholipid bilayer interspersed with proteins and cholesterol (Fig. 3).

The main lipids in MFGM can be divided into phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS), and sphingolipids such as sphingomyelin (SM) and cholesterol. Most relevant MFGM proteins comprise mucin 1, xanthine oxidase (XO) cluster of differentiation 36 (CD36), periodic acid and Schiff (PAS) 6/7, adipophilin, butyrophilin (BTN), and proteose peptone 3 (PP3) (Lu et al., 2016; Fong et al., 2007; Le et al., 2009). The outer phospholipid bilayer is divided into liquid-ordered domains, rich in sphingolipids and cholesterol, and liquid-disordered domains of glycerophospholipids (Lopez et al., 2010). A substantial amount of PC and SM likely occurs in the outer part of the bilayer whereas PE is located in its inner domain (Fig 3E). Some of the polar lipids associate strongly with the MFGM structure while others are more loosely bound. Cholesterol interacts mainly with SM and contribute to the packing of the lipids. Glycoproteins and glycolipids are heterogeneously distributed on MFGM (Gallier et al., 2012), whereas PAS 6/7, mucin 1, XO and PP3 are found at the external part of the trilayer structure. The inner monolayer consists of PI and PS and electron-dense coating of proteins, which interconnects different layers of MFGM. For instance, BTN is most likely located at the internal part of the membrane which interacts covalently with XO (Heid and Keenan, 2005).

Overall, the organization of MFGM components govern the functional properties of the bio-membrane and the chemical reactions that occur on the surface of fat globules.

2.2 Effect of processing on milk fat globules

Milk fat globules are subjected to physical and biochemical changes upon handling and processing of milk. Their biological and technological functionality depends greatly on the quality of the interface in terms of composition and organization (MFGM vs. serum proteins) (Garcia-Moreno et al., 2014; Berton-Carabin et al., 2013). Therefore, any change occurring on the surface of the fat globules might affect their interactions, stability, shelf-life and many of the physical properties associated with dairy products (Jukkola and Rojas 2017).

The fat globules in milk are subjected to colloidal instabilities, including creaming, aggregation and coalescence. The physical-chemical principles governing emulsion and milk stability involve the classical theories of electrostatic and steric stabilization. Electrostatic stabilization results from the presence of electrostatic charges on the surface of the droplets, whereas steric stabilization

arises from the presence of macromolecular barriers on the droplet surface. Under weak electrostatic and steric forces, the particles tend to aggregate and flocculate by van der Waals and other interactions (McClements, 2009). However, at physiological pH the particles in milk (fat globules and proteins) carry a net negative electrostatic charge and are thus a strong source for stabilization of milk, including its fat globules. In order to fully exploit its potential, it is important to understand the physicochemical and colloidal properties of MFGM and the challenges related to its processing.

According to the Stokes' law, droplets move under gravitational forces depending on their diameter, density and shear viscosity (Stokes, 1843; Einstein, 1906):

$$V_{stokes} = \frac{g(\rho_2 - \rho_1)d^2}{18\eta_1} \quad [1]$$

where, g is the gravitational field, d is the droplet diameter, ρ is density, η is the shear viscosity and the subscripts 1 and 2 denote the continuous and dispersed phases, respectively. The sign of the Stokes velocity determines whether the droplets cream (+) or sediment (-). Smaller droplets are more stable compared to larger ones, whereas coalescence or aggregation increases the rate of instability. Typically, emulsifiers such as MFGM, are denser than water, whereas oil has a lower density (McClements et al., 2016). Accordingly, the presence of an emulsifier layer tends to lessen the difference in density between the droplets and the surrounding medium, thereby reducing the creaming velocity (Eq.1). However, such effect requires a relatively small droplet size and a thick interfacial layer, both of which can be disrupted upon milk processing and storing.

In addition to colloidal instabilities, MFGM is subjected to interactions with lipid molecules and proteins, for instance upon pasteurization and homogenization, which affect its surface properties. Furthermore, MFGM can be released from fat globules upon exposure to air during churning, agitation, freezing, thawing and upon interaction with polar solvents used to suspend the system. More specifically, the loss of membrane is caused by instabilities such as coalescence, rupture of fat globules or migration. The released MFGM fragments are enriched in the aqueous phase of milk such as in skimmed milk, buttermilk and butter serum (Rombaut et al., 2006; Bourlieu et al., 2018) along with some valuable components (Michalski et al., 2005). Moreover, released MFGM causes other surface-active components, mainly serum proteins, to adsorb to the surface of the fat globules and form a new coverage. As discussed before, MFGM is responsible for many of the functional and nutritional properties of milk fat. Therefore, any loss of MFGM from the fat globule surface will directly affect the functional properties of milk (Scott et al., 2003). For instance, the type of fat globule surface (MFGM versus serum proteins) have been shown to affect their digestion and bioavailability (Gallier et al., 2017).

Despite the potential benefits of MFGM, it is far from being industrially used in dairy products, given that technological processes often disrupt MFGM and affects its functionality (Holzmüller and Kulozik, 2016; Corredig and Dalgleish, 1998). Furthermore, several efforts have been applied to examine the isolation

of MFGM, but the efficiency of such isolation has remained limited, mainly because of process-induced depletion and the presence of impurities (serum proteins). The effectiveness of the process is reduced considerably if fat globules are damaged before MFGM isolation, by creating exposed MFGM fragments that bind caseins, making it difficult for them to be washed out completely.

MFGM isolation has been examined using centrifugal separation – a mechanical processing based on differences in particle velocities under centrifugal fields. MFGM isolation is commonly performed through a four-step process including separation of the fat globules, cream washing (often by using multiple cycles), discharge of MFGM from washed cream and collection of MFGM material. Despite its usefulness, centrifugal separation induces high shear stress that can remove surface proteins and phospholipids from MFGM and cause fat globule coalescence (Zheng et al., 2014; Britten et al., 2008). Moreover, such separation leads to the enrichment of low-density particles, such as fat globules in milk, but is not selective to proteins. In order to yield high MFGM recovery, processing often requires large volumes of aqueous washing solutions.

2.3 Microfiltration

Unlike centrifugal separation, microfiltration is a pressure-driven process that separates components by size exclusion through a porous separation membrane (Fig. 4). Microfiltration has been adopted for fractionation of milk components, for instance for fractionation of milk proteins and removal of bacteria (i.e., cold pasteurization) (Beolchini, 2005; Hoffman et al., 2006; Hurt and Barbano, 2010; Piry et al., 2012; Adams and Barbano, 2013). Furthermore, microfiltration was reported to produce less damage to shear-sensitive milk components, providing an alternative method for MFGM separation compared to centrifugal separation. However, skim milk is often used for milk membrane filtration processes and, due to fat-induced membrane fouling, microfiltration of fat-containing dairy fluids is mainly limited to defatting of whey (Golbandi et al., 2013).

Figure 4. Schematic illustration of the microfiltration (MF) process. Yellow droplets represent fat globules in the stream whereas the blue circles represent proteins.

Membrane filtration has been applied to fractionate milk fat populations into small and large globules (Goudedranche et al., 2000; Michalski et al., 2006) and for separation of anhydrous milk fat into fractions with different melting points (Abbas et al., 2006). However, microfiltration of dairy, fat-rich fluids is limited by membrane fouling. Fouling poses a major challenge in all applications related to membrane filtration of milk. Moreover, fouling and concentration polarization are related to the deposition of a layer on the membrane's surface and to the narrowing of the pores, which negatively affect the selectivity and filtration performance by means of flux reduction (Guerra et al., 1997; Fouladitajar et al., 2015). Concentration polarization, the formation of a dense, multicomponent layer at the walls of the filtration membrane, has remained one of the most important challenges in pressure-driven membrane processes (Tashvigh et al., 2015). One way to prevent particle deposition and minimize fouling is to utilize a higher shear at the membrane-liquid interface, which can be accomplished by adopting high cross-flow velocities (4–8 m/s) (Guerra et al., 1997). In addition to shear stress, microfiltration performance can be controlled by means of the transmembrane pressure (TMP) and permeate flux (Gésan-Guizieu et al., 1999). Furthermore, to enhance filtration performance, diafiltration (DF) is commonly used in dairy processing, which mainly involves washing out permeating compounds with DF medium. Herein, the “retained” solution in microfiltration, e.g. cream and milk, is called retentate while the permeating fraction is called “permeate”.

2.4 Ideal butter process

In the traditional buttermaking process, cream is churned into butter upon which the side stream, buttermilk, is separated from the solid fraction of butter. The volume of the buttermilk side stream is roughly half of the initial volume of the cream used for buttermaking. The composition of the buttermilk resembles that of skimmed milk, comprised of small traces of fat, protein, lactose and milk minerals. Moreover, the cream churning process disrupts the milk fat globules, and the resulting MFGM fragments are released into the aqueous phase of buttermilk. Therefore, buttermilk that is rich in functional constituents from MFGM is of great relevance to industry and consumers. Despite the numerous attempts to fractionate and concentrate MFGM fragments from buttermilk (Corredig et al., 2003; Rombaut et al., 2006; Fauquant et al., 2014), isolation of such components is challenging because of the complexes they form and the size similarity with proteins (Dewettinck et al., 2008).

We propose a new method, *ideal butter*, for producing MFGM material without the aid of chemicals and avoiding side streams. In particular, raw bovine cream is microfiltered prior to buttermaking to separate proteins and milk fat globules (Figure 1 and 4). Then, the microfiltered cream is churned according to the normal butter making procedure and MFGM is released from the fat globule surface into the resultant (ideal) buttermilk. The *ideal buttermilk* may contain MFGM without other milk components, such as proteins, allowing it to be

utilized without the need for further purification steps. The *ideal butter* process provides a scalable method for MFGM isolation for the dairy industry where membrane filtration techniques are already adopted.

