Effect of Ingredient Interactions and Heat Treatment on the Structure and Stability of Dairy based Oil-in-Water Emulsions

A thesis presented in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy in Food Technology
at Massey University, Palmerston North, New Zealand

By

Yichao Liang

BSc. (Hons)

March 2014

Abstract

Oil-in-water emulsions are an important basis of many pharmaceutical, nutraceutical and food products such as mayonnaise, dressings, coffee creamers, chocolate, infant formulae, parenteral emulsions and enteral medical beverages. The effects of protein adsorption, addition of other food components (i.e. polysaccharides, carbohydrates and minerals), processing conditions (i.e. high pressure, heat and shear) and environmental conditions (i.e. pH, ionic strength and solvent quality) on the physicochemical properties of protein-stabilized oil-in-water emulsions (i.e. heat stability, creaming stability, viscosity and flow behavior) have been covered in past studies. In addition, there is increasing information on the heat stability and the viscosity of emulsions containing high concentrations of protein. However, there is very limited understanding of the inter-particle interactions in high solids emulsion systems containing high amounts of protein, oil or carbohydrate. A lack of studies that have investigated the types of interfacial proteins and non-adsorbed proteins, the aggregation state of the proteins, the particle size distributions of protein particles and oil droplets and the presence of a third component, especially under high temperature heating (i.e. 120−140 °C) conditions, has limited the understanding of how processing and ingredients influence the properties of proteins and, in turn, the physical stability and the rheological properties of protein-stabilized emulsions.

To address this issue, the research described here was aimed at determining the importance of the mechanisms of instability, such as depletion flocculation, bridging flocculation, aggregation, creaming and coalescence, in high solids emulsion systems and at understanding the colloidal forces that drive specific material/rheological properties and the storage stability of complex high solids systems. Five main techniques were used to characterize the structure-material properties of protein-stabilized emulsions: light scattering particle sizing, Turbiscan, rheology, polyacrylamide gel electrophoresis and confocal microscopy. The behaviors of interfacial proteins, non-adsorbed proteins and protein-coated oil droplets before and after heat treatment, in the presence of a third food

ingredient (polysaccharides or sugars) and with pre-homogenization protein preheat treatment were investigated.

As a prelude to addressing the mechanisms of instability of emulsions containing high concentrations of protein, before and after heat treatment (120 °C for 10 min), a stability map approach was developed. The model emulsions were successfully categorized into four model systems based on droplet−protein, protein−protein and droplet−droplet interactions with respect to the roles of the types of adsorbed and non-adsorbed proteins. Emulsions stabilized by whey proteins or containing high whey protein concentrations were shown to be highly sensitive to calcium content and heat treatment. Non-adsorbed whey proteins were involved not only in the heat-induced destabilization of whey-protein-stabilized emulsions but also when the interfacial layer was replaced by sodium caseinate (NaCas). In this case, NaCas-stabilized droplets were incorporated in the gel network, probably through heat-exchanged interfacial interaction. Gels were formed after heat treatment when NaCas was added to whey protein emulsions; this gel formation was dependent on the casein to whey protein ratio. Replacement of the continuous phase intact whey proteins with hydrolyzed whey proteins improved the heat stability of the emulsions. With one notable exception, surface-active fractions from hydrolyzed whey proteins were seen to promote droplet coalescence in Tween-20-stabilized emulsions at high heating temperatures. Model emulsions containing relatively high concentrations of micellar or non-micellar caseins were found to be heat stable. However, rapid enhanced creaming, which was associated with the depletion flocculation, occurred in these emulsions. The non-adsorbed protein concentration, the aggregation state and the polydispersity of the protein determined the extent of depletion flocculation.

Other studies on the effect of the depletion interaction potential and the continuous phase viscosity on the creaming stability of weakly flocculated NaCas-stabilized emulsions showed that an increase in the non-adsorbed protein concentration promoted a stronger depletion interaction potential, which could change emulsion destabilization to restabilization because of the formation of a space-filling droplet network. At higher NaCas concentrations (≥ 6% w/w), there was a significant delay in the formation of the space-filling droplet network, which was attributed to the particularly small diffusive

motion of flocculated droplet clusters and their inability to redisperse under a strong depletion interaction potential. Although the addition of maltodextrin or xanthan gum influenced the creaming behavior, neither additive could be considered to be an inert thickening agent. The reduced network formation caused by the addition of maltodextrin was not an effect of viscosity, but rather a breakdown of the caseinate nano-particles that induced the formation of the network. Once the maltodextrin concentration exceeded 15% w/w, the droplets were entirely non-interacting and would presumably cream at a rate predicted by modified Stokes' law for concentrated emulsion ($\emptyset$ > 0.2) (Tadros, 2004). Adding xanthan gum to the NaCas-stabilized emulsions resulted in local domains that were rich in either xanthan gum or NaCas and emulsion. The effect of these local domains was that the droplets experienced a stronger depletion interaction potential and the high viscosity of the combined xanthan gum and emulsion phases resulted in uniform phase separation across the emulsion rather than gravimetric separation.

Depending on the heating duration, heat treatment (120 °C) of NaCas-stabilized emulsions (2−8% w/w, protein) resulted in a weakened depletion interaction potential and a more rapid formation of the space-filling droplet network. Heat treatment of NaCas-stabilized oil droplets had relatively limited influence on the phase separation kinetics. Heat-induced degradation of non-adsorbed NaCas molecules was the dominant factor determining the creaming stability of NaCas-stabilized emulsions. The particle size, viscosity and molecular weight of continuous phase NaCas decreased as the heating time increased. A deviation of the size of the NaCas nano-particles from the optimum value (~ 20 nm in diameter) resulted in changes in the strength of the depletion interaction potential and the range of attraction. A decrease in the continuous phase viscosity also contributed to rapid formation of the droplet network at higher NaCas concentrations (> 6% w/w).

The droplet break-up and the heat stability of milk protein concentrate (MPC)-stabilized emulsions were determined to understand the impact of carbohydrate type and concentration during emulsification and subsequent heat treatment. The addition of different carbohydrates (up to 30% w/w) slightly increased the droplet diameter to similar extents. However, the addition of 30% w/w maltodextrin significantly decreased the

droplet diameter, which was attributed to the marked decrease in the dispersed/continuous phase viscosity ratio. Generally, added carbohydrates reduced the heat stability maximum in heat coagulation time–pH profiles of MPC-stabilized emulsions. The pH at the heat stability maximum was shifted towards more acidic values by an increased concentration of glucose, maltose, sucrose or trehalose but towards more alkaline values by an increased concentration of maltodextrin. The extent of destabilization also varied between carbohydrates, with trehalose being particularly effective in retaining the original heat stability of the MPC-stabilized emulsion. Reducing carbohydrates (glucose, maltose and maltodextrin) decreased the heat stability maximum more considerably than non-reducing carbohydrates (sucrose and trehalose).

Studies on the effect of pre-homogenization heat treatment on the physicochemical, microstructural and rheological properties of protein-stabilized emulsions showed that preheating the MPC and the whey protein concentrate denatured the whey proteins, leading to a reduction in subsequent heat-induced interactions between adsorbed proteins, between non-adsorbed proteins and between adsorbed and non-adsorbed proteins. This was attributed to fewer reactive groups being available for secondary heat-induced interactions after pre-homogenization heating. Extensive heat-induced aggregation during post-homogenization heat treatment resulted in delayed creaming because of the high viscosity at low shear rates. The presence of non-micellar caseins, the homogenization pressure and the order of heat treatment of the proteins were shown to significantly influence the physical stability and the rheological properties of protein-stabilized emulsions.

