

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. MASSEY UNIVERSITY

Colloidal interactions in an alternate make cheese

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

Luo, Jing

[2017]

Abstract

The role of emulsion structure and interactions on the material and technical functionality of an alternate make cheese (AMC) was investigated. Lab scale cheese samples (25 g comprising 23 wt.% fat and 20 wt.%) were prepared by recombining model emulsions with a separate protein phase under controlled temperature, shear speed and residence time in a rapid visco analyser (RVA). Sodium caseinate and Tween 20 were used respectively to stabilize fat globules for the model emulsions. Preliminary experiments were carried out for samples prepared using either calcium caseinate or sodium caseinate as protein phase. Structural characterisation of samples showed emulsion structure and distribution within these phases to be dependent on protein type. It was inferred that the calcium from calcium caseinate, as indicated by the increased fat globule size distribution after cheese making. In comparison, the size of fat globules covered with sodium caseinate appeared relatively stable in cheese produced form cheese curd. Based on these observations, caseinates were subsequently replaced by cheese curd as the protein phase for the remainder of the study.

For cheese samples prepared with low fat cheese curd, fat droplets stabilised with sodium caseinate were hypothesised as binding with the surrounding protein matrix, and thereby these fat globules could be considered as 'active fillers'. Confocal laser scanning microscopy supported this hypothesis showing homogeneously dispersed fat droplets within the protein network. This emulsion system did not show fat-protein phase separation in baking (170 °C 10 minutes) as droplets were prevented from coalescing as a consequence of entrapment within the protein phase.

Fat globules covered with Tween 20 were hypothesised as behaving as 'inactive fillers', with the adsorbed layer not anticipated to form bonds with the surrounding protein network. Confocal and scanning electron microscopy instead showed localised domains of fat droplets within the protein structure that underwent partial coalescence on cooling of the cheese after manufacture. Cheeses comprising Tween stabilised droplets exhibited phase separation on baking and visible oil-off on the surface of cheese arising

i

from extensive coalescence taking place within the localised regions of fat due to melting of the partially coalesced structures. Additional rheological analysis of cheeses was carried out to determine the effect of droplet-protein interactions on the material properties of the cheese samples. Notably, findings were presented in relation to a non-fat control cheese. Findings showed that, at temperatures below 30 °C when fat was crystallized, both inactive and active fillers had a higher relative modulus to the non-fat sample. However, at elevated temperature without fat crystals, inactive fillers resulted in a relative reduction in storage modulus when compared to the non-fat cheese, while active fillers increased relative storage modulus.

Model cheeses prepared with either sodium caseinate or Tween 20 stabilised emulsions were then compared to cheese samples comprising non-homogenised cream as the emulsion phase. Structural analysis of samples determined that cheeses comprising fat globules stabilized with native milk fat globule membrane behaved in a manner analogous to samples prepared with the Tween stabilised emulsion, indicating the presence of inactive droplets. However, it was also observed that increasing the residence time of cheese production within the RVA caused a transition of the interaction behaviour of the emulsion from inactive to active, as evidenced by corresponding changes to structural, material and functional properties of the cheese.

Further exploration of this transition determined that the mechanical work applied during cheese preparation was sufficient to homogenise fat droplets during extended shearing, resulting in a reduction to fat droplet size. Droplet homogenisation during shearing was also found to have disrupted the native milk fat globule membrane, allowing protein adsorption to take place. It was also determined that whey proteins were the predominant interfacial fraction adsorbed as a consequence of extended shearing, and were considered responsible for the transition of droplets from inactive to active. Combined findings have shown that the material and functional properties of an alternate make cheese composition could be strongly influenced by the interactions of the emulsion phase with the surrounding protein network. These interactions could, in turn, be manipulated through formulation and/or process design, providing greater control over product properties.

ii

Acknowledgements

Firstly, I would like to express my deepest gratitude to my supervisors: Prof. Matt Golding, Dr. Graeme Gillies and Dr. Mita Lad. Many thanks for your time and advices.

It is my pleasure to do a PhD research under the supervision of Prof. Matt Golding, who is well-known for his passion on research and very kind attitude to people. Much appreciation for his comments on the improvement of my thesis. As he said, people need to enjoy the moment when they are reading the thesis.

Dr. Graeme Gillies is the supervisor at Fonterra Research and Development Centre. From him I learned how to think as a scientist, how to work efficiently and how to do impressive presentations. I enjoyed the time discussing with him, and I always obtained much more than what had expected. Thanks for his selfless contribution on his knowledge.