To conclude, the objectives of the present study included the examination of microfiltration of fat-containing dairy fluids, such as whole milk and cream, for its potential in MFGM isolation. Moreover, the filtration process was incorporated in conventional butter making process to convert the current buttermilk side stream into a functional one, rich in MFGM. The open questions considered in this thesis included:

1. Is microfiltration suitable for the separation of fat globules from other dairy constituents?
2. How does the filtration process and permeation of proteins affect the colloidal stability of milk?
3. Can the filtered cream be churned into butter? Does the absence of proteins affect the phase inversion process?
4. How much of the original MFGM can be recovered in cream? Is there a difference between microfiltration and centrifugal separation methods?
5. What is the functionality of MFGM-rich ideal buttermilk, and could it be used as a natural emulsifier in dairy emulsions?

Question 1 is covered in **Publication 1**, whereas **Publication 2** aims to elucidate Questions 1-3. **Publication 3** examines Question 4 and also addresses Questions 2. Question 5 is considered in **Publication 4**.

The hypotheses of this work include:

- a) The overall filtration performance depends on the nature (relative to their original condition) of the milk or cream in the feed. The interactions between milk fat globules and proteins and their accumulation on the surface of the filtration membrane increases with heat treatment. Increased shear decreases colloidal stability and filtration throughput due to desorption of MFGM, increased particle size and formation of free fat.
- b) MFGM is disrupted by the butter churning process, leading to phase inversion, from o/w to w/o. Loss of proteins decreases cream stability and facilitates the phase inversion due to a more limited competitive adsorption and stabilization of the interface.
- c) MFGM is expected to be surface active, even after its disruption into membrane fragments present in buttermilk. Such fraction can be deployed in emulsions without the need of additional emulsifiers.

The following section describes the experimental approaches adopted to address these hypotheses.

3. Experimental

This section outlines the most important materials and experimental methods employed in this research work. After describing the materials, the most relevant methods adopted in the publications are explained. Emphasis is given to the materials and methods that are most essential to the discussion and conclusions of the thesis.

3.1 Materials

3.1.1 Milk based components

Milk and cream were collected from dairy plant (Valio Ltd., Riihimäki, Finland) and used as a raw material for filtrations. Raw bovine milk (fat 4.4%, protein 3.5%, dry matter 13.4%, pH 6.8) was used in **Publication 1**, whereas cream (fat 41 %, protein 2.0 %, dry matter 45%, pH 6.7) was adopted in rest of the publications. Moreover, raw and pasteurized (75 °C, 20 s) creams were compared in **Publication 2**, whereas only pasteurized cream was used in **Publication 3**. Raw cream was again applied for microfiltration in **Publication 4** followed by pasteurization after separation to minimize the effect of heat treatment on filtration performance.

Non-pasteurized skim milk ultrafiltration permeate (SMUF) was collected from a dairy plant (Valio Ltd., Äänekoski, Finland) and used as a diafiltration media in **Publication 1**.

Bovine casein (Sigma-Aldrich, Merck KGaA, Germany) was employed in model emulsion experiments in **Publication 4**.

3.1.2 Other materials

Ultrapure water and 50 mM saline were used as diafiltration media in **Publication 1**. Deionized water was used for diafiltration in **Publication 2-4**.

Fluorescent stains were utilized for imaging in a confocal laser scanning microscope (CLSM) in **Publication 2** and **4**. Nile red (Sigma-Aldrich, St. Louis, US), acridine orange (Sigma-Aldrich, St. Louis, US) and wheat germ agglutinin (WGA) Alexa fluor 488 conjugate (Thermo-Fisher Scientific, Eugene, OR, US)

were used for staining fat, protein and glycosylated components in the sample, respectively.

Commercially available sunflower oil Lecithin (Unilec SF-DP, UAE) and sodium azide (Sigma-Aldrich, Merck KGaA, Germany) were used for the emulsion studies in **Publication 4**. All the chemicals used were of analytical grade.

3.2 Methods

3.2.1 Microfiltration

Two types of microfiltration units were adopted by the thesis. First, a benchtop system was employed in **Publication 1** (Sartoflow Study, Sartorius Stedim Biotech GmbH, Goettingen, Germany) equipped with single channel tubular ceramic membrane (Membralox T1-70, Pall Corporation, Port Washington, NY) with 1.4 μm pore diameter and 0.005 m^2 filtration surface area. Then, a uniform trans-membrane pressure (UTMP) filtration system (Tetra Alcross®, Tetra Pak, Denmark) equipped with multichannel tubular ceramic membrane (Membralox, Pall Corporation, France), 1.4 μm pore diameter and 0.24 m^2 filtration surface area was used in **Publication 2, 3** and **4**.

Before and after each filtration, the system was cleaned according to a three-step washing procedure including base-acid-base steps at temperatures 75°C, 50°C, 75°C, respectively. In particular, the temperature of the washing solution was raised accordingly, and the washing solution was added to the feed. After 25 min, the permeate valve was opened and circulated to the feed for a total washing time of 40 min per cycle, followed by flushing with water after each washing cycle. Pure water flux was determined at the end of each washing procedure by circulating the retentate and permeate to the feed using a feed pump (Fig. 4) and recording the pressure values upon standardized flow conditions. Finally, the water was displaced with 50°C deionized water or other diafiltration solution to eliminate contamination with tap water.

A constant-volume type diafiltration was adopted for raw milk microfiltration by diafiltrating the milk with saline, water or SMUF as the permeate was collected so that the net amount of feed remained constant (**Publication 1**). The amount of diafiltration was allowed to reach 200% of feed weight before ending the process. TMP and permeate flux was constantly measured for further analysis.

Water was used as a diafiltration media for cream microfiltration. First, a stage-wise diafiltration procedure was adopted in **Publication 2** by diluting the cream with 50% of water of the feed weight and concentrating the cream to its initial volume. Overall, four cycles were completed, starting with dilution and ending with concentration. Hence, 50%, 100%, 150% and 200% diafiltration points were obtained for sample withdraw. Two different TMPs, 0.4 bar and 0.6

bar, were used in the experiment and kept constant upon processing. A stage-wise three cycle diafiltration was adopted in **Publication 3** in ratio 1:10 for each cycle. The filtration was operated at 0.6 bar TMP. Finally, to produce MFGM material, unpasteurized cream was diafiltered 1:10 with water followed with pasteurization at 75°C (**Publication 4**). The different diafiltration methods applied are summarized in table 1.

Table 1. Outline of the diafiltration (DF) methods operated in the thesis.

Publication	1	2	3	4
<i>Feed</i>	Raw milk	Raw and pasteurized cream	Pasteurized cream	Raw cream
<i>Type of DF</i>	Continuous	Stagewise	Stagewise in cycles	Continuous
<i>DF % of feed weight</i>	200	200	3 x 900 = 2700	900

The cross-flow velocity was adjusted >5 m/s and the average flux was calculated as flow per total membrane surface area. The filtration temperature was kept 50-55°C in all of the experiments and three replicates were performed for each filtration. After the treatments, creams were stored 5-8°C for further processing or analysis.

3.2.2 Centrifugal separation

The magnitude of MFGM rupture under influence of microfiltration processing and shear was compared to that of traditional centrifugal separation. Centrifugal separation was implemented in three washing cycles similar to that of microfiltration in **Publication 3**. In particular, bovine cream was diluted 1:10 with water and separated (~200 L/h) into skim and cream fractions using a disk stack centrifugal separator (Frau CN2S, Italy) equipped with 51 disks (55 x 51 mm, 52°). The washing-separation process was repeated three times, and samples were collected after each cycle. The temperature was kept 50-55°C upon the whole process and the experiments were conducted in triplicate.

3.2.3 Butter making

Cream and filtered cream were churned into butter to evaluate the effect of protein content on butter formation (**Publication 2**). A precise time-temperature ripening process was performed to allow partial crystallization of the fat before churning. In particular, creams were heated to 19°C in a temperature-controlled water bath (Incubator PT 1000, Aerne analytic, Germany) for two hours, followed by cooling to churning temperature of 12 °C. Creams were churned (ELBA 30, Elecrem, France) into butter immediately after the crystallization process. The phase inversion and formation of butter granules were visually controlled through a transparent window on the churn. After churning, buttermilk was

drained out and the granules were worked into butter. Churning time, temperature, buttermilk and butter amount were recorded, and samples were taken from each fraction. Three replicates were performed for each experiment.

MFGM material in **Publication 4** was produced by churning of the microfiltered cream followed by freeze drying (Alpha 1-4 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) of the resulting buttermilk.

3.2.4 Emulsion preparation

Model food emulsions were formulated with 2% (w/v) MFGM-rich ideal butter-milk powder and 10% sunflower oil (**Publication 4**). The emulsions were supplemented with casein (0.1%) and/or calcium (30 mM). The pH of the water phase was adjusted to 5.8, 6.8 or 7.8 and 0.05% sodium azide was added to prevent microbial spoilage. Finally, the emulsions were sonicated (5x20 s, 40%, Ultrasonic Sonifier S-450, Branson Ultrasonics Corporation, USA) on ice bath. The headspace of the emulsion vials was flushed with nitrogen gas and left for storage at ambient temperature, protected from light. Three replicates were performed for each center point (pH 6.8).

3.2.5 Analysis

Particle sizing and Zeta Potential

The particle size distribution of the samples was determined by a light scattering method using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) according to (Ye et al., 2011). A refractive index of 1.458 and 1.473 was adopted for milk fat (**Publication 1, 2 and 3**) and sunflower oil (**Publication 4**), respectively. The measurements were performed after dilution in water.

