IFS, Massey University, New Zealand

&

Fonterra, New Zealand

Investigations of the behaviour of pectin in casein micelle systems and their analogues

Thesis presented by:

Aurélie Suzanne Bernadette CUCHEVAL

For the degree of Doctor of Philosophy in Physics

Research conducted at the Institute of Fundamental Sciences, Massey University of Palmerston North

Supervision by: **Dr. Martin A.K Williams**

Co-supervision by: **Dr. Yacine Hemar and Dr. Don Otter**

May 28, 2009

Abstract

Firstly, the effect of pectin on acid milk gels in concentrated, quiescent systems was investigated by passive microrheology using two complementary techniques: diffusive wave spectroscopy (DWS) and multiple particle tracking (MPT). DWS, by allowing probing the mechanical properties of the network at high frequency, gave information on its microstructure. The addition of high methoxyl pectins was shown to change the network structure which has been explained by bridging of the casein micelles by the polymer as the system was undergoing acidification. On the other hand, the presence of low methoxyl pectin in the acid milk gel was shown to have no effect on the microstructure of the network at low concentration of polymer (0.1%w/w) which has been attributed to the sensitivity of this low DM pectin to calcium: LM pectin are trapped by calcium and not able to interact with casein micelles anymore. Multiple particle tracking was used to probe the effect of pectin on the heterogeneity of the system by following the distribution of the displacements of added micro beads at a given time lag during the gelation using the Van Hove distribution. Furthermore, the surface chemistry of the probes was modified in an attempt to control their location in the system. Finally, the mean square displacements of the casein micelles obtained by DWS and, of κ-casein coated particles obtained by MPT were shown to give good agreement for the same acid milk system.

Having established that the interaction between casein micelles and low methoxyl pectin is prevented by the pectin sensitivity to calcium, the effect of the pectin fine structure was investigated on the interaction between κ-casein and pectin by surface plasmon resonance (SPR). The amount of pectin binding on a κ-casein coated gold surface was shown to be strongly dependant on the pectin fine structure. It was concluded that small negative patches on the pectin backbone, likely to comprise of around two consecutive unmethylesterified galacturonic acid, are the most effective for pectin binding to κ-casein. The effect of the direct interaction between pectin and κ-casein on ‘calcium-free casein micelle mimics’ in pectin solution was then investigated using coated latex beads. A pectin structure with a limited number of negative patches on its backbone was also shown to limit the potential for destabilization via bridging.

Acknowledgements

I better first start with the VIPs…Bill Williams, Don Otter and Yacine Hemar. Yacine; thanks for always being here, always being available for questions…and answering fast a bit in a ‘Lucky Luck’ way…thanks for all your good ideas… and thanks as well for believing in me and for your encouragements… Don; thanks for all interesting and helpful discussions and good ideas…and thanks for your milk knowledge. Bill, if I say Super Bill, you will tell me to not take the mickey, but it is in a good way….thanks for all your help, your passion for pectin, your hidden passion for milk….and your enthusiasm all the way. I enjoyed all these discussions in your office…even what you call ‘wibbles’ …now I know the proper way to communicate with (swearing) English people.

I would also like to thank all the members of the Biopolymer group for ideas exchange, discussions…and good moments. Medhat Al-Ghobashy, I better confess, I am really impressed by your lab organization and your knowledge of techniques, thanks a lot to have let me in your lab and initiate me to the Biacore. Dr RRR Vincent, work with you was fun even if we didn’t agree too often…your physics knowledge (in a no stamp collection way) was much appreciated…and thanks for your special way to always see the positive side of each problem…thanks Bro. Motoko, thanks to have taken time to show me the NMR techniques and to have shared with me the PME experience. Erich, going with you and Bill to the synchrotron was cool (far from ‘boring’), thanks for your help to prepare the samples there…Erich and Steve, thanks for the ‘social pressure’, you know what I mean…

I would like to thank all the people from Fonterra who helped me, especially Rob Hunter, Skelte Anema and Steve Taylor.

Chère ‘familie’, que pouvais je faire de pire que de partir aux ‘antipodes’ (comme papa aime a le répéter) et pourtant vous m’avez supporté tout du long, et vous êtes même venu en expédition organisée…merci, merci beaucoup...

Moncheri, pour les nombreux aller-retour à la fac à pas d’heure, les multiples problèmes et fins du monde que je te ressasse, pour t’avoir ignoré quelquefois, tu dessers une médaille pas de crapauds mais de charming prince!

