Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
Behaviour of Milk Protein Ingredients and Emulsions Stabilised by Milk Protein Ingredients

In the Simulated Gastrointestinal Tract

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

Master of Food Technology

Massey University, Manawatu, New Zealand

Xin Wang

2017
Milk clotting behaviours in the stomach impact the digestion rates of protein and fat. A variety of milk protein products are applied as functional ingredients in many foods. This research was conducted to investigate the digestion behaviours of various commercial dairy ingredients and lipids in emulsions stabilised by these ingredients using a dynamic in vitro digestion model, i.e., a human gastric simulator (HGS), with a focus on the effect of different structures of clots formed in dairy ingredients during gastric digestion on hydrolysis of proteins and/or lipids.

Skim milk powder (SMP), milk protein concentrate (MPC) 4851, MPC 4861, sodium caseinate, whey protein isolate (WPI) and heated (90°C, 20 min) WPI were used in the present study. Results showed that SMP and MPC 4851, which contained casein micelles, formed ball-like clots with a relatively dense network after 10 min of gastric digestion. These clots did not disintegrate after 220 min of digestion. MPC 4861 and sodium caseinate generated clots at around 40 min, and a loose, fragmented structure was observed at the end of the gastric digestion due to a lacking micellar structure of caseins. No clot was observed in WPI or heated WPI after 220 min gastric digestion, although aggregation occurred at around 40 min in heated WPI. These differences in coagulation behaviours apparently affected the rate of gastric emptying and protein hydrolysis by pepsin in the gastric system. In SMP and MPC 4851, the gastric emptying and hydrolysis of caseins was much slower than that observed in MPC 4861 and sodium caseinate. The most rapid gastric emptying of proteins was observed in the WPI samples both with and without heating. This is attributed to the formation of varied structured clots at different times under the gastric conditions.

The effect of protein concentration on the gastric behaviour of these dairy ingredients in solution was then examined, with a particular emphasis on the structure of clots. SMP and MPC 4851 have been selected as model protein ingredients. Their gastric behaviours were investigated over a protein concentration range of 0.5-5.0% (w/w). The results showed that the digestion behaviour of SMP and MPC 4851 followed a similar pattern. The rate of pH changes in the emptied digesta during digestion was protein concentration dependent. With an increase in protein concentration, the decrease in pH slowed. The protein concentration had no apparent impact on the casein clotting time.
Abstract

Clots were formed in the first 10 min of digestion in all samples. However, in both SMP and MPC 4851, when protein concentration was lower than 2.0% (w/w) the clots consisted of small protein pieces with a loose, porous and open structure after a 220 min digestion. Whereas a cheese ball-like clot with a denser network was observed at the end of gastric digestion when the protein concentration varied from 2.0% to 5.0% (w/w). Such a difference in the structure apparently affected the rate of protein hydrolysis. A more rapid hydrolysis ($P < 0.05$) of the clotted protein was observed when protein concentration was lower than 2.0% (w/w) compared to the samples containing a higher proportion of protein (2.0%-5.0%, w/w).

To study the effect of different coagulation behaviours on the digestion of oil droplets in oil-in-water emulsions, these dairy ingredients (with the exception of SMP) were used to prepare an oil-in-water emulsion (20.0% soy oil and 4.0% protein, w/w). They were digested under the dynamic gastric conditions using the HGS. The gastric digesta was emptied at 20 min intervals. Then all digesta were mixed to investigate the lipid digestion under the small intestinal conditions. Changes in physicochemical properties of emulsions, involving the particle size, the microstructure, the oil content of the emptied gastric digesta and the amount of free fatty acids (FFAs) released during the small intestine stage, were determined using an *in vitro* small intestinal digestion model.

