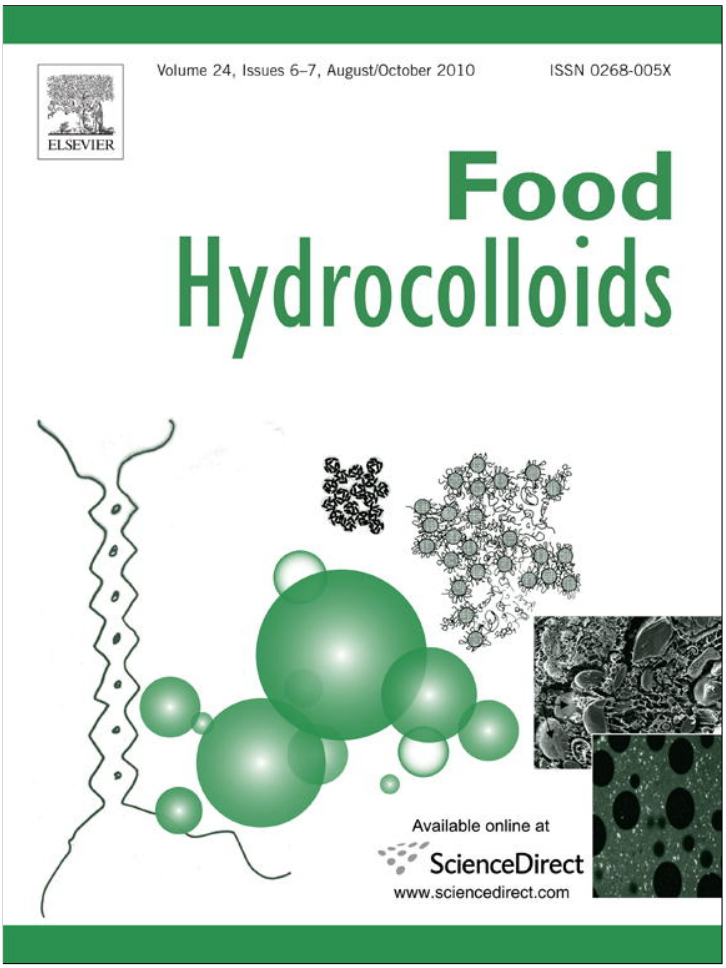

Food Hydrocolloids 24 (2010) 641–651

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Impact of phase separation of whey proteins/hydroxypropylmethylcellulose mixtures on gelation dynamics and gels properties

Federico Jara [a,b], Oscar E. Pérez [a,c,*], Ana M.R. Pilosof [a,c]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Buenos Aires, Argentina
[b] Research fellow of Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina
[c] Member of Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

ARTICLE INFO

Article history:
Received 10 September 2009
Accepted 18 March 2010

Keywords:
Incompatibility fractionation
Whey proteins
Phase separation
Gelation

ABSTRACT

This work constitutes a study of the impact of phase separation behaviour on the gels properties of a low viscosity hydroxypropylmethylcellulose and whey protein concentrate (WPC) mixed system. The phase separation was characterized by drawing the limit of thermodynamic compatibility, i.e. binodal curve, at pH 6.5 and room temperature (25 °C). Gelling properties were studied under thermodynamic compatibility (WPC 12% (w/w)/E50LV 0.25% (w/w) mixed system) and incompatibility conditions (WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed systems)

Under thermodynamic compatibility the WPC/E50LV mixed system shows gelling parameters similar to WPC. Confocal scanning laser microscopy (CSLM) micrographs showed a regular pattern of microdomains of proteins imbibed into E50LV matrix.

Confocal microscopy of WPC/E50LV mixture under thermodynamic incompatibility offered details about the constitution of continuous and non-continuous phase and characteristics of non-continuous phase domains. Related to gelling parameters, the solid character upon heating was reinforced in mixed systems since they reflected the concentrating effect arising from phase separation. On the other hand, the solid character of gels upon cooling correlated with the component constituting the continuous phase, and the gelation temperature was similar to polysaccharide-rich phase predicted gelation temperature.

Regarding to textural properties, the presence of the polysaccharide diminished the hardness of the mixed gels inducing less resistance to small and large deformation. WPC 20% (w/w)/E50LV 4% (w/w) mixed gel presented an interesting particulated macrostructure. This result would find application in food design and technology if the E50LV concentration is chosen to finely control the rate and extent of WPC aggregation-gelation-particulation. These results could be used in microparticulation or microencapsulation application of whey proteins.

1. 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 (Simonet, Garnier, & Doublier, 2000). 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 (Ercelebi & Ibanoglu, 2007).

Concerning to these interactions, the term `biopolymer compatibility' implies miscibility of different biopolymers on a molecular level. The terms `incompatibility' and `limited compatibility' means a limited co-solubility of biopolymers that are usually miscible either in a dilute solution or when they form a soluble complex (Tolstoguzov, 2007). 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. From a thermodynamic point of view, for small molecules the enthalpic drive for segregation is normally outweighed by the entropic advantage of intimate mixing (Fitzsimons, Mulvihill, & Morris, 2008). For polymers, however, where at equivalent mass concentration, there are far fewer molecules free to move independently, entropy of mixing is much less significant, and mixed systems often

* Corresponding author at: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Buenos Aires, Argentina. Fax: +54 11 45763366.
E-mail address: operez@di.fcen.uba.ar (O.E. Pérez).

0268-005X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2010.03.005

resolve spontaneously into two phases, each enriched in one polymer and depleted in the other (Antonov, Losinskaya, Grinberg, Dianova, & Tolstoguzov, 1979; Tolstoguzov, 1997)

Thermodynamic incompatibility gives same interesting benefits as the reduction in the critical concentration to gel in mixed systems (Baeza & Pilosof, 2001; Tolstoguzov, 1995); the formation of non-equilibrium trapped structures that finds application in terms of texture and flavour (Norton and Frith, 2001), thickeners, fat substitutes, carriers of nutritional and taste components, structural components in food products or matrices for controlled drug release (Lorén & Hermansson, 2003).

Whey is a by-product of cheese production which is used mainly as animal feed or released into the wastewater treatment process, although it is rich in valuable components. It contains lactose, minerals (e.g., calcium, magnesium, phosphorus), vitamins, non-casein proteins (except glycomacropeptide), and traces of milk fat (Yorgun, Balcioglu, & Saygin, 2008). Whey proteins have many technological applications. The main proteins present are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serumalbumin (BSA) (Cayot & Lorient, 1997) 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).

Hydroxypropylmethylcellulose (HPMC) is used in the food industry, printing technology, and has pharmaceutical applications because is nontoxic and possesses good mechanical properties. In the pharmaceutical industry, HPMC has acquired special interest for controlled drug-release matrices (Ford, 1999; McCristal, Ford, & Rajabi-Siahboomi, 1997). 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 substitutes 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 (Ford & Mitchell, 1995; Fyfe & Blazek, 1997; Tritt-Goc & Pislewski, 2002) and gelation (Sarkar & Walker, 1995; Yoguchi, Urakawa, Kitamura, Ohno, & Kajiwara, 1995), there is scarce literature concerning their behaviour in ternary systems, i.e. systems formed by water, proteins and HPMC.

The present work is part of an integral project undertaken to characterize the behaviour of WPC/HPMC mixed systems. Important aspects regarding to their potential application, in the scientific as well in the technological fields, are (i) the definition of the limit between compatible and incompatible mixed systems, under well defined conditions (temperature, pH, ionic strength), (ii) the extent of the incompatibility, (iii) their ability to generate new food structures and textures, (iv) the possibility of study in a dynamic way the mechanisms involved in the biopolymers concomitant thermal gelation, (v) the application of a set of techniques to elucidate the behaviour of these complex systems.

In a previous a work (Pérez, Wargon, & Pilosof, 2006) we have studied the influence of a hydroxypropylmethycellulose of high molecular weight (E4M), i.e. when dissolved gives high viscosity solutions, on whey protein concentrate gelling properties under thermodynamic incompatibility conditions (pH 7 and 25 °C). An interesting founding was obtained regarding to the morphology of some mixed gels that an exhibited core–shell structure where the core was constituted by gelled HPMC. The elastic modulus of mixed gels was much higher than that of single WPC gels at the same concentration, while hardness and relative viscoelasticity were slightly decreased.

Considering the findings from Pérez et al. (2006), we propose in the present work to characterize the phase separation behaviour of mixtures of WPC and E50LV, a low viscosity HPMC, and to study the impact of this segregation on the dynamics of gelation and gel properties of the mixed systems.

2. Materials and methods

2.1. Materials

Commercial food grade HPMC (E50LV; Dow Chemical, Findlay, USA) was kindly supplied by Colorcon SA (Buenos Aires, Argentine). 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, Argentine). 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.

