Food Chemistry 138 (2013) 630–637

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phase behaviour of oat β-glucan/sodium caseinate mixtures varying in molecular weight

Jacob K. Agbenorhevi [a], Vassilis Kontogiorgos [a,*], Stefan Kasapis [b]

[a] Department of Chemical and Biological Sciences, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK
[b] School of Applied Sciences, RMIT University, City Campus, Melbourne, Vic 3001, Australia

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 2 September 2012
Accepted 2 October 2012
Available online 12 November 2012

Keywords:
β-Glucan
Phase separation
Mixed systems
Sodium caseinate
Rheology

ABSTRACT

The isothermal phase behaviour at 5 °C of mixtures of sodium caseinate and oat β-glucan isolates varying in molecular weight (MW) was investigated by means of phase diagram construction, rheometry, fluorescence microscopy and electrophoresis. Phase diagrams indicated that the compatibility of the β-glucan/sodium caseinate system increases as β-glucan MW decreases. Images of mixtures taken at various biopolymer concentrations revealed phase separated domains. Results also revealed that at the state of thermodynamic equilibrium, lower MW samples yielded considerable viscosity in the mixture. At equivalent hydrodynamic volume of β-glucan in the mixtures, samples varying in molecular weight exhibited similar flow behaviour. A deviation dependent on the protein concentration was observed for the high MW sample in the concentrated regime due to the size of β-glucan aggregates formed. Results demonstrate that by controlling the structural features of β-glucan in mixtures with sodium caseinate, informed manipulation of rheological properties in these systems can be achieved.

1. Introduction

The phase behaviour of protein–polysaccharide mixtures contributes significantly to the stability, structural, rheological and textural characteristics of food products. It is well established in the literature that phase separation depends on the molecular characteristics of biopolymers. The interplay of parameters such as molecular weight, chain conformation and charge density as well as the mixing conditions (biopolymer concentration and mixing ratio, temperature, cooling rate) and solvent pH and ionic strength will determine the thermodynamics of phase separation (de Kruif & Tuinier, 2001; Doublier, Garnier, Renard, & Sanchez, 2000; Perez, Carrara, Sanchez, Rodríguez Patino, & Santiago, 2009; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Syrbe, Bauer, & Klostermeyer, 1998; Tolstoguzov, 2003; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

Mixed linkage (1→3)(1→4)-β-D-glucans found in cereals (oats, barley, rye and wheat) have received significant consumer and research attention because of their health benefits, including lowering cholesterol levels and glycaemic index response. β-Glucans are linear cell wall homopolysaccharides of consecutively linked (1→4)-β-D-glucosyl units that are separated by single (1→3) bonds. The structure, molecular weight and concentration of β-glucans are known to influence their physical and functional properties in solution or when used as ingredients in various formulated food products. These parameters as well as their physical properties, applications and physiological effects have been discussed previously in detail (Brennan & Cleary, 2005; Lazaridou & Biliaderis, 2007; Lazaridou, Biliaderis, & Izydorczyk, 2003; Wood, 2007).

Approval of oat β-glucans as functional bioactive ingredients has stimulated new product development activity over the years (Brennan & Cleary, 2005). Incorporation of β-glucans into milk or dairy products presents a potential application of this polysaccharide as a delivery method so as to provide the associated health benefits to consumers. However, the required amount ($\geqslant$0.75 g per serving) for health claims, makes it difficult in the development of food formulations as β-glucans exhibit thermodynamic incompatibility when mixed with milk proteins that results in phase separation (Kontogiorgos, Tosh, & Wood, 2009a, 2009b; Lazaridou & Biliaderis, 2009). It is important, therefore, to understand the phase behaviour of β-glucans with food proteins before we attempt to make products with desired rheological properties.

Determination of phase diagrams is often employed to describe the thermodynamic compatibility of mixed biopolymer systems. In our previous study, the phase behaviour of mixtures of β-glucan with whey proteins under different solvent conditions was investigated and it was shown that a decrease of pH from 7.0 to 3.0 and sucrose addition enhanced miscibility (Kontogiorgos et al., 2009b).

The present work, builds on our previous investigation assessing the effect of β-glucan molecular weight in mixtures with casein

* Corresponding author. Tel.: +44 1484 472488; fax: +44 1484 472182.
E-mail address: v.kontogiorgos@hud.ac.uk (V. Kontogiorgos).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.073

proteins. The molecular weight and nature of biopolymers (caseins as opposed to our previous work with whey proteins) is expected to influence the extent of miscibility and concentration levels at which phase separation occurs.

This study, therefore, aims to investigate the phase behaviour and morphology of mixtures of sodium caseinate with β-glucan isolates varying in molecular weight by means of phase diagram construction, rheometry, electrophoresis and fluorescence microscopy.

2. Materials and methods

2.1. Materials and sample preparation

Sodium caseinate (90% w/w protein, 6.1% moisture, 3.7% ash, 0.1% lactose), fluorescent brightener-28, rhodamine B, sodium azide (NaN_3) and trifluoroacetic acid (TFA) were purchased from Sigma–Aldrich (Poole, Dorset, UK).

The initial β-glucan isolate (denoted as OBG) was obtained by aqueous extraction from oat flour and three samples of lower molecular weights (H05, H10 and H15) were produced from the initial isolate by controlled acid hydrolysis and characterised as described previously in detail (Agbenorhevi, Kontogiorgos, Kirby, Morris, & Tosh, 2011). The β-glucan samples, OBG, H05, H10 and H15 had molecular weights of 2800×10^3, 252×10^3, 172×10^3 and 142×10^3 g mol^{-1}, respectively.