Dr. Mita Lad is another supervisor at Fonterra Research and Development Centre. At most of time she was the first one to write back when I need a help. Thanks for her supervision and priority on this project.

Secondly, I would like to show my sincerely gratitude to Prof. Peter Munro, Dr. Christina Coker and Dr. Steve Taylor for their great support to this PGP project on cheese. Thanks to bring in this PGP project and organize PGP reviews. It was very thoughtful to arrange training courses during PGP reviews. Thank the finance support from the PGP funding and the technical support from Fonterra Research and Development Centre.

As one of PGP projects I had chances to discuss with researchers at Fonterra Research and Development Centre. I would like to thank Ms. Elizabeth Nickless, Dr. Peter Wiles, Dr. Skelte Anema, Dr. Siew Kim Lee, Dr. Sheelagh Hewitt, Dr. Palatasa Havea, Dr. David Reid, Mr. Andrew Broome, Mr. Michael Loh, Ms. Amy Yang, Ms. Sally Hewson, Ms. Weiping Liu, Dr. Steve Dybing, Dr. Abraham Chawanji, Mr. Ivan Simpson and Dr. Philip Watkinson for their advices and technical supports. Studying with other young researchers in PGP groups, I gained sincere friendships with Ms. Orianne Thionnet, Mr. Prateek Sharma, Dr. Tzvetelin Dessev, Ms. Xiaoli Sun, Ms. Seo Won Yang, Dr. Collin Brown and Dr. Sina Hosseiniparvar. I will miss this period of studying together. People in PGP built a very healthy research group where people enjoyed sharing and communicating. In particular, I would like to thank Ms. Orianne Thionnet for her accompanying at the office. Thank Mr. Prateek Sharma for his organization of drinking parties. Thank Dr. Tzvetelin Dessev for his advices on the calculation. Thanks Dr. Sina Hosseiniparvar for his time of discussion.

Finally, I would like to acknowledge my families for their significant support throughout my PhD study. It was not easy to do the PhD research at the time raising a baby. Much appreciate the considerable support from my husband Ran Gao, who was my strongest backup. I became stronger when I was in a difficult time, and Gao was always there to encourage me. I am grateful to my parents and parents-in-law for their help looking after my baby Lucas. I am afraid my success on this PhD study couldn't live without the support from my families.

Table of Contents

Abstracti
Acknowledgements iii
Table of Contentsv
List of figuresix
List of tablesxvi
Acronymsxvii
Chapter 1: Introduction1
1.1 Objectives and research questions1
1.2 Thesis structure2
Chapter 2: Literature review5
2.1 Colloidal interactions in traditional cheeses5
2.1.1 Molecular interactions in cheese5
2.1.2 The dynamics of cheese emulsion structure during manufacture8
2.2 Structural parameters influencing cheese functionality and material properties12
2.2.1 Effect of fat structuring12
2.2.1.1 Fat globule interface12
2.2.1.2 Fat globule size16
2.2.1.3 Fat melting points
2.2.2 Effect of cheese manufacture21
2.3 Colloidal interactions in an alternate make cheese (AMC)24
2.3.1 From emulsion gels to cheese system24
2.3.2 Fat structuring in AMC27
2.3.3 Analysis of AMC functionalities
2.3.3.1 Fat globule dispersion in cheese
2.3.3.2 Cheese melting properties

Chapter 3: Materials and methods	33
3.1 Materials	33
3.1.1 Cheese materials	33
3.1.2 Materials for analytical methods	34
3.1.2.1 Walstra (dissociating) solution	34
3.1.2.2 Fluorescent staining of samples for CLSM	34
3.1.2.3 Buffers for protein composition analysis	35
3.2. Equipment	35
3.3. Sample preparation	36
3.3.1 Cream emulsion preparation	
3.3.1.1 Emulsified cream emulsions	36
3.3.1.2 Modified natural cream	37
3.3.2 Cheese-making	
3.3.2.1 Model cheese	
3.3.2.2 Alternate make cheese (AMC)	40
3.4. Analysis of the cream emulsion	43
3.4.1 Particle size distribution	43
3.4.2 CLSM	43
3.4.3 Protein composition in serum	44
3.5. Analysis on cheese samples	46
3.5.1 Particle size distribution	46
3.5.2 CLSM	46
3.5.3 Rheology	47
3.5.4 Cheese melting (Schreiber test)	
3.5.5 Moisture content in cheese-like samples	
3.5.6 Total shear work calculation	50
Chapter 4: Behavior of a viscous protein matrix with emulsified lipids	53