2.2. Preparation of stock solutions

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) using a magnetic stirrer within a cell (50 ml) having external temperature control from a thermostatic bath (model Polystat 12108-15, Cole–Parmer Instrument, Vernon Hills, USA) for 2 h in order to ensure a complete dissolution of the components. After preparation, stock solutions were stored at 4 °C. They were heated up to room temperature prior to the experiments.

2.3. Preparation of binary solutions

Binary solutions of WPC/HPMC were prepared by mixing carefully weighed amounts of stock solutions in order to obtain systems with concentrations of WPC 6–20% (w/w) and E50LV 0.25–4% (w/w). Systems were mixed with a magnetic stirrer at 25 °C for 4 h to allow an homogeneous distribution of the components. The pH of mixtures was 6.5 (pH meter model A920, ORION RESEARCH, Beverly, USA) and it was not adjusted.

2.4. Phase diagram determination

Phase diagram was established at pH 6.5 and 25 °C, and it was constructed by determining the transition from single to two-phase system of blends with WPC 6–20% (w/w) and E50LV 0.5–4% (w/w). In order to ensure phase separation the mixtures were centrifuged in 15 ml graduated plastic tubes at 13,000 × g in a centrifuge model 5810 R from Eppendorf (Hamburg, Germany) at room temperature (25 °C), up to complete phase separation The volumes of separated lower phases (V_L) and upper phase (V_U) were visually determined. The protein concentration in lower phase, rich in protein, was determined by the Kjeldhal method as outlined in AOAC (1995). Briefly, samples were digested using a heating block model 1009 Digester from Tecator AB (Höganäs, Sweden) and their total nitrogen content was determined using an automatic distillation unit model Kjeltec Auto 1030 Analyzer, also from Tecator AB. Final protein concentration was calculated using a factor of 6.25. The protein content of upper phase (X_U) was determined by the difference between the total protein content in the sample (also determined by Kjeldhal method) and that determined in the lower phase (X_L). The polysaccharide concentration in the lower, rich protein phase (Y_L), and in the upper, rich

polysaccharide phase (Y_S), were calculated according to the following equation (Pérez et al., 2006),

$$Y_{L,U} = [-(\alpha Y_0 + Y_0)/(\alpha X_0 + X_0)]\alpha X_{L,U} + (\alpha Y_0 + Y_0) \quad (1)$$

which represents the straight line passing through initial concentration coordinates of each mixture (X_0, Y_0), and through coordinates defining the upper phase composition (X_U, Y_U) and the lower phase composition (X_L, Y_L). The slope (α) is defined by volume ratio of phases (V_L/V_U). The complete assay was performed in triplicate, and the average and standard deviation of all variables were recorded. Then, the binodal curve was obtained by gathering together each tie-line points corresponding to the initial concentration of each mixed system, using OriginPro software v8.0 from OriginLab Corp. (Northampton, USA).

2.5. Differential scanning calorimetry

Differential scanning calorimetry was used to determine the onset temperature (T_{onset}) and peak temperature (T_{peak}) involved in HPMC chains dehydration. Thermal analysis was performed using a DSC 822 from Mettler–Toledo calorimeter (Schwerzenbach, Switzerland). The instrument was calibrated with indium (156.6 °C), lead (327.5 °C) and zinc (419.6 °C). Thermograms were evaluated using the STARe Thermal Analysis System software v3.1 also from Mettler–Toledo. The thermal parameters were determined by heating 60 μl of each sample in 160 μl capacity pans from 5 to 100 °C at 10 °C/min. An empty pan was used as reference. The average and standard deviation from at least two replicates were reported.

2.6. Dynamics of gelation and viscoelasticity

Dynamic oscillation measurements were performed in a MCR 300 controlled stress rheometer from Paar Physica (Graz, Austria). WPC solutions, E50LV solutions or their mixtures were poured onto the bottom plate of a parallel plate measuring system, gap 1 mm. The temperature of the bottom plate was controlled with a Peltier system model Viscotherm VT2, also from Paar Physica, and liquid silicone was applied to the exposed surfaces of the sample to prevent evaporation. 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'), the loss modulus (G'') and the damping factor ($\tan\delta$) were recorded over time. The temperature at which the storage modulus (G') and the loss modulus (G'') crossed over was taken as the gelation temperature (T_{gel}). Experiments were performed at least in triplicate, and the average and standard deviation of all variables were reported.

2.7. Confocal scanning laser microscopy

Images of WPC/E50LV aqueous mixtures were recorded by an FV300 CLSM from Olympus Corp. (Tokyo, Japan) equipped with a vertical microscope model BX61 also from Olympus, used in the single photon mode with an Ar/HeNe visible light laser. The following Olympus objective lenses were used: UplanFl 10X/0.3NA/dry and UplanFl 10X/0.5NA/dry. Non-covalent labeling of protein was performed with few drops of rhodamine B solution 0.02% (w/v) (excitation wave length 560 nm; emission maximum 625 nm). Digital image files were acquired in multiple.tif format in 1024 × 1024 and 512 × 512 pixel resolutions by FluoView™ image acquisition software v3.3 also from Olympus Corp.

2.8. Texture properties

WPC and WPC/E50LV mixed gels for texture analysis were prepared in glass cylinders (13 mm diameter × 55 mm height) containing 2 ml of sample. Then, these samples were heated for 20 min at 90 ± 0.1 °C and stored at 4 °C overnight. A texture profile analysis was performed at 25 °C in a Texture Analyzer model TA-XT2i from Stable Microsystems (Godalming, UK) using a cylindrical probe (P/36R 36 mm diameter). Cylindrical specimens of the gels (13 mm diameter × 11 mm height) were compressed to 30% of the initial height at a compression rate of 0.5 mm/s. For each sample the hardness and springiness were recorded. The assay was performed in triplicate, and the average and standard deviation were reported.

2.9. Statistical analysis

All mean and standard deviations were calculated using Excel 2007 software form Microsoft Corp. (Redmond, USA).

3. Results and discussion

3.1. WPC/E50LV phase diagram

Protein and polysaccharide mixtures at neutral pH are generally unstable and upon a critical biopolymer concentration they separate in a protein-rich and a polysaccharide-rich phase (Tolstoguzov, 2003). Fig. 1 shows the phase diagram, for the ternary system WPC-E50LV-water, at 25 °C and pH 6.5.

It can be seen that the binodal curve is very close to axis which means that the compatibility zone is relatively small and phase separation takes place in a broad range of concentrations. Moreover, phase diagram is asymmetrical reflecting the competition between both biopolymers for water, which in turn is caused by differences in molecular weight and hydrophilicity (Zhuravskaya, Kiknadze, Antonov, & Tolstoguzov, 1986a; Zhuravskaya, Kiknadze, Antonov, & Tolstoguzov, 1986b). The tie lines connect the binodal points representing coexisting phase compositions and the measured volumes of each phase are proportional to the segment

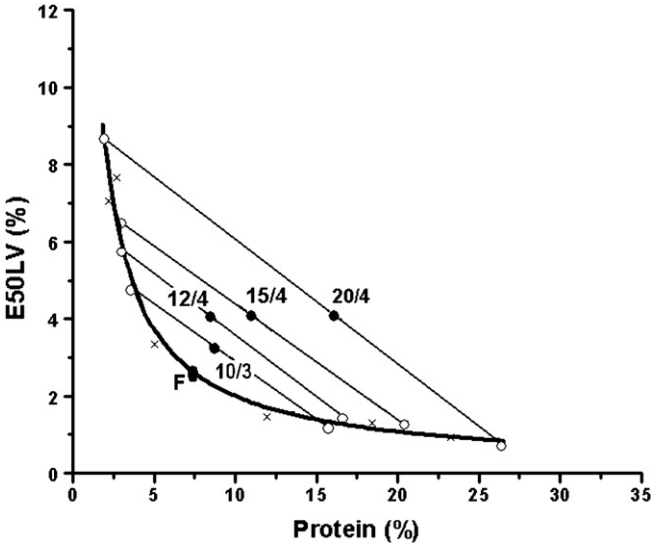

Fig. 1. Phase diagram for WPC/E50LV mixtures at room temperature (25 °C) and pH 6.5. The solid line is the binodal curve; 10/3, 12/4, 15/4 and 20/4 represent original concentrations (% (w/w)) of WPC/E50LV in these mixed systems; F: phase separation threshold.

