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**MASSEY
UNIVERSITY**

***FORMATION AND STABILITY OF
OIL-IN-WATER CASEINATE EMULSIONS***

**A THESIS
PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
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**BY
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Dedication

To my Parents

ABSTRACT

The main objective of this study was to gain a better understanding of the formation, stability and microstructure of oil-in-water emulsions stabilized by commercial sodium (ALANATE 180) and calcium caseinates (ALANATE 380). The study also determined the effects of heat treatment and NaCl addition on the formation and stability of these emulsions.

Emulsions were prepared using various concentrations of sodium or calcium caseinate solutions (0.5 to 5.0%) and 30% soya oil. Surface protein coverage (mg/m^2) in freshly prepared emulsions was determined from analysis of the aqueous phase after centrifugation of emulsions at 45,000 g for 40 minutes, using the Kjeldahl method. SDS-PAGE was used to identify the adsorbed protein components in the cream phase. Creaming stability was determined after storage of emulsions for 24 hours at 20°C by a low speed centrifugation method. The microstructure of these emulsions was determined using confocal laser scanning microscopy. The aggregation state of caseins in sodium and calcium caseinate solutions was determined by successive centrifugation, gel permeation chromatography and multi-angle laser light scattering techniques.

For emulsions stabilized with sodium caseinate, the surface protein concentration increased gradually with protein concentration up to 3%, but the increase was much smaller at higher concentrations. By comparison, the surface protein coverage in emulsions stabilized with calcium caseinate showed an almost linear increase with protein concentration (0.5 to 5.0%). At all protein concentrations, the surface protein coverage of emulsions stabilized with calcium caseinate was higher than that of sodium caseinate emulsions. β -Casein was adsorbed in preference to other caseins in emulsions made using $\leq 2.0\%$ sodium caseinate, but α_s -casein (α_{s1} - + α_{s2} -) appeared to adsorb in preference to other caseins when emulsions were made using $> 2.0\%$ sodium caseinate. In calcium caseinate-stabilized emulsions, α_s -casein was found to

adsorb in preference to other caseins at all protein concentrations used.

Heat treatment (121°C for 15 min) of sodium caseinate emulsions or heat treatment of sodium caseinate solutions prior to emulsion formation, at all caseinate concentrations, resulted in an increase in surface protein coverage and altered the proportions of individual caseins at the droplet surface. The surface protein coverage of emulsions formed with calcium caseinate solutions increased markedly when the emulsions were heated (121°C for 15 min) or when calcium caseinate solutions were heated prior to emulsion formation. The preferential adsorption of α_s -casein, observed in the unheated calcium caseinate emulsions, diminished after heating, which was due to polymerization of α_s -casein during heating and/or degradation of this casein.

In sodium caseinate emulsions, the surface protein coverage and the composition of emulsion droplets were influenced by the presence of NaCl prior to emulsion formation. The surface protein coverage in emulsions made with 1 and 3% sodium caseinate increased with an increase in NaCl concentration up to 40 mM, with a large increase in the adsorption of α_s -casein at the droplet surface. Addition of NaCl beyond 40 mM had no further effects on surface coverage and composition. Similar trends were observed when NaCl was added to the emulsions after they were formed. By contrast, in calcium caseinate emulsions, the surface protein coverage decreased with increase in NaCl concentration up to 40 mM, but with further increase in NaCl concentration the surface protein coverage increased slightly. In these emulsions, the composition of the interface remained largely unaffected by NaCl addition; α_s -casein was adsorbed in preference to other caseins.

Creaming stability of calcium caseinate emulsions, after storage at 20°C for 24 hours, increased with an increase in protein concentration. However, the creaming stability of sodium caseinate emulsions decreased markedly as the protein concentration was increased above 2%. This decrease in stability was attributed to the reversible flocculation arising from a 'depletion flocculation'

mechanism. This flocculation in turn resulted in enhanced creaming at high caseinate concentrations. In sodium caseinate emulsions, the appearance of the droplets in the confocal micrographs was dependent on the concentration of protein used for making emulsions. Emulsions formed with low concentrations of sodium caseinate (0.5 and 1.0%) appeared to be homogenous with no sign of flocculation. However the emulsions made with > 2% sodium caseinate showed some irregular flocs, which appeared to be forming a network structure at higher concentrations of protein. In contrast, confocal micrographs of emulsions formed with calcium caseinate at all protein concentrations showed individual droplets. The creaming stability of these emulsions improved, when the emulsions were heated or when emulsions were made using heated sodium or calcium caseinate solutions. The presence of 200 mM NaCl prior to emulsion formation resulted in improved creaming stability and a reduced degree of flocculation.