Aggregation of MPC 4851-stabilised emulsion took place after 5 min of digestion in the HGS with the largest size. The aggregates remained in the stomach and did not disappear during the whole gastric digestion. The hydrolysis of the aggregated network by pepsin was largely slowed by the reduced ability of the simulated gastric fluid (SGF, containing pepsin) to diffuse into the larger sized aggregates. MPC 4851-stabilised emulsion thus resulted in the slowest release of oil droplets into the small intestine. In comparison, MPC 4861 and sodium caseinate-stabilised emulsions aggregated in the stomach at approximately 40 min, forming smaller sized aggregates. These aggregates disintegrated at the mid and late-stages of digestion in these two emulsions. Therefore, MPC 4861 and sodium caseinate-stabilised emulsions had a more rapid delivery of oil droplets into the small intestine. In relation to the WPI-stabilised emulsions both with and without heating, the aggregations formed at a similar time to that which was observed in MPC 4861 and sodium caseinate-stabilised-emulsions; i.e., at approximately 40 min. However, they had the smallest sized aggregates amongst all samples and they
Abstract
disintegrated quickly with further digestion. WPI-stabilised emulsions both with and
without heating had the fastest gastric emptying and hydrolysis by pepsin in the early and
mid-stages of the gastric digestion process. Thus, the highest level of oil content contained
in the emptied gastric digesta was produced from both WPI-stabilised emulsions. In the
mixed gastric digesta, which were subjected to the small intestinal digestion, the oil
contents contained in the different emulsion samples varied. This difference impacted the
extent of lipid digestion by pancreatic lipase. The sample with a higher oil content
released a greater amount of FFAs compared to the sample with a lower oil content. The
extent of lipid digestion of different emulsion samples adhered to the following pattern:
MPC 4851-stabilised emulsion < MPC 4861-stabilised emulsion < sodium caseinate-
stabilised emulsion, WPI-stabilised emulsions both with and without heating.

Overall, the gastric behaviours of dairy ingredients either in solutions or
emulsions were affected by the formation of structured clots/aggregates. The differences
in clotting/aggregation times and their structures were greatly dependent on the
component and structure of protein, the processing prior to digestion and the
susceptibility to proteases. These differences in protein coagulation/aggregation
behaviour impacted the rates of protein hydrolysis and gastric emptying. The oil content
and protein composition of the gastric digesta transferred into small intestine and the
extent of lipid digestion in small intestine were also affected. These results are important
in an application perspective. They provide useful information for the design and
development of healthier food products by allowing greater control over the manipulation
of protein bioavailability, which subsequently provides greater control over lipid
metabolism.
Acknowledgment

First and foremost, I would like to thank my supervisor Associate Professor Aiqian Ye, who has been supportive of my research and provided me with encouragement, direction, assistance, insightful comments and extensive personal and professional guidance throughout my Master study, and taught me a great deal about both scientific research and life in general. He also helped me to coordinate my project especially in writing this report. As my supervisor and mentor, he has taught me more than I will ever know. He has shown me, by his example, what a good scientist should be.

I would like to express my deepest appreciation to Professor Harjinder Singh for providing me the possibility to complete my research in Riddet Institute with a financial assistance.

My special thanks go to my teammate Quanquan Lin, who gave me selfless help, encouragement and sharing her pearls of wisdom with me during the course of this research.

Furthermore, I would also like to acknowledge with much appreciation Ms Maggie Zou, Ms Janiene Gilliland, Mr. Chris Hall, and Mr. Steve Glasgow, who gave me the permission to use all required equipment and the necessary materials to complete my research, as well as providing timely assistance for reagents ordering, laboratory induction, safety advice, and training and guidance of the use of instruments. A special gratitude I give to Mr. Jian Cui, who has provided me training, technical support and scientific suggestions in my overall practical work in the laboratory. I am especially indebted to Dr. Matthew Savoian, Ms Jordan Taylor and Ms Niki Minards for their valuable help and training in using Laser Scanning Confocal Microscopy (LSCM).

I am grateful to Ms Ansley Te Hiwi, Ms Terri Palmer, Ms Hannah Hutchinson and Dr. Michael Parker for their administrative assistances. I would like to thank Mr. Matt Levin for his assistance in information systems. I am also thankful Mr. John Henley-King.