Overall, the results of this research have advanced our understanding of how the protein concentration, the aggregation state of caseins, the heat-induced physicochemical changes of proteins and the attractive force impact on the physical stability, rheological properties and microstructures of model protein-stabilized emulsions. This information may have important implications for developing tailor-made milk protein ingredients that allow controlled functionalities in the processing and storage of dairy emulsions and for making strategic plans to control/manipulate the properties of high solids colloid systems.

Acknowledgements

First and foremost, I would like to sincerely thank my chief supervisor Assoc Prof. Matt Golding at Massey for his inspiration, passion, guidance and encouragement, which he gave me during my doctoral study. His appreciation of my effort in each stage during the study including preparation of manuscripts for publication, conference presentations and preparation of thesis and his confidence and determination on the final goals positively drive me to keep up the good work.

I would like to express my sincerely gratitude to my co-supervisor Asst. Prof. Hasmukh Patel (South Dakota State University, Brookings, USA) for his encouragement, enthusiasm and freedom during my first one and half year doctoral study, for his constant support in the preparation of conference presentations. His deep thinking and understanding in the end applications of milk protein ingredients has advanced and influenced my way of thinking in both fundamental science and commercial applications.

I would like to thank Dr. Graeme Gillies (Fonterra Research & Development Centre). Thanks to him for kindly accepting the Fonterra supervisor role after Asst. Prof. Hasmukh left during the last one and half year of doctoral study. His knowledge and expertise on the subject and his thorough supervision, constant support even when I didn't ask for help, guidance for experiments, constructive suggestions on physic and mathematical equations and his enormous help in the preparation of manuscript and thesis reviewing. He always motivates me to produce the best possible results in preparing publication materials based on the findings of the caseinate emulsion systems. I am indeed thankful to my co-supervisor Dr. Lara Matia-Merino (Massey University) for her valuable scientific inputs and advice on experimental plans, coordination of using equipment in Food Science Characterization Laboratory and preparation of publications through the entire doctoral study period and her advices on job seeking. I am also truly thankful to Dr. Aiqian Ye (Riddet Institute) for his constant supervision, advice on the experimental methods and critical review of my publications throughout the whole doctoral study.

Acknowledgements

I would like to express my deep sense of gratitude to Dr. Ran Gao, Dr. Skelte Anema, MD. Sergey Ukraintsev and Dr. Abraham Chawanji for their friendly encouragements, kindly supports, helpful technical discussions and good advices during my studying and living in Palmerston North. I would like to thank Dr. Julita van Oosten-Manski (now FrieslandCampina Ltd) and Dr. Edeline Wong (Fonterra Research & Development Centre) for their assistance and advices on the rheological methods' set up and analysis in my initial stage of doctoral study. I am thankful to Mr. Michael Loh, Dr. Abraham Chawanji (Fonterra Research & Development Centre), Dr. Dimtry, Dr. Jianyu Chen and Mr. Doug Hopcroft (Manawatu Microscopy & Imaging Centre) for their training and assistance of confocal scanning laser microscopy and transmission electron microscopy. I would like to thank Dr. Kerianne Higgs for her assistance and guidance for RP-HPLC experiments. I am sincerely thankful to Dr. Claire Woodhall, Dr. Sheelagh Hewitt, Dr. Thérese Considine, Dr. Nick Robinson and Dr. Steve Taylor for being proof-reader of my publications and conference abstracts. I am thankful to Ms. Christine Ramsay for her administrative assistance with conference registrations.

I am grateful to all technicians, assistants and staff members of Fonterra Research & Development Centre especially to Dr. Dave Elgar, Dr. Jos Schalk, Dr. Jonathan Depree, Dr. Nilesh Saija, Dr. Emmanuelle Riou, Dr. Esra Cakir-Fuller, Dr. Aurelie Cucheval, Ms. Ping Gao, Ms. Yvonne van der Does, Ms. Sue Adam, Ms. Nguyen, Ms. Christina Streicher, Ms. Lucile Tercinier, Ms. Jing Luo, Ms. Carolina Saavedra-Melo, Mr. Ivan Simpson, Mr. Bert Fong, Mr. Rob Hunter, Mr. Kevin Ma, Mr. Thomas Fuller and Mr. Hemang Bhatt for their company and support. I would to thank the members of the Food Science Laboratory for providing a fun and active working atmosphere.

I would like to thank the Ministry of Science and Innovation (MSI) and Fonterra Co-operative Group Limited for funding this work. I am sincerely thankful to Mr. Kevin Palfreyman for his help in preparing this project contract with MSI and Ms. Liz Ricketts and Ms. Maret Sinclair for their help in administrative assistance with technical reports.

Acknowledgements

Last but not least, I would like to thank my partner Chia-Eng Chong, who enjoyed discussions and chats on everything in the hours when the lights in the lab were off, and for providing moral support throughout my study. I would like to express my deepest sense of gratitude to my parents, for their endless encouragement, motivations and blessings. I would like to say without the help from all those aforementioned people, it would have been impossible for me to achieve the good work in my PhD study.

Dedication

The author wishes to dedicate this dissertation to my parents and my girlfriend.

Table of Contents

Abstract i
Acknowledgements v
Dedication ix
Table of Contents xi
List of Publications xix
List of Presentations xx
List of Tables xxii
List of Figures xxiv
List of Abbreviations xxxviii
Chapter 1: Introduction 1
1.1 Introduction 1
1.2 Aim and thesis structure overview 3
Chapter 2: Literature Review 7
2.1 Introduction 7
2.2 Protein-stabilized oil-in-water emulsions 9
2.2.1 Caseins and casein micelles 10
2.2.1.1 Individual caseins 10
2.2.1.2 Casein micelles 17
2.2.2 Whey proteins 19
2.2.3 Mixed proteins 23
2.3 Mechanisms of emulsion stability 24
2.3.1 Attraction in colloidal interactions 24
2.3.1.1 van der Waals' forces 26
2.3.1.2 Hydrophobic interactions 27
2.3.1.3 Depletion interaction 28
2.3.2 Repulsion in colloidal interactions 29
2.3.2.1 Steric stability 29
2.3.2.2 Electrical double layer repulsion 30
2.3.3 Destabilization mechanisms of protein-stabilized oil-in-water emulsions 34

2.3.3.1 Creaming....34
2.3.3.2 Coalescence....36
2.3.3.3 Partial coalescence....37
2.3.3.4 Flocculation and aggregation....38
2.3.4 Impact of heat treatment on protein-stabilized oil-in-water emulsions....41
2.3.4.1 Impact of pre- and post-homogenization heat treatment on interfacial proteins....42
2.3.4.2 Impact of heat treatment on non-adsorbed proteins....45
2.4 Impact of droplet size and volume fraction on heat stability, creaming stability and rheological properties of protein-stabilized emulsions....49
2.4.1 Influence of droplet size....49
2.4.1.1 Formation of emulsion droplet and creaming stability....49
2.4.1.2 Influence of droplet size on heat stability....51
2.4.1.3 Influence of droplet size and droplet size distribution on emulsion rheology....52
2.4.2 Influence of volume fraction....55
2.4.2.1 Influence of volume fraction on heat stability....55
2.4.2.2 Influence of volume fraction on emulsion rheology....56
2.5 Impact of attractive and repulsive forces on the heat stability, creaming stability and rheological properties of protein-stabilized emulsions....60
2.5.1 Effect of attractive force on heat stability, creaming stability and rheological properties....60
2.5.1.1 Impact of depletion force on the heat stability of protein-stabilized emulsions....60
2.5.1.2 Impact of depletion force on the creaming stability and rheology of protein-stabilized emulsions....61
2.5.2 Effect of repulsive force on the heat stability of protein dispersion....63
2.5.2.1 Modulation of repulsive force through controlled protein aggregation....63
2.5.2.2 Modulation of repulsive force through protein−surfactant, protein−protein and protein−polysaccharide interactions....64

