

**Preliminary communications**

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**THERMODYNAMIC INCOMPATIBILITY OF WHEY PROTEINS  
WITH POLYSACCHARIDES IN AQUEOUS MEDIA**

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Phase diagrams of aqueous whey protein (WP) and polysaccharide (PS) mixtures, including carboxymethylcellulose (CMC),  $\kappa$ -carrageenan (C) and locust bean gum (LG) are presented in this paper at pH from 5.0 to 7.0 with 0.1 to 0.5 M NaCl. Thermodynamic incompatibility of WP–CMC, WP–LG and WP–C systems increased as pH was close to the isoelectric point of WP ( $pI=5.2$ ). Increasing salt concentration (0.1 to 0.5 M) increased the incompatibility of WP–LG and WP–CMC systems. However, the effect of NaCl on thermodynamic incompatibility of WP–C was the opposite, i.e. increasing salt concentration decreased the thermodynamic incompatibility of the system. The type of the polysaccharides was the critical factor, which affected the compatibility of WP–PS systems. The thermodynamic compatibility of WP–PS systems decreased in the following order: locust gum > carboxymethylcellulose >  $\kappa$ -carrageenan.

**Keywords:** whey proteins, polysaccharides, thermodynamic incompatibility, phase diagrams

Combinations of biopolymers (proteins and polysaccharides) are commonly used in food products for the purpose of obtaining the desired stability, performance and consistency. The gelation of mixed aqueous solutions of whey proteins and polysaccharides is expected to increase the potential application of whey proteins, as a wider range of textural characteristics can be obtained. However, in order to control product texture and to design processing, it is important to gain knowledge about incompatibility of whey proteins-polysaccharides systems.

By systematic studies of phase separation in ternary protein-polysaccharide systems, conducted by a group of scientists (ANTONOV et al., 1994; ANTIPOVA & SEMENOVA, 1995; GRINBERG & TOLSTOGUZOV, 1997), the thermodynamic incompatibility of proteins and polysaccharides has been found as a very general phenomenon. They conclude that, under certain conditions, any protein-polysaccharide-water system is spontaneously separated into two liquid phases of protein and polysaccharide, inhibiting complexing between proteins and polysaccharides and promoting association between macromolecules of the same type, i.e. self-association of biopolymers. Conditions for incompatibility depend on the structure and composition

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of the given biopolymer pair and pH and ionic strength of the system (HOSKINS et al., 1996). These observations, however, were obtained by analysing the phase state of about 100 protein-polysaccharide-water systems, where the sources of proteins were water soluble proteins used by the food industry, namely gelatine, soy bean protein, casein, bovine serum albumin and ovalbumin. Isolated whey proteins were not used in these analyses. On the other hand, several anionic and neutral polysaccharides have been used in the phase separation studies of the protein-polysaccharide systems.

Mixing behaviour of native whey protein-polysaccharide systems has been studied by SYRBE and co-workers (1995, 1998). Phase separation was observed with mixtures of native whey proteins and neutral polysaccharides, such as maltodextrins, dextrans and methylcellulose, although restricted to pH values from 5 to 7 and high polymer concentrations. On the other hand, anionic polysaccharide-whey protein solutions, including high-methoxyl pectin, sodium alginate or sodium carboxymethylcellulose yielded homogeneous mixtures at pH 6 to 7 in 1% NaCl solutions. However, the authors have no clear answer, whether this behaviour is due to a real compatibility or whether the phase separation threshold has not been reached.

The aim of this study was to investigate the behaviour of aqueous whey protein-polysaccharide mixtures, including carboxymethylcellulose,  $\kappa$ -carrageenan and locust bean gum at pH from 5.0 to 7.0 in 0.1 to 0.5 M NaCl solutions by establishing phase diagrams of mixed systems. The choice of experimental conditions in our study is based on the following suggestions: pH range and NaCl concentrations relevant to food are 4.2–6.5 and 0.1–0.5 M, respectively.