I am also immensely grateful to all the staffs and research fellows whom I have had pleasure to work during this project at Riddet Institute and Massey Institute of Food
Acknowledgment

Science and Technology. I also would like to express my appreciation to my friends, Nan Luo, Yu Cheng, Xiaqi Sang, Zhigao Niu, Siqi Li, Lisanne Fermin, Sewuese Okubanjo, Geeshani Somaratne, Feng Ming Chian, Chih-Chieh Chuang and Nicole Chen for their encouragements and supports.

Finally, I wish to thank my parents for their encouragement, generosity and financial support. I would not complete my study without them. Their love and guidance are with me in whatever I pursue.
# Table of Contents

Abstract ............................................................................................................................. i  
Acknowledgment ............................................................................................................... v  
Table of Contents .......................................................................................................... vii  
List of Tables .................................................................................................................. xi  
List of Figures ................................................................................................................. xiii  
List of Abbreviations ........................................................................................................ xix  

Chapter 1: Introduction ................................................................................................. 1  

Chapter 2: Literature Review ......................................................................................... 5  

2.1 The human gastrointestinal tract ........................................................................... 5  

2.1.1 Stomach ............................................................................................................. 5  

2.1.1.1 The pH and ionic strength of gastric fluid .............................................. 6  

2.1.1.2 Enzyme .................................................................................................... 7  

2.1.2 Small intestine .................................................................................................. 7  

2.1.2.1 pH and ionic strength .......................................................................... 7  

2.1.2.2 Bile salts ............................................................................................ 8  

2.1.2.3 Pancreatic lipase ................................................................................. 9  

2.2 Milk protein ......................................................................................................... 10  

2.2.1 Casein ............................................................................................................. 10  

2.2.1.1 Casein micelle structure .................................................................... 11  

2.2.1.2 The stability of casein micelles ........................................................... 13  

2.2.1.3 Enzymatic coagulation of caseins ...................................................... 14  

2.2.2 Whey protein ................................................................................................. 15  

2.2.2.1 β-lactoglobulin ................................................................................. 15  

2.2.2.2 α-lactalbumin .................................................................................... 16  

2.2.2.3 Heating induced denaturation of whey protein .................................. 16  

2.2.3 Milk protein products ..................................................................................... 18  

2.2.3.1 Skim milk powder ............................................................................. 19  

2.2.3.2 Milk protein concentrate .................................................................... 21  

2.2.3.3 Sodium caseinate .............................................................................. 21
# Table of Contents

2.2.3.4 Whey protein isolate ............................................................... 22
2.2.4 The digestion behaviours of milk protein during gastric digestion .... 22
2.3 Emulsion ..................................................................................... 24
  2.3.1 Emulsion formation ................................................................. 25
  2.3.2 Emulsion stability ................................................................. 26
    2.3.2.1 Gravitational separation .................................................. 26
    2.3.2.2 Flocculation ................................................................. 28
    2.3.2.3 Coalescence ................................................................. 29
  2.3.3 Protein emulsifier ................................................................. 29
    2.3.3.1 MPC ................................................................. 31
    2.3.3.2 Sodium caseinate ....................................................... 32
    2.3.3.3 Whey protein isolate .................................................... 33
  2.3.4 The digestion behaviours of milk protein-stabilised emulsions ....... 34
    2.3.4.1 Milk protein-stabilised emulsions in the gastric environment ...... 35
    2.3.4.2 Milk protein-stabilised emulsions in the intestinal environment .. 36