The zeta potential of the milk fat globules was measured by using a dynamic light scattering instrument (Zetasizer, Nano series ZS90, Malvern Instruments) according to (Michalski et al., 2001). Samples were diluted in water (**Publication 1**) or saline (**Publication 3**) and measured in a Malvern Dip Cell.

Three readings were made from each individual sample and the measurements were run in triplicate.

Composition

Dry matter content was analysed gravimetrically by drying the samples overnight at 105 ° (Le et al., 2011). Total fat content was analysed according to the extraction method of Röse-Gottlieb (ISO 1211, IDF 1:2010). The Kjeldahl method was used to determine the total nitrogen and a conversion factor 6.38 was adopted to calculate the protein content in the sample, according to the standards (ISO 8968-1:2014, IDF 20-1:2014). Alternatively, the protein content was obtained spectrophotometrically using the Bradford dye-binding method

(Protein Assay, Bio-Rad Laboratories Inc., Hercules, CA) with standard protein curve of skim milk powder (**Publication 1**).

Protein identification was performed by gel electrophoresis (SDS-PAGE). Thin layer chromatography (TLC) and gas chromatography were adopted to separate lipid classes and analyse their concentration, respectively (**Publication 3**).

Microstructural analysis

Confocal laser scanning microscopy, CLSM (Leica TCS SP2, Leica Microsystems, Germany) was adopted to estimate the process-induced changes on the fat globules (**Publication 2**). Samples were dual stained with Nile red (0.05% in acetone) and acridine orange (0.5% in deionized water) to evaluate the interactions with fat globules and proteins, according to Gallier et al. (2010) and Garcia et al. (2014). Briefly, the samples were diluted to 1:10 in phosphate-buffered saline, PBS. Then, 10 μL of Nile red and acridine orange was added per 1 ml of sample with a staining time of at least 20 min (protected from light). Then, the sample was mixed (50:50) with 0.5% agarose (Top Vision LM, Thermo-Fisher Scientific, Spain) and approximately 10 μL of the mix was pipetted on microscopy glass. Finally, a cover glass was sealed to inhibit drying and the sealed sample was allowed to set in dark for 20 min prior to imaging. Comparable staining procedure was adopted for staining fat and glycoproteins, except WGA (50 μL , 1 mg/ml in PBS) was applied instead of protein dye according to Lopez et al. (2010). Argon laser excited the samples at 488 nm and emission wavelengths were collected between 550–650 nm, 515–530 nm and 493–540 nm for Nile red, acridine orange and WGA, respectively. A 63X oil-immersion lens (Leica, Germany) was used for objecting the samples. Dual-stain images were produced by merging two individual images from different channels.

The microstructure of emulsions was assessed with CLSM (**Publication 4**) by staining the oil droplets with Nile Red according to the staining procedure described above. At least 10 individual images were collected for each sample.

4. Results and Discussion

This section highlights the most important results of the thesis and their relevance. First, the role of different factors on milk and cream microfiltration performance is reported. Secondly, a description of the process-induced changes on the milk fat globules is provided. Moreover, the effects of microfiltration and centrifugal separation on MFGM are discussed. Then, the functional properties of MFGM are evaluated based on its emulsifying properties. Finally, a discussion of the ideal butter process and the outcome of the thesis is presented. A detailed presentation of each of the components of the thesis is provided in Publications 1-4.

4.1 Factors affecting whole milk and cream microfiltration

4.1.1 Diafiltration media

The effect of diafiltration with water, skim milk ultrafiltration permeate (SMUF) and saline (50 mM) on permeate flux was examined upon raw milk microfiltration (**Publication 1**). The permeate flux obtained at the beginning of the filtration remained at the same level with saline and water. In contrast, it decreased with time when SMUF was used (Fig. 5). The decreased flux in SMUF diafiltration was associated with the higher dry matter content and viscosity – both critical parameters in microfiltration. Moreover, the dilute concentration of the saline prevented the reduction of the flux, previously attributed to saline diafiltration (Jimenez-Lopez et al., 2011; Carpintero-Tepole et al., 2014). High concentrations of saline may dissociate the casein micelles, which increases milk viscosity and affects the filtration performance.

The differences in filtration performance can be explained by the change in ionic equilibrium. The ionic strength of milk was reduced with water and low concentration saline diafiltration. As a consequence, the fat globules and caseins were subjected to increased repulsive forces (Liu and Guo, 2008; Carpintero-Tepole et al., 2014). Increased electrostatic repulsion most likely limited packing of the concentration polarization layer and inhibited fouling on the filtration membrane. Hence, the concentration polarization layer was looser if particles were adsorbed under electrostatic repulsion forces. Overall, water was suggested as diafiltration media for further studies, as previously proposed for the washing of milk fat globules upon centrifugal separation (Le et al., 2009; Kathirarachchi et al., 2014).

Figure 5. Permeate flux for unpasteurized and pasteurized milk and cream upon microfiltration processing time. Diafiltration (DF) with water was adopted in all experiments, except when skim milk ultrafiltration permeate (SMUF) or 50 mM saline was used for diafiltering milk. Diafiltration was performed with amount of 200% of the feed volume unless indicated otherwise (900% or 3 x 900%). TMP = transmembrane pressure in bars.

The permeate flux decreased with increasing filtration time and cycles, as explained by concentration polarization. Moreover, concentration of the feed increased at the end of each filtration cycle, thus decreasing permeate flux (Fig. 5). The highest permeate flux for cream microfiltration was recorded when high amounts of diafiltration water were used. Evidently, dilution of cream with water decreased concentration and increased permeate flux.

Microfiltration at a transmembrane pressure <1 bar is advisable to avoid compression of the deposit layer (Gesán-Guiziou et al., 1999). However, profound differences in permeate fluxes were observed between a TMP of 0.4 and 0.6 bar (**Publication 2**). Moreover, filtration with lower TMP increased the filtration time significantly by lowering the permeate flux (Fig. 5). Filtration with lower TMP was attributed to the formation of a concentration polarization layer and reversed flow (hysteresis), whereas the higher TMP (0.6 bar) increased filtration throughput without the compression expected at pressures above 1 bar.

4.1.2 Heat treatment

Raw and pasteurized (75°C, 20 s) creams were used for microfiltration to evaluate the effect of heat treatment on filtration performance (**Publication 2**). Pasteurized cream was found to reduce the permeate flux, which affected the

overall processing time and, subsequently, exposure to shear (Fig. 5). Moreover, the permeate flux was found to decrease already at the beginning of the filtration, suggesting that the relatively mild pasteurization modified the milk fat globule surface by protein adsorption.

Heat treatment is known to cause protein conformational changes and increased hydrophobicity (Qi et al., 1997, Kelly and Zydney, 1994). Moreover, heating cream may result in aggregation and in the decline of filtration flux. Hence, it was expected that adsorption of proteins not only enlarged the size of the fat globules but also made them more hydrophobic, thus facilitating the concentration polarization on the filtration membrane. Point of notice is that the relatively low pasteurization temperature was likely insufficient to denature the whey proteins (Laiho et al., 2015). However, minor changes in the proportion of inert (native) to interactive (denatured) whey proteins, and their attachment to casein micelles, may have an influence on deposition layer formation and viscosity. In addition, some of the MFGM proteins are known to denature at temperatures above 60 °C (Sharma et al., 2015). Previously, the hydrophobicity of MFGM was reported to increase by protein adsorption (Haddadian et al., 2017). Therefore, the hydrophobicity of unpasteurized cream was likely lower compared to that of pasteurized cream, which facilitated filtration performance and protein permeation. The mechanism of protein association on the surface of fat globule was attributed to a combination of association types. In particular, protein adsorption may result from protein-protein interactions with MFGM proteins. Furthermore, the attractive interactions between polar lipid head groups and charged protein amino acids may promote protein-lipid interaction. Likewise, van der Waals forces and steric effects may take place at the interface and hydrophobic effects may result from the hydrophobic domains of the proteins and phospholipids.

4.1.3 pH

Diafiltration with water was found to affect cream and milk pH by increasing it from about 6.7 to 7.2 (**Publication 1** and **2**). Moreover, the pH of cream was found to fluctuate upon microfiltration (**Publication 2**). In particular, the pH temporarily decreased when water was added in the stage-wise diafiltration procedure, whereas it increased gradually upon the concentration phase (Fig. 6). The pH of the deionized water adopted in the filtration was 6.1. Therefore, the decrease in pH immediately after increasing water addition was attributed to the lower pH of water and dilution of the pH-affecting components. The increase in pH was explained by permeation of proteins and soluble minerals, as well as dissolution of colloidal calcium phosphate (Gonzalez-Jordan et al., 2015). More specifically, a decrease in protein and mineral concentration upon diafiltration reduced the buffering capacity, which would otherwise inhibit fluctuations in pH. Furthermore, a decrease in ionic strength favoured dissolution of colloidal calcium phosphate to balance the salt equilibrium. Overall, loss of buffering capacity and increase in soluble phosphates gave rise to cream pH upon each diafiltration stage. Previous studies support such interpretation of

dissociation of colloidal phosphates in milk and increase in pH upon diafiltration with water (Alexander et al., 2011).

The natural adjustment of cream pH upon microfiltration affected the filtration performance. Increased cream pH was observed to enhance permeate flux, thus decreased filtration time (**Publication 2**). In contrast, a decrease in pH reduced permeate flux, explained by fouling of the filtration membrane. Interestingly, the delayed permeate flux was restored and the membrane fouling layer diminished if the filtration pH was raised from 6.5 to 7.1 by addition of NaOH. Such phenomenon indicates a substantial impact of electrostatic interactions on cream microfiltration performance: higher pH values increase colloidal repulsion and reduce aggregation. Previous observations support our findings (Jimenez-Lopez et al., 2011). Moreover, casein micelle deposition was reported to decrease under low ionic strength conditions upon skim milk microfiltration (Jimenez-Lopez et al., 2011) and higher pH was found to reduce flux decline upon bovine serum albumin microfiltration (Kelly and Zydney, 1994).

