

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.

Effects of urea addition on the structural and material properties
of caseinate solutions and emulsions

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

Master of
Food Science and Technology

at Massey University, Manawatū,
New Zealand.

XueXin Wen

2014

Abstract

The effects of urea addition on the self-assembly of caseinate sub-micelles and subsequent impact on the structural and material properties of caseinate solutions and caseinate stabilised oil-in-water emulsions (30 vol% oil) were studied.

Sub-micellar particle size and distribution were measured using dynamic light scattering. Sodium caseinate in solution showed a bimodal particle size distribution centred at 20 and 120 nm (radius). The increasing addition of urea (1.1 to 6.6 M) was seen to cause a reduction in the size of caseinate sub-micelle, which was attributed to a dissociation of the sub-micellar fraction arising from a reduction in hydrophobic interactions.

For sodium caseinate solutions above estimated sub-micellar close-packing phase volumes (>10 wt% protein concentration), increasing addition of urea was seen to significantly lower solution viscosity with more pronounced changes observed with increasing protein concentration. All the urea-treated solutions were Newtonian.

For sodium caseinate stabilised emulsions, where protein concentrations were above the critical threshold for onset of depletion flocculation, the addition of urea was seen to result in a reduction in depletion free energy of the emulsions. The effects of reduced depletion interaction on emulsion stability, structural and rheological properties were found to be dependent not only on the concentration of added urea, but also on the protein concentration in the emulsions. For 2 and 4 wt% caseinate concentrations, depleted droplets reduced with increasing urea concentration and the emulsion stability increased as well. For 6 wt% protein concentration, the stability decreased with the addition of 1.1 and 3.3 M urea, and it was re-established when the urea concentration was up to 6.6 M. This was attributed to the synergic effects on the depletion potential and continuous phase viscosity which were induced by urea, and it was supported by the results of creaming profiles, rheological properties and microstructure. Likewise, urea had similar effects on the emulsions stabilised by potassium caseinate.

Acknowledgement

First of all, I would like to express my sincere gratitude and appreciation to my supervisor associate professor Matt Golding, for his novel ideas, inspiring advices and guidance. With his excellent supervision, I really enjoy doing my project, which evokes my interest in the dairy industry.

Moreover, I would like to offer my special thanks to the people provided great support in my experiments. I would like to show my appreciation to Mrs Michelle Tamehana, Mr Steve Glasgow, Mr Garry Radford and Mr Warwick Johnson for their technical support and useful advices. I would also like to thank Mr Chris Hall and Miss Janiene Gilliland for their support and suggestions when I was using the equipment in Riddet Institute. I would like to express my gratitude to Yvonne van der Does (Fonterra) for her cooperation in using Turbiscan in my project.

I really appreciate all of my friends in New Zealand, especially Soffalina, Anges, Ruby, Desmond, Andrew and Gina for their encouragement and support during my study. I thank Yichao Liang for the valuable discussion about the research and the proofreading of the thesis.

Finally I would like to thank my family for their unconditional love for supporting the fulfilment of my study.

I really have a good time doing research in Massey University, and I am grateful to anyone who has ever helped me in this wonderful journey.

Table of Contents

1 Contents

1. Introduction	1
2 Literature Review	3
2.1 General introduction of milk protein	3
2.2 Casein.....	3
2.2.1 Characteristics of casein.....	3
2.2.2 Individual caseins.....	4
2.2.3 Casein micelle structure	5
2.2.4 Casein manufacture.....	10
2.3 Caseinate	12
2.3.1 Characteristics of caseinate.....	12
2.3.2 Self-assembly behaviour in caseinate solution	14
2.3.3 Functional properties of caseinates	16
2.3.4 General methods of caseinate manufacture.....	18
2.3.5 Applications in food industry.....	20
2.4 Emulsions.....	24
2.5 Emulsion stability	24
2.5.1 Creaming.....	25
2.5.2 Coalescence.....	25
2.5.3 Flocculation.....	26
2.5.4 Ostwald ripening.....	26
2.5.5 Phase inversion	27