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TABLE OF CONTENTS

DEDICATION	
ABSTRACT	i
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	vi
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	
2.1 Introduction	4
2.2 General characteristics of milk proteins	4
2.3 Association properties of caseins	11
2.4 General methods of casein manufacture	14
2.5 General methods of caseinate manufacture	20
2.6 Effect of heat processing on caseinate solutions	23
2.7 Emulsion formation	25
2.8 Adsorption behaviour of proteins at the oil-in-water interface	27
2.9 Emulsion stability	39
2.10 Factors affecting emulsion stability	50
CHAPTER 3: MATERIALS AND METHODS	
3.1 Materials	56
3.2 Preparation of emulsions	56
3.3 Emulsion characterization	56
3.4 Determination of surface protein concentration and composition	58
3.5 Turbidity measurements	60
3.6 Sedimentation of calcium caseinate dispersions	60
3.7 Chemical analysis	60
3.8 Determination of viscosity	60
3.9 Determination of microstructure	61
3.10 Size exclusion chromatography	61

3.11	Electrophoresis	62
3.12	Multi-angle laser light scattering (MALLS)	65
3.13	Creaming stability	70

CHAPTER 4: CHARACTERIZATION OF PROTEINS IN COMMERCIAL SODIUM AND CALCIUM CASEINATES

4.1	Introduction	71
4.2	Results	72
4.2.1	Composition of commercial sodium and calcium caseinates	72
4.2.2	Characterization of proteins in commercial sodium and calcium caseinates	73
4.2.3	Effect of protein concentration on the aggregation state of protein in sodium or calcium caseinate solutions	77
4.3	Discussion	87

CHAPTER 5: ADSORPTION BEHAVIOUR OF SODIUM AND CALCIUM CASEINATES IN OIL-IN-WATER EMULSIONS

5.1	Introduction	91
5.2	Results and discussion	92
5.2.1	Emulsion formation	92
5.2.2	Particle size distribution	92
5.2.3	Surface protein coverage	98
5.2.4	Composition of caseins at the oil/water interface (cream phase)	100
5.2.5	Competitive adsorption: analysed by size exclusion chromatography (SEC)	109
5.2.6	Adsorption behaviour of mixtures of calcium and sodium caseinates in emulsions	115

CHAPTER 6: STABILITY OF OIL-IN-WATER EMULSIONS FORMED WITH SODIUM AND CALCIUM CASEINATES

6.1	Introduction	125
6.2	Results and discussion	125
6.2.1	Confocal microscopy	128
6.2.2	Viscometry	135
6.2.3	Creaming stability of mixtures	151
6.3	General Discussion	155

CHAPTER 7: EFFECT OF HEAT TREATMENT (RETORT CONDITIONS) ON THE FORMATION AND STABILITY OF CASEINATE EMULSIONS

7.1	Introduction	159
7.2	Emulsion formation	159
7.3	Results and discussion	160
7.3.1	Particle size distribution and droplet diameter	160
7.3.2	Surface protein coverage	163
7.3.3	SDS-PAGE	167
7.3.4	Stability of emulsions	181

CHAPTER 8: THE EFFECT OF ADDITION OF NaCl ON THE FORMATION AND STABILITY OF CASEINATE EMULSIONS

8.1	Introduction	195
Part A:	The effect of addition of NaCl on the formation and stability of sodium caseinate emulsions.	
8.2	Results	197
8.2.1	Emulsion formation	197
8.2.2	Particle size distribution and droplet diameter	197
8.2.3	Surface protein coverage	197
8.2.4	Composition of caseins at the oil/water interface (cream	

phase).	200
8.2.5 Surface concentration	204
8.2.6 Stability of emulsions	207
8.3 Effect of NaCl on the adsorption behaviour of caseins in emulsions made with varying concentrations.	213
8.3.1 Droplet diameter	213
8.3.2 Surface protein coverage	213
8.3.3 Composition of caseins at the oil/water interface (cream phase)	216
8.3.4 Estimated surface concentrations	218
8.3.5 Stability of emulsions	220
8.4 Discussion	225
Part B: The effect of addition of NaCl on the formation and stability of calcium caseinate emulsions.	
8.5 Results	229
8.5.1 Emulsion formation	229
8.5.2 Particle size distribution and droplet diameter	229
8.5.3 Surface protein coverage	229
8.5.4 Composition of caseins at the oil/water interface (cream phase)	232
8.5.5 Stability of emulsions	232
8.6 Effect of NaCl on the emulsions made with various calcium caseinate concentrations	232
8.6.1 Particle size distribution and droplet diameter	234
8.6.2 Surface protein coverage	237
8.6.3 Stability of emulsions	237
8.7 Discussion	240
CHAPTER 9: General Conclusions and recommendations	243
REFERENCES	249