Abbreviations

MPT	Multiple Particle Tracking
DWS	Diffusive Wave Spectroscopy
HG	Homogalacturonan
DM	Degree of Methylesterification
HM pectin	High-Methoxy pectin
LM pectin	Low-Methoxy pectin
DB	Degree of Blockiness
RGI	RhamnoGalacturonan I
RGII	RhamnoGalacturonan I
AFM	Atomic Force Microscopy
PME	Pectin Methyl-Esterase
f-PME	Fungal Pectin Methyl-Esterase
p-PME	Plant Pectin Methyl-Esterase
NMR	Nuclear Magnetic Resonance
PL	Pectin Lyase
PG	PolyGalacturonase
M_w	Molecular weight
PGA	PolyGalacturonic Acid
$G'(\omega)$	Elastic modulus
$G''(\omega)$	Viscous modulus
f	Frequencies
k	Boltzmann constant
T	Temperature
$\langle r^2(\tau)\rangle$	Mean Square Displacement

PEG	PolyEthylene Glycol
τ	Time lag
$g_1(\tau)$	Field autocorrelation function
$g_2(\tau)$	Intensity autocorrelation function
l^*	Light mean free path
z_0	Penetration depth
L	DWS (cell) sample thickness
T0, T1, T2	Treatment 0, 1, 2
SPR	Surface Plasmon Resonance
RU	Resonance Unit
D	Diffusion coefficient
DAm	Degree of amidation
GDL	Glucono-δ-lactone
pC	Cirtical pH
a	Radius of particle
LMA pectin	Low Methoxyl Amidated pectin
CE	Capillary Electrophoresis
HG	Homogalacturonan
Dabs	Absolute Degree of blockiness

Table

ABSTRACT **3**

ACKNOWLEDGEMENTS **5**

CHAPTER 1 .... **13**

BACKGROUND **13**

1 PECTIN 14

1.1 Pectin structure *15*

1.2 Pectin fine structure modification *18*

1.3 Enzymatic deesterification: pectinesterase *18*

1.3.1 Plant Pectinesterase 18

1.3.2 Fungal pectinesterase 19

1.4 Alkaline deesterification *19*

1.5 Backbone degradation by polygalacturonase (PG) *19*

1.6 Pectin gels *20*

1.6.1 Calcium gels 21

1.6.2 Acid gels 22

2 MILK PROTEIN 23

2.1 Milk composition *23*

2.1.1 General 23

2.1.2 Milk lipids 24

2.2 Milk protein composition and structure *24*

2.2.1 Caseins 24

Individual caseins 24

Casein micelles 26

2.2.2 Whey protein: 27

3 ACID MILK GELS 28

3.1 General *28*

3.2 Effect of acidification on micellar state *30*

3.3 Rheology and microstructure of acid milk gels *31*

3.4 Rearrangement *32*

3.5 Models applied to acid milk gels and gelation *33*

3.5.1 Adhesive sphere model 33

3.5.2 Fractal models 34

4 PECTIN-CASEIN MICELLE INTERACTION 35

4.1 The Effect of pectin on the properties of acid milk gels and rennet induced gels *35*

4.1.1 Rennet gels 36

4.1.2 Acid milk gels 36

4.2 Interaction of pectin with casein micelles & casein micelle 'aggregates' and the localisation of pectin in acid milk systems *38*

4.2.1 Interaction with casein micelle 38
4.2.2 Interaction with casein aggregates 39
4.2.3 Casein micelle / aggregates sterically stabilized by a pectin layer supported by serum pectin or pectin / casein mixed gel...40
4.3 The interaction of pectin with casein molecules in the absence of calcium........................41
4.3.1 Acidified sodium caseinate gels with pectin 41
4.3.2 Thermodynamic compatibility of pectin / casein mixtures 42
4.3.3 Oil in water emulsions stabilized by sodium caseinate containing pectin....................................43
5 INSTRUMENTATION 44
5.1 Method to study pectin fine structure: capillary electrophoresis (CE)..............................44
5.1.1 CE general 44
5.1.2 Capillary electrophoresis studies of pectins 45
5.2 Microrheology 47
5.2.1 MPT 48
5.2.2 DWS 49
5.3 Surface Plasmon Resonance 50
6 AIMS OF THE THESIS 51
REFERENCES 53

CHAPTER 2..67

PECTIN FINE STRUCTURE: MEASUREMENTS OF THE INTERMOLECULAR CHARGE DISTRIBUTIONS FOR PECTIN WITH RANDOM AND BLOCKY PATTERNS OF METHYLESTERIFICATION 67

