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.
STUDIES ON THE EFFECTS OF HEAT AND HIGH PRESSURE TREATMENTS ON FAT GLOBULE SURFACE LAYERS IN RECOMBINED MILK

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology at Massey University, Manawatu, New Zealand

VISAKA ANANTAWAT

2011
ABSTRACT

The present study examined the effects of heat treatment, high pressure (HP) treatment or combined heat and HP treatments, either before or after homogenization, on recombined milk systems. The main focus was to explore the changes induced by these treatments on the surface layers of recombined fat globules, milk proteins and rheological properties of acid gels.

Heat treatments caused denaturation of whey proteins; the degree of denaturation was dependent on temperature, holding time and to a lesser extent on the placement of heat treatment. Recombined milks that underwent heat treatment before or after homogenization had similar levels of whey protein denaturation. The amounts of caseins and denatured whey proteins adsorbed on the surface of fat globules of recombined milk heated before homogenization were significantly lower than those heated after homogenization, indicating different interaction mechanisms in these two systems.

Increases in treatment pressure used in HP treatment resulted in decreased amounts of caseins, while whey proteins adsorbed onto the surface layers of fat globules increased. This was probably due to the dissociation of casein micelles under HP treatment and the interactions between HP-induced denatured whey proteins and casein particles on the surface layers of fat globules.

Combined heat and HP treatments induced changes on adsorbed caseins and whey proteins on fat globule surface layers. HP treatment induced additional denaturation of whey proteins in heated milks, resulting in slightly increased amounts of denatured whey protein adsorbed onto the surface layers.

Gelation pH, final $G'$ and yield stress values of acid gels prepared from recombined milks heated before or after homogenization were dependent on temperature, holding time and the placement of heat treatment. These changes were attributed to the extent of denaturation of the whey proteins and their interactions with casein particles adsorbed onto the fat globule surface and in the serum. Differences in acid gels prepared from recombined milks heated before and after homogenization were
attributed to the relative proportions of caseins and whey proteins at the surface layers of fat globules resulting in different interactions with protein strands in the gel network.

The acid gels prepared from recombined milks HP-treated either before or after homogenization had shorter gelation times, higher gelation pH, final $G'$ and yield stress values compared with untreated recombined milk and the effects were dependent on treatment pressure, temperature, holding time and the placement of HP treatment. The denaturation of whey proteins and their interactions with casein particles were responsible for these changes. In HP-treated recombined milks the proportions of caseins and denatured whey proteins adsorbed onto the surface layers of fat globules had significant effects on the acid gel structure. When HP treatment was applied after homogenization, the proteins on the surface layer were present as a layer which might provide better sites for the interactions with the protein strands in the gel matrix.

The application of these processing treatments to recombined milk could provide new avenues to the dairy industry for manufacturing novel products with enhanced texture and nutritional properties.
ACKNOWLEDGEMENTS

I wish to express sincere gratitude to my chief supervisor, Professor Harjinder Singh, and to my co-supervisor, Dr. Skelte Anema, for providing me with excellent guidance, support, encouragement and advice throughout the course of my Ph.D. study. Their wide knowledge, logical way of thinking and professional guidance provided a good basis for the present thesis.

I would like to acknowledge the Royal Thai Government for the scholarship for my Ph.D. study and Riddet Institute for additional financial support. I would like to thank Steve Glasglow, Gary Redford, Michelle Tamehana, Janiene Gilliland, Warwick Johnson and Chris Hall for their assistance with laboratory techniques. The excellent editorial support from Professor Andy Rao and Dr. Julia Raynor (Student Learning Centre, Massey University) is highly appreciated and gratefully acknowledged.

I am indebted to my friends, Alistair and Bunjan Broughton. The long chats and discussions with you were fun and relaxing, and helped greatly throughout my Ph.D. study. I also appreciate their inspiration and great efforts in helping me to prepare this thesis. Their detailed and constructive comments are greatly appreciated.

