

D – FOOD ADDITIVES AND CONTAMINANTS

The Effect of Chemical Modifications on Rheological Properties of Collagen from of Baltic Cod (*Gadus morhua*) Skin Stabilized by Interaction with κ -carrageenan

R. TYLINGO* and M. SADOWSKA

Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdansk University of Technology, Gdansk, Poland, *E-mail: robertt@chem.pg.gda.pl

Abstract: The κ -carrageenan-collagen complex was precipitated by κ -carrageenan from solution of collagen sourced from Baltic cod (*Gadus morhua*) skin in 0.5M acetic acid. The influence of glutaraldehyde or EDC [1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride] concentration on rheological properties of κ -carrageenan-collagen complex was examined. At 5 to 30mM glutaraldehyde content in 0.5 κ -carrageenan-collagen dispersion a rapid increase in its viscosity was observed, which remained unchanged over 120 min. Further increase in glutaraldehyde concentration to 30mM caused enhanced viscosity and shorten time of complex stability, which not exceed 45 min. The level of dispersion viscosity caused by glutaraldehyde cross-linking ranged from 70 to 420 mPa·sec. For comparison, at EDC concentrations from 30 up to 180mM the viscosity of suspension changed after 200 min from 130–280 mPa·sec. Glutaraldehyde not influenced on mechanical resistance of the membranes as well as elongation at the break. However, EDC cross-linking significantly increased the flexibility of the membranes and decreased the stress needed at break. It was observed that at 30 mM concentration of EDC the tensile strength of the membranes was reduced from about 21 MPa to 11 MPa, and the elongation at the break was enhanced from about 0.4 to 20%. At 60 mM concentration of EDC the stress at break was reduced to 1.4 MPa, whereas the elongation at break was about 17%. Cross-linking of the membranes decreases their hydrophilic properties.

Keywords: fish collagen; κ -carrageenan; cross-linking

INTRODUCTION

Precipitation of collagen in fibryllar form by κ -carrageenan occurs only in case of acidified solutions, because interactions between both biopolymers are caused by electrostatic interactions. Modification of pH value leads to reversibility of the process and reiterated solubility of the collagen [1]. Furthermore, collagen extracted from skins of fish living in cold waters show lower denaturation temperature as compared with collagen from the mammals skin. The phenomenon influences on thermal stability of the collagen products. Therefore properties of collagen should be improved by reduction of its solubility and increased shrinkage

temperature. Different physical processes such as drying, heating and cross-linking associated with aging and irradiation were investigated [2]. The most widely used reagent for crosslinking of collagen is transglutaminase. Traditionally glyoxal, polyepoxy compounds, glutaraldehyde, formaldehyde, heksametylene diisocyanate, acyl azide [3] was applied as chemical cross-linking agents. However, recently EDC has been widespread used for cross-linking process [4–7]. The choice of cross-linking agent and its concentration depends on desired modification of collagen – based materials, including: water holding capacity, solubility in relation to pH and temperature medium, biodegradability and mechanical properties.

The aim of this study was to investigate the usefulness of glutaraldehyde and EDC for modification of mechanical properties and water holding capacity of membranes containing collagen from Baltic cod skin.

EXPERIMENTAL

Collagen was extracted from whole skins of Baltic cod (*Gadus morhua*) using 0.5M acetic acid according to procedures described previously [8]. Collagen membranes were formed from 1% solution of κ -carrageenan-collagen. The final concentrations of cross-linking reagents in dispersion were 15mM or 30mM glutaraldehyde, and 30mM or 60mM EDC, respectively. After cross-linking the dispersion was extensively stirred, and poured onto moulds and dried. Dry and weighted film were immersed in 20 cm³ of McIlvaine's buffer at pH 7 and room temperature for 30 min. After removal from the buffer and dripping on Buchner funnel the samples were weighted. The swelling value (*S*) was calculated according to the formula: $S = (w_2 - w_1)/w_1$ (g H₂O/g membrane) where: w_1 – weight of the dry membrane and w_2 – weight of the swollen membrane. Rheological properties of 0.5% dispersion of carrageenan/collagen mixture were characterized using Brookfield's viscosimeter (DV-III+) with a mouthpiece for small volumes.

Mechanical properties of the membranes were characterized using an Instron (5543) universal mechanical tester controlled by computer program Merlin.

RESULTS AND DISCUSSION

Influence of cross-linking of carrageenan collagen mixture solution on its viscosity

Both cross-linking agents caused a rapid increase in the viscosity of the dispersion of carrageenan/collagen. At presence of glutaraldehyde the final viscosity remains at constant level. However, the preparations cross-linked by EDC show a small increase of viscosity after prolonged incubation time (Figures 1 and 2).