Figure 6: Fluctuation in temporal permeate flux upon cream microfiltration as affected by pH and addition of diafiltration water (indicated with water droplet icons).

4.1.4 Protein permeation

Besides permeate flux, the filtration performance was evaluated in terms of protein permeation: the higher the protein permeation, the more effective the separation efficiency and the higher the MFGM purity in buttermilk. Moreover, permeate flux correlated strongly with protein permeation and the highest protein mass flow was achieved with the highest permeate flux (**Publication 2**).

As for permeate flux, heat treatment of cream was found to lower protein permeation, explained by adsorption of proteins on the surface of fat globules and

aggregation (section 4.1.2). Moreover, the highest protein permeation was achieved when unpasteurized cream or milk was used as a raw material for filtration (Fig. 7).

The permeation of proteins between diafiltration stages was most effective upon the first dilution-concentration stage, followed by a gradual decline upon the following stages. Theoretically, the maximum separation of cream serum proteins is 98-99% (since MFGM-proteins comprise 1-2% of milk proteins (Jukola and Rojas, 2017)), whereas 93% protein separation was achieved in the experiments in the thesis (**Publication 3**). Hence, it can be argued if the maximum separation efficiency is achievable with microfiltration or other separation-based method. Moreover, the protein permeation upon diafiltration showed a gradual decrease in the diafiltration stages, indicating that addition of more diafiltration steps would not result in a higher permeation (**Publication 1 and 2**). The protein separation remained incomplete despite the extensive diafiltration used (Fig. 7). In addition, increasing the amount of diafiltration solution is not economically nor ecologically meaningful. Especially when the filtration performance is evaluated based on the volume of diafiltration solution needed per unit mass of separated protein. Furthermore, increasing the diafiltration stages and processing time enhance MFGM disintegration, thus decrease MFGM yield (**Publication 3**). Hence, it is advisable to optimize the microfiltration process in terms of diafiltration volume and type of addition as well as MFGM purity and yield.

Lower concentration of the milk increased filtration performance in terms of protein separation, compared to that of cream. Moreover, approximately 7 times more diafiltration water was used for cream in order to achieve a protein permeation similar to that of milk (Fig. 7). Hence, concentration of the feed plays a significant role in protein permeation. The type of diafiltration adopted for milk and cream filtrations vary (continuous or stagewise) and different filtration rigs are often employed for milk processing. Therefore, the results for protein permeation undermine a rigorous comparison. However, it is well known that both concentration and diafiltration contribute significantly to filtration performance (Fouladitajar et al., 2015). Furthermore, the dilution of the feed affected the efficiency of the protein permeation. In particular, only 47% protein permeation was achieved in raw cream microfiltration when the cream was diluted with a diafiltration volume of 200% of the feed volume at the beginning of the filtration (**Publication 4**, Fig. 7). In contrast, 78% permeation was achieved when the same amount of diafiltration (200%) was operated in smaller sections (50% each). Compared to the filtration surface area, a high dilution decreases the efficiency of protein permeation by increasing the filtration volume. Hence, the permeating compounds are less likely to encounter the filtration pores and permeate through them. Overall, it is advisable to limit any extensive dilution, within the limits of the concentration effect to flux, to allow effective protein permeation. The efficiency of the microfiltration in terms of protein permeation and diafiltration volume remains as a subject for optimization.

Figure 7. Ratio of protein permeation (mass in permeate/total mass) upon milk and cream microfiltration as influenced by the type (continuous or stagewise) and amount of diafiltration used (%) compared to the feed volume.

4.2 Changes in the physicochemical properties of the milk fat globules

4.2.1 Particle size

The measured particle size distribution of the feed followed that reported for milk and cream (Yao et al., 2016, Michalski et al., 2001). Moreover, the size of the fat globules varied between ~1-15 μm and a smaller fraction of casein micelles was prominent around ~0.1 μm . Microfiltration with 1.4 μm membrane cut-off is therefore expected to remove particles with mean sizes below 1.4 μm , comprising up to 30% of the number of fat globules in milk (Kessler, 2002).

As expected, heat treatment was determined to increase the mean particle size of cream due to protein association (Fig. 8). In addition, diafiltration solution affected the shielding effect *via* particle repulsion (section 4.1.1). The shielding was strong when water was used for diafiltration, thus protecting the fat globules from coalescence. Furthermore, SMUF decreased the apparent particle charge due to high counterion concentration, which screened repulsive forces and caused the fat globules to coalesce. Similar observation was previously reported in washing fat globules by centrifugal separation (Holzmüller et al., 2016). Moreover, loss of small fat globules upon microfiltration is expected to increase the mean particle size. However, longer processing of cream generally decreased its mean particle size: the longer the microfiltration processing time, the more markedly the reduction in mean particle size (Fig. 8). The decrease in size was attributed to shearing or deflocculation of the milk fat globules under the increased shear field and flow conditions (**Publication 3**).

The process-induced size reduction was partly inhibited when high amounts of diafiltration solution were employed. In particular, diafiltration with 200%

water volume resulted 5-6 h processing time in cream microfiltration and $\sim 1 \mu\text{m}$ reduction in fat globule size (**Publication 2**). However, when more than 10 x (2700%) diafiltration water was adopted, under a similar processing time, the size of the fat globules was reduced only by $0.3 \mu\text{m}$ (**Publication 3**). The higher dilution of the cream was expected to increase the separation distance between surface-active material (proteins) and the fat globules. Therefore, the interaction of the proteins and fat globules upon shearing was not fast enough to allow stabilization at the interface, hence restricting the size reduction.

Previously, microfiltration was reported to cause less damage to milk fat globules compared to that of other separation methods, such as centrifugal separation (Brans et al., 2004). However, microfiltration was shown to reduce milk fat globule particle size with increasing processing time (**Publication 2**). Therefore, to elucidate the difference of these processes, microfiltration and centrifugal separation were compared in terms of protein separation; in addition, their effect on milk fat globule size was examined in **Publication 3**. Despite their similar performance in protein separation, a drastic difference in milk fat globule particle size was observed. Interestingly, centrifugal separation increased the mean particle size, whereas microfiltration resulted in the opposite effect (Fig. 8). This observation was attributed to the different flow mechanisms. Moreover, the estimated energy dissipation rate for microfiltration and centrifugal separation was $\sim 1200 \text{ W/kg}$ and, and $\sim 60 \text{ W/kg}$, whereas the turbulent eddy sizes were $\sim 6 \mu\text{m}$ and $\sim 13 \mu\text{m}$, respectively (**Publication 3**). Hence, the flow conditions in centrifugal separation favoured aggregation and coalescence, whereas those of microfiltration induced deflocculation and shearing.

Figure 8. Changes in the mean milk fat globule particle size upon processing. The effect of heat treatment (raw and heat), diafiltration (DF) with water and skim milk ultrafiltration permeate (SMUF), microfiltration (MF) or centrifugal separation (CS) process and diafiltration amount (high and low) is plotted versus processing time.

4.2.2 Zeta potential

The surface charge of the milk fat globules was assessed by means of electrophoretic mobility and zeta potential measurements. The zeta potential of the fat globules decreased upon processing (Table 2), implying an increase in colloidal stability due to increased repulsion (Schramm, 2014). However, increase in negative zeta potential might indicate a damage of MFGM surface or incorporation of serum proteins (Michalski et al., 2002; Le et al., 2009). Furthermore, accumulation of free fatty acids (FFA) on MFGM has been shown to decrease the zeta potential (Bourlieu et al., 2012). In addition, incorporation of air and washing have been shown to release MFGM components and lower the zeta potential (Le et al., 2009).

Table 2. The effect of separation processes on milk fat globule surface charge as measured by the zeta potential (ζ). Diafiltration (DF) with saline (50 mM), skim milk ultrafiltration permeate (SMUF) and water was applied for microfiltration of milk, whereas water was used for washing cream upon microfiltration and centrifugal separation. Samples were collected in fractions with increasing processing as indicated by numerals.

	Milk, ζ (mV)			Cream, ζ (mV)	
	Saline DF	SMUF DF	Water DF	Microfiltration	Centrifugal separator
Feed	-29.9±1.4	-29.2±2.4	-27.6±3.0	-18.8±1.6	-18.8±1.6
Fraction 1	-31.4±2.6	-31.3±2.2	-30.3±1.7	-22.8±1.7	-22.0±0.5
Fraction 2	-33.4±1.7	-31.0±2.1	-29.8±3.3	-23.9±1.7	-24.1±1.6
Fraction 3	-33.6±3.1	-33.5±2.4	-31.2±2.4	-22.8±1.7	-27.3±1.0
Fraction 4	-36.4±2.8	-33.9±1.4	-31.5±1.9		

Diafiltration with different solutions was found to affect the zeta potential of the milk fat globules, **Publication 1**. In particular, the decrease in zeta potential was strongest in diafiltration with saline, followed by SMUF and water, respectively (Table 2). The difference was explained by ionic strength of the diafiltration solutions. In particular, SMUF retains the ionic equilibrium and mineral composition of milk with 6.75 g/L salt concentration (Le et al., 2009), whereas saline comprised only 2.92 g/L NaCl. Therefore, the increase in negative zeta potential in SMUF diafiltration resulted from changes on the MFGM surface, such as partitioning of MFGM components or incorporation of FFA from free fat. Instead, the decrease of zeta potential in saline diafiltration was attributed to the changes in ionic balance of milk. In particular, lower salt concentration and addition of NaCl promoted calcium binding to chloride, which increased the amount of soluble phosphate and lowered of the zeta potential. Diafiltration with water limited the colloidal damage due to enhanced electrostatic double layer and fat globule repulsion, as no counter-ions were involved. Instead, the increase in negative zeta potential was attributed to the slow dissolution of colloidal phosphates. Overall, water was suggested for diafiltration to avoid fat globule coalescence and losses in MFGM material.

