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# Phase behaviour of casein micelles and barley beta-glucan polymer molecules in dietary fibre-enriched dairy systems

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ABSTRACT

Enrichment of colloidal dairy systems with dietary fibre frequently causes quality defects because of phase separation. We investigate phase separation in skimmed milk enriched with Glucagel (a commercial product made from barley that is predominantly comprised of the polysaccharide $\beta$-glucan). The driving force for phase separation was depletion flocculation of casein micelles in the presence of molecules of the polysaccharide. Depending on the volume fraction of casein micelles and the concentration of Glucagel, the stable system phase separated either as a transient gel or as a sedimented system. The rate at which phase separation progressed also depended on the volume fraction of casein micelles and the concentration of Glucagel. To confirm the role of depletion flocculation in the phase separation process, enzymatic reduction in the molecular weight of $\beta$-glucan was shown to limit the range of attraction between micelles and allow the stable phase to exist at a higher $\beta$-glucan concentration for any given volume fraction of casein micelles. These phase diagrams will be useful to dairy product manufacturers striving to improve the nutrient profile of their products while avoiding product quality impairment.



## 1. Introduction

Milk is a classic colloidal system [1,2], behaving as a semi-dilute dispersion of hard spheres (casein micelles), occupying a volume fraction ($\varphi$) of approximately 0.11 in a continuous phase of water, lactose, salts and whey proteins [3,4]. The casein micelles, which are the most abundant (~80%) protein source in normal bovine milk [5], have a mean diameter of 200 nm [6]. Steric stabilisation of the micelles is achieved by surface coverage of the casein micelles with molecules of $\kappa$-casein whose C-terminals extend into the continuous phase. In the relatively high ionic strength of milk, the carboxylic charges are screened by counter-ions, thus the polyelectrolyte brush of the casein micelles has been called a "salted brush" [7].

Given milk's complex colloidal nature, attempts to manipulate the quality of dairy products, either from a nutrient enhancement perspective [8–10] or from a product development perspective [11,12], are frequently stymied by a lack of knowledge of how the colloidal particles interact with ingredients added to milk systems. For example, in the development of a novel dairy product, the dairy liqueur [11], attaining an understanding of how the colloidal nature of casein micelles was affected by ethanol was pivotal to the development of formulations that permitted long-term shelf stability for the liqueur [10,13].

Milk's nutritional benefits have been studied extensively because milk is a source of essential nutrients and energy for mammalian young [14,15]. However, milk is totally devoid of dietary fibre. As a result, research on fibre supplementation of dairy products has been conducted by a number of research groups [9,16–18] in order that dietary reference intake targets for fibre that have been recommended by bodies such as the National Academy of Sciences Research Council might be met in servings of dairy products.

One dairy products sector that is a good candidate for fibre enrichment is the yogurt sector, and fortification of both set and drinkable yogurts has been evaluated [9,16,18]. In yogurt, the stable colloidal dispersion of casein micelles is destabilised by disruption of the salted brush [7,19,20]. The resulting soft solid arises from the formation of a disordered particle gel [21–23]. In the manufacture of yogurt drinks, the particle gel structure of the set yogurt is destroyed mechanically, typically by homogenisation [19,24], and sometimes with the addition of more liquid material [19]. The comminuted gel particulates are colloidally stabilized when added molecules of the charged polysaccharide high-methoxyl pectin adsorb to the casein micelles to provide steric and electrostatic repulsion [25–27].

The mixed-linkage polysaccharide $\beta$-glucan is a favoured molecule for fibre enrichment strategies because of its demonstrated health benefits [28]. However, when $\beta$-glucan has been added to yogurt or drinking yogurts, multiple quality defects were observed [9,18,29]; in particular, extremely high whey separation was evident when threshold concentrations of $\beta$-glucan were exceeded

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(usually less than 1%). The resulting yogurts were unacceptable in quality, both from an appearance and a mouthfeel (texture) perspective [9,18,29].

The aim of this study was to investigate the hypothesis that the appalling quality defects that arise in fibre-enriched yogurts and drinking yogurts are not due to fibre interference with particle gel formation, but are due to prior events occurring in the milk. Specifically, we will determine how milk's native casein micelles, as with other colloidal particles, are destabilized by depletion flocculation in the presence of non-adsorbing polymers [30,31]. In our case, the non-adsorbing polymers are the added dietary fibre molecules of $\beta$-glucan. During depletion flocculation, segregation of the dispersed casein micelles occurs when the concentration of non-adsorbing fibre polymers exceeds a certain threshold [32,33]. The unbalanced osmotic potential leads to solvent exclusion from the overlap volume between the casein micelles, and the flocculated particles precipitate, separating the system into two phases: a bottom phase rich in particles and a top phase rich in polymer. Numerous phase separation scenarios can occur depending on the volume fraction of particles and the concentration of polymer molecules [34–38]. Thus, to test the hypothesis, a systematic study of the time-dependent phase behaviour of fibre-enriched skimmed milk systems was conducted, manipulating the volume fraction of casein micelles, the concentration of $\beta$-glucan (using a commercial dietary fibre product) and the $\beta$-glucan's molecular weight.

