

## Rheological characterization of hydrogels based on gelatin/ $\kappa$ -carrageenan mixtures

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### ABSTRACT

In this study, influence of concentration of  $\kappa$ -carrageenan on rheological properties and thermal stability of gelatin/ $\kappa$ -carrageenan hydrogels has been studied. It was shown that an increase of the concentration of  $\kappa$ -carrageenan leads to growth of the thermal stability of gelatin/ $\kappa$ -carrageenan gels. The strength of gelatin gels increases with the increase of  $\kappa$ -carrageenan concentration. The models of gelatin/polysaccharide complexes and mechanisms of their formation are discussed. Properties of hydrogels, modified by  $\kappa$ -carrageenan, change due to the formation of complexes caused generally by electrostatic interaction between positively charged groups of gelatin and negatively charged sulfate groups of  $\kappa$ -carrageenan. Structure of modified hydrogels was studied.

### INTRODUCTION

Native gelatin is widely used as a gelation agent in food production. The applicability of gelatin is limited by physical and chemical properties of gelatin gels, particularly, thermal stability and strength of the gels. In some cases, modification of gelatin is required. The gelatin can be modified by additives of surfactants and biopolymers. One of the most popular and perspective methods of gelatin modification is using of polysaccharides as a co-gelation agents. The action of polysaccharides is to

form polyelectrolytes complexes in aqueous solutions in the result of interaction between charged groups of gelatin and macro ions of polysaccharide<sup>1</sup>. One of the promising polysaccharides for creating functional food products is  $\kappa$ -carrageenan which is non-toxic and biodegradable. Numerous studies demonstrate that addition of polysaccharides effects on rheological properties of gelatin-based gels<sup>2</sup>. This is achieved by formation of polyelectrolyte complexes in aqueous phase and, in so doing, the yield stress and viscosity of gels growth with increasing concentration of polysaccharide.

This current work is aimed to study of rheological properties of gels formed from gelatin and  $\kappa$ -carrageenan mixtures at different polysaccharide/gelatin ratio. The impact of  $\kappa$ -carrageenan on thermal and rheological properties of  $\kappa$ -carrageenan/gelatin gels was investigated. The mechanism of interaction between components including formation of polyelectrolyte complexes is discussed.

### MATERIALS AND METHODS

An alkaline gelatin (225 Bloom) from bovine skin with average molecular weight of  $1 \cdot 10^5$  g/mol was used (Sigma Aldrich, Germany). pH at the isoelectric point of gelatin is 4.9. The sample of  $\kappa$ -carrageenan from the red algae with average molecular weight of  $6.8 \cdot 10^5$  g/mol was used (Sigma Aldrich).

Aqueous solutions of gelatin and  $\kappa$ -carrageenan were prepared separately. The samples were preliminary swelled in distilled water at 20 °C for 1 h. Then they were dissolved at 50 °C and 80 °C respectively. After that, aqueous solutions of biopolymers were mixed together in a given ratio  $Z = m_{\kappa\text{-carrageenan}}/m_{\text{gelatin}}$ . Concentration of  $\kappa$ -carrageenan was ranging from 0.01 to 1 wt. %. Concentration of gelatin in mixtures was 1wt. %. Gels were obtained from aqueous solutions at 14 °C for 5 h. Rheological properties of gelatin and modified gels were determined using the Physica MCR 302 rheometer with a cone-plate measuring cell. The cone diameter was 50 mm and the cone to plate angle was 1°. The gap between the cone apex and plate was 0.100 mm. The amplitude dependencies were obtained at 14 °C at constant frequency of 1 Hz at amplitude ranging from 0.1 to 1000 %. Frequency dependencies were measured at constant amplitude of 1 % and at constant temperature of 14 °C in the frequency range from 0.05 to 200 s<sup>-1</sup>. The melting and gelling temperatures of gelatin/ $\kappa$ -carrageenan based gels were measured at a constant frequency of 1 Hz and a constant applied strain of 1 %. The samples were heated from 14 to 50 °C and subsequently cooled to 5 °C at constant rate of 2 °C/min. FT-IR spectra of samples were registered with IR-Fourier spectrometer Nicolet 700 (Thermo Scientific, Madison WI, USA) in the middle range of 400-4000 cm<sup>-1</sup> IR radiation. Gel microstructure was studied by the scanning electron microscopy (SEM) using a microscope S405-A (Hitachi, Tokyo, Japan) supplied with the SEM LEO-420 software.