Stock dispersions of sodium caseinate (14% w/v) and β-glucans (1.0% w/v OBG, 1.2 % w/v H05, 1.8 % w/v H10, 1.8 % H15) were made using 0.1 M Sorensen's phosphate buffer (pH 7.0) with 0.02% w/v sodium azide as preservative. Sodium caseinate was dispersed under mild heating at 40 °C in the buffer solution whereas β-glucan was dispersed at 90 °C in a sealed vial under continuous stirring for 3 h. The concentration of β-glucan stock solutions differs because of the maximum concentration that could be prepared with each isolate. Stock solutions were subsequently treated and mixed at different volume ratios as described previously (Kontogiorgos et al., 2009b).

2.2. Phase analysis and phase diagram construction

The sodium caseinate/β-glucan mixtures were centrifuged (Alergra X-15R, Beckman Coulter, Inc.) at 3000*g* for 30 min at 5 °C until equilibrium phase separation was reached as evidenced by the constant composition of the phases. The resulting equilibrium phase was analysed for protein and β-glucan content.

The protein content was determined according to the Bradford protein assay (Bradford, 1976). The β-glucan concentration was determined using a modified phenol–sulphuric acid method for phase-diagram construction (Agbenorhevi & Kontogiorgos, 2010). Briefly, 0.2 ml TFA was added in 5 ml of diluted mixtures and vortexed immediately to precipitate the proteins out of solution. The resulting mixture was left to stand for 40 min at room temperature and immediately centrifuged at 6000*g* for 10 min. An aliquot of the supernatant was taken and a phenol–sulphuric acid method was then carried out to determine the total polysaccharide concentration. All mixtures were replicated at least twice and the biopolymer concentration in the phases was analysed in triplicates. This yields six replicates for each of the equilibrium phases analysed and average values are reported.

After the equilibrium phase analysis for biopolymer concentration, values obtained were used to determine the phase diagram. It is important to note that, after centrifugation the resulting mixture was made up of one single liquid phase and a biopolymer precipitate. The liquid layer was analysed for biopolymer concentration and used to determine the phase diagram. It was not possible to determine tie lines, as systems did not yield two distinct liquid phases (a protein and polysaccharide enriched, respectively). GraphPadPrism v.5 (GraphPad Software, San Diego, USA) was used to create the final binodal curve performing non-linear regression on the experimental data by fitting a hyperbolic function of the form $y = 1/x^b$. Statistical significance tests were performed on SPSS (v. 20, IBM SPSS Statistics, USA) at $\alpha = 0.05$.

2.3. Viscosity measurements

The flow behaviour of the mixed systems was determined at 20 °C using a Bohlin Gemini 200HR-nano rotational rheometer (Malvern Instruments, Malvern, UK) equipped with cone-and-plate geometry (55 mm diameter, cone angle 2°). The flow curves were measured at shear rate range of 0.01–1000 s^{-1} on freshly prepared samples. Statistical significance tests were performed on SPSS (v. 20, IBM SPSS Statistics, US) at $\alpha = 0.05$.

2.4. Fluorescence microscopy

Fluorescence microscopy observations were carried out on freshly prepared β-glucan/sodium caseinate mixtures pre-stained with fluorescent brightener-28 (0.01% w/v) and rhodamine B (0.02% w/v) using the procedure and equipment as reported previously (Agbenorhevi & Kontogiorgos, 2010).

2.5. Gel electrophoresis

All chemical reagents (MES SDS buffer kit) and gels (NuPAGE® Novex 12% Bis-Tris Gel 1.0 mm, 10 well) used for SDS–PAGE analysis were purchased from Invitrogen Ltd. (Paisley, UK). Stock solutions of each pure casein subunit (α-, β-, κ-casein), sodium caseinate and the various mixtures of sodium caseinate and β-glucans were prepared using a phosphate buffer pH 7.0 as in all other experiments. Sample solution (5 μl), NuPAGE® LDS sample buffer (5 μl, 4×), NuPAGE® reducing agent (2 μl, 10×) and deionised water (8 μl) were vortex-mixed and heated at 70 °C for 10 min.

Electrophoresis was performed using the XCell *SureLock*™ Mini-Cell (Invitrogen Ltd., Paisley, UK) according to the NuPAGE® Bis-Tris Mini Gel protocol. A continuous buffer system (prepared by adding 50 ml of 20× NuPAGE MES SDS running buffer to 950 ml of deionised water) was used. The various treated samples, standards and the protein molecular weight markers were then loaded (10 μl) into separate wells of the gel and electrophoresis was run for 35 min at 200 V. The Novex® Sharp Protein Standard pre-stained molecular weight marker was used comprising a mix of proteins ranging from 3.5 to 260 kDa. After the electrophoresis was completed, gels were stained using SimplyBlue™ Safe-Stain.

3. Results and discussion

3.1. Phase diagram and morphology of mixed systems

To investigate the concentration levels at which β-glucan of different molecular weight in mixtures with sodium caseinate may exhibit compatibility (co-solubility) or incompatibility (phase-separation), phase diagrams were determined (Fig. 1) following the analytical determination of the biopolymer concentrations in the equilibrium phase at 5 °C. The solid curve represents the binodal, which demarcates the compatible (one phase) from the incompatible (two phase) region. Thus the area below the binodal shows the concentration levels at which the β-glucan/sodium caseinate mixtures are at equilibrium (i.e., stable) whereas the area above the binodal represents the concentration zones where phase separation occurs. Therefore, the results clearly indicate that the

