D8

# FUNCTIONALITY OF CARRAGEENAN AND SALT-SOLUBLE MEAT PROTEINS IN MODEL SYSTEMS

ZORAIDA DEFREITAS, JOSEPH G. SEBRANEK, DENNIS G. OLSON, AND JIM M. CARR
Departments of Animal Science, and Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, USA
Jim Carr is with Sanofi Bio-Industries, Waukesha, WI.

## INTRODUCTION

The current consumer trends toward low calorie foods has increased the food industry's interest in developing low-fat meat products with acceptable sensory characteristics. During the manufacturing of low-fat products, fat is partially replaced by non-meat ingredients such as carrageenan, which help improve water retention, consistency, and texture. Most of the research on carrageenan/protein interactions has been conducted on dairy systems. Little is known about the molecular mechanisms by which carrageenan (CGN) interacts with meat proteins. The objectives of this study were to evaluate model systems for possible interactions between three CGN types (κ, ι or λ) and salt-soluble meat proteins (SSMP), and to study the molecular forces involved during the SSMP and CGN interactions. This paper consists of three major parts, each part containing two or three separate experiments.

## MATERIALS AND METHODS

Part 1. Functionality of Dry Carrageenan in SSMP Gels. SSMP were extracted as described by Camou et al. (1989). 0.5 % CGN (κ, ι, and λ) was added to 5 % SSMP extract to make SSMP/CGN gels. The mixtures were homogenized, heated until their internal temperature reached 70 °C, and cooled overnight at 2 °C. Gel strength (hardness) and water losses were recorded after compressing the gels 20 % of their height with an Instron machine and centrifuging at low speed to collect the water released from the gels. CGN and SSMP alone were used as control gels

Sodium chloride (0.05 M), sodium thiocyanate (NaSCN, 0.05 M), urea (0.05 M), 2-mercaptoethanol (MeSH, 0.02 M) and propyl glycol (PG, 10 %), were added to 1% and 5% SSMP solutions to study the molecular forces involved during gel formation. SSMP and SSMP/κ-CG gels were made as described above. Gel strength, water losses, protein concentration and characterization (SDS-PAGE) of these gels were evaluated. Two ionic strength solutions (0.47$\mu$ and 0.38$\mu$) were used to make κ-CGN gels. The above reagents were added to the solutions, followed by the addition of 0.5 % κ-CGN.

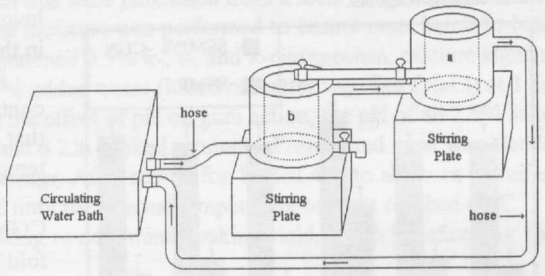



Fig. 1 Temperature control system designed to mix carrageenan in solution with salt-soluble meat protein extracts. Glass-jackets represented by the letters "a" and "b."

Part 2. Functionality of Carrageenan in Solution-SSMP Gels. SSMP/κ-CGN gels were made by using a temperature control system (Fig. 1). CGN solutions and SSMP extracts were added to beakers "a" and "b," respectively, and stirred until the temperature dropped to 40 °C. When both solutions reached 40 °C, 50 mL of 1% CGN solution (0.26 μ) was added to 50 mL of SSMP (2%, 3% and 5%) extract and stirred for few seconds. Control gels (κ-CGN and SSMP gels) were made in similar way. The solutions were heated until their internal temperature reached 70 °C, and cooled overnight at 2 °C. The gels were evaluated for changes in gel strength, water losses and ultrastructure (SEM).

Part 3. Effect of CGN on the thermal transition characteristics of meat proteins. SSMP and myofibrillar proteins (MP) were extracted following Camou et al. (1989) and Lever-Garcia (1988) methods, respectively. SSMP, SSMP/κ-CGN and κ-CGN gels were prepared with 5% SSMP and two CGN concentrations (0 and 0.5 %). MP, MP/κ-CGN and κ-CGN gels were made of 5 and 10 % MP and 0, 0.5 and 2 % CGN. The mixtures were homogenized for 30 sec. and kept overnight in a cooler at 2 °C. Ground lean pork muscles were prepared according with the procedure described by Shand et al. (1994) and included two ionic strength conditions (0.18 and 0.52) and three types of CGN (κ, ι, and λ). A Perkin-Elmer DSC-7 was used to measure thermal transition temperature maxima ($T_{max}$) and enthalpy (ΔH) of meat proteins and CGN.