Colloidal interactions in the alternate make cheese

4.1 Overview	53
4.2 Sodium caseinate (NaCas) stabilised emulsion droplets	54
4.2.1 Oil-in-water emulsions	54
4.2.2 Oil-in-viscous protein matrix emulsions	55
4.3 Tween 20 stabilized emulsion droplets	59
4.3.1 Oil-in-water emulsions	59
4.3.2 Oil-in-viscous protein matrix emulsions	60
4.4 Viscous emulsions manufactured from natural cream	66
4.5 Conclusion	69
Chapter 5: Emulsion interactions and structuring in Alternate Make Cheese (AMC)	73
5.1 Overview	73
5.2 Microstructure of fat dispersion in protein matrix	75
5.3 Fat globule size distribution	80
5.4 AMC rheological properties during heating	86
5.5 Cheese melting properties	93
5.5.1 Flowing extent after baking	93
5.5.2 Oil-off in baking	96
5.5.3 Water loss in baking	99
5.6 Conclusions	101
Chapter 6: Influence of cheese manufacture and protein adsorption on Alternate Made Cheese (AMC)	
6.1 Overview	104
6.2 Impact of processing temperature	106
6.2.1 Microstructure	106
6.2.2 Particle size distribution	109
6.2.3 Cheese melting	111
6.3 Effect of shear speed	113
6.3.1 Microstructure	115

Colloidal interactions in the alternate make cheese

6.3.2 Particle size distribution	119
6.3.3 Cheese melting	122
6.4 Total shear work impacting on cheese modification	123
6.5 Effect of protein adsorption at fat interface on fat filler properties	129
6.5.1 AMC made from preheated natural cream	
6.5.2 AMC made from preheated natural cream within NaCas	
6.5.3 AMC made from natural cream loading within proteins dissociated fro	
6.6 Cheese rheological properties	
6.7 Conclusion	141
Chapter 7: General conclusions	
7.1 Cheese melting	
7.2 Phase separation	145
7.4 Recommendations	146
References	
Appendix I: Capillary number and Reynold number in the RVA mixing	155

List of figures

Figure 2.7: Confocal micrographs of semi-hard type cheese made of milk with different milk fat fractions (AMF5, AMF41 and butter oil as control) and large (LFG, $d_{50,3} \sim 2.75 \mu m$) or small (SFG, $d_{50,3} \sim 1 \mu m$) fat globules after 4 weeks of ripening at 4 °C. Protein is labeled in red, the fat is in blue and black areas reveal gas holes or the aqueous phase. Scale bar represents 20 μm (Schenkel, Samudrala et al. 2013).

Figure 2.11: Capillary numbers for droplet break-up in planar elongation flow (left) and simple
shear flow (right) (Jackson and Tucker III 2003). The star points show the 4 μm active fat globules
in protein matrix of AMC blended at 1000 rpm 60 °C

Figure 4.1: Comparison of the fat globule size distribution in oil-in-water emulsions before (\diamondsuit)
and after (X) RVA processing (800 rpm 10 minutes 70 °C). Fat globules were fully covered with
NaCas55

 Figure 4.9: Small strain rheological properties of the viscous CaCas matrix emulsions in a heating from 4 °C to 80 °C. Tween 20 was used as the interfacial ingredients. The viscous emulsion without biphasic structure (■) was compared to over-sheared samples of biphasic structure (●).

Figure 4.11: CLSM images of the viscous emulsions manufactured from natural cream and CaCas powder. The RVA processing was 800 rpm 25 minutes at 60 °C. Red is fat and green is protein..68

Figure 4.14: Conclusion of active fillers and inactive fillers locating in the protein matrix. Yellow circles are fat globules; Blue area is the serum pocket; squared area presents CaCas matrix.72

 Figure 5.5: Fat globule size distribution before and after 30 minutes AMC_Tween producing. The samples are cream emulsion used for AMC_Tween making (\diamond), fresh AMC_Tween without cooling (X) and AMC_Tween after 5 days storage at 4 °C (\triangle)......83

Figure 5.10: Cheese melting after 10 minutes baking at 170 °C for AMC made from natural cream in 10 minutes (a) and 30 minutes (b). The cheese area before baking was indicated by yellow dotted circles, which is the same area for both samples, and the large yellow dashed circles indicate cheese flowing in baking. The arrows indicate directional flowing by difficult melt.......96