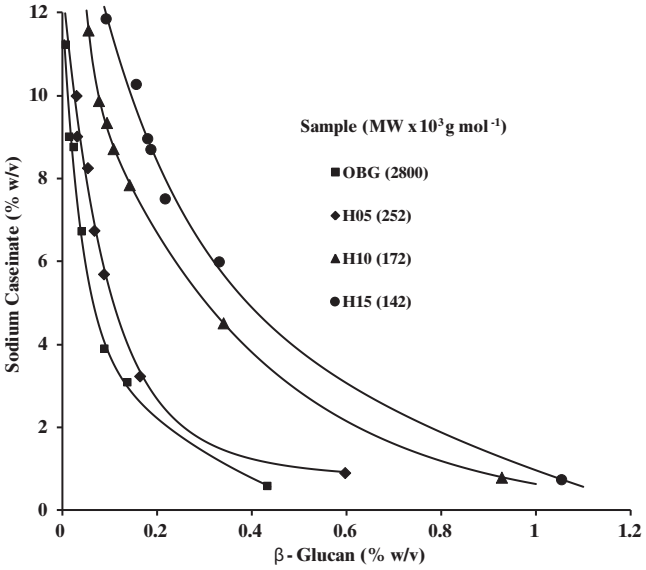

Fig. 1. Phase diagram of binary mixtures of sodium caseinate and β-glucans varying in molecular weight (MW). Solid line represents the binodal, which sets the boundaries of the compatible (below the curve) from the incompatible (above the curve) regimes. Compatibility increases significantly ($p < 0.05$) with decreasing MW.

compatibility region increases significantly as the molecular weight of β-glucan decreases in the order of OBG < H05 < H10 < H15 that shows that the molecular weight of β-glucan samples has a remarkable effect on miscibility with casein proteins.

Polymer thermodynamics allow for qualitative explanation of the observed phase separation in terms of reduced entropy of mixing. The general relationship that describes the free energy of mixing (ΔG_m) is given by (Sperling, 2006):

$$\Delta G_m = \Delta H_m - T\Delta S_m \quad (1)$$

where ΔH_m is the enthalpy of mixing and ΔS_m the entropy of mixing. In the equilibrium phase of the mixtures ΔG_m is negative leading to miscibility whereas in the two-phase regime is positive and the mixtures phase-separate. As all β-glucan samples used here are structurally similar (glucose homopolymers) the energetic component (ΔH_m) of equation 1 should not play dominant role on the free energy at equivalent biopolymer concentrations. Therefore, the phase separation should be attributed to changes in the MW of β-glucans, as is the only parameter that is modified in the mixtures. Increase of the degree of polymerisation reduces the entropy of mixing and the entropic contribution to the free energy ($-T\Delta S_m$) becomes negligible (Rubinstein & Colby, 2003; Sperling, 2006).

Since the experiments were performed at neutral pH, and β-glucan is a neutral polysaccharide, variation in the thermodynamic compatibility with sodium caseinate as a function of molecular weight could be explained by the excluded volume effect. High molecular weight β-glucan yields a large excluded volume, which is inaccessible to sodium caseinate thus reducing the entropy of mixing. In contrast, low molecular weight samples have increased molecular flexibility and reduced hydrodynamic volume thereby creating free volume for the protein molecules even at relatively high β-glucan concentrations, resulting in extensive thermodynamic compatibility. In other words, the smaller molecular size of β-glucan chains in the system gives rise to increased entropy of mixing and consequently enhance the mixing stability (Doublier et al., 2000; Semenova, 2007; Syrbe et al., 1998; Tolstoguzov, 1997). Furthermore, changes in MW influence the morphology of β-glucan (Agbenorhevi et al., 2011) that may also play a significant role on the phase separation behaviour. β-Glucan aggregation may result in density changes leading to further discrepancies from the theory as it is usually assumed to be equal for the polymers in the mixture (Lloyd, 2007). To seek for further evidence on the mechanism of phase separation of the systems in the present work, the

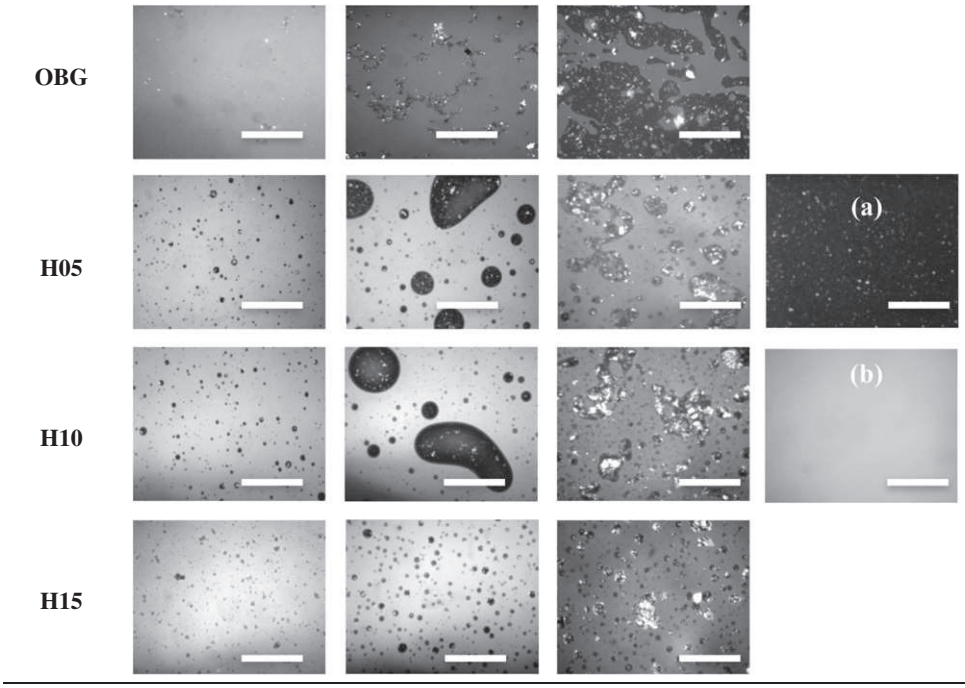

Fig. 2. Typical microstructure of β-glucan/sodium caseinate mixtures at different regions of the phase diagram in the out of equilibrium mixtures (above the binodal). (a) β-glucan only and (b) protein (sodium caseinate) only. Scale bar: 100 μm.

