INFLUENCE OF NACL ON RHEOLOGICAL AND MICROSTRUCTURAL PROPERTIES OF HEAT INDUCED WHEY PROTEINS-CARBOXYMETHYLCELLULOSE GELS

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Abstract
In recent years several studies have been made on the gelation of whey proteins (WP) in combination with polysaccharides, focusing mainly on the effect of starch or starch derived gelling polysaccharides, carrageenans, pectins. Only few studies have been performed on the mixed gels made from proteins and carboxymethylcellulose (CMC). The aim of the present research was to characterize the influence of NaCl on the rheological and microstructural properties of mixed whey proteins-carboxymethylcellulose (WP-CMC) gels at pH 6.2. The mixed WP-0.2% CMC gels strength exhibited maximum at 20mM added NaCl followed by a decrease with further addition up to 100mM NaCl. At higher CMC concentration in mixed WP-CMC gels (1.0%) the addition of of NaCl (from 20 mM to 100 mM) caused the decrease of G^*. The results of the time of gelation, inflection temperature and cooling ratio showed the impact of salt on protein-protein interactions in the protein phase of mixed WP-CMC gels.

Keywords: mixed gels, whey proteins, starch, salt influence.

INTRODUCTION

Mixed whey proteins and polysaccharides gels are of particular interest since synergism between these biopolymers can lead to a microstructure very different from that of pure gels and thus to related texture that can improve product quality. In recent years several studies have been made on the gelation of whey proteins (WP) in combination with polysaccharides, focusing mainly on the effect of starch or starch derived gelling polysaccharides [Aguilera and Rojas 1997, Aguilera and Rojas 1996, Manoj and all. 1997, Chronakis and all. 1996, Aguilera and Baffico 1997] carrageenans [Shim and Mulvaney 2001, Capron and all. 1999, Ould Eleya and Turgeon 2000, Neiser and all. 2000, Ould Eleya and Turgeon 2000, Hemar and all. 2002, Capron and all. 1999, Olsson and all. 2000], pectins [Olsson and all. 2002, Mleko and all. 1997]. Only few studies have been performed on the mixed gels made from proteins and carboxymethylcellulose (CMC) [Renard 1998, Castelain 1996].

CMS is an inert binding and thickening agent used to adjust or improve the texture of many food products, such as jellies, paste fillings, spread able processed cheeses, salad dressings and cake fillings. CMC is obtained by treating alkaline cellulose with chloroacetic acid. The properties of CMS, especially its gelling temperature depends on the degree of substitution and CMC concentration in solution. The increasing content of CMC causes the decrease in gelling temperature.

The properties of proteins-polysaccharides gels are mainly governed by pH and ionic strength of the system. Investigations conducted on the influence of salts on the molecular, microstructural and physical properties of thermally induced WP gels revealed the following general trends: at low ionic strength and far from isoelectric point fine-stranded networks are formed [Foegeding 1995]; at high ionic strength and close to the isoelectric point of proteins particulate networks are formed [Langton and Hermansson 1992, Neiser and all. 1998]. However, there are no reports on the role of salt on the rheology and microstructure of mixed gels, containing WP and polysaccharides.

Studies of Neiser at all. [1998] were focused on the influence of pH and ionic strength on the gel strength of heat set gels, containing globular blood protein (BSA) and sodium alginate. The strengthening effect of salt at low ionic strength was recorded and explained by attraction between alginate and positively charged areas on the BSA molecule, which was possible due to the shielding of long range repulsion between molecules. Authors suggested that the decrease in gel strength at high ionic strength was due to a phase separation and a more particulate gel structure.

