Carrageenans in meat systems

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Carrageenans in meat systems

DeFreitas, Zoraida, Ph.D.
Iowa State University, 1994
Carrageenans in meat systems

by

Zoraida DeFreitas

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirement for the Degree of
DOCTOR OF PHILOSOPHY

Departments: Food Science and Human Nutrition
Animal Science
Co-majors: Food Science and Technology
Meat Science

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For the Major Departments
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For the Graduate College
Iowa State University
Ames, Iowa

1994
DEDICATION

To my husband, Dan, for his love, support and understanding throughout all my doctorate work. To my parents, João and Carmelita for their love and for teaching me endurance and perseverance, without which I would not be able to accomplish this goal. To my sister and brother, Isabel and Juan for their encouragement from overseas.
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GENERAL INTRODUCTION

Over the past two decades, there has been a growing concern over the consumption of fats and cholesterol in the American population. This new trend has been mostly influenced by diet and health concerns and government recommendations. In 1979, the Surgeon General’s Report on Nutrition and Health urged Americans to improve their health by: reducing dietary intake of saturated fats, cholesterol, salt and sugar; increasing their consumption of complex carbohydrates; and taking only enough energy to maintain desirable body weight (Keeton, 1991). Many consumers are currently limiting the amount of fat and calories from fat in their diets. This public demand for low fat foods has increased research and industry interest in developing lower fat meat products with acceptable sensory attributes. The meat industry has responded to some of these diet issues by reducing the amount of fat in processed meats and by producing leaner cuts. However, much work remains to be done in this area. Fat has several important roles in meat products; it provides desirable texture, flavor and juiciness. Reduction of fat, therefore, results in a product with lower overall palatability.

Interest is growing in the use of hydrocolloids as water binding and texture modifying agents in low-fat processed meats. Carrageenans are sulfated polysaccharides that are produced from a variety of seaweeds in the class Rhodophyceae. The functionality of carrageenans in meat systems is related to their thermal reversible gelation properties. Addition of carrageenans to low-fat meat products improves water retention, consistency, "sliceability", and texture but the molecular mechanisms involved are not well understood.
Interactions between polysaccharides and proteins generally involve changes in functional properties of food systems. The use of model systems in which protein and polysaccharide composition and interactions can be evaluated may help to explain the functional changes that occur during meat product manufacturing. In general, the forces involved in protein-polysaccharide interactions are electrostatic in nature; other forces such as hydrogen, hydrophobic or covalent bindings may also be involved. Carrageenan has been reported to react with milk proteins, that is, $\kappa$-casein interacts with $\kappa$-carrageenan to form a stable complex. However, interactions between carrageenans and salt soluble meat proteins have not yet been reported.

Many extrinsic factors can affect the gelation properties of proteins and polysaccharides. For instance, changes in ionic strength, and pH have been proven to either increase or decrease the protein-protein interaction of heat-induced meat proteins, and the gelation of carrageenans. More information is also needed on the effect of carrageenans in the freeze-thaw stability of meat products, at various pH values and ionic strength conditions. Carrageenan, when added to a meat system, improves water holding capacity. Therefore, it is expected that under freeze-thaw conditions, it would serve as a stabilizing agent.

The objectives of this study were to evaluate, with the use of model systems, the interactions of three carrageenan types (kappa, iota, and lambda) with salt soluble meat proteins, the forces involved in such interactions and the effect of carrageenan on the thermal denaturation of salt soluble meat proteins. The extracted salt soluble meat proteins used in these experiments contained sarcoplasmic and myofibrillar fractions, which are commonly extracted in the manufacture of processed meats. The effects of pH, phosphate and salts on
the performance of carrageenans in the freeze-thaw stability of cooked-pork sausage were also investigated.

**Dissertation Organization**

This dissertation consists of a general introduction, a literature review and three papers to be submitted to the Journal of Food Science, and a general summary. The first paper examines the functionality of carrageenans and salt soluble meat proteins in model systems. The second paper uses differential scanning calorimetry to study meat/carrageenan samples. The third paper examines the effect of salt, phosphate and carrageenan on the freeze/thaw stability of cooked pork sausages. Each paper consists of an abstract, introduction, materials and methods, results and discussion, conclusions and references.

**Reference**

LITERATURE REVIEW

Meat Proteins

The amount of protein contained in lean muscle tissue is approximately 20%, but it varies with age, sex, specie, genetic makeup and the state of nutrition of the animal from which it is obtained (Morrisey et al., 1987). Many proteins have been separated from muscle and classified based on their organization, distribution, solubility and function.

On the basis of their solubility, meat proteins are classified as sarcoplasmic, myofibrillar and stromal proteins. Sarcoplasmic proteins consist of proteins that are soluble at low ionic strength (0.1 μ) (Morrisey et al., 1987), located in the sarcoplasm (muscle-cell cytoplasm) and account for approximately 30 to 34 % of the total protein. They are very similar to the proteins that are present in the cell cytoplasm except for the presence of myoglobin and glycolitic proteins (Asghar et al., 1985). Myofibrillar proteins constitute among 50-55% of the total protein content of the total muscle proteins. They are located in the myofibrils, which are the contractile elements of muscle. Myofibrillar proteins are usually extracted from muscle with high ionic strength solutions, but once extracted some of them are soluble at low ionic strength. The major proteins in this salt-soluble fraction are myosin, actin, tropomyosin, troponin, and α-actinin. Stromal proteins comprise 10 to 15% of the total protein content (Morrisey et al., 1987). This group of proteins contain mainly three fibrillar proteins: collagen, reticulin and elastin that are commonly referred as connective tissue (Asghar et al., 1985). The biological function of connective tissue is to cover the body as well as connecting the muscle organs, and other tissue structures to the skeleton and to each other.
On the basis of their physiological function in the muscle, myofibrillar proteins are divided into two subgroups: contractile and regulatory proteins; and intermediate filaments and proteins (Asghar et al., 1985). Myosin (actomyosin in the post rigor state) and actin are the major components of the thick and thin filaments, respectively. They are directly involved in the contraction-relaxation cycle of live muscle. Myosin proteins represent 52-53% of the total myofibrillar proteins. Myosin is composed of two large polypeptide chains, "heavy chains," each having a molecular weight of about 200,000 kdaltons and three or four small chains, "light chains." The light chains vary in their solubilization properties and molecular weights: Alkali light chain 1 or LC-1 has a molecular weight of 20.7 to 25 kdaltons and is isolated with alkali treatments; the "DTNB-light chain," or LC-2 (19 to 20 kdalton) is isolated with 5,5'-dithiobis(2-nitrobenzoic acid), and the LC-3 or alkali light chain-2, of molecular weight 16 to 16.5 kdalton, is soluble in alkali. Studies conducted on isolated myosin molecules have shown three important intrinsic properties of myosin. It is an enzyme with ATPase activity, it has the ability of binding actin and it can form aggregates with itself to form filaments (Bandman, 1987). Actin is the major constituent of the thin filament and accounts for 22% of the myofibrillar protein. Depending on environmental conditions, actin can be found in two forms. In low ionic strength solutions, actin exists as a single-chain globular protein (G-actin). Under high salt conditions, G-actin polymerizes to form a fibrous protein (F-actin) with a 6-nm wide double-stranded, right handed helical structure (Morrissey et al., 1987). The actins are designated as α-actin if present in skeletal and cardiac muscle tissues, while in non-muscle tissues of higher vertebrates they are called β- and γ-actin. Regulatory proteins are the myofibrillar proteins that are involved in the contraction-relaxation
cycle. They have been divided into two subgroups; the major (tropomyosin and the troponins) and the minor (M-protein, C-protein, F-protein, H-protein, X-protein, I-paramyosin, actinins, α-actinin, β-actinin, γ-actinin, Eu-actinin, and Z-protein) regulatory proteins. Tropomyosin is a long protein molecule (941 nm long) that consists of two polypeptides, α- and β-chain. The molecular weights of the α-chain, β-chains and tropomyosin have been reported to be 34 kdalton, 36 kdalton and 66 to 68 kdalton, respectively. Tropomyosin regulates muscle contraction by steric blocking and unblocking of actin-S-1 myosin interaction (Asghar et al., 1985; Eskin, 1990). Troponin is an elongated molecule of 80,000 kdalton and is composed of three subunits. Each of these subunits has specific function in living muscle. The subunit that binds Ca^{2+} ions is called troponin C (17 to 18 kdalton), the subunits that binds to tropomyosin is named troponin T (20 to 21 kdalton) and the actomyosin ATPase inhibitory subunit is termed troponin I (30 to 33 kdalton). The minor regulatory proteins are believed to be concerned with the regulation of filamentous structures and have been shown to be distributed in the M-line (e.g., M-protein), Z-line (e.g., α-actinin, Eu-actinin, and Z-protein) and I-band (e.g., β-actinin, γ-actinin) (Asghar et al., 1985).

**Protein Gelation**

Protein gelation has been extensively used as a model system to study the mechanisms responsible for texture and water holding characteristics of meat products. In the study of meat protein gelation, the complexity of the system has to be established. Extensive studies have indicated that myosin was the main protein responsible for binding meat pieces together,
thus many researchers have used myosin as a model system (Fukazawa et al., 1961a; Fukazawa et al., 1961b; Yasui et al., 1979; Ishioroshi et al., 1979; Ishioroshi et al., 1980; Dudziak et al., 1988). More complex systems, such as meat batters and intermediate systems that include salt-soluble protein and myofibril suspensions, have also been used to study the chemical/physical events that occur during the manufacturing of processed meat products (Trout and Schmidt, 1987; Camou et al., 1989; Foegeding et al., 1991).

Heat-induced gelation has been simplified by Ferry (1948) into a two-step process that involves; the initial denaturation of native protein (P$_N$) into uncoiled polypeptides, followed by aggregation of denatured proteins (P$_D$) into a cross-linked gel network during heating (Fig. 1). In this mechanism, $x$ represents the number of protein molecules involved in gelation.

$$xP_N \xrightarrow{\text{heat}} xP_D \xrightarrow{\text{heat and/or cooling}} (P_D)_x$$

Fig. 1. Ferry's gelation mechanism (Morrissey et al., 1987)

The aggregation step must occur slowly, to allow the denatured protein molecules to orient and interact, thus the gel matrix can be formed (Acton and Dick, 1984). Quick aggregation of protein molecules results in random protein-protein interactions that promote coagulation, characterized by syneresis, low elasticity and high opacity (Morrissey et al., 1987). During the gelation process water is immobilized within a network to form a firm and
stable structure that is resistant to flow under pressure (Glicksman, 1982). Hence, gelation involves not only protein-protein interactions but also protein-solvent interactions.

An expanded Ferry's model in which aggregation has been divided into series of steps leading to a final structure was suggested in the model of Foegeding and Hamann (Fig. 2). In this model, proteins unfold and aggregate to form a viscous solution. At the gel point, the gel formed exhibits viscoelastic properties and changes with time to form an equilibrium matrix or the final gel structure (Smith, 1994).

![Diagram of gelation process]

Fig. 2. Heat-induced gelation of thermally irreversible proteins (Smith, 1994)

It is generally accepted that polypeptide chains cross-link to form five or six crystalline regions per molecule during gelation. These interactions may involve multiple hydrogen bonds, disulfide or peptide bonds and may also be electrostatic and hydrophobic in nature (Asghar et al., 1985). Other molecules can move in between the links or strands conferring elasticity to the gel.
Role of muscle proteins in gelation

Myosin Several studies have shown myosin as the most important component of the salt-soluble meat proteins involved in protein gelation (Fukazawa et. al, 1961a, 1961b, 1961c; Samejima et al., 1969). Myosin is composed of tail and head portions that play different roles on the gelation properties and binding of muscle and are susceptible to proteolytic enzyme action. Trypsin and chymotrypsin cleave the molecule into two parts in a region of the tail that lacks α-helical conformation. The main part (150,000 Kdalton of molecular weight) is called light meromyosin (LMM) and the remaining part containing the head (350,000 Kdalton molecular weight) is known as heavy meromyosin (HMM). The latter can be further cleaved under control conditions into segments. The main globular part is named subfragment 1 (S-1) and the remaining fibrous part is called subfragment-2 (S-2) (Asghar et al., 1985).

Samejima et al. (1981) compared the heat-induced gelation of subfragment 1 and the myosin rod to that of intact myosin. They observed that upon heating at 65 °C for 20 min, myosin and myosin rod formed firm gels, while S-1 showed very poor gelation properties. Scanning electron microscopy studies corroborated these observations. A network was observed in intact myosin and myosin rod gels, whereas bead-like protein aggregates were visible in S-1 precipitates. It has been found that myosin and actomyosin show two transition temperatures during heat-induced gelation (Ishioroshi et. al, 1979; Samejima et al. 1981). Ishioroshi et al. (1979) suggested two kinds of conformation changes in the myosin molecule each with a different temperature of transition \( T_{m1} = 43 °C \) and \( T_{m2} = 55 °C \). The first transition was attributed to the aggregation of globular head portions (S-1) at about 43
°C, which corresponded to Tm1 values of myosin and its subfragments. These interactions are closely associated with the oxidation of SH groups residing in that region. The second transition temperature (55 °C) was mainly due to the formation of the network by the thermal unfolding of the tail helical portion of the myosin molecules during heating, and corresponded to Tm2 of the thermogelling reaction of myosin (Samejima et al., 1981). The network formation by tail-tail interactions is apparently stabilized by hydrophobic interactions since hydrophobic residues are exposed during the thermal unfolding of the helix (Ishioroshi et al., 1981). Therefore, it was suggested that the unfolding of the helical tail portion of the myosin molecule may play a role in the heat-induced gelation of myosin.

Sano et al. (1988) also proposed two steps at two temperature ranges for the thermal gelation of myosin, that is, 30-44 °C and 51-80 °C. In an attempt to elucidate the roles of the head and the tail portions of the molecule in the thermal gelation of myosin, Sano et al. (1990) conducted further experiments using HMM and LMM as model systems. Their results indicated that HMM was not able to form a gel, but it aggregated at a temperature range above 50 °C, which corresponded to that of myosin. On the contrary, LMM formed a gel in the temperature range below 45 °C, which also corresponded to that of myosin in this temperature range. In conclusion, these researchers proposed that during the first development of gel elasticity, which occurred at 30-44 °C range, thermal gelation changes of myosin were attributed to the tail portion of the molecule (LMM), and that during the second development of the gel in the 51-80 °C range to the head portion of the molecule (HMM).

Samejima et al. (1984) studied the role of myosin heavy chain (MHC) in the heat-induced gelation of myosin extracted from rabbits. Their observations suggested that myosin
gelation was dependent on the behavior of MHC and that the presence of myosin low chain did not contribute to the strength of the system. MHC formed a firm gel at temperature range of 60 °C, similar to the gelation temperature for intact myosin. Furthermore, MHC gelation showed two thermal transition temperatures, which corresponded to the ones observed by Samejima et al. (1981) for intact myosin.

Myosin binding strength is affected by concentration, nature of myosin, extraction and gelation conditions. Yasui et al. (1979) observed that heat-induced gelation of myosin started at 30 °C and ended at 60 °C to 70 °C and that the formation of the network was highly dependent on pH, being pH 6.0 the optimum for development of gel rigidity. Different isoforms of myosin exhibit different behavior. Amato et al. (1989) compared the functionality of dark and light chicken muscle at two ionic strength conditions. Their results showed that red muscles had better functionality than white muscles at their natural pH. Red muscles had higher stress values, indicating higher strength, than gels made from white muscles. Red muscle also had higher water retention properties than white muscle.

**Actomyosin** Actin alone does not exhibit any binding properties, but in the presence of myosin it reveals a synergistic effect, primarily due to the formation of actomyosin, a complex between actin and myosin (Asghar et al. 1985). Samejima et al. (1969) studied the heat gelling properties of myosin, actin, actomyosin and myosin-subs units in a saline model system. The data reported by these researchers indicated that heat gelling properties of these protein solutions were not parallel with those of more complex model systems (stroma and myofibrillar proteins). F-actin did not have an effect on heat-gelling properties, but it improved the gel strength when myosin was present in the solution. Yasui et
al. (1980) suggested that the enhancing effect of F-actin on the heat-induced gel formability of myosin was introduced by the binding of myosin to actin. This effect disappeared by the addition of ATP or inorganic phosphate, which is known to dissociate actomyosin to myosin and actin (Ishiroshi et al. 1980). Sano et al. (1989b) studied the role of F-actin in the gelation of fish muscle proteins. They observed that the addition of F-actin to myosin solutions resulted in gels with higher elasticity modulus, that is, it increased the viscosity of the cooked gels. However, F-actin alone showed no gel formation and turned into a curdled matter upon heating (Sano et al., 1989a).