## 1. Materials and methods

Whey protein isolate (WPI) was supplied by courtesy of Arla Foods (Denmark, Viby J), carboxymethylcellulose (CMC) and locust bean gum (LG) by Danisco Ingredients (Denmark, Brabrand), and  $\kappa$ -carrageenan (C) by Chemcolloids Ltd. (Great Britain, Cheshire). WPI had the following characteristics: crude protein content ( $N \times 6.38$ ) 93.5%; fat content: 0.2%; ash content: 2.3%; moisture content: 4.0%, pH 6.5–7.0 and pI 5.2.

A phase-volume ratio method was used for plotting phase diagrams of protein-polysaccharide systems (POLYAKOV et al., 1985). Phase diagrams for protein-polysaccharide systems show the position of binodal curve, which separates the regions of single-phase (under the binodal curve) and two-phase systems (above the binodal curve). The binodal points can be obtained by the system composition phase-volume ratio relationship.

Whey protein (WP) solutions were obtained either by dissolving whey protein isolate in water or specified NaCl solutions (0.1 to 0.5 M) and stirring them for 30 min at room temperature. The proper pH was adjusted by adding 0.1 M NaOH or 1.0 M HCl. Polysaccharide (PS) solutions were either dissolved in water or specified NaCl solutions (0.1 to 0.5 M) by stirring them at 60 °C for 5–6 h. The adjustment of pH was

performed under the same conditions as for the WP solutions. WP and PS stock solutions were prepared separately at the following concentrations: 20% WP, 2% PS. The stock solutions were then mixed at different volume ratios and stirred at room temperature for 5 min. The pH of the mixtures was checked and, where necessary, adjusted to the pH of the stock WP and PS solutions. The mixtures were kept at 20 °C for 12 h to attain equilibrium.

After that the WP–CMC and WP–C mixtures were centrifuged at 1000 g for 1 h. The WP–LG mixtures were centrifuged at 100 g for 30 min. Special transparent test tubes were employed for centrifugation. For the mixtures, which were separated into two phases after centrifugation, the phase-volume ratio ( $r^+$ ) was calculated as follows:

$$r^+ = V^+ / (V^+ + V^-),$$

where  $V^+$  and  $V^-$  are the volumes of the lower and the upper phases, respectively.

The mixture compositions were expressed by values of apparent weight fractions:

$$y = C_A / C_A^0 \text{ and} \\ 1-y = C_B / C_B^0$$

where  $C_A$  and  $C_B$  – weight concentrations of WP and PS in the mixtures, respectively;  $C_A^0$  and  $C_B^0$  – weight concentrations of WP and PS in the initial solutions, respectively.

Dependency  $r^+=f(y)$  was also determined. Phase diagrams were obtained by graphical extrapolation of the determined dependence of  $r^+=f(y)$  by plotting the phase-volume ratio corresponding to the concentrations of the components in the mixture (POLYAKOV et al., 1980).

## 2. Results and discussion

The effect of pH on the phase behaviour of WP–PS systems is shown in Fig. 1. In these experiments the pH range was 5.0–7.0 for WP–C systems and 5.0–6.0 for WP–CMC and WP–LG systems with no addition of NaCl. As we can see in Fig. 1, the binodal curves of all systems at pH 5.0 are located closer to the axes in comparison to those at pH 6.0. This means that incompatibility of WP with all types of polysaccharides becomes more pronounced by shifting the pH of the system towards the isoelectric point of whey proteins (pI=5.2).

To explain the latter phenomenon WP–PS mixtures are to be considered as systems in which the charged  $\text{NH}_3^+$  and  $\text{COO}^-$  groups are on the protein molecules and  $\text{OSO}_3^-$  or  $\text{COO}^-$  groups on the anionic polysaccharides molecules. The carboxylated or sulphated polysaccharides, in our case CMC or C, respectively, have their isoelectric points around or below pH 3. In the pH range used here, the polysaccharides, both anionic and neutral, can be regarded as molecules with minimal self-association.