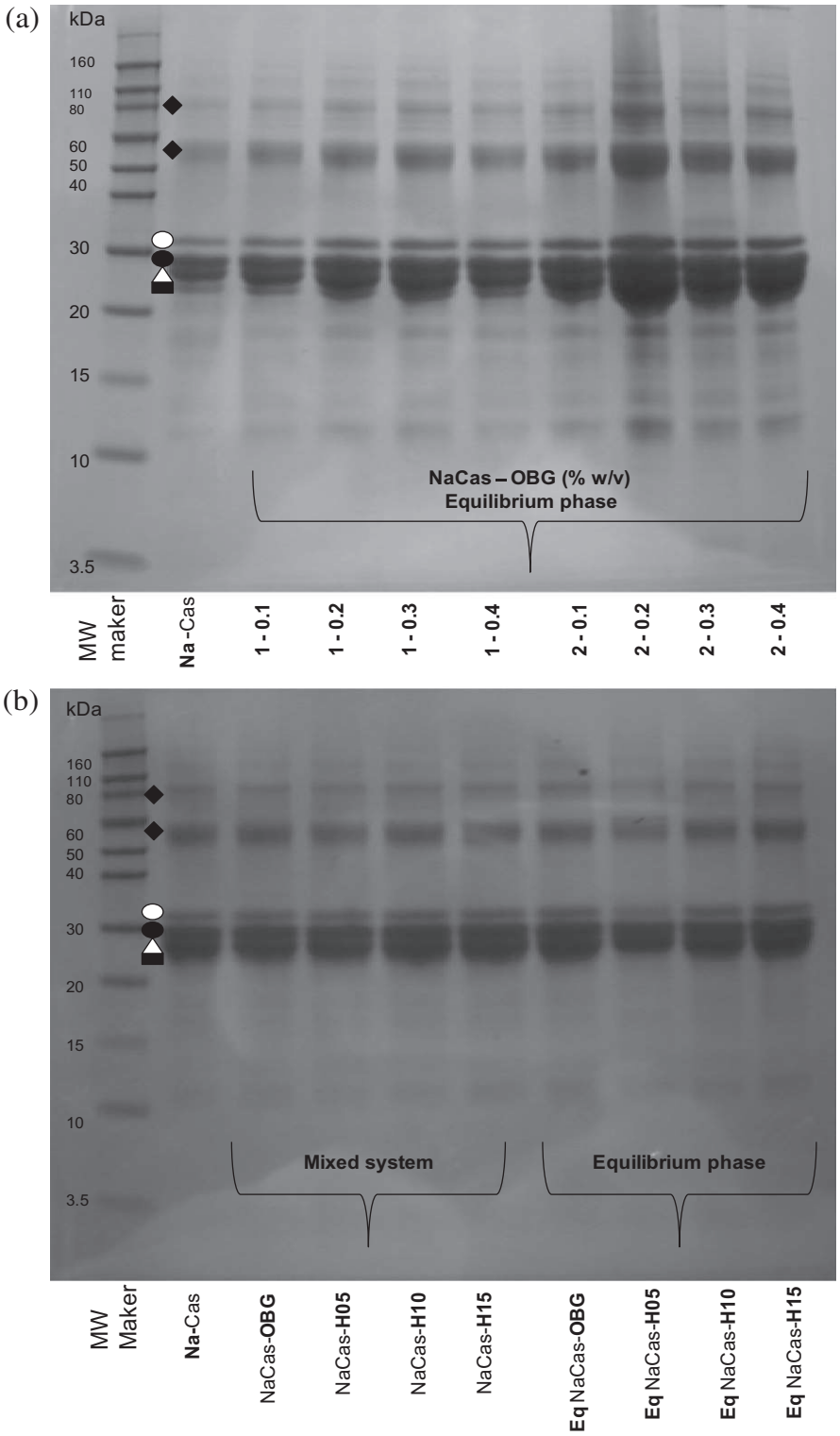

Fig. 3. Typical electrophoretic patterns of sodium caseinate/β-glucan mixtures (a) at different biopolymer concentrations and (b) different molecular weight β-glucan samples and constant protein concentration. Electrophoresis was performed under reducing conditions and NaCas denotes sodium caseinate. The symbols indicate the bands representing the various caseins: ♦ unreduced disulphide linked casein aggregates/contaminants; ○ α_{s2}-casein; ● α_{s1}-casein, △ β-casein; ■ κ-casein.

morphology of the mixtures was probed by fluorescence microscopy.

Single β-glucan solutions exhibited spots of intense fluorescence that were distributed evenly throughout the sample (Fig. 2a). On the other hand, single sodium caseinate solutions did not show any distinct attributes at all concentrations studied (Fig. 2b). Fig. 2 shows images of morphology at various concentration regimes (Fig. 1) typical of phase-separated biopolymer mixtures (Fig. 2). Morphology varied depending on the concentration and molecular weight of the biopolymers in the mixture. In the protein-rich phase, β-glucans appear as droplets whereas in the bicontinuous regime, droplets apparently increase in number espe-