It has been suggested by some authors that an optimal myosin/actin ratio was needed for actomyosin to develop maximum heat-induced gel formability. However, others have indicated that a higher portion of myosin in the actomyosin system resulted in better binding ability within the range between mole ratios of myosin and actin of about 3 to 8 (Yasui et al., 1980). This discrepancy was solved by Yasui et al. (1982), who observed that a specific myosin to actin ratio was essential to develop stronger gels. The molar ratio of myosin to actin was reported to be 2.5, which corresponded with the weight ratio of myosin to actin about 15 to 1. Further studies have indicated that the synergistic effect of actin and myosin is either ionic dependent or determined by the state of myosin per se at different ionic strength (Ishiroshi et al. 1983; Asghar et al. 1985). For instance, addition of F-actin (0.5 to 3.0 mg/ml) to a myosin system at low ionic strength (0.2 M KCl) resulted in lower heat-induced gel strength of the myosin system (Ishiroshi et al. 1983).

**Tropomyosin and troponins** Many researchers have observed no effect of tropomyosin and troponins on the gel strength of actomyosin. Samejima et al. (1982)
observed no significant differences in gel strength treated with or without tropomyosin. In a later study, Samejima et al. (1985) found soluble tropomyosin and troponins after heat treatments, which suggested that tropomyosin was a heat stable protein and had little gelation value at normal cooking temperature. However, other researchers have reported a significant increase in binding quality and water holding capacity by the addition of native tropomyosin to actomyosin system in the presence of pyrophosphate and MgCl₂ than in tropomyosin-free systems (Nakayama and Sato, 1971; Asghar et al. 1985).

The gelation of meat proteins during heating is critical for the development of the characteristic texture of comminuted meats and depends on environmental conditions, such as; salt concentration, pH and temperature of the system.

**Factors that affect protein gelation**

**Protein concentration** Heat-induced gel strength of myosin increased proportionally with protein concentration, independently of ionic strength, and the presence of actin in the system (Asghar et al., 1985). Similar results were observed by Ishioroshi et al. (1979), who evaluated the effect of protein concentration (0.1-1%) on the gelation of myosin. They found that heat-induced gel strength increased proportionally to its concentration at pH 6.0 and 65 °C. Cofrades et al. (1993) studied the effect of protein concentration on the apparent viscosity of natural actomyosin from hake, pork and chicken muscles. Their observations showed that in diluted solutions the effect of protein-solvent interaction predominated and there was a linear relationship between viscosity and protein
concentration. However, when protein concentrations were higher, protein-protein interaction was increased and a non-linear relationship was found at that concentration.

Not only meat-protein gel strength was affected by protein concentration but also water-holding capacity. In a study conducted by Camou et al. (1989), the effect of heating rate and protein concentration (10-50 mg/mL) on gels made with salt soluble meat proteins were evaluated. Gels made with protein concentrations lower than 20 mg/mL had greater water loss and lower protein in the expelled water, while higher protein concentration yielded more stable gels.

**Salt concentration**  MacFarlane et al. (1977) studied the effect of salt concentration (0-1.4 M NaCl) on the binding strength of myosin, actomyosin, and sarcoplasmic proteins. The binding strength, that is, the force needed to pull two pieces of meat apart, of myosin was superior to that of actomyosin at ionic strengths up to 1.0 M. At higher salt concentrations (1.2 M to 1.4 M), actomyosin binding ability was found to match myosin in this respect. Other researchers have also reported this ionic strength effect on myosin behavior. For instance, Turner et al. (1979) measured the effect of extracting crude myosin from beef muscle with various salt and phosphate concentrations. They observed that salt concentration increased the binding strength of crude myosin primarily by solubilizing the protein (Siegel and Schmidt, 1979). It has been suggested that salt increases the binding ability of myosin due to its ability to dissolve myosin by enhancing electrostatic repulsions (Hamm, 1960) and to dissociate myosin aggregates (Huxley, 1963). These effects would augment the number of molecular interactions induced by heating to form a firm gel. Also, it would induce an increase on water holding capacity of myosin binders and the muscle surfaces being bound
Ishioroshi et al. (1979) observed that at low ionic strength, rabbit myosin molecules were assembled into filaments which upon heating formed ordered three-dimensional structures. At high ionic strength (>0.3 M), however, the molecules were released from the myofibrils and existed as monomers in solution (Ishiroshi, 1983). The latter array was more likely to be affected by random collision caused by brownian motion. One possible explanation for the weakening of the gels in high salt concentration is the weakened of the electrostatic interactions between F-actin and myosin. Under these conditions, the F-actin effect on the gel strength of myosin is diminished (Asghar et al., 1985).

Offer and Trinick (1983) have reported the mechanism involved in the disruption of myofibrils by sodium chloride. Their results indicated that myofibrils were able to swell to at least twice their original volume in salt conditions normally used in the meat industry. The salt concentration required for maximum swelling of myofibrils was greater (0.8M) in the absence of pyrophosphate than in its presence (0.4M). The same concentrations required for maximum swelling have been reported by other researchers for maximum water uptake in muscle. Therefore, Offer and Trinick (1983) suggested that myofibrils were the site of water retention in meat processing. Swelling of myofibrils was found to be accompanied by extraction of the middle portion of the A-band in the absence of pyrophosphate, while, the A-band was completely removed in the presence of pyrophosphate. They also reported that structural constraints in the myofibril, such as the Z-line or M-line had to be disrupted by salt to allow the filaments to expand.

The swelling effect of NaCl in myofibrils has been attributed to the chloride ions, since experiments done with sodium acetate caused no swelling. Cl⁻ ions are attracted to the amino
group of the myofibrillar proteins, causing an increase in electrostatic repulsive forces, which would lead to swelling. High concentration of salt also causes dissociation of protein molecules because of the competitive effect of Cl⁻ ions for electrostatic interactions. In addition, high NaCl concentration causes a shift in the isoelectric point (pI) to a lower pH value. This shift in pI would increase the negative charge of the carboxyl groups, increasing repulsion between them and therefore augmenting the water holding sites (Offer and Trinick, 1983).

**Salt type**  Sodium reduction has been recommended in western diets, however its replacement results in products with poor sensorial attributes and water holding ability. Not much information is available on the influence of different cations on heat induced gelation of myosin. Studies conducted with myosin solutions treated with Na⁺ or K⁺ showed no significant difference in gel strength between these two treatments (Asghar et al., 1985). However, studies conducted using more complex systems, such as meat batters and frankfurters, indicated different behavior. Hand et al. (1982) studied the effect of NaCl, MgCl₂ and LiCl alone or in combination on pork/beef or mechanically deboned turkey frankfurters. Replacement of NaCl with either KCl or MgCl₂ resulted in a product with poor palatability. However, no differences in firmness among treatments (100% NaCl, 100% KCl and 100% MgCl₂) were observed, up to three weeks of storage. After six weeks of storage, the 100% KCl treatment was less firm than control. Barbut and Mittal (1988) studied the effect of NaCl, KCl and MgCl₂ alone or in combination on the rheological and gelation properties of meat batters. They observed breakdown of the emulsions containing 100% MgCl₂, which they attributed to the destabilizing effect of divalent cations. It was suggested
that divalent cations decrease water holding capacity by forming cross-bridges between peptide chains. Also, divalent cations can participate in coordinate bonds with carboxyl, sulfhydryl, and hydroxyl groups of proteins and phosphoryl groups of nucleotides (Asghar et al. 1985). Emulsions with 100% KCl had the minimum gelation modulus of rigidity, an effect that has also been observed by Trius (1994).

**pH** Ishioroshi et al. (1979) have reported pH 6.0 and 60-70 °C as the optimum pH and temperature for heat-induced myosin gelation, respectively. At pH 6.0, solutions of NaCl or KCl containing myosin exhibited greater gel strength than those formed at other pH values. Gels made at higher pH were translucent in appearance, while gels formed at pH 5.0 and 5.5 appeared "spongy" and those made at pH 4.0 and 4.5 were described as "particulate." These observations agreed with protein-protein interaction studies conducted by Ziegler and Acton (1984). These researchers investigated the kinetics of the protein-protein interaction of bovine natural actomyosin that occur during heat-induced gelation. At pH 5.5, the apparent energy of activation ΔHa (17.1 Kcal/mole) for the heat-induced protein solution was significantly lower than activation energies found for pH 6.0 (24.3 kcal/mole) and pH 7.5 (26.9 Kcal/mole). This indicated that at pH 5.5, protein-protein interactions will occur at lower temperature (16 °C) than at pH 6.0, in which aggregation does not occur until the temperature approached 37 °C. Maximum interaction occurs at pH 5.5 and 6.0 as compared to the changes observed at pH 6.5 and 7.5. These results also agreed with the findings published by Acton et al. (1981), who reported maximum gelling strength for natural and crude actomyosin at pH 5.0 to 5.5 and at pH 5.5, respectively.
The effects of pH (4.5, 5.5, 6.5 and 7.5) on the rheological properties of chicken breast salt-soluble proteins during heat-induced gelation were investigated by Wang et al. (1990). Their results indicated that unfolding and aggregation of native protein molecules during heating were influenced by pH. No major rheological transitions were recorded during heating proteins at pH 4.5, because proteins coagulated and failed to gel. This behavior is typical for myosin at pH close to its pI. At its pI, the net protein charge is zero, which increases the electrostatic attraction among molecules. Electrostatic attraction minimizes the unfolding of the proteins during heating, therefore preventing gel formation. Lower transition temperatures were observed at pH 5.5 than pH 6.5 and 7.5, due to lower protein thermostability at this pH. At pH 6.5 and 7.5, gels were more elastic in comparison to the other pH studied (Wang et al., 1990).

The optimal pH for gelation has been reported to be dependent on the relative concentration of actin and myosin in the system. As the ratio of myosin-to-actin decreases, the optimal pH for heat-induced gelation changes from pH 6.0 to pH 5.5 (Acton et al. 1983). Siegel and Schmidt (1979) did not find a significant effect of pH on binding ability of myosin extracted from prerigor bovine trapezius muscles. They suggested that the absence of any significant effect of pH in their system could be explained by the buffering effect of the muscle surfaces on the myosin binders, which could have eliminated any differences in pH.

The effect of pH on the apparent viscosity of natural actomyosin extracted from hake, pork and chicken muscle was studied by Cofrades et al. (1993). These researchers indicated that pH had a major influence on the effect of concentration in the apparent viscosity of actomyosin. The higher the pH, the greater the effect of protein concentration, hence the
higher the viscosity of the solution. As protein pH varied from its pI, protein molecules swell, making hydrophilic groups available, which increases the friction in the system. At high protein concentration, this pH effect will favor molecular interaction which explains the great influence of pH at higher protein concentrations.

The effect of pH in water retention has been described by various authors. At the pI of meat (pH 5.3-5.5), water retention is very low, therefore, cooking losses are expected to increase. Trout and Schmidt (1987) reported lower gel cooking losses at pH 6.0 than at pH 5.5, either in the presence or absence of phosphates.

**Temperature** The influence of heating temperature on myosin gelation in model systems is well documented. Asghar et al. (1985) have reported that myosin gel strength increased exponentially from 25 to 60 °C, when myosin solutions were heated at pH 6-7 range. However, these changes remained constant from 60-70 °C either at pH 6.0 or 7.0, indicating that the temperature optimum for gelation was 60-70 °C (Yasui et al., 1979). Gels that were heated at 60 °C had a sponge-like network with relatively large holes, while those gels heated at 50 °C had lacy networks. At temperatures as low as 40 °C, some changes in the molecules have been detected by means of light scattering absorbance, which may be indicative of protein-protein interactions (Asghar et al. 1985). Conformational changes for myosin at 37 °C and for actomyosin as low as 20 °C have also been detected by means of viscometric techniques (Acton et al. 1983).

Siegel and Schmidt (1979) studied the effect of temperature on the binding ability of myosin. The ability of myosin to bind pieces together was not observed at temperatures lower
than 45 °C. However, binding linearly increased as temperature approached 80 °C, and remained constant from 85 °C to 90 °C.

Most of the data published in heat-induced gelation have been collected using constant temperature in the range of 55 °C to 60 °C; little has been reported on the effect of heating rate on gel strength of muscle proteins. Foegeding et al. (1986) examined the effect of heating rate on the formation of myosin gels. Their results indicated that increasing heating rate to a final temperature of 70 °C at a rate of 12 °C/hr produced stronger gels than at 50 °C/hr, or at constant heating for 20 min. This suggested that slow heating rates may favor more protein-protein interaction to occur, while faster heating rates would promote more nucleation and less growth. It was also suggested that a constant temperature more random protein-protein interactions are developed. Further studies (Saliba et al., 1987) on the structural failure and nondestructive rheological analyses of frankfurter batters supported the previous findings by Foegeding et al. (1986). Failure shear stress values increased as heating rate was decreased. Similar results were obtained by Camou et al. (1989), who observed decreased gel strength when the heating rate was increased up to 85 °C/hr.

**Phosphates** Phosphates constitute a very large group of compounds that have been defined as "those chemical substances in which the anion consists of PO₄ tetrahedra that may be linked together by the sharing corners" (Molins, 1991). The members of the series having one phosphorus atom are called orthophosphates. The dimers (two P atoms) are the pyrophosphates, followed by the triphosphates or triopolyphosphates (three P atoms) and by the tetraphosphates (four P atoms). In the food industry, sodium salts are the most frequently
used, sodium tripolyphosphate (STPP) being the most popular, although sodium acid pyrophosphate and tetra sodium pyrophosphates (TSPP) are also widely used.

There are several ways in which phosphates may alter the structure and characteristics of meat proteins. Indirectly, phosphates can induce changes in the environment in which proteins exist. These changes may involve alteration in pH and ionic strength as well as chelation of metal cations. Phosphates may also adsorb into proteins causing diverse effects on protein characteristics such as hydration, swelling, gelation and protein-protein interactions (Molins, 1991). Some of these changes induced by polyphosphates have been found to improve meat functionality and properties, such as increase water-holding capacity (Bendall, 1954; Shults et al., 1972; Shults and Wierbicki, 1973; Trout and Schmidt, 1986; Barbut et al., 1988; Paterson et al., 1988) and meat binding, improve emulsification and color development, retard lipid oxidation and provide certain antimicrobial effects (Trout and Schmidt, 1983).

The effects of polyphosphates on the solubility of myosin are still controversial. Some authors attributed these effects to the changes in pH induced by phosphates, while others proposed specific phosphate-myosin interactions (Molins, 1993; Yasui et al., 1964a; Yasui et al., 1964b; Bendall, 1954). Proteins are least soluble at their isoelectric point (net charge balance equal to zero) because the protein structure contracts upon itself. When phosphates are added, the net charge of the proteins becomes positive or negative depending upon the type of phosphate used. Repulsion between groups and chain with the same charge forces the protein molecule to open up, thus exposing more sites to surrounding water molecules. These changes make protein molecules more soluble, increasing water holding capacity and decreasing viscosity of protein solutions. The latter is beneficial in meat batter stability because lower viscosity of the
batter reduces heat generation during chopping, which helps in maintaining low temperature during batter formation (Molins, 1991).

Yasui et al. (1964a) classified the effects of polyphosphates on the solubility of myosin B into two types. Type I included low molecular weight polyphosphates (pyrophosphate, PP and TP), which showed great affinity with myosin B in the presence of monovalent cations and formed divalent-metal phosphates complexes with the protein. In addition, these phosphates also behaved similarly to ATP, favoring the dissociation of actomyosin into actin and myosin. Type II consisted of high molecular weight polyphosphates, which solubilized myosin B by a direct binding where no salt was present. In the presence of monovalent cations, these polyphosphates acted like normal salts, by increasing the ionic strength of the system.

Further studies conducted by Yasui et al. (1964b) demonstrated the specific effect of phosphates on the viscosity of myosin B solutions. PP and TP increased their affinity with myosin B in the presence of monovalent and divalent cations. Furthermore, these inorganic polyphosphates, also showed the specific reaction with myosin B similar to that of ATP. Similar results were obtained by Bendall (1954). He studied the effect of various polyphosphates and sodium chloride on the swelling and water holding ability of lean meat. Among all polyphosphates examined, PP was suggested to have the largest effect on meat swelling, which was also increased by increasing ionic strength. PP was thought to facilitate the extraction of myosin and actin from muscle at high ionic strength by splitting the link between actin and myosin.
Phosphates have been also shown to increase the gel strength of meat batters. Whiting (1987) studied the effect of various salts and water soluble components on the water and fat exudation and gel strength of meat batters. He observed an increased in gel strength of meat batters when phosphate was used. Siegel and Schmidt (1979) used crude myosin, myosin, actomyosin and myosin heavy chain preparations to study the effect of phosphate on the ability of myosin to bind pieces together. Phosphate and salt linearly increased the ability of myosin to bind meat pieces by solubilizing the proteins. These researchers attributed this effect to the selective binding of phosphate to proteins and to increase solubilization and water holding capacity. On the other hand, Trout and Schmidt (1986) have suggested that combinations of pH and ionic strength were the main factors involved in improving gel strength. The changes in muscle protein functionality induced by salt and phosphate are caused in part by changes in protein structure, which are mainly due to electrostatic and hydrophobic interactions.