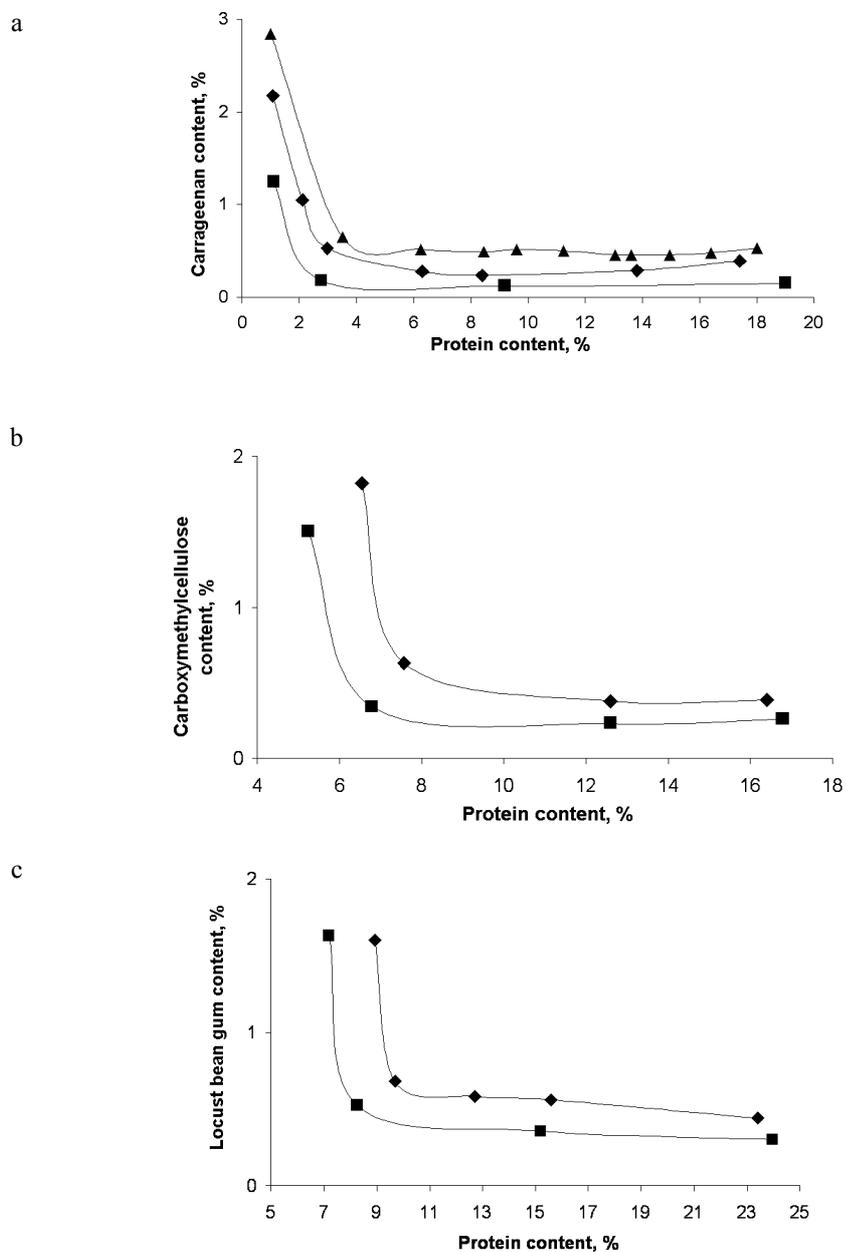


Fig. 1. Phase diagrams for systems. a: WP-C system: ■: pH = 5.0, ◆: pH = 6.0, ▲: pH = 7.0, b: WP-CMC system: ■: pH = 5.0, ◆: pH = 6.0, c: WP-LG system: ■: pH = 5.0, ◆: pH = 6.0

Therefore, the incompatibility of the WP–PS systems at pH 5.0–7.0 is directly related to the protein self-association, which is stronger at the isoelectric point of whey proteins ( $pI=5.2$ ). Thus, at pH 5.0 WP–PS systems are more incompatible.

