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Acid-induced gelation of milk protein concentrates with added pectin: Effect of casein micelle dissociation

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Abstract

The dynamics of the formation of the acid gel network for mixtures of milk protein concentrate (MPC) and low methoxyl amidated (LMA) pectin were studied using rheological measurements. The results as a function of pectin content and casein micelle integrity, from neutral pH to approximately pH 4.2, together with the microstructural changes observed in some of these systems, are presented.

The gelation profiles of a mixture of 4% w/v MPC and LMA pectin (0–0.075% w/v) after the addition of 1.2% w/v glucono-δ-lactone showed a gradual decrease in the shear modulus with the incorporation of pectin. The effects of casein micelle integrity on casein–pectin interactions were studied, by preparing MPC dispersions containing various levels of micellar casein. A gradual change in the shear modulus, from a disrupting effect of pectin added to MPC, in which the casein micelles are intact, to a clear synergistic effect of pectin added to dissociated casein systems, was found in the acid-induced milk gels.

Keywords: Acid milk gels; Pectin; Milk protein concentrates; Rheology; Calcium; CSLM

1. Introduction

The rheology and the structure of milk protein gels have been the subject of many studies during the last decade, as recently reviewed by a number of authors (Lucey, 2002; van Vliet, Lakemond, & Visschers, 2004). Whereas the aggregation and gelation of casein micelles as a result of acidification have been studied frequently (Lucey & Singh, 2003), the presence of hydrocolloids during the acid-induced gelation presents another degree of complexity of milk protein systems that needs to be further explored. The kinetics and the dynamics of the formation of casein gels can be controlled by varying the amount of glucono-δ-lactone (GDL) to produce different rates of acidification (Horne, 2001). The first stages of acidification are critical for the development of the network and the aggregation process appears to be more complicated than simply reaching a critical pH for the collapse of the hairy κ-casein layer on the micelles, as believed until recently (De Kruif, 1997).

Pectin is an anionic carboxylated polysaccharide that is frequently used in the food industry. The proportion of carboxyl groups esterified with methanol and with acid amide units and the ester distribution along the polymer determine the functionality of pectin (May, 2000; Morris, 1998; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Pectin is widely used in many dairy products as a gelling/thickening agent (acid and non-acid milk desserts) and as a stabilizer ingredient (acid milk drinks, milk/juice blends). In particular, the gelation of low methoxyl pectin, over a wide range of pH and solids content, is mainly the result of strong interactions between calcium ions and blocks of galacturonic acid (Braccini & Perez, 2001).

Previous rheological and microstructural studies have shown that low methoxyl amidated (LMA) pectin is a challenging polysaccharide to investigate when it is present during the acidification of casein-based systems (Matia-Merino, Lau, & Dickinson, 2004). The fact that (1) ionic calcium is released into the serum phase during the

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acidification of casein micelles (Law & Leaver, 1998; Singh, Roberts, Munro, & Teo, 1996), creating favourable gelling conditions for the pectin, and (2) adsorption of pectin around the casein particles through electrostatic interaction seems to occur at or below pH 5.0 (Tuinier, Rolin, & de Kruif, 2002) makes casein micelles and pectin an interesting biopolymer mixture that could result in different gel networks upon acidification.

Through its role in the ionization of the protein and pectin molecules, pH is the most significant factor affecting the electrostatic protein–pectin interactions. At neutral pH, where both casein micelles and pectin carry negative charges, the interaction is minimal (Oakenfull & Scott, 1998) and the non-adsorbing pectin induces depletion flocculation above a certain critical concentration. At a lower pH, when the pectin is adsorbed onto the casein micelles, the stability of the system may vary. Depending upon the amount of pectin, bridging flocculation and depletion flocculation can also induce phase separation of a casein and pectin mixture (Maroziene & de Kruif, 2000; Syrbe, Bauer, & Klostermeyer, 1998).

In general, the relative concentration of a biopolymer mixture is crucial for a subsequent gelation process because the relative kinetics of phase separation and gelation will determine the morphology of a mixed gel (Morris, 1990; Tolstoguzov, 1990). This can be seen specifically for LMA pectin added to caseinate-stabilized emulsion systems prior to acidification (Matia-Merino & Dickinson, 2004).