The process-induced MFGM damages were estimated based on changes in zeta potential upon microfiltration and centrifugal separation (**Publication 3**). The zeta potential was found to decrease under both processes. However, the decrease was more pronounced in centrifugal separation compared to that of microfiltration, which reached a plateau after the first washing cycle (Table 2). Therefore, it was expected that the decrease in zeta potential upon microfiltration was caused by the decrease in mineral composition, which made the fat globules more negatively charged. Upon microfiltration, the zeta potential did not decrease in further washing cycles, since all the minerals were permeated. In contrast, the zeta potential continued to decrease upon centrifugal separation, implying changes in MFGM structure and possible losses of MFGM components or formation of FFA upon fat globule damage.

The challenges related to MFGM damage evaluation from zeta potential evolution has been noted because of its non-linear relationship with the surface coverage and partitioning of MFGM components (Evers, 2004). Hence, a decrease in zeta potential does not necessarily indicate increased repulsion, as shown in this study. Therefore, estimation of the changes occurring in MFGM is further discussed in the following sections.

4.3 Evaluation of MFGM functionality

As discussed previously, the reduction in particle size and zeta potential can be taken as indicative of the disruption of native milk fat globules and changes in MFGM. Such changes and their effect on MFGM functionality were further assessed by microstructural evaluation, compositional and surface activity analysis and emulsification experiments.

4.3.1 Microstructural observations

Confocal laser scanning microscopy (CLSM) revealed microstructural changes in the milk fat globules upon processing (**Publication 2**). Moreover, the CLSM images confirmed a decrease in milk fat globule particle size with increasing filtration processing time (Fig. 9), as was also observed by the particle size measurements (4.2.1). A long processing time was proposed to cause fat globule rupture, due to increased shear exposure, whereas only limited decrease in size was noticed with shorter processing time.

The expected release of MFGM material was evaluated by studying WGA-labelled glycosylated components in fat globule membranes. Figure 9 reveals that a significant quantity of MFGM material remained in the fat globules after the filtration process. Most likely, diafiltration with water protected the fat globules from rupture and evident losses of MFGM material due to increased repulsion. Nevertheless, it is likely that water diafiltration releases some membrane components due to its solubilizing effect and associated interactions. Moreover, the triple-layer structure of MFGM might pose challenges in the observation of surface component desorption by exposing glycosylated components from the inner layers upon desorption phenomena. Hence, any changes in the molecular scale cannot be assessed appropriately.

The relatively mild pasteurization treatment was confirmed to induce protein adsorption on the surface of fat globules (Fig. 9B). Pasteurization at 55-60°C is known to cause protein adsorption on the surface of fat globule (Sharma et al., 2015; Houlihan et al., 1992; Singh, 2006). However, the protein interaction was no longer apparent after microfiltration, due to several possible factors: i) most of the proteins permeated upon processing, and ii) low protein concentration and lack of sensitivity of the CLSM limited the assessment. Furthermore, iii) MFGM might comprise limited available binding sites for protein association (Ye et al., 2004). If such bindings sites are occupied or released upon processing, no further association occurs. Overall, the microstructural estimation indicates that changes in MFGM structure can be minimized if unpasteurized cream is used for filtration and the process is operated under optimal efficiency in terms of processing time. Further methods are needed to estimate the extent of MFGM damage.

Figure 9. Confocal laser scanning microscopy images of (A) raw cream (B) pasteurized cream, microfiltered cream with (C) short processing time and (D) long processing time. Fat droplets stained with Nile red (red), proteins with acridine orange (green) and glycosylated compounds with WGA (cyan).

4.3.2 Partitioning of MFGM components

Both protein and polar lipid fraction in MFGM were analysed to evaluate the process-induced changes in MFGM composition. Proteins were analysed by electrophoresis to compare the relative amount of MFGM proteins and serum proteins and to determine the partitioning of membrane-associated proteins upon processing (**Publication 3** and **4**).

Figure 10 shows an increase in the intensity of the bands for high-molecular-weight proteins, indicating that MFGM proteins were concentrated upon processing, not released. Previously, centrifugal separation was reported to release MFGM proteins, such as XO, due to coalescence upon washing (Holzmüller et al., 2016). However, results obtained here suggest that the cream protein fraction became concentrated with high-molecular-weight MFGM proteins, such as XO, Muc1, BTN, adipophilin and PAS6/7. As expected, the intensity of casein decreased when unpasteurized cream was used as raw material instead of pasteurized cream (**Publication 4**), due to incorporation of serum proteins on MFGM upon pasteurization (Hansen et al., 2018).

Figure 10. SDS-PAGE protein profiling of (A) processed creams from microfiltration (MF) and centrifugal separation (C) upon 1-3 washing cycles, and of (B) milk fat globule membrane (MFGM) material derived from buttermilk. The protein concentration of each lane was ~10 µg. Numerals in (A) indicate the relative intensity of the bands. Std = molecular weight standard, Rf = reference cream, Muc1 = mucin 1, XO = xanthine oxidase, LP = lactoperoxidase, LF = lactoferrin, BTN = butyrophilin, Adipo. = adipophilin, PAS6/7 = periodic acid and shiff 6/7, CNs = caseins, β-Lg = betalactoglobulin, PP3 = proteose peptone 3, a-La = α-lactalbumin.

The mass balance of polar lipids was conducted to quantify the loss in MFGM material. Figure 11 shows the partitioning of polar lipids from cream to permeate and skim fractions after microfiltration and centrifugal separation, respectively. Moreover, the loss of polar lipid mass was strongest upon the first washing cycle, indicating losses of the smallest fat globules, which comprise relatively large surface area and thus high amount of polar lipid (Rombaut et al., 2006). In particular, small fat globules, with diameter <1 µm, comprise ~25% of total

fat globules in milk, and, among them, up to half of the original milk polar lipids may permeate (Kessler, 2002). Overall, the decrease in polar lipid mass after three washing cycles was found higher for centrifugal separation compared to that of microfiltration (Fig. 11).

Figure 11. Polar lipid mass balance for cream (A) microfiltration and (B) centrifugal separation and their respective relative amounts of polar lipids from fat in permeate and skim fraction. Percentages correspond to permeation of polar lipids after 1, 2 and 3 washing cycles and in cumulative total.

The ratio of polar lipid-to-fat was calculated to determine if the polar lipids permeated among intact fat globules, as damaged fragments or individual molecules. After the first washing cycle of microfiltration and centrifugal separation, 7% and 6% of the permeated fat comprised polar lipids, respectively. Such, ratio was expected to result from partitioning of the smallest, intact fat globules (**Publication 3**). The ratio of polar lipid to fat decreased with increasing washing cycles upon microfiltration due to decrease in small fat globule permeation and potential release of polar lipid material from MFGM (Fig. 11). In contrast, the ratio remained unchanged at the end of the washing cycle upon centrifugal separation, implying that more polar lipids may have been disintegrated upon processing. Previously, centrifugal separation was noted to release polar lipid material from cream (Zheng et al., 2014). Overall, it can be expected that both processes caused desorption of MFGM polar lipid material.

The loss of polar lipids is expected to shift the original ratio of individual polar lipids in MFGM. In previous reports, centrifugation was reported to release loosely attached polar lipids, such as PE and PC, whereas SM was reported to bear higher resistance to rupture (Zheng et al., 2014; Cheng et al., 2017). Hence,

a thorough analysis of the molecular composition of individual polar lipids within different fractions remains a subject for further development.

4.3.3 Surface activity and emulsifying properties

Dynamic surface tension measurements indicated that the MFGM material derived from ideal buttermilk was surface active (**Publication 4**). Moreover, MFGM lowered the surface tension to approximately the same value (55 mN/m) as commercial phospholipid emulsifiers (lecithin) at equilibrium. The results indicated a potential of MFGM material in the formulation of emulsions.

The emulsifying properties of MFGM were examined in model food emulsions in the presence or absence of casein and calcium. The emulsions produced with MFGM by low shear sonication resulted in mean droplet size of 1.2 μm . The droplets were possibly formed differently and destabilized at $\text{pH} < 6$ due to decreased repulsive surface charge. The stability increased at higher pH or in the presence of casein (Fig. 12). The stabilizing effect of casein was partly anticipated since proteins and phospholipids have been reported to have synergistic effects on emulsion behaviour (McClements and Jafari, 2018; Xue et al., 2014). However, compared to that of pure phospholipid emulsifier, lecithin, for MFGM the effect of casein on emulsion stability was limited. Moreover, the interaction of casein was more pronounced with lecithin where lipids display less repulsion and their phase state is expected to be more favourable for interaction compared to that of MFGM (Obeid et al., 2019). Overall, the interaction between casein and MFGM was anticipated to be limited and very specific (Lopez et al., 2010).

MFGM-based emulsions were destabilized by addition of calcium, due to decrease in electrostatic shielding and coalescence (Fig. 12). Interestingly, the negative effect of calcium was offset by casein when both were added together. Three possible explanations can explain these observations: Firstly, calcium induced flocculation of the emulsion droplets in the presence of casein, thus inhibiting droplet coalescence and increased stability. Secondly, adsorption of calcium-casein complexes on MFGM may have increased the surface coverage and steric repulsion of the emulsion droplets (Dickinson, 2010). Lastly, MFGM glycoproteins may have inhibited the calcium-induced protein flocculation due to their calcium binding ability and by blocking the calcium binding sites on casein molecules (McCarthy et al., 2014; Müller-Buschbaum et al., 2007; Ye et al., 2012).