Figure 2 illustrates the effect of NaCl concentration on the phase diagrams of WP–CMC mixtures at pH 6.0. As the salt concentration increased from 0.2 M to 0.5 M NaCl, the incompatibility of WP with CMC increased as well (binodal curve at 0.5 M NaCl was closer to the axes). According to TOLSTOGUZOV (1994), higher salt concentrations screen the electrostatic interactions between biopolymers and promote the self-association of protein molecules.

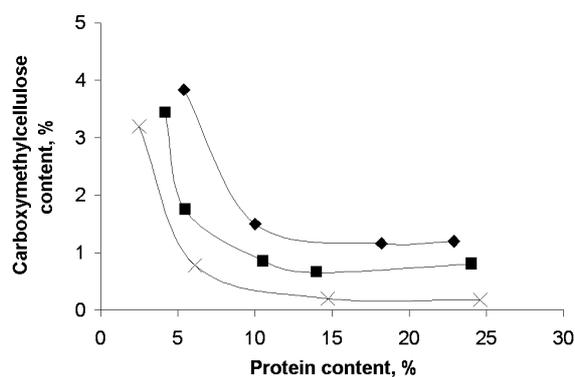


Fig. 2. Phase diagrams of WP–CMC system at pH 6.0. ◆: 0.2 M NaCl; ■: 0.3 M NaCl; ×: 0.5 M NaCl

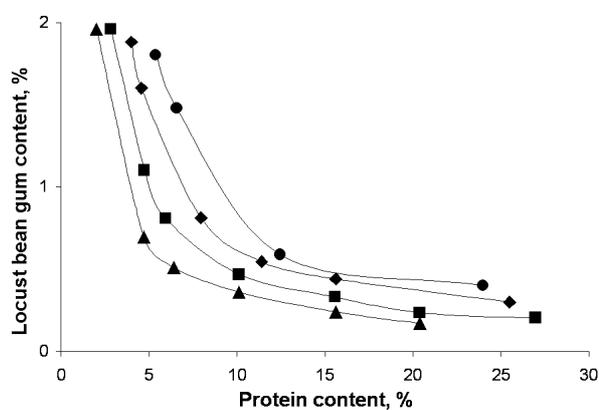


Fig. 3. Phase diagrams of WP–LG system at pH 6.0. ●: 0.1 M NaCl; ◆: 0.2 M NaCl; ■: 0.3 M NaCl; ▲: 0.4 M NaCl

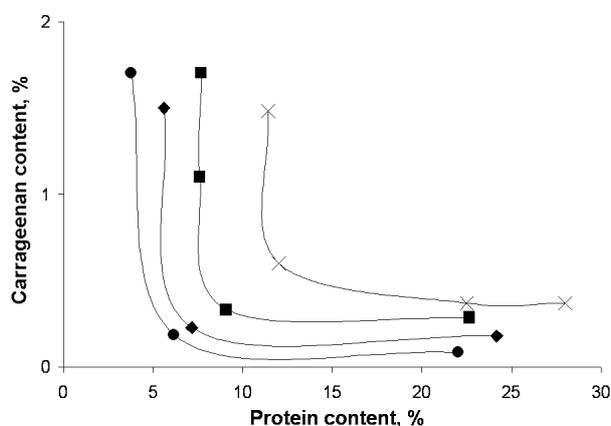


Fig. 4. Phase diagrams of WP-C system at pH 6.0. ●: 0.1 M NaCl; ◆: 0.2 M NaCl; ■: 0.3 M NaCl; ×: 0.5 M NaCl

The experiments conducted with the protein-neutral polysaccharide system, i.e. WP-LG, showed that phase separation occurred over a wide range of NaCl concentrations at pH 6.0 in the system. The increasing salt content caused the incompatibility of the WP-LG system to increase (Fig. 3). The smaller area under the binodal curve at higher NaCl concentration indicated increasing incompatibility of WP-PS systems. The effect of NaCl concentration on WP-C phase diagrams was different from those of WP-CMC and WP-LG systems. The compatibility of the WP-C system was more pronounced when the concentration of NaCl increased from 0.1 to 0.5 M (Fig. 4).

