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 Fat Globules and Membrane Proteins under Different Processing Environments as Related to Milk Powder Manufacture

Massey University

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN FOOD TECHNOLOGY

BY

AIQIAN YE

RIDDET CENTRE AND INSTITUTE OF FOOD, NUTRITION
AND HUMAN HEALTH
MASSEY UNIVERSITY, PALMERSTON NORTH

2003

ABSTRACT

The objective of the first part in this study was to gain a better understanding of the protein components of the milk fat globule membrane (MFGM). In the second part, the influence of processing factors on the fat globules and the MFGM during the manufacture of whole milk powder were examined. Relationships between the state of the MFGM in whole milk powders and their reconstitutions properties were also explored.

The MFGM proteins, isolated from early-, mid- and late-season fresh whole milks, were characterized using one- and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and non-reducing conditions. SDS-PAGE under reducing conditions showed the presence of about 40 protein bands, ranging in molecular weight from 15 to 200 kDa. The major MFGM proteins e.g., xanthan oxidase, butyrophilin, PAS 6 and PAS 7 constituted 60-70% of total MFGM proteins while 20-30% were minor proteins. Two-dimensional SDS-PAGE indicated that xanthine oxidase and butyrophilin might be complexed via intermolecular disulfide bonds in the natural MFGM. The examination of MFGM proteins heated at > 60 °C in the absence of skim milk proteins (caseins and whey proteins) showed that xanthine oxidase and butyrophilin interacted further to form very high molecular weight protein complexes, whereas PAS 6 and PAS 7 were relatively heat stable and did not form complexes.

Heat treatment of fresh whole milk in the temperature range 65-95 °C caused incorporation of β-lactoglobulin (β-lg) into the MFGM. Small amounts of α-lactalbumin (α-la) and κ-casein were also observed in the MFGM material of heated milk. The amounts of β-lg and α-la that associated with the MFGM increased with an increase in temperature up to 80 °C, and then remained almost constant. The maximum values for β-lg and α-la association with the MFGM were ~1.0 mg/g fat and ~0.2 mg/g fat, respectively. Association of β-lg and α-la with the MFGM was described by a first-order reaction (65-85 °C for β-lg and 70-80 °C for α-la) in the low temperature range and by a second-order reaction in the high temperature range (85-95 °C for β-lg and 80-95 °C for α-la). Arrhenius plots showed an abrupt change in temperature dependence of

the rate constants at 85 °C for β-lg and 80 °C for α-la. Of the major original MFGM proteins, xanthine oxidase and butyrophilin were not affected by the heat treatment of whole milk, whereas PAS 6 and PAS 7 decreased during heating. Interestingly, this behaviour is in contrast to that shown by these proteins in systems containing no skim milk proteins.

The changes in fat globule size and MFGM proteins during the manufacture of whole milk powder were determined using light scattering, SDS-PAGE, confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM).

Heat treatment of whole milk by direct stream injection (DSI) prior to evaporation caused a decrease in the fat globule size and an increase in the MFGM protein, through the association of caseins and whey proteins with the MFGM material.

Evaporation of milk by a multiple-effect falling film evaporator caused a gradual decrease in the fat globule size and an increase in the MFGM protein after each effect. Caseins dominated the total MFGM protein, indicating the adsorption of casein micelles to the newly formed surface of the fat globules during evaporation. When whole milk was preheated (95 °C for 20 s) before evaporation, the amounts of total MFGM protein were higher (~6 mg/m^2 compared to ~4 mg/m^2 for the non-preheated whole milk) because of association of whey proteins with the native MFGM proteins and casein micelles.

The average fat globule size decreased further upon homogenisation of the concentrated milk. The amount of MFGM protein (mg/m^2) of concentrated milk also increased after homogenisation, the extent of the increase being dependent upon the temperature and pressure of homogenisation. Furthermore, heat treatment of concentrated milk to 79 °C either before or after homogenisation also increased the amount of MFGM protein. However, at the same homogenisation temperature and pressure, the amounts of whey proteins in the MFGM of the concentrated milk that was heated after homogenisation were higher than the concentrated milk that was heated followed by homogenisation.

The amounts of the major native MFGM proteins did not change during homogenisation, indicating that the skim milk proteins did not displace the native MFGM proteins but adsorbed onto the newly formed surface.

The fat globule size of homogenized concentrated milk decreased after spray drying, while the amount of MFGM protein (mg/m^2) decreased slightly. Some "uncovered fat" was observed on the surface of powder particles. It is possible that the proteins do not adsorb to all newly formed fat surfaces during spray drying.

The reconstitution properties of whole milk powders produced using different processing treatments were determined. High homogenization pressure and temperature used before spray drying resulted in poor reconstitution properties of the powder, particularly when the heat treatment was carried out after homogenization. It is suggested that the proteins adsorbed at the fat globule surfaces during homogenisation of the concentrated milk and their subsequent aggregation during heat treatment play a key role in determining the reconstitution properties of whole milk powders.