2.5.2.3 Alteration of thermodynamic properties by protein−carbohydrate and carbohydrate−water interactions and their impact on the physicochemical properties of protein-stabilized emulsions 71
2.5.2.4 Manipulation of Ca^{2+} ion activity by addition of polyphosphates 72
2.6 Concluding remarks 74
Chapter 3: Materials and Methods 77
3.1 Materials 77
3.1.1 Milk protein ingredients 77
3.1.2 Corn oil 78
3.1.3 Chemicals 78
3.2 Methods 78
3.2.1 Preparation of protein solutions 79
3.2.2 Preparation of milk protein stabilized emulsions 80
3.2.3 Particle size measurement of protein solutions and milk protein stabilized emulsions 80
3.2.4 Determination of surface protein concentration and composition 82
3.2.4.1 Resolving gel 83
3.2.4.2 Stacking gel 84
3.2.4.3 Electrode buffer 84
3.2.4.4 SDS sample buffer 85
3.2.5 Characterization of heat stability of emulsion 85
3.2.6 Emulsion rheology 86
3.2.7 Emulsion stability (cream layer thickness) 88
3.2.8 Microstructure of emulsions 90
Chapter 4: Structure and Stability of Heat-treated High-Protein-Stabilized Oil-in-Water Emulsions: A Stability Map Characterization Approach 93
4.1 Abstract 93
4.2 Introduction 94
4.3 Materials and methods 96
4.3.1 Materials 96
4.3.2 Preparation of model emulsion systems 96

4.3.3 Characterization of heat stability of the model emulsions....98
4.3.4 Determination of surface protein composition....98
4.3.5 Particle size determination....99
4.3.6 Emulsion stability....99
4.3.7 Rheological properties of model emulsions....99
4.3.8 Microsturcture of model emulsions....99
4.3.9 Statistical analysis....100
4.4 Results and Discussion....101
4.4.1 Model emulsions with interactions between adsorbed proteins, interactions between non-adsorbed proteins and cross-interactions between adsorbed and non-adsorbed proteins....101
4.4.2 Model emulsions with no interactions between adsorbed proteins but with protein−protein interactions between non-adsorbed proteins....106
4.4.3 Model emulsions with no interactions between adsorbed proteins and between non-adsorbed proteins but with droplet−droplet interactions....110
4.4.4 Model emulsions with no apparent interactions between adsorbed proteins, cross-interactions between adsorbed and non-adsorbed proteins and between non-adsorbed proteins....120
4.5 Conclusions....124
Chapter 5: Physical Stability, Microstructure and Rheological Properties of Sodium-Caseinate-Stabilized Oil-in-Water Emulsions as Influenced by Depletion Interaction Potential and Viscosity....127
5.1 Abstract....127
5.2 Introduction....128
5.3 Materials and methods....129
5.3.1 Materials....129
5.3.2 Preparation of model emsulsions....129
5.3.3 Particle size determination....129
5.3.4 Calculation and application of depletion interaction potential....132
5.3.5 Emulsion stability....133
5.3.6 Rheology measurements....134

5.3.7 Microstructure of model emulsions 135
5.3.8 Statistical analysis 135
5.4 Results and discussion 135
5.4.1 Influence of non-adsorbing biopolymers on the phase separation of caseinate-stabilized emulsions 135
5.4.2 Influence of non-adsorbing biopolymers on the microstructure of caseinate-stabilized emulsions 143
5.4.3 Influence of non-adsorbing biopolymers on the mechanical properties of caseinate-stabilized emulsions 146
5.5 Conclusions 154
Chapter 6: Effect of Heat Treatment on the Phase Separation Behavior of Sodium-caseinate-stabilized Oil-in-Water Emulsions 157
6.1 Abstract 157
6.2 Introduction 158
6.3 Materials and methods 159
6.3.1 Materials 159
6.3.2 Preparation of model emsulsions 159
6.3.3 Heat treatment of caseinate-stabilized emulsions and corresponding oil-free phase 160
6.3.4 Determination of protein content of heated NaCas solutions and oil-free phase of NaCas stabilized emulsions 160
6.3.5 Particle size determination of heated NaCas solution 160
6.3.6 Particle size determination of NaCas-stabilized droplets 161
6.3.7 Quantification of total native caseins in NaCas solutions after heating 161
6.3.8 Emulsion stability 161
6.3.9 Rheology measurements 161
6.3.10 Calculation of depletion interaction potential 161
6.3.11 Statistical analysis 162
6.4 Results and discussion 162
6.4.1 Impact of heat treatment at 120 °C on the phase separation behavior of NaCas-stabilized emulsions 162

6.4.2 Impact of heat treatment at 120 °C on the physicochemical properties of NaCas in the continuous phase 165
6.4.3 Prediction of the phase separation behavior of heated NaCas-stabilized emulsions 169
6.4.4 Rheological properties of heated NaCas-stabilized emulsions 175
6.5 Conclusions 181
Chapter 7: Effect of Carbohydrate Type and Concentration on the Heat-induced Behavior of Milk-Protein-Concentrate-Stabilized Oil-in-Water Emulsions 183
7.1 Abstract 183
7.2 Introduction 184
7.3 Materials and methods 186
7.3.1 Materials 186
7.3.2 Prepration of model emulsions 187
7.3.3 Determination of heat coagulation time (HCT) 189
7.3.4 Determination of heat-induced changes of model emulsions 189
7.3.5 Determination of particle size distributions of protein solutions and emulsions 189
7.3.6 Calcium-ion activity measurement 189
7.3.7 Rheological properties of model emulsions 190
7.3.8 Microstructure of model emulsions 190
7.3.9 Statistical analysis 190
7.4 Results and discussion 191
7.4.1 Effect of carbohydrate type and concentration on the droplet size formation of model MPC-stabilized emulsions 191
7.4.2 Heat stability of MPC solutions with added carbohydrate 194
7.4.3 Heat stability of model MPC emulsions with added carbohydrate 200
7.4.4 Correlation between particle size, microstructure and rheological properties of heated MPC emulsions with added carbohydrate 203
7.5 Conclusions 214

Chapter 8: Effects of Pre- and Post-heat Treatments on the Physicochemical, Microstructural and Rheological Properties of Milk-Protein-Stabilized Oil-in-Water Emulsions 217
8.1 Abstract 217
8.2 Introduction 218
8.3 Materials and methods 219
8.3.1 Materials 219
8.3.2 Preparation of model emulsions 219
8.3.3 Determination of effects of heat treatment on emulsions 219
8.3.4 Determination of heat coagulation time (HCT) of emulsions 220
8.3.5 Determination of particle size distributions of protein dispersions and emulsions 220
8.3.6 Determination of surface protein concentration and composition 221
8.3.7 Emulsion stability 221
8.3.8 Rheological properties of model emulsions 221
8.3.9 Microstructure of model emulsions 221
8.3.10 Statistical analysis 221
8.4 Results and discussion 222
8.4.1 Particle size of milk protein dispersions and whey protein denaturation level of MPC dispersions 222
8.4.2 Particle size distribution of emulsions 223
8.4.3 Protein load and protein composition of emulsions 224
8.4.4 Heat stability of the model emulsions 227
8.4.5 Microstructure of model emulsions 228
8.4.6 Rheological properties of model emulsions 231
8.4.7 Creaming stability of model emulsions 235
8.5 Conclusions 238
Chapter 9: Physicochemical Properties and Rheological Behaviors of Whey-Protein-Stabilized Oil-in-Water Emulsions as Influenced by Pre− and Post−homogenization Heat Treatments 239
9.1 Abstract 239