The similar stability in the viscosity of carrageenan collagen dispersion was observed in the presence of 15 and 20 mM glutaraldehyde and was increased from about 70 cP to 300 cP. Whereas EDC at concentration of 60 mM increased the viscosity to 230 cP. At enhanced concentrations of the reagents the viscosity changes proceed in different manner, as immediately after adding the cross-linking agents to measuring cells the outer surface is cross-linked. The heterogeneous system is formed and after some time is unified as an effect of rotation of the gauge plunger of the viscometer. Different results of the influence of glutaraldehyde cross-linking of the pig skin collagen were obtained by Ho *et al.* [9]. An increase of the viscosity in the dispersion treated in glutaraldehyde in the range concentrations from 5 to 30mM was not dramatic. It was not until 20 min that the viscosity was established, which was a sign that the cross-linking process was completed. The same viscosity was obtained using 20mM glutaraldehyde cross-linking within

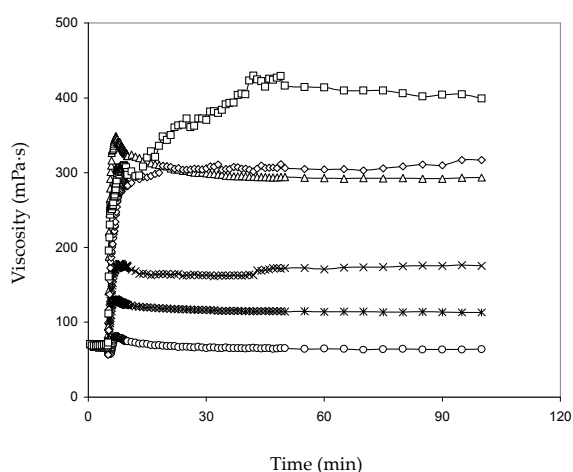


Figure 1. The influence of glutaraldehyde concentration on the viscosity of 0.5% κ -carrageenan-collagen dispersion (w/w) —□— 5; —*— 7.5; —x— 10; —△— 15; —◇— 20; —□— 30 (mM)

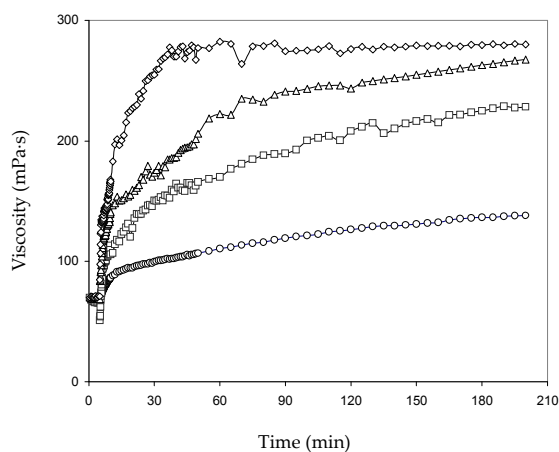


Figure 2. The influence of EDC concentration on the viscosity of 0.5% κ -carrageenan-collagen dispersion (w/w) —□— 30; —□— 60; —△— 90; —◇— 180 (mM)

60 min. The 12mM glutaraldehyde cross-linking process of the pig skin collagen in 0.1% dispersion was completed within 24 h by SHEU *et al.* [10]. This discrepancy between the results in the present study and results which were obtained by the others authors might be explained by differences in origin, concentration of collagen in examined systems and thus different ratio collagen glutaraldehyde.

Influence of chemical cross-linking of κ -carrageenan-collagen dispersion on mechanical properties of the membranes

It was observed that glutaraldehyde cross-linking at concentration, 15 to 30mM did not affect the tensile strength of the membranes (Table 1, Figure 3). Until the rupture deformation was directly proportional to the applied stress. When, concentration of glutaraldehyde was increased the elongation of the membranes at break de-

creased, whereas their fragility was increased. Contradictory results of the effect of EDC cross-linking on mechanical properties were obtained (Figure 4). At increased concentration of EDC a large increase in the flexibility of the membranes were observed whereas their tensile strength was decreased (Table 1). Stress-strain curves of EDC cross-linked membranes were comparable to the one of polyethylene sheeting.

Differences in mechanical properties of the membranes might be explained by different mechanism of κ -carrageenan-collagen cross-linking using EDC or glutaraldehyde. EDC is not involved in creation of the covalent bonds but activate carboxylic acid groups which subsequently react with adjacent amine groups forming amide bonds [11]. Additionally EDC might activate carboxylic and sulphate acid groups, which contain. Whereas glutaraldehyde participate in formation of covalent bonds.

Table 1. The influence of cross-linking agent on mechanical proprieties of the κ -carrageenan-collagen membranes

Cross-linking agent	Concentration (mM)	σ^* (Mpa)	ε^{**} (%)
None	–	20.6 ± 1.80	0.4 ± 0.10
Glutaraldehyde	15	20.6 ± 1.80	0.4 ± 0.10
	30	19.1 ± 3.82	0.2 ± 0.05
EDC	30	11.4 ± 1.29	20.3 ± 3.87
	60	1.4 ± 0.23	16.9 ± 2.29