ACKNOWLEDGMENT

I wish to express my sincere gratitude to my chief supervisor, Professor Harjinder Singh, for the constant encouragement throughout my research study, his never ending patience in helpful and his generous help in the preparation of this manuscript. His expertise and advice throughout the project are gratefully appreciated.

I also wish to extend my gratitude to my co-supervisor, Dr. Mike Taylor and Dr. Skelte Anema for providing important guidance and support during this work. I wish to specially thank Professor P.F. Fox and Professor D. Horne for valuable advice when they visited Massey University.

I wish to thank the Fonterra Co-operative Group for providing the funding for the project.

I would like to thank Dr. Tasa Havea for guidance in making 2D PAGE and Mrs Evelyn Lupton for assistance during the electron microscopy work. Many thanks to the help provided by Dr. David Oldfield in the calculation work of kinetics. Particular thank to Dr. Hong Chen for help in collecting the samples from manufacture plant. I also wish to express my gratitude to Jun (Amy), my wife, for assistance in the test of properties of powder.

Thank also to these people who assisted in different aspects of this project. Professor Paul Moughan, Professor Peter Munro, Mr. Rod Bennett, Dr. Yacine Hemar, Dr. Rogerio Pereira, Mr. Kelvin Goh, Ms. Karen Pickering, Dr. John Flanagan from Riddet Centre and Institute of Food, Nutrition and Human Health, Dr. Gill Norris from Biochemistry Institute, Mr. Mervyn Birtles from Institute of Veterinary Animal and Biomedical Sciences, Massey University. In particular, I thank Ms Geedah Reid, Mr Garry Radford, Mr Steve Glassgow, Mr Byron McKillop, Mrs Michelle Tamehana and Ms Maggie Zou from the Institute of Food, Nutrition and Human Health, Ms Liz Nickless from the Institute of Molecular Biosciences, Massey University; Mr Paul Webby, Mr Colin Knight, Ms Robyn Hirst and Mrs Giao Truong from Fonterra Research Centre for their valuable support during the experimental work.

Thank to all the postgraduate students and researchers I have met during my study for their friendship and help, in particular, Maya Sugiarto, Ping Gao, Mei-Li Chiang, Abby Thompson, Yong Hee, Caro Ma. The following families are thanked for friendship, guidance, encouragement and making my stay enjoyable in Palmerston North: ChunLi and Joseph Zhou, Julia and Chen Hong, Lily and Zheng Hao, Kuen and Chen Ping.

Last, but not least, I want to thank, with all my heart, every one in my family (my parents, sisters) who have always been there with me through out the project. I cannot express strongly enough my gratitude to my wife for her support, encouragement and tolerance. I also thank my son (Jack) and daughter (Rebecca) for being the inspirations for doing all this work, in every day over the past three years.