Figure 5.12: Water loss after 10 minutes baking at 170 °C was compared in AMCs within four types of fat fillers: manipulated fat globules covered with NaCas (\blacklozenge) or Tween 20 (\bigcirc); the

anhydrous milk fat without emulsifier (x) and fat globules from natural cream (▲). X-axis is residence time of AMCs in RVA processing101
Figure 5.13: Schematic diagrams of fat and water dynamic dispersion in cheese melting. Melting behaviour in AMC within active or inactive fat fillers are described and concluded
Figure 6.1: Comparison of total shear work in cheese produced at 60 °C (\Box) and 70 °C ($lacksquare$) 106
Figure 6.2: CLSM images of AMC made from natural cream in 20 minutes (a), 30 minutes (b) and 40 minutes (c) at 70 °C. The photos were taken using the lenses of x 40 (left) and x 25 (right). Red is fat and green is protein, black is serum or air
Figure 6.3: The change of average fat globule size in cheese to the total shear work made at 60 °C or 70 °C. Fresh cheeses without cooling (60 °C: \Box , 70 °C: Δ) are compared to the cheeses after 7 days storage at 4 °C (60 °C: \blacksquare , 70 °C: \blacktriangle)
Figure 6 4: Fat globule size distribution of AMC after 7 days storage at 4 °C. Three cheese samples were made at 70 °C in 20 minutes (▲, 26.8 kJ/kg total shear work), 30 minutes (▲, 42.9 kJ/kg total shear work) and 40 minutes (▲, 77.5 kJ/kg total shear work), and another cheese sample was made at 60 °C in 30 minutes (■, 78.9 kJ/kg total shear work)
Figure 6.5: Cheeses were baked at 170 °C for 10 min. The amount of free oil released to cheese surface is decreasing when total shear work is increasing. Cheeses were made at 60 °C (\Box) and 70 °C (\blacktriangle), respectively. Three images of molten cheese are attached: cheese (a) was 30 minutes made at 60 °C within 78.9 kJ/kg total shear work; cheese (b) was 30 minutes made at 70 °C within 42.9 kJ/kg total shear work; and cheese (c) was 40 minutes made at 70 °C within 71.3 kJ/kg. The cheese area before baking was indicated by yellow dotted circles, which is the same area for all samples, and the large yellow dashed circles indicate cheese flowing in baking. The arrows indicate directional flowing by difficult melt
Figure 6.6: The cheeses made from the maximum shear rate of 600 rpm (◆) and 1200 rpm (○), respectively, at 60 °C. (a) Comparison of total shear work in cheese production. The total shear work was calculated after the first 5 minutes when temperature is constant at 60 °C. (b) Comparison of viscosity shown in the RVA. Yellow: shear speed profile; Red: cheese produced at 600 rpm; Blue: cheese produced at 1200 rpm
Figure 6.7: CLSM images of cheese made at 600 rpm 60 °C for 10 min (a), 20 min (b) and 40 min (c). The photos were taken using lenses of x 40 (left) and x 25 (right). Red is fat and green is protein, black is serum or air
Figure 6.8: CLSM images of cheese made at 1200 rpm 60 °C for 10 min (a), 15 min (b) and 20 min (c). The photos were taken using lenses of x 40 (left) and x 25 (right). Red is fat and green is protein, black is serum or air
Figure 6.9: The fat globule size distribution is compared between cheeses made at low shear speed (600 rpm) and cheeses made at high shear speed (1200 rpm) at 60 °C. Figure (a) and (b)

Figure 6.13: CLSM images of dissociated cheese in Walstra solution without Tween. The images are shown in two separated photos. Fat membrane is in red on left photos and proteins are in green on right photos. Image (a) is from cheese made in 10 minutes at 800 rpm (8.3 kJ/kg total shear work); Image (b) is from cheese made in 10 minutes at 1200 rpm (19.0 kJ/kg total shear work); Image (c) is from cheese made in 15 minutes at 1200 rpm (35.0 kJ/kg total shear work).

Figure 6.15: Cheeses were produced using preheated natural cream adding NaCas. The cream was premixed with 4 wt.% NaCas in RVA for 30 minutes at 800-1000 rpm 60 °C, and then cheese

was produced using this cream in 10 minutes at 800 rpm 60 °C. (a) Fat globule size distribution is compared in fresh uncooled cheese (X), 7 days 4 °C stored cheese (X) and the cream (X) used for cheese producing; (b) CLSM images were taken using lenses of x40 (left photo) and x25 (right photo) on cheese in 7 days storage at 4 °C; (c) Molten cheese in 10 minutes baking at 170 °C, where cheese area is compared before and after baking which were marked by yellow circles.