The aim of the present research was to characterize the influence of NaCl on the rheological and microstructural properties of mixed whey proteins-carboxymethylcellulose (WP-CMC) gels at pH 6.2. Mixed gels with constant WP content (10 %) and two concentrations of CMC – 0.2 and 1.0 % were chosen to be studied, since in our previous work these concentrations showed different effect on the properties of WP-CMC gels.It was found that low concentrations of CMC (0.2-0.3 %) caused the strengthening effect of WP-CMC gels due to the excluded volume effect in the phase-separated network. At high CMC concentrations (0.7-1.0%) the decrease in gel strength with increasing CMC concentration was recorded [Leskauskaite and Treciokiene 2003].

MATERIALS AND METHODS

Materials

The WPI (Lacprodan DI-9224, Arla Food Ingredients, Nr. Vium, DK) contained 93.5% protein, < 4.5% ash, < 0.2% fat, < 0.2% lactose and < 6% moisture (manufacturers specifications). The CMC (CL 110, Danisco Cultor, Århus, DK) had a pH value of 7.0 – 8.0 in a 1% solution and contained 10% moisture (manufacturers specifications).

Solutions of WP and CMC

Stock solutions of WP (20%) were prepared in distilled water at room temperature. The pH was adjusted to 6.2 using 1M HCl, 4M HCl or 0.1N NaOH solutions. Stock solutions containing 2% (w/w) CMC were prepared separately with distilled water by stirring at 50 ^{o}C for 5-6 h, followed by cooling to 20 ^{o}C. Adjustment of pH was made as for the WP solutions. WP and CMC stock solutions were mixed in different ratios to obtain WP-CMC solutions with final concentrations of 10% with regards to whey protein and from 0.1 to 1.0% in terms of dry polysaccharide. pH of the mixtures were checked and if necessary adjusted. The WP-CMC mixtures were equilibrated at room temperature for 12 h.

Small deformation rheology

The viscoelastic properties during thermal gelation were monitored using a controlled stress rheometer (Bohlin CVO, Bohlin Ltd., Cirencester, UK). The complex modulus, G*, defined as $\sqrt{G'^2 + G''^2}$, where G' is the storage modulus and G" the loss modulus, was used as an indicator of the total stiffness of the gels and the phase angle, δ, where tan(δ)= G"/G', was used to characterize the balance between viscous and elastic properties of the gel. The mixed WP-CMC solutions (13 ml) were transferred to the measuring system (Couette geometry, diameter 25 mm). Dynamic oscillatory measurements were performed at a frequency of 1 Hz and a strain of 0.02. The sample was heated from 20^{o}C to 80^{o}C with a gradient of 2^{o}C/min, kept for 30 min at 80 ^{o}C, cooled with a gradient of 1^{o}C/min from 80 ^{o}C to 5 ^{o}C and held at this temperature for 30 min. After that the sample was melted with a gradient of 1^{o}C/min from 5 ^{o}C to 60 ^{o}C. To avoid evaporation, the samples were covered with a thin layer of oil.

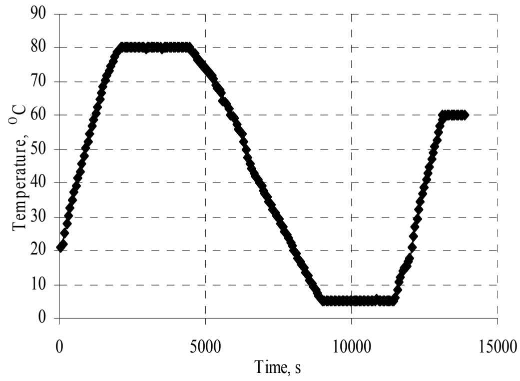


Figure1 **-** Example of gelation curve for mixtures of WP-CMC.

A typical curve is show in ***Figure 1***. The following parameters were extracted from the curves and used for statistical analysis:

t_g = time of gelation, i.e. the time when the phase angel dropped below 45^{o}

T_{ic} = Inflection temperature, i.e. the temperature where the rate of change in G* during cooling from 80 to 5^{o}C was maximal.

