Rheological properties of protein/polysaccharide blends as affected by time-dependent phase separation

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ABSTRACT

The use of mixed protein/polysaccharide systems offers the possibility to control or improve the functional and structural properties of food products due to their synergistic interactions. Protein/polysaccharide blends tend to separate into two phases due to the phenomenon of thermodynamic incompatibility. In the present work, we studied the rheological properties of gels prepared from a mixture of whey protein concentrate (WPC) and hydroxypropylmethylcellulose (HPMC) as affected by the degree of phase separation. The phase separation kinetics of a mixed system containing 4% wt/wt HPMC and 15% wt/wt WPC was determined, measuring the change of the lower phase volume over time. Dynamic rheological tests were conducted in a controlled stress rheometer (Paar Physica MCR 300), on mixed gels with different degrees of phase separation (0, 7, 16, 34 and 70%) and single WPC and HPMC gels, determining the elastic modulus (G') and gelation temperature (Tgel).

Tgel values for WPC and HPMC were 82.0 ± 1.0 ºC and 54.0 ± 2.0 ºC, respectively. The initial system (0% of phase separation) presented a Tgel of 76.3 ± 2.0 ºC, which tends to the average value of the Tgel of individual components affected by their concentrations (76 ºC). At low phase separation degrees (7 or 16%) the Tgel was determined by the HPMC rich-phase. When the system reaches a 70% of phase separation, it presented a Tgel of 79.4 ± 4.0 ºC, similar to that obtained for single WPC (80 ºC). On the other hand, the elastic modulus (G') of mixed gels did not show any correlation with the degree of phase separation, although there was a synergistic effect due to concentration of the components during the separation process.

Controlling the degree of phase separation before gelling a mixed WPC/HPMC system allows obtaining gels with specific gelation temperature or elastic properties.

Keywords: thermodynamic incompatibility; gelling; hydroxypropyl methylcellulose; whey proteins; dynamic rheology.

INTRODUCTION

Proteins and polysaccharides are frequently present together imparting a wide range of desirable features in food systems. They contribute to the structure, texture and stability of food through their thickening or gelling ability [1]. However, the overall properties of these mixed systems depend not only on the properties of the proteins and polysaccharides, but also on nature and strength of protein/polysaccharide interactions [2]. Concerning to these interactions, the term “thermodynamic compatibility” implies the miscibility of the biopolymers. On the other hand, the term “thermodynamic incompatibility” means that biopolymer are immiscible, while a “limited thermodynamic compatibility” means a co-solubility of biopolymers which is usually in dilute mixed solutions [3]. Incompatibility of unlike macromolecules is rather the rule than an exception. Sufficiently concentrated solutions of biopolymers slightly differing in chemical composition and conformation are usually immiscible. These mixed systems often resolve spontaneously into two phases, each enriched in one polymer and depleted in the other [4, 5]. Thermodynamic incompatibility gives some interesting benefits as the reduction in the critical concentration to gel in mixed systems [6, 7]; the formation of non-equilibrium trapped structures that finds application in terms of texture and flavour [8], thickeners, fat substitutes, carriers of nutritional and taste components, structural components in food products or matrices for controlled drug release [9].
Whey is a by-product of cheese production. However, it is rich in valuable components, it contains lactose, minerals, vitamins, non-casein proteins (except glycomacropeptide), and traces of milk fat [10]. Whey proteins have many technological applications. The main proteins present are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA) [11], and they account for 75% of total whey proteins. These proteins are responsible for the hydration capacity, gelling, foaming and emulsifying properties of whey protein concentrates (WPC) and isolates (WPI).

The usefulness of HPMC is essentially based upon four key attributes: efficient thickening, surface activity, film forming ability, and the capacity to form thermal gels that melt upon cooling. These interesting properties are given by methyl substituents along the cellulose backbone that constitute strong hydrophobic zones and hydroxypropyl groups that are more hydrophilic. Although several works have been focused on certain properties of HPMC as water affinity [12-14] and gelation [15, 16], there is scarce literature concerning their behaviour in ternary systems, i.e. systems formed by water, proteins and HPMC.

MATERIALS & METHODS

Commercial food grade HPMC (E50LV; Dow Chemical, Findlay, USA) was kindly supplied by Colorcon S.A. (Buenos Aires, Argentina). It was used without further purification. According to the supplier, this cellulose derivative has 29.1% methyl groups, 9.2% hydroxypropyl groups, being the methyl/hydroxypropyl ratio 3.2%. The viscosity, measured on 2% (w/v) aqueous solution at 20 °C, was 41 mPa.s⁻¹. Its averaged molecular weight was 18 kDa, and it had a moisture content of 1.6%.

WPC powder was kindly given by Milka-Frank (Santa Fe, Argentina). Its composition was: protein 78.9%; lactose 5%; fat 6%; ash 4.3% and moisture 5.6%. All other chemical reagents used were of analytical grade. WPC80 (40%, w/w) and HPMC (10%, w/w) stock solutions were prepared by adding each component (as powders) to distilled water and mixing (WPC at 25 °C and HPMC at 85 °C) for 2 h in order to ensure a complete dissolution of the components. Mixed systems of WPC 15% (w/w)/HPMC 4% (w/w) were prepared by mixing carefully weighed amounts of stock solutions with a magnetic stirrer at 25 °C for 4 h to allow a homogeneous distribution of the components. The pH of mixtures was 6.5 and it was not adjusted.