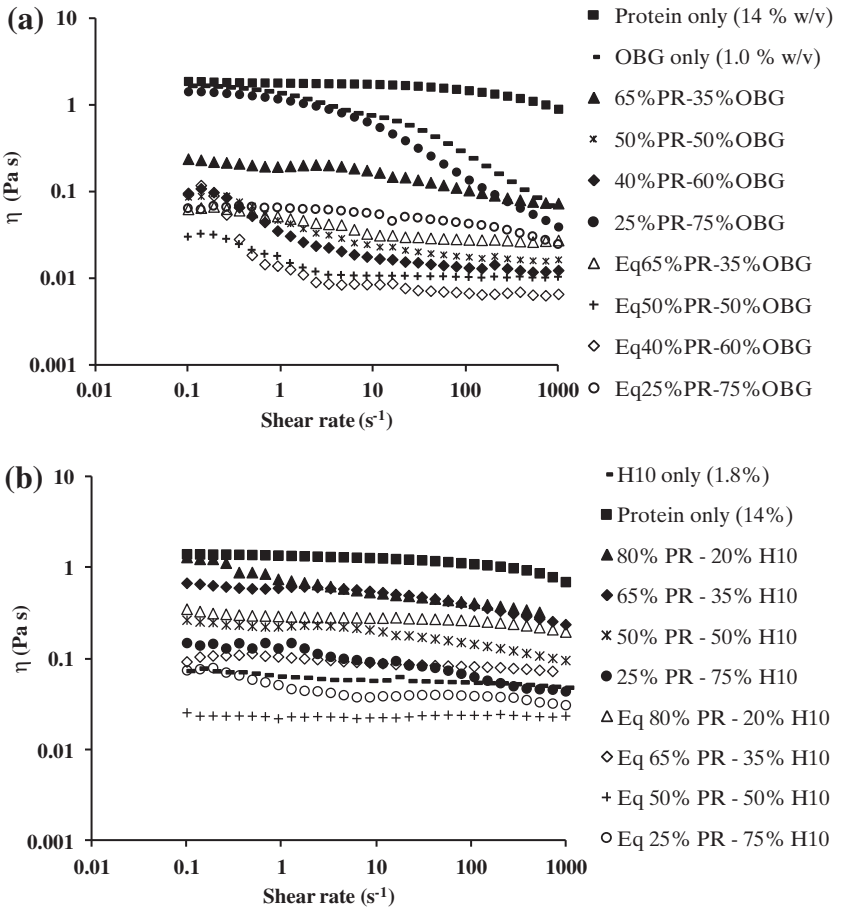

Fig. 4. Flow curve of β-glucan/sodium caseinate mixtures at different biopolymer concentrations: (a) mixtures of OBG and (b) mixtures with H10. Percentage values represent the mixing ratio of the stock solutions. PR: Protein, Eq denotes the mixtures at equilibrium phase. Viscosity of mixtures differs significantly ($p < 0.05$) as a function of biopolymer concentration. Mixture at equilibrium phase had significantly lower viscosity ($p < 0.05$) than that after mixing.

cially for the lower molecular weight samples. In the polysaccharide-rich phase, β-glucan aggregates become more pronounced in size and irregularity especially for the higher molecular weight materials. As it can be clearly seen, there is a remarkable change in the morphology of the mixtures with decreasing β-glucan molecular weight. High MW sample (OBG) shows distinct morphology from the low MW counterparts in the bicontinuous and polysaccharide-continuous regime. Such morphology suggests that apart from the thermodynamic considerations outlined above, the state of aggregation should also influence the phase behaviour of the mixtures through density differences. It should be stressed that the present images were taken in freshly prepared out of equilibrium mixtures. Therefore, kinetic considerations of β-glucan aggregation should be also regarded as it has been previously shown to influence phase separation in β-glucan/whey protein mixtures varying in MW (Kontogiorgos et al., 2009a). Finally, different β-glucan samples at equivalent concentration levels become more dispersed in the protein domain, form small droplets and achieve homogeneity in the equilibrium phase as MW decreases (Fig. 2).

The present results suggest that the mechanism of phase separation in these systems should be attributed to entropy changes as β-glucan MW changes as well as to the extent of polysaccharide aggregation. In order to investigate if changes of β-glucan MW alter protein composition in the equilibrium phases, therefore, affecting the overall thermodynamics of demixing, electrophoretic observations were carried out to examine the distribution of proteins in the respective phases.

3.2. Electrophoretic observations

Electrophoretic patterns of sodium caseinate revealed the presence of all casein subunits (α-, β-, and κ-casein) between ~20 and ~30 kDa (Fig. 3) when compared with the standards and as previously reported in literature (Macierzanka et al., 2011). However, high molecular weight protein bands around 60 kDa and the faint bands around 80 kDa are probably due to unreduced disulphide linked casein aggregates or contaminants something that has also been previously observed (Macierzanka et al., 2011). Keeping β-glucan concentration constant while varying the sodium caseinate concentration, showed that the bands become more prominent for the respective mixtures with higher protein concentration. However, by holding constant sodium caseinate concentration, but varying the concentration of β-glucan, did not reveal any significant differences in electrophoretic patterns of the protein compositions (Fig. 3a).

Therefore, changing β-glucan concentration does not influence the protein composition while the experimental conditions (pH, temperature, etc.) are maintained. Furthermore, at the same concentration levels of biopolymers in the mixture, but different β-glucan MW the electrophoretic patterns of both mixed system and equilibrium phase were similar (Fig. 3b). This reveals that varying the MW of β-glucans in the mixture does not induce any significant changes to the composition of the proteins under the experimental conditions used. Furthermore, it is probable that the centrifugation of the mixtures did not cause any significant precipitation of the protein subunits since the resulting equilib-