9.2 Introduction....240
9.3 Materials and methods....241
9.3.1 Materials....241
9.3.2 Preparation of model emulsions....241
9.3.3 Determination of effects of heat treatment on emulsions....242
9.3.4 Determination of particle size distributions of model emulsions....242
9.3.5 Determination of surface protein concentration and composition....243
9.3.6 Emulsion stability....243
9.3.7 Rheological properties of model emulsions....243
9.3.8 Statistcial analysis....243
9.4 Results and discussion....244
9.4.1 Effect of preheat treatment on the denaturation of whey protein dispersions....244
9.4.2 Particle size distribution of model emulsions....245
9.4.3 Protein load of model emulsions....246
9.4.4 Rheological behaviors of model emulsions....248
9.4.4.1 Dynamic oscillatory rheology....248
9.4.4.2 Shear flow behavior....250
9.4.5 Creaming stability of model emulsions....252
9.5 Conclusions....257
Chapter 10: Summary and Recommendations for Future Work....259
10.1 Overall conclusions....259
10.2 Industrial significance for the dairy industry....265
10.3 Recommendations for future work....267
10.4 Concluding remarks....271
Reference....273
Appendix: Publications....299
Paper One....299
Paper Two....313
Paper Three....325
Paper Four....337

List of Publications

This work has been published in part in the following papers:

1. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding (2013). Structure and stability of heat-treated concentrated dairy-protein-stabilized oil-in-water emulsions: a stability map characterization approach, *Food Hydrocolloids*, *33*, 297−308.

2. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding (2013). Effect of pre- and post-heat treatments on the physicochemical, microstructural and rheological properties of milk-protein-concentrate-stabilized oil-in-water emulsions, *International Dairy Journal*, *32,* 184−191.

3. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye, and M. Golding (2014). Physical stability, microstructure and rheology of sodium-caseinate-stabilized emulsions as influenced by protein concentration and non-adsorbing polysaccharides, *Food Hydrocolloids*, *36*, 245−255.

4. Y.C. Liang, L. Matia-Merino, H. Patel, A.Q. Ye, G. Gillies and M. Golding. Effect of sugar type and concentration on the heat-induced behavior of milk-protein-concentrate-stabilized oil-in-water emulsions. *Food Hydrocolloids*, *41*. 332−342.

5. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye, and M. Golding. Phase separation behavior of sodium-caseinate-stabilized emulsions as influenced by heat treatment. *Draft intended for Food Hydrocolloids*.

6. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye, and M. Golding. Modulation of the heat stability, creaming stability and rheological properties of high protein oil-in-water emulsions. A review. *Submitted for editorial review*.

List of Presentations

This work has been presented in part in the following presentations at scientific conferences:

1. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Physicochemical properties of model milk protein emulsions as influenced by the dispersed and continuous phases, 7th NIZO conference: Flavour and Texture: Innovations in Dairy, Papendal, The Netherlands, September 21-23, 2011. Finalist of student oral competitions.

2. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. The physicochemical properties of milk-protein-concentrate-stabilized emulsion as influenced by pre- and post-processing treatments, 7th NIZO conference: Flavour and Texture: Innovations in Dairy, Papendal, The Netherlands, September 21-23, 2011.

3. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Effect of heat treatment on the properties of milk-protein-concentrate-stabilized oil-in-water emulsions prepared with a preheated protein continuous phase, 2011 IDF Annual Meeting, Parma, Italy, October 15-19, 2011.

4. Y.C. Liang, H. Patel, M. Golding, L. Matia-Merino, A.Q. Ye. Physical stability of milk-protein-concentrate based dispersions and emulsions as influenced by heating pH and presence of small molecule components, 2012. NZIFST Annual Conference: Let's Talk, Hamilton, New Zealand, June 26-28, 2012.

5. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Development of a model system to understand the mechanisms of instability and to predict the shelf-life of oil-in-water emulsions, 2012 ADSA®-ASAS Joint Annual Meeting, Phoenix, USA, July 15-19, 2012. Finalist of graduate student oral competitions.

6. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Structural properties of milk protein concentrate (MPC) dispersions and emulsions as influenced by presence of small molecule components, 2012 ADSA®-ASAS Joint Annual Meeting, Phoenix, USA, July 15-19, 2012. Finalist of graduate student poster competitions.

7. Y.C. Liang, H. Patel, M. Golding, L. Matia-Merino, A.Q. Ye. Particle-particle system characterization of model milk-protein-stabilised emulsions, 2012. NZIFST Annual Conference: Let's Talk, Hamilton, New Zealand, June 26-28, 2012. Finalist of graduate student oral competitions.

8. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Microstructure, physical stability and rheological properties of concentrated-milk-protein-stabilized oil-in-water emulsions, 2013 NZIFST Annual Meeting: Time For Action, Hawke's Bay, New Zealand, July 2-4, 2013. Finalist of graduate student oral competitions.

9. Y.C. Liang, G. Gillies, L. Matia-Merino, A.Q. Ye, H. Patel and M. Golding. Influence of depletion flocculation and continuous phase viscosity on the stability of sodium-caseinate-stabilized emulsions, 2013 ADSA®-ASAS Joint Annual Meeting, Indianapolis, USA, July 8-12, 2013. Selected finalist of graduate student oral competitions.

10. Y.C. Liang, G. Gillies, H. Patel, L. Matia-Merino, A.Q. Ye, G. Gillies and M. Golding. Impact of processing and storage temperatures on the physical stability of sodium-caseinate-stabilized emulsions, 2013 ADSA®-ASAS Joint Annual Meeting, Indianapolis, USA, July 8-12, 2013.

11. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Influence of the properties of interfacial proteins and heat treatment on the stability and structure of oil-in-water emulsions, 2013 IFT Annual Meeting, Chicago, USA, July 13-16, 2013. Selected finalist of student oral competitions.

12. Y.C. Liang, H. Patel, L. Matia-Merino, A.Q. Ye and M. Golding. Influence of sonication on droplet size and physicochemical properties of milk-protein-concentrate-stabilized oil-in-water emulsions, 2013 IFT Annual Meeting, Chicago, USA, July 13-16, 2013. 2nd place in final graduate poster competition.

List of Tables

Number *Page*

Table 2.1. Ingredients and their roles in a typical high protein beverage formulation. 8
Table 3.1. Composition of milk protein ingredients (in g/100g) 78
Table 4.1. Distributions of casein and whey protein in the protein- and surfactant-stabilized model emulsions (8.5% w/w protein, 10% w/w oil). 100
Table 4.2. Mean effective particle sizes of model emulsions (8.5% w/w protein, 10% w/w oil) before and after heat treatment (120°C for 10 min). 101
Table 4.3. Mean primary particle sizes of model emulsions (8.5% w/w protein, 10% w/w oil) before and after heat treatment (120°C for 10 min). 102
Table 5.1. The concentration of non-adsorbed caseinate particles, xanthan and maltodextrin as a function of the overall fraction of biopolymer in the entire emulsion. 131
Table 6.1. The total solids content (by CEM microwave solids analyzer) and the total nitrogen content (by the Kjeldahl method) as a function of heating time of 30% w/w oil-in-water emulsions formed with 2−6% w/w NaCas. 165
Table 7.1. Molar concentration for different carbohydrate types at concentrations used in this study. 188
Table 7.2. Primary droplet diameters, $d_{3,2}$ and $d_{4,3}$ (μm), of MPC-stabilized oil-in-water emulsions (10% w/w protein, 10% oil w/w) with and without the addition of different types of carbohydrate. 194
Table 8.1. Particle sizes of MPC and NaCas dispersions before and after heating (90 °C, 5 min) and the denaturation level of whey proteins in the oil-free aqueous phase. 222
Table 8.2. Mean primary particle sizes, mean effective particle sizes and surface protein concentration of 30% w/w oil-in-water emulsions formed with 3% w/w MPC and NaCas before and after heat treatment (120 °C, 10 min). 225

Table 9.1. Protein denaturation of different whey protein dispersions (4.5%, w/w) before and after preheat treatment (90 °C for 5 min), determined by RP-HPLC. 244