It has been established that casein micelles may dissociate to various degrees when the pH of milk is decreased. The combined effect of low temperature and low pH is more than additive in causing dissociation of casein and solubilization of the colloidal calcium phosphate (CCP) from the casein micelles (Anema & Klostermeyer, 1997; Dalgleish & Law, 1988; Law & Leaver, 1998; Le Graet & Gaucheron, 1999; Singh et al., 1996; Udabage, McKinnon, & Augustin, 2000). CCP can be removed from milk in a controlled manner by using an acidification–dialysis technique (Pyne & McGann, 1960) that involves the acidification of cold milk (5 °C) to pH values in the range 4.8–6.7, followed by equilibrium dialysis against excess skim milk. This results in milks that are reduced in CCP content (and consequently that have different levels of micellar casein), but otherwise are identical in composition to skim milk. The impact of these changes in casein micelle structure and CCP on the interactions of casein micelles with LMA pectin is unknown.

In this paper, we report on the effect of casein micelle integrity, prior to acidification, on casein–pectin interactions. The dynamics of the formation of the acid gel network, along with the microstructural changes, were studied through rheological measurements of mixtures of milk protein concentrate (MPC) and LMA pectin as a function of pectin content and casein micelle integrity in MPC dispersions, from neutral pH to approximately pH 4.2.

2. Materials and methods

2.1. Materials

A freshly made batch of MPC powder was obtained from Fonterra Co-operative Group Ltd, New Zealand. The typical composition of the MPC (% w/w) was: 82% total protein (71.3% casein, 10.9% whey protein), 1.5% fat, 2.3% lactose, 6.6% moisture and 7.0% ash (21.7 g calcium/kg, 13.4 g phosphorus/kg, 0.57 g sodium/kg and 0.95 g magnesium/kg). The pectin, GRINDSTED® Pectin LA 410, was provided by Danisco; it is a low ester amidated pectin (approximately 31% degree of esterification and approximately 19% degree of amidation) with high calcium reactivity and is standardized with sugars. GDL and Rhodamine B were obtained from Sigma Chemical Co. Ltd (St. Louis, MO, USA).

2.2. Preparation of reconstituted MPC and compositional analysis

Reconstituted MPC samples were prepared by dissolving MPC powder in Milli-Q water (Millipore Corp., Bedford, MA, USA) with continuous stirring at 60–62 °C for 2 h. Sodium azide (0.02%) was added to the milk dispersion as a preservative. The samples were stirred overnight before use. Under these conditions, MPC was completely soluble in water and no denaturation of whey proteins was observed.

The calcium levels in the milk powder samples, milk dispersions and serum phases were determined by inductively coupled plasma emission spectroscopy (Alkanani, Friel, Jackson, & Longerich, 1994). The total protein, casein and whey protein contents were calculated from the total nitrogen, non-casein nitrogen and non-protein nitrogen contents using methods described previously (Hill, 1993).

2.3. Adjustment of pH, dialysis and ultracentrifugation

MPC samples (10% w/v) were cooled to approximately 8 °C and adjusted to pH 5.6 ± 0.005 or pH 5.1 ± 0.005 by the slow addition of 1 M/0.1 M HCl under vigorous stirring. A stable pH reading was achieved after approximately 2 h. Sub-samples (100 ml) were then dialysed against reconstituted MPC (20 times volume, three changes, 36–48 h) to restore the soluble mineral components and the pH to levels similar to those in the original MPC dispersion (Pyne & McGann, 1960). After dialysis, all samples were allowed to equilibrate at room temperature and the pH was adjusted to 7.0 by the slow addition of 2 M NaOH. Particle size, protein and mineral content and rheological measurements were carried out on these samples.

Dialysed MPC samples (or the original MPC dispersions) were also subjected to ultracentrifugation (100,000 *g*, for 1 h at 20 °C) in a Beckman L8-80 M ultracentrifuge,