The phase separation kinetics of the samples was determined by measuring the change of the lower phase volume over time. Dynamic oscillation measurements were performed in a MCR 300 controlled stress rheometer from Paar Physica (Graz, Austria). The samples were poured onto the bottom plate of a parallel plate measuring system (gap 1 mm) and the temperature was controlled with a Peltier system model Viscotherm VT2 (Paar Physica). During gelation experiments, the frequency was 1 Hz and the strain was kept constant at 0.01%, a value found to be in the linear viscoelastic region. The samples were heated from 20 to 90 °C at a rate of 10 °C/min, then kept at 90 °C during 15 min, which was enough time to allow G’ equilibration and then cooled to 20 °C at 25 °C/min. The storage modulus (G’) was recorded over time. The temperature at which the storage modulus (G’) and the loss modulus (G’”) crossed over was taken as the gelation temperature (Tgel). Experiments were performed at least in triplicate, and the average and standard deviation were reported.

RESULTS & DISCUSSION

Figure 1 shows the phase separation kinetics of the mixed system containing 4% wt/wt HPMC and 15% wt/wt WPC as the change of the lower phase volume over time. The percentage degree of phase separation was determined as (V(t)/Vmax)100, where V(t) is the volume of the lower phase at time t and Vmax is the maximum volume reached by the lower in the full segregated system. Five time points which correspond to 0, 7, 16, 34 and 70 % of phase separation degree, were selected to analyze the rheological parameters during heat gelation.

The confocal microscopy image showing the microstructure mixed system at 0 % phase separation and 25 °C can be seen in Figure 2. The interpretation of the image is based on the difference in the intensity of the fluorescent signal between the protein and the polysaccharide. It can be seen that the mixed system behaves as a water/water emulsion with a continuous phase rich in WPC (red) and a dispersed phase rich in HPMC (black).

Firoozmand, Murray and Dickinson [17] performing small deformation rheological experiments on gelatine/oxidized starch mixtures found that the microstructure and the rheology of these mixtures depend
both on the system composition and on the components distribution within system microstructure. In turn, these factors are related to the formation of the polymer network by intermolecular annealing processes taking place during thermal processing. In the present work however, a major source of complexity during the early stages of thermal processing is the time-dependent character of the biopolymer local concentrations: this means that the compositions of the protein-rich and polysaccharide-rich domains in the resulting gel microstructure are not precisely known. Likewise, Jara and Pilosof [18] determined, by thermal analysis and confocal microscopy, that thermal properties of WPC/HPMC partially phase-separated co-dried mixtures depended mainly on the morphology and degree of phase separation of the corresponding aqueous mixture.

\[ \text{Figure 1. Phase separation kinetics for the mixed system WPC (15% w/w)/HPMC (4% w/w) determined by the change of the lower phase volume over time.} \]

\[ \text{Figure 2. Confocal microscopy of the mixed system immediately after mixing WPC (15% w/w)/HPMC (4% w/w),} \]

Gelation temperatures (Tgel) determined by the crossover of G’ and G” moduli upon time for the single components and the mixed system at the five time points of the phase separation are shown in Figure 3. Tgel values for WPC and HPMC were 82.0 ± 1.0 °C and 54.0 ± 2.0 °C, respectively. At 0% of phase separation, the mixed system presented a Tgel of 76.3 ± 2.0 °C, which tends to the average value of the Tgel of individual components affected by their concentrations (76 °C). At low phase separation degrees (7 or 16%) the Tgel
was determined by the HPMC rich-phase. When the system reaches a 70% of phase separation, it presented a Tgel of 79.4 ± 4.0 °C, similar to that obtained for single WPC (80 °C) (Figure 3).

Figure 3. Gelation temperature (Tgel) of the mixed system WPC (15% w/w)/HPMC (4% w/w) over phase separation degree, compared with Tgel of single components. (WPC: dot line; HPMC: dash dot line; WPC: – – – – ; error bar: standard deviation)

Figure 4 shows the solid character of the mixed system upon heating (G’) compared to single components. The elastic modulus (G’) of mixed gel showed a synergistic effect, which is increased with the advance of phase separation. This is due to concentration of the WPC at the continuous phase during the separation process [1, 19].

Figure 4. Elastic modulus (G’) evolution of the mixed system WPC (15% w/w)/HPMC (4% w/w) over phase separation degree, compared with G’ of single components. (WPC: dot line; HPMC: dash dot line; WPC: – – – – ; error bar: standard deviation)
CONCLUSION

At the beginning of phase separation, the mixed system showed an average gelation temperature of the T_{gel} corresponding to single components affected by the percentage of each one in the system. However, the confocal microscopy showed that this mixed system behaves as a water/water emulsion with a continuous phase rich in WPC and a dispersed phase rich in HPMC. At low phase separation degrees (7 or 16 %) T_{gel} was determined by the HPMC rich phase. When the system was almost completely phase separated (70%), the mixed system showed a T_{gel} similar to that obtained for single WPC. The elastic character of the gels (G') showed a synergistic effect that increases with increasing degree of phase separation. Jara et al. [20] found that solid character of WPC/HPMC gels kept correlation with the component constituting the continuous phase, while gelation temperature reflected the gelation of the phase with the lowest T_{gel}, i.e. the polysaccharide-rich phase. Thus, the difference between the rates of phase separation and gelation phenomena will have a great influence on the final gel structure. Therefore, the study of the relationship between phase separation and gelification of WPC/HPMC mixed systems results of high interest to control texture and functional properties of mixed gels.

REFERENCES