**Protein Extraction**

Protein extraction techniques are designed to obtain the greatest amount of myofibrillar proteins (MP) from muscle. By grinding and chopping, it is possible to disrupt the muscle membranes and sarcolemma, thus facilitating the release of myofibrils and myofibrillar filaments. Gillett et al. (1977) studied the effect of various parameters (stirring time, temperature and salt concentration) on the extraction of meat proteins in a model system. They observed an increase of soluble protein with mixing time and high NaCl concentration and an optimum temperature of 7.5 °C. This is in agreement with the work of
other researchers who have shown a linear relationship between salt concentration and extractable protein up to 10% NaCl. However, in practical applications, concentrations higher than 2.5% should be avoided due to flavor considerations. Turner et al. (1979) reported greater protein extraction using high salt concentrations in the presence of phosphates (1 M salt and 0.25% tripolyphosphate) than with low salt concentrations.

The addition of sodium chloride (NaCl) during MP extraction produces a shift in the pI of the myofibrillar proteins to a lower pH value, creating a larger net negative charge. Repulsion between these negatively charged groups causes the protein to open up, thus increasing their water holding capacity (Acton et al., 1983). Polyphosphates are also effective in increasing the water holding capacity of meat, particularly at higher pH. In the presence of 2% NaCl and 0.3% Na-pyrophosphate, myofibrils have been reported to swell significantly. The effect of phosphate is to facilitate the extraction of myosin and actin from muscle at high ionic strength (0.5-0.6 μ). Offer and Trinick (1983) reported maximum swelling of myofibrils at 0.4 M NaCl and 10mM sodium pyrophosphate. Equivalent swelling of myofibrils was previously observed in solutions containing 0.8M NaCl (Asghar et al., 1985). Common extraction procedures for myofibrillar proteins in meat systems are performed with ionic strength 0.6 to 1.3 and pH range of 5.8 to 6.2. In buffered solutions these parameters changes between studies but seldom fall below of pH 6.0 and ionic strength of 0.5-0.6 (Acton et al., 1983).
**Differential Scanning Calorimetry**

Differential Scanning Calorimetry (DSC) has been used increasingly in the study of thermal denaturation of meat proteins. DSC is based on the principle that whenever a material undergoes physical or chemical change, heat is either absorbed or liberated (Parsons and Patterson, 1986). The purpose of this technique is to measure the difference in enthalpy change that occurs between a sample and an inert reference material when both samples are heated at the same time (Lund, 1983). The changes in enthalpy reflect chemical and physiological changes taking place in the sample, such as protein denaturations (Findlay and Barbut, 1990; Quinn et al., 1980; Starbursvik and Martens, 1980), starch gelatinization, and phase transitions of lipids.

Thermal transitions represent the point where conformational changes occur in proteins. A typical thermal transition thermogram for muscle is composed of three major transition peaks, which represent myosin subunits (55 °C), sarcoplasmic proteins and collagen (67 °C) and actin (80 °C) (Findlay and Barbut, 1990). Wright et al. (1977) observed three discernible peaks in post-rigor rabbit muscles, which corresponded to the thermal transitions obtained with myosin (60 °C), sarcoplasmic proteins (67 °C) and actin (80 °C).

It is well established that factors such as pH, salt concentration, sarcomere length, and postmortem conditions influence the thermal properties of meat (Findlay and Barbut, 1990). Quinn et al. (1980) used a DSC to monitor the changes in heat stability of beef proteins during processing meat into sausage batter. They observed that addition of sodium chloride drastically altered the stability of meat proteins, indicated by lower transition temperatures of the major peaks. Similar results were observed in chicken muscle by Shiga et al. (1988).
When NaCl was added to the chicken, the endothermic peak shifted to lower transition temperatures. A shift in the thermal transitions of actin to lower temperature was observed by Park and Lanier (1989) upon addition of salt to fish muscle. Furthermore, enthalpies of denaturation for actin and myosin were decreased with further processing. A study conducted by Stabursvik and Martens (1980) comparing DSC thermograms of beef samples with different pH (5.45-6.75), showed that pH had a destabilizing effect on muscle proteins, changing the shape and position of the denaturation peaks. Stabursvik et al. (1984) also compared the denaturation of myofibrillar proteins from normal and pale, soft and exudate (PSE) pork tissues in situ using DSC techniques. Thermograms of myofibrillar tissue from normal pork showed three major peaks with temperature maxima at 58 °C and 66 °C, associated with myosin denaturation and at 78 °C, associated with actin denaturation. In PSE thermograms, the peak at 58 °C was markedly reduced, and appeared as a shoulder, indicating that partial denaturation of myosin had occurred before thermal denaturation in the calorimetry.

Recently, DSC has been used to examine the thermal properties of protein-polysaccharide mixtures. Ensor et al. (1991) reported that the addition of algin/calcium (0.4%/0.075%) to crude myofibrillar, sarcoplasmic, and connective tissue proteins had a thermal destabilization effect on these meat proteins, which may indicate the occurrence of physical changes of the protein. Interaction between other polysaccharides (Alginate, pectate, carboxymethyl cellulose and dextrin) and meat proteins (myoglobin and bovine serum albumin) were also studied. These researchers observed that the thermal stability of myoglobin was decreased upon addition of pectin, alginate or CMC. The addition of other
polysaccharides (i.e., carrageenans) to meats has also been reported to affect the thermal
transition of meat proteins. Shand (1991) studied the effect of adding κ-carrageenan to
ground beef under various ionic strengths. She observed that the addition of 2% κ-
carrageenan decreased $T_{\text{max}1}$, $T_{\text{max}2}$, and $T_{\text{max}3}$ values by an average of 0.9 °C, 0.5 °C
and 2.6 °C, respectively. The destabilizing effect was more pronounced when carrageenan
was added to the high higher ionic strength samples.

Carrageenan

The term carrageenan has been derived from the older term carrageenin originally used
to designate extractives of *Chondrus* and *Gigartina* species. Today, other related species
with similar structure are also called carrageenans, the genera *Furcellaria*, *Eucheuma*,
*Hypnea*, *Iridea* and *Polyides* (Towle, 1973). Carrageenan is officially described in the Food
Chemical Codex III, as the product "obtained by extraction with water or aqueous alkali from
certain members of the Class Rhodophyceae," one of the four major categories in which
marine seaweeds or algae have been classified. It is a hydrocolloid consisting mainly of the
potassium, sodium, magnesium, calcium and ammonium sulfate esters of galactose and 3,6-
anhydrogalactose copolymers. These polymers are essentially linear chain, composed of
galactose units that are alternately linked glycosidically $\alpha$ 1,3 and $\beta$ 1,4. The predominant
monosaccharide units for this galactan are shown in Table 1. Their sulfate content is the
major feature used to distinguish the red seaweed extracts. Furcelans must contain 8-19%
sulfate and carrageenans must have 18 to 40% sulfate. The percentage of sulfate also varies
Table 1. Monomer units of carrageenans

<table>
<thead>
<tr>
<th>Galactan</th>
<th>Predominant Monosaccharide Units</th>
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<tbody>
<tr>
<td>Iota-carrageenan</td>
<td>D-galactose 4-sulfate</td>
</tr>
<tr>
<td></td>
<td>3,6-anhydro-D-galactose 2-sulfate</td>
</tr>
<tr>
<td>Kappa-carrageenan</td>
<td>D-galactose 4-sulfate</td>
</tr>
<tr>
<td></td>
<td>3,6-anhydro-D-galactose</td>
</tr>
<tr>
<td>Lambda-carrageenan</td>
<td>D-galactose 2-sulfate</td>
</tr>
<tr>
<td></td>
<td>D-galactose 2,6-disulfate</td>
</tr>
<tr>
<td>Mu-carrageenan</td>
<td>D-galactose 4-sulfate</td>
</tr>
<tr>
<td></td>
<td>D-galactose 6-sulfate</td>
</tr>
<tr>
<td></td>
<td>3,6-anhydro-D-galactose</td>
</tr>
<tr>
<td>Nu-carrageenan</td>
<td>D-galactose 4-sulfate</td>
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<tr>
<td></td>
<td>D-galactose 2,6-disulfate</td>
</tr>
<tr>
<td></td>
<td>3,6-anhydro-D-galactose</td>
</tr>
</tbody>
</table>

Adapted from Glicksman (1983b)

within carrageenans; \( \kappa, \iota \) and \( \lambda \) values are 25-30%, 28-35% and 32-39% sulfate, respectively (Glicksman, 1983a).

Types of carrageenans

The three major carrageenans are kappa, iota, and lambda carrageenans. The latter is not a gelling agent (Lockwood, 1985). These fractions are derived from minor fractions or precursors; mu (\( \mu \), precursor of \( \kappa \)), nu (\( \nu \), precursor of \( \iota \)) and theta (\( \theta \) formed from \( \lambda \)) (Glicksmann, 1983b; Towle, 1973). They differ chemically in their degree and manner of sulfation, presence of a position of 3-6 anhydrolactase residues linked 1-4, different pyranose ring conformations (Reeves C1 or 1C); and different cations associated with the sulfate groups (Fig. 3).
Kappa Carrageenan

\[ \text{D-galactose-4-sulfate} \quad \text{3,6-anhydro-D-galactose} \]

Iota Carrageenan

\[ \text{D-galactose-4-sulfate} \quad \text{3,6-anhydro-D-galactose-2-sulfate} \]

Lambda Carrageenan

\[ \text{D-galactose-2-sulfate} \quad \text{D-galactose-2,6-disulfate} \]

Fig. 3. Carrageenan structure (Glicksman, 1983b)
**kappa-CGN**  \(\kappa\)-carrageenan is composed of alternating 1,3 linked galactose-4-sulfate and 1,4 linked 3,6-anhydro-D-galactose units (Fig. 3). This is formed in the red algae by enzymatic action. Dekinkase is the enzyme responsible of removing the sulfate at C6 in the 1,4 linked galactose-6-sulfate of \(\mu\)-carrageenan to form the 3,6 anhydride (Glicksmann, 1983b). The amount of 3,6 anhydro-D-galactose usually found in nature is 28%, however amounts up to 35% can be obtained by appropriate processing conditions. An increase of anhydride content, affects the gelling capacity of carrageenan (Towle, 1973; Glicksmann, 1983b).

**iota-CGN** The structure of \(\iota\)-carrageenan is the same as that of \(\kappa\)-carrageenan, except that the 3,6 anhydro-D-galactose unit is sulfated at the C2 position (Fig. 3). The precursor fraction of \(\iota\) is \(\nu\), which contain no anhydride but 6-sulfate in the 1,4-linked galactose. By eliminating the sulfate at the sixth carbon position, the ring is closed to make the 3,6 anhydrogalactose ring that forms iota-carrageenan. \(\iota\)-carrageenan gels are less sensitive to potassium than \(\kappa\)-carrageenan due to its higher content of sulfate (25-50%). On the other hand, as sulfation of C2 positions increases, the sensitivity to calcium becomes predominant (Glicksman, 1983). The presence of the 2-sulfate group increases its hydrophobicity resulting in a marked difference in physical properties between \(\kappa\) and \(\iota\)-carrageenan.

**lambda-CGN** This non-gelling carrageenan is mainly composed of alternating units of 1,3 linked galactose and 1,4 linked galactose 6-sulfate (Fig. 3). Differently from kappa and iota, the 1,3 linked galactose unit is not sulfated but the second carbon is usually 70% sulfatated (Glicksman, 1983b). In the presence of potassium ion, \(\iota\)-carrageenan does not...
precipitate. It remains a mixture of highly sulfated galactans with small amount of contaminating polysaccharides, such as floridean starch and xylan (Towle, 1973).

Production

There are several steps to the production of carrageenan (Fig. 4). First of all, the geographic location or distribution of the algae must be determined to assess the availability in the particular location. Harvesting of carrageenan is done by traditional manual operations.

![Diagram of production process](Fig. 4. Process for commercial production of carrageenan (Glicksman, 1983b))

Much of the raw material is harvested by workers, others are obtained as drift weed cast upon the beaches following storms and other violent weather changes. After harvesting the seaweed is dried mechanically or under the sun, the algae are baled and shipped to the processing plant. At the plant, the seaweed is washed to remove dirt and sand and then
macerated and extracted in hot (100 °C) alkaline solution (Fig. 4). The alkaline solution has two functions: it helps the extraction of the polysaccharide from the plant and catalyzes the formation of 3,6 anhydro-D-galactose residues by eliminating the 6 sulfate groups from the monomer. After cooking, three extraction methods (washing, concentration and alcohol precipitation) can be used, however the method that provides the best quality is by alcohol precipitation. Significant amount of some red seaweeds species are being grown commercially using tank aquaculture and seaweed cultivation farms, to expand the supply of seaweeds (Glicksman, 1983b).

Properties

Solubility All the carrageenans are soluble in hot water and temperatures above 70 °C. Only λ-carrageenan and sodium salts of κ and τ-carrageenans are soluble in cold water (Glicksmann, 1983b). The temperature of solubility depends on the concentration of carrageenan, cations associated with it and cations present in the environment (Moirano, 1977). The hydrophobicity of the molecule plays an important role on solubility, that is the presence of sulfate half-ester groups and galactopyranosyl unit makes the molecules cold water soluble. For instance, λ-CGN that has high percentage of sulfate esters and is void of 3,6-anhydrogalactopyranosyl units, is cold water soluble in all salt forms. In contrast, κ-carrageenan, having large number of hydrophobic 3,6 anhydrogalactopyranosyl units and less hydrophilic sulfate-ester groups, is cold water soluble only in the sodium salt form. Some organic compounds retard the hydration of carrageenans, such as some salts, sugar and polysaccharides (Table 2). Hydration of carrageenan is also affected by pH. Lower pH levels
increase the solubility of carrageenan, while pH levels of 6 and above retard it (Glicksman, 1983b; Towle, 1973; and Moirano, 1977).

**Gelation** In the presence of gel-inducing cations and upon cooling, kappa-carrageenanans form brittle gels characterized by syneresis. Syneresis may be defined as the loss of fluid from the interior of a gel due to shrinkage of the gel and expulsion of the fluid to the surface (Moirano, 1977). Iota-carrageenan forms elastic gels. κ- and τ-carrageenan gels are thermally reversible, i.e., they melt on heating and gel again on cooling. Lambda is not
capable of forming a gel (Table 2). \(\kappa\)-carrageenan gels can become more elastic in the presence of other hydrocolloids, for example the addition of iota-carrageenan to kappa-carrageenan solutions makes more elastic gels and less prone to syneresis (Igoe, 1982). Kappa-carrageenan also exhibits a synergistic effect with locust gum, that is, the gel strength of the two polysaccharides is markedly enhanced when there are combined in solution. Also, the gels are transformed from a brittle to a more elastic structure and present less syneresis (Table 2).

The gel strength and gelling temperature of \(\kappa\)-carrageenan are affected by the type of cations present (Glicksmann, 1983b). Zabik and Aldrich (1968) reported that high concentrations of KCl favored the formation of gels at higher temperatures. Smidrod and Grasdalen (1982) reported evidence of specific binding of \(K^+\) by \(\iota\)- and \(\kappa\) carrageenan during gelation. It has been suggested that ions, particularly \(K^+\) and \(Rb^+\) by acting as solvent structure-makers or breakers influence gelation of carrageenans (Oakenfull, 1987).

**Stability** All carrageenans are stable under neutral and alkaline pH, however carrageenans in solution are hydrolyzed at pH 3.5 (Table 2). The hydrolysis is greatly accelerated by heat under low pH conditions. Carrageenan hydrolyses results from a cleavage of the 1,3-glycosidic linkage and is accelerated by the presence of the strained ring system of the anhydride (Moirano, 1977). In the gelled state, however, iota-carrageenans are very resistant to hydrolysis.
Mechanism of gelation

One of the most distinctive properties of a gel is that its composition resembles a pure liquid and it also can approach that of a solid. The general explanation for this change in physical state is the association of polymer molecules resulting in a three-dimensional framework that contains solvent in the interstices. The gelation mechanism of carrageenans is extremely sensitive to polysaccharide structure. Double helix formation is completely blocked by the presence of D-galactose 6-sulfate or 2,6-disulfate in place of a 3,6 anhydride residue. The presence of one molecule of D-galactose 6-sulfate per 200 residues results in a kinked helix that prevents the double helix from forming. Closure of the ring to form the 3,6-anhydride, constrains the 1,4 pyranose unit to the 1C form with resultant removal of the kink. Therefore, the introduction of 3,6 anhydride is a chain straightening process, leading to greater regularity in the polymer and increasing capability of forming a double helix (Moirano, 1977).