The emulsion microstructure was evaluated based on CLSM images. Moreover, flocculation was apparent in lecithin-based emulsion when supplemented with calcium and casein, whereas it was indistinct in the MFGM emulsions. The calcium-induced protein flocculation has been reported and cannot be ignored (McCarthy et al., 2014; Ye and Singh, 2001). Hence, it was expected that the heterogeneity of MFGM interface allowed partial interaction with casein, thus causing limited flocculation.

Figure 12. (A) MFGM (ideal buttermilk) emulsion microstructure supplemented with (B) casein, (C) calcium or (D) both and their respective mean drop size diameter in (i) freshly prepared samples and (ii) after 7 days of storage. Stability of the emulsions is elucidated by the photo images after 4 months of storage.

The stability of the MFGM-based emulsions, upon long-term storage, was found highest in the absence of calcium or calcium and casein, which led to emulsion clarification due to coalescence and flocculation (Fig. 12). Overall, MFGM was shown to be a potential natural and nutraceutical emulsifier, especially in dairy products where components such as casein and calcium play a significant role. However, further research is needed to estimate MFGM's emulsifying ability and emulsion characteristics prepared by different emulsifying processes, e.g. high-pressure homogenization.

4.4 Ideal butter process

The churning processes of microfiltered cream and unfiltered cream (traditional) were compared to evaluate the effect of microfiltration on buttermaking (**Publication 2**). The loss of proteins in microfiltered cream was expected to affect churning time (Smith et al., 2000). Moreover, the stability of milk was found to decrease upon microfiltration and protein permeation (**Publication 1**). The decrease in stability was explained by the reduction of the viscosity of

the continuous phase, which according to Stoke's law (Eq. 1) affects creaming (Kessler, 2002). The effect of proteins on stability, due to altered viscosity, was suggested previously (Sajedi et al., 2014). Therefore, any loss in cream protein could enhance the phase inversion upon churning.

Interestingly, no significant difference was recorded between the churning times of cream and microfiltered cream (Table 3). This unexpected observation relates to the variation of churning temperature (11.9-12.7°C), which was found to correlate negatively with churning time. Previously, the size of milk fat globules was reported to affect milk crystallization behaviour and the small fat globules were reported to delay crystallization (Michalski et al., 2004). Hence, one could expect that the loss of the smallest fat globules upon microfiltration affect crystallization and churning. This subject remains to be elucidated for scale-up processing.

Table 3. Comparison of traditional and ideal buttermaking process and their effect on butter and buttermilk composition.

	Butter	Ideal butter	Butter-milk	Ideal Buttermilk
<i>Churning time, min</i>	3.40±0.29	3.41±0.32	-	-
<i>Yield, kg</i>	4.28±0.34	4.23±0.59	4.55±0.11	4.59±0.50
<i>Protein, %</i>	0.60±0.03	0.21±0.02	3.28±0.10	0.68±0.10
<i>Dry matter, %</i>	85.70±0.50	85.50±0.93	9.97±0.27	2.09±0.17

Butter yield was unaffected by the permeation of proteins. In particular, corresponding values of butter and buttermilk were obtained from cream with or without microfiltration (Table 3). However, small differences in the fat composition took place, since loss of MFGM (and small fat globules) upon processing might decrease the amount of some nutraceutical components, such as CLA (Michalski et al., 2005). As expected, microfiltration of cream lowered the protein content, correspondingly in ideal butter and ideal buttermilk. The dry matter content in ideal buttermilk was much lower compared to that of buttermilk (Table 3).

The dry matter of ideal buttermilk was expected to be enriched with MFGM fragments. Furthermore, the freeze-dried ideal buttermilk consisted of 73.5% fat, 16.5% protein and 1.08% ash. Most of the protein was expected to result from MFGM (based on the protein profiling, Fig. 10 B). In contrast, the fat fraction was not expected to consist solely of MFGM polar lipids but also of triacylglycerols from small intact fat globules and FFA present in buttermilk.

Previously, it was argued that the quality of the MFGM depends on its source. In particular, butter serum was reported to contain more SM compared to buttermilk, possibly resulting in different functional or nutraceutical properties (Bourlieu et al., 2018). In turn, buttermilk is usually considered as low-value side stream and exploitation of the MFGM from buttermilk would be economically beneficial. Moreover, ideal buttermilk offers a readily available source of MFGM, without the need for further separation processes.

Overall, microfiltration may be considered a scalable method to integrate in butter making process, since it is already widely adopted by the dairy industry

for instance in processing cheese and whey (Heino et al., 2010). However, the quality of MFGM derived from different sources and their functionality remains a subject for elucidation. Furthermore, the ideal butter process should be optimized with respect to the intended MFGM application and feasibility.

5. Concluding Remarks

Ideal butter, a novel process for enhancing MFGM exploitation from butter side streams, was developed and examined in this study. As a starting point, it was hypothesized that bovine cream can be microfiltered for separation of proteins and fat globules. Furthermore, the filtered cream can be churned to butter and the resulting buttermilk can be enriched with functional MFGM fragments, without need for further purification.

Microfiltration was proven suitable for protein separation from milk and cream. However, the overall filtration performance was affected by the nature of the cream in the feed. In particular, heat treatment increased accumulation of serum protein on milk fat globules, hence decreasing their permeation upon filtration. Moreover, pasteurization increased the hydrophobicity of the cream and facilitated concentration polarization on the surface of the filtration membrane.

Cream stability decreased upon protein permeation. Furthermore, diafiltration with SMUF decreased colloidal repulsion and favoured coalescence. In contrast, pure water was found to increase electrostatic repulsion and limited particle deposition on the filtration membrane, due to a reduction in ionic strength. Therefore, water was suggested for diafiltration media for cream microfiltration. The quality of water is expected to affect the mineral balance of cream and overall performance of the filtration.

Unexpectedly, the pH of the cream increased upon diafiltration with water (despite its relatively low pH). Such effect was explained by the reduction in the buffering capacity upon diafiltration and by the decreased ionic strength, favouring dissolution of colloidal calcium phosphate. Moreover, a higher pH enhanced filtration performance by increasing permeate flux. Such phenomenon indicates a considerable influence of electrostatic interactions on cream microfiltration performance.

Microfiltration yielded 93% separation of cream proteins and the resultant protein fraction was enriched with MFGM proteins. Long processing time increased shear exposure and loss of polar lipids (20%), explained by permeation of small fat globules and disruption of MFGM. Microfiltration can be considered beneficial compared to centrifugal separation in terms of reduced water consumption. Unlike initially assumed, microfiltration induced higher energy dissipation compared to that of centrifugal separation. Such conditions favour fat globule deflocculation and shearing whereas coalescence and flocculation were dominant in centrifugal separation.

Microfiltered creams were churned into butter. Equal amounts of butter and buttermilk were produced by the ideal butter process compared to that of traditional butter process. The loss of proteins in cream did not affect the phase inversion, e.g., churning process. The ideal butter and buttermilk yielded significantly lower protein content, most from the residual protein in MFGM. The detailed mechanisms of the effect of protein on churning process needs to be examined under carefully optimized conditions.

MFGM-rich ideal buttermilk powder was found to be surface active and capable of forming food emulsions by low sonication method. Such material is of interest in formulating nutraceutical food emulsions, including infant formulas. A detailed information of MFGM mass balance upon ideal butter process and its quality in different fractions (butterserum or buttermilk) are topics for further consideration. Overall, the ideal butter method developed in this study brings value to dairy processing through novel and scalable cream fractionation method, side stream valorisation, and exploitation of MFGM.

References

- Abbas, H., M. M. Hossain and X. D. Chen. 2006. *A laboratory investigation of the anhydrous milkfat fractionation using a membrane technique*. Sep. Purif. Technol. 48:167-175.
- Adams, M. C. and D. M. Barbano. 2013. *Serum protein removal from skim milk with a 3-stage, 3× ceramic isoflux membrane process at 50°C*. J. Dairy Sci. 96:2020-2034.
- Alexander, M., M. Nieh, M. A. Ferrer and M. Corredig. 2011. *Changes in the calcium cluster distribution of ultrafiltered and diafiltered fresh skim milk as observed by small angle neutron scattering*. J. Dairy Res. 78:349-356.
- Beolchini, F. 2005. *Microfiltration of bovine and ovine milk for the reduction of microbial content: Effect of some operating conditions on permeate flux and microbial reduction*. Sep. Sci. Technol. 40:757-772.
- Berton-Carabin, C., C. Genot, C. Gaillard, D. Guibert and M. H. Ropers. 2013. *Design of interfacial films to control lipid oxidation in oil-in-water emulsions*. Food Hydrocoll. 33:99-105.
- Bourlieu, C., F. Rousseau, V. Briard-Bion, M. -. Madec and S. Bouhallab. 2012. *Hydrolysis of native milk fat globules by microbial lipases: Mechanisms and modulation of interfacial quality*. Food Res. Int. 49:533-544.
- Bourlieu, C., D. Cheillan, M. Blot, P. Daira, M. Trauchessec, S. Ruet, J. Gassi, E. Beaucher, B. Robert, N. Leconte, S. Bouhallab, F. Gaucheron, G. Gésan-Guiziou and M. Michalski. 2018. *Polar lipid composition of bioactive dairy co-products buttermilk and butterserum: Emphasis on sphingolipid and ceramide isoforms*. Food Chem. 240:67-74.
- Brans, G., C. G. P. H. Schroën, R. G. M. van der Sman and R. M. Boom. 2004. *Membrane fractionation of milk: State of the art and challenges*. J. Membr. Sci. 243:263-272.
- Britten, M., S. Lamothe and G. Robitaille. 2008. *Effect of cream treatment on phospholipids and protein recovery in butter-making process*. Int. J. Food Sci. Tech. 43:651-657.
- Carpintero-Tepole, V., E. Brito-de la Fuente, E. Martínez-González and B. Torrestiana-Sánchez. 2014. *Microfiltration of concentrated milk protein dispersions: The role of pH and minerals on the performance of silicon nitride microsieves*. LWT - Food Sci. Technol. 59:827-833.
- Cheng, K., M. Ropers and C. Lopez. 2017. *The miscibility of milk sphingomyelin and cholesterol is affected by temperature and surface pressure in mixed langmuir monolayers*. Food Chem. 224:114-123.
- Contarini, G. and M. Povolo. 2013. *Phospholipids in milk fat: Composition, biological and technological significance, and analytical strategies*. Int. J. Molec. Sci. 14:2808-2831.
- Corredig, M. and D. Dalgleish. 1998. *Effect of heating of cream on the properties of milk fat globule membrane isolates*. J. Agric. Food Chem. 46:2533-2540.