Last, but not least, I am very gratefully to my caring husband, Satit Anantawat, and my lovely son, Eksarat Anantawat, for their great patience, companionship, understanding, and for providing excellent moral support throughout the course of my Ph.D. I believe that the useful advice and blessing from my father, my sister, my brother and their families have always been a motivational factor for me. I am very grateful to my friends at Walailak University for their excellent support, motivation and encouragement. I deeply appreciate the love and concern they have shown throughout this period. Without the help from all these people, this achievement would not have been possible.
TABLE OF CONTENTS

ABSTRACT i

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF FIGURES ix

LIST OF TABLES xxii

CHAPTER 1 INTRODUCTION ................................. 1

CHAPTER 2 LITERATURE REVIEW .......................... 3

2.1. Milk and milk composition ........................................... 3

2.2. General characteristics of milk proteins ......................... 4

2.2.1. Caseins ............................................................ 4

2.2.2. Whey Proteins ................................................... 6

2.3. General characteristics of milk fat globules ..................... 11

2.4. Recombined milk...................................................... 15

2.5. High pressure processing of milk ................................. 20

2.6. Acid-induced milk gelation ......................................... 36

2.7. Objectives ............................................................ 43
<table>
<thead>
<tr>
<th>CHAPTER 3</th>
<th>MATERIALS AND METHODS</th>
<th>44</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.</td>
<td>Materials</td>
<td>44</td>
</tr>
<tr>
<td>3.2.</td>
<td>Methods</td>
<td>44</td>
</tr>
<tr>
<td>3.2.1.</td>
<td>Recombined milk preparation</td>
<td>44</td>
</tr>
<tr>
<td>3.2.2.</td>
<td>Heat treatment</td>
<td>45</td>
</tr>
<tr>
<td>3.2.3.</td>
<td>High pressure treatment</td>
<td>45</td>
</tr>
<tr>
<td>3.2.4.</td>
<td>Chemical analysis</td>
<td>49</td>
</tr>
<tr>
<td>3.2.5.</td>
<td>Statistical analysis</td>
<td>57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 4</th>
<th>HEAT-TREATED RECOMBINED MILKS</th>
<th>59</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.</td>
<td>Introduction</td>
<td>59</td>
</tr>
<tr>
<td>4.2.</td>
<td>Results and discussion</td>
<td>60</td>
</tr>
<tr>
<td>4.2.1.</td>
<td>Untreated recombined milk</td>
<td>60</td>
</tr>
<tr>
<td>4.2.2.</td>
<td>Heat-treated recombined milk</td>
<td>64</td>
</tr>
<tr>
<td>4.3.</td>
<td>Conclusions</td>
<td>78</td>
</tr>
<tr>
<td>CHAPTER 5</td>
<td>ACID-GELATION OF HEATED RECOMBINED MILKS</td>
<td>80</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>5.1.</td>
<td>Introduction</td>
<td>80</td>
</tr>
<tr>
<td>5.2.</td>
<td>Results and discussion</td>
<td>81</td>
</tr>
<tr>
<td>5.2.1.</td>
<td>Gelation pH and gelation time</td>
<td>81</td>
</tr>
<tr>
<td>5.2.2.</td>
<td>Viscoelastic properties during acidification</td>
<td>83</td>
</tr>
<tr>
<td>5.2.3.</td>
<td>Viscoelastic properties after acidification</td>
<td>87</td>
</tr>
<tr>
<td>5.2.4.</td>
<td>Large deformation rheology of acid gels</td>
<td>90</td>
</tr>
<tr>
<td>5.2.5.</td>
<td>Factors contributing to the differences in acid gelation properties of recombined milks heated before or after homogenization</td>
<td>92</td>
</tr>
<tr>
<td>5.3.</td>
<td>Conclusions</td>
<td>96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 6</th>
<th>HIGH PRESSURE TREATED RECOMBINED MILKS</th>
<th>97</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1.</td>
<td>Introduction</td>
<td>97</td>
</tr>
<tr>
<td>6.2.</td>
<td>Results and discussion</td>
<td>97</td>
</tr>
<tr>
<td>6.2.1.</td>
<td>Whey protein denaturation</td>
<td>98</td>
</tr>
<tr>
<td>6.2.2.</td>
<td>Diameter ($d_{32}$) and protein load of HP-treated recombinant milk fat globules</td>
<td>104</td>
</tr>
<tr>
<td>6.2.3.</td>
<td>Composition of fat globule surface layers in HP-treated recombinant milk</td>
<td>108</td>
</tr>
<tr>
<td>6.2.4.</td>
<td>Proteins adsorbed directly at the interface of fat globules</td>
<td>117</td>
</tr>
<tr>
<td>6.2.5.</td>
<td>Possible structures of proteins and fat globules in HP-treated recombinant milk</td>
<td>124</td>
</tr>
<tr>
<td>6.3.</td>
<td>Conclusions</td>
<td>133</td>
</tr>
</tbody>
</table>
CHAPTER 7 ACID-GELATION OF HIGH PRESSURE-TREATED RECOMBINED MILKS ................................. 136
7.1 Introduction ................................................................................... 136
7.2 Results and discussion ................................................................. 136
  7.2.1. Gelation pH and gelation time ................................................. 136
  7.2.2. Viscoelastic properties during acidification ................................ 138
  7.2.3. Viscoelastic properties after acidification ................................. 143
  7.2.4. Large deformation rheology of acid gels ................................. 146
  7.2.5. Factors contributing to the differences in acid gelation properties of recombined milks HP-treated before or after homogenization .................................................. 149
7.3 Conclusions ................................................................................... 157