Table 9.2. Mean primary and effective particle sizes of 30% w/w oil-in-water emulsions formed with 3% w/w whey proteins before and after heat treatment and mean effective particle sizes after heat treatment. 246

Table 9.3. Surface protein load on unheated and heated (120°C, 10 min) 30% w/w oil-in-water emulsions formed with 3% w/w unheated and preheated protein dispersions. 247

List of Figures

Number *Page*

Figure 2.1. Schematic illustration of the linear α_{s1}-casein structure, as simulated by self-consistent field (SCF) calculations; adapted from Akinshina, Ettelaie, Dickinson, & Smyth (2008). 11

Figure 2.2. Schematic illustration of observed and speculative structures of self-associated individual caseins. Redrawn from Horne (2002). 12

Figure 2.3. Schematic illustration of the primary sequence of β-casein, as simulated by self-consistent field (SCF) calculations; adapted from Dickinson (2006). 13

Figure 2.4. Schematic illustration of the primary sequence of κ-casein, as simulated by self-consistent field (SCF) calculations; adapted from Ettelaie, Khandelwal, & Wilkinson (2014). 15

Figure 2.5. Schematic illustration of the conformation of the interfacial layer formed by flexible and globular proteins. Redrawn based on Dickinson (1998b), Ettelaie et al. (2014) and McClements (2005). 16

Figure 2.6. Schematic illustration of interfacial structures with different proportions of casein micelles and non-micellar caseins, based on a study of oil-in-water emulsions prepared using milk protein concentrate (MPC) (Ye, 2011). 19

Figure 2.7. Schematic illustration of the three-dimensional structure of β-lg, showing the relative positions of the five Cys residues. Adapted from Edwards et al. (2008). 21

Figure 2.8. Schematic illustration of the electrical double layer at the surface of protein-stabilized emulsion droplet with negative surface charge (Malvern Instruments Ltd, 2004). 32

Figure 2.9. Schematic illustration of emulsion instability in oil-in-water emulsions (redrawn from McClements, 2005) 36

Figure 2.10. Schematic illustration of depletion-induced weakly attractive spheres. α is the radius of the large sphere, h is the surface−surface distance and r is the hydrodynamic radius of the non-adsorbed polymers, based on Asakura & Oosawa (1954). 40

Figure 2.11. Schematic illustration of three types of emulsification equipment that can be used to produce fine emulsion droplets: two-valve high pressure homogenizer; microfluidizer; ultrasonicator (Piorkowski & McClements, 2014). 50

Figure 2.12. Zero-shear relative viscosity of polydisperse oil-in-water emulsions stabilized by casein micelle (CM), NaCas nano-particle (NaCas), whey protein (WP) and aggregated whey protein (AWP), as predicted by Equation 2.13 with $\emptyset_m = 0.71$ (Schaertl & Sillescu, 1994). The R_h of milk proteins used in the calculation is 100 nm for CM (de Kruif et al., 2012), 10 nm for NaCas (Radford & Dickinson, 2004), 2 nm for WP (Zhai et al., 2011b) and 50 nm for aggregated whey protein (Schmitt et al., 2007). 53

Figure 2.13. Predicted zero-shear relative viscosities of suspensions of polydisperse spheres for different PSDs, with the infinite particle size ratio based on data from Genovese (2012). The arrow indicates that higher solids contents can be packed in a suspension for a given relative viscosity.....55

Figure 2.14. Comparison of the relative zero-shear viscosities of casein micelle (CM) and NaCas dispersions as a function of casein concentration with those of a predicted monodisperse hard sphere dispersion with $\emptyset_m \sim 0.64$ (dotted line) and a polydisperse hard sphere dispersion with $\emptyset_m \sim 0.78$ (dashed line), as calculated using the Quemada equation (de Kruif, van Iersel, Vrij, & Russel, 1985). The voluminosity of the casein micelle was estimated to be 4.4 g/cm^3 (de Kruif, 1998).....59

Figure 2.15. Schematic illustration of the structure, stability and depletion force relationship in NaCas emulsions with the addition of non-adsorbed NaCas and non-adsorbed polysaccharide. 62

Figure 2.16. Schematic illustration of the modulation of interfacial layer properties at the oil/water interface through protein−surfactant, protein−protein and protein−polysaccharide interactions. 66

Figure 3.1. Schematic of the cone and plate geometry (A) and cup and bob geometry (B). 87

Figure 3.2. Schematic representation of Turbiscan Classic MA 2000.....89

Figure 3.3. Schematic illustration of a confocal laser scanning microscopy (CLSM). 90

Figure 4.1. Schematic diagrams of the proposed model systems: top left system, no interactions between droplets but non-adsorbed proteins interact with each other (i.e. an unstable system with a partly liquid and a partly gel-like structure); top right system, no droplet−droplet interactions and no protein−protein interactions at the oil−water interface and in the continuous phase (i.e. a stable system with no coagulation and no phase separation); bottom left system, interactions between adsorbed proteins, between non-adsorbed proteins and between adsorbed and non-adsorbed proteins (i.e. a whey protein emulsion gel); bottom right system, interactions between droplets but no protein−protein interactions at the oil−water interface and in the continuous phase (i.e. a phase separation induced by depletion flocculation).....95

Figure 4.2. Flow chart of the preparation of model emulsions (8.5% w/w protein, 10% w/w oil).....98

Figure 4.3. Visual appearance and microstructure of heated (120 °C, 10 min) model emulsions (8.5% w/w protein, 10% w/w oil): (A) sole WPC-stabilized emulsion; (B) Tween-20-stabilized emulsion containing WPC, with the top image showing the gelled phase and the bottom image showing the serum layer expelled from the gel; (C) NaCas-stabilized emulsion containing WPC. Red: oil droplet; Green: protein. Scale bar = 10 μm. 104

Figure 4.4. Heat stability map of WPI-stabilized 10% w/w oil-in-water emulsions (pH 6.8-7.0) with different $CaCl_2$ contents after heating for 10 min at 90°C. Depending on the visual appearance and measured viscosity after heating, samples may be assigned to different categories: ○, stable against heat

(remained liquid); ▲, unstable against heat (formed precipitate or gel-like paste). 105

Figure 4.5. Droplet size distribution of the serum phase that was expelled from the gel phase in a heated (120 °C, 10 min) Tween-20-stabilized emulsion containing 8.5% w/w WPC. 109

Figure 4.6. SDS-PAGE patterns of the interfacial proteins (A) under non-reducing conditions and (B) under reducing conditions (*β*-mercaptoethanol was added). Lane 1: skim milk standard; lane 2: unheated NaCas stock emulsion; lane 3: unheated NaCas-stabilized emulsion containing WPC; lane 4: heated NaCas-stabilized emulsion containing WPC. The heated emulsion was collected after heating at 90 °C for 10 min. 111

Figure 4.7. Phase separation was detected in the unheated 8.5% w/w NaCas-stabilized 10% w/w oil-in-water model emulsion. Changes in *BS* profile as a function of sample height with storage time at room temperature. The shaded areas indicate the extent of creaming (top), the extent of the change in *BS* (%) between 0 and 144 h (middle) and *BS* (%) after 144 h of storage (bottom).....113

Figure 4.8. Variation in backscattering expressed as Δ*BS* (%) of (A) unheated model emulsions. In set: variation in classified layer thickness of stock NaCas emulsion during storage. (B) heated (120 °C, 10 min) model emulsions (8.5% w/w protein, 10% w/w oil) monitored over 144 h at 20 °C for: a stock NaCas emulsion (solid line); a sole NaCas-stabilized emulsion (■); an NaCas-stabilized emulsion containing MPC I (▲); an NaCas-stabilized emulsion containing MPC II (×); a Tween-20-stabilized emulsion containing MPC I (●); a Tween-20-stabilized emulsion containing MPC II (♦). 114