Figure 6.16: The change of protein composition in serum from non-fat cheese. The serums were collected from non-fat cheese produced in 7 minutes (red), 10 minutes (blue) and 30 minutes (green). Skim milk was used as the control sample in the measurement. A: α -lactalbumin; B: β lactoglobulin; C: β-casein; D: α- casein.....135

Figure 6.17: CLSM images of cream diluted by the EDTA solution without Tween. The cream was after sonication with the serum from 30 minutes non-fat cheese. The images are shown in two separated photos. Fat membrane is in red on the left photos and proteins are in green on the

Figure 6.18: Cheeses were produced using the cream after sonication with serum collected from non-fat cheese. The cheese was produced in 15 minutes at 800 rpm 60 °C. (a) Fat globule size distribution is compared in cheese (fresh cheese, ■; 7 days 4 °C stored cheese, ■; and the cream,) used for cheese producing; (b) CLSM images were taken using lenses of x 40 (left photo) and x 25 (right photo) on cheese in 7 days storage at 4 °C.....137

Figure 6.19: Small strain rheological properties are compared in cheese heated up from 4 °C to 80 °C. G' is cheese storage modulus and Gm' is cheese matrix storage modulus, which was measured on non-fat cheese of the same ratio of water to protein. Cheeses were made by varied constant shear speed, producing temperature and preheated natural cream. Cheese made in 40 minutes 600 rpm 60 °C (◆) within 45.3 kJ/kg total shear work; cheese made in 10 minutes 1200 rpm 60 °C (\bigcirc) within 19.0 kJ/kg total shear work; cheese made in 15 minutes 1200 rpm 60 °C (\bullet) within 35.0 kJ/kg total shear work; cheese made in 30 minutes 1000 rpm 70 °C (**A**) within 42.8 kJ/kg total shear work; cheese made in 10 minutes 800 rpm 60 °C and the cream was precooked with 4 % NaCas (X); cheese made in 15 minutes 800 rpm 60 °C and the cream was after

Figure 6.20: Schematic diagrams of the impact factors to transit inactive fat fillers to active fat

List of tables

Table 2.1: Approximate composition of cheese varieties including AMC (Walstra, Wouters et al. 2006)
Table 2.2: References on different sizes of native fat globules separated from a raw whole bovine milk
Table 2.3: Physical, chemical and sensory properties and functionality of cheese manufactured using native small fat globule milk (SFG) or large fat globule milk (LFG).

Table 3.1: information of the equipments	35
Table 3.2: cream emulsion ingredients and details of preparation including pressures and passing times on the two-stage homogenizer	•
Table 3.3: RVA profile parameters of the model cheese production in chapter 4	40
Table 3.4: RVA profile parameters in the AMC producing	42

Table 5.1: The temperature of G' and G" crossover point in small strain rheological analysis......87

Acronyms

	AMC	Alternate make cheese
		Alternate make cheese produced from emulsified fat fully covered with
	AMC_NaCas	sodium caseinate
		Alternate make cheese produced from emulsified fat fully covered with
	AMC_Tween	Tween 20
	AMC_NC	Alternate make cheese produced from fat globules with native milk fat
		globule membrane
	AMC_AMF	Alternate make cheese produced from anhydrous milk fat without
	AIVIC_AIVIF	emulsifiers
	AMF	Anhydrous milk fat
	β-ΜΕ	β-Mercaptoethanol
	Са	Capillary number
	CaCas	Calcium caseinate
	CLSM	Confocal laser scanning microscopy
	cm	Centimetre
	d(0.1)	Volume-weighted diameter of 10 % smallest droplets
	d(0.9)	Volume-weighted diameter of 10 % largest droplets
	d _{4,3}	Volume-weighted average mean diameter
	EDTA	Ethylene diamine tetra acetic acid
	FG	Fast Green
	FO	Free oil
	g	Gram
	G'	Storage modulus
	G"	Loss modulus
	G _m '	Storage modulus of non-fat cheese
	Hz	Hertz
	LFCC	Low fat cheese curd
	m	Mass (in equations); or meter (after numbers)

min	minutes
mm	Millimetre
mg	Microgram
nm	Nanometre
NaCas	Sodium caseinate
NaOH	Sodium hydroxide
NR	Nile Red
PEG	Polyethylene glycol
Re	Reynold number
RhPe	1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine
KIIFE	rhodamine B sulfonyl)
RVA	Rapid visco analyzer
rpm	Revolve per minute
SDS-PAGE	Sodium dodecyl sulfate-poly acrylamide electrophoresis
vol	volume
Tween	Tween 20
Tris	Tris(hydroxymethyl)aminomethane
μΙ	Microliter
WPI	Whey protein isolate
wt	Weight
w/v	Weight/ volume