1 INTRODUCTION..68
2 EXPERIMENTAL SECTION 70
2.1 Samples 70
2.2 Capillary electrophoresis..73
3 RESULTS AND DISCUSSION...74
3.1 Electrophoretic mobility measurement 74
3.1.1 Comparison of mobility measurements with literature 75
3.1.2 Mobility of RGI compared with HG 76
3.1.3 Mobility of HGs compared with pectins 76
3.1.4 Calculation of the dependence of mobility on charge density...77
3.2 Intermolecular Distributions for Randomly Demethylesterified Monodisperse Homogalacturonans..79
3.3 Intermolecular and intramolecular distributions for blocky pectin 83
3.3.1 Influence of the DM decrease on the methylester group intermolecular distribution...................84
3.3.2 Digest pattern by PGII 86
3.3.3 PGII digestibility of the substrates as a function of the DM decrease induced by PME 87
CONCLUSION 92
REFERENCES..93

CHAPTER 3 .... **97**

DIFFUSING WAVE SPECTROSCOPY INVESTIGATIONS OF ACID MILK GELS CONTAINING PECTIN **97**

ABSTRACT 98
1 INTRODUCTION 98
2 MATERIALS AND METHODS 101
2.1 Materials *101*
2.1.1 Acid milk gel preparation 101
2.2 Methods *102*
2.2.1 Diffusing wave spectroscopy 102
2.2.2 Bulk rheology 104
2.2.3 Confocal microscopy 104
2.2.4 ^{31}P NMR 105
3 RESULTS AND DISCUSSION 105
3.1 Acid milk gel *105*
3.2 Addition of pectin *110*
4 CONCLUSIONS 119
ACKNOWLEDGEMENT 120
REFERENCES 121

CHAPTER 4 .... **125**

MULTIPLE PARTICLE TRACKING INVESTIGATIONS OF ACID MILK GELS USING ADDED TRACER PARTICLES WITH DESIGNED SURFACE CHEMISTRIES; AND COMPARISONS WITH DIFFUSING WAVE SPECTROSCOPY STUDIES **125**

ABSTRACT 126
1 INTRODUCTION 127
2 MATERIALS AND METHODS 129
2.1 Acid milk gel preparation *129*
2.2 Modification of the beads surface chemistry *130*
2.2.1 Coatings 130
2.3 Methods *132*
2.3.1 DWS 133
2.3.2 MPT 134
2.3.3 Confocal microscopy 135
3 RESULTS 135
3.1 MPT carried out in an acid milk gel with uncoated probes *136*
3.2 MPT carried out in an acid milk gel with κ-casein coated probes *138*
3.3 Comparison of the MPT measured ensemble-MSDs for bare and κ-casein coated probes 140
3.4 MPT carried out in an acid milk gel with PEG coated probes *141*

3.5 Comparison between MPT and DWS methodologies ... *142*
3.6 Effect of pectin on the casein network ... *144*
4 CONCLUSIONS ... 146
ACKNOWLEDGMENTS ... 147
REFERENCES ... 148

CHAPTER 5 ... 151

DIRECT MEASUREMENTS OF INTERFACIAL INTERACTIONS BETWEEN PECTIN AND κ-CASEIN AND IMPLICATIONS FOR THE STABILIZATION OF CALCIUM-FREE CASEIN MICELLE MIMICS ... 151

ABSTRACT ... 152
1 INTRODUCTION ... 153
2 MATERIALS AND METHODS ... 155
2.1 Materials ... *155*
2.1.1 Control of surface chemistry ... 155
Covalent immobilization of κ-casein onto an SPR sensor chip ... 155
Passive adsorption of κ-casein onto the surface of latex beads ... 156
2.1.2 Sample preparation and interaction study ... 156
2.2 Methods ... *158*
2.2.1 SPR ... 158
2.2.2 DWS ... 158
2.2.3 Microscopy ... 160
3 RESULTS AND DISCUSSION ... 160
3.1 Interaction of κ-casein with pectin studied by SPR ... *160*
3.1.1 State of the immobilized κ-casein on the sensor chip surface ... 160
Comparison with the conformation on the casein micelle surface ... 160
Effect of pH on the immobilized κ-casein conformation ... 162
3.1.2 Effect of pectin fine structure on the interaction ... 164
3.2 Interaction of "model casein micelles" with pectin ... *168*
3.2.1 κ-casein coated probes versus naked particles in water ... 169
3.2.2 κ-casein coated probes versus naked particles in HM pectin solutions ... 172
3.2.3 Effect of pectin fine structure on the interaction ... 177
4 CONCLUSION ... 181
ACKNOWLEDGMENTS ... 183
REFERENCES ... 183

CONCLUSION AND FURTHER WORK ... 187

1 SUMMARY ... 187
2 MAIN CONCLUSIONS AND FURTHER SUGGESTED WORK ... 188

Chapter 1

Background