T_{im} = Inflection temperature, i.e. the temperature where the rate of change in G* during melting from 5 to 60°C was maximal.
G_i = The stiffness of the gel at T_i
R_c= The cooling ratio, defined as $((G_{80}-G_i)/(80-T_i))/((G_i- G_5)/(T_i-5))$. This ratio reflect the increase in G* in the interval from 80 to T_i compared to the increase from T_i to 5°C
R_m= The melting ratio, defined as $((G_i-G_{60})/(T_i-60))/((G_5-G_i)$ This ratio reflect the decrease in G* in the interval from 5 to T_i compared to the decrease from T_i to 60°C.

Confocal microscopy

Confocal Laser Scanning Microscopy (CLSM) was performed with a Leica TCS-SP system fitted with an inverted microscope and an ArKr laser (Leicia, Heidelberg, D). Fluoresceine isothiocyanate (FITC) was used to label the protein. Samples (10 mm x 10 mm x 1 mm) were cut from gels made in the same way as for the oscillatory measurements and 1-2 droplets of 1 % FITC dispersion in water was added to the surface of the gels. The pieces were placed between a slide and a coverslip and sealed to prevent evaporation. An excitation wavelength of 484 nm was used.

RESULTS AND DISCUSSION

Rheology and microstructure of final gels

The gel strength was expressed by complex modulus G^* at the end of cooling period. The pure WP gels strength exhibited maximum at 20 mM added NaCl followed by a decrease with further addition up to 100 mM NaCl (***Figure 2***). This is in accordance with the results of Chantraporuchai and McClements [2002] who found high gel elastic modulus at NaCl concentration 60 mM. When the NaCl concentration increased up to 200 mM authors observed the decrease of gel elastic modulus. It was proposed that WP gel network changed from strand-like structures to particulate-like structures due to the weakening of electrostatic interactions between proteins.

Our observed influence of NaCl on the complex modulus of mixed WP-0.2% CMC gels was the same as in the case of pure WP gels. It can be also explained in the terms of the impact of salt on protein-protein interactions in the protein phase of mixed gel. NaCl screened the electrostatic interactions in the system; so that protein molecules could closely approach each other strengthening the protein phase in the phase separated mixed gel network due to the increase of proteins strand-strand interactions. When the NaCl concentration was increased further, the electrostatic repulsion between the proteins molecules was screened more effectively therefore protein aggregates formed particulates in the protein phase. The decrease of G^* of WP-0.2% CMC gels at 100 mM NaCl could be contributed to the increase in protein particles size which caused the lower connectivity of phase separated network.

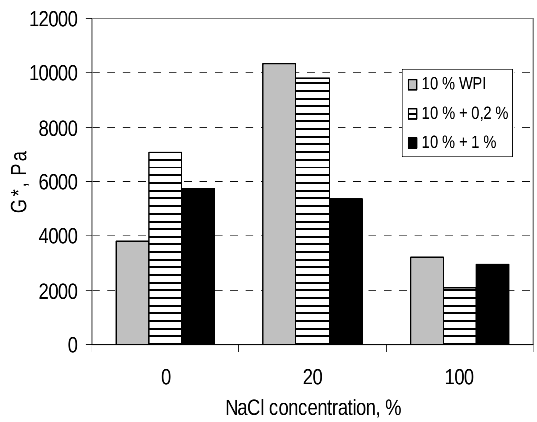
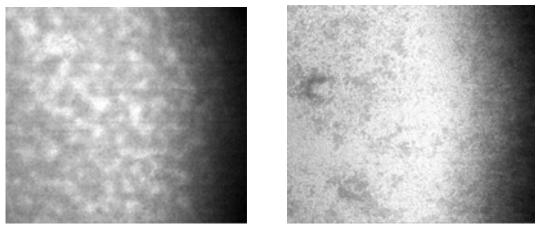
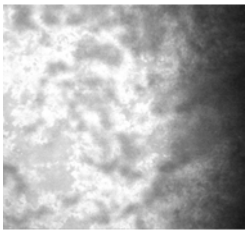


Figure 2 - Final complex modulus (G*) of mixed WP-CMC gels.