CHAPTER 8 HEAT AND HIGH PRESSURE-TREATED RECOMBINED MILKS ........................................ 158
8.1. Introduction ................................................................................... 158
8.2. Results and discussion ................................................................. 158
  8.2.1. Whey protein denaturation ...................................................... 159
  8.2.2. Composition of fat globule surface layers in heat/HP-treated recombined milk ........................................ 161
  8.2.3. Acid gelation properties .......................................................... 167
8.3. Conclusions ................................................................................... 182

CHAPTER 9 OVERALL CONCLUSION AND RECOMMENDATIONS ..................................................... 183
9.1 Introduction ................................................................................... 183
9.2 Comments and conclusions .......................................................... 184
9.3 Concluding remarks ...................................................................... 187
9.4 Recommendations for further study .............................................. 188
APPENDIX A  DATA FOR CHAPTER 4................................................................. 190
APPENDIX B  DATA FOR CHAPTER 6............................................................ 194
APPENDIX C  DATA FOR CHAPTER 8............................................................ 198
REFERENCES.......................................................... ........................................ 201
LIST OF FIGURES

Figure 2.1. Submicelle model of casein micelle; A: a submicelle; B: protruding chain of κ-casein (and possibly β-casein); C: calcium phosphate; D: κ-casein; E: phosphate groups, from Walstra (1999) ................................................................. 5

Figure 2.2. Nanocluster model of casein micelles, from de Kruif and Holt (2003) .... 5

Figure 2.3. Dual bonding model of casein micelle, from Horne (1998) .................. 6

Figure 2.4. Structure of a β-Lg subunit. Ribbons denote the secondary structure with arrows for beta strands and spirals for alpha-helices, from Swaisgood (2004) .... 7

Figure 2.5. Structure of α-La, from Swaisgood (2004) ............................................. 8

Figure 2.6. Fat globules in recombined milk. Fat globules (F), casein micelle (C) and surface layer (S), from Sharma (1993) ................................................................. 16

Figure 2.7. Schematic diagram of basic equipment design for HP treatment of foods, from Huppertz, Kelly and Fox (2002) ................................................................. 24

Figure 2.8. Monoblock casting technology for moderate pressure/size vessels, wire-winding technology for vessels and yoke used in larger size and higher pressure applications, from Torres & Velazquez (2005). ......................................................... 24

Figure 2.9. High hydrostatic pressure pump or pressure intensifier, from Torres & Velazquez, (2005). ................................................................................................. 24

Figure 2.10. Typical pressure-temperature curves for HP treatment of foods, from Balasubramaniam, (2003) ................................................................. 26

Figure 2.11. Dynamic experiment showing response of elastic solid, liquid and viscoelastic material, from Ferry (1980) ........................................................................ 39
Figure 2.12  A systematic presentation of acid gels made from unheated (A, B) and heated (C) reconstituted skim milks with fat globules stabilized by different materials. In unheated system, the gel matrix is formed by association of casein particles. In heated system, the gel matrix is formed by association of denatured whey protein attached to casein micelles. Fat globules stabilized by interacting materials (SMP) are incorporated into the casein-based gel network. Fat globules stabilized by heated WPC interact with each other as the isoelectric point of whey protein is approached, from Cho et al. (1999). ............................................................... 42