Figure 4.9. Confocal micrographs of model emulsions (8.5% w/w protein, 10% w/w oil): (A) unheated emulsion; (B) heated (120 °C, 10 min) emulsion. Scale bar is 10 μm. 115

Figure 4.10. Creaming (after 24 h of storage at 20 °C) of unheated 10% w/w oil-in-water model emulsions containing 1~8% w/w MPC I as the continuous

phase and (A) NaCas-stabilized oil droplets and (B) Tween-20-stabilized oil droplets. The arrow shows the creaming trend. 116

Figure 4.11. TEM images of selected heated (120 °C, 10 min) model emulsions: (A) NaCas-stabilized emulsion containing MPC I; (B) NaCas-stabilized emulsion containing MPC II. The images were captured at 19 000× magnification. Scale bar is 1000 nm. CM = casein micelle.....117

Figure 4.12. (A) Flow behavior of unheated model emulsions: stock NaCas emulsion (+); sole NaCas-stabilized emulsion (■); NaCas-stabilized emulsion containing MPC I (▲); NaCas-stabilized emulsion containing MPC II (×); Tween-20-stabilized emulsion containing MPC I (●); Tween-20-stabilized emulsion containing MPC II (♦). (B) Relative viscosity of unheated NaCas-stabilized 30% w/w oil-in-water emulsions containing 0, 0.5 and 1% w/w MPC as a function of shear stress at 20 °C. η_s is the corresponding viscosity of MPC in the continuous phase. 120

Figure 4.13. Confocal micrographs of model emulsions (8.5% w/w protein, 10% w/w oil): (A) unheated emulsion; (B) heated (120 °C, 10 min) emulsion. Scale bar is 10 μm. 121

Figure 4.14. TEM image of heated (120 °C, 10 min) NaCas-stabilized emulsion containing WPH. The image was captured at 19 000× magnification. Scale bar is 1000 nm.....123

Figure 4.15. Particle size distributions of Tween-20-stabilized emulsions (10% w/w oil); control heated at 120 ºC for 10 min (solid line); containing 1% w/w WPH heated at 120 ºC for 0.5 min (dotted line), for 1 min (dashed line), for 2 min (dashed dotted line) and for 3 min (long dashed line). 124

Figure 5.1. Flow chart of the preparation of model emulsions (1−10% w/w protein, 0.025−0.2% w/w xanthan and 2.5−20% w/w maltodextrin respectively). ...130

Figure 5.2. Droplet size distribution in a 60% w/w oil-in-water stock emulsion stabilized by 3% w/w NaCas. Dashed line represents the droplet population in a network based on the calculation from Section 5.3.4 using an osmotic pressure of 5 Pa, note below diameters of 0.5 μm only ≈ 50%

of the droplets participate in the network above diameters of 3 μm ≈ 80% of the droplets participate in the network. 136

Figure 5.3. Variation in clarified layer thickness as monitored by Turbiscan over 160 h at 20 °C for 30% w/w oil-in-water emulsions containing 1, 1.5, 2, 4, 5 and 6% w/w NaCas. The black dashed line shows a prediction for the creaming of concentrated emulsions with non-interacting droplets (McClements, 2005). Inset: visual appearance of NaCas-stabilized oil-in-water emulsions after storage for 2 weeks at 20 °C. A small amount of 2% methylene blue stain was added for better observation of the movement of the creaming boundaries. The solid lines are theoretical fits, as predicted by Manley, Skotheim, Mahadevan, & Weitz (2005), giving $h_0 - h(t) = \Delta h(1 - e^{\frac{-t}{\tau}})$, where h_0 is the initial height, Δh is the total change in height and τ is the time scale for the collapse. 138

Figure 5.4. The predicted and experimental determined depletion interaction potential of NaCas-stabilized 30% w/w oil-in-water emulsions as a function of protein concentration. 139

Figure 5.5. Variation in clarified layer thickness as determined by Turbiscan over 160 h at 20 °C for 1.5% w/w NaCas-stabilized 30% w/w oil-in-water emulsions containing 0.01, 0.025, 0.05 and 0.075% w/w xanthan. Inset: visual appearance of 1.5% NaCas-stabilized emulsions containing xanthan after 2 weeks of storage at 20 °C. 140

Figure 5.6. Variation in clarified layer thickness as determined by Turbiscan over 160 h at 20 °C for 1.5% w/w NaCas-stabilized 30% w/w oil-in-water emulsions containing 2.5, 5, 7.5 and 10% w/w maltodextrin. Inset: visual appearance of 1.5% NaCas-stabilized emulsions containing maltodextrin after 2 weeks of storage at 20 °C. Arrows indicate the barely visible boundary between the cream phase and the stationary phase. 141

Figure 5.7. Variation in the backscattered light intensity (%) of 1.5% w/w NaCas-stabilized 30% w/w oil-in-water emulsions as a function of maltodextrin concentration after 2 weeks of storage at 20 °C. The visual appearance of

the 1.5% NaCas emulsion is added to distinguish the cream layer from the clarified layer. 142

Figure 5.8. Representative confocal micrographs of NaCas-stabilized 30% w/w oil-in-water emulsions containing (A) 2% w/w, (B) 4% w/w, (C) 5% w/w, (D) 6% w/w and (E) 8% w/w NaCas as a function of time. Scale bar = 20 μm. .144

Figure 5.9. Confocal micrographs of 1.5% w/w NaCas stabilized 30% w/w oil-in-water emulsions containing (A) 0% w/w polysaccharide, (B) 5% w/w maltodextrin, (C) 10% w/w maltodextrin, (D) 15% w/w maltodextrin, (E) 0.05% w/w xanthan, (F) 0.075% w/w xanthan and (G) 0.1% w/w xanthan after 4 h and 1 week. Scale bar = 20 μm. 145

Figure 5.10. Viscosity of 30% w/w oil-in-water emulsions containing increasing concentrations of NaCas relative to their low shear rate viscosities (η_S) in the continuous phase as a function of shear stress at 20 °C. Samples were pre-sheared to eliminate any shear history and there was no equilibration time during measurement. The dashed line is the prediction of the zero shear relative viscosity of a hard sphere dispersion of $\emptyset$ = 0.3, as calculated using the Krieger-Dougherty equation $\frac{\eta}{\eta_0} = (1 - \frac{\emptyset}{\emptyset_m})^{-[\eta]\emptyset_m}$, where $\emptyset$ is the volume concentration of particles and $\emptyset_m$ is the maximum packing (~ 0.71 for a polydisperse system) (Schaertl & Sillescu, 1994). 147

Figure 5.11. Viscosity of 30% w/w oil-in-water emulsions containing 1.5% and 6% w/w NaCas relative to their low shear rate viscosities (η_S) in the continuous phase as a function of shear stress measured at 0 h and 6 h at 20 °C. 148

Figure 5.12. Viscosities of 1.5% w/w NaCas-stabilized 30% w/w oil-in-water emulsions containing various concentrations of maltodextrin relative to their low shear viscosities (η_S) in the continuous phase as a function of shear stress at 20 °C. The dash line indicates the viscosity of an emulsion with non-droplet-droplet and/or protein-protein interactions. 149

