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Phase behaviour, rheological properties, and microstructure of oat β-glucan-milk mixtures

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ABSTRACT

Incorporation of high molecular weight oat β-glucan into milk to obtain calorie-reduced and cholesterol-lowering dairy products is challenged by the thermodynamic incompatibility and phase separation of milk proteins and β-glucan. Fine tuning the concentrations of the biopolymers in the mixture may result in dairy matrices with unique structure and texture. The thermodynamic incompatibility behaviour may follow Asakura and Oosawa (1958) theory for depletion interactions between non-interacting polysaccharides and colloidal hard spheres. A phase behaviour diagram was developed to describe the separation of the mixtures into a protein-rich (lower) and a β-glucan-rich (upper) phase, but unlike previous literature, the concentration of lactose and ions in the soluble phase was kept constant. Analysis of the protein composition in the lower phase demonstrated that whey proteins did not play a role in phase separation. The experimental phase boundary agreed well with the phase boundary calculated using Vrij's theory (1976) for mixtures of β-glucan and casein micelles demonstrating that phase separation was driven by depletion interactions. Confocal images showed the formation of different structures (droplet-like or bi-continuous) at different points of the phase behaviour diagram. The flow behaviour of the mixtures with concentrations higher than the binodal curve was not only governed by the presence of β-glucan chains, but also by the formation of these structures.

1. Introduction

The current need for carefully designed diets able to reduce blood glucose and serum cholesterol has resulted in an increased demand for foods containing soluble dietary fibre. Oat β-glucans are of particular interest, as they have been shown to modulate postprandial glycaemic response and attenuate serum cholesterol levels in patients with hypercholesterolaemia (Wood, 2007). The consumption of β-glucan has been associated with a reduction in glucose absorption rate (Tosh, 2013) and increase in excretion of cholesterol and cholesterol metabolites (predominantly bile acids) (Theuwissen & Mensink, 2007). These beneficial effects are caused by the ability of β-glucan to develop high viscosity in the gastrointestinal tract (Lazaridou & Biliaderis, 2007; Tosh, Brummer, Wolever, & Wood, 2008; Wolever et al., 2010).

The use of β-glucan in dairy matrices as a health promoting ingredient has been under investigation for more than a decade (Konuklar, Inglett, Warner, & Carriere, 2004; Lazaridou, Vaikousi, & Biliaderis, 2008; Schmidt, 2007; Tudorica, Jones, Kuri, & Brennan, 2004). The thermodynamic incompatibility between milk proteins and β-glucan (Lazaridou et al., 2008; Volikakis, Biliaderis, Vamvakas, & Zerfiridis, 2004) influences stability, texture and quality of the dairy products containing this polysaccharide. Indeed, mixtures containing a sufficient amount of β-glucan required for a health claim would show undesirable appearance and texture because of the incompatibility between the polymers. In Canada and the USA, the concentration required for the health claim is 0.75 g per serving (see for example, FDA, 1997). To date, despite its consumption being associated with many health benefits, β-glucan is only used as a fat replacer and prebiotic agent in calorie-reduced yoghurt and cheese products, but not in concentrations sufficient to be nutritionally significant (Bekers et al., 2001; Konuklar et al., 2004).

Studies on the phase behaviour of whey protein isolate or sodium-caseinate with β-glucan have demonstrated limited compatibility between these biopolymers (Kontogiorgos, Ritzoulis, Biliaderis, & Kasapis, 2006; Kontogiorgos, Tosh, & Wood, 2009). However, no research is available on the effect of β-glucan addition

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to milk systems, where the original ionic conditions are maintained. Such studies would lead to a better understanding of the principles behind the instability of the mixture under conditions relevant to the development of dairy products.

Phase separation in mixtures of casein micelles and non-interacting polysaccharides are driven by depletion interactions, and theoretical phase boundaries at the spinodal region have been related to the experimental phase boundary obtained after complete separation of protein and polysaccharide into two phases (Asakura & Oosawa, 1958; de Bont, van Kempen, & Vreeker, 2002; Tuinier & de Kruif, 1999). The spinodal region is located in the range of protein and polysaccharide where the change in osmotic compressibility is equal to zero. The present work tested the applicability of this model to β-glucan-milk systems, whereby the ionic environment and the lactose concentration were maintained constant. Vrij's thermodynamic theory (Vrij, 1976) was used to measure the changes in osmotic pressure close to the surface of the casein micelles in the presence of non interacting polymers. In the colloidal limit, where the radius of the particles is much larger than the radius of the polymer, the concentration of polymer in the binodal region (the limit where phase separation will occur) is below the coil overlap concentration and the depletion thickness is close to the radius of gyration of the polymer (polymer overlap results in a larger depletion thickness around particles) (Fleer, Scheutjens, & Vincent, 1984). Assuming that at the spinodal region, which is located quite closely to the binodal region, the change in osmotic compressibility is equal to zero, it is possible to derive the volume fraction of colloidal particles after phase separation. This allows development of a theoretical phase boundary and determination of the critical concentration of β-glucan that causes macroscopic phase separation. The expressions of Vrij's theory (Vrij, 1976) for depletion interactions can be employed to obtain the theoretical phase separation boundaries (de Bont et al., 2002; Tuinier & de Kruif, 1999). The agreement with theoretical prediction and experimental data will demonstrate that depletion interactions are the driving force for phase separation in the mixture (de Bont et al., 2002; Tuinier & de Kruif, 1999).