The Statistical Analysis System (SAS, 1991) was used to determine means, standard errors and analysis of variance. Least significant difference P(<0.05) was used to test differences between means. All experiments were replicated at least twice.

## RESULTS AND DISCUSSION

Part 1. Functionality of Dry Carrageenan in SSMP Gels. Addition of CGN (κ, ι, and λ) to SSMP significantly (P<0.05) affected the physical properties of SSMP gels (Table 1). κ- and ι-CGN increased (P<0.05) the gel strength of SSMP while λ-CGN was detrimental to gelation. The reason for the behavior of λ-CGN in the presence of SSMP is unknown. However, it may suggest that highly negative-charged λ-CGN molecules could change the orientation of the SSMP proteins, resulting in the formation of weaker gels. The force needed to compress SSMP and κ-CGN gels independently did not add up to the total force required to compress SSMP/κ-CGN gels. This may indicate that a protein/hydrocolloid interaction between SSMP and κ-CGN had occurred. No significant (P>0.05) difference was observed between pure ι- and λ-CGN in terms of gel strength. All CGN gels had lower gel strength than SSMP and SSMP/λ-CGN gels (Table 1). Addition of CGN (κ, ι and λ) significantly (P<0.05) decreased the percentage of water loss from the samples after centrifugation, indicating that all CGNs improved water retention of SSMP gels (Table 1). The effect of various stabilizing/destabilizing reagents on the formation and hardness of gels may be correlated with molecular forces contributing to the formation or maintenance of the network structure of the gels. The effect of these reagents (NaCl, NaSCN, urea, 2-MeSH and PG) on the gel strength of SSMP/κ-CGN gels made with 1 % protein extract and 0.5 % κ-CGN is shown in Fig. 2. Gel strength was significantly (P<0.05) affected by

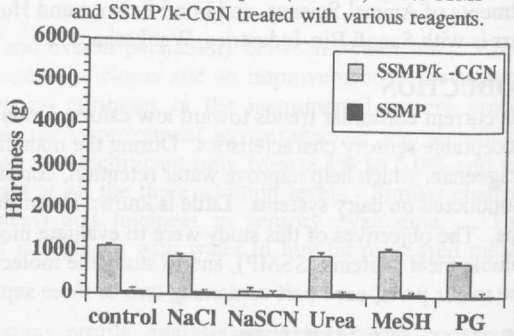
Table 1. Physical properties of SSMP/CGN gels.[1]

| Treatment | Hardness (g) | Water losses (%) |
|---|---|---|
| SSMP | 1333.8±162.5$^{c}$ | 6.04±2.87$^{a}$ |
| SSMP/κ-CGN | 4442.3±504.9$^{a}$ | 2.10±1.19$^{b}$ |
| SSMP/ι-CGN | 1846.2±291.8$^{b}$ | 2.69±0.88$^{b}$ |
| SSMP/λ-CGN | 896.0±208.6$^{d}$ | 2.95±1.19$^{b}$ |
| κ-CGN | 597.2±203.4$^{e}$ | nd |
| ι-CGN | 18.6±8.4$^{f}$ | nd |
| λ-CGN | 9.90±3.1$^{f}$ | nd |
| Std error | 149.6 | 0.99 |

[1] Means in the same column with different superscripts are significantly different (P<0.05).

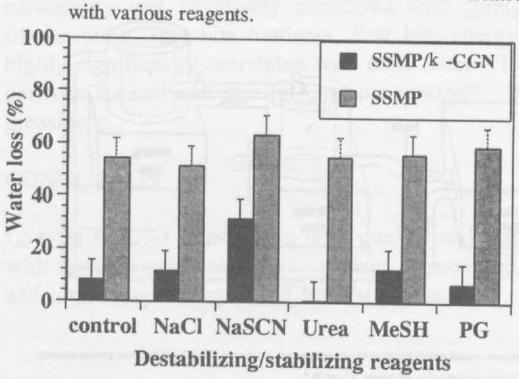
type of gel (SSMP and SSMP/κ-CGN) and type of reagent. SSMP gels were significantly ($P<0.05$) softer than SSMP/κ-CGN gels, except those treatments containing NaSCN, which were as soft as SSMP gels. The number of disulfide bonds within the sample appeared to have no effect on the stability of heat-induced gels. These results are in agreement with the data published by Gordon and Barbut (1992), who observed no significant impact of disulfide formation on the stability of meat batters. NaSCN and NaCl also decreased ($P<0.05$) κ-CGN gel strength. NaSCN is known to affect electrostatic interactions that are important in the formation of κ-CGN and SSMP gel matrixes. It is not clear whether hydrogen bonding or electrostatic interactions were involved in the increase of SSMP and SSMP/κ-CGN gel strength since κ-CGN gels showed similar behavior as SSMP/κ-CGN gels in the presence of these reagents. These results may suggest that the changes in gel strength were due to κ-CGN instability rather than to the weakness of SSMP and κ-CGN interaction.