Figure 3.1.  Experimental protocol for the preparation of recombined milk with heat and/or HP treatment before homogenization................................................................. 46

Figure 3.2.  Experimental protocol for the preparation of recombined milk with heat and/or HP treatment after homogenization. ............................................................ 47

Figure 3.3.  The photograph of a pilot-scale indirect UHT unit............................... 48

Figure 3.4.  The photograph of “Food Lab” high-pressure food processor............. 48

Figure 4.1  SDS-PAGE patterns under reducing conditions of recombined milk (A) and creams washed with SMUF (B) or SMUF containing dissociating agents (C). Sample dilution = 1:30. .......................................................................................... 61

Figure 4.2.  Schematic illustration of the state of protein and fat globules in recombined milk. The relative sizes of individual components are not to scale. .......... 64

Figure 4.3.  Native-PAGE patterns of recombined milks without heat treatment (RM) and with heat treatments after homogenization at 72°C for 15 s, 140°C for 5 s, and 72 - 100°C for 0 (A), 10 (B), 20 (C) and 30 (D) min. Heat treatments were also applied to milks before homogenization at 72°C for 15 s, 140°C for 5 s, and at 72 - 100°C for 30 min. ........................................................... 65

Figure 4.4.  Denaturation of whey proteins as a function of holding time at different temperatures. Recombined milks were heated after homogenization........... 66

Figure 4.5  Denaturation of whey protein as a function of heating temperature for 30 min holding time either before or after homogenization. ................................. 67
Figure 4.6. Protein load (mg·m⁻²) on fat globules of recombined milks heated after homogenization................................................................. 69

Figure 4.7. SDS-PAGE patterns under reducing conditions of membrane materials isolated from recombined milks: untreated (RM); heated either before or after homogenization at 72°C for 15 s, 140°C for 5 s, and 72 - 100°C for 0 (A), 10 (B), 20 (C) and 30 min (D). Creams were washed with SMUF........................................ 70

Figure 4.8. SDS-PAGE patterns under reducing condition of membrane materials isolated from recombined milks heated either before or after homogenization at 80 and 90°C for 0 (A), 10 (B), 20 (C) and 30 min (D). Creams were washed with SMUF containing dissociating agents................................................................. 71

Figure 4.9. Changes in the amounts of caseins and whey proteins on the surface layers of fat globules isolated from recombined milks heated at 76 - 100°C for 0 - 30 min after homogenization. Dash lines represent those of untreated recombined milk. ........................................................................................................ 72

Figure 4.10. Changes in the amount of caseins and whey proteins on the surface layers of fat globules isolated from recombined milk heated at 72-100°C for 30 min either before or after homogenization. Dash lines represent those of untreated recombined milk ........................................................................................................ 74

Figure 4.11. Changes in caseins and whey proteins on the surface layers of fat globules isolated from recombined milk heated at 80 - 90°C for 0-30 min either before or after homogenization. Dash lines represent those of untreated recombined milk........................................................................................................ 75

Figure 4.12. Transmission electron micrograph images of skim milk heated at 90°C for 30 min. The scale bar on the figure represents 100 nm, from Anema (2010b)........................................................................................................................................ 76

Figure 4.13. Schematic illustration of casein micelles and whey proteins in heated reconstituted milk. The relative sizes of individual components are not to scale........................................................................................................ 77
Figure 4.14. Schematic illustration of caseins, whey proteins and fat globules of recombined milk heated before homogenization. The relative sizes of individual components are not to scale.................................................................77

Figure 4.15. Schematic illustration of caseins, whey proteins and fat globules of recombined milk heated after homogenization...............................................................78

Figure 5.1. Changes in pH with time after GDL addition of recombined milks: (●) untreated; heated before homogenization at (○) 72°C for 15 s and (▼) 140°C for 5 s, or heated after homogenization for 30 min at (△) 76°C, (■) 90°C and (□) 100°C. .............................................................................................................................81

Figure 5.2. Changes in storage modulus as a function of time after GDL addition. Recombined milks were heated either before or after homogenization at different heating profiles........................................................................................................84

Figure 5.3. Denaturation of β-Lg and characteristics of acid gels as a function of heating temperature. Recombined milks were heated for 30 min either before or after homogenization. Dash lines represent those characteristic of untreated recombined milk. .............................................................................................................86