This resumption was confirmed by CLSM micrographs of mixed WP-0.2% CMC gels with 100 mM salt added (***Figure 3***).



a) b)



c)

Figure 3 - The influence of NaCl on the microstructure of mixed WP-CMC gels at pH 6.2: a) 10% WP - 1% CMC 0 mM, b) 10% WP - 1% CMC 20 mM, c) 10% WP - 1% CMC 100 mM. The scale: 1 mm - 20 μm.

At higher CMC concentration in mixed WP-CMC gels (1.0%) the addition of even small concentrations of NaCl (20 mM) caused the decrease of G^*. Further increase of added NaCl determined the formation of very weak mixed WP-1.0%CMC gels. The CLSM micrographs of these gels show the structure with loosely connected proteins aggregates clusters with large pores between them.

Seeking to find explanation for the above-mentioned phenomenon we analyzed the changes of viscoelastic properties during different periods of gel formation.

Time of gelation

The gelation of pure WP solution as well as mixed WP-CMC solutions occurred during heating period from 20 to 80 ^oC.

The time of gelation of pure WP solution without added NaCl was 2145 ± 28 s, while the time of gelation slightly decreased with increasing concentration of CMC (***Table 1***). The results also showed that the time of gelation was dependent on NaCl content in the WP-CMC system. At 100 mM NaCl content the gelation occurred more rapidly than at 20 mM NaCl content in pure WP gels. The same tendency was observed in mixed WP-CMC gels containing 0.2 % CMC and 1.0 % CMC.

In the case of pure CMC solution gelation took place during cooling period from 80 to 5 ^oC. The gelation temperature of 1 % CMC solution in distilled water was detected at 53 ^oC and was not influenced by NaCl content .

Table 1

The influence of CMC and NaCl content on the time of gelation of mixed WP-CMC gels

CMC content in mixed WP-CMC gel	Time of gelation (s) of WP-CMC gels at NaCl content during heating		
	0 mM	20 mM	100 mM
0 %	2145 ± 28	1834 ± 21	1713 ± 20
0.2 %	1816 ± 20	1752 ± 18	1642 ± 22
1.0 %	1741 ± 17	1702 ± 15	1613 ± 18

Changes of viscoelastic properties of gels during cooling

The influence of NaCl on the viscoelastic properties of pure WP and mixed WP-CMC gels was examined during cooling step from 80 to 5 ^oC. The maximum rate of increase in G^* for different samples was noticed at different inflection temperatures (***Table 2***). For pure WP gels without addition of NaCl and with 20 mM of NaCl the inflection temperature T_{ic} was observed at 47.7 ±0.9 ^oC. The same temperature was recorded for the mixed gels containing 0,2 % of CMC. However when concentration of NaCl was 100 mM, the maximum rate of increase in G^* was detected at 25.1 ^oC in the case of pure WP gels, whereas mixed WP-0,2%CMC gels showed no maximum rate at this salt concentration. The same tendencies were specified for mixed WP-CMC gels with higher content of CMC (1.0 %). However for these gels we found different temperature at which the maximum rate of increase in G^* arised, i.e 28.2 ±0.7 ^oC.

Table 2

The influence of CMC and NaCl content on the inflection temperature of mixed WP-CMC gels

CMC content in mixed WP-CMC gel	Inflection temperature (^oC) during cooling		
	0 mM	20 mM	100 mM
0 %	47,7 ± 0,9	47,7 ± 0,9	25,1 ± 0,5
0.2 %	47,7 ± 0,9	47,7 ± 0,9	-
1.0 %	28,2 ± 0,7	28,2 ± 0,7	28,2 ± 0,7

Thereby, there were differences between increase in gel stiffness during initial cooling stage and further cooling stage. However, for pure WP gels and WP-0.2%CMC gels the initial cooling stage was from 80 to 47.7 ^{o}C and for WP-1.0%CMC gels the initial cooling stage was longer, i.e. from 80 to 28.2 ^{o}C.