Rees (1969) proposed a double helix model for carrageenan gelation based on structural studies by x-ray diffraction. On cooling, a transition from a random-coil conformation in the sol state to the gel state (Gel I) occurs, where the chains are cross-linked by isolated double helices (Fig. 5). During further cooling, the aggregation of double helices leads to a three-dimensional gel structure (Gel II), which intersticials are occupied by water and sometimes other small molecules and ions (Rees, 1972). Dea et al. (1972) suggested that each chain was caused to combine with more than one partner by structural irregularities or "kinks," to form a network rather than propagating the same double helical association alone.
Fig. 5. Mechanism proposed by Rees (1969) for gelation by \( \tau \)- and \( \kappa \)-carrageenans.

the entire molecular length. This model is supported by detail comparison of gel properties and theoretical rotation changes attributed to helix formation.

Rees et al. (1969) showed more evidence that supports the double helix model after studying the changes in optical rotation that occur during gel setting. Changes in optical rotation are to be expected when helices form and aggregate. When a hot \( \kappa \)-carrageenan solution is cooled, its optical rotation and similar properties change very slowly until 40°C or the "gel point" is reached. Changes in optical rotation begin to increase sharply without apparent textural changes. As the temperature reaches 37.0 °C, textural measurements show a sol-gel transformation but without major changes in optical rotation. At 33.8 °C, changes in gel texture become visible and there is an inflection in the optical rotation curve. At this point, the gel becomes turbid and loses its clear appearance, this stage is also called the "haze point." Snoeren and Payens (1976) observed that these transition temperatures were linearly dependent on ionic strength.
Further studies on carrageenan gelation led Robinson et al. (1980) to postulate a more complete mechanism (Fig. 6). In the newer model, "domain model," carrageenan chains associate to form "ordered domains" by the formation of intermolecular double helices. The formation of these domains does not produce a gel network; gelation only occurs with the aggregation of the domains, which is mediated by specific binding of gel promoting cations such as potassium, rubidium and calcium (Oakenfull, 1987).

Smidsrød and Grasdalen (1980) have proposed another model to explain the mechanism of carrageenan gelation (Fig. 7). These authors suggested that an intramolecular conformation change results upon cooling and gelation occurs by formation of cross-linkages in which specific cations act as bridges. By comparing the addition of tetramethylammonium iodide and chloride to solutions of κ-carrageenan, Smidsrød and Grasdalen (1982) found that iodine (20 mM) induced the formation of ordered single helices, presumably by binding itself
to the polysaccharide. Meanwhile, the presence of polysaccharide random coils were still evident in solutions containing up to 150 mM chloride.

**Protein-polysaccharide Interactions**

Understanding the interactions that occur between polysaccharides and proteins is very important in the study of functional properties of food systems. Charged polysaccharides have the ability to interact with other polysaccharides, proteins and lipids, changing the properties of food. Edible gels may be produced by three kinds of interactions between proteins and polysaccharides. The simplest kind is that between a protein with a net positive charge with a sulfated or carboxylated polysaccharide. Much more specific is the interaction between two polymers bearing the same net charge. And a third kind, which includes highly selective linking of polysaccharide to protein by covalent bonds (Stainsby, 1980).

When two polymers of opposite net charge are mixed, such as in the case of an anionic polysaccharide at or above its pI and a protein below its pI, the enthalpy of mixing is exothermic and the chain-chain interactions lead to complex formation. This phenomenon
involves interactions that are primarily electrostatic in nature (Ledward, 1994). Electrostatic interactions between positively charged proteins and negatively charged polysaccharides are dependent on pH and ionic strength. For example, at pH 6.0 the electrostatic interaction between myoglobin, which possesses a positive charge at this pH, and anionic polysaccharides are stronger than with bovine serum albumin that carries a net negative charge (Imeson et al. 1977; Hansen, 1982). It has been suggested that a carboxylate group of polysaccharides interacts with a positively charge protein residue, such as ε-amino, α-amino guanidinium and imidazole and that the strength of the interaction is related to the distribution of these groups and the overall charge on the protein (Ledward, 1994; Samant et al., 1993). Following denaturation, the buried basic groups are liberated, increasing the number of reactive sites. Also, the denatured state permits configuration adjustments to maximize interactions and yield stable complexes (Ledward, 1994).

Sulfated polysaccharides are negatively charged polymers over a wide range of pH, therefore they are able to form complexes with positively charge proteins (Glicksman, 1983b). Lin (1977) described four mechanisms by which sulfated polysaccharide reacted with proteins. Above the pI of the protein, the proteins are negatively charged therefore polyvalent metal ions act as bridges between the negatively charged carboxyl group of the proteins and the negatively charged ester sulfates of the polysaccharides. At pH below the pI, proteins are positively charged, thus direct interactions between ester sulfate of the polysaccharide and the protonated amino groups on the protein take place. This author also suggested intermediate or transitional degrees of association where protein and polysaccharide charges are not extremely opposed to each other. Similar mechanisms were also suggested by Carr (1993).
Carrageenan molecules could interact directly with positively charged groups on the protein surface (Fig. 8a) or indirectly with negatively charged proteins through cation bridges (Fig. 8b). Upon denaturation, the number of sites increases as well as the flexibility of the random coil maximizing protein-polysaccharide interactions. Snoeren et al. (1975) and Snoeren et al. (1976) have proposed an electrostatic interaction between two macromolecules bearing the same overall charge. Although contradictory, highly unequal distributions of the polar residues in the molecules provide regions capable of electrostatic interaction.

\[ \text{H} \quad \text{H} \]
\[ \text{R - C - COOH} + \text{R'OSO}_2\text{O}^- \rightarrow \text{R - C - NH}_3^+ \text{O}_2\text{OSOR'} \quad (a) \]
\[ \text{NH}_3^+ \quad \text{COOH} \]

\[ \text{H} \quad \text{H} \]
\[ \text{R - C - COO}^- + \text{Ca}^{++} + \text{R'OSO}_2\text{O}^- \rightarrow \text{R - C - COO} \cdot \text{Ca} \cdot \text{O}_2\text{OSOR'} \quad (b) \]
\[ \text{NH}_2 \quad \text{NH}_2 \]

Fig. 8. Mechanisms of carrageenan and protein interaction (Carr, 1993)

Carrageenans have been used extensively as a stabilizing agent in the dairy industry. Consequently, a lot of research has been done to understand the effects and interactions of carrageenans in dairy systems (Grindrod and Nickerson, 1968; Hansen, 1968; Payens, 1972; Snoeren et al., 1976; Hood and Allen, 1977; Skura and Nakai, 1981; Schmidt and Smith, 1992a; Schmidt and Smith, 1992b; Xu et al., 1992). The addition of hydrocolloids to milk
systems is known to cause profound changes in the aggregation state of the casein micelles. Interactions between κ-casein and κ-carrageenan have been observed even at pH values above the pI of the proteins and polysaccharide, in which κ-casein and κ-carrageenan carry net negative charges (Ledward, 1994). Grindrod and Nickerson (1968) using polyacrylamide gel electrophoresis (PAGE) demonstrated that κ-casein interacted with carrageenan in presence of Ca++. These researchers observed, after using (PAGE) to separate milk proteins, that all protein bands were present in the gel except for κ-casein. They suggested that a complex between κ-casein and carrageenan was formed and that this complex was too big to migrate inside the gel. The complex was easily broken when PAG-urea-mercaptoethanol was used to separate these proteins. It is likely that the calcium ions neutralize the sulfate groups on the polysaccharide, permitting the chain to form a “gel-like” structure, which physically serves to inhibit protein aggregation (Ledward, 1994). Thus the stabilization of calcium sensitive proteins such as casein by sulfated polysaccharides like carrageenans is favored (Hansen, 1982; Samant et al., 1993). Other researchers have also reported the formation of a carrageenan/κ-casein complex in the presence of Ca++ (Schmidt and Smith, 1992b) and the stabilization against precipitation of αs-casein by carrageenan. Skura and Nakai (1981) reported that calcium salt bridges between κ-carrageenan and αs-casein were involved in the formation of the κ-carrageenan and αs-casein complex.

Electron microscopy techniques have also been utilized to study protein/hydrocolloid interactions. Hood and Allen, (1977) used transmission electron microscopy to study the influence of kappa and lambda carrageenan on the ultrastructure of milk gels and sols. Their
observation suggested that casein-carrageenan interactions occur at the micelle surface and that these interactions would help to stabilize the web-like matrix that is formed by the carrageenan among the micelles. Carrageenan and κ-casein networks have been observed by Snoeren et al. (1976) using transmission electron microscopy and by Payens, (1972) by a light scattering technique. Chakraborty and Hansen (1971) examined protein hydrocolloid complexes in the presence of calcium by electron microscopy and suggested that the complexes between carrageenan and αs-casein appeared as dense protein bodies entrapped in a polysaccharide network. Interactions such as a highly selective linking where polysaccharides are bound to proteins through covalent bonds have also been suggested (Hansen, 1982).

In meat systems most of the research involving the use of carrageenans has primarily focused on practical applications (Foegeding and Ramsey, 1987; Bater et al., 1992a; Bater et al., 1992b; and Bater et al., 1993), therefore, not very much is known about the molecular changes that may occur in meats when gums are used. Recently, a mathematical modeling of thermal gelation of myofibrillar beef proteins and their interactions with selected hydrocolloids has been used to predict the gel strength and the water holding capacity of these proteins in the presence of various hydrocolloids (carrageenan, guar, locust bean and xanthan gum). Carrageenan was found to contribute to the protein-gum system by increasing the gel strength and water holding capacity of the cooked gels over the control treatment, by 184.4% and 180.7%, respectively. As predicted by the model system, protein-hydrocolloid gels had a higher gel strength and water holding capacity than control, except for xanthan gum that was found to inhibit gel gelation (Lever-Garcia, 1988).
The nature of the forces involved during protein and/or polysaccharide heat-induced gelations can be investigated by adding reagents that either promote or prevent interactions. Salts such as NaCl, NaSCN and urea can be used to study these interactions. NaCl and NaSCN have charge neutralization effects while urea disrupts electrostatic and hydrogen bonds. Other reagents such as propylene glycol and 2-mercaptoethanol have also been used. Propylene glycol affects water structure by disrupting hydrophobic interactions and promoting hydrogen and electrostatic bonds. 2-mercaptoethanol separates polypeptide chains linked by disulfide bonds (Rodwell, 1990) by reducing the inner- and intramolecular disulfide bonds (Cheftel et al., 1985). Utsumi and Kinsella (1985) were able to determine the forces involved in soy protein gelations by using stabilizing/destabilizing reagents.

Bernal et al. (1987) studied the nature of the forces involved in the intermolecular interaction of myofibrillar proteins/polysaccharide/calcium systems in the presence of various stabilizer/destabilizer reagents (0.05 M NaCl, 0.05 M NaSCN, 10% propyl glycol, 0.02 M 2-mercaptoethanol and 0.05 M urea). Although, the formation of κ-casein/carrageenan complex in presence of calcium has been reported, interactions between carrageenan and myofibrillar proteins were not observed by Bernal et al. (1987). These authors suggested that carrageenan may help to improve the water holding capacity of meat emulsions by holding the water in the interstitial spaces of the gel network rather than by true interactions with proteins in the formation of the network. However, the carrageenan composition used in this study was unknown, and the presence of calcium and sodium may explain for the failure of the carrageenan preparation to gel.
Carrageenans in Meats

The current consumer trend towards low-fat meat products has prompted the food industry in developing low-calorie foods with acceptable sensory characteristics (Brewer et al., 1992). However, reduction of fat without changing sensory properties is not an easy task. Fat has a great impact in juiciness, texture and flavor characteristics. Addition of water to partially replaced fat has been effective in improving texture and juiciness of low-fat meat products. According to the Code of Federal Regulations (CFR, 1991), addition of water to frankfurters and similar cooked sausages is allowed to a certain extent. This regulation says: "the finished product shall not contain more than 30 percent fat. Water or ice, or both may be used ..... but the sausage shall contain no more than 40 percent of a combination of fat and added water."

In water-added products, retention of water in the product is a concern.

Polysaccharides' gums are often used to hold water and enhance the texture properties of low-fat meat products. Garcia-Cruz and Scamparini (1992) studied the effect of various hydrocolloids in a model system. Their results indicated greater cooking stability when xanthan gum, locust gum and guar gum were used. In this study, the protective effect of carrageenan was lower than the other hydrocolloids at concentrations of 1%. However, at higher carrageenan concentrations (2-6 %) cooking stability was increased 100 %.

Carrageenans are added to low-fat meat products to improve water retention, consistency, sliceability, and texture. The functionality of carrageenans in meat products is due to its ability to form gel and hold water.
The effect of frozen storage on functionality of meats was evaluated by Miller et al. (1980). Frozen storage was shown to affect the water binding ability, emulsifying capacity, and cooking loses of beef and pork lean muscles. Da Ponte et al. (1985) investigated the effect of various anionic and neutral hydrocolloids on the stability of frozen stored minced fillets. They observed an improved water-holding capacity and cook drip loss when hydrocolloids were used. Further studies on the protective effect of carrageenan were conducted by Da Ponte et al. (1987). These researchers studied the functionality of iota carrageenan, carboxymethyl cellulose and xanthan gum in formulated minced fish products. Their results showed a marked stabilizing effect on texture and weight loss during frozen storage upon the addition of gums.

In the meats area, the introduction of McLean “Deluxe” by the McDonald's Corp was the most publicized and commercialized use of carrageenan in low-fat meat products. The McDonald's Corp., Oak Brook, IL, adapted the AU Lean formulation developed by a team of researchers at Auburn University, AL. The objective was to develop a product with less than 10% fat and with sensory characteristics similar to high-fat patties. To achieve this goal, several changes were made such as, meat particle size, addition of spices and flavor enhancers and inclusion of iota carrageenan. Iota carrageenan was chosen, among all carrageenans, because of its greater water-holding capacity, cold solubilization (some of them) and good freeze/thaw stability (Egbert et al., 1991). After extensive analyses that included compositional, physical, ultrastructural and sensory evaluation, Huffman et al., (1991) reported that a reduction of calories from fat in AU Lean patties was achieved while sensory properties of the low-fat product were maintained.
Since the development of AU Lean, a lot of research has been conducted to study the incorporation of carrageenan in meat products. Barbut and Mittal (1989) investigated the effect of potassium and calcium chloride on the gelation and rheological properties of reduced fat pork meat sausages containing either iota- or kappa-carrageenan. An increase on sausage rigidity was observed by the addition of KCl and CaCl₂ to kappa and iota treatments, respectively. These authors suggested that KCl and CaCl₂ affected the structure of the carrageenans resulting in sausages with higher gel strength values. They observed that kappa-carrageenan treatments became more brittle and had higher gel strength when potassium was present. Also, iota-carrageenan treatments formed a more condensed helical structure in the presence of calcium. These results indicated that addition of low concentrations of KCl (0.4%) and CaCl₂ (0.2%) could increase the effectiveness of kappa- and iota-carrageenans in reduced fat products, respectively.

The addition of 0.5% kappa-carrageenan to oven-roasted turkey breasts manufactured with 70% added brine resulted in better yield, sliceability, water retention and higher rigidity (Bater et al., 1992a). The ability of carrageenan in increasing rigidity was also shown by Foegeding and Ramsey (1987). These researchers observed an increase in water holding capacity and firmness in those products with carrageenan added. Among the gums studied, k-carrageenan was the most effective in increasing hardness, while iota-carageenan was very effective in increasing water holding capacity. In earlier studies, Foegeding and Ramsey (1986) studied the effect of iota-carrageenan, kappa-carrageenan, guar gum, locust bean gum, xanthan gum, methylcellulose, and a locust bean gum/kappa carrageenan mixture on water retention and texture characteristics of low-fat, high moisture meat batters. They found that
kappa- and iota-carrageenans were the most beneficial gums in manufacturing low-fat frankfurters. Kappa- and iota-carrageenan decreased cooking loss and improved texture without significant changes in sensory properties.

Shand et al. (1993) studied the effect of two levels of kappa-carrageenan (0.5% and 1%) and three levels of NaCl (1-3%) on the yield and texture of structured beef rolls with 33% added water. The addition of k-carrageenan improved cook yield and textural properties at all levels of sodium chloride. Furthermore, reduction of salt to 1% and incorporation of carrageenan resulted in products with better characteristics than controls. These researchers, therefore, suggested the use of kappa-carrageenan to improve the overall characteristics of low-salt beef systems.

Bater et al. (1993) used κ-carrageenan in combination with other hydrocolloids to improve the quality characteristics of cured turkey thigh meat with 60% brine added. Incorporation of 0.5% k-carrageenan increased cooked yield, visual appearance, sliceability and bind and decreased freeze and thaw purge compared to control. However, combinations of 0.5% κ-carrageenan and 2% nonfat dry milk or starch yielded higher binding values than those obtained with 0.5% κ-carrageenan alone.