TABLE OF CONTENTS

ABSTRACT i

ACKNOWLEDGMENTS iv

TABLE OF CONTENTS vi

CHAPTER 1. INTRODUCTION 1

CHAPTER 2. LITERATURE REVIEW 3

2.1. General characteristics of milk components 3

2.2. General characteristics of milk proteins 3

2.2.1. Caseins 4

2.2.2. Casein micelle 5

2.2.3. Dissociation of casein micelles 11

2.2.4. Whey proteins 11

2.3. General characteristics of milk fat globules 14

2.3.1. Size distribution of milk fat globule 15

2.3.2. Milk fat globule membrane 15

2.3.3. Proteins of milk fat globule membrane 17

2.3.4. Isolation of MFGM material 18

2.3.5. Molecular organization and structure of the MFGM 19

2.4. Effect of heat treatment on the fat globules and the MFGM protein 20

2.4.1. Size of fat globules 20

2.4.2. Association of serum proteins with the MFGM 20

2.4.3. Change in the native MFGM proteins 24

2.4.4. Quantities of β-lg, α-la associated with the MFGM 25

2.5. Effect of evaporation on the fat globules and the MFGM protein 26

2.6. Effect of homogenization on the milk fat globules and the MFGM protein 27

2.7. Properties of modified fat globules and the MFGM proteins 29

2.8. Fat globules and the MFGM in the milk powder 31

2.9. Role of fat globules and the MFGM proteins in influencing functional properties of the whole milk powder 33

2.10. The aim and objectives of the thesis 35

CHAPTER 3. MATERIALS AND METHODS 36

3.1. Materials 36

3.2. Heat treatment of whole milk samples 36

3.3. Manufacture of whole milk powder 37

3.3.1. Preheat treatment of milk 37

3.3.2. Evaporation 37

3.3.3. Homogenization of milk 37

3.3.4. Spray drying of concentrated milk 39

3.4. Isolation of milk fat globule membrane material 40

3.5. Characterization of MFGM protein components 41

3.6. Protein and fat content 43

3.7. Average fat globule size 43

3.8. Confocal laser microscopy 44

3.9. Transmission electron microscopy 44

3.10. Determination of functional properties of whole milk powder 45

3.10.1. Solubility index 45

3.10.2. Cold (25 °C) sediment 45

3.10.3. Hot (85 °C) sediment 45

3.10.4. Hot coffee sediment 45

3.10.5. Dispersibility 46

3.11 Statistical analysis 46

CHAPTER 4. CHARACTERIZATION OF PROTEIN COMPONENTS OF NATURAL AND HEAT-TREATED MILK FAT GLOBULE MEMBRANES 47

4.1. Introduction 47

4.2. Results and discussion 48

4.2.1. Characterization of the MFGM proteins isolated from whole milk 48

4.2.2. Characterization of the MFGM proteins from heated washed cream 57

CHAPTER 5. INTERACTIONS OF WHEY PROTEINS WITH MILK FAT GLOBULE MEMBRANE PROTEINS DURING HEAT TREATMENT OF WHOLE MILK **63**

5.1. Introduction 63

5.2. Interactions of milk serum proteins with milk fat globule membrane proteins during heat treatment of whole milk 65

5.2.1. Results 65

5.2.2. Discussion 78

5.3. Kinetics of heat-induced association of β-lg and α-la with milk fat globule membrane in whole milk 81

5.3.1. Results 81

5.3.2. Order of reaction and kinetic parameters 84

5.3.3. Effect of temperature on the rate constant 88

5.3.4. Thermodynamic considerations 88

5.3.5. Discussion 93

CHAPTER 6. CHANGES IN FAT GLOBULE MEMBRANE PROTEINS DURING CONCENTRATION OF WHOLE MILK IN A PILOT-SCALE MULTIPLE-EFFECT EVAPORATOR **98**

6.1. Introduction 98

6.2. Preliminary studies 100

6.2.1. Milk fat globule size 100

6.2.2. Total MFGM protein concentration and protein composition 102

6.2.3. Microscopic structures 104

6.3. Main trials 106

6.3.1. Experimental milk samples 106

6.3.2. Change in milk fat globule size distributions 110

6.3.3. Changes in total protein concentration at the milk fat globule surface 110

6.3.4. Protein composition of the fat globule surface 111

6.3.5. Microscopic structures 118

6.4. Analysis of industrial samples 124
6.5. Discussion 128
6.5.1. Disruption of fat globules during evaporation of milk 128
6.5.2. Change in MFGM during evaporation of milk 130

CHAPTER 7. CHANGES IN THE FAT GLOBULE MEMBRANE DURING HOMOGENIZATION AND HEAT TREATMENT OF CONCENTRATE PRIOR TO SPRAY DRYING 133
7.1. Introduction 133
7.2. Milk fat globule size 135
7.3. Change in surface protein concentration 135
7.3.1. Heating the concentrate before homogenisation 135
7.3.2. Heating the concentrate after homogenisation 138
7.4. Protein composition of the fat globule surface of the concentrate after homogenisation 140
7.4.1. Cream washed with SMUF solution 140
7.4.1.1. Heating before homogenisation 140
7.4.1.2. Heating after homogenisation 143
7.4.2. Cream washed with a dissociation buffer 146
7.4.3. Native MFGM proteins after homogenisation 147
7.5. Ratio of individual adsorbed caseins to total caseins at the fat globule surface 149
7.6. CSLM and electron microscopy 152
7.7. Discussion 159

CHAPTER 8. EFFECT OF SPRAY DRYING ON THE MILK FAT GLOBULE AND MFGM PROTEINS DURING THE MANUFACTURE OF WHOLE MILK POWDER 163
8.1. Introduction 163
8.2. Changes in fat globule size distributions 164
8.3. Changes in the total protein concentration and the protein composition of the fat globule surface 167
8.4. Confocal micrographs of the fat globules in the whole milk powders 168

8.5. Discussion 177

CHAPTER 9. ROLE OF FAT GLOBULES AND MFGM PROTEINS IN INFLUENCING THE RECONSTITUTION PROPERTIES OF WHOLE MILK POWDER 180

9.1. Introduction 180

9.2. Functional properties of whole milk powders 181

9.3. Fat globule size distributions of reconstituted whole milk powders 183

9.4. Confocal micrographs of reconstituted powders 183

9.5. Surface protein coverage and composition of the fat globules in the powders 190

9.6. Discussion 194

CHAPTER 10. OVERALL DISCUSSION AND CONCLUSIONS 197

10.1. Changes in the fat globule size and the MFGM proteins during the manufacture of whole milk powder 197

10.2. Adsorption behaviour of casein micelles at the fat globule surface during processing 201

10.3. Association of whey proteins with the MFGM during heat treatment 205

10.4. Behaviour of the native MFGM proteins during processing 208

10.5. Relationship between the reconstituted properties of powder and the MFGM proteins 208

10.6. Further studies 210

REFERENCES 213