# A comprehensive study on the relative importance of disulphide and non-covalent interactions between proteins on the heat-induced aggregation and functional property of acid milk gels

A thesis presented in partial fulfilment of the requirements

for the degree of

**Doctor of Philosophy in Food Technology**

At Massey University, Albany, New Zealand

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2014

# Abstract

Understanding the interactions between the milk proteins during heat treatment of milk can be employed to manipulate the functional properties of dairy products. The ability to control the functional properties can be beneficial to the dairy industry. When being heated, milk proteins interact via two main types of bonding: disulphide bonds and non-covalent interactions. They are both considered to be important in the properties of heated milks and the resulting milk products. This research aimed to investigate the relative importance of each interaction type on the heat-induced aggregation between the proteins in milk and the functional properties of a milk product in a model food system.

Experiments involved adding low concentrations of a disulphide-bond reducing agent or a thiol blocking reagent to milk systems to either enhance or inhibit the thiol-disulphide exchange reactions between the proteins. The reagent was added to unheated milks, heated milks and unheated milks followed by heating. The effect of modifying the extent of thiol-disulphide exchange reactions between the proteins on the level of proteins participating in intermolecular disulphide bonds, on the degree of interactions between the casein micelles/casein proteins and the whey proteins were investigated. The treated milks were acidified to form acid milk gels of which the rheological properties and the microstructure were examined.

Results demonstrated that the proportion of proteins participating in intermolecular disulphide bonds can be controlled by systematically modifying the thiol-disulphide exchange reactions between the milk proteins. It was shown that the initial interactions between the proteins in milk upon heating were non-covalent and disulphide bonds were subsequently formed to strengthen the bonding between the proteins in the heat-induced aggregates. When the milks were made to acid gels, both types of protein interactions in the milk were equally important in influencing the storage modulus (G') values of the resulting gels with the higher the degree of connections, the higher the G' values. On the other hand, disulphide bonds played a more important role than non-covalent interactions in determining the yield properties of the acid gels. The yield stress values can be increased by increasing the proportion of disulphide bonds in the milk system before acidification or by enhancing the formation of disulphide bonds between the particles during the formation of acid gels.

# Acknowledgements

There are a number of people who I would like to acknowledge and thank for their assistance during the completion of my degree.

First and foremost, I would like to express my sincere respect and appreciation to my supervisors: **Dr Skelte Anema** (Fonterra), **Dr Fanny Guyomarc'h** (INRA), **Dr Palatasa Havea** (Fonterra) and **Associate Professor Marie Wong** (Massey University). Your patience, support, guidance and advice throughout the years have been invaluable. It was your high expectation of me that encouraged me to push myself as hard as I have.

I would like to thank **Michael Loh** for his training and support on Confocal Microscopy, **Dr Graeme Gillies** for useful discussions about rheology, and **Dr Claire Woodhall** for her excellent editorial support for various publications from this work. A special thank goes to **Dr Steve Taylor** for his kind support during my stay at Fonterra Research and Development Centre (FRDC).

The financial support of the New Zealand Foundation for Research, Science and Technology (FCGL0810) and Fonterra Co-operative Group is gratefully acknowledged. I would like to also acknowledge the Claude McCarthy Fellowship, the Japan Society for the Promotion of Science, and São Paolo School of Advanced Science on Advance in Molecular Structuring of Food Materials for sponsoring my attendance at conferences.

In my daily work, I have been blessed with helpful and cheerful members of the Food Science Lab: **Alastair, Aurelie, Carolina, Christina C., Dianne, Esra, Edeline, Kevin, Kendison, Lucile, Rob, Sheelagh and Yvonne**. There are many others at FRDC who have also helped me along the way, too many to name, but you know who you are and I am grateful to you.

Special thanks to **Christina Streicher**, **Lu Lin** and **Catherine Davis** for always being by my side in both the good times and the best.

Thank you also to **Dr Siew Kim Lee**, my New Zealand-based aunts and cousins who always cared about my progress and my well-being during my stay in New Zealand.

Last, but not least, I am especially grateful to my **Mum** and **Dad** for your sacrifices that have enabled me to complete my degree in New Zealand. It was your love, support and understanding that have motivated me throughout the years. I am also deeply thankful to my partner **Tuan** for providing excellent moral support throughout the course of my PhD.