Trius (1994) studied the effect of various extrinsic parameters, such salt type, ionic strength, carrageenan and phosphate on the performance of carrageenans in beaker sausage. Effect of meat pH on the functionality of carrageenan was also studied. One of the studies focused on the effect of three types of salts (NaCl, KCl and CaCl2) on the cooking losses and textural properties of beaker sausages treated with kappa-, iota- or lambda- carrageenan. Trius (1994) found that the use of CaCl2 resulted in excessive cooking losses and very poor
textural qualities in all the carrageenan treatments as well as the control. Iota- and lambda-
carrageenan sausages had greater water retention in the presence of KCl. This salt, however,
had a negative effect on the ability of kappa-carrageenan to retain water. In a second study,
Trius (1994) investigated the effect of phosphate and meat pH on the functionality of
carrageenan (kappa, iota and lambda). Addition of sodium tripolyphosphate or higher pH
decreased cooking losses and increased firmness. However, softer texture was observed in
lambda-carrageenan sausages. At low pH and in absence of phosphate, kappa- and iota-
carrageenan significantly decreased cooking losses.

Literature Cited

Meat Conf. 37:36-42.


18:99-121.

Amato, P. M., Hamann, D. D., Ball, R. H., Jr., and Foegeding, E. A. 1989. Influence of
poultry species, muscle groups, and NaCl level on strength, deformability, and water


Trius, A. Performance of different carrageenans in meat batters and model systems. MS. thesis. Iowa State University, Ames, IA.


FUNCTIONALITY OF CARRAGEEANAN AND SALT SOLUBLE MEAT PROTEINS IN MODEL SYSTEMS

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ABSTRACT

Protein-carrageenan gels were made using a combination of salt-soluble meat proteins (SSMP) and carrageenans (κ-, ι-, and λ-CGN). The effects of CGN on the rheological properties, water loss and ultrastructure of SSMP gels were evaluated. Also, the natures of the forces involved in the formation and maintenance of SSMP/κ-CGN gels were investigated. Of all CGN studied, κ-CGN showed the greatest effect on gel strength, while λ-CGN was detrimental to SSMP gelation. κ-CGN increased significantly the gel strength of SSMP, suggesting that a molecular interaction may have occurred. However, no indication of specific molecular interactions between κ-CGN and SSMP was observed. Cryo-SEM studies suggested that improvement of water holding capacity and texture of combined SSMP/κ-CGN gels may be due to physical rearrangement of the κ-CGN and SSMP molecules rather than to a molecular interaction.

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INTRODUCTION

The current consumer trend toward low calorie foods has increased the food industry's interest in developing low-fat meat products with acceptable sensory characteristics (Brewer et al., 1992). During the manufacturing of low-fat products, fat is partially replaced by non-meat ingredients such as hydrocolloids (carrageenan, starch, maltodextrins, etc.) which help improve the rheological properties and stability of low-fat meat products (Foegeding and Ramsey, 1987; Bater et al., 1992a; Bater et al., 1992b; and Bater et al., 1993).

Carrageenans (CGN) cover a whole range of sulfated polysaccharides extracted from various red seaweeds. There are three major types of CGNs; kappa- (κ, gelling), iota- (ι, gelling), and lambda-CGN (λ, non-gelling). They differ chemically in their degree and manner of sulfation, presence of a position of 3-6 anhydrogalactose residues linked 1-4, different pyranose ring conformations and different cations associated with the sulfate groups (Towle, 1973). The functionality of CGN in meat systems has been related to its thermally reversibly properties. CGN dissolves throughout meat during thermal processing and gels on cooling (Carr, 1993).

Polysaccharide-protein interactions are well documented for non-meat proteins (Schmidt and Smith, 1992a; Schmidt and Smith, 1992b; Ozaka et al., 1984; Stainsby, 1980; Hood and Allen, 1977; Snoeren et al., 1975; Grindrod and Nickerson, 1968; Hansen, 1968). However, few studies have been conducted of the interaction of meat proteins and hydrocolloids (Bernal et al., 1987; Lever-Garcia, 1988). Bernal et al. (1987) studied the forces involved in the interaction of myofibrillar proteins/polysaccharide/calcium systems with
various stabilizer/destabilizer reagents (0.05 M NaCl, 0.05 M sodium thiocyanate [NaSCN], 10% propyl glycol [PG], 0.02 M 2-mercaptoethanol [MeSH] and 0.05 M urea). Although, the formation of κ-casein/CGN complexes in presence of calcium have been reported, interactions between CGN and SSMP were not observed by Bernal et al. (1987). These authors suggested that CGN helped water holding capacity of meat emulsions by holding the water in the interstitial spaces of the gel network rather than by true interactions with proteins. However, the failure of carrageenan to gel in their system may have obscured their findings.

The objectives of this study were to evaluate model systems for study of possible interactions between CGNs (κ, τ, or λ) and SSMP and to determine the molecular forces involved during the SSMP and CGN interactions. For the purpose of this research, two model systems were used; dry CGN and CGN in solution.

MATERIALS AND METHODS

Functionality of Dry Carrageenan in Salt Soluble Meat Protein Gels

Experiment 1: Effect of κ-, τ-, and λ-carrageenan on the physical characteristics of heat-induced SSMP gels

Protein Extraction

Semimembranosus muscles from pigs were obtained from the Meat Laboratory at Iowa State University. The meat was trimmed of excess fat, ground through a 4.5 mm plate (Jupiter model 863, Germany), vacuum packed, boxed, and frozen at -40 °C until further use.
Before the experiment, the frozen samples were allowed to thaw overnight in a 2 °C walk-in cooler. The meat pH was determined after mixing 10 g of ground muscle with 100 ml of deionized-distilled water for one minute by means of a pH meter (Accumet 925 pH/ion Meter, Fisher Scientific).

One part of meat and two parts of a 0.58 M saline (0.49 M NaCl, 17.8 mM Na₅P₃O₁₀ and 1mM NaN₃, pH 8.3, 2 °C) solution were blended for 30 sec in a laboratory blender (Waring commercial, New Hartford, CT). The slurry was kept at 2 °C for 1 hr and then centrifuged at 12,000 x g at 2 °C for 1 hr (Beckman model J21C centrifuge, Beckman Instruments Inc., St. Louis, MO). The protein extract was strained through three layers of cheese cloth (Camou et al., 1989). Protein concentrations of meat and supernatant were determined by a nitrogen analyzer (model FP-428, LECO corporation, St. Joseph, MI). Moisture and fat determinations were performed using AOAC official methods (AOAC Official Methods of Analysis, 1990).

**Gel Preparation**

Salt-soluble meat protein solutions were diluted to 5% protein with a saline solution of the same ionic strength (ca. 0.47 μ) and pH (6.00) as the protein extract. The ionic strength of the meat extract was calculated taking into account the ionic strength of muscle, 0.26 μ (Bendall, 1954), and the μ of extracting solution (0.58 μ). kappa-, iota- or lambda-CGN (0.5 g) was added to 99.5 mL SSMP extract (ca. 0.47μ) and to 99.5 mL of saline solution (ca. 0.47 μ) to make SSMP/CGN and CGN (κ, ι, λ) dispersions, respectively. The control treatment consisted of SSMP with non-CGN added. All treatments were stirred and homogenized for 30 seconds at medium speed (set 5) by means of a Polytron homogenizer.
(Model PT 10/35, Brinkmann Instruments, Inc., Westbury, N. Y.). Forty mL of the sample were added, in duplicate, to 150 mL beakers. The beakers were allowed to equilibrate at room temperature for 1 hr, then placed in a water bath at 70 °C and heated until the internal temperature of the gel reached 70 °C. The gels were removed and placed in an ice bath and held overnight at 2 °C.

**Gel Evaluation**

**Rheological characteristics**  An Instron Universal Testing machine (model 4502, Instron Corp., Canton, MA) equipped with a 100 N load cell and a cyclic computer software program (cyclic 2, Instron Corp., Canton, MA) was used to measure texture characteristics. The gels were compressed two times to 20% of their original height, at a constant speed of 50 mm/min. An anvil of 35 mm of diameter was used to compress the gels. Peak load, hysteresis and elasticity values obtained after compressing were recorded.

**Water losses**  The gels were placed in centrifuge tubes, weighed and centrifuged at low speed (476 x g for 15 min) to prevent disruption of the gel caused by centrifugal forces (Camou, 1989). The liquid in the supernatant was decanted and saved for further analyses. The percent water loss was calculated as the difference of the total gel weight and the water expelled by the gel.

**Statistical Analyses**

The experiment was replicated three times and results were analyzed using a completely randomized design with seven treatments. 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.
Experiment 2: Molecular Forces involved in protein-carrageenan interactions

Gel Preparation

SSMP and SSMP/κ-CGN gels  SSMP solutions were diluted to 1 and 5% protein concentrations with a saline solution of the same ionic strength and pH as the meat extract. The ionic strength of the meat extract was calculated taking into account the ionic strength of muscle, 0.26 μ (Bendall, 1954), and the μ of extracting solution (0.58 μ). The treatments consisted of a factorial combination of stabilizing/destabilizing reagents with six levels, control (non-reagent), 0.05 M NaCl, 0.05 M NaSCN, 10% PG, 0.02 M 2-MeSH and 0.05 M urea and κ-CGN with two levels, 0 and 0.5%. The reagents were solubilized in SSMP extracts followed by the addition κ-CGN. All treatments were stirred and homogenized for 30 sec at medium speed (set 5) by means of a Polytron homogenizer. Forty mL of the samples were added in duplicate to 150 mL beakers. The beakers were allowed to equilibrate at room temperature, then placed in a water bath set at 70 °C and heated until their internal temperature reached 70 °C. Gels were cooled in an ice bath and held overnight at 2 °C.

κ-CGN gels  Two different ionic strength solutions (0.47 μ and 0.38 μ) were made using NaCl and Na₅P₃O₁₀. These solutions represented the ionic strengths of the protein extracts in which the value of μ for the muscle (0.26) was either considered (0.47 μ) or neglected (0.38 μ). This consideration is very important since CGN gelation, particularly κ-CGN, is highly affected by ionic strength (Zabik and Aldrich, 1968) and the actual ionic strength of the meat extract is difficult to determine. The reagents (0.05 M NaCl, 0.05 M NaSCN, 10% propylene glycol, 0.02 M 2-mercaptoethanol and 0.05 M urea) were added to the solutions, followed by the addition of 0.5 % κ-CGN. The dispersions were homogenized,
and heated as previously described during preparation of SSMP and SSMP/κ-CGN gels.

**Gel Evaluation**

**Rheological characteristics and water losses**  These characteristics were determined as described above.

**Protein concentration**  The amount of protein present in the SSMP extract and in the supernatant after centrifugation at 476 x g for 10 min was quantified with a nitrogen analyzer.

**Electrophoresis**  Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 10% acrylamide [bis-acrylamide/acrylamide, 1:10 (w/v)] slab gels (pH 8.8) and 5% stacking gel (pH 6.8). Protein samples were incubated at 50 °C for 15 min in a sample buffer containing 60 mM Tris-HCl (pH 6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, and 0.1 % pyronin. The amount of protein loaded on the polyacrylamide gel was 30 μg. Electrophoresis was conducted at room temperature with 30 mA per gel for about 5 h using Iris-glycine-SDS buffer (pH 8.3) as electrode buffer. Gels were stained with Coomassie brilliant blue R250 (Bollag and Edelstein, 1991). Molecular weights were determined by comparing mobilities of proteins in the samples with mobilities of a high and low molecular weight standard mixtures (Sigma, St. Louis, MO).

**Statistical Analyses**

The experiments were replicated twice and results were analyzed using a 2 x 6 factorial design with either two CGN levels (0 and 0.5 %) or two ionic strengths (0.38 and 0.47 μ) and six treatments (control, NaCl, NaSCN, urea, MeSH and PG). 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.

**Functionality of Carrageenan in Solution in Salt Soluble Meat Protein Gels**

**Experiment 1: Effect of protein concentration on the gelation of salt-soluble meat proteins with carrageenan added**

**Protein Extraction**

The extraction procedure was the same described in experiment 1.

**Gel Preparation**

Salt-soluble meat protein extracts were diluted to 2%, 3% and 4% protein concentration with a 0.33 M NaCl and 12.2 mM \( \text{Na}_5\text{P}_3\text{O}_{10} \) solution of the same ionic strength (0.38 \( \mu \)) and pH (6.00) as the protein extract. The CGN solutions were prepared dissolving 1.2 g of CGN in 118.8 mL hot saline solution (0.2 M NaCl and 12.2 mM \( \text{Na}_5\text{P}_3\text{O}_{10} \), 0.26 \( \mu \)). A temperature control system (Fig. 1), composed of two closed jackets, hoses and a circulating water bath (Haake type F 423, Berlin, Germany), was designed to control the temperature of the solutions.

The preparation of the SSMP/\( \kappa \)-CGN gels was as follows; CGN solutions and SSMP extracts were added to beakers "a" and "b," respectively, and stirred until the temperature dropped to 40 °C (Fig. 1). When both solutions reached 40 °C, 50 mL of the 1% CGN solution (0.26 \( \mu \)) was added to 50 mL of SSMP extract and stirred at medium speed for a few
seconds. Control gels (κ-CGN and SSMP gels) were made in similar way. κ-CGN gels were made by combining 50 mL of 1% κ-CGN solution (0.26 μ) and 50 mL of saline solution (0.38 μ). The SSMP gels resulted from the addition of 50 mL of a saline solution (0.26 μ) and 50 mL of SSMP extract. Approximately, 40 mL of the mixture was added to 150 mL beakers, in duplicate. The solutions were allowed to equilibrate at room temperature for 1 hr, then placed in a water bath at 70 °C until their internal temperature reached 70 °C. The gels were removed and placed in an ice bath and held overnight at 2 °C.

**Gel Evaluation**

**Rheological characteristics and water losses** These characteristics were determined as described above.

**Statistical Analyses**

The experiments were replicated three times and results were analyzed using a 2x3 factorial design with two CGN levels (0 and 0.5 %) and three protein levels (1.0, 1.5 and 2.0 %). 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.

**Experiment 2: Effect of carrageenan on the ultrastructure of heat-induced meat protein gels**

Two-percent protein gels (SSMP, SSMP/κ-CGN) and 0.5% κ-CGN gels were made as described above. The gels were cut into 3 mm cubes, fixed to a specimen holder with
tissue glue and cryofrozen by plunging the unit into nitrogen slush at -210 °C in a EMScope SP2000A cryo-unit with vacuum (EMScope, Ashford, Kent, U.K.). After cryofixation, samples were fractured, sublimated for 45 min at -80 °C and sputter-coated with gold for 4 min (Xu et al., 1992). The observations were made in a JEOL JSM-35 scanning electron microscope with a cold stage operated at 15 kV. The experiment was replicated twice.

**Experiment 3: Molecular forces involved in maintaining the gels**

A 2 x 2 x 4 factorial design with two pH levels (4.5 and 6.5), two gel types (SSMP and SSMP/k-CGN) and four reagents (control, NaCl, 2-MeSH and PG) was used to study the forces involved in maintaining the gels.

Salt-soluble meat protein gels were made with 4 % protein extract, since 2 % protein gels were too soft to handle. SSMP/k-CGN gels (2 %) were made using the temperature control system, as described previously. The gels were cut into 3.77 cm² surface cylinders using a metal cork of 0.3 cm diameter. The cylinders were placed in 50 mL beakers containing sodium phosphate buffers of pH 4.5 and 6.5 (controls) and one of the following reagent; 0.5M NaCl, 0.2 M 2-mercaptoethanol, and 20% propylene glycol. The samples were held at room temperature for 24 hr in continuous agitation and centrifuged at 10,000 g for 15 min. Two mL of supernatant were collected. The protein concentration was measured using a nitrogen analyzer.

**Statistical Analyses**

The experiments were replicated two times and results were analyzed using a 2x2x4 factorial design with two pH levels (4.5 and 6.5), two gel types (MP and MP/k-CGN) and
four reagents (control, NaCl, 2-MeSH and PG). 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.

RESULTS AND DISCUSSION

Functionality of Dry Carrageenan in Salt Soluble Meat Protein Gels

**Experiment 1:** Effect of $\kappa$-, $\iota$-, and $\lambda$-carrageenan on the physical characteristics of heat-induced salt-soluble protein gels

The raw meat had the following composition: Moisture $72.94 \pm 0.66$ %, protein $23.6 \pm 0.14$ %, fat $3.87 \pm 0.57$ and pH $5.30 \pm 0.01$.

**Gel Evaluation**

**Rheological evaluation** The gel strength, elasticity and hysteresis of the gels were determined. Peak load (hardness) indicated the energy under the curve at the highest peak after the first compression. Elasticity was calculated as the ratio of energies at the first and second peaks of the cyclic program (Lefebvre and Theraudin, 1992). Elasticity values of 1 represented gels that were 100 % elastic. Hysteresis was defined as the deformation process where the loading and unloading parts (energies) do not coincide, but form a loop (Muller, 1973; Prentice, 1984). The higher the hysteresis value, the higher the percent of loading energy, therefore the less elastic the material.