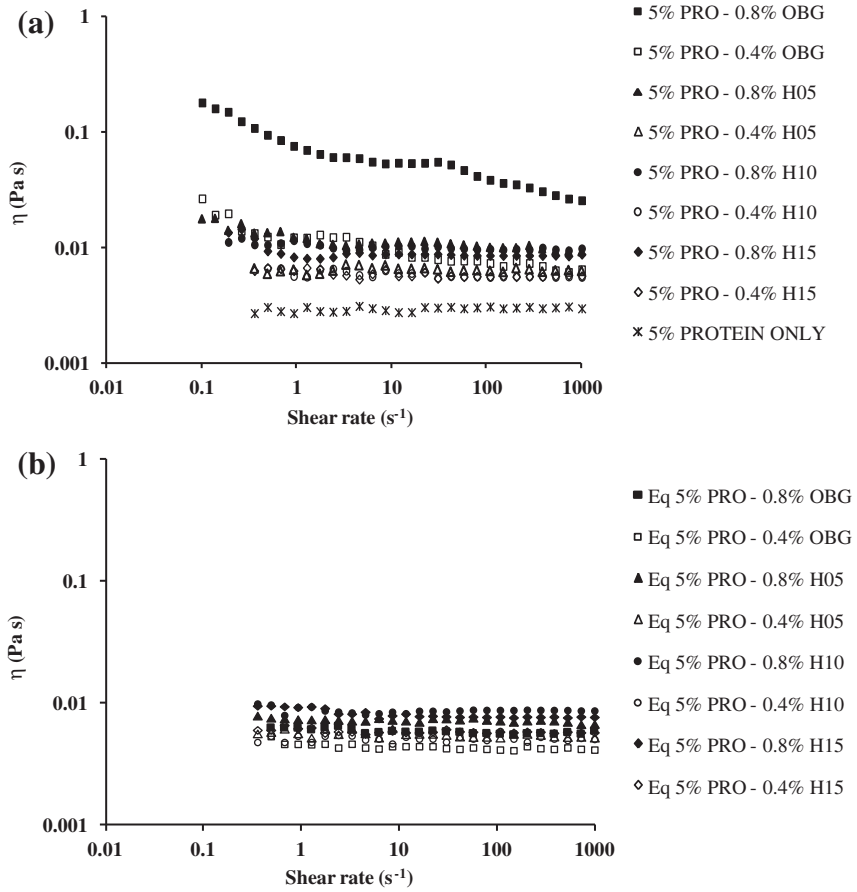

Fig. 5. Flow behaviour of mixed systems at same protein (PRO) level with varying concentration and MW of β-glucan samples (OBG, H05, H10, H15). (a) After mixing; viscosity increases with increasing MW and concentration of β-glucan after mixing ($p < 0.05$). (b) Equilibrium phase after centrifugation. "Eq" denotes the mixtures at equilibrium phase. At equilibrium phase, comparable samples with lower MW had higher viscosity ($p < 0.05$).

rium phase exhibited similar bands as the initial mixed state. Present results reveal similarities in phase behaviour with those of a previous study involving high molecular weight β-glucan ($\sim 1.3 \times 10^6$ g mol^{-1}) and whey proteins (Kontogiorgos et al., 2009b). Electrophoretic profiles showed that whey proteins (α-lactalbumin, β-lactoglobulin) also do not precipitate selectively at pH 7.0. Addition of sucrose and changes in the polysaccharide concentration did not induce any observable differences in the electrophoretic patterns of the system. However, for the system at pH 3.0, it was shown that the concentration of α-lactalbumin and bovine serum albumin decreased in the upper phase in comparison to that of pH 7.0 showing the remarkable influence of pH on the properties of mixtures. Changes in protein composition and conformation were reported to partly account for increased miscibility at pH 3.0 due to a decrease in excluded volume of the protein component (Kontogiorgos et al., 2009b). Changing pH is also expected to play a role on the phase behaviour of the present mixture as a result of conformational changes of caseinate. However, electrophoretic patterns under the present conditions show evidence that the phase separation mechanism of the mixed systems is dominated by modifications in the β-glucan MW. It is clear that changes in polysaccharide MW will play important role on the flow behaviour of the mixed systems that is discussed in the next section.

3.3. Flow behaviour of mixtures

The viscosity of sodium caseinate solutions, increases as the concentration increases, with the flow behaviour being mainly Newtonian at all shear rates for the concentrations (0.1–14% w/v) studied. For β-glucan solutions, viscosity also rises with increasing concentration, and at equivalent concentration levels, viscosity was higher for the higher molecular weight samples (Agbenorhevi et al., 2011). In the sodium caseinate/β-glucan mixed systems, however, the viscosity also varied depending on the concentration of the two biopolymers. Fig. 4 shows typical flow curves of the β-glucan/sodium caseinate mixtures at different biopolymer concentrations. In the protein-rich phase, the flow behaviour was mainly influenced by the sodium caseinate whereas in the polysaccharide-rich phase, the flow behaviour was largely determined by β-glucan.

At intermediate biopolymer concentrations, a mixed flow pattern was evident as previously reported (Kontogiorgos et al., 2009b). Noticeably, the flow pattern remains the same but the viscosity of the binary mixtures at equilibrium (one-phase regime) was lower than that after mixing (two-phase regime) when the system was under kinetic control. This is because centrifugation caused some protein and polysaccharide to precipitate resulting in less total biopolymer concentration in the final one-phase system, hence yielding lower viscosity. As confirmed by the microscopic images, mixed systems at equilibrium have finer β-glucan aggregates present, which influence the flow behaviour.

At the same protein concentration in the mixtures, the viscosity increases with increasing MW and concentration of β-glucans (Fig. 5). This is attributed to the higher entanglement of the molecules and larger aggregates formation as the MW increases. However, it was interesting to note that at equilibrium comparing similar levels in mixture, the lower MW samples yielded identical or higher viscosity. This is also in agreement with the microscopic observations as the lower MW samples were more dispersed in the protein domain with smaller aggregates, hence achieving better homogeneity in the equilibrium phase. In other words, centrifuga-

