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

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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 methoxy 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 methoxy 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 ‘famille’, 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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPT</td>
<td>Multiple Particle Tracking</td>
</tr>
<tr>
<td>DWS</td>
<td>Diffusive Wave Spectroscopy</td>
</tr>
<tr>
<td>HG</td>
<td>Homogalacturonan</td>
</tr>
<tr>
<td>DM</td>
<td>Degree of Methylesterification</td>
</tr>
<tr>
<td>HM pectin</td>
<td>High-Methoxy pectin</td>
</tr>
<tr>
<td>LM pectin</td>
<td>Low-Methoxy pectin</td>
</tr>
<tr>
<td>DB</td>
<td>Degree of Blockiness</td>
</tr>
<tr>
<td>RGI</td>
<td>RhamnoGalacturonan I</td>
</tr>
<tr>
<td>RGII</td>
<td>RhamnoGalacturonan I</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>PME</td>
<td>Pectin Methyl-Esterase</td>
</tr>
<tr>
<td>f-PME</td>
<td>Fungal Pectin Methyl-Esterase</td>
</tr>
<tr>
<td>p-PME</td>
<td>Plant Pectin Methyl-Esterase</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PL</td>
<td>Pectin Lyase</td>
</tr>
<tr>
<td>PG</td>
<td>PolyGalacturonase</td>
</tr>
<tr>
<td>Mw</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>PGA</td>
<td>PolyGalacturonic Acid</td>
</tr>
<tr>
<td>$G'(\omega)$</td>
<td>Elastic modulus</td>
</tr>
<tr>
<td>$G''(\omega)$</td>
<td>Viscous modulus</td>
</tr>
<tr>
<td>$f$</td>
<td>Frequencies</td>
</tr>
<tr>
<td>$k$</td>
<td>Boltzmann constant</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
</tr>
<tr>
<td>$&lt;r_2(\tau)&gt;$</td>
<td>Mean Square Displacement</td>
</tr>
</tbody>
</table>
PEG  PolyEthylene Glycol
\( \tau \)  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-\( \delta \)-lactone
pC  Critical pH
a  Radius of particle
LMA pectin  Low Methoxyl Amidated pectin
CE  Capillary Electrophoresis
HG  Homogalacturonan
Dabs  Absolute Degree of blockiness
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