*Stress; **Strein

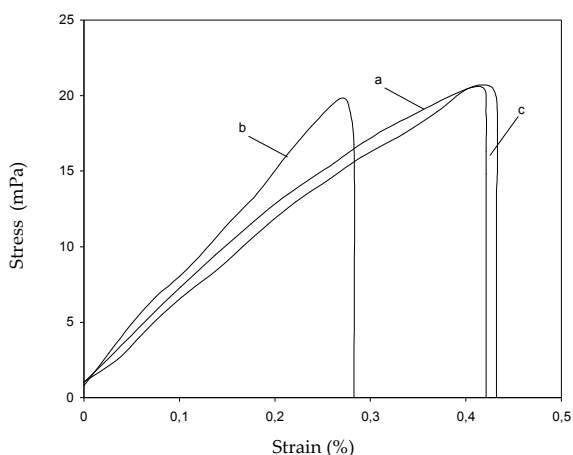


Figure 4. The influence of EDC concentration on mechanical proprieties of membranes: a – 30mM; b – 60mM

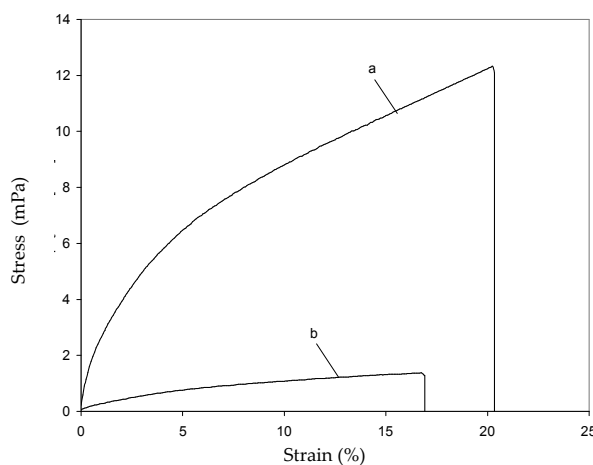


Figure 3. The influence of glutaraldehyde concentration on mechanical proprieties of membranes: a – 0.15%; b – 0.30%; c – membrane non cross-linked

Influence of chemical cross-linking of the membranes on their hydrophilic properties

Different cross-linking mechanisms connected with differences in rheological properties of chemical cross-linked membranes influences on their water uptake ability (Table 2). EDC cross-linked membranes showed relatively lower water uptake ability in comparison with glutaraldehyde treated membranes. It was observed that membranes treated with glutaraldehyde at pH 6.8 showed three times lower water uptake ability in comparison with the membranes cross-linked using co dispersion method. Whereas EDC cross-linking did not effect their water uptake ability.

Table 2. The influence of cross-linking agent and the cross-linking method of the κ -carrageenan-collagen membranes on water uptake ability (Water Holding Capacity – WHC)

Cross-linking agent	Concentration (mM)	WHC (g H ₂ O/g of membrane) codispersion
None	–	18.3
Glutaraldehyde	15	16.2
EDC	60	1.8

CONCLUSION

Comparison of rheological properties of EDC and glutaraldehyde cross-linked carrageenan-collagen dispersion, showed that these cross-linking agents caused an increase in the viscosity of the dispersion. However, the cross-linking by glutaraldehyde was faster. For comparison the EDC treatment influence on higher decrease in water uptake ability of the membranes than that achieves with

glutaraldehyde. The highest differences were also observed in elongation at break of the membranes treated with EDC and glutaraldehyde. It seems, that there is no correlation between stress at break and elongation at break of the membranes. EDC was more effective cross-linking agent because better improve functional properties of the membranes than glutaraldehyde. In apposite to EDC, glutaraldehyde is toxic, formed membranes had yellow colour and gave off unpleasant pungent odour.

References

- [1] SADOWSKA M., KOŁODZIEJSKA I. (2004): Food Chem., in press
- [2] SIONKOWSKA A. (2000): Polymer Degradation and Stability. **68**: 147–151.
- [3] LEE C.H., SINGLA A., LEE Y. (2001): Int. J. Pharmaceut., **221**: 1.
- [4] OLDE DAMINNK L.H.H., DIJKSTRA P.J., VAN LUYN M.J.A., VAN WACHEM P.B., NIEUWENHUIS P., FEIJEN J. (1996): Biomaterials, **17**: 679.
- [5] PARK S.N., PARK J.C., KIM H.O., SONG M.J., SUH H. (2002): Biomaterials, **23**: 1205.
- [6] PARK S.N., LEE H.J., LEE K.H., SUH H. (2003): Biomaterials, **24**: 1631.
- [7] TENGVALL P., JANSSON E., ASKENDAL A., THOMSEN P., GRETZER CH. (2003): Colloids and Surfaces B: Biointerfaces, **28**: 261.
- [8] SADOWSKA M., KOŁODZIEJSKA I., NIECIKOWSKA C. (2003): Food Chem., **81**: 257.
- [9] HO H.O., LIN C.W., SHEU M.T. (2001): J. Contr. Release, **77**: 97.
- [10] SHEU M.T., HANG J.C., YEH G.C., HO H.O. (2001): Biomaterials, **22**: 1713..
- [11] ZEEMAN R., DIJKSTRA P.J., VAN WACHEM P.B., VAN LUYN M.J.A., HENDRIKS M., CAHALAN P.T., FEIJEN J. (1999): Biomaterials, **20**: 921.