## RESULTS AND DISCUSSION

The "melt transition temperature" is determined as the crossover point of storage and loss modulus. Figure 1 demonstrates the dependence of the melting and gelling temperatures of hydrogels on the  $\kappa$ -

carrageenan/gelatin ratio. The sol-gel transition temperature sharply increases at the  $\kappa$ -carrageenan/gelatin ratio of 0.05. Gelling temperature of modified gels increased as  $\kappa$ -carrageenan/gelatin ratio increased. The addition of  $\kappa$ -carrageenan strongly affected the thermo-stability of  $\kappa$ -carrageenan/gelatin hydrogels what can be related with formation of polyelectrolyte complexes between positively charged gelatin and negatively charged  $\kappa$ -carrageenan with following transformation of supramolecular structure of hydrogels.

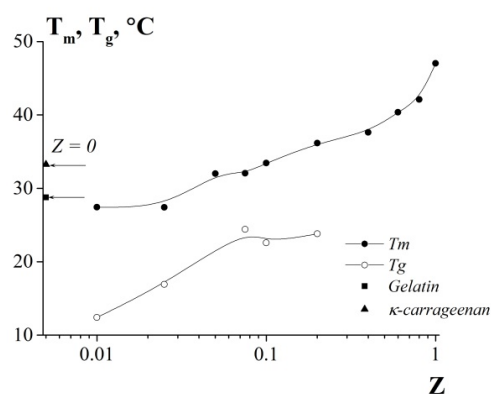


Figure 1. Dependencies of gelling temperature ( $T_g$ ) and melting temperature ( $T_m$ ) of carrageenan/gelatin based gels on  $Z$ .  $\gamma = 1\%$ ,  $\omega = 6.28\text{ c}^{-1}$ .

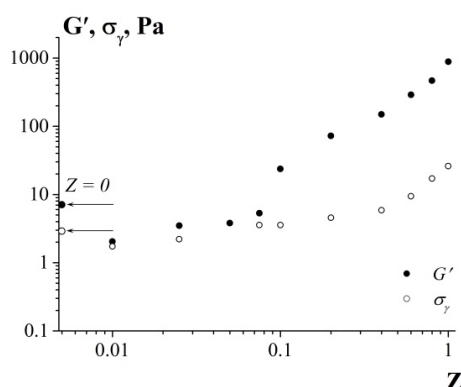


Figure 2. Dependencies of the yield stress  $\sigma_\gamma$  and the storage modulus  $G'$  on the  $\kappa$ -carrageenan/gelatin ratio  $Z$ .

The measurements of the viscoelastic properties of the systems after gelation (at 14 °C) show that they are physical gels. The storage modulus practically does not depend on frequency in the linear domain of viscoelastic behavior. Similar behavior is characteristic the solid-like materials. This applies for both modified and native gelatin gels. The storage modulus of modified gels exceeds modulus of individual components at all frequencies (Figure 2). When carrageenan was incorporated, carrageenan/gelatin gels became more resistant to applied force as shown by the increased gel strength.

Intermolecular interaction between gelatin and  $\kappa$ -carrageenan is confirmed by the FT-IR spectroscopy data. The shift of Amide I and Amide II lines to the low frequency region appeared in the result of interaction between positively charged amide groups in a polypeptide chain of gelatin and negatively charged sulfate groups in  $\kappa$ -carrageenan with following formation of polyelectrolyte complexes. The shift of the Amide I band into the low frequency range suggests that the conformation state of gelatin macromolecules changes with an increase of the order structures as the result of complex formation. Evolution of the conformation state obliged to modification and an increase in intermolecular contacts might cause an increase of the strength and viscoelastic parameters of modified gels.

Supramolecular structure of native gelatin formed in the result of the self-organization process is characterized by transversal collagen like helix (Fig. 3a). The size of helix strongly depends on gelatin concentration. Network is formed by fibrils. Furthermore, gel structure includes discrete zones distributed along fibrils inside a network, which presumably consist mainly of non-helical pieces of macromolecules. Addition of even small quantity  $\kappa$ -carrageenan leads to significant changes in the gel structure (Fig. 3b).

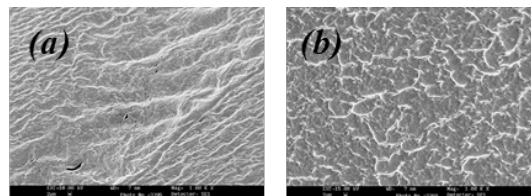


Figure 3. Micrographs of gels structure: (a) – 20 wt% of native gelatin, (b) - the same gel with adding of  $\kappa$ -carrageenan ( $C_{\text{car}} = 0.5$  wt %) obtained at 1000x magnification.

These changes are initiated by interactions between helix zones in gelatin with polysaccharide due to the formation of  $\kappa$ -carrageenan-gelatin complexes. One can believe that contacts with  $\kappa$ -carrageenan in gels based on carrageenan-gelatin mixtures are stronger than hydrogen bonds formed by gelatin that leads to the formation of the interactions between helix zones in macromolecules. This leads to the increase of viscoelastic properties.

#### ACKNOWLEDGMENTS

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