**Chapter 3: Materials and Methods** ................................................. 39

  3.1 Materials .................................................................................... 39
    3.1.1 Dairy ingredients ............................................................... 39
    3.1.2 Soybean oil ......................................................................... 39
    3.1.3 Chemicals .......................................................................... 39
    3.1.4 Enzymes ............................................................................. 40
    3.1.5 Simulated gastric fluid (SGF) .............................................. 40
    3.1.6 Simulated intestinal fluid (SIF) .......................................... 40
  3.2 Methods ..................................................................................... 41
    3.2.1 Preparation of protein solution (Chapter 4 and 5) ................... 41
    3.2.2 Preparation of emulsions ...................................................... 41
      3.2.2.1 Protein solution preparation (Chapter 6) ......................... 41
      3.2.2.2 Emulsion preparation .................................................. 41
    3.2.3 *In vitro* gastric digestion .................................................. 41
      3.2.3.1 Human gastric simulator (HGS) ..................................... 43
      3.2.3.2 pH measurement ........................................................ 44
      3.2.3.3 Weight of clot ............................................................ 44
3.2.3.4 Measurement of oil content (Chapter 6) ........................................ 44
3.2.4 In vitro intestinal digestion (Chapter 6) ........................................ 46
  3.2.4.1 Measurement of free fatty acid release ........................................ 46
3.2.5 Particle size measurements ................................................................. 47
3.2.6 Confocal laser scanning microscopy .................................................... 48
3.2.7 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
  ............................................................................................................. 49
  3.2.7.1 Preparation of stock solutions ...................................................... 49
  3.2.7.2 Gel preparation ........................................................................ 50
  3.2.7.3 Sample preparation .................................................................... 51
  3.2.7.4 Running of electrophoresis, staining and destaining .................... 51
3.2.8 Statistical analysis ................................................................................ 52

Chapter 4: Behaviours of Different Milk Protein Ingredients during in Vitro Gastric
Digestion ............................................................................................................ 53
  4.1 Abstract .................................................................................................... 53
  4.2 Introduction ............................................................................................. 54
  4.3 Results ..................................................................................................... 58
    4.3.1 pH profiles ....................................................................................... 58
    4.3.2 Coagulation behaviour of different milk protein ingredients ............ 59
    4.3.3 Protein hydrolysis ........................................................................... 62
    4.3.3.1 SDS-PAGE pattern of clots ......................................................... 62
    4.3.3.2 SDS-PAGE patterns of emptied digesta ....................................... 63
  4.4 Discussion .................................................................................................. 68
  4.5 Conclusions ............................................................................................. 73

Chapter 5. The Dynamic Gastric Digestion Behaviours of Skim Milk Powder and
Milk Protein Concentrate: the Influence of Protein Concentration .................. 75
  5.1 Abstract .................................................................................................... 75
  5.2 Introduction ............................................................................................. 76
  5.3 Results and Discussion ........................................................................... 77
    5.3.1 Skim milk powder ........................................................................... 77
      5.3.1.1 Results .................................................................................... 77
      5.3.1.2 Discussion .............................................................................. 88
List of Tables

<table>
<thead>
<tr>
<th>Number</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1. Average characteristics of casein micelles (Fox, 2003)</td>
<td>12</td>
</tr>
<tr>
<td>Table 2.2. Thermal denaturation temperature of whey proteins (De Wit, 1984)</td>
<td>17</td>
</tr>
<tr>
<td>Table 2.3. Food applications of skim milk powder (SMP) of different heat classes (Kelly &amp; Fox, 2016)</td>
<td>20</td>
</tr>
<tr>
<td>Table 3.1. The composition of dairy ingredients</td>
<td>39</td>
</tr>
<tr>
<td>Table 6.1. The volume ($d_{4,3}$) and surface ($d_{3,2}$) mean diameters of original emulsions stabilised by different dairy ingredients (20.0% soybean oil and 4.0% protein, w/w)</td>
<td>115</td>
</tr>
<tr>
<td>Table 6.2. The volume ($d_{4,3}$) mean diameters and oil content of the mixed gastric digesta emptied from emulsions made with different dairy ingredients</td>
<td>135</td>
</tr>
</tbody>
</table>
Table of Contents
List of Figures