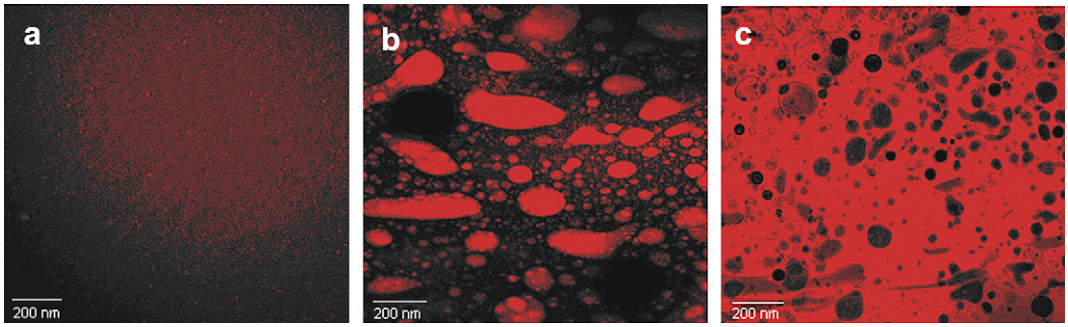

Fig. 2. CSLM pictures of WPC 12% (w/w)/E50LV 0.25% (w/w) (a), WPC 12% (w/w)/E50LV 4% (w/w) (b), and WPC 20% (w/w)/E50LV 4% (w/w) (c) aqueous mixed systems at room temperature (25 °C) and pH 6.5 (image width 200 μm).

length that determines it. The point F is the phase separation threshold for a given biopolymer pair, i.e. the minimal total concentration of biopolymers required for phase separation to occur. In this case, F values corresponded to 7.5% (w/w) of protein and 2.5% (w/w) of E50LV.

In general terms, a phase diagram describes the behaviour of two polymer-one solvent systems with different ratios of these components. Thus, it allows making the illustration of the complex interactions in protein–polysaccharide–water ternary systems (Clark, 2000; Doublier, Garnier, Renard, & Sanchez, 2000). More specifically, the binodal curve constitutes the limit between compatible and incompatible mixtures. The position of this limit depends on the intensity of the interactions (i) between biopolymer$_1$-biopolymer$_2$, (ii) between equal biopolymer molecules, and (iii) between biopolymers and solvent, i.e. differences in the biopolymers hydrophilicity. At a moderate total biopolymer concentration, the separation of the mixed system into two phases depends on the biopolymer charge, pH, ionic strength, being the entropy the main factor driving the phenomena (Alves, Antonov, & Gonçalves, 1999).

Others factors that have remarkable influence on the binodal curve position, bringing it closer to the concentration axes (increasing the incompatibility), are: i) the molecular weight and molecular structural stiffness increase; ii) the globular proteins denaturation provoked by hydrophobicity and the enhancement of molecular size by peptidic chains aggregation (pre-gelation), iii) the higher salts concentrations with the concomitant ionic strength increase, and iv) the higher temperature (Tolstoguzov, 2007). The displacement of the curve could also be influenced by the polysaccharide, since the linear types are less compatible than the branched (HPMC belongs to this last category) (Grinberg & Tolstoguzov, 1997).

3.2. Phase behaviour of selected mixtures for gelation studies

From the phase diagram three mixed systems were selected to study the gelation behaviour: WPC 12% (w/w)/E50LV 0.25% (w/w) (non phase-separated system, under the binodal curve), WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) (both phase-separated systems, above the binodal).

Confocal microscopy images showing the microstructure of these mixed solutions upon mixing at 25 °C can be seen in Fig. 2. The interpretation of the CLSM images is based on the difference in the intensity of the fluorescent signal between the protein and the polysaccharide. Fig. 2a displays the microscopic appearance of WPC 12% (w/w)/E50LV 0.25% (w/w) mixed system, which is located inside the compatible region (below the binodal of Fig. 1). A homogeneous pattern (non separated domains) can be observed. Moreover, as expected, this system did not show any macroscopic phase separation.

The other two mixtures which lay in the incompatibility region (above the binodal) separated into two phases, a polysaccharide-rich upper phase and a protein-rich lower phase. Table 1 shows the polysaccharide and protein composition of WPC and E50LV-rich phases, as well as their percentage volumes. The WPC 12% (w/w)/E50LV 4% (w/w) mixed system segregated into an E50LV-rich phase accounting for by 64% of total volume and a WPC-rich phase with 36% of volume. Contrarily, the WPC 20% (w/w)/E50LV 4% (w/w) mixed system segregated into an E50LV-rich phase representing 33% of total volume and a WPC-rich phase representing 67% of total volume.

The microstructure during the demixing process of these systems, shown in Fig. 2b–c, corroborates that phase-inversion took place. Thus, it can be seen that the microstructure of WPC 12% (w/w)/E50LV 4% (w/w) mixed system consisted of protein domains in a continuous dark E50LV phase (Fig. 2b), while WPC 20% (w/w)/E50LV 4% (w/w) mixed system showed a microstructure with E50LV inclusions (dark domains) into a continuous WPC phase (Fig. 2c). Typically the minor (lower volume) phase will exist as droplets in the major phase. Hence, as the composition of the system changes, a phase-inversion occurs where the continuous and droplets phase are swapped (Norton & Frith, 2003). Strictly speaking, there is a region of phase-inversion (or a range of concentrations) over which a more complex, bicontinous or droplet-within-droplet structures are seen. Moreover, the extent of this phase-inversion region for biopolymer mixtures is

Table 1
Composition and volume of segregated phases from WPC/E50LV mixed systems.

Initial mixed system composition (% (w/w))		WPC-rich-phase			E50LV-rich-phase		
WPC	E50LV	Protein (% (w/w))	E50LV (% (w/w))	Volume (%)	Protein (% (w/w))	E50LV (% (w/w))	Volume (%)
12	4	15.7 ± 0.2	1.30 ± 0.02	36.0 ± 0.5	3.0 ± 0.1	5.7 ± 0.2	64.0 ± 0.4
20	4	26.5 ± 0.3	0.80 ± 0.01	67.0 ± 0.2	2.0 ± 0.1	8.7 ± 0.2	33.0 ± 0.2

Mean ± SD, $n = 3$.

Table 2
Gelation parameters of WPC obtained by dynamic rheometry.

WPC (% (w/w))	T_{gel} (°C)	G'_{eq} (Pa)
12	82.2 ± 1.0	151.1 ± 1.2
15	81.6 ± 2.0	632.2 ± 2.0
20	81.0 ± 2.0	2184.4 ± 0.5
32	75.5 ± 1.0	26558.4 ± 1.3

Mean ± SD, $n = 3$.
T_{gel} is the gelation temperature.
G'_{eq} is the plateau value of G' at 90 °C.

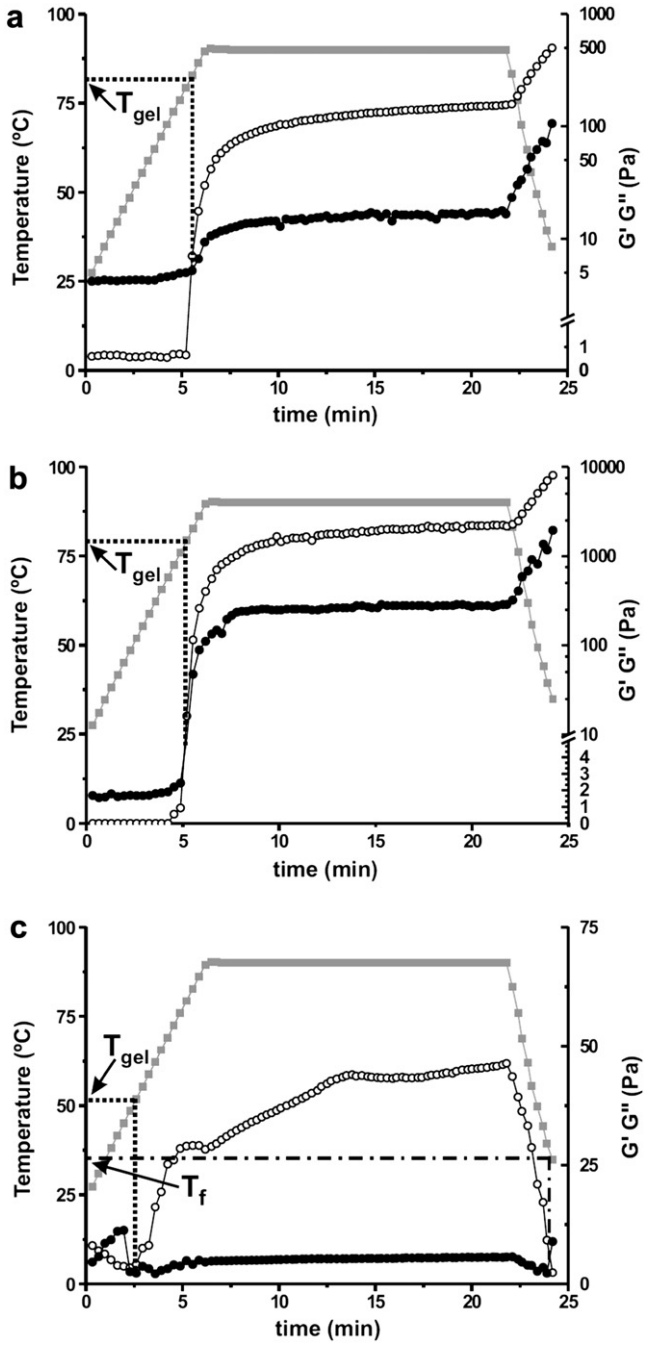

Fig. 3. Evolution of storage modulus (G') and loss modulus (G'') upon heating and cooling of WPC 12% (w/w) solution (a), WPC 20% (w/w) solution (b), and E50LV 4% (w/w) solution (c). Arrows indicate the gelation temperature (T_{gel}) or melting point (T_f) in the case of E50LV. Temperature: (■); G': (○); G'': (●).

generally much broader than in the case of O/W emulsion (Norton & Frith, 2001).