Figure 5.13. (A) Relative viscosity (performed at 1 Hz, 0.5% strain, 20 °C) as a function of time for various emulsions normalized by the time taken for a

droplet to diffuse an arbitrary distance, in this case 20 nm was used in equation (5.6). Emulsions stabilized by various concentrations of NaCas: 1.5% w/w (solid line); 2% w/w (round dot); 3% w/w (square dot); 4% w/w (dash); 5% w/w (dash dot); 6% w/w (long dash); 8% w/w (long dash dot); 10% w/w (long dash dot dot). (B) Relative viscosity (performed at 1 Hz, 0.5% strain, 20 °C) as a function of time for 1.5% w/w NaCas-stabilized emulsions containing various concentrations of maltodextrin: 2.5% w/w (round dot); 5% w/w (square dot); 7.5% w/w (dash); 10% w/w (dash dot); 15% w/w (long dash). (C) Relative viscosity (performed at 1 Hz, 0.5% strain, 20 °C) as a function of time for 1.5% NaCas-stabilized emulsions containing various concentrations of xanthan: 0.01% w/w (round dot); 0.025% w/w (square dot); 0.05% w/w (dash); 0.075% w/w (dash dot); 0.1% w/w (long dash). 151

Figure 5.14. Variation of *G'* (▲) and *G"* (■) as a function of time for (A) 3% w/w and (B) 5% w/w NaCas emulsions at 20 °C (performed at 1 Hz, 0.5% strain). 153

Figure 5.15. Schematic illustration of structure, stability and depletion force relationship in NaCas emulsions with addition of non-adsorbed NaCas and non-adsorbed polysaccharide. 156

Figure 6.1. Flow chart of the preparation of model emulsions (2−8% w/w protein, 30% w/w oil). 159

Figure 6.2. Variation in classified layer thickness as determined by Turbiscan over 168 h at 20 °C for heated NaCas-stabilized 30% w/w oil-in-water emulsions containing 1% w/w (A), 2% w/w (B), 4% w/w (C) and 6% w/w (D) NaCas. Inset: visual appearance of NaCas-stabilized emulsions after 1 week of storage at 20 °C. 163

Figure 6.3. (A) Representative SDS-PAGE patterns of unheated (lane 1) and heated NaCas in the continuous phase of 6% w/w NaCas-stabilized 30% w/w oil-in-water emulsions (lanes 2−7). Each lane was loaded with approximately 25 μg of protein. The major casein proteins are α_{s2}-casein, α_{s1}-casein, β-casein and κ-casein. Some degradation products were indicated in the

zones marked B and C. (B) Normalized casein hydrolysis (determined by SDS-PAGE) in NaCas after heating at 120 °C as a function of time. NaCas at different concentrations that corresponded to the continuous phases of 3% w/w (□), 4% w/w (○) and 6% w/w (●) NaCas-stabilized 30% w/w oil-in-water emulsions was heated.....166

Figure 6.4. (A) Relative viscosity (η_t/η_0) and (B) Relative change in hydrodynamic diameter (D_t/D_0) of NaCas heated at 120 °C as a function of time. NaCas at different concentrations that corresponded to the continuous phases of 3% w/w (□), 4% w/w (○) and 6% w/w (●) NaCas-stabilized 30% w/w oil-in-water emulsions was heated.....168

Figure 6.5. Variation in classified layer thickness, as determined by Turbiscan over 196 h at 20 °C, for 4% w/w NaCas-stabilized 30% w/w oil-in-water emulsions (A) and 6% w/w NaCas-stabilized 30% w/w oil-in-water emulsions (B) containing unheated NaCas and NaCas heated at 120 °C as a function of time. The dashed lines indicate the predicted creaming rates. .170

Figure 6.6. Estimation of non-adsorbed NaCas concentrations (close symbols) and the corresponding depletion interaction potentials (open symbols) based on SDS-PAGE (A) and viscosity (B) for 3% w/w (■, □), 4% w/w (▲, Δ) and 6% w/w (●, ○) NaCas-stabilized 30% w/w oil-in-water emulsions after heat treatment at 120 °C as a function of time (min). 172

Figure 6.7. Normalized classified layer thickness of 4% w/w NaCas-stabilized 30% w/w oil-in-water emulsions (A) and 6% w/w NaCas-stabilized 30% w/w oil-in-water emulsions (B) containing unheated NaCas and NaCas heated at 120 °C as a function of storage time at 20 °C. The classified layer thickness was normalized by the corresponding continuous phase viscosity of the emulsion.....174

Figure 6.8. Relative viscosities of unheated and heated (120 °C, up to 30 min) (A) 2% w/w, (B) 3% w/w, (C) 4% w/w, (D) 5% w/w and (E) 6% w/w NaCas-stabilized 30% w/w oil-in-water emulsions as a function of shear stress at 20 °C. Samples were pre-sheared to eliminate any shear history and there was no equilibration time during measurement. The solid line is the

prediction of the zero-shear relative viscosity of a polydisperse hard sphere dispersion of $\emptyset$ = 0.3, as calculated using the Krieger−Dougherty equation $\frac{\eta}{\eta_0} = (1 - \frac{\emptyset}{\emptyset_m})^{-[\eta]\emptyset_m}$. 177

Figure 6.9. Relative viscosity (performed at 1 Hz, 0.5% strain, 20 °C) as a function of time for 2% w/w (A), 4% w/w (B) and 6% w/w (C) NaCas-stabilized emulsions containing unheated NaCas (solid line), and NaCas heated at 120 °C for 10 min (round dot), 20 min (square dot), 30 min (dash), 40 min (dash dot), 50 min (long dash) and 60 min (long dash dot). 181

Figure 7.1. Schematic illustration of the effect of initial heating pH on the type of casein particle at high temperature, on the casein−whey protein interactions, on the type of rate determining reaction and on the HCT (Anema & Li, 2003; van Boekel et al., 1989a; Vasbinder & de Kruif, 2003). 186

Figure 7.2. Flow chart of the preparation of model emulsions (10% w/w protein, 10% w/w oil and 0−30% w/w carbohydrate). 188

Figure 7.3. Effect of carbohydrate content (A) maltodextrin, (B) sucrose on the volume-weighted droplet size ($d_{4,3}$) (square symbol) and η_D/η_C ratio (viscosity of dispersed phase/viscosity of continuous phase which was determined within the Newtonian region between shear rate 1 to 1000 s^{-1}) (round symbol) of MPC-stabilized oil-in-water emulsions (10% w/w oil). ..192

Figure 7.4. HCT−pH profiles of 10% w/w MPC solutions: with no added carbohydrate (■) and with addition of (A) 10% w/w, (B) 20% w/w, (C) 30% w/w glucose (♦), maltose (□), sucrose (▲), trehalose (●), and maltodextrin (✳). Visual images showed the color of the heated MPC solutions containing glucose and maltodextrin as a function of pH, respectively. 198

Figure 7.5. (A) HCT−pH profiles of MPC solutions at protein concentrations of 10% w/w (solid line), 12.5% w/w (round dot), 15% w/w (square dot), and 20% w/w (dash dot). (B) The effect of protein concentration on the heat stability maximum of MPC solutions with no added carbohydrate (■) and

with the addition of 10−30% of glucose (♦), maltose (□), sucrose (▲), trehalose (●), and maltodextrin (∗). Heat stability maximum was obtained from Figure. 4. The effective protein concentration of MPC solutions was calculated based on the protein concentration in the water phase in which the weight of carbohydrate was excluded. Inset: heat stability maximum obtained from Figure. 5A was plotted as a function of MPC concentration. 199

Figure 7.6. Particle size of 10% w/w MPC solutions with and without added carbohydrate after heat treatment (120 °C) at pH 6.8. Plotted are intensity-based effective mean diameters. The patterned filled bars indicate the samples contain large aggregates and the arrows indicate the effective particle diameters of the aggregates are outside the detection limit. 200

Figure 7.7. HCT−pH profiles of MPC-stabilized oil-in-water emulsions (10% w/w protein, 10% w/w oil): with no added carbohydrate (■) and with the addition of (A) 10% w/w, (B) 20% w/w, (C) 30% w/w glucose (♦), maltose (□), sucrose (▲), trehalose (●), and maltodextrin (∗). 203