According to MORRIS (1986), monovalent ions such as  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  have a substantial effect on the molecular structure of  $\kappa$ -carrageenan. These positive ions cause the gelation of  $\kappa$ -carrageenan when domains of polysaccharides associate side by side with cations in between and form a network. This phenomenon supports the hypothesis that increasing concentration of  $\text{Na}^+$  ions in WP-C system induces the formation of three-dimensional network of  $\kappa$ -carrageenan molecules, which stabilizes the WP-C system.

The effect of the polysaccharide type on the WP-PS thermodynamic incompatibility is shown in Fig. 5. Phase diagrams developed at pH 6.0 with no addition of NaCl showed that the type of PS affected the incompatibility of these systems. As seen in Fig. 5, the most incompatible system was WP-C (sulphate containing PS) and the most compatible one WP-LG (containing neutral PS).

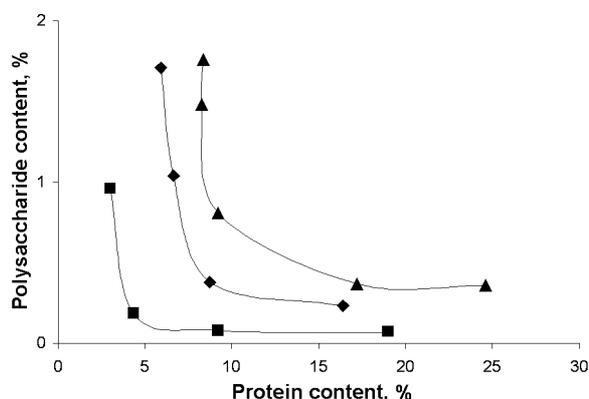


Fig. 5. Phase diagrams of WP-PS systems at pH 6.0 with no added NaCl. ◆: WP-CMC; ▲: WP-LG; ■: WP-C

Our results are different from those described by TOLSTOGUZOV (1997). In these studies bovine serum albumin (BSA), gum arabic, BSA-dextran and BSA-dextran sulphate systems were examined. According to their results, the compatibility of these systems decreased in the following order: carboxyl-containing polysaccharides (gum arabic) > neutral polysaccharides (dextran) > sulphate-containing polysaccharides (dextran sulphate). The differences between our results and those described in the literature (TOLSTOGUZOV, 1997) may be explained by the different structure of polysaccharides used in the experiments. Carboxyl groups containing gum arabic macromolecules have a branched structure with a smaller molecular size compared to CMC, known as a polysaccharide with linear structure. Differences between the molecular size of CMC and gum arabic were illustrated by evaluating the viscosity of polysaccharide solutions. The viscosity of CMC solution was noticeably higher than that of the gum arabic solution (TOLSTOGUZOV, 1995). It can be assumed that the linear polysaccharide, in our case CMC, shows a smaller compatibility region in the protein-polysaccharide systems. However, further work is necessary to prove this hypothesis.

### 3. Conclusions

1. The thermodynamic incompatibility of WP-CMC, WP-LG, WP-C increased when pH was close to pI of whey proteins.
2. Increasing NaCl concentration (from 0.1 to 0.5 M) at pH 6.0 caused an increase in thermodynamic incompatibility of WP-LG and WP-CMC systems. However, the effect of NaCl on thermodynamic incompatibility of WP-C was the opposite, i.e. increasing NaCl concentration caused the decrease of thermodynamic incompatibility of the latter system.

3. The type of polysaccharide was the most decisive factor, which predetermined the compatibility of WP–PS systems. The thermodynamic compatibility of WP–PS systems decreased in the following order: locust bean gum > carboxymethylcellulose > carrageenan.

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