<table>
<thead>
<tr>
<th>Number</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 2.1.</strong> The external and internal anatomy of the stomach of human (Tortora &amp; Derrickson, 2008).</td>
<td>6</td>
</tr>
<tr>
<td><strong>Figure 2.2.</strong> Main components of milk proteins showing partitioning into casein and whey fractions (Cheison &amp; Kulozik, 2017).</td>
<td>10</td>
</tr>
<tr>
<td><strong>Figure 2.3.</strong> The casein micelle schematic diagram (Walstra &amp; Jenness, 1984).</td>
<td>13</td>
</tr>
<tr>
<td><strong>Figure 2.4.</strong> Functional protein ingredients manufactured from skim milk [derived from Singh (2005)].</td>
<td>19</td>
</tr>
<tr>
<td><strong>Figure 3.1.</strong> The schematic diagram of digestion process.</td>
<td>42</td>
</tr>
<tr>
<td><strong>Figure 3.2.</strong> Image of a HGS (A) and schematic illustration of a latex stomach chamber (B). (1) SGF; (2) plastic tubes for secretion; (3) pump; (4) latex stomach chamber; (5) mesh bag; (6) roller; (7) belt; (8) pulley; (9) shaft; (10) angle gear; (11) Lovejoy joint; (12) fan heater for temperature control. Picture derived from Ye et al. (2016b).</td>
<td>44</td>
</tr>
<tr>
<td><strong>Figure 4.1.</strong> Changes under gastric digestion in pH of the different dairy ingredient solutions with 3.0% (w/w) protein.</td>
<td>59</td>
</tr>
<tr>
<td><strong>Figure 4.2.</strong> Images of clots obtained from 200 g of dairy ingredient solution containing 3.0% (w/w) protein after 220 min of <em>in vitro</em> gastric digestion.</td>
<td>60</td>
</tr>
<tr>
<td><strong>Figure 4.3.</strong> Wet weight of clots obtained from 200 g of dairy ingredient solution containing 3.0% (w/w) protein after 220 min of <em>in vitro</em> gastric digestion. Different lowercase letters indicate significant difference ($P &lt; 0.05$) in the wet weight of the clots.</td>
<td>61</td>
</tr>
<tr>
<td><strong>Figure 4.4.</strong> Dry weight of clots obtained from 200 g dairy ingredient solution containing 3.0% (w/w) protein after 220 min of <em>in vitro</em> gastric digestion. Different lowercase letters indicate significant difference ($P &lt; 0.05$) in the dry weight of the clots.</td>
<td>62</td>
</tr>
<tr>
<td><strong>Figure 4.5.</strong> SDS-PAGE patterns of clots collected from different dairy ingredients after 220 min of gastric digestion in the HGS. M, trim milk; SM, skim milk powder;</td>
<td></td>
</tr>
</tbody>
</table>
List of Figures

MPC, milk protein concentrate 4851; D-MPC, milk protein concentrate 4861; SC, sodium caseinate. .......................................................... 63

Figure 4.6. SDS-PAGE patterns of the emptied digesta collected from different dairy ingredients during 220 min of gastric digestion in the HGS. M, trim milk. BSA, bovine serum albumin. (A) skim milk powder; (B) MPC 4851; (C) MPC 4861; (D) sodium caseinate; (E) WPI; (F) WPI heated (90°C, 20 min). .......................................................... 66

Figure 5.1. pH of emptied digesta obtained from the SMP samples with different protein concentrations (0.5-5.0%, w/w) as a function of digestion time. Blank refers to a control experiment carried out without addition of SMP.......................................................... 78

Figure 5.2 Images of the clots obtained from 200 g of SMP samples containing a different level of protein (0.5-5.0%, w/w) after 220 min of in vitro gastric digestion. ...................................................................................... 79

Figure 5.3. Wet weight of the clots obtained after 220 min of in vitro gastric digestion of SMP samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the wet weight of clots. ...................................................................................... 80

Figure 5.4. Dry weight of the clots obtained after 220 min of in vitro gastric digestion of SMP samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the dry weight of clots. ...................................................................................... 81

Figure 5.5. The curd weight ratio after 220 min of in vitro gastric digestion of the SMP samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the curd weight ratio (g dry matter in the clot/g protein in the initial sample). . 82

Figure 5.6. The microstructure of the clot obtained from 200 g of SMP samples containing (A) 0.5%, (B) 2.0%, and (C) 5.0% (w/w) protein after 220 min of in vitro gastric digestion. The scale bar in all images is 50 μm. .................. 83