Figure 5.4. Storage modulus ($G'$) and loss modulus ($G''$) of acid gels at 30°C as a function of frequency. Recombined milks were heated either before or after homogenization........................................................................................................88

Figure 5.5. Comparison between the final storage modulus ($G'$) at 30°C and 5°C. Recombined milks were heated either before or after homogenization........................................89

Figure 5.6. Comparison between the final storage modulus ($G'$) and final loss modulus ($G''$) at 30°C and 5°C. Recombined milks were heated either before or after homogenization..................................................................................................................90

Figure 5.7. Shear stress as a function of strain for acid gels made from recombined milks heated either before or after homogenization........................................91
Figure 5.8. Schematic illustration of acid gels made from untreated recombined milk. The relative size of individual components are not to scale. ................................ 93

Figure 5.9. Schematic illustration of acid gel made from recombined milk heated before homogenization. The relative sizes of individual components are not to scale.................................................................................................................. 94

Figure 5.10. Transmission electron micrograph images of heated (90°C/30 min) skim milk taken before and during acidification, from Anema (2010b)......................... 95

Figure 5.11. Schematic illustration of protein gel network of acid gel made from recombined milk heated after homogenization. .............................................................. 95

Figure 6.1. Native-PAGE patterns of HP-treated recombined milks. HP treatments were carried out after homogenization at 10, 20 and 40°C for (A) 5, (B) 15, (C) 30 and (D) 60 min. RM represents native-PAGE of untreated recombined milk. ................................................................................................................................ 99

Figure 6.2. Denaturation of β-Lg (filled symbols) and α-La (open symbols) as a function of pressurizing time. HP treatments were carried out on recombined milks after homogenization at 200 (●, ○), 400 (▼, △), and 600 (■, □) MPa. .......... 100

Figure 6.3. Native-PAGE patterns of HP-treated recombined milks. HP treatments were carried out at 20°C for 30 min before or after homogenization at (A) 200, (B) 400, and (C) 600 MPa. RM represents native-PAGE of untreated recombined milk............................................................................................................ 102

Figure 6.4. Denaturation of β-Lg (●, ○) and α-La (▼, △) as a function of treatment pressures at 20°C for 30 min. HP treatments were applied to milks either before (filled symbols) or after (open symbols) homogenization.................. 103

Figure 6.5 Protein load (mg·m⁻²) on HP-treated recombined milk fat globules as a function of treatment pressures. HP treatments were carried out after homogenization at 10 - 40°C. Dash lines represent the protein load of untreated recombined milks.................................................................................................................. 105
Figure 6.6. Protein load on HP (for 30 min/20°C)-treated (before or after homogenization) recombined milk fat globule as a function of treatment pressures. Dash line represents protein load of untreated recombined milk........................................107

Figure 6.7. SDS-PAGE patterns of surface layers isolated from HP-treated recombined milk fat globules. HP-treatments were applied to recombined milks after homogenization at 10, 20 and 40°C for (A) 5, (B) 15, (C) 30, and (D) 60 min. RM represents the surface layers of fat globules of untreated recombined milk.................................................................................................................................109

Figure 6.8. The amounts of αs- and β-caseins on the surface layers of HP-treated recombined milk fat globules as a function of pressurizing times. HP treatments were carried out after homogenization at (●) 200, (▲) 400, and (■) 600 MPa. Dash lines represent the amounts of αs- and β-caseins on the surface layers of fat globules isolated from untreated recombined milk.........................................................110

Figure 6.9. The amounts of κ-casein and β-Lg on the surface layers of fat globules isolated from HP-treated recombined milks as a function of pressurizing times. HP treatments were carried out after homogenization at (●) 200, (▲) 400, and (■) 600 MPa. Dash lines represent the amounts of κ-casein and β-Lg on the surface layers of fat globules isolated from untreated recombined milks..............................112

Figure 6.10. The amount of α-La on the surface layers of HP-treated recombined milk fat globules. HP treatments were carried out after homogenization at (●) 200, (▲) 400, and (■) 600 MPa. Dash lines represent the amount of α-La on the surface layers of fat globules isolated from untreated recombined milks...............................113