Addition of carrageenans ($\kappa$, $\iota$, and $\lambda$) to SSMP significantly ($P<0.05$) affected the
physical properties of SSMP gels (Table 1). κ- and τ-CGN significantly 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 τ- and λ-CGN in terms of gel strength. All CGN had lower gel strength alone than SSMP and SSMP/λ-CGN gels (Table 1). CGN had no effect (P>0.05) on final elasticity of the SSMP gels (Table 1), in spite of the marked differences observed among CGN gels. As expected, τ-CGN had the highest elasticity value (0.78) while κ-CGN was found to be very inelastic, a property that has been observed by Towle (1973). The hysteresis characteristics of the samples in our experiment were only affected (P>0.05) by the addition of CGN. κ-CGN had the highest hysteresis value (P<0.05), indicating that they were the most brittle gels.

**Water loss** Water loss determinations were only done on the SSMP and SSMP/CGN gels since CGN gels were difficult to handle. Addition of CGN (κ, τ 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. The ability of plant gums to contribute to viscosity or to form gels may help explain the decrease in water
loss observed by the authors. However, greater water holding capacity of SSMP/λ-CGN gels did not reflect an increase in gel strength (Table 1). This is because λ-CGN increases viscosity but does not form a gel.

**Experiment 2: Molecular forces involved in protein-carrageenan interactions**

The raw meat had the following composition: Moisture 74.83 ± 0.45 %, protein 22.05 ± 1.32 %, fat 2.85 ± 0.04 and pH 5.65 ± 0.27.

**Rheological evaluation** 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/CGN gels made with 1 % and 5 % protein extract and 0.5 % κ-CGN is shown in Fig. 2. Gel strength was significantly (P<0.05) affected by 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 addition of the destabilizing reagents to 1 % SSMP did not significantly (P>0.05) change the gel strength of SSMP, however it changed the properties of SSMP/κ-CGN gels (Fig. 2a). No significant (P>0.05) differences in gel strength were observed among gels treated with 2-MeSH, Urea, and control (non-treated) gels, however, SSMP/CGN gels that contained NaCl, NaSCN and PG were significantly (P<0.05) weaker than control (Fig. 2a). NaSCN gels were the softest of all treatments. 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. Whiting (1987) reported no significant effect of MeSH on the gel strength of pork/beef meat batters.

Pure κ-CGN gels treated with these reagents at two ionic strengths (Fig. 3a) behaved similarly to the SSMP/κ-CGN gels. NaSCN and NaCl significantly (P<0.05) decreased κ-CGN gel strength. NaSCN is known to affect electrostatic interactions that are important in the formation of κ-CGN and SSMP gel matrixes. A study conducted by Utsumi and Kinsella (1985) on the forces involved during the formation of soy protein gels indicated that the addition of NaCl and NaSCN produce weakening of the gels. Bernal et al. (1987) observed similar effect of NaCl and NaSCN on alginate gels. However, no differences in viscosity were observed by these researchers in carrageenan/meat protein gels treated with the former destabilizing agents. Nevertheless, their conclusions were obscured by the fact that the CGN samples used in the study were unable to gel.

The addition of κ-CGN to 5% protein extract increased significantly (P<0.05) the gel strength of the SSMP gels (Fig. 2b). Among all the treatments used, PG was the only treatment to have an effect on SSMP gel strength. These results showed that SSMP and SSMP/κ-CGN gels became harder (P<0.05) with the increased involvement of hydrogen bonds and electrostatic interactions. The presence of PG diminishes hydrophobic bonds, but enhances the contribution of hydrogen bonds and electrostatic interactions by lowering the electrostatic constant. At 5% protein concentration, the reagents effect was not as clear as at
1 % protein concentration.

It is not clear whether hydrogen bonding or electrostatic interactions were involved in the increase of SSMP and SSMP/κ-CGN gel strength since 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 CGN instability rather than to the weakness of SSMP and CGN interaction.

The elasticity and hysteresis of the SSMP gels made with 1% protein extract were significantly (P<0.05) affected by the addition of CGN (Fig. 4). However, no significant (P>0.05) effect was attributed to reagent type. SSMP gels were significantly (P<0.05) more elastic than SSMP/κ-CGN gels as demonstrated by their higher and lower elasticity (Fig 4a) and hysteresis values, respectively (Fig 4b). Non-significant (P>0.05) differences in elasticity and hysteresis values were observed between SSMP and SSMP/κ-CGN gels made with 5% protein and treated with the stabilizing/destabilizing reagents.

**Water loss**  As expected, the addition of κ-CGN to SSMP significantly (P<0.05) increased the water holding capacity of both 1 % and 5 % SSMP/κ-CGN gels (Fig. 5). 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. 5a). These effects were not observed when 5% SSMP concentrations were used to make the gels. Higher protein concentration in the solutions may have masked the effect of these reagents (Fig. 5b).
Protein loss Because NaSCN and urea interfered with the analysis, these treatments were not included in the assay. The protein concentration of the water released by the SSMP and SSMP/κ-CGN gels after centrifugation was not significantly (P>0.05) affected by the addition of κ-CGN. These results may suggest that κ-CGN was not involved in the stabilization of these proteins (Fig. 6). As expected, higher protein concentration was obtained in gels with 5% protein.

Electrophoresis SDS-PAGE was used to monitor the protein profiles in the expelled water of heat-induced gels after compression, and the selective separation of the SSMP treated with various destabilizing reagents and κ-CGN. Figure 7 shows the SDS-PAGE profile of high and low molecular weight standard mixtures (lane 1-2) and SSMP in the expelled water of heat-induced SSMP and SSMP/κ-CGN gels untreated (lane 3-4), treated with NaCl (lane 5-6), NaSCN (lane 7-8) and MeSH (lane 9-10). Figure 8 shows the fractions of SSMP in the expelled water of heat-induced SSMP and SSMP/κ-CGN gels untreated (lane 1-2), treated with urea (lane 3-4) and PG (lane 5-6) and the molecular patterns of a SSMP extract and high and low molecular weight standard mixtures (lane 8-9).

The protein profile of SSMP extract changed drastically after heat treatment (Fig 6-7). Myosin heavy chain was the major protein removed from the expelled water, suggesting that this protein was involved in gel formation and contributed to gel strength. The proteins that were not bound to the gel network were released after compression. Typical proteins released in the supernatant were albumin (66 Kd), tropomyosin β (approx. 36 Kd) and tropomyosin α (approx. 34 Kd) and myosin LC1 (approx. 20 Kd). The presence of the former proteins in
the supernatant supports the finding published by Samejima et al. (1982) and Camou et al. (1989). Samejima et al. (1982) reported that tropomyosin was highly heat stable and did not contribute to gel formation. They also found that myosin light chains remained soluble after heat treatment, indicating that light chains do not contribute to gel formation. These observations supported the idea that upon heating myosin, myosin heavy chains aggregate and gel while light chains dissociate and solubilize (Samejima et al., 1984). Similar results were observed by Camou (1989) who found some actin, tropomyosin (β and α-isoforms), and MLC1 in the expelled water. Porcine albumin, a sarcoplasmic protein, which remained soluble after heat treatments, was also reported by Camou (1989). In our studies, some actin (45 Kd) was observed in the SDS-PAGE when 5% SSMP extract was used to make the SSMP and SSMP/κ-CGN. Actin was not observed in the gels made with 1% SSMP, suggesting that all actin molecules were involved in the gel.

No differences in protein profiles among control, NaCl, NaSCN, urea, and PG treatments were observed, regardless of the addition of κ-CGN. MeSH reduced the disulfide bonds of albumin (Fig. 8), resulting in low molecular weight fractions that could not be separated with SDS-PAGE (Haard, 1985). This effect was observed independently of the presence of κ-CGN.

The addition of κ-CGN to 1 % and 5% SSMP extracts did not affect the protein profiles of the water expelled from the gels, suggesting that κ-CGN did not modify the protein in the network formation.
Functionality of Carrageenan in Solution in Salt Soluble Meat Protein gels

A temperature control system was used to mix CGN and SSMP in solution (Fig. 1). At room temperature κ- and τ-CGN gelled, therefore dissolving CGN in the SSMP extract was impossible. 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. The optimum temperature for mixing the solutions was 40 °C. Temperatures higher and lower than 40 °C caused denaturation of meat protein and gelation of κ-CGN, respectively.

The effect of three major CGN on the gel strength of SSMP was the focus of preliminary studies. κ-, τ-, λ-CGN solutions were prepared as described in materials and methods. The addition of λ-CGN to SSMP was very detrimental to gelation, resulting in gels softer than control (SSMP gels). These results were also observed in dry carrageenan experiments. The gelling temperature of τ-CGN at the ionic strength and CGN concentrations used was higher than 40 °C. In preliminary studies it was observed that ionic strength higher than 0.3 increased the temperature of gelation. Hence, the CGN solutions gelled before the desired temperature was reached, making it impossible for the τ-CGN solution to dissolve in the SSMP extract. Therefore, κ-CGN was the only CGN used for this portion of the study.
Experiment 1: Effect of protein concentration on the gelation of SSMP with CGN added

The raw meat had the following composition: Moisture 75.09 ± 0.07 %, protein 24.2 ±0.3 %, fat 1.24 ± 0.37 % and pH 5.4 ± 0.07.

Gel Evaluation

Rheological characteristics The effect of protein concentration on the gel strength of SSMP and SSMP/κ-CGN gels is shown in Fig. 9. Protein concentrations up to 2 % in the SSMP gels did not significantly (P>0.05) improve gel strength. Furthermore, the addition of 0.5 % CGN to SSMP extract, at the three protein concentrations used, did not significantly (P>0.05) increase the gel strength of the SSMP/κ-CGN gels. However, significant differences between SSMP and SSMP/κ-CGN gels were obtained, despite protein concentration (Fig. 9a). Addition of κ-CGN to SSMP resulted in gels with higher gel strength than CGN or SSMP independently. SSMP/κ-CGN (2119.0±92.03) gels were approximately several fold harder than κ-CGN (1204.17 ± 49.04) and SSMP (26.67±8.58) 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 Water losses were significantly (P<0.05) different between gel types (Fig. 9b). SSMP/κ-CGN gels hold significantly (P<0.05) more water than SSMP gels. 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. This was not so for SSMP gels, which behaved similarly at all protein levels.

**Experiment 2: Effect of CGN on the ultrastructure of heat-induced SSMP gels**

The microstructure of the gels (Fig 10) showed distinctive differences, which tended to correspond to the differences in hardness and water holding capacity showed earlier in the protein concentration study (Fig. 9). The micrograph of SSMP/κ-CGN gels showed a well structured matrix with a highly interconnected network of strands (Fig. 10e,f) that may offer more resistance to applied stress and higher water holding capacity (Fig 9). 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 (Fig. 10a,b). Although, large open spaces were also observed in κ-CGN gels, the network seemed to be well interconnected and smooth (Fig. 10c,d). 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.

**Experiment 3: Molecular forces involved in maintaining the gels**

The effect of various destabilizing reagents on solubilization of proteins from SSMP/κ-CGN gels may reflect the forces contributing to the maintenance of the gel. The amount of protein released into solution, should indicate the type of interaction. In our study, the
amount of protein obtained from the supernatant was very small. Furthermore, no significant
difference (P>0.05) in protein concentration was found among treatments, regardless the
presence of CGN (data not shown).

CONCLUSIONS

Two model systems were used to evaluate the interaction between SSMP and CGN. The first system involved the addition of dry CGN to SSMP. Rheological properties of SSMP
gels were affected by the addition of CGN. Among all CGN used, κ-was the most effective in
increasing the gel strength of SSMP gels, while, λ-CGN was detrimental to gelation. All
CGNs were effective in improving the water holding capacity of the gels. Addition of
destabilizing agents to SSMP, SSMP/κ-CGN gels did not indicate the presence of a specific
molecular interaction between these polymers. The changes observed in SSMP/κ-CGN gels
were similar to those obtained in pure κ-CGN gels, indicating that these changes were related
to the instability of κ-CGN rather that interaction between SSMP and CGN.

The second model system evaluated the effect of adding κ-CGN in solution to SSMP.
Gel strength and water loss determinations of SSMP and SSMP/κ-CGN under various protein
concentrations showed an increased gel strength and water holding ability of SSMP upon
addition of κ-CGN. Protein concentration had no effect on gelation of SSMP in the presence
of κ-CGN, suggesting that no interaction occurred between SSMP and κ-CGN.

The ultrastructure changes of SSMP in the presence of κ-CGN was studied by cryo-
scanning electron microscopy. Cryo-SEM was helpful to show differences in the ultrastructure of SSMP, SSMP/κ-CGN and κ-CGN gels, which may help explain the rheological properties of these gels.

The results obtained in this study suggest 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 carrageenan under various conditions, which may be utilized to explain the functionality of carrageenan in low-fat meat products.

REFERENCES


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."
Table 1. Physical properties of carrageenan/salt-soluble meat protein gels.¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness (g)</th>
<th>Elasticity</th>
<th>Hysteresis² (%)</th>
<th>Water losses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSMP</td>
<td>1333.8±162.5</td>
<td>0.50±0.04</td>
<td>80.6±1.86</td>
<td>6.04±2.87</td>
</tr>
<tr>
<td>SSMP/κ-CGN</td>
<td>4442.3±504.9</td>
<td>0.44±0.04</td>
<td>86.0±3.18</td>
<td>2.10±1.19</td>
</tr>
<tr>
<td>SSMP/τ-CGN</td>
<td>1846.2±291.8</td>
<td>0.47±0.03</td>
<td>75.0±2.67</td>
<td>2.69±0.88</td>
</tr>
<tr>
<td>SSMP/λ-CGN</td>
<td>896.0±208.6</td>
<td>0.45±0.04</td>
<td>91.6±12.95</td>
<td>2.95±1.19</td>
</tr>
<tr>
<td>κ-CGN</td>
<td>597.2±203.4</td>
<td>0.36±0.04</td>
<td>95.3±8.57</td>
<td>nd</td>
</tr>
<tr>
<td>τ-CGN</td>
<td>18.6±8.4</td>
<td>0.78±0.12</td>
<td>37.5±18.16</td>
<td>nd</td>
</tr>
<tr>
<td>λ-CGN</td>
<td>9.90±3.1</td>
<td>0.89±0.11</td>
<td>23.7±17.56</td>
<td>nd</td>
</tr>
</tbody>
</table>

Std error: 149.6  0.03  6.67  0.99

¹ Means in the same column with different superscripts are significantly different (P<0.05).
² Percent of loading energy during first compression.
Fig. 2. Energy at the first peak (hardness) of 1% (a) and 5% (b) salt-soluble meat proteins (SSMP) and SSMP/κ-CGN gels treated with sodium chloride (NaCl), sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG).
Fig. 3. Energy at the first peak (hardness) and percent of loading energy at the first compression (hysteresis) of κ-CGN gels treated made at two ionic strength and treated with sodium chloride (NaCl), sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG).
Fig. 4. Ratio of the energies at the first and second peaks (elasticity) and percent of loading energy at the first compression (hysteresis) of 1% salt soluble meat proteins (SSMP) and SSMP/κ-CGN treated with sodium chloride (NaCl), sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG).
Fig. 5. Percent of water loss of 1% (a) and 5% (b) salt-soluble meat proteins (SSMP) and SSMP/κ-CGN treated with sodium chloride (NaCl), sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG).
Fig. 6. Protein concentration in the supernatant of 1% (a) and 5% (b) salt-soluble meat proteins (SSMP) and SSMP/κ-CGN treated with sodium chloride (NaCl), sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG).
Fig. 7. SDS polyacrylamide gel electrophoresis of 1% salt-soluble meat protein (SSMP) and SSMP/κ-CGN gels treated with various stabilizing/destabilizing reagents. Thirty μg of protein were added to each well. Lane 1 and 2 contain standard protein markers of 29 to 205 Kilodalton, and 14.2 to 66 Kilodaltons, respectively. Lanes 3, 5, 7, 9 contain the protein fractions of SSMP untreated, treated with NaCl, NaSCN, and MeSH, respectively. Lanes 4, 6, 8, 10 contain the protein fractions of SSMP/κ-CGN untreated, treated with NaCl, NaSCN and MeSH, respectively.
Fig. 8. SDS polyacrylamide gel electrophoresis of 1% salt-soluble meat protein (SSMP) and SSMP/κ-CGN gels treated with various stabilizing/destabilizing reagents. Thirty μg of protein were added to each well. Lanes 1, 3, and 5 contain the protein fractions of SSMP untreated, treated with urea, and PG, respectively. Lanes 2, 4, and 6 contain the protein fractions of SSMP/κ-CGN untreated, treated with urea, and PG, respectively. Lane 7 contains protein fractions of 1% SSMP extract. Lane 8 and 9 contains standard protein markers of 29 to 205 Kilodalton, and 14.2 to 66 Kilodaltons, respectively.
9. Energy at the first peak (hardness) and water loss of salt soluble meat protein (SSMP) and SSMP/κ-CGN gels at various protein concentrations.
Fig 10. Scanning electron microscopy of salt-soluble meat protein (SSMP), \(\kappa\)-CGN, and SSMP/\(\kappa\)-CGN gels.