Similar results were found for mixed systems of gelatin/maltodextrin (Norton & Frith, 2003), high methoxyl pectin/whey protein isolate (Kim, Decker, & McClements, 2006), locust bean gum/caseinate (Norton & Frith, 2001) and skimmed milk/locust bean gum (Schorsch, Jones, & Norton, 1999).

3.3. Gelation dynamics of single components

Considering single WPC solutions upon heating, an increase of G'_{eq} (the plateau value of G' at 90 °C) was observed with increasing concentration from 12 to 32% (w/w) (Table 2). Concerning to this, Fernandes (1994) found that WPC solutions showed a gradual increase of G' with concentration and a small G' dependence on oscillation frequency. Table 2 also shows that gelation temperatures (T_{gel}) determined by the cross over of G' and G'' moduli upon time (Fig. 3a–b), decreased by 7 °C when WPC concentration increased from 12 to 32% (w/w). This result agrees well with former studies (Puyol, Perez, & Horne, 2001). Aggregation of protein molecules at neutral pH takes place throughout thiol–disulfide reactions leading to S–S bridges formation (Hoffmann & VanMil, 1999). These reactions leading to gel formation are favored at higher protein concentrations (Verheul, Pedersen, Roefs, & Kruif, 1999).

E50LV is a kind of hydroxypropylmethylcellulose of low viscosity; in turn hydroxypropylmethylcellulose belongs to a bigger family of cellulose derivatives, the so-called methylcelluloses (MC). These compounds comprise cellulose ethers with methyl substitution and with or without additional functional substituents (Coffey, Bell, & Henderson, 1995). A complete characterization of HPMC gelation was previously done by Pérez et al. (2006), who used several techniques in order to describe the thermal events occurring upon heating HPMC solutions (pH 7). In short, most of the works in the literature agrees with a two-step mechanism for the gelation process. The first, called pregel-regime, involves hydrophobic interactions that lead to clusters formation. Kato, Yokoyama and Takahashi (1978) have proposed that the pregel-regime is mainly determined by the aggregation of the most hydrophobic domains of HPMC chains, i.e. trimethylglucose units that act as cross-linking loci on heating. This association results in the increase of the size of hydrophobic aggregates.

The second stage or gel-regime corresponds to the gelation (i.e. network formation) that occurs at higher temperatures and it is commonly associated with phase separation (Kobayashi, Huang, & Lodge, 1999; Yoguchi et al., 1995). According to Kato et al. (1978) the second stage involves hydrophobic association of less hydrophobic domains, i.e. di and mono-methylglucoses. Clusters grow up to form crystallites, which would cause phase separation (Yoguchi et al., 1995). The presence of crystallites had been

Table 3
Events occurring upon heating of E50LV 4% (w/w) solution.

E50LV		
Cloud-point	(°C)	30.0 ± 1.0
Parameters describing the dynamic of gelation	T_{gel} (°C)	55.1 ± 1.0
	T_m (°C)	38.2 ± 0.5
	G'_{eq} (Pa)	47.2 ± 2.0
DSC parameters	T_{onset} (°C)	56.5 ± 0.4
	T_{peak} (°C)	65.6 ± 0.4

Mean ± SD, $n = 3$.
T_{gel} is the gelation temperature.
T_m is the melting temperature.
G'_{eq} is the plateau value of G' at 90 °C.
T_{onset} is the temperature at the start of the endothermic transition.
T_{peak} is the peak temperature of the endothermic transition.

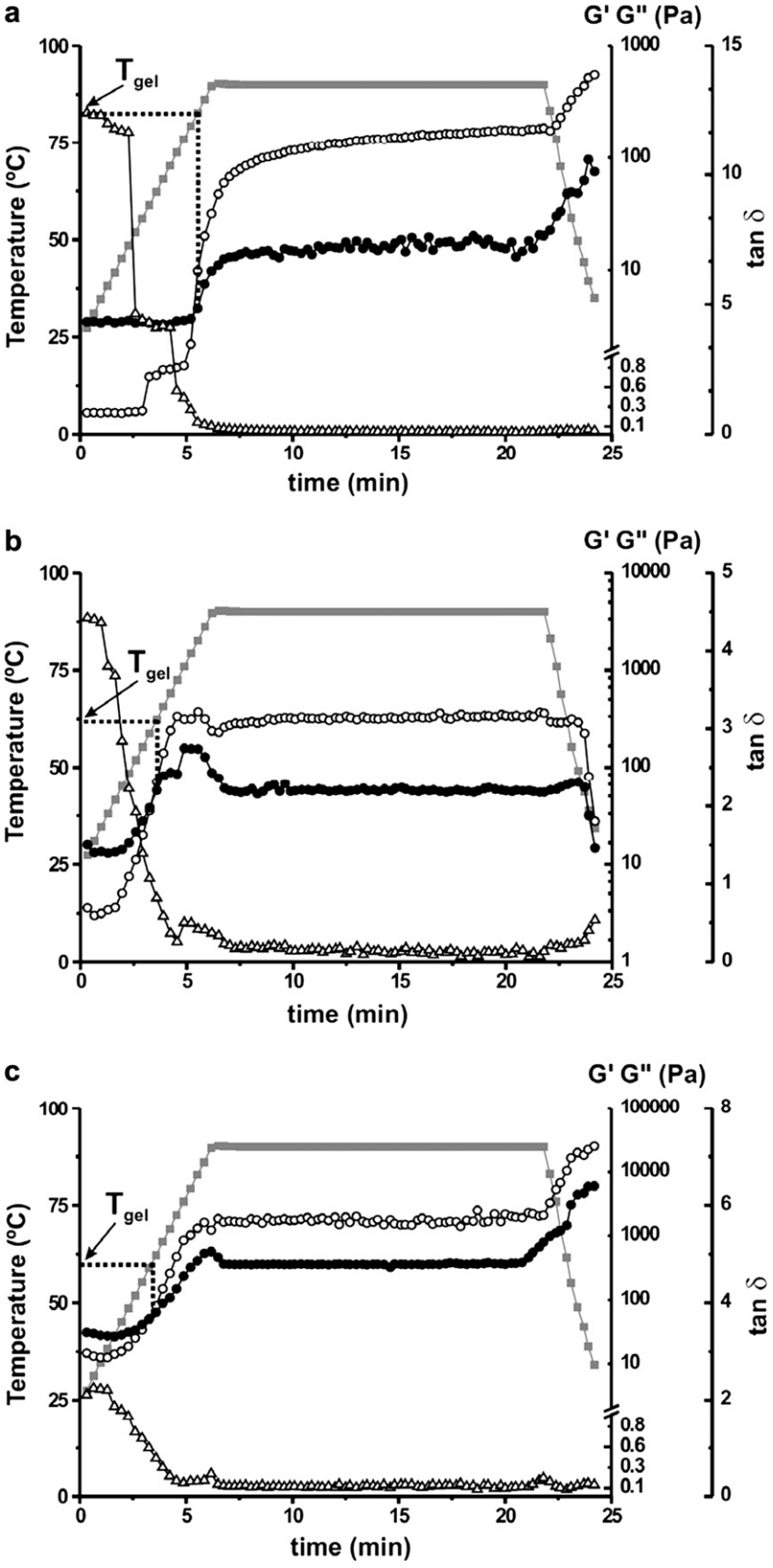

Fig. 4. Evolution of storage modulus (G'), loss modulus (G'') and damping factor (tan δ) upon time and temperature for the mixed systems: WPC 12% (w/w)/E50LV 0.25% (w/w) (a), WPC 12% (w/w)/E50LV 4% (w/w) (b), and WPC 20% (w/w)/E50LV 4% (w/w) (c). Arrows indicate the gelation temperature (T_{gel}). G': (◯); G'': (●); tan δ: (△); temperature: (▒).