The calculated cooling ratio R_c indicated that for all gels the major increase in gel stiffness took place before T_{ic} ($R_c > 0.5$) (***Figure 4***). The addition of NaCl caused the decrease of R_c in mixed WP-CMC gels. In the case of pure WP gels the addition of 20 mM of salt also caused the decrease of R_c. Further increase in NaCl content up to 100 mM gave the increase of R_c.

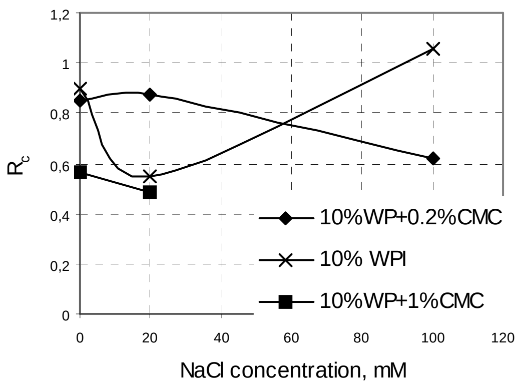


Figure 4 **-** The cooling ratio (R_c) as a function of NaCl content.

It should be mentioned that for pure CMC gels the even increase in G^* was estimated during the cooling stage at all concentrations of NaCl.

Changes of viscoelastic properties of gels during melting

The changes of viscoelastic properties of pure WP, pure CMC and mixed WP-CMC gels with different NaCl content were observed during melting from 5 to 60 ^{o}C. At this step the maximum rate of decrease in G^* was observed for all gels within a narrow temperature interval. An average inflection temperature at this step was 26.9 ± 1.9 ^{o}C.

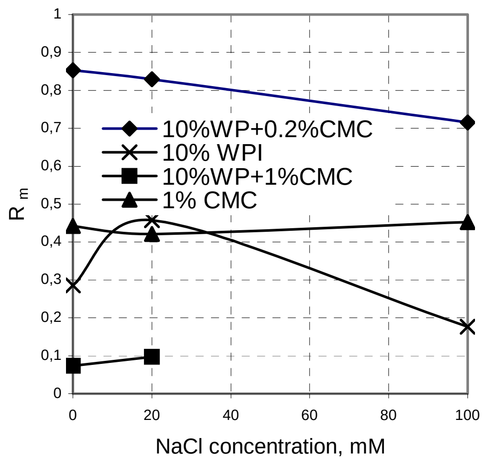


Figure 5 **-** The melting ratio (R_m) as a function of NaCl content.

The melting ratio R_m was calculated. The results presented in ***Figure 5*** indicated the major decrease in gel stiffness after T_{im} ($R_m < 0.5$) in the case of pure WP and mixed WP-1.0%CMC gels slight increase of R_m at 20mM and the significant decrease of R_m at 100 mM estimate the influence of salt on the properties of pure WP gels. When CMC content in gels was 0.2 % the major decrease of G^* occurred during the second melting stage from 26.9 to 60^{o}C. The addition of NaCl caused the even decrease of R_m.

CONCLUSIONS

The mixed WP-0.2% CMC gels strength exhibited maximum at 20 mM added NaCl followed by a decrease with further addition up to 100 mM NaCl. At higher CMC concentration in mixed WP-CMC gels (1.0%) the addition of of NaCl (from 20 mM to 100 mM) caused the decrease of G^*. The results of the time of gelation, inflection temperature and cooling ratio showed the impact of salt on protein-protein interactions in the protein phase of mixed WP-CMC gels. When the NaCl concentration was 100 mM, the electrostatic repulsion between the proteins molecules was screened and protein aggregates formed particulates in the protein phase.

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