A and B: SSMP, 10 \(\mu\)m and 5 \(\mu\)m, respectively.
C and D: \(\kappa\)-CGN, 30 \(\mu\)m and 10 \(\mu\)m, respectively.
E and F: SSMP/\(\kappa\)-CGN, 10 \(\mu\)m and 5 \(\mu\)m, respectively.
Fig 10. Continued

C and D: κ-CGN, 30 μm and 10 μm, respectively.
Fig 10. Continued

E and F: SSMP/κ-CGN, 10 \( \mu \text{m} \) and 5 \( \mu \text{m} \), respectively.
EFFECT OF CARRAGEEANAN ON THE THERMAL STABILITY OF MEAT PROTEINS

A paper to be submitted to the Journal of Food Science

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ABSTRACT

Differential scanning calorimetry (DSC) was used to investigate the interactions between carrageenans and meat proteins. Salt-soluble meat proteins (SSMP), myofibrillar proteins (MP) and ground pork were used as model systems. No thermal curves were detected by DSC in SSMP gels. Addition up to 2% of carrageenan (CGN) to MP caused a very small change in the thermal denaturation of the meat proteins. The thermal properties of ground pork, CGN and mixtures were examined under two ionic strength conditions. Three transition temperatures were obtained in ground pork samples, which were characteristic of myosin (59.40 °C), sarcoplasmic proteins (67.85 °C) and actin (82.45 °C). High ionic strength mixtures had lower thermal transition peaks. CGN did not cause major shifts in the transition temperature of meat proteins, suggesting that a molecular interaction between CGN and meat proteins did not occur.

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INTRODUCTION

Differential scanning calorimetry is an important tool for studying the thermal denaturation of meat proteins in muscle tissue in situ (Lund, 1983). The major contractile proteins, actin and myosin can be distinguished from each other and from sarcoplasmic proteins by this technique. Transition temperatures for typical muscle can range from 54-58 °C, 67 °C and 71-83 °C for myosin and its subunits, sarcoplasmic proteins, and actin, respectively (Wright et al., 1977; Findlay and Barbut, 1990). The thermal properties of these proteins may be affected by factors such as pH and salt (Findlay and Barbut, 1990). Addition of sodium chloride to meats and also changes in pH alter the stability of proteins, lowering their temperature of denaturation (Quinn et al., 1980; Stabursvik et al., 1984; Shiga et al., 1988; Ensor et al., 1991). The thermal denaturation changes of meat proteins that occur upon the addition of non-meat ingredients may indicate shifts in the physical state of the proteins or interactions between meat proteins and non-meat ingredients, which could affect some physical attributes of cooked meat products, such as juiciness and texture.

The use of polysaccharide gums such as alginate and carrageenan in meat products has been of great interest to meat processors due to the increasing consumer demand for leaner and more economical meat products. Polysaccharides have also been reported to affect the thermal transition temperatures of meat proteins. The addition of alginates to crude myofibrillar, sarcoplasmic and connective tissue proteins, lowered the thermal destabilization temperature of these proteins by 7.5, 23.6 and 8.6 °C, respectively (Ensor et al., 1991). Other studies have shown a small destabilizing effect of meat proteins upon the addition of 2 % κ-
CGN to beef chunks. This destabilizing effect observed by Shand (1994) may be a consequence of conformational changes induced by the presence of \( \kappa \)-CGN or possibly by an interaction between \( \kappa \)-CGN and meat proteins.

Changes in the thermal properties of meat proteins induced by non-meat ingredients, such as carrageenans, may suggest the presence of a protein/carbohydrate interaction. Thus, the purpose of this study was to examine the effect of three types of CGN (\( \kappa \), \( \iota \), and \( \lambda \)) on the heat denaturation of meat proteins in meat model systems.

**MATERIALS AND METHODS**

**Experiment 1: Effect of CGN on the thermal transition characteristics of meat proteins in a model systems**

The effect of CGN on the thermal transition changes of meat proteins extracted from ground pork was studied. Salt-soluble meat proteins (SSMP) and myofibrillar proteins (MP) were combined with various concentrations of \( \kappa \)-CGN and analyzed for thermal stability using differential scanning calorimetry.

**Protein Extraction**

Semimembranosus muscles from pigs were obtained from the Meat Laboratory at Iowa State University. The meat was trimmed of excess fat, ground through a 4.5 mm plate, vacuum packaged, boxed, and frozen at -40 °C until further use. Prior to the experiment, the frozen ground samples were allowed to thaw overnight in a 2 °C walk-in cooler. The meat
pH was determined after mixing 10 g of ground muscle with 100 ml of deionized-distilled water for one minute by means of a pH meter (Accumet 925 pH/ion Meter, Fisher Scientific).

**Salt-soluble meat proteins**  One part of meat and two parts of 0.56 M solution (0.49 M NaCl, 17.8 mM Na₅P₃O₁₀ and 1mM Na₂N₃, pH 8.3) were blended for 30 sec in a laboratory blender. The mixture was kept at 2 °C for 1 hr then centrifuged at 12,000 x g at 2 °C for 1 hr. The protein extract was strained through a cheese cloth (Camou et al., 1989). Protein concentration of supernatant was measured with a nitrogen analyzer (model FP-428, LECO corporation, St. Joseph, MI) and diluted to 5 % protein concentration (0.16 μ and 0.33 μ) with a NaCl and Na₅P₃O₁₀ solution. The pH of the samples was adjusted to 6.00.

**Myofibrillar proteins**  MP were extracted following the procedure described by Lever-Garcia (1988). Six-hundred g of meat was blended with 1200 mL of 0.1 M NaCl and 0.05 M phosphate buffer pH 7.1 in a blender at low speed for 30 sec. The solution was stirred at high speed for 60 min and centrifuged at 9000 x g for 15 min at 0 °C in an automatic refrigerated centrifuge. The supernatant containing fat and sarcoplasmic proteins was discarded and the pellet resuspended in 2000 mL of phosphate buffer. The extraction procedure was repeated three times.

Protein concentrations were determined by a nitrogen analyzer. The final pellet was diluted to 5 and 10 % protein concentration with a NaCl and Na₅P₃O₁₀ solution of 0.58 μ.

**Preparation of Protein/Carrageenan Mixtures**

**Salt soluble meat proteins**  SSMP, SSMP/κ-CGN and κ-CGN gels were prepared with 5% SSMP and two levels of CGN (0 and 0.5%). The dispersions were homogenized for
30 sec at medium speed (set 5) using a Polytron homogenizer (Model PT 10/35, Brinkmann Instruments, Inc., Westbury, N. Y.). The samples were held overnight at 2 °C.

**Myofibrillar proteins**  Three sets of samples were made; MP, MP/κ-CGN and κ-CGN. The MP and κ-CGN concentrations were 5 and 10% and 0, 0.5 and 2 %, respectively. CGN was added to the MP solution and to salt/phosphate to make MP/κ-CGN and κ-CGN mixtures, respectively. The mix was stirred and homogenized for 30 sec. at medium speed (set 5) by means of a Polytron homogenizer. The samples were kept overnight in a walk-in cooler at 2 °C.

**Experiment 2:** Effect of carrageenan on thermal transition of ground pork

**Preparation of Meat/Carrageenan Mixtures**

Semimembranosus muscles from pigs were obtained from the Meat Laboratory at Iowa State University. The meat was trimmed of excess fat, ground through a 4.5 mm plate, vacuum packaged, boxed, and frozen at -40 °C until further use. Prior to the experiment, the frozen ground samples were allowed to thaw overnight in a 2 °C walk-in cooler. The meat pH was determined after mixing 10 g of ground muscle with 100 ml of deionized-distilled water for one minute by means of a pH meter (Accumet 925 pH/ion Meter, Fisher Scientific). Protein, fat and moisture composition of raw meat was done by means of a nitrogen analyzer, soxhlet fat extraction and gravity oven, respectively.

The ionic strength of the samples was calculated considering the ionic strength of muscle, which has been reported to be approximately 0.26 μ (Bendall, 1954).
Treatments were prepared according with the procedure described by Shand et al. (1994) and included two ionic strength conditions and three carrageenan types (Table 1). The mix was stirred and homogenized for 30 seconds at medium speed (set 5) by means of a Polytron homogenizer. Control samples included meat mixtures with or without salt added.

**Differential Scanning Calorimetry**

A Perkin-Elmer (Norwalk, CT) DSC-7 equipped with an IBM computer and cooling system was used. Calibration of the DSC was done on a monthly basis using indium ($\Delta H_{\text{fusion}} = 6.80 \text{ cal/g; } 156.4 \degree \text{C}$) as standard. Samples were placed in stainless-steel pans, weights were recorded and entered into the computer for DSC analysis. Air was sealed in another pan and used as reference. Heating rate was 10 °C/min 25-110 °C. Energy input was measured in milliwatts (mW). All the thermal curves were normalized for weight differences. Thermal transition temperature maxima ($T_{\text{max}}$) and enthalpy ($\Delta H$) were determined using an internal curve integration program of the DSC instrument. For reproducibility, the temperature of peak maxima was taken as the transition temperature (Hastings et al., 1985).

**Statistical Analysis**

A 2 x 3 factorial design with two ionic strength levels and three CGN types ($\kappa$, $\iota$, and $\lambda$) were used to study the effect of CGN types on the thermal denaturation characteristics of ground pork. The Statistical Analysis System (SAS, 1991) was used to decide means, standard errors and analysis of variance. Least significant difference ($P<0.05$) was used to measure differences among treatments. The experiment was replicated twice.
RESULTS AND DISCUSSION

Experiment 1: Effect of CGN on the thermal transition characteristics of meat proteins in a model systems

Salt Soluble Meat Proteins

The protein concentration obtained by this preparation was in the order of 5 %, which was similar to protein concentrations previously extracted by other researchers (Camou, 1989). Salt-soluble proteins included myosin, actin and sarcoplasmic proteins. At the concentration extracted by this methodology, the typical myosin and actin peaks could not be distinguished. Therefore, this model system was not suitable for investigating CGN/meat protein interactions.

Myofibrillar proteins

Protein concentrations as high as 10 % were obtained using the procedure of Lever-Garcia (1988). This procedure included the extraction of sarcoplasmic proteins from ground muscle with a low ionic strength solution, resulting in a white thick paste composed of myofibrillar proteins and collagen. The peaks obtained with the DSC were characteristic to the transition temperatures of myosin, collagen and actin (Stabursvik and Martens, 1980) Collagen was only evident in those samples made with higher protein concentration (Table 2). Actin transition peaks were shifted to a lower temperature due to the presence of salt in the MP sample. This effect has been very well documented by several authors (Quinn et al., 1980; Shiga et al., 1988) and was also seen in our study of ground pork.

The addition of κ-CGN to MP at concentrations as high as 2% caused a slight
decrease in the temperature of transition of myosin. Lower concentrations of CGN (0.5 %) did not induce thermal changes of meat proteins. Furthermore, at lower meat protein concentrations (5 % MP), CGN concentration did not have an effect on the stability of MP (Table 2). It has been suggested that DSC techniques could be used to study interactions between protein and polysaccharides. Changes in protein structure and thermal stability are good indicators of protein-polysaccharide interactions. Therefore, shifts in MP transition temperatures in the presence of CGN may suggest an interaction between these polymers. However, the change in $T^\text{max}$ observed upon addition of 2 % CGN to 10 % MP is too small to be the likely result of an interaction.

The transition temperature of pure $\kappa$-CGN in solution was affected by its concentration. At 0.5 % CGN concentration no peak was observed, however a transition temperature of 54.45 °C was detected when 2 % CGN was added (Table 2). Similar temperature of transition for 2 % $\kappa$-CGN (53.9 ± 1.9) was observed by Shand et al (1994). These authors referred to this temperature as the "average melting temperature of $\kappa$-CGN" and indicated that above this temperature, destabilization of the $\kappa$-CGN double-helix occurs, resulting in solubilization of the CGN.

Experiment 2: Effect of carrageenan on thermal transition of ground pork

The protein, fat and moisture concentrations for meat were 23 %, 3.88 %, and 72.54 %, respectively. Thermal curves for meat samples without salt and CGN revealed three transition temperatures; at 59.40 °C ($T_{\text{max}}$), at 67.85 °C ($T_{\text{max}2}$) and at 82.45 °C ($T_{\text{max}3}$) (Table
3). Similar transition temperatures for myosin and actin have been reported by several researchers. For beef at pH 5.4, the transition temperatures reported by Stabursvik and Martens (1980) were 58 °C and 65 °C for myosin; 66 °C for sarcoplasmic proteins, 67 °C for collagen and 80 °C for actin. For porcine *M. longissimus dorsi* at pH 5.4, Stabursvik et al. (1984) reported three major peaks with temperatures maximal at 58 °C and 66 °C associated with myosin denaturation and at 78 °C, associated with actin denaturation. Quinn et al. (1980) showed temperatures of transition for pork muscle at 59 °C, 66.5 °C and 81 °C for myosin, sarcoplasmic proteins, and actin, respectively.

Ionic strength significantly (P<0.05) affected thermal transition of meat proteins. Addition of 2 % NaCl to ground meat decreased the temperature of transition of the first and third peaks by approximately 4 °C and 9 °C, respectively. The second transition temperature was not significantly affected (P>0.05) by ionic strength (Table 3). Similar results were obtained by Quinn et al. (1980), who observed that the addition of salt (0.23 M NaCl) to pork muscle caused the disappearance of a 81 °C peak and the formation of a lower temperature peak at 76 °C. These thermal transition peaks were characteristics of actin under these ionic conditions. Also, the temperature of the first peak was decreased by 1.5 °C. Shiga et al. (1988) also found a shift in *T*<sub>max</sub> for SSMP in the presence of NaCl. Actin *T*<sub>max</sub> decreased from 76.8 °C to 68.3 °C upon addition of 3 % NaCl.

Two percent of CGN was used in this experiment because preliminary studies using 0.5 % CGN in myofibrillar systems did not show any changes in the behavior of the proteins (Table 2). Although, changes in thermal transitions for myosin in the presence of 2 % CGN were not very drastic and probably do not indicate an interaction, the followed experiment
was conducted to investigate the behavior of CGN in a more complex system. The addition of carrageenan, particularly κ-CGN, had a small destabilizing (P<0.05) effect on the third transition temperature of meat at low ionic strength. However, at high ionic strength, the same effect was not seen. An influence on thermal transition temperature was not observed at the first peak for meat/iota and meat/kappa mixtures. Shand observed a very small negative effect on the transition temperature of beef with the addition of 2% κ-CGN at various ionic strength conditions. In our study, the κ-CGN destabilizing effect was only observed at the third peak under low ionic conditions. Although statistically significant, the difference in thermal transitions is too small to suggest a major protein/CGN interaction. As it is typical for DSC thermograms of meat proteins, only a single enthalpy peak occurs in the range of 50-90 °C. The enthalpy values did not differ (P>0.05) among treatments and were not affected by ionic strength.

Studies conducted by DeFreitas et al. (1994a, 1994b) indicated that addition of 0.5% λ-CGN to SSMP and pork sausages resulted in gels and sausages with softer texture characteristics than control treatments (non-CGN added). These results may suggest that λ-CGN caused a destabilizing effect of λ-CGN on the gelation of meat proteins. However, thermal destabilization of meat proteins upon addition of 2% λ-CGN was not observed in DSC thermograms (Table 3), therefore this effect could not be explained by this technique.

The thermal temperatures of transition for carrageenan are shown in table 4. Transition temperatures of κ-CGN and τ-CGN were significantly (P<0.05) increased by the addition of NaCl. Similar results have been reported by Shand et al. (1994) in their study of
κ-CGN in meat mixtures. These authors observed a $T_{\text{max}}$ of $59.2 \pm 1.1 \degree C$ for 2% κ-CGN gels with 3% NaCl added, compared to a $T_{\text{max}}$ of $52.6 \pm 2.6 \degree C$ for κ-CGN in water. The κ-CGN transition temperatures observed in our study were $54.6 \pm 0.55 \degree C$ and $62.6 \pm 2.48 \degree C$ for low and high ionic strength treatments, respectively. 1-CGN gels did not show a peak at lower ionic strength conditions, however at higher ionic strength a $T_{\text{max}}$ of $82.20 \pm 1.51$ was detected. These results may suggest that in processed meats, 1-CGN may be not completely solubilized, therefore decreasing its functionality. As it was expected for λ-CGN gels (nongelling CGN), no peaks were obtained at the ionic conditions studied. At high ionic strength, transition temperatures of κ-CGN was higher (63.11 °C) than at low ionic strength. Furthermore, 1-CGN showed a higher transition peak than κ-CGN. A similar increase of κ-CGN transition temperature at high ionic strength conditions was observed by Shand (1991).