# List of publications and presentations

**Publications in international journals**

Nguyen, H. A. N., Anema, S. G., Havea, P., Guyomarc'h, F. & Wong, M. (2012) Effect of adding low levels of β-mercaptoethanol on the disulphide bonds of κ-casein and β-lactoglobulin solutions. *International Dairy Journal*, *26*, 78-82.

Nguyen, H. A. N., Wong, M., Anema, S. G., Havea, P. & Guyomarc'h, F. (2012) Effects of adding low levels of disulphide reducing agent on the disulphide interactions of β-lactoglobulin and κ-casein in skim milk. *Journal of Agricultural and Food Chemistry, 60*, 2337-2342.

Nguyen, H. A. N., Wong, M., Havea, P., Guyomarc'h, F. & Anema, S. G. (2013) The proteins interactions and rheological properties of skim milk heated in the presence of low levels of reducing agent. *Food Chemistry, 138*, 1604-1609.

Nguyen, H. A. N., Wong, M., Guyomarc'h, F., Havea, P. & Anema, S. G. (2014) Effects of non-covalent interactions between the milk proteins on the rheological properties of acid gels, International Dairy Journal, http://dx.doi.org/10.1016/j.idairyj.2014.03.001

**Conference presentations (oral and poster presentations)**

Nguyen, H. A. N., Guyomarc'h, F., Wong, M., Anema, S. G. & Havea, P. (2013) Effects of modifying the protein interactions in heated skim milk on the properties of acid milk gels. Poster presentation, Sao Paulo School of Advanced Science: Advances in Molecular Structuring of Food Materials workshop, Sao Paulo, Brazil.

Nguyen, H. A. N., Guyomarc'h, F., Wong, M., Havea, P. & Anema, S. G. (2012) Protein interactions and rheological properties of skim milk heated in the presence of a reducing agent. Poster presentation, Chemical Reactions in Food conference, Prague, Czech Republic.

Nguyen, H. A. N., Wong, M., Guyomarc'h, F., Havea, P. & Anema, S. G. (2012) Effects of modifying thiol-disulphide interactions in skim milk on the rheological properties of acid gels. Poster presentation, Food Colloids conference, Copenhagen, Denmark.

Nguyen, H. A. N., Havea, P., Guyomarc'h, F., Wong, M. & Anema, S. G. (2012) Behaviour of whey protein and k-casein upon addition of low concentrations of β-mercaptoethanol to skim milk. Poster presentation, HOPE meeting, Tuskuba, Japan.

Nguyen, H. A. N., Anema, S. G., Havea, P., Guyomarc'h, F. & Wong, M. (2011) Interactions of β-lactoglobulin and κ-casein after addition of low levels of a disulphide reducing agent. Oral and poster presentations, NIZO conference, Papendal, the Netherlands.

Nguyen, H. A. N., Anema, S. G., Havea, P., Guyomarc'h, F. & Wong, M. (2011) β-Lactoglobulin and κ-casein disulphide interactions after controlled addition of a disulphide reducing agent. Poster presentation, NZIFST conference, Rotorua, New Zealand.

# Table of content

# List of figures

# List of Tables

# List **of Symbols**

| | |
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| **G'** | Storage modulus |
| **G"** | Loss modulus |
| **pI** | Isoelectric point |
| **pKa** | The negative base-10 logarithm of the acid dissociation constant ($K_a$) of a solution. The lower the pKa values, the stronger the acid. |
| **tan δ** | Loss tangent, ratio of G"/G' |

# List of Abbreviations

| | |
|---|---|
| **APS** | Ammonium persulphate |
| **CCP** | Colloidal calcium phosphate |
| **Cys** | Cysteine residue |
| **DSC** | Differential scanning calorimetry |
| **DTT** | Dithiothreitol |
| **GDL** | Glucono-δ-lactone |
| **MF** | Micro-fluidic |
| **NEM** | N-ethylmaleimide |
| **PAGE** | Polyacrylamide gel electrophoresis |
| **SDS** | Sodium dodecyl sulphate |
| **SH** | Thiol group |
| **TEM** | Transmission electron microscopy |
| **TEMED** | Tetramethylethylenediamine |
| **Tris-base** | Tris (hydroxymethyl) methylamine |
| **WPE** | Whey protein enriched |
| **WPF** | Whey protein free |
| **WPI** | Whey protein isolate |
| **UV** | Ultraviolet |