2.6	Caseinate-stabilised oil-in-water emulsions.....	27
2.6.1	Self-assembly in caseinate-stabilised emulsions.....	28
2.6.2	Factors affecting the creaming stability of caseinate-stabilised emulsions.....	31
2.6.3	Creaming profiles of caseinate-stabilised emulsions	35
2.6.4	Rheological properties of caseinate-stabilised emulsions.....	36
2.6.5	Microstructure of caseinate-stabilised emulsions	37
2.7	The dissociation of caseins aggregates	37
2.8	Research questions.....	38
3	Materials and Methods	40
3.1	Materials	40
3.2	Sample preparation	40
3.2.1	Preparation of caseinate solutions.....	40
3.2.2	Preparation of caseinate-stabilised emulsions.....	41
3.3	Analyses of the samples.....	41
3.3.1	Particle size of caseinates.....	41
3.3.2	Droplet size of caseinate-stabilised emulsions.....	42
3.3.3	Rheological measurements of caseinate solutions	42
3.3.4	Rheological measurements of caseinate-stabilised emulsions	43
3.3.5	Creaming stability of caseinate-stabilised emulsions.....	43
3.3.6	Microstructure of caseinate-stabilised emulsions	44
4	Effects of urea on sodium caseinate solutions.....	45
4.1	Particle size of sodium caseinate	45
4.1.1	Sample preparation and particle size of sodium caseinate.....	45
4.1.2	Effects of urea on the self-assembly in sodium caseinate solutions.....	47

4.2	Rheological properties of sodium caseinate solutions with urea	50
4.2.1	Shear rate dependence.....	50
4.2.2	Concentration dependence	51
4.2.3	Temperature dependence.....	52
4.2.4	Highly concentrated sodium caseinate solutions	53
4.3	Discussion	54
4.3.1	Sample preparation	54
4.3.2	Particle size of sodium caseinate.....	56
4.3.3	Disassociation effects of urea on sodium caseinate solutions.....	56
4.3.4	Highly concentrated sodium caseinate solutions	57
5	Effects of urea on sodium caseinate stabilised emulsions	59
5.1	Droplet size of sodium caseinate stabilised emulsions	59
5.2	Creaming stability of sodium caseinate stabilised emulsions with urea	62
5.2.1	Visual creaming profiles	62
5.2.2	Turbiscan backscattering.....	64
5.3	Rheological properties of sodium caseinate stabilised emulsions with urea.....	70
5.3.1	Shear rate dependence.....	70
5.3.2	Time dependence	72
5.4	Microstructure of sodium caseinate stabilised emulsions with urea	75
5.5	Discussion	76
6	Effects of urea on potassium caseinate stabilised emulsions	80
6.1	Characteristics of potassium caseinate.....	80
6.1.1	Particle size of potassium caseinate	80
6.1.2	Viscosity of potassium caseinate solutions	80

6.1.3	Droplet size of potassium caseinate stabilised emulsions	81
6.2	Creaming stability of potassium caseinate stabilised emulsions with urea.....	84
6.2.1	Visual creaming profiles	84
6.2.2	Turbiscan backscattering.....	86
6.3	Rheological properties of potassium caseinate stabilised emulsions with urea	92
6.3.1	Shear rate dependence.....	92
6.3.2	Time dependence	94
6.4	Microstructure of potassium caseinate stabilised emulsions with urea.....	97
6.5	Discussion.....	98
7	Overall discussions.....	100
8	Conclusions and Recommendations.....	102
8.1	Effects of urea on caseinate solutions	102
8.2	Effects of urea on emulsions stabilised by caseinates.....	102
8.3	Applications of findings to industry.....	103
8.4	Recommendations for future work	103
	References.....	105

List of Figures

Figure 2.1 Models of casein - part A.....	7
Figure 2.2 Models of casein - part B.....	9
Figure 2.3 Processes for the manufacture of caseins.	11
Figure 2.4 Method for the manufacture of sodium caseinate.....	21
Figure 2.5 Methods for the manufacture of different caseinate types.....	22
Figure 2.6 The mechanism of depletion flocculation of emulsion droplets by micelles.....	29
Figure 2.7 Schematic diagram showing the interplay between the various interparticle energies.	30
Figure 4.1 Size distributions by intensity of 1 wt% sodium caseinate solutions with EDTA.....	46
Figure 4.2 Size distributions by number of 1 wt% sodium caseinate solutions with EDTA.	47
Figure 4.3 Size distributions by intensity of 1 wt% sodium caseinate solutions made with 200 mM EDTA and urea.	48
Figure 4.4 Size distributions by number of 1 wt% sodium caseinate solutions made with 200 mM EDTA and urea.	49
Figure 4.5 Shear rate dependence of the apparent viscosity of sodium caseinate solutions from 5 to 20 wt% with urea (T = 25 °C).	51
Figure 4.6 Apparent viscosity of sodium caseinate solutions from 5 to 20 wt% with and without urea (T = 25 °C, shear rate = 10 s ⁻¹).	52
Figure 4.7 Temperature dependence of apparent viscosity of sodium caseinate solutions with and without urea (shear rate = 0.1 s ⁻¹).	53
Figure 5.1 Droplet size distributions of oil-in-water emulsions (30 vol% oil) stabilised with sodium caseinate.	61
Figure 5.2 Visual creaming profiles (after 24, 48 h of storage at room temperature) of oil-in- water emulsions (30 vol% oil) made with sodium caseinate and urea.....	63
Figure 5.3 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 2 wt% sodium caseinate and urea after preparation, 24 and 48 h of storage.	64