Figure 6.11. SDS-PAGE patterns of surface layers isolated from fat globules of HP-treated recombined milks. HP treatments were carried out before or after homogenization at 20°C for 30 min at (A) 200, (B) 400, and (C) 600 MPa. RM represents the surface layers of fat globules of untreated recombined milk.........................115
Figure 6.12. The amount of proteins on the surface layers of fat globules isolated from HP-treated recombined milks as a function of treatment pressures. HP treatments were applied to milks before or after homogenization at 200 - 600 MPa for 30 min at 20°C. ........................................................................................................ 116

Figure 6.13. SDS-PAGE patterns under reducing conditions of proteins at the interface of fat globules isolated from HP-treated recombined milks. HP-treatments were carried out after homogenization for (A) 5, (B) 15, (C) 30, and (D) 60 min. RM represents the interface of fat globules isolated from untreated recombined milk. ........................................................................................................ 118

Figure 6.14. The ratio of αs- and β-caseins at the interface of HP-treated recombined milk fat globules to those of untreated recombined milk as a function of pressurizing time. HP treatments were carried out after homogenization at (●) 200, (▽) 400, and (■) 600 MPa. ........................................................................................................ 119

Figure 6.15. The ratio of κ-casein and β-Lg (compared with those of untreated recombined milk fat globules) at the interface of HP-treated recombined milk fat globules. HP treatments were carried out after homogenization at (●) 200, (▽) 400, and (■) 600 MPa. ........................................................................................................ 121

Figure 6.16. The ratio of α-La (compared to that of untreated recombined milk fat globules) at the interface of HP-treated recombined milk fat globules. HP treatments were carried out after homogenization at (●) 200, (▽) 400, and (■) 600 MPa. ........................................................................................................ 123

Figure 6.17. SDS-PAGE patterns under reducing conditions of proteins at the interface of fat globules isolated from HP-treated recombined milks. Creams were washed with SMUF containing dissociating agent. HP-treatments were carried out either before or after homogenization at 20°C for 30 min at (A) 200, (B) 400, and (C) 600 MPa. RM represents proteins at the interface of fat globules isolated from untreated recombined milk. ........................................................................................................ 124
Figure 6.18. The ratio of individual proteins (compared to those of untreated recombined milk) at the interface of HP-treated recombined milk fat globules. HP treatments were carried out before or after homogenization at 200 - 600 MPa for 30 min at 20°C. ................................................................................................................125

Figure 6.19. Illustration of caseins and whey proteins in reconstituted milk HP-treated at 200 MPa. The relative sizes of the individual components are not to scale. .................................................................................................................................128

Figure 6.20. Illustration of casein particles and whey proteins in reconstituted milk HP-treated at 400 - 600 MPa. The relative sizes of the individual components are not to scale. ............................................................................................128

Figure 6.21. Schematic illustration of recombined milk undergoing HP treatment at 200 MPa before homogenization. The relative sizes of the individual components are not to scale. ............................................................................................130

Figure 6.22. Schematic illustration of recombined milk undergoing HP treatment at 600 MPa before homogenization. The relative sizes of the individual components are not to scale. ............................................................................................131

Figure 6.23. Schematic illustration of recombined milk undergoing HP treatment at 200 MPa after homogenization. The relative sizes of the individual components are not to scale. ............................................................................................133

Figure 6.24. Schematic illustration of recombined milk undergoing HP treatment at 600 MPa after homogenization. The relative sizes of the individual components are not to scale. ............................................................................................134

Figure 7.1. Changes in storage modulus ($G'$) as a function of time after GDL addition. The acid gels were made from recombined milks HP-treated after homogenization at 20°C at 200 MPa (open symbols) and 600 MPa (filled symbols) for ($\bigcirc$, $\bullet$) 15, ($\triangle$, $\blacktriangle$) 30 and ($\square$, $\blacksquare$) 60 min. The closed diamonds ($\blacklozenge$) represent $G'$ of untreated recombined milk. The change in $G'$ of recombined milk HP-treated at 200 MPa for 15 min at 20°C ($\bigcirc$) was on the same curve as that of untreated recombined milk (bottom curve). .................................................................. 138
Figure 7.2. Changes in storage modulus ($G'$) as a function of time after GDL addition. The acid gels were made from recombined milks HP-treated before (filled symbols) or after (open symbols) homogenization at 20°C for 30 min at (●, ○) 200, (▼, △) 400 and (■, □) 600 MPa. The closed diamonds (♦) represent $G'$ of untreated recombined milk............................................................ 139