Figure 5.7. The SDS-PAGE pattern under reducing conditions of the clots collected from the SMP samples with a different protein concentration (0.5-5.0%, w/w) after
Figure 5.8. SDS-PAGE patterns of emptied digesta collected from the SMP samples containing (A) 0.5%, (B) 1.0%, (C) 2.0%, (D) 3.0%, (E) 4.0% and (F) 5.0% (w/w) protein during 220 min of gastric digestion in the HGS. M. trim milk. BSA, bovine serum albumin. ................................................................. 84

Figure 5.9. Changes in pH of emptied digesta obtained from MPC 4851 samples with a different protein concentration (0.5-5.0%, w/w) as a function of digestion time. Blank refers to a control experiment carried out without addition of MPC 4851………………………………………………………………… 91

Figure 5.10. Images of clots obtained from 200 g of MPC 4851 samples containing a different level of protein (0.5-5.0%, w/w) after 220 min of gastric digestion in the HGS……………………………………………………… 92

Figure 5.11. Wet weights of the clots obtained after 220 min of in vitro gastric digestion of the MPC 4851 samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the wet weight of clot…………………………………………………………… 93

Figure 5.12. Dry weights of the clots obtained after 220 min of in vitro gastric digestion of MPC 4851 samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the dry weight of the clot…………………………………………………... 93

Figure 5.13. The curd weight ratio of MPC 4851 samples containing different protein concentrations (0.5-5.0%, w/w). Different lowercase letters indicate significant difference ($P < 0.05$) in the curd weight ratio (g dry weight of the clot/g protein in the initial sample) .............................................................. 94

Figure 5.14. The microstructure of the clots obtained from 200 g of MPC 4851 samples containing (A) 0.5%, (B) 2.0%, and (C) 5.0% (w/w) protein after 220 min of gastric digestion. The scale bar in all images is 50 μm......................... 95

Figure 5.15. The SDS-PAGE pattern of the clots collected from the MPC 4851 samples containing different protein concentrations (0.5-5.0% w/w) after 220 min of gastric digestion in the HGS. M, trim milk. BSA, bovine serum albumin… 96
Figure 5.16. SDS-PAGE patterns of the emptied digesta collected from the MPC 4851 samples containing (A) 0.5%, (B) 1.0%, (C) 2.0%, (D) 3.0%, (E) 4.0% and (F) 5.0% (w/w) during 220 min of gastric digestion in the HGS. M. trim milk. BSA, bovine serum albumin

Figure 6.1. Changes in pH of digesta emptied from different milk protein ingredients-stabilised emulsions during 220 min of gastric digestion

Figure 6.2. The aggregates collected from MPC 4851-stabilised emulsion after 220 min of gastric digestion in the HGS

Figure 6.3. The changes in mean diameters volume \( d_{4,3} \) of emptied digesta obtained from different milk protein ingredients-stabilised emulsions (20.0% soybean oil and 4.0% protein, w/w) during 220 min of gastric digestion in the HGS. (A) MPC 4851-stabilised emulsion; (B) MPC 4861-stabilised emulsion; (C) sodium caseinate-stabilised emulsion; (D) WPI-stabilised emulsion; (E) WPI-stabilised emulsion heated (90°C, 20 min). Different lowercase letters indicate significant difference \( (P < 0.05) \) on the mean diameters volume \( d_{4,3} \) of digesta emptied from emulsions between different digestion time points within the same emulsifier.

Figure 6.4. Particle size distribution of emptied digesta obtained from 220 min gastric digestion of different protein stabilised emulsions (20.0% soybean oil and 4.0% protein, w/w) in the HGS: (A) MPC 4851-stabilised emulsion; (B) MPC 4861-stabilised emulsion; (C) sodium caseinate-stabilised emulsion; (D) WPI-stabilised emulsion; (E) WPI-stabilised emulsion heated (90°C, 20 min).

Figure 6.5. Oil content (g oil/100 g emptied gastric digesta) of the emptied gastric digesta at different digestion time points. Different capital letters indicate significant difference \( (P < 0.05) \) on the oil content between the gastric digesta emptied from different dairy ingredients-stabilised emulsions within the same digestion time point. Different lowercase letters indicate significant difference \( (P < 0.05) \) on the oil content of emptied digesta between different digestion time points within the same emulsifier.