confirmed by X-ray diffraction (Kato et al., 1978) and by microcalorimetry (Yoguchi et al., 1995). In this last case, two endothermic peaks were observed in the thermograms when cooling HPMC gels. The peak close to 60 °C belongs to the dissolution of two separated phases and that close to 50 °C to the melting of crystalline clusters. Pregel to gel regime transition have been observed around 50 °C by Kobayashi et al. (1999). Further theorical studies support the exposed interpretation of HPMC gelation (Tanaka, 1996). Different experimental techniques were used in the present work to characterize the phenomena occurring upon heating E50LV solutions; these events were not affected by polysaccharide concentration. Table 3 summarizes the thermal parameters determined for 4% (w/w) E50LV aqueous solution. According to Kobayashi et al. (1999), during the first stages of heating a transition from transparent to turbid solutions is observed. The temperature corresponding to this transition, the cloud-point, is 30 °C and indicates the beginning of aggregation by means of hydrophobic interactions. In solution, HPMC is hydrated with water molecules surrounding the hydrophobic groups forming the so-called cage-like structures (Kobayashi et al, 1999). There are little polymer-polymer interactions other than simple entanglements. As the temperature increases, molecules adsorb translational energy and gradually lose their hydration water, resulting in a decrease in solutions viscosity. Eventually, polymer–polymer associations take place, due to hydrophobic interactions, causing cloudiness in solution and then an infinite network structure formation (Sarkar & Walker, 1995).

By DSC, E50LV chains dehydration could be determined as an endothermic transition starting (T_{onset}) at 56.5 °C (Table 3). Thermograms displayed a broad endothermic transition (data not shown), which has been attributed to HPMC polydispersity (Pérez et al, 2006; Sarkar & Walker, 1995). The higher dehydration rate, which corresponds to the peak temperature (T_{peak}) of the endotherms was observed at 65.6 °C.

Fig. 3c shows the evolution of G' and G'' upon time for 4% (w/w) E50LV solution. First of all, a decrease in G' values were observed which is related to cellulose chains dehydration, i.e. cage-like structure disorganization by increasing the hydrophobic groups interactions. Temperature corresponding to a fast increase of G' values indicated the T_{gel}. It was found to be about 55 °C, which was coincident with the onset for E50LV chain dehydration observed by DSC (Table 3). This result indicates that concomitantly to E50LV dehydration, hydrophobic cluster formation occurs leading to a primary gel network formation at T_{gel}. This temperature coincides with the gel point detected by a tilting-test, which determines the temperature where the gel structure is fully developed and the resulting gel does not flow upon tilting (Pérez et al., 2006). After the gel point, G' increased up to a G'_{eq} value of 47.2 Pa (Table 3).

Finally, the E50LV melting temperature (T_m) was observed at around 38 °C (Table 3) as a cross over of G' and G'' curves upon cooling (Fig. 3c).

3.4. Gelation dynamics of mixed systems

3.4.1. Gelation temperatures

Firoozmand, Murray and Dickinson (2009) 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 (2009) 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.

Thus, in the absence of a working model concerning to the relationship between the polymers composition, their distribution within mixed system microstructure and the rheological behaviour, any quantitative analysis or discussion about this seems to be unjustified. However, we could infer some qualitative trends concerning to the relationship between the gelation temperature (T_{gel}) and the biopolymer ratio in the WPC/HPMC mixed systems. Then, we applied simple mathematical models (linear equations) to estimate a value of this parameter from the mixture composition, and compare it with the experimental values.

The experimental gelation temperatures (T_{gel}) of the three mixed systems are indicated in Fig. 4a–c. For the homogeneous mixture WPC 12% (w/w)/E50LV 0.25% (w/w) the gelation temperature was related to its composition using the following equation,

$$T_{gel}(\mathrm{MIX}) = X_{\mathrm{WPC}} \cdot T_{gel}(\mathrm{WPC}) + X_{\mathrm{E50LV}} \cdot T_{gel}(\mathrm{E50LV}) \quad (2)$$

where T_{gel} (MIX) is the gelation temperature of the mixed system, T_{gel} (WPC) and T_{gel} (E50LV) are the gelation temperatures of the individual components, and X_{WPC} and X_{E50LV} are their weight fractions.

The estimated T_{gel} was 81.6 °C which is close to the experimental value, 79.3 °C (Table 4). For each one of the phase-separated mixed systems, two gelation temperatures were estimated: one for the WPC-rich phase and another for the E50LV-rich phase on the basis of their protein and polysaccharide compositions (Table 1). The equations used were the following,

$$T_{gel}(E) = X_{\mathrm{WPC}}(E) \cdot T_{gel}(\mathrm{WPC}) + X_{\mathrm{E50LV}}(E) \cdot T_{gel}(\mathrm{E50LV}) \quad (3)$$

$$T_{gel}(W) = X_{\mathrm{WPC}}(W) \cdot T_{gel}(\mathrm{WPC}) + X_{\mathrm{E50LV}}(W) \cdot T_{gel}(\mathrm{E50LV}) \quad (4)$$

Table 4
Gelation temperature of WPC/E50LV mixed systems.

Initial mixed system composition (% (w/w))		Experimental	Predicted		
WPC	E50LV	T_{gel} (°C)[a]	T_{gel} (MIX) (°C)	T_{gel} (E) (°C)	T_{gel} (W) (°C)
12	0.25	79.3 ± 0.2	81.6	–	–
12	4	60.5 ± 0.4	–	65.9	80.5
20	4	60.0 ± 2.0	–	61.1	81.6

T_{gel} (MIX) is the T_{gel} value estimated with equation (2).
T_{gel} (E) and T_{gel} (W) are the T_{gel} estimated using equations (3) and (4) for the E50LV and WPC rich phases, respectively.
[a] Mean ± SD, $n = 3$.

where T_{gel} (E) and T_{gel} (W) are the gelation temperatures of E50LV and WPC-rich phases, respectively, T_{gel}(WPC) and T_{gel}(E50LV) are the gelation temperatures of the individual components, and X_{WPC}(W), X_{E50LV}(W) are the weight fractions of WPC and E50LV in the WPC-rich phase, while X_{E50LV}(E), X_{WPC}(E) are the weight fractions of E50LV and WPC in the E50LV-rich phase. Thus, the estimated T_{gel} of the WPC-rich phase for both mixed systems was around 80 °C, while the estimated T_{gel} for E50LV-rich phase was 65.9 °C or 61.1 °C for the WPC 12% (w/w)/E50LV 4% (w/w) or WPC 20% (w/w)/E50LV 4% (w/w) mixed systems, respectively (Table 4). It can be seen that the experimental T_{gel} which were around 60 °C for both mixed system were similar to the estimated T_{gel} of the E50LV-rich phase. These results point out that the experimental T_{gel} is the one that corresponds to the segregated phase with the lower T_{gel}, i.e. the incipient E50LV-rich phase.

Therefore, these results point out certainly the existence of a quantitative relationship between the protein/polysaccharide composition, the phase separation microstructure morphology, and the gelation temperature of the WPC/HPMC mixed systems.

3.4.2. Evolution of elastic and viscous moduli upon heating and cooling

The rheological behaviour of WPC 12% (w/w)/E50LV 0.25% (w/w) mixed system (located below the binodal curve of Fig. 1) can be seen in Fig. 4a. It was similar to that obtained for WPC 12% (w/w) (Fig. 3a) because the low E50LV concentration does not produce any significant influence on the rheological properties of the formed gel.

The dynamic behaviour observed for WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed systems (both located above the binodal curve) is shown in Fig. 4b–c, respectively. WPC 20% (w/w)/E50LV 4% (w/w) mixed system (Fig. 4c) presented a behaviour like that observed for the WPC (Fig. 3b). Contrarily, the rheological behaviour of WPC 12% (w/w)/E50LV 4% (w/w) mixed system (Fig. 4b) was similar to E50LV behaviour (Fig. 3c). Such a behaviour can be accounted for by the nature of the continuous phase since it was constituted by WPC in the WPC 20% (w/w)/E50LV 4% (w/w) mixed system (Fig. 2c) or by E50LV in the WPC 12% (w/w)/E50LV 4% (w/w) mixed system (Fig. 2b). Although this last mixed system did not show any melting of E50LV upon cooling, a decrease of G' with a simultaneously increase of tan δ were observed (Fig. 4b), being this rheological behaviour indicative of an incipient melting of E50LV.