Figure 7.3. Denaturation of β-Lg and characteristics of acid gels as a function of pressurizing time. Recombined milks were HP-treated at 200 and 600 MPa after homogenization. Dash lines represent the denaturation and characteristics of untreated recombined milks................................................................. 140

Figure 7.4. Denaturation of β-Lg and characteristics of acid gels as a function of treatment pressures. Recombined milks were HP-treated either before or after homogenization at 20°C for 30 min. Dash lines represent the denaturation and characteristics of untreated recombined milks................................................................. 141

Figure 7.5. Storage modulus ($G'$, filled symbols) and loss modulus ($G''$, open symbols) of acid gels at 30°C as a function of frequency. Acid gels were made from milks HP-treated either before (●, ○) or after (▼, △) homogenization at 200 MPa for 30 min at 20°C. The squares (■, □) represent $G'$ and $G''$ of acid gel made from untreated recombined milk................................................................. 143

Figure 7.6. Comparison between the final storage modulus ($G'$) at 30°C and 5°C. Acid gels were made from recombined milks HP-treated either before or after homogenization at different treatment pressures and times at 20°C.......................................... 144

Figure 7.7. Comparison between the final storage modulus ($G'$) and final loss modulus ($G''$) at 30°C (filled symbols) and 5°C (open symbols). HP treatments were carried out either before (●, ○) or after (△, ▼) homogenization at different treatment pressures and times at 20°C. The squares (■, □) represent the $G'$ and $G''$ of acid gel made from untreated recombined milk................................................................. 145
Figure 7.8. Shear stress as a function of strain for acid gels made from recombined milks HP-treated after homogenization at 20°C at 200 MPa (open symbols) and 600 MPa (filled symbols) for (○, ●) 15 min, (▲, ▼) 30 min, and (□, ■) 60 min respectively. The closed diamonds (◆) represent shear stress of acid gel made from untreated recombined milk. ................................................................. 146

Figure 7.9. Shear stress as a function of strain for acid gels made from milk HP-treated before (filled symbols) or after (open symbols) homogenization at 20°C for 30 min at (●, ○) 200 MPa, (▼, △) 400 MPa, and (■, □) 600 MPa respectively. The closed diamonds (◆) represent shear stress of acid gel made from untreated recombined milk. ........................................................................ 147

Figure 7.10. Change in yield stress as a function of pressurizing time and treatment pressure. (A) Recombined milks were HP-treated at 200 MPa (●) and 600 MPa (○) after homogenization. (B) Milk samples were HP-treated at different pressure for 30 min before (●) and after (○) homogenization. Dash line represents the yield stress of untreated recombined milks. ..................................................................... 148

Figure 7.11. Transmission electron micrograph (TEM) images of HP (400 MPa/30 min)-treated skim milk before and during acidification. The scale bar on each figure represents 100 nm, from Anema (2010b). .............................................. 150

Figure 7.12. Possible acid gel formation in milk HP-treated at 200 MPa before homogenization, showing the interactions between casein micelles and other milk proteins. The relative sizes of individual components are not to scale. ......................... 153

Figure 7.13. Schematic illustration of structure of acid gels made from milk HP-treated before homogenization at 600 MPa for 30 min at 20°C. The relative sizes of individual components are not to scale. ................................................................. 154

Figure 7.14. Schematic illustration of structure of acid milk gel made from recombined milks HP-treated at 200 MPa for 30 min at 20°C after homogenization. The relative of the individual components are not to be scale. ....... 155
**Figure 7.15.** Schematic illustration of structure of acid milk gel made from recombined milks HP-treated at 600 MPa for 30 min at 20°C after homogenization. The relative of the individual components are not to scale. .......................... 156

**Figure 8.1.** Experimental protocols to prepare recombined milks with heat/HP treatments before or after homogenization. ............................................................................................ 158

**Figure 8.2.** Native-PAGE patterns of heat/HP-treated recombined milks. HP treatments were carried out either before or after homogenization at (A) 0, (B) 200, (C) 400, and (D) 600 MPa. RM represents native-PAGE of untreated recombined milk.................................................................................................................. 159

**Figure 8.3.** Denaturation of whey proteins as a function of treatment pressures (for 30 min at 20°C). ................................................................................................................ 160