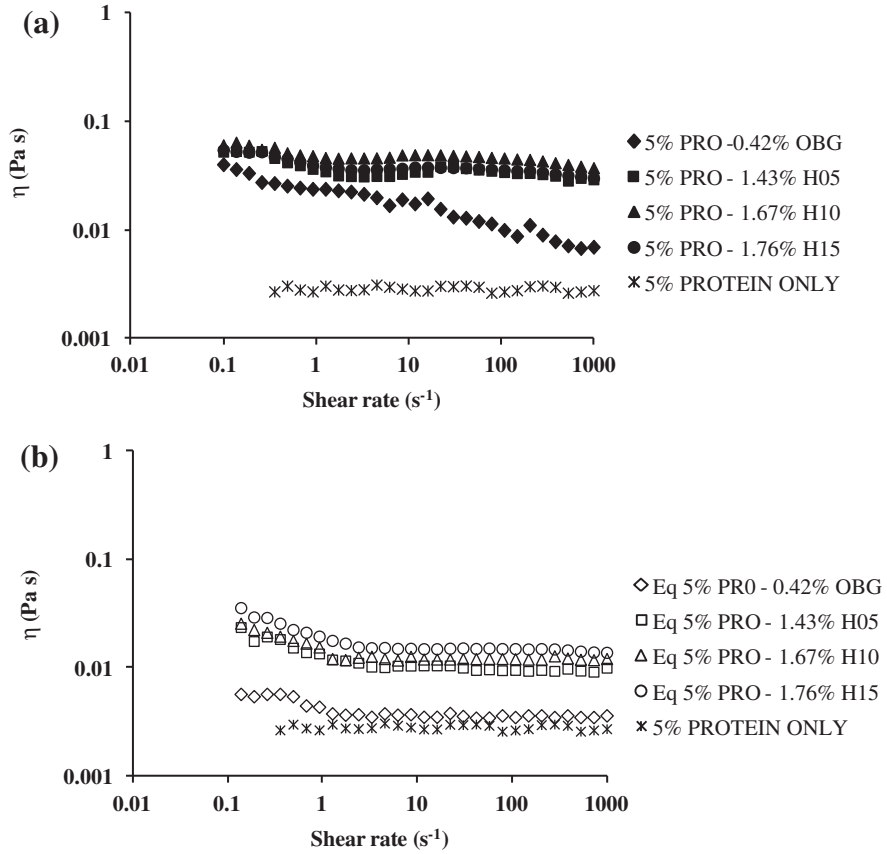

Fig. 6. The flow curve of mixtures of sodium caseinate (PRO) and β-glucan isolates (OBG, H05, H10, H15) at concentration levels of equivalent hydrodynamic volume, c[η] = 3. (a) After mixing, (b) equilibrium phase after centrifugation. "Eq" denotes the mixtures at equilibrium phase. Viscosity of the mixtures with lower MW β-glucan (H15, H10, H05) was significantly higher ($p < 0.05$) in comparison to those with high MW β-glucan (OBG).

tion caused more aggregates of higher MW β-glucans to sediment out resulting in a lower viscosity.

Apart from the general phenomenology of viscosity of the various mixtures, a more fundamental approach would be to investigate the mixtures at the same hydrodynamic volume of the polysaccharide, as MW itself will not determine viscosity any longer. Such a treatment will reveal if there is any specific structure–function relationship on the mechanism that β-glucan influence the viscosity of the mixtures.

3.4. Effect of hydrodynamic volume

From our previous study, intrinsic viscosity ([η]) values of samples OBG, H05, H10 and H15 are known to be 7.2, 2.1, 1.8 and 1.7 dL g^{-1}, respectively (Agbenorhevi et al., 2011). As shown in Fig. 5, the viscosity of the mixed system was also higher for the higher molecular weight samples (OBG > H05 > H10 > H15) at the same concentration levels. Therefore, the flow behaviour was investigated at the same hydrodynamic volume (the volume of the hydrated polymer coil in solution) to ascertain whether a similar trend is observed. In order to create β-glucan solutions of equivalent hydrodynamic volume, the coil overlap parameter ($c[\eta]$) was used that is the product of intrinsic viscosity and concentration and serves as an index of the total degree of volume occupied by the polymer in solution. The experiments were therefore performed at concentration levels of the β-glucan that yield equivalent hydrodynamic volume.

At the concentration levels in mixture with sodium caseinate where $c[\eta] = 1$ (i.e., at the same hydrodynamic volume where the concentration is below their respective critical concentration (c^*) ($c < c^*$ for each β-glucan sample), the viscosity was similar at all shear rates for all the samples despite the variation in molecular weight. However, when the hydrodynamic volume was increased to $c[\eta] = 3$ (i.e., at $c > c^*$), the viscosity of the out of equilibrium mixtures showed an equivalent increase for the lower molecular weight samples (H05, H10 and H15) that exhibited Newtonian flow (Fig. 6a). The results for the low MW samples are in agreement with our previous rheological characterisation of pure β-glucan solutions (Agbenorhevi et al., 2011) that showed increase of specific viscosity, which is independent of the molecular weight of β-glucan, a typical behaviour of disordered polysaccharides. However, departure from this behaviour was observed with the high MW sample (OBG) that showed distinct pseudoplastic behaviour at the same $c[\eta]$ as the low MW counterparts. These deviations in flow patterns can be attributed to the formation of β-glucan aggregates that increases with increasing molecular weight, which also correlates with the morphology of pure β-glucan solutions as previously examined by atomic force microscopy (Agbenorhevi et al., 2011). Furthermore, the equilibrium mixtures showed an expected decrease in viscosity (Fig. 6b) as the polysaccharide is distributed to the phases after centrifugation thus lowering its overall concentration.

As the size of the macromolecules and their aggregates change, the viscosity of the mixed systems is influenced differently. Under force of deformation or shear stress, the larger aggregates progressively breakdown with increasing shear rate and hence account for the exhibited shear thinning behaviour. It can, therefore, be stated that the aggregate size and morphology was mainly responsible for the variation in the rheological deviation of the mixtures containing high MW β-glucans. That was the case even at constant protein concentration and equivalent hydrodynamic volume of β-glucans of different molecular weights. It is interesting to note that by

keeping the hydrodynamic volume of β-glucan constant while the protein concentration varies, results in greater viscosity as protein concentration increases. For instance, the viscosity of mixture with 5% w/v protein was higher than that with 2.5% w/v protein, other things being equal (not shown). This suggests a synergistic contribution of the concentration of both biopolymers on the flow behaviour of the mixed system. Thus, the higher concentration of sodium caseinate and β-glucan, the more viscous the resulting mixture reflecting limited mobility of the biopolymer components.