## 2. Materials and methods

### 2.1. Materials

A commercial product made from hulless barley, Glucagel (PolyCell Technologies, Crookston, MN), that was determined to be primarily comprised of $\beta$-glucan [39] was used as the source of the $\beta$-glucan polymer. Low heat skim milk powder (SMP) was the source of casein micelles and dairy solids (protein 35.9% (w/w), lactose 52.1% (w/w) and fat 0.66% (w/w)). Lichenase, or endo-(1-3)-(1-4)-$\beta$-D-glucan 4-glucanohydrolase (EC 3.2.1.73) from *Bacillus subtilis*, was acquired from Megazyme (Bray, Ireland). Lichenase contaminants were $\alpha$-amylase at less than 0.0001%. The lichenase (1000 U/mL) was diluted in sodium phosphate buffer (pH 6.5) to achieve a specific activity of 50 U/mL.

### 2.2. Methods

#### 2.2.1. Sample preparation

The Glucagel and SMP were dissolved separately in two equal volumes (500 mL) of deionized water. Various concentrations of Glucagel solutions were prepared by dissolution at 90 °C for 40 min using magnetic stirring [40,41]. The highest concentration of Glucagel ($c_G$) used during this preparation step was 25 g/L. Higher $c_G$ was not used to avoid insoluble agglomerate formation [41]. Solutions were cooled to ~30 °C and their volumes restored to 500 mL with deionized water.

Various volume fractions of casein micelles ($\varphi$) were prepared by reconstitution of different weights of SMP in 500 mL of deionized water at room temperature using magnetic stirring for 2 h. Volume fractions were determined from the volume of skimmed milk solutions of different concentration and using conversion factors previously determined for milk systems [42].

Colloid dispersions and polymer solutions were warmed to 85 °C, and the Glucagel solution was added to the SMP dispersion with stirring for 5 min at 85 °C. After holding at 85 °C for 30 min, the mixture was cooled to 42 °C in a tank with circulating cold water. Heat treatment of milk is employed to enhance the textural characteristics of some dairy products [43,44]. Thus, the fibre-enriched milk systems were subject to manufacturing processes that are relevant for yogurt production [19].

The mean pH of the freshly prepared samples (at 42 °C) was 6.52 ± 0.02, which is close to the pH of natural milk, approximately 6.6 [5]. Samples were supplemented with preservatives (sodium azide (0.1 g, BDH, B30111) and tetracycline (0.125 g, Sigma, T3258-25C)) to restrict growth of microorganisms during storage (standard plate count < 30 cfu/mL at 720 h). Addition of preservatives slightly reduced the pH of the milk (by ~0.01). Samples were poured into four previously sanitized 100 mL graduated cylinders and covered with Parafilm. Cylinders were stored at 4 °C for 720 h (approximate shelf life of yogurt under refrigeration conditions [45]).

#### 2.2.2. Measurements of phase separation

The effect of volume fraction of casein micelles and Glucagel concentration on the phase behaviour of the samples was determined as a function of time. The extent of phase separation was determined by measuring the volume of any emerging top or bottom phase in the graduated cylinder and expressing as a percentage of the total volume [25]. For one given production run of a fibre-enriched milk system, the mean value for the phase-separated volume of the four cylinders was determined, with the mean and standard deviation from two runs representing the final result.

#### 2.2.3. Enzymatic hydrolyses of $\beta$-glucan

Glucagel solutions (25 g/L) were prepared as in Section 2.2.1 and cooled to 20 °C. A 10 μL (0.5 U) aliquot of lichenase was added to 200 mL of the solution and stirred magnetically for 2 min with the end of the stirring period considered as the starting point of the enzymatic incubation time (0 min). After various incubation periods, solutions were removed from the water bath at 20 °C and lichenase was inactivated by immersing the flask in water at 100 °C for 2 h with constant stirring [46]. After enzyme inactivation, solutions were cooled to ~30 °C with magnetic stirring. A total of 11 incubation times was analysed (0–165 min). To attain lower concentrations than the 25 g/L stock solutions, the cooled (~30 °C) enzyme-hydrolysed Glucagel solutions were diluted with deionized water.

#### 2.2.4. Construction of phase diagram of samples with hydrolysed $\beta$-glucan

For fibre samples hydrolysed with lichenase, the effect of Glucagel concentration on the phase behaviour of fibre-enriched milk was determined only for samples with a casein micelle volume fraction (in the final sample) of 0.142. Colloidal systems were prepared as in Section 2.2.1 except that the enzyme-treated Glucagel solution was prepared according to Section 2.2.3, and smaller volumes of the resulting fibre-enriched milk samples were analysed. Smaller samples facilitated preparation of a larger number of treatments, and since the time-dependent evolution of phase separation was not evaluated for the enzyme-treated Glucagel milk systems, the systems were simply divided into one of two categories: stable (no separation during storage for 720 h at 4 °C) or unstable (separated).

## 3. Results and discussion

### 3.1. Phase behaviour

To create the phase diagram for milk supplemented with $\beta$-glucan, a series of samples with $0.062 \leqslant \varphi \leqslant 0.225$ and $2.5 \leqslant c_G \leqslant 12.5$ g/L were prepared and monitored over the course of 720 h storage at 4 °C. Three different types of behaviour were observed: equilibrium type (1), referred to as stable, and two types of