Interaction of chitosan oligomers with whey proteins – production of stable nano- and micro-particles

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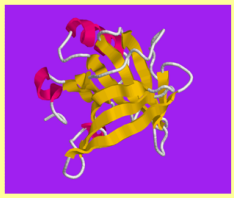
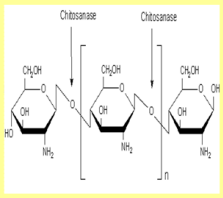
Introduction

Stable micro- and nanometre sized particles can be used as carriers to protect sensitive compounds from detrimental effects during processing or storage. The milk protein, β-lactoglobulin (β-lg) has electro-negative hydrophilic domains at pH values > 5.2 and an excess of positive charges below pH 5.2. Unlike β -lg, chitosan has positively charged hydrophilic domains at pH values below its pKa of ~ 6.5. In aqueous solution, the properties of chitosan depend on its molecular weight, degree of deacetylation, as well as on the pH and ionic strength of the solution. Previous studies have shown that chitosan can interact with proteins to form complexes. The objective of this work was to examine the effect of pH on the interaction between two polymers: (i) the dairy protein (β-lg) and (ii) oligomers of the polysaccharide chitosan (Mw of 3.9 to 1,000kDa) or its basic unit glucosamine (Mw = 215.6Da).



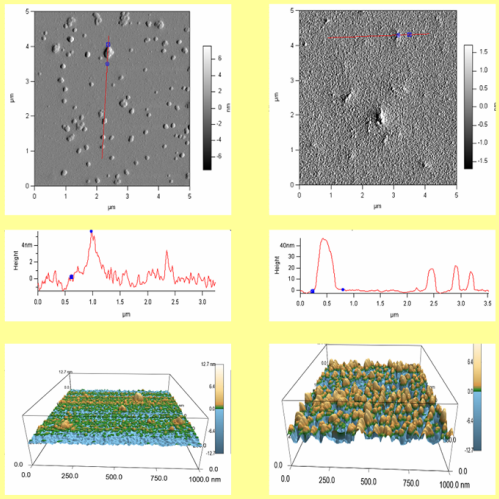


β -lg (left) and Chitosan: polymer of b-(1-4)-D-glucosamine units

MATERIALS & METHODS

β-Lg-enriched powder was prepared in Moorepark Food Research Centre. Glucosamine was obtained from Sigma Aldrich. 22 or 1000 kDa chitosan was obtained from Marinard Biotech . Chitosan oligomers were obtained from the University of Agriculture (Poland). Mixtures of 1% (w/w) β-lg and 0.1% (w/w) glucosamine or chitosan in water were prepared in the pH range 4.0 – 8.0 and at 22°C.

Viscosity measurements (mPa s) of dispersions was measured using a Vibro Viscometer (A & D Co. Ltd. Tokyo, Japan) at a frequency of 30 Hz.
Particle size analysis was performed at scattering angle of 12° (Zetasizer Nano system)
Zeta potential measurements (in mV) were determined using laser doppler electrophoresis (Nano-S Zetasizer, Malvern Instruments) An applied potential of 150 V and a modulation frequency of 250 Hz were used.
Insoluble protein levels (%) were determined after centrifugation at 14,000 × g for 10 min. Levels of insoluble protein were calculated by subtracting the concentration of β-lg in the supernatant from the concentration in a reference sample of β -lg (non-centrifuged).
Atomic force microscopy (AFM) AFM images were taken on air-dried samples using a MFP-3D atomic force microscope.



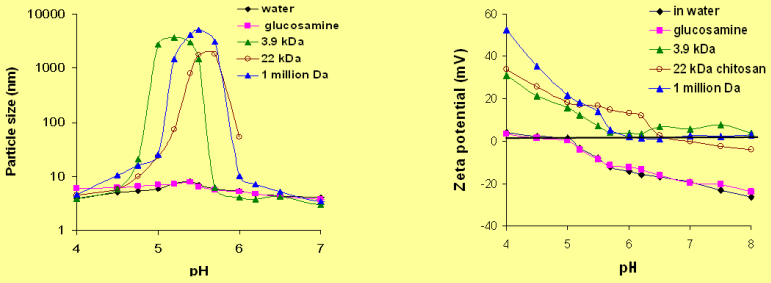
AFM image 1% β-lg and 0.1% 22 kDa chitosan at pH 4.0 and 5.0

The AFM image shows the formation of larger particles of 20 – 40 nm in diameter, which were not evident at pH 4.0.

RESULTS & DISCUSSION

Particle size/ zeta potential

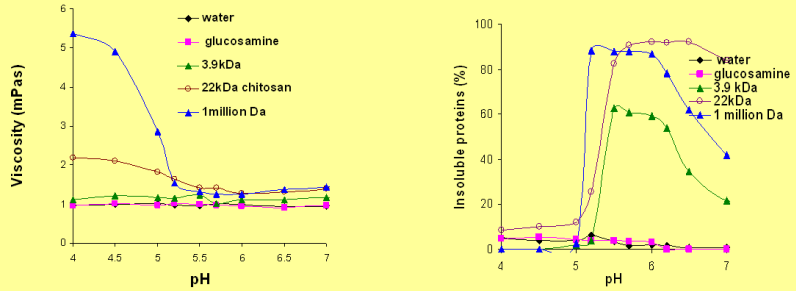
In the presence of 0.1% glucosamine, the particle size (3-7 nm depending on pH) and zeta potential (~ +25 mV at pH 3.0 to ~ -25 mV at pH 8.0) of 1% β -lg was unchanged over the pH range, which indicated little protein-glucosamine interactions.
In contrast, the particle size of 1% β-lg / 0.1% chitosan mixtures was significantly increased at pH values ≥ 5.0. The particle size of these dispersions increased to ~ 900 nm and the zeta potential remained positive (+ 17.5 mV) at pH 5.6. Precipitation occurred at higher pH values. These results are indicative of interaction between the protein and chitosan at pH values ≥ 5.0.



Particle size and Zeta Potential of 1% β -lg or mixtures of 1% β -lg and 0.1% glucosamine/chitosan oligomers

Viscosity

The viscosity of β-lg and mixtures of β-lg and glucosamine was low (~1mPas) throughout the pH range. In contrast the mixtures of β-lg and chitosan oligomers, in the pH range 4-5 inclusion of the larger Mw chitosan oligomers resulted in the highest viscosities (up to 5mPas). With pH increases to 5 or higher the viscosities of the mixtures containing the larger Mw chitosan oligomers dropped indicating interaction with the β-lg.



Viscosity (mPas) and Insoluble Proteins (%) of 1% b-lg or mixtures of 1% b-lg and 0.1% glucosamine/chitosan oligomers

Insoluble protein levels (%)

No significant change in insoluble protein levels were observed for β-lg. Complexes of β -lg with 22kDa and 1 million Da chitosan were formed at pH values ≥ 5.0, with maximum complexation observed at pH 6.2 (~85%). With further increases in pH above 6.5 for β -lg dispersed with chitosan, the amount of insoluble protein was reduced to 20-50% at pH 8.0.

CONCLUSIONS

The results indicate that no interaction occurs between β -lg and the basic unit glucosamine. In contrast, complex interactions were seen with different Mw chitosan and β -lg where they formed particles in the pH range 5.0-6.2.
Future work will involve the use of these novel protein polysaccharide particles as encapsulating materials and texturising agents in foods.

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