- Corredig, M. and D. Dalgleish. 1997. *Isolates from industrial buttermilk: Emulsifying properties of materials derived from the milk fat globule membrane*. J. Agric. Food Chem. 45:4595-4600.
- Corredig, M., R. R. Roesch and D. G. Dalgleish. 2003. *Production of a novel ingredient from buttermilk*. J. Dairy Sci. 86:2744-2750.
- Corredig, M. and D. G. Dalgleish. 1998. *Characterization of the interface of an oil-in-water emulsion stabilized by milk fat globule membrane material*. J. Dairy Res. 65:465-477.
- Dewettinck, K., R. Rombaut, N. Thienpont, T. T. Le, K. Messens and J. Van Camp. 2008. *Nutritional and technological aspects of milk fat globule membrane material*. Int. Dairy J. 18:436-457.
- Dickinson, E. 2010. *Flocculation of protein-stabilized oil-in-water emulsions*. Colloids Surf. B: Biointerfaces. 81:130-140.
- Einstein, A. 1906. *Zur theorie der brownischen bewegung*. Ann Phys Leipzig. 324:371-381.
- Evers, J. M. 2004. *The milkfat globule membrane—methodologies for measuring milkfat globule (membrane) damage*. Int. Dairy J. 14:747-760.
- Fauquant, J., E. Beaucher, C. Sinet, B. Robert and C. Lopez. 2014. *Combination of homogenization and cross-flow microfiltration to remove microorganisms from industrial buttermilks with an efficient permeation of proteins and lipids*. Innov. Food Sci. Emer. Tech. 21:131-141.
- Fong, B. Y., C. S. Norris and A. K. H. MacGibbon. 2007. *Protein and lipid composition of bovine milk fat globule membrane*. Int. Dairy J. 17:275-288.
- Fouladitajar, A., F. Z. Ashtiani, B. Dabir, H. Rezaei and B. Valizadeh. 2015. *Response surface methodology for the modeling and optimization of oil-in-water emulsion separation using gas sparging assisted microfiltration*. Environ. Sci. Poll. Research. 22:2311-2327.
- Gallier, S., D. Acton, M. Garg and H. Singh. 2017. *Natural and processed milk and oil body emulsions: Bioavailability, bioaccessibility and functionality*. Food Structure. 13:13-23.
- Gallier, S., K. C. Gordon and H. Singh. 2012. *Chemical and structural characterisation of almond oil bodies and bovine milk fat globules*. Food Chem. 132:1996-2006.
- Gallier, S., D. Gragson, R. Jimenez-Flores and D. Everett. 2010. *Using confocal laser scanning microscopy to probe the milk fat globule membrane and associated proteins*. J. Agric. Food Chem. 58:4250-4257.
- Gallier, S., K. Vocking, J. A. Post, B. Van De Heijning, D. Acton, E. M. Van Der Beek and T. Van Baalen. 2015. *A novel infant milk formula concept: Mimicking the human milk fat globule structure*. Colloids Surf. B: Biointerfaces. 136:329-339.
- Garcia, C., C. Antona, B. Robert, C. Lopez and M. Armand. 2014. *The size and interfacial composition of milk fat globules are key factors controlling triglycerides bioavailability in simulated human gastro-duodenal digestion*. Food Hydrocoll. 35:494-504.
- Garcia-Moreno, P. J., A. F. Horn and C. Jacobsen. 2014. *Influence of casein-phospholipid combinations as emulsifier on the physical and oxidative stability of fish oil-in-water emulsions*. J. Agric. Food Chem. 62:1142-1152.
- Gésan-Guiziou, G., E. Boyaval and G. Daufin. 1999. *Critical stability conditions in crossflow microfiltration of skimmed milk: Transition to irreversible deposition*. J. Membr. Sci. 158:211-222.

- Gesan-Guiziou, G., G. Daufin, E. Boyaval and O. Le Berre. 1999. *Wall shear stress: Effective parameter for the characterisation of the cross-flow transport in turbulent regime during skimmed milk microfiltration*. Lait. 79:347-354.
- Golbandi, R., M. A. Abdi, A. A. Babaluo, A. B. Khoshfetrat and T. Mohammadlou. 2013. *Fouling study of TiO₂-boehmite MF membrane in defatting of whey solution: Feed concentration and pH effects*. J. Membr. Sci. 448:135-142.
- Gonzalez-Jordan, A., P. Thomar, T. Nicolai and J. Dittmer. 2015. *The effect of pH on the structure and phosphate mobility of casein micelles in aqueous solution*. Food Hydrocoll. 51:88-94.
- Goudedranche, H., J. Fauquant, J.L. Maubois. 2000. *Fractionation of globular milk fat by membrane microfiltration*. Le Lait, INRA Edit. 80(1):93-98.
- Guerra, A., G. Jonsson, A. Rasmussen, E. Waagner Nielsen and D. Edelsten. 1997. *Low cross-flow velocity microfiltration of skim milk for removal of bacterial spores*. Int. Dairy J. 7:849-861.
- Haddadian, Z., G. T. Eyres, A. Carne, D. W. Everett and P. Bremer. 2017. *Impact of different milk fat globule membrane preparations on protein composition, xanthine oxidase activity, and redox potential*. Int. Dairy J. 64:14-21.
- Hansen, S. F., B. Petrat-Melin, J. T. Rasmussen, L. B. Larsen, M. S. Ostfeld and L. Wiking. 2018. *Placing pasteurisation before or after microfiltration impacts the protein composition of milk fat globule membrane material*. Int. Dairy J. 81:35-41.
- Heid, H. W. and T. W. Keenan. 2005. *Intracellular origin and secretion of milk fat globules*. Eur. J. Cell Biol. 84:245-258.
- Heino, A., J. Uusi-Rauva and M. Outinen. 2010. *Pre-treatment methods of edam cheese milk. effect on cheese yield and quality*. LWT - Food Sci. Technol. 43:640-646.
- Hoffman, W., C. Kiesner, I. Clawin-Rädecker, D. Martin, K. Einhoff, P. C. Lorenzen, H. Meisel, P. Hammer, G. Suhren and P. Teufel. 2006. *Processing of extended shelf life milk using microfiltration*. Int. J. Dairy Tech. 59:229-235.
- Holzmüller, W. and U. Kulozik. 2016. *Technical difficulties and future challenges in isolating membrane material from milk fat globules in industrial settings – A critical review*. Int. Dairy J. 61:51-66.
- Holzmüller, W., M. Müller, D. Himbert and U. Kulozik. 2016. *Impact of cream washing on fat globules and milk fat globule membrane proteins*. Int. Dairy J. 59:52-61.
- Houlihan, A., P. Goddard, S. Nottingham, B. Kitchen and C. Masters. 1992. *Interactions between the bovine-milk fat globule-membrane and skim milk components on heating whole milk*. J. Dairy Res. 59:187-195.
- Hurt, E. and D. M. Barbano. 2010. *Processing factors that influence casein and serum protein separation by microfiltration*. J. Dairy Sci. 93:4928-4941.
- Jimenez-Lopez, A. J. E., N. Leconte, F. Garnier-Lambrouin, A. Bouchoux, F. Rousseau and G. Gésan-Guiziou. 2011. *Ionic strength dependence of skimmed milk microfiltration: Relations between filtration performance, deposit layer characteristics and colloidal properties of casein micelles*. J. Membr. Sci. 369:404-413.
- Jukkola, A. and O. J. Rojas. 2017. *Milk fat globules and associated membranes: Colloidal properties and processing effects*. Adv. Colloid Interface Sci. 245:92-101.
- Kathiriarachchi, K., M. Leus and D. W. Everett. 2014. *Oxidation of aldehydes by xanthine oxidase located on the surface of emulsions and washed milk fat globules*. Int. Dairy J. 37:117-126.
- Kelly, S. and A. Zydny. 1994. *Effects of intermolecular thiol-disulfide interchange reactions on bsa fouling during microfiltration*. Biotechnol. Bioeng. 44:972-982.