Fig. 5 shows the solid character (G') evolution upon heating time of each WPC/E50LV mixed systems jointly with G' for single components. Upon time, G' tends to an equilibrium value (G'_{eq}), which was reached around 18 min for each sample.

For the mixed system located in the miscibility region, WPC 12% (w/w)/E50LV 0.25% (w/w), the G' behaviour was mainly determined by WPC due to E50LV concentration in this mixed systems is too low to produce significant modification on the solid character of the formed gel (Fig. 5a).

The behaviour for the mixed systems located within the incompatibility region is showed in Fig. 5b (WPC 12% (w/w)/E50LV 4% (w/w)) and Fig. 5c (20% WPC (w/w)/E50LV 4% (w/w)). A synergistic effect on G' can be seen for WPC 12% (w/w)/E50LV 4% (w/w)

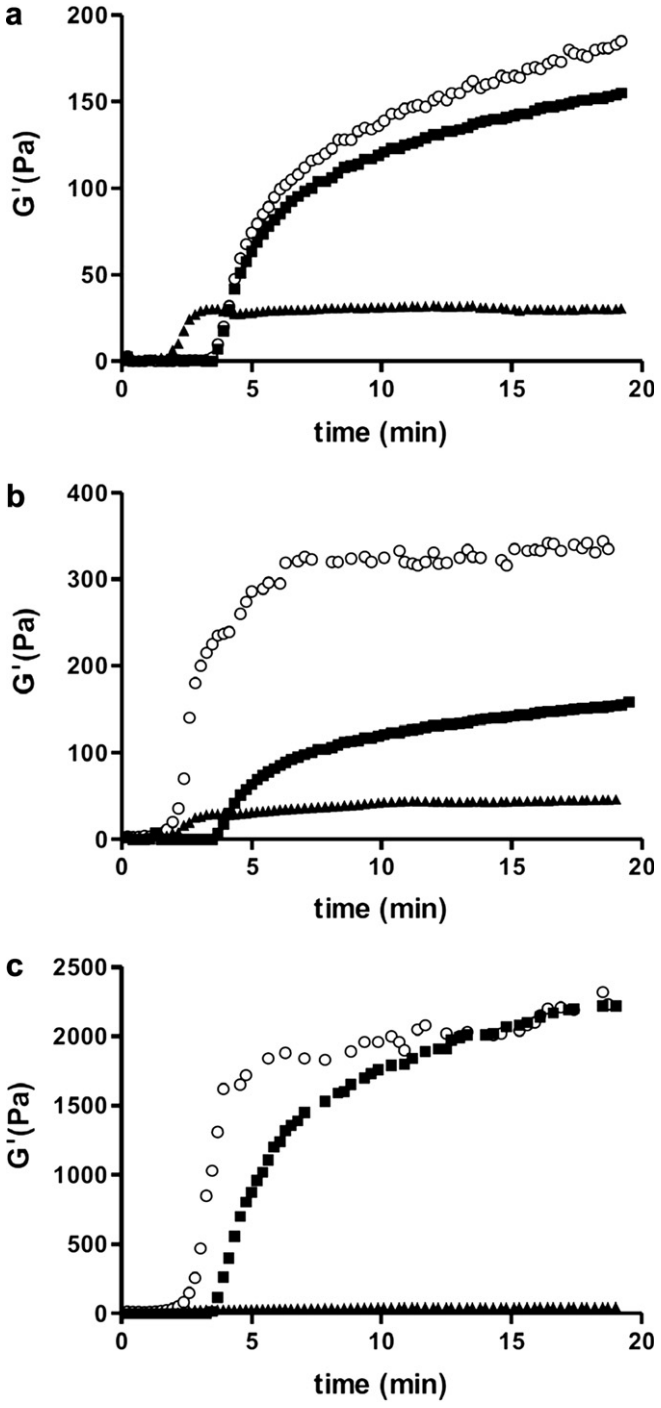

Fig. 5. G' time evolution of mixed systems: WPC 12% (w/w)/E50LV 0.25% (w/w) (a), WPC 12% (w/w)/E50LV 4% (w/w) (b), and WPC 20% (w/w)/E50LV 4% (w/w) (c) compared to single components. WPC: (■); E50LV: (▲); mixed systems: (◯)

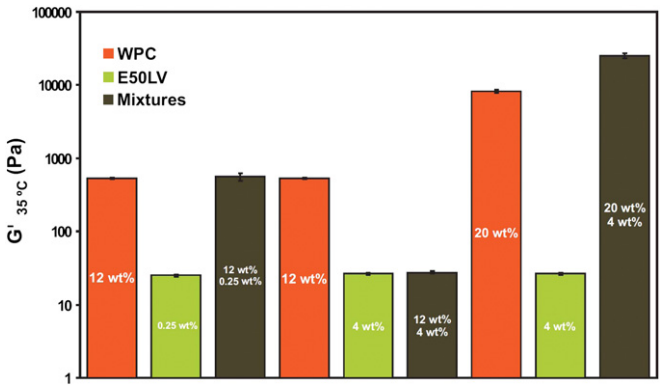

Fig. 6. Solid character upon cooling ($G'_{35\,°C}$) for WPC 12% (w/w)/E50LV 0.25% (w/w), WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed gels compared to single WPC gel. Error bar indicate the standard deviation of $G'_{35\,°C}$.

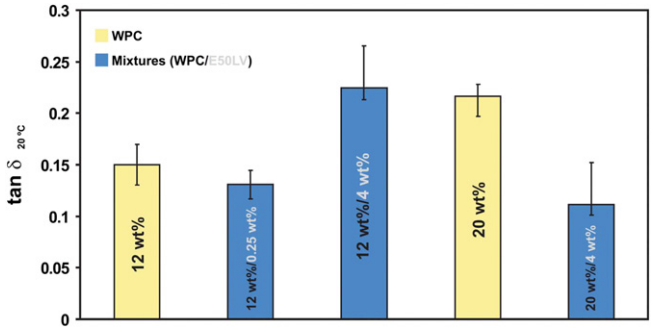

Fig. 7. Damping factor upon cooling (tan $\delta_{20°C}$) for WPC 12% (w/w)/E50LV 0.25% (w/w), WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed gels compared to single WPC gel. Error bar indicate the standard deviation of tan $\delta_{20°C}$.

mixed system. Thus, G'_{eq} values increased twice for this mixed system (303.2 Pa) in comparison to 12% (w/w) single WPC gelling solution (151.1 Pa) (Fig. 5b). In the case of 20% WPC (w/w)/E50LV 4% (w/w) mixed system the G'_{eq} did not show any synergistic effect since it was 2184.4 Pa while single 20% (w/w) WPC gave 2132.1 Pa. However this mixed system showed a synergistic effect onto G' at the beginning of gelation (Fig. 5c). The synergistic interactions would be attributed to segregative phase separation taking place before gelation (Pérez et al., 2006; Simonet et al., 2000). Besides phase separation, other important factor contributing to the synergistic effect seen on G'_{eq} values would be the thermal gelation of polysaccharide, reinforcing the global solid character of the mixed gelling system. Contrarily, the lack of synergistic effects would obey to the high initial protein bulk concentration that allows a fast aggregation–gelation even before a significant phase separation could occur, and the further contribution of the gelling polysaccharide to the solid character upon heating is not really important (Tolstoguzov, 1995).

Fig. 6 shows the solid character of WPC/E50LV mixed systems upon cooling ($G'_{35\,°C}$) compared to single components. In the WPC 12% (w/w)/E50LV 0.25% (w/w) mixed system $G'_{35\,°C}$ had a behaviour similar to G'_{eq}. Because it was mainly determined by WPC due to the low E50LV concentration. On the other hand, for segregated mixed systems $G'_{35\,°C}$ was principally determined by the nature of the continuous phase which was constituted by the WPC-rich phase in WPC 20% (w/w)/E50LV 4% (w/w) or HPMC-rich phase in WPC 12% (w/w)/E50LV 4% (w/w) mixed systems, respectively (Fig. 2).

It is worth to notice that $G'_{35\,°C}$ for WPC 12% (w/w)/E50LV 0.25% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed systems was higher than G'_{eq} (Figs. 5 and 6) which indicates the network strengthening occurring upon cooling, with the development of reversible physical interactions, i.e. hydrogen bonds and ionic interactions (Paraskevopoulou & Kiosseoglou, 2000).

On the contrary, the $G'_{35\,°C}$ of WPC 12% (w/w)/E50LV 4% (w/w) mixed system was lower than G'_{eq} indicating a gel network weakening as a consequence of the incipient E50LV melting (Figs. 5 and 6). Concerning to this, Tolstoguzov (1997) pointed out that the solid character of the mixed gel would be ruled by the polysaccharide when it was the continuous phase of the demixed system.