Figure 6.6. Confocal microscopy images of digestion residues of different milk protein ingredients-stabilised emulsions in the stomach at different times during
List of Figures

gastric digestion from 0 to 220 min. All samples were stained with Nile Red (for oil) and Fast Green (for protein). The scale bar in all images is 50 μm. .................................................................................................................... 127

Figure 6.7. Confocal microscopy images of the emptied digesta from different milk protein ingredients-stabilised emulsions at different time points during gastric digestion from 0 to 220 min. Oil is stained red, protein is stained green. The scale bar in all images is 50 μm. .................................................................................................................... 130

Figure 6.8. SDS-PAGE patterns under reducing conditions of the emptied digesta obtained at the different time points during 220 min of gastric digestion from: (A) MPC 4851-stabilised emulsion; (B) MPC 4861-stabilised emulsion; (C) sodium caseinate-stabilised emulsion; (D) WPI-stabilised emulsion; (E) heated (90°C, 20 min) WPI-stabilised emulsion. BSA, bovine serum albumin; M, trim milk. .................................................................................................................... 134

Figure 6.9. Confocal microscopy images of initial mixed-digesta (0 min) in the SIF of the different milk protein ingredients-stabilised emulsions. All samples were stained with Nile Red (for oil) and Fast Green (for protein). The scale bar in all images is 50 μm. .................................................................................................................... 136

Figure 6.10. Changes in the average droplet diameter ($d_{4,3}$) of the mixed gastric digesta emptied from emulsions stabilised by different dairy ingredients during digestion in the SIF. .................................................................................................................... 137

Figure 6.11. The changes in volume ($d_{4,3}$) mean diameters (-1) and the size distributions (-2) of oil droplets in the emptied digesta from different milk protein ingredients-stabilised emulsions (20.0% soybean oil and 4.0% protein, w/w) during 120 min of intestinal digestion: (A) MPC 4851-stabilised emulsion; (B) MPC 4861-stabilised emulsion; (C) sodium caseinate-stabilised emulsion; (D) WPI-stabilised emulsion; (E) WPI-stabilised emulsion with heating (90°C, 20 min). Different lowercase letters indicate significant difference ($P < 0.05$) on the volume ($d_{4,3}$) mean diameters of oil droplets in emptied digesta between different digestion time points within the same emulsifier type. 139

Figure 6.12. Amount of free fatty acids released (μmol FFA/mL mixed gastric digesta) from emulsion made with different dairy ingredients (titrated by 0.25 M NaOH) in pH-stat during 120 min intestinal digestion. ............................. 141
Figure 6.13. Amount of free fatty acids (%) released from per gram oil contained in the mixed gastric digesta from emulsions made with different dairy ingredients (titrated by 0.25M NaOH) in pH-stat during 120 min intestinal digestion.
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-La</td>
<td>α-lactalbumin</td>
</tr>
<tr>
<td>β-Lg</td>
<td>β-lactoglobulin</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CCP</td>
<td>Colloidal calcium phosphate</td>
</tr>
<tr>
<td>HGS</td>
<td>Human gastric simulator</td>
</tr>
<tr>
<td>MPC</td>
<td>Milk protein concentrate</td>
</tr>
<tr>
<td>WPI</td>
<td>Whey protein isolate</td>
</tr>
<tr>
<td>WPNI</td>
<td>Whey protein nitrogen index</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate-poly acrylamide electrophoresis</td>
</tr>
<tr>
<td>SGF</td>
<td>Simulated gastric fluid</td>
</tr>
<tr>
<td>SIF</td>
<td>Simulated intestinal fluid</td>
</tr>
<tr>
<td>$d_{4,3}$</td>
<td>Average volume-weighted diameter</td>
</tr>
<tr>
<td>$d_{3,2}$</td>
<td>Average surface-weighted diameter</td>
</tr>
<tr>
<td>PI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight/weight</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight/volume</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume/volume</td>
</tr>
</tbody>
</table>