- Kessler, H. G. ed. 2002. *Food and Bio Process Engineering: Dairy Technology*. 5th ed. Verlag A. Kessler, München.
- Laiho, S., D. Ercili-Cura, P. Forssell, P. Myllärinen and R. Partanen. 2015. *The effect of dynamic heat treatments of native whey protein concentrate on its dispersion characteristics*. Int. Dairy J. 49:139-147.
- Le, T. T., J. Van Camp, R. Rombaut, F. van Leeckwyck and K. Dewettinck. 2009. *Effect of washing conditions on the recovery of milk fat globule membrane proteins during the isolation of milk fat globule membrane from milk*. J. Dairy Sci. 92:3592-3603.
- Le, T. T., J. van Camp, P. A. L. Pascual, G. Meesen, N. Thienpont, K. Messens and K. Dewettinck. 2011. *Physical properties and microstructure of yoghurt enriched with milk fat globule membrane material*. Int. Dairy J. 21:798-805.
- Liu, W., A. Ye, W. Liu, C. Liu and H. Singh. 2013. *Stability during in vitro digestion of lactoferrin-loaded liposomes prepared from milk fat globule membrane-derived phospholipids*. J. Dairy Sci. 96:2061-2070.
- Liu, Y. and R. Guo. 2008. *pH-dependent structures and properties of casein micelles*. Biophys. Chem. 136:67-73.
- Lopez, C., V. Briard-Bion, O. Ménard, E. Beaucher, F. Rousseau, J. Fauquant, N. Leconte and B. Robert. 2011. *Fat globules selected from whole milk according to their size: Different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains*. Food Chem. 125:355-368.
- Lopez, C., M. Madec and R. Jimenez-Flores. 2010. *Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins*. Food Chem. 120:22-33.
- Lu, J., X. Wang, W. Zhang, L. Liu, X. Pang, S. Zhang and J. Lv. 2016. *Comparative proteomics of milk fat globule membrane in different species reveals variations in lactation and nutrition*. Food Chem. 196:665-672.
- McCarthy, N. A., A. L. Kelly, J. A. O'Mahony and M. A. Fenelon. 2014. *Sensitivity of emulsions stabilised by bovine β -casein and lactoferrin to heat and CaCl_2* . Food Hydrocoll. 35:420-428.
- McClements, D. J. 2009. *Biopolymers in food emulsions*. in *Modern Biopolymer Science – Bridging the Divide between Fundamental Treatise and Industrial Application*. S. Kapis, I. T. Norton and J. B. Ubbink eds. Elsevier, US.
- McClements, D. J. and C. E. Gumus. 2016. *Natural emulsifiers – biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physico-chemical basis of functional performance*. Adv. Colloid Interface Sci. 234:3-26.
- McClements, D. J. and S. M. Jafari. 2018. *Improving emulsion formation, stability and performance using mixed emulsifiers: A review*. Adv. Colloid Interface Sci. 251:55-79.
- Michalski, M., V. Briard and F. Michel. 2001. *Optical parameters of milk fat globules for laser light scattering measurements*. Lait. 81:787-796.
- Michalski, M. C., N. Leconte, V. Briard-Bion, J. Fauquant, J. L. Maubois and H. Goudéranche. 2006. *Microfiltration of raw whole milk to select fractions with different fat globule size distributions: Process optimization and analysis*. J. Dairy Sci. 89:3778-3790.
- Michalski, M., V. Briard and P. Juaneda. 2005. *CLA profile in native fat globules of different sizes selected from raw milk*. Int. Dairy J. 15:1089-1094.
- Michalski, M., B. Camier, J. Gassi, V. Briard-Bion, N. Leconte, M. Famelart and C. Lopez. 2007. *Functionality of smaller vs control native milk fat globules in emmental cheeses manufactured with adapted technologies*. Food Res. Int. 40:191-202.

- Michalski, M., F. Michel, D. Sainmont and V. Briard. 2002. *Apparent ζ -potential as a tool to assess mechanical damages to the milk fat globule membrane*. Colloids Surf. B: Biointerfaces. 23:23-30.
- Michalski, M., M. Ollivon, V. Briard, N. Leconte and C. Lopez. 2004. *Native fat globules of different sizes selected from raw milk: Thermal and structural behavior*. Chem. Phys. Lipids. 132:247-261.
- Müller-Buschbaum, P., R. Gebhardt, S. V. Roth, E. Metwalli and W. Doster. 2007. *Effect of calcium concentration on the structure of casein micelles in thin films*. Biophysical J. 93:960-968.
- Obeid, S., F. Guyomarc'h, E. David-Briand, F. Gaucheron, A. Riaublanc and C. Lopez. 2019. *The phase and charge of milk polar lipid membrane bilayers govern their selective interactions with proteins as demonstrated with casein micelles*. J Colloid Interface Sci. 534:279-290.
- Phan, T. T. Q., T. T. Le, P. Van der Meeren and K. Dewettinck. 2014. *Comparison of emulsifying properties of milk fat globule membrane materials isolated from different dairy by-products*. J. Dairy Sci. 97:4799-4810.
- Phan, T., T. Le, D. Van de Walle, P. Van der Meeren and K. Dewettinck. 2016. *Combined effects of milk fat globule membrane polar lipids and protein concentrate on the stability of oil-in-water emulsions*. Int. Dairy J. 52:42-49.
- Piry, A., A. Heino, W. Kuehn, T. Grein, S. Ripperger and U. Kulozik. 2012. *Effect of membrane length, membrane resistance, and filtration conditions on the fractionation of milk proteins by microfiltration*. J. Dairy Sci. 95:1590-1602.
- Qi, X., C. Holt, D. McNulty, D. Clarke, S. Brownlow and G. Jones. 1997. *Effect of temperature on the secondary structure of beta-lactoglobulin at pH 6.7, as determined by CD and IR spectroscopy: A test of the molten globule hypothesis*. Biochem. J. 324:341-346.
- Rombaut, R., J. V. Camp and K. Dewettinck. 2006. *Phospho- and sphingolipid distribution during processing of milk, butter and whey*. Int. J. Food Sci. Tech. 41:435-443.
- Rombaut, R., V. Dejonckheere and K. Dewettinck. 2006. *Microfiltration of butter serum upon casein micelle destabilization*. J. Dairy Sci. 89:1915-1925.
- Sajedi, M., A. Nasirpour, J. Keramat and S. Desobry. 2014. *Effect of modified whey protein concentrate on physical properties and stability of whipped cream*. Food Hydrocoll. 36:93-101.
- Schramm, L. L. 2014. *Emulsions, Foams, Suspensions, and Aerosols*. Wiley-VCH, Somerset, DE.
- Scott, L., S. Duncan, S. Sumner and K. Waterman. 2003. *Physical properties of cream reformulated with fractionated milk fat and milk-derived components*. J. Dairy Sci. 86:3395-3404.
- Sharma, P., I. Oey and D. W. Everett. 2015. *Interfacial properties and transmission electron microscopy revealing damage to the milk fat globule system after pulsed electric field treatment*. Food Hydrocoll. 47:99-107.
- Singh, H. 2006. *The milk fat globule membrane—A biophysical system for food applications*. Curr. Opin. Colloid Interface Sci. 11:154-163.
- Smith, A. K., Y. Kakuda and H. D. Goff. 2000. *Changes in protein and fat structure in whipped cream caused by heat treatment and addition of stabilizer to the cream*. Food Res. Int. 33:697-706.
- Stokes, G. G. 1843. *On some cases of fluid motion*. Trans.Camb.Phil.Soc. 8:105-137.
- Tashvigh, A. A., A. Fouladitajar and F. Zokaee Ashtiani. 2015. *Modeling concentration polarization in crossflow microfiltration of oil-in-water emulsion using shear-induced diffusion; CFD and experimental studies*. Desalination. 357:225-232.

- Xue, J. and Q. Zhong. 2014. *Thyme oil nanoemulsions coemulsified by sodium caseinate and lecithin*. J. Agric. Food Chem. 62:9900-9907.
- Yao, Y., G. Zhao, Y. Yan, H. Mu, Q. Jin, X. Zou and X. Wang. 2016. *Milk fat globules by confocal raman microscopy: Differences in human, bovine and caprine milk*. Food Res. Int. 80:61-69.
- Ye, A., J. Cui and H. Singh. 2011. *Proteolysis of milk fat globule membrane proteins during in vitro gastric digestion of milk*. J. Dairy Sci. 94:2762-2770.
- Ye, A., J. Lo and H. Singh. 2012. *Formation of interfacial milk protein complexation to stabilize oil-in-water emulsions against calcium*. J. Colloid Interface Sci. 378:184-190.
- Ye, A., H. Singh, D. James Oldfield and S. Anema. 2004. *Kinetics of heat-induced association of β -lactoglobulin and α -lactalbumin with milk fat globule membrane in whole milk*. Int. Dairy J. 14:389-398.
- Ye, A. and H. Singh. 2001. *Interfacial composition and stability of sodium caseinate emulsions as influenced by calcium ions*. Food Hydrocoll. 15:195-207.
- Zheng, H., R. Jiménez-Flores and D. W. Everett. 2014. *Lateral lipid organization of the bovine milk fat globule membrane is revealed by washing processes*. J. Dairy Sci. 97:5964-5974.

Standards

- ISO-IDF. (2010). *Milk—Determination of fat content*. International Standard ISO 1211 and IDF 1:2010. ISO, Geneva, Switzerland and IDF, Brussels, Belgium.
- ISO-IDF. (2014). *Milk and milk products – Determination of nitrogen content – Part 1: Kjeldahl principle and crude protein calculation*. International Standard ISO 8968-1:2014 and IDF 20-1:2014. ISO, Geneva, Switzerland and IDF, Brussels, Belgium.

Have you ever wondered what gives milk its white color? It's the several hundreds of different lipid and protein particles that provide the characteristic white appearance. But what other functions do they provide? Could these specific components be separated and used individually as added value ingredients? To replace food additives? To increase biological functions of infant formulas? This thesis dives into the science behind the most versatile fraction of milk, *Milk Fat Globule Membranes*, and aims to separate it from processing side streams. Perhaps the future food applications could be designed for optimal functionality and health by utilizing these minor components of milk.

ISBN 978-952-60-8549-4 (printed)

ISBN 978-952-60-8550-0 (pdf)

ISSN 1799-4934 (printed)

ISSN 1799-4942 (pdf)

Aalto University
School of Chemical Engineering
Department of Bioproducts and Biosystems
www.aalto.fi

**BUSINESS +
ECONOMY**

**ART +
DESIGN +
ARCHITECTURE**

**SCIENCE +
TECHNOLOGY**

CROSSOVER

**DOCTORAL
DISSERTATIONS**