The values of the damping factor upon cooling (tan $\delta_{20°C}$) of the three WPC/E50LV mixed systems compared with single components are shown in Fig. 7. This parameter is a measurement of the relative viscoelasticity of the system. It presented a behaviour according to the results obtained for the solid character upon cooling, $G'_{35\,°C}$ (Fig. 6). Thus, the WPC 12% (w/w)/E50LV 0.25% (w/w) mixed system presented a relative viscoelasticity similar to WPC 12% (w/w) gel. The WPC 12% (w/w)/E50LV 4% (w/w) mixed system was less elastic (more viscous) than WPC 12% (w/w) gel as a consequence of the incipient E50LV melting. Finally, the WPC 20% (w/w)/E50LV 4% (w/w) mixed system was more elastic (solid-like) than WPC 20% (w/w) gel because of the strengthening of the gel network described above. Again, this behaviour observed for the

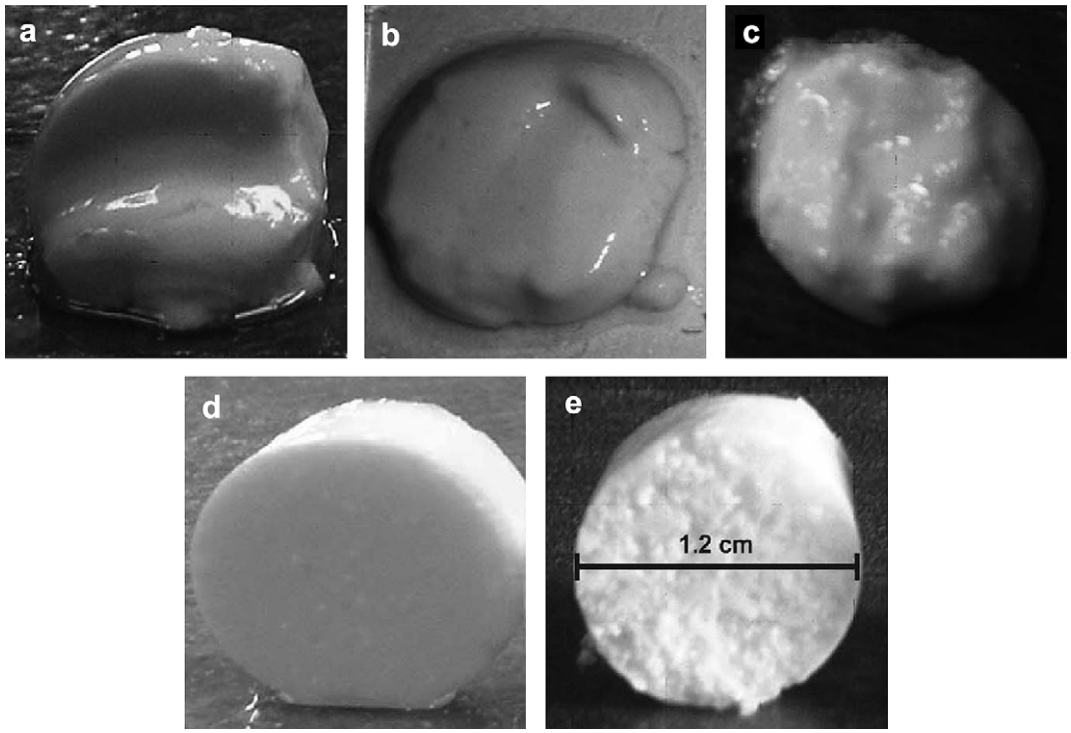

Fig. 8. Macroscopic view of WPC 12% (w/w) gel (a), WPC 12% (w/w)/E50LV 0.25% (w/w) mixed gel (b), WPC 12% (w/w)/E50LV 4% (w/w) mixed gel (c), WPC 20% (w/w) gel (d), and WPC 20% (w/w)/E50LV 4% (w/w) mixed gel (e).

two last mixed systems is related to the nature of the continuous phase which was constituted by E50LV in the first mixed system and by WPC in the second

3.5. Macrostruture and textural properties of mixed gels

The macroscopic aspect of WPC/E50LV mixture gels and WPC single gels are shown in Fig. 8. It is noteworthy that none of these gels presented syneresis. Fig. 8b shows the appearance of WPC 12% (w/w)/E50LV 0.25% (w/w) gel. It is a self-supporting, cohesive and non-opaque gel, similar to single WPC 12% (w/w) gel (Fig. 8a). Increasing the polysaccharide concentration, i.e. WPC 12% (w/w)/E50LV 4% (w/w), a non-self-supporting gel was obtained (Fig. 8c). As it was mentioned before, E50LV-rich-phase constituted the continuous phase in this mixed system (Fig. 2b). Thus, the incipient E50LV melting that took place upon cooling (Fig. 4b) produced the weakening of the gel matrix.

Contrarily, the continuous phase of WPC 20% (w/w)/E50LV 4% (w/w) mixed system was constituted by the protein, being the solid character of the gel determined by WPC. Thus, the resulting gel (Fig. 8e) was a self-supporting-opaque gel similar to single WPC 20% (w/w) gel (Fig. 8d). However, the mixed gel showed a particulate macrostructure because the gelled protein-continuous-phase kept trapped the gelled E50LV domains upon cooling. This gel was more firm than the other mixed gels.

Textural assays of mixed gels, showed that the presence of 0.25% (w/w) of E50LV did not modify their hardness or springiness (Fig. 9a and b, respectively), since these textural parameters were similar to those of WPC 12% (w/w) gel. Moreover, independently whether E50LV formed the continuous phase (WPC 12% (w/w)/E50LV 4% (w/w)) or the dispersed phase (WPC 20% (w/w)/E50LV 4% (w/w)) the hardness of mixed gels strongly decreased as compared to single WPC gels (Fig. 9a). As was suggested by Rosell and Foegeding (2007), HPMC could interfere with protein association and its further aggregation during heating, likely occupying the space of the protein in the network. Finally, the springiness of mixed systems was slightly affected by E50LV (Fig. 9b).

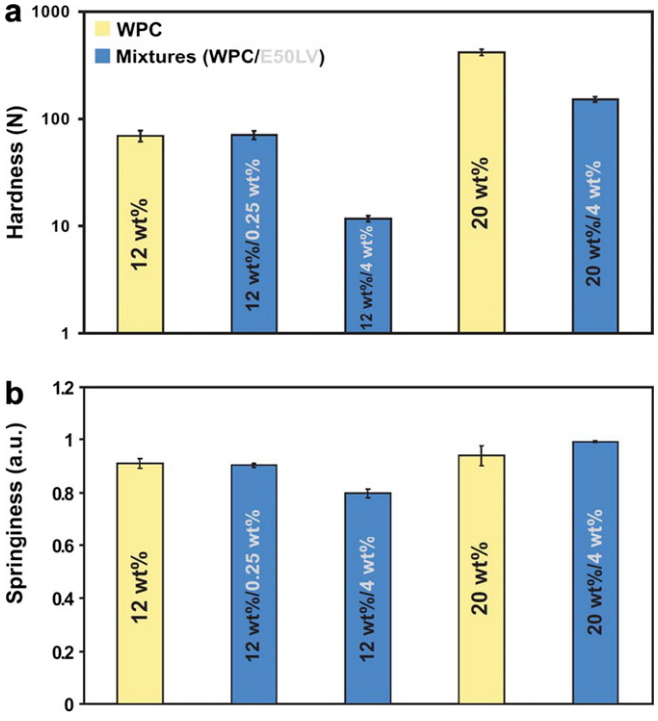

Fig. 9. Hardness (a) and springiness (b) of WPC 12% (w/w)/E50LV 0.25% (w/w), WPC 12% (w/w)/E50LV 4% (w/w) and WPC 20% (w/w)/E50LV 4% (w/w) mixed gels compared to single WPC gel. Error bar indicate the standard deviation of hardness or springiness, respectively.

4. Conclusions

This study has shown that the ternary systems WPC/HPMC/water behave as a water-in-water emulsion, where the microstructure is clearly dictated by the phase volume ratio. In turn, we found that the microstructure determinates the rheological behaviour of the mixed systems. The solid character of 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 in the final gel structure.

Finally, the knowledge of whey proteins/HPMC interactions would be a key element for formulating and the controlling quality of manufactured foods, and the development of novel food processes. Thus, our findings may have a variety of novel application within the food industry, for example to create novel textures or appearances, for producing reduced fat products, or for microencapsulation and realease of bioactive food components.