**Figure 8.4.** SDS-PAGE patterns under reducing conditions of membrane materials isolated from heat/HP-treated recombined milks. HP-treatments were carried out at 20°C for 30 min at (A) 0, (B) 200, (C) 400, and (D) 600 MPa on milk samples that has been heated at 80 or 90°C (for 30 min) either before or after homogenization. RM represents the fat globule surface material of untreated recombined milk. The creams were washed with SMUF. ................................................................. 162

**Figure 8.5.** The amounts of caseins on the surface layers of heat/HP-treated recombined milk fat globules. HP treatments were applied to recombined milks that had been heated for 30 min either before or after homogenization. Dash lines represent casein on surface layers of untreated recombined milk......................... 163

**Figure 8.6.** The percentages of whey proteins on the surface layers of heat/HP-treated recombined milk fat globules. HP treatments for 30 min were applied to recombined milks that were heated at 80°C or 90°C for 30 min. Dash lines represent whey proteins on surface layer of untreated recombined milk.................. 164
Figure 8.7. SDS-PAGE patterns under reducing conditions of membrane materials isolated from heat/HP-treated recombined milks. The creams were washed with SMUF containing dissociating agents. HP treatments were carried out at 20°C for 30 min at (A) 0, (B) 200, (C) 400, and (D) 600 MPa on milk samples that have been heated at 80 or 90°C either before or after homogenization. RM represents the surface materials of untreated recombined milk. ................................................................. 166

Figure 8.8. Changes in gelation pH as a function of treatment pressures for 30 min at 20°C. HP treatments were applied to milks heated at 80°C or 90°C for 30 min either before or after homogenization. At 90°C, the change in gelation pH is similar for milk sample HP-treated before or after homogenization, i.e., the same line in the Figure. Dash lines represent gelation pH of untreated recombined milk.... 168

Figure 8.9. Changes in storage modulus, $G'$, with time after GDL addition of heat/HP-treated recombined milks. The milk samples were heat/HP treated either before or after homogenization. .................................................................................... 170

Figure 8.10. Final $G'$ values at 30°C as a function of treatment pressures for 30 min at 20°C. HP treatments were applied to the milks heated at 80°C or 90°C for 30 min either before or after homogenization. Dash line represent final $G'$ values of untreated recombined milk. .........................................................................................171

Figure 8.11. Shear stress as a function of strain of acid gels made from untreated (■) and heat/HP-treated recombined milks. Milk samples were heated at 80°C or 90°C for 30 min either before or after homogenization and HP-treated at 20°C for 30 min at 0 (●), 200 (○), 400 (◇), 600 (△) MPa. ..........................................................................................173

Figure 8.12. Yield strain and stress as a function of treatment pressures for 30 min at 20°C. HP treatments for 30 min were applied to the milks heated at 80°C or 90°C for 30 min either before or after homogenization. Dash lines represent yield strain and stress of untreated recombined milk..............................................................174

Figure 8.13. Schematic illustration of heat/HP-treated reconstituted milk. The relative sizes of individual components are not to scale..................................................................176
Figure 8.14. Schematic illustration of recombined milk heat/HP-treated before homogenization. The relative sizes of individual components are not to scale. .......... 177

Figure 8.15. Schematic illustration of acid milk gel made from recombined milk heat/HP-treated before homogenization. The relative sizes of individual components are not to scale. ................................................................. 178

Figure 8.16. Schematic illustration of recombined milk heat/HP-treated at 200 and 600 MPa after homogenization. The relative sizes of individual are not to scale................................................................. 180

Figure 8.17. Schematic illustration of acid milk gel made from recombined milk heat/HP-treated at 200 and 600 MPa after homogenization. The relative sizes of individual components are not to scale................................................................. 181
LIST OF TABLES

Table 2.1  *Typical milk composition, from Bylund (1995) and Fox (2003)* ............3

Table 4.1  *Some characteristics of recombined milk and cream washed with SMUF* .................................................................60

Table 4.2  *Individual proteins in recombined milk and creams washed with SMUF and SMUF containing dissociating agents* ..................62

Table 5.1  *The acid gelation properties of heated recombined milks.* .................................82

Table 7.1  *Acid gelation properties of HP-treated recombined milks* ......................137

Table 8.1  *Acid gelation properties of heat/HP-treated recombined milks* .............167