The present research aimed at developing a phase behaviour diagram of β-glucan and milk proteins in unheated skim milk. In addition, the microstructures formed with mixing, and their effect on the rheological properties of the mixtures with varying concentration of the two polymers, were investigated. Unlike previous reports, where the interactions between milk proteins and β-glucan were studied using isolated proteins or reconstituted skim milk powder (see for example, Lazaridou & Biliaderis, 2009), in the present work phase separation behaviour was observed under conditions as close as possible to those present in untreated skim milk, with minimal changes to serum composition. It is well established that the ionic environment of the casein micelles has to be maintained to obtain true information on their structure function behaviour (Dalgleish, Horne, & Law, 1989), and that phase separation of protein polysaccharide systems is affected by the concentration of sugar present (Schorch, Jones, & Norton, 1999; Spyropoulos, Portsch, & Norton, 2010). To preserve the integrity of the casein micelles and maintaining the serum composition constant, the protein concentration was adjusted by diluting skim milk concentrated by membrane filtration in its own serum phase, the permeate fraction obtained from filtration.

2. Material and methods

2.1. Milk, permeate and β-glucan stock preparation

Fresh milk was collected from the research station at the University of Guelph (Ponsonby Research Station, Ontario, CA), and after addition of sodium azide (0.02% w/v) (Fisher Scientific, Mississauga, ON, Canada) it was skimmed by centrifugation at 6000 g for 25 min at 4 °C (model J2-21, Beckman Coulter, Mississauga, ON, Canada). The residual fat globules were then removed by filtering four times through glass fibre filters (Whatman, Fisher Sci.). A working batch of fresh, four times concentrated milk (4 ×, based on volume reduction) was prepared, using a laboratory scale (0.1 m^2) ultrafiltration unit equipped with regenerated cellulose cartridge (PLGC, 10 kDa nominal cut-off, Millipore Corp., Bedford, MA, USA). Permeate (the serum transmitted through the ultrafiltration membrane) was used to prepare the β-glucan stock solution and to adjust milk protein concentrations. By using a batch of concentrated milk and re-diluting it with its own serum, it was possible to effectively maintain the ionic composition amongst all the mixtures at the various casein micelle volume fractions.

High molecular weight β-glucan was isolated from oat bran produced at the POS Pilot Plant (Saskatoon, SK, Canada) as previously described (Wood, Weisz, Fedec, & Burrows, 1989). In brief, β-glucan was extracted from dehulled oat bran at pH 10 following two precipitation steps with 20% w/v ammonium sulphate and two precipitation steps with 50% w/v, 2-propanol. The isolate contained about 80% (dry matter) β-glucan, about 4% w/w protein, 10% w/w moisture, and the residual was mainly composed of starch and pentosans (Wood et al., 1989). A stock solution of the high molecular weight β-glucan (1.2%w/v) was prepared by dispersing it at 90 °C for 3 h in permeate (higher concentrations would result in non homogeneous dispersions). To ensure the complete solubilisation of β-glucan in permeate, the β-glucan concentration was measured using a flow injection analysis unit (FIAstar 5010 Analyzer, Foss Analytical, Hillerod, Denmark) equipped with a fluorescent detector (calcoflour white M2R New, C.I., 40622 fluorescent brightener 28, American Cyanamide Co., Bound Brook, NJ, USA) (Tosh et al., 2010). The concentration of β-glucan was determined using a standard curve built with pure β-glucan (Megazyme, Bray Co. Wicklow, Ireland).

To be able to predict and compare experimental and theoretical phase boundaries, the radius of gyration of β-glucan and its molecular weight were determined by high performance size exclusion chromatography (Shimadzu SCL-10A vp, Shimadzu Scientific Instruments Inc, Columbia, MD, USA), equipped with light scattering, refractive index, and viscometric detectors in series (Viscotek, Houston, TX, USA) as previously reported (Tosh, Wood, Wang, & Weisz, 2004). Pullulan (Shodex Std. P-82, Showa Denko K.K. Kawasaki, Japan) was used as standard.

Aliquots of β-glucan (2–4 mg) were solubilised in water at 90 °C at a concentration of 0.1 mg/mL. The samples were passed through a Shodex Ohpak Kb 806M column (Showa Denko K.K., Tokyo, Japan), followed by an Ultrahydrogel linear column (Waters, Milford, CT, USA) in 100 mM $NaNO_3$ buffer containing 5 mM NaN_3, and the flow rate of 0.6 mL/min at 40 °C. Molecular weight and intrinsic viscosity were determined using the TriSEC3.0 software (Viscotek) using a refractive index increment of 0.146 mL/g (Tosh et al., 2004).

2.2. Construction of the phase diagram

Samples were prepared in 15 mL polypropylene centrifuge tubes by mixing appropriate volume ratios of 4 × concentrated skim milk and 1.2% β-glucan dispersed in permeate. After 18 h under quiescent storage at room temperature (25 °C), the volume of each separated phase was recorded, and the concentrations of protein and β-glucan quantified in the two phases. Protein content was determined (Leco, FP528, St. Joseph, MI, USA) using a conversion factor of 6.38. The β-glucan concentration was measured using the specific fluorescence reaction with calcofluor as described above.