Figure 7.8. Effective particle size distributions of MPC-stabilized oil-in-water emulsions (10% w/w protein, 10% w/w oil) with and without 30% w/w sucrose unheated and heated (at pH 6.8) at 120 °C for the times indicated. Shaded region indicates how aggregates are described in other figures. 205

Figure 7.9. Aggregation time lines of the heat-induced physicochemical changes of model MPC emulsions in terms of particle size, microstructure and rheological properties. Viscosity index of unheated and heated (120 °C, pH 6.8) MPC-stabilized oil-in-water emulsions (10% w/w protein, 10% w/w oil) with and without 30% w/w added carbohydrate at 20 °C: (A) glucose (♦) and maltose (■); (B) sucrose (▲); (C) control (■) and trehalose (●). Confocal micrographs show the fat signal only, scale is identical in all images with 20 μm scale bars. Data labels indicate the essential features size and volume taken from particle size distributions of Figure 7.8. The lines are drawn to guide the eye. 208

Figure 7.10. Effective particle size distributions of unheated MPC-stabilized oil-in-water emulsions at pH 6.8 (10% w/w protein, 10% w/w oil): control (solid line); containing 10% w/w maltodextrin (dotted line), containing 20% w/w maltodextrin (dashed line), and containing 30% w/w maltodextrin (dashed dotted line). 209

Figure 7.11. Effect of pH on the logarithm of the free calcium ion concentration of MPC-stabilized emulsions with no added carbohydrate (■) and with the addition of (a) 10% w/w, (b) 20%, w/w (c) 30% w/w glucose (♦), maltose (■), sucrose (▲), trehalose (●), and maltodextrin (✳).....210

Figure 7.12. Oscillation rheology of MPC-stabilized emulsions (10% w/w protein, 10% w/w oil) with and without added glucose and maltodextrin (30% w/w) as a function of time during a heating and cooling cycle (from 20 to 90 to 20 °C) at pH 6.8: (♦) glucose; (▲) maltodextrin. The solid line indicates the heating and cooling cycle. The dashed line indicates gel formation when $G' > 1$ Pa.....214

Figure 8.1. Flow chart of the preparation of model emulsions (3% w/w protein, 30% w/w oil). 220

Figure 8.2. Particle size distribution of unheated MPC I and MPC II dispersions (pH 6.8) determined by Zetasizer at 20 °C. 223

Figure 8.3. SDS-PAGE patterns of proteins adsorbed at the oil−water interface of (A) unheated and (B) heated 30% w/w oil-in-water emulsions formed with 3% w/w unheated and preheated protein dispersions. Lane 1, skim milk standard; lane 2, MPC I emulsion; lane 3, MPC II emulsion; lane 4, NaCas emulsion; lane 5, MPC I emulsion formed with preheated protein; lane 6, MPC II emulsion formed with preheated protein; lane 7, NaCas emulsion formed with preheated protein. 226

Figure 8.4. HCT profiles of 30% w/w oil-in-water emulsions stabilized by 3% w/w unheated protein and by 3% w/w preheated protein. Inset: HCT (min) of MPC I emulsion. 227

Figure 8.5. Confocal laser scanning micrographs of 3% w/w (a) unheated and (b) heated (120 °C, 10 min) milk-protein-stabilized emulsions in a 63x

objective. (A) 30% w/w oil-in-water emulsions stabilized by 3% w/w unheated protein; (B) 30% w/w oil-in-water emulsions stabilized by 3% w/w preheated protein. The scale bars in A and B represent 10 μm. 230

Figure 8.6. Apparent viscosities of (A) unheated and (B) heated (120 °C, 10 min) 30% w/w oil-in-water emulsions stabilized by 3% w/w unheated protein and by 3% w/w preheated protein measured at 20 °C. PreH = preheated protein solution. 233

Figure 8.7. Apparent viscosities of 30% w/w oil-in-water emulsions stabilized by (A) MPC I unheated solution and (B) MPC I preheated solution before and after heating at 120 °C for 1, 3 and 10 min measured at 20 °C. PreH = preheated protein solution. 234

Figure 8.8. Apparent viscosities of heated 30% w/w oil-in-water model emulsions stabilized by 3% w/w unheated protein and by 3% w/w preheated protein, determined at a shear rate of $100s^{-1}$. 235

Figure 8.9. Variation in classified layer thickness as determined by Turbiscan over 120 h at 20 °C for heated 30% w/w oil-in-water emulsions stabilized by 3% w/w unheated and preheated proteins. PreH = preheated protein solution. The black dashed line shows a prediction for the creaming of concentrated emulsions with non-interacting droplets using the equation for concentrated emulsion adapted in McClements (2005), where $\emptyset_{max} \approx 0.71$ was used for a polydisperse emulsion. 237

Figure 9.1. Flow chart of the preparation of model emulsions (3% w/w protein, 30% w/w oil). 242

Figure 9.2. Evolution of storage modulus (G') of 30% w/w oil-in-water emulsions stabilized by 3% w/w protein as a function of time during a heating and cooling cycle (20 to 90 to 20 °C). (■) WPC I emulsion stabilized by unheated protein; (♦) WPC I emulsion stabilized by preheated protein; (▲) WPC II emulsion stabilized by unheated protein; (×) WPC II emulsion stabilized by preheated protein; (*) WPI emulsion stabilized by unheated protein; (●) WPI emulsion stabilized by preheated protein. The solid line indicates the heating and cooling cycle. 249

Figure 9.3. Effect of homogenization pressure on the particle size distribution of the oil-free phase of WPC II emulsions and on the HCT at 140 °C. 250

Figure 9.4. Apparent viscosity of (A) unheated and (B) heated (120°C, 10 min) 30 % w/w oil-in-water emulsions stabilized by 3% w/w unheated protein and by 3% w/w preheated protein (shear rate was increased from 0.05 to 200 s^{-1} at 20 °C). PreH = preheated protein. 252

Figure 9.5. Variation in backscattering, monitored over 672 h (28 days), for unheated and heated (120 °C for 10 min) 30% w/w oil-in-water emulsions stabilized by 3% w/w unheated and preheated protein solutions expressed as classified layer thickness at room temperature: (A) unheated WPC I emulsions; (B) unheated WPC II emulsions; inset, evolution of sediment layer; (C) unheated WPI emulsions; (D) heated WPC I emulsions; (E) heated WPC II emulsions; inset, evolution of sediment layer; (F) heated WPI emulsions. PreH=preheated protein. 256

Figure 10.1. The relationship between the colloidal interactions, emulsion structure, flow behavior and physical stability of the protein-stabilized oil-in-water emulsions. 264

List of Abbreviations

α-lac:	α-lactalbumin
β-lg:	β-lactoglobulin
BSA:	Bovine serum albumin
BS:	Backscattering
CaCas:	Calcium caseinate
CCP:	Colloidal calcium phosphate
CLSM:	Confocal laser scanning microscopy
CM:	Casein micelle
CMC:	Critical micelle concentration
$d_{3,2}$:	Surface area moment mean diameter
$d_{4,3}$:	Volume-weighted average mean diameter
DLS:	Dynamic light scattering
EDTA:	Ethylene diamine tetra acetic acid
MPC:	Milk protein concentrate
MD:	Maltodextrin
NaCas:	Sodium caseinate
RP-HPLC:	Reverse phase high performance liquid chromatography
SDS-PAGE:	Sodium dodecyl sulfate-poly acrylamide electrophoresis

SLS:	Static light scattering
SMP:	Skim milk powder
TEM:	Transmission electron microscopy
Tris:	Tris(hydroxymethyl)aminomethane
TS:	Transmission
UF:	Ultrafiltration
UHT:	Ultra high heat treatment
WPC:	Whey protein concentrate
WPH:	Whey protein hydrolyzate
WPI:	Whey protein isolate
w/w:	Weight/weight
w/v:	Weight/volume
Γ:	Surface coverage