Figure 5.4 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 4 wt% sodium caseinate and urea after preparation, 24 and 48 h of storage.	65
Figure 5.5 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 6 wt% sodium caseinate and urea after preparation, 24 and 48 h of storage.	66
Figure 5.6 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 2 wt% sodium caseinate and urea in 24 h of storage.	67
Figure 5.7 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 4 wt% sodium caseinate and urea in 24 h of storage.	68
Figure 5.8 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 6 wt% sodium caseinate and urea in 24 h of storage.	69
Figure 5.9 Shear rate dependence of apparent viscosity of oil-in-water emulsions (30 vol% oil) made with sodium caseinate and urea.	71
Figure 5.10 Time dependence of complex viscosity of oil-in-water emulsions (30 vol% oil) made with sodium caseinate and urea.	73
Figure 5.11 A close up of the first 10 min of the time dependence rheological properties of Figure 5.10.	74
Figure 5.12 Confocal micrographs of oil-in-water emulsions (30 vol% oil) made with sodium caseinate and urea (scale bar is 40 μ m).	75
Figure 6.1 Apparent viscosity of 20 wt% potassium and sodium caseinate solutions.	81
Figure 6.2 Droplet size distributions of oil-in-water emulsions (30 vol% oil) stabilised with potassium caseinate.	83
Figure 6.3 Visual creaming profiles (after 24, 48 h of storage at room temperature) of oil-in-water emulsions (30 vol% oil) made with potassium caseinate and urea.	85
Figure 6.4 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 2 wt% potassium caseinate and urea after preparation, 24 and 48 h of storage.	86
Figure 6.5 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 4 wt% potassium caseinate and urea after preparation, 24 and 48 h of storage.	87
Figure 6.6 Creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 6 wt% potassium caseinate and urea after preparation, 24 and 48 h of storage.	88

Figure 6.7 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 2 wt% potassium caseinate and urea in 24 h of storage.	89
Figure 6.8 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 4 wt% potassium caseinate and urea in 24 h of storage.	90
Figure 6.9 Dynamic creaming profiles (Turbiscan) of oil-in-water emulsions (30 vol% oil) made with 6 wt% potassium caseinate and urea in 24 h of storage.	91
Figure 6.10 Shear rate dependence of apparent viscosity of oil-in-water emulsions (30 vol% oil) made with potassium caseinate and urea.	93
Figure 6.11 Time dependence of complex viscosity of oil-in-water emulsions (30 vol% oil) made with potassium caseinate and urea.	95
Figure 6.12 A close up of the first 10 min of the time dependence rheological properties of Figure 6.11.	96
Figure 6.13 Confocal micrographs of oil-in-water emulsions (30 vol% oil) made with potassium caseinate and urea (scale bar is 40 μ m).	97

List of Table

Table 2.1 The characteristics of individual caseins.....	5
Table 2.2 Applications of caseinates in food industry*.....	23
Table 2.3 Key physical factors affecting the stability and rheology of emulsions.....	27
Table 3.1 Product analysis of caseinates.....	40
Table 4.1 Apparent viscosity of highly concentrated sodium caseinate solutions with urea	54
Table 5.1 Droplet size of sodium caseinate oil-in-water emulsions (30 vol% oil) with urea	60
Table 6.1 Particle size of caseinates.....	80
Table 6.2 Droplet size of potassium caseinate stabilised oil-in-water emulsions (30 vol% oil) with urea	82