Acknowledgements

The authors acknowledge the support from the Universidad de Buenos Aires, the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

References

Alves, M. M., Antonov, Y. A., & Gonçalves, M. P. (1999). The effect of structural features of gelatin on its thermodynamic compatibility with locust bean gum in aqueous media. *Food Hydrocolloids, 13*, 157–166.

Antonov, Y., Losinskaya, N., Grinberg, V., Dianova, V., & Tolstoguzov, V. (1979). Phases equilibria in water–protein–polysaccharide systems III. Water bean globulins–polysaccharide systems. *Colloid and Polymer Science, 257*, 1159–1179.

AOAC (Association of Official Analytical Chemists). (1995). *Official methods of analysis* (AOAC 16th ed.). Arlington: AOAC Press.

Baeza, R., & Pilosof, A. M. R. (2001). Mixed biopolymer gel systems of β-lactoglobulin and non-gelling gums. In E. Dickinson, & R. Miller (Eds.), *Food colloids, fundamentals of formulation* (pp. 392–403). Cambridge: RSC.

Cayot, P., & Lorient, D. (1997). Structure–function relationships of whey proteins. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their applications* (pp. 225–256). New York: Marcel Dekker Inc.

Clark, A. H. (2000). Direct analysis of experimental tie line data (two polymer-one solvent systems) using Flory–Huggins theory. *Carbohydrate Polymers, 42*, 337–351.

Coffey, D., Bell, D., & Henderson, A. (1995). Cellulose and cellulose derivatives. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 123–153). New York: Marcel Dekker Inc.

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

Ercelebi, E., & Ibanoglu, E. (2007). Influence of hydrocolloids on phase separation and emulsion properties of whey protein isolate. *Journal of Food Engineering, 80*, 454–459.

Fernandes, P. (1994). Viscoelastics characteristics of whey protein systems at neutral pH. *Food Hydrocolloids, 8*, 277–285.

Fitzsimons, S., Mulvihill, D., & Morris, E. (2008). Co-gels of whey protein isolate with crosslinked waxy maize starch: analysis of solvent partition and phase structure by polymer blending laws. *Food Hydrocolloids, 22*, 468–484.

Ford, J., & Mitchell, K. (1995). Thermal analysis of gels and matrix tablets containing cellulose ethers. *Thermochimica Acta, 248*, 329–345.

Ford, J. (1999). Thermal analysis of hydroxypropylmethycellulose and methylcellulose: powders, gels and matrix tables. *Internacional Journal of Pharmaceutics, 179*, 209–228.

Fyfe, C., & Blazek, A. (1997). Investigation of hydrogel formation from hydroxypropylmethylcellulose (HPMC) by NMR Spectroscopy and NMR Imaging Techniques. *Macromolecules, 28*(4), 313–317.

Grinberg, V. Y., & Tolstoguzov, V. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids, 11*(2), 145–158.

Firoozmand, H., Murray, B. S., & Dickinson, E. (2009). Microstructure and rheology of phase-separated gels of gelatin + oxidized starch. *Food Hydrocolloids, 23*, 1081–1088.
Hoffmann, M., & VanMil, P. (1999). Heat-induced aggregation of β-lactoglobulin: as a function of pH. *Journal of Agricultural and Food Chemistry, 47*, 1898–1905.
Jara, F. L., & Pilosof, A. M. R. (2009). Glass transition temperature of protein/polysaccharide co-dried mixtures as affected by the extent and morphology of phase separation. *Thermochimica Acta, 487*, 65–73.
Kato, T., Yokoyama, M., & Takahashi, A. (1978). Melting temperatures of thermally reversible gels IV. Methyl cellulose–water gels. *Colloid and Polymer Science, 266*, 15–21.
Kim, H., Decker, E., & McClements, J. (2006). Preparation of multiple emulsions based on thermodynamic incompatibility of heat-denaturated whey protein and pectin solutions. *Food Hydrocolloids, 20*, 586–595.
Kobayashi, K., Huang, C., & Lodge, T. (1999). Thermorreversible gelation of aqueous methylcellulose solutions. *Macromolecules, 32*, 7070–7077.
Lorén, N., & Hermansson, A. (2003). Structure evolution during phase separation and gelation of biopolymer mixtures. In E. Dickinson, & T. van Vliet (Eds.), *Food colloids, biopolymers and materials* (pp. 298–308). Cambridge: RSC.
McCristal, C., Ford, J., & Rajabi-Siahboomi, A. (1997). A study on the interaction of water and cellulose ethers using differential scanning calorimetry. *Thermochimica Acta, 294*, 91–98.
Norton, I. T., & Frith, W. J. (2001). Microstructure design in mixed biopolymer composites. *Food Hydrocolloids, 15*, 543–553.
Norton, I., & Frith, W. (2003). Phase separation in mixed biopolymer systems. In E. Dickinson, & T. van Vliet (Eds.), *Food colloids, biopolymers and materials* (pp. 282–296). Cambridge: RSC Press.
Paraskevopoulou, A., & Kiosseoglou, V. (2000). Small deformation measurements of single and mixed gels of low cholesterol yolk and egg white. *Journal of Texture Studies, 31*, 225–244.
Pérez, O., Wargon, V., & Pilosof, A. M. R. (2006). Gelation and structural characteristics of incompatible whey proteins/hydroxypropylmethylcellulose mixtures. *Food Hydrocolloids, 20*, 966–974.
Puyol, P., Perez, M., & Horne, D. (2001). Heat-induced gelation of whey protein isolates (WPI): effect of NaCl and protein concentration. *Food Hydrocolloids, 15*, 233–237.
Rosell, C., & Foegeding, A. (2007). Interactions of hydroxypropylmethylcellulose with gluten proteins: small deformation properties during thermal treatment. *Food Hydrocolloids, 21*, 1092–1100.
Sarkar, N., & Walker, L. (1995). Hydration–dehydration properties of methylcellulose and hydroxypropylmethylcellulose. *Carbohydrate Polymers, 27*, 177–185.
Sarkar, N., & Walker, L. (1995). Hydrationedehydration properties of methylcellulose and hydroxypropylmethylcellulose. *Carbohydrate Polymers, 27*, 177–185.
Schorsch, C., Jones, M., & Norton, I. (1999). Thermodynamic incompatibility and microstructure of milk protein/locust bean gum/sucrose systems. *Food Hydrocolloids, 13*, 89–99.
Simonet, F., Garnier, C., & Doublier, J. L. (2000). Partition of proteins in the aqueous guar/dextran two-phase system. *Food Hydrocolloids, 14*, 591–600.
Tanaka, F. (1996). Phase formation of associating polymers: gelation, phase separation and microphase formation. *Advances in Colloid and Interface Science, 63*, 23–40.
Tolstoguzov, V. (1995). Some physico-chemical aspects of protein processing in foods. Multicomponent gels. *Food Hydrocolloids, 9*(4), 317–322.
Tolstoguzov, V. (1997). Multicomponent biopolymer gels. *Food Hydrocolloids, 11*, 159–170.
Tolstoguzov, V. (2003). Some thermodynamic considerations in food formulation. *Food Hydrocolloids, 17*, 1–23.
Tolstoguzov, V. (2007). Ingredient interactions in complex foods: aggregation and phase separation. In J. McClements (Ed.), *Understanding and controlling the microstructure of complex foods* (pp. 186–206). Cambridge: CRC Press.
Tritt-Goc, J., & Pislewski, N. (2002). Magnetic resonance imaging study of the swelling kinetics of hydroxypropylmethylcellulose (HPMC) in water. *Journal of Controlled Release, 80*, 79–86.
Verheul, M., Pedersen, J., Roefs, S., & Kruif, K. (1999). Association behavior of native β-lactoglobulin. *Biopolymers, 49*, 11–20.
Yoguchi, Y., Urakawa, H., Kitamura, S., Ohno, S., & Kajiwara, K. (1995). Gelation mechanism of methylhydroxypropylcellulose in aqueous solution. *Food Hydrocolloids, 9*(3), 173–179.
Yorgun, M., Balcioglu, I., & Saygin, O. (2008). Performance comparison of ultrafiltration, nanofiltration and reverse osmosis on whey treatment. *Desalination, 229*, 204–216.
Zhuravskaya, N. A., Kiknadze, E. V., Antonov, Y. A., & Tolstoguzov, V. B. (1986a). Concentration of proteins as a result of the phase separation of water–protein–polysaccharide systems. Parts 1. Phase equilibria in water-milk proteins-polysaccharide systems. *Die Nahrung, 30*(6), 591–599.
Zhuravskaya, N. A., Kiknadze, E. V., Antonov, Y. A., & Tolstoguzov, V. B. (1986b). Concentration of proteins as a result of the phase separation of water–protein–polysaccharide systems. Part 2. Concentration of milk proteins. *Die Nahrung, 30*(6), 601–613.