Fig. 2. Energy at the first peak (hardness) of 1% SSMP and SSMP/k-CGN treated with various reagents.




Addition of κ-CGN to SSMP significantly ($P<0.05$) increased the water holding capacity of SSMP/κ-CGN gels. This is due to the ability of κ-CGN to form a gel. A significant ($P<0.05$) increase in water loss was obtained upon addition of NaSCN to SSMP and SSMP/κ-CGN gels made with 1% protein (Fig. 3). The amount of protein expelled after centrifugation was not affected by the addition of κ-CGN, which also suggested that κ-CGN was not involved in stabilizing the proteins. Electrophoresis studies indicated no differences in the protein profiles of SSMP and SSMP/κ-CGN gels.

Fig. 3. Water losses of 1% SSMP/k-CGN and SSMP treated with various reagents.




Part 2. Functionality of Carrageenan in Solution-SSMP Gels. The temperature control system was designed to maintain the same temperature in both solutions, so that they could be mixed before CGN changed its state (Fig. 1). The optimum temperature for mixing the solutions was 40 °C.

Addition of κ-CGN to SSMP resulted in gels with higher gel strength than κ-CGN or SSMP independently. SSMP/κ-CGN (2119.0±92.03 g) gels were several fold harder than κ-CGN (1204.17 ± 49.04 g) and SSMP (26.67±8.58 g) gels, which suggested a possible protein-hydrocolloid interaction. However, if an interaction between the protein and hydrocolloid was the cause of increased in gel strength, higher protein concentration should also affect the interaction by increasing the gel strength. This effect was not observed in the study. Water losses were significantly ($P<0.05$) different between gel types. SSMP/κ-CGN and SSMP gels held 92 % and 62% water, respectively. These results were expected, due to the ability of κ-CGN to hold water. As protein concentration increased, water losses significantly ($P<0.05$) decreased in SSMP/κ-CGN gels (62-54 %). This was not so for SSMP gels, which behaved similarly at all protein levels.

The microstructure of the gels observed by SEM showed distinctive differences, which tended to correspond to the differences in hardness and water holding capacity observed earlier in the protein concentration study. The micrograph of SSMP/κ-CGN gels showed a well structured matrix with a highly interconnected network of strands that may offer more resistance to applied stress and higher water holding capacity. However, the structure of SSMP gels appeared to be discontinuous with poor linkage between the protein strands. This might have been the result of a very highly aggregated structure that led to large open spaces within the matrix and conferred poor water holding capacity and poor texture. Although, large open spaces were also observed in κ-CGN gels, the network seemed to be well interconnected and smooth. Consequently, the gels are harder and hold more water than SSMP gels alone. These observations may be helpful to explain functionality differences among the gels.

Part 3. Effect of CGN on the thermal transition characteristics of meat proteins. The protein concentration obtained during extraction of SSMP was in the order of 5%. At this concentration, the typical myosin and actin peaks commonly detected by DSC could not be distinguished. MP extraction yielded protein concentrations as high as 10%. The DSC measurements showed the presence of three transitional temperatures which were characteristic of meat proteins. However, no changes in thermal denaturation of the proteins were observed after adding up to 2% CGN to the extract. Addition of 2 % NaCl to ground pork, decreased the temperature of transition of the first and the second peaks by 4 °C and 9 °C, respectively. The second transition temperature was not significantly affected by ionic strength. CGN did not significantly change the thermal transition temperatures of meat proteins, suggesting that a molecular interaction between CGN and meat proteins did not occur. Ionic strength also affected the temperature of transition of CGN. Higher transition points were observed at high ionic strength.

## CONCLUSIONS

The results obtained in this study suggested that improvement of water holding capacity and texture of the heat-induced SSMP gels upon the addition of κ-CGN were due to physical entrapment of the protein and water, conferring greater water holding and stronger gel strength, rather than to molecular interaction between protein and hydrocolloid. This study provided valuable information on the behavior of CGN under various conditions, which may be utilized to explain the functionality of carrageenan in low-fat meat products.

## REFERENCES

Camou, J. P., Sebranek, J. G., and Olson, D. G. 1989. J. Food Sci. 54:850-854.
Lever-Garcia, C. 1988. Sc.D. thesis. Michigan State University, MI.
Gordon, A., and Barbut, S. 1992 Food Structure 11:133-146.
Shand, P. J. A. 1994. J. Food Sci. 59:711-715.