The present report highlights that viscosity of β-glucan/sodium caseinate mixtures is largely independent of polysaccharide particularly when is kept low below $\sim 300 \times 10^3$ g mol^{-1}. Such an observation may have significant technological implications in creating novel food formulations. This indicates that utilisation of oat β-glucans with a specific MW is not crucial as long as the hydrodynamic volume is kept to the desired technologically important value. Such an approach should help meet the recommended amount required for health claims of a product while still achieving suitable bulking effect.

4. Conclusion

The isothermal phase behaviour at 5 °C of mixtures of sodium caseinate with oat β-glucan isolates varying in molecular weight was investigated. Results showed distinct phase behaviour with variable biopolymer concentration in mixtures. Phase diagrams documented that with decreasing MW of the β-glucan component, thermodynamic compatibility with sodium caseinate increases. Images of the mixtures taken at various biopolymer concentrations revealed phase separation in the presence of β-glucan aggregates, whose size depends on MW and concentration. Electrophoretic separation of the mixtures did not reveal significant difference among the samples varying in concentration and MW of β-glucans that indicates no significant precipitation of the protein components under the experimental conditions studied.

At equivalent hydrodynamic volume of the β-glucan component in the mixture, all the samples exhibited similar flow behaviour. A deviation dependent on the protein concentration was observed for the very high MW sample in the concentrated regime due to the size of the β-glucan aggregates formed. The study shows that it is possible to maximise thermodynamic compatibility and achieve comparable flow behaviour of the β-glucan/sodium caseinate mixed system by using lower MW β-glucan samples and controlling the hydrodynamic volume of the polysaccharide.

Acknowledgements

We are very grateful to Dr. Tim Foster for his useful suggestions.

References

Agbenorhevi, J. K., & Kontogiorgos, V. (2010). Polysaccharide determination in protein/polysaccharide mixtures for phase diagram construction. *Carbohydrate Polymers, 81*, 849–854.

Agbenorhevi, J. K., Kontogiorgos, V., Kirby, A. R., Morris, V. J., & Tosh, S. M. (2011). Rheological and microstructural investigation of oat-glucan isolates varying in molecular weight. *International Journal of Biological Macromolecules, 49*, 369–377.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72*, 248–254.

Brennan, C. S., & Cleary, L. J. (2005). The potential use of cereal (1→3, 1→4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science, 42*, 1–13.

de Kruif, C. G., & Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids, 15*, 555–563.

Doublier, J.-L., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein–polysaccharide interactions. *Current Opinion in Colloid and Interface Science, 5*, 202–214.

Kontogiorgos, V., Tosh, S. M., & Wood, P. J. (2009a). Kinetics of phase separation of oat-glucan/whey protein isolate binary mixtures. *Food Biophysics, 4*, 240–247.

Kontogiorgos, V., Tosh, S. M., & Wood, P. J. (2009b). Phase behaviour of high molecular weight oat β-glucan/whey protein isolate binary mixtures. *Food Hydrocolloids, 23*, 949–956.

Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties technological applications and physiological effects. *Journal of Cereal Science, 46*, 101–118.

Lazaridou, A., & Biliaderis, C. G. (2009). Concurrent phase separation and gelation in mixed oat β-glucans/sodium caseinate and oat β-glucans/pullulan aqueous dispersions. *Food Hydrocolloids, 23*, 886–895.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels. *Food Hydrocolloids, 17*, 693–712.

Lloyd, M. R. (2007). *Polymer blends: A comprehensive review*. Munich: Hanser Gardner Publications.

Macierzanka, A., Bordron, F., Rigby, N. M., Mills, E. N. C., Lille, M., Poutanen, K., & Mackie, A. R. (2011). Transglutaminase cross-linking kinetics of sodium caseinate is changed after emulsification. *Food Hydrocolloids, 25*, 843–850.

Perez, A. A., Carrara, C. R., Sanchez, C. C., Rodríguez Patino, J. M., & Santiago, L. G. (2009). Interactions between milk whey protein and polysaccharide in solution. *Food Chemistry, 116*, 104–113.

Rubinstein, M., & Colby, R. H. (2003). *Polymer physics*. New York: Oxford University Press Inc..

Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and technofunctional properties of protein–polysaccharide complexes: A review. *Critical Reviews in Food Science and Nutrition, 38*, 689–753.

Semenova, M. G. (2007). Thermodynamic analysis of the impact of molecular interactions on the functionality of food biopolymers in solution and in colloidal systems. *Food Hydrocolloids, 21*, 23–45.

Sperling, L. H. (2006). *Introduction to physical polymer science* (4th ed.). New Jersey: John Willet & Sons, Inc..

Syrbe, A., Bauer, W. J., & Klostermeyer, H. (1998). Polymer science concepts in dairy systems – An overview of milk protein and food hydrocolloid interaction. *International Dairy Journal, 8*, 179–193.

Tolstoguzov, V. B. (2003). Some thermodynamic considerations in food formulation. *Food Hydrocolloids, 17*, 1–23.

Tolstoguzov, V. (1997). Protein–polysaccharide interactions. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 171–198). New York: Marcel Dekker, Inc..

Turgeon, S. L., Beaulieu, M., Schmitt, C., & Sanchez, C. (2003). Protein–polysaccharide interactions: Phase-ordering kinetics, thermodynamic and structural aspects. *Current Opinion in Colloid and Interface Science, 8*, 401–414.

Wood, P. J. (2007). Cereal β-glucans in diet and health. *Journal of Cereal Science, 46*, 230–238.