CONCLUSIONS

Changes in transition temperatures may indicate conformational changes due to protein-carbohydrate interaction. Differential scanning calorimetric studies of salt-soluble meat proteins (SSMP), myofibrillar proteins (MP), ground pork, and carrageenans (κ, 1, λ) revealed a very small destabilizing effect of meat proteins by the addition of κ- and 1-CGN to ground pork. However, this effect was too small to be attributed to protein-carbohydrate interaction. Greater changes in transition temperatures of meat proteins were observed upon addition of salt. Three peaks, which were characteristic of myosin, collagen or sarcoplasmic
proteins and actin, were obtained in MP and ground pork mixtures. 2% κ-CGN caused a small destabilizing effect of the third transition peak.

κ- and λ-CGN gels showed higher transition temperatures at higher ionic strength. Particularly, λ-CGN had a $T_{\text{max}}$ of 82.2 °C, which may suggest that at the normal ionic conditions of processed meats, this CGN may need higher temperature to reach its melting point. No thermal peaks were observed in λ-CGN gels.

Further research on the thermal behavior of carrageenans under various ionic strength conditions and under the presence of different salts may prove useful on the selection of a particular gum for a given meat application.

REFERENCES


J. Food Sci. 59:711-715.

Shiga, K., Kami, T., and Fuji, M. 1988. Relation between gelation behavior of ground 
chicken muscle and soybean proteins and their differential scanning calorimetric 

tissue as studied by differential scanning calorimetry. J. Sci. Food Agric. 1034-1042.

Stabursvik, E., Fretheim, K., and Froystein, T. 1984. Myosin denaturation in pale, soft, and 
exudative (PSE) porcine muscle tissue as studied by differential scanning calorimetry. 
J. Sci. Food Agric. 35:240-244.

Wright, D. J., Leach, I. B., and Wilding, P. 1977. Differential scanning calorimetric studies 
Table 1. Definition of ground meat treatments used for the DSC study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IS$^1$</th>
<th>Meat (g)</th>
<th>Water (g)</th>
<th>NaCl (g)</th>
<th>CGN$^2$ (g)</th>
<th>Total weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>0.18</td>
<td>35</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\iota$-CGN</td>
<td>0.18</td>
<td>35</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\kappa$-CGN</td>
<td>0.18</td>
<td>35</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\lambda$-CGN</td>
<td>0.18</td>
<td>35</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat</td>
<td>0.52</td>
<td>35</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\iota$-CGN</td>
<td>0.52</td>
<td>35</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\kappa$-CGN</td>
<td>0.52</td>
<td>35</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat plus $\lambda$-CGN</td>
<td>0.52</td>
<td>35</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
</tbody>
</table>

$^1$ Ionic strength.

$^2$ Carrageenan.
Table 2. Differential scanning calorimetry $T_{\text{max}}$ measurements of Myofibrillar proteins (MP)/carrageenan (CGN) gels at various concentrations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_{\text{max}1}$</th>
<th>$T_{\text{max}2}$</th>
<th>$T_{\text{max}3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP (5)</td>
<td>59.6</td>
<td>np$^2$</td>
<td>75.3</td>
</tr>
<tr>
<td>MP/CGN(5/0.5)$^1$</td>
<td>60.5</td>
<td>np</td>
<td>75.6</td>
</tr>
<tr>
<td>MP/CGN (5/2)</td>
<td>59.4</td>
<td>np</td>
<td>75.6</td>
</tr>
<tr>
<td>MP (10)</td>
<td>60.4</td>
<td>68.5</td>
<td>76.8</td>
</tr>
<tr>
<td>MP/CGN (10/0.5)</td>
<td>59.1</td>
<td>68.9</td>
<td>75.5</td>
</tr>
<tr>
<td>MP/CGN (10/2)</td>
<td>58.6</td>
<td>68.9</td>
<td>75.1</td>
</tr>
<tr>
<td>CGN (0.5)</td>
<td>np</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>CGN (2)</td>
<td>54.45</td>
<td>np</td>
<td>np</td>
</tr>
</tbody>
</table>

$^1$ Numbers in parenthesis indicate concentrations of MP and CGN, respectively.

$^2$ No peak found.
### Table 3. Differential scanning calorimetry $T_{\text{max}}$ and enthalpy ($\Delta H$) measurements of meat, and meat/carrageenan (CGN) mixtures under various ionic strength (IS) conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IS</th>
<th>$T_{\text{max1}}$ Mean</th>
<th>Std.</th>
<th>$T_{\text{max2}}$ Mean</th>
<th>Std.</th>
<th>$T_{\text{max3}}$ Mean</th>
<th>Std.</th>
<th>$\Delta H$, J/g Mean</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>0.18</td>
<td>59.40 0.14a,b</td>
<td></td>
<td>67.85 0.21a</td>
<td></td>
<td>82.45 0.21a</td>
<td></td>
<td>1.52 0.45a</td>
<td></td>
</tr>
<tr>
<td>Meat plus $\tau$-CGN</td>
<td>0.18</td>
<td>58.55 0.35b</td>
<td></td>
<td>65.95 0.78b</td>
<td></td>
<td>81.60 0.42a,b</td>
<td></td>
<td>1.98 0.26a</td>
<td></td>
</tr>
<tr>
<td>Meat plus $\kappa$-CGN</td>
<td>0.18</td>
<td>58.50 0.42b</td>
<td></td>
<td>67.15 0.78a,b</td>
<td></td>
<td>80.20 0.28b</td>
<td></td>
<td>2.39 0.22a</td>
<td></td>
</tr>
<tr>
<td>Meat plus $\lambda$-CGN</td>
<td>0.18</td>
<td>59.95 0.20a</td>
<td></td>
<td>67.90 0.28a</td>
<td></td>
<td>81.90 0.15a,b</td>
<td></td>
<td>2.10 0.83a</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>0.52</td>
<td>55.15 0.64d</td>
<td></td>
<td>68.05 0.64a</td>
<td></td>
<td>73.70 0.28c</td>
<td></td>
<td>1.59 0.21a</td>
<td></td>
</tr>
<tr>
<td>Meat plus $\tau$-CGN</td>
<td>0.52</td>
<td>56.30 0.28c</td>
<td></td>
<td>68.30 0.84a</td>
<td></td>
<td>74.90 0.14c</td>
<td></td>
<td>2.45 0.70a</td>
<td></td>
</tr>
<tr>
<td>Meat plus $\kappa$-CGN</td>
<td>0.52</td>
<td>56.55 0.35c</td>
<td></td>
<td>67.90 0.28a</td>
<td></td>
<td>74.25 0.08c</td>
<td></td>
<td>2.24 0.42a</td>
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<tr>
<td>Meat plus $\lambda$-CGN</td>
<td>0.52</td>
<td>55.05 0.92d</td>
<td></td>
<td>67.85 0.78a</td>
<td></td>
<td>73.85 1.34c</td>
<td></td>
<td>2.05 0.34a</td>
<td></td>
</tr>
</tbody>
</table>

Std. error 0.34 0.44 0.47 0.12

1 Means within the same column sharing the same superscript are not significantly ($P>0.05$) different.
Table 4. Differential scanning calorimetry $T_{\text{max}}$ and enthalpy ($\Delta H$) measurements of carrageenan (CGN) mixtures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IS</th>
<th>$T_{\text{max}}$, $^\circ$C</th>
<th>Mean$^1$</th>
<th>Std.</th>
<th>Mean</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-CGN</td>
<td>0.18</td>
<td>54.60</td>
<td>0.55$^c$</td>
<td>0.52</td>
<td>0.17$^a$</td>
<td></td>
</tr>
<tr>
<td>i-CGN</td>
<td>0.18</td>
<td>np$^2$</td>
<td>np</td>
<td></td>
<td>np</td>
<td></td>
</tr>
<tr>
<td>$\lambda$-CGN</td>
<td>0.18</td>
<td>np</td>
<td>np</td>
<td></td>
<td>np</td>
<td></td>
</tr>
<tr>
<td>K-CGN</td>
<td>0.52</td>
<td>62.60</td>
<td>2.48$^b$</td>
<td>0.57</td>
<td>0.11$^a$</td>
<td></td>
</tr>
<tr>
<td>i-CGN</td>
<td>0.52</td>
<td>82.20</td>
<td>1.51$^a$</td>
<td>0.24</td>
<td>0.00$^b$</td>
<td></td>
</tr>
<tr>
<td>$\lambda$-CGN</td>
<td>0.52</td>
<td>np</td>
<td>np</td>
<td></td>
<td>np</td>
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</tr>
<tr>
<td>Std. error</td>
<td></td>
<td>0.49</td>
<td>0.07</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

$^1$ Means within the same column sharing the same superscript are not significantly (P>0.05) different.

$^2$ no peak found.
EFFECT OF SALT, PHOSPHATE, pH AND CARRAGEENAN ON THE FREEZE/THAW STABILITY OF COOKED PORK SAUSAGES

A paper to be submitted to the Journal of Food Science

Z. DeFreitas, J. G. Sebranek, D. G. Olson, and J. M. Carr

ABSTRACT

Two experiments were conducted to investigate the effect of CGN, sodium tripolyphosphate, chloride salts and meat pH on the freeze/thaw stability of cooked pork sausages. Sixteen treatment combinations with four CGN levels, two salt types and two phosphate levels were used in the first experiment. The second experiment included eight treatment combinations with four CGN and two pH levels. STPP decreased thaw drip (TD) and increased hardness of all treatments regardless of the type of salt or CGN. KCl did not affect the texture of control samples in the presence of STPP, but decreased the functionality of κ- and λ-CGN. Meat pH significantly (P<0.05) increased the hardness and decreased the TD of all CGN treatments, except for λ-CGN which remained unchanged. κ- and λ-CGN were effective in improving the stability of sausages prepared with low pH meats.

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INTRODUCTION

In 1979, the Surgeon General's Report urged the American population to improve their health by reducing dietary intake of saturated fats, cholesterol, salt and sugar; increasing the consumption of complex carbohydrates; and maintaining desirable body weight (Keeton, 1991). In response to these recommendations and to studies relating sodium and fat intake to cardiovascular disease (Keeton, 1991; Sebranek et al., 1983), researchers have attempted to find substitutes for sodium chloride and fat that would provide similar functional properties.

The effects of sodium chloride in processed meats are well known. NaCl increases water holding capacity, stabilizes meat emulsions, and enhances flavor. Other chloride salts, such as MgCl₂, CaCl₂, and KCl have been studied as possible substitutes for NaCl. Among all these salts, KCl appeared to be the most suitable substitute for NaCl when flavor characteristics are the major concern (Terrell and Olson, 1981). These researchers suggested that 50% replacement of NaCl for KCl would yield products with similar functionality.

Barbut and Mittal (1989) reported that the addition of KCl to low-fat pork batters increased the functionality of κ- and τ-carrageenans (CGN). On the other hand, Trius (1994) observed greater cooked losses when KCl was used in combination with κ-CGN. However, an increase in water retention was obtained in τ- and λ-CGN sausages treated with KCl.

The production of low-fat meat products is rapidly increasing, due to the current consumer interests in reduced calorie foods. Polysaccharide gums are often used to enhance functionality of low-fat meat products. Addition of 0.5 % κ-CGN to oven-roasted turkey
breasts increased the yield, improved sliceability and rigidity and decreased expressible juice (Bater et al., 1992).

Carrageenans are a family of sulfated linear polysaccharides of D-galactose and 3,6-anhydro-D-galactose extracted from various red seaweeds. They have been classified in three types according to structural characteristics and gelation properties: kappa (gelling), iota (gelling) and lambda (non-gelling) (Glicksman, 1983). CGN dissolves throughout meat during thermal processing and gels on cooling. Several functional properties have been attributed to CGN in meat products, including increased yields and purge control, enhanced texture of finished products, improved sliceability, and increased product juiciness (Carr, 1993).

The use of phosphates in processed meats has been very effective in increasing water holding capacity and improving texture of meat products. Nonetheless, the ability of phosphates to retain water in processed meats subjected to freeze/thaw treatments, particularly when high volumes of brine are injected, has been unsatisfactory (Acton, 1983). Bater et al. (1993) found that κ-CGN was very effective in decreasing the freeze and thaw purge of cured turkey thigh meat. However, the effect of carrageenan on the freeze/thaw stability of red meat products made at various ionic, pH and phosphate conditions has not been yet reported.

The objective of these experiments was to evaluate the effect of carrageenans (κ, ι and λ) on the freeze/thaw stability of cooked pork sausages made with two types of salts (KCl and NaCl), two phosphate levels (0 and 0.5 %) and two pH values (<5.5 and >6.1).
MATERIALS AND METHODS

Experiment 1: Effect of salt, phosphate and carrageenan on the freeze/thaw stability of cooked pork sausages

Pork cushions were obtained from a local packing plant (Carriage House, Ames, IA). The meat was trimmed of excess fat and connective tissue and ground through a 1/8 inch plate (Biro grinder, The Biro Mfg. Co., Marblehead, OH). Protein measurements were determined with a nitrogen analyzer (model FP-428, LECO corporation, St. Joseph, MI). Moisture and fat analysis were performed using official analytical methods (AOAC, 1990).

Preparation of the Sausages

Sixteen brine solutions were prepared by dissolving or dispersing dry ingredients in distilled water (30 % of meat weight). The dry ingredients used for the brine solutions included food-grade sodium chloride (Morton International, Chicago IL), potassium chloride (Fisher Scientific, Pittsburgh, PA), sodium tripolyphosphate (STPP, formula 24) and κ-, τ- and λ-CGN (Sanofi, Bio-Industries, Waukesha, WI). The mixing sequence was as follows: 0.5 % sodium tripolyphosphate was completely dissolved in distilled water, followed by the addition of sodium chloride (0.34 μ) or potassium chloride (0.34 μ) and 0.5 % carrageenan. Control treatments included sausages without carrageenan (Table 1). The brines were held overnight at 2 °C.

The treatments were randomly assigned to the experimental units (3.63 Kg ground meat) using a random permutation table of sixteen numbers (Cochran and Cox, 1950). The
experiment was a factorial combination of two salt types (NaCl and KCl), two levels of STPP (0 % and 0.5%) and four carrageenan treatments (non-CGN, κ-, τ- and λ-CGN). The sausages were prepared by blending the meat and brine for a total of three minutes in a mixer (Higashimoto Kimai, Tokyo, Japan). The meat mixtures were stuffed with a piston stuffer (Hollymatic Corp., Countryside, IL) into presoaked 4 x 24 opaque CMVP fibrous casings (Teepak, Kansas City, MO), then the casings were closed and clipped. The sausages were steam cooked to an internal temperature of 70 °C, showered for 10 min in an Alkar thermal processing unit (Alkar, DEC International, Lodi, WI), equipped with a direct digital control system. The sausages were placed in a cooler (2 °C) for two days, boxed, and frozen at -40 °C in a blast freezer (Vollrath Refrigeration, Inc., River Falls, WI) for 72 hr.

After frozen storage, slices of 2.5 cm thickness were cut using a Butcher-boy band saw (Lasar, Mfg. company Inc., Los Angeles, CA), placed in Ziploc® bags and held in a walk-in cooler for 48 hr at 2 °C to thaw. Four slices of sausage per treatment were used to measure thaw drip and texture measurements.

Sausage Evaluation

**Thaw drip**  Empty bags and total weights (bag plus frozen meat) were recorded before thawing. After 48 hr of storage at 2 °C, the meat slices were removed from the Ziploc® bags, blotted dry with towels and weighed for purge determinations. The percentage weight loss after thawing based on initial frozen weight was recorded as thaw drip.

**Texture measurements**  The meat slices were held at room temperature to equilibrate. Three core samples of 2.5 cm diameter from each slice were cut for evaluation. An Instron Universal Testing machine (model 4502, Instron Corp., Canton, MA) equipped
with a 1 KN load cell was used to measure hardness. Hardness was defined as the maximum force occurring during the compression cycle (Sanderson et al., 1988). An anvil of 35 mm of diameter was used to compress the sample to 45% of the original height, at a crosshead speed of 300 mm/min. A preconditioning program (20 g preload) was used to correct for small differences in height.

**pH measurements**  Meats mixtures were analyzed for pH differences by homogenizing 10 g of sample with 100 mL distilled water (Polytron homogenizer, Kinematica Gmbh, Luzer, Switzerland). The pH of the resultant suspension was measured with an Accumet 925 pH/ion meter (Fisher Scientific, Pittsburgh, PA).

**Statistical Analyses**

The experiment was replicated twice and was arranged in a 2 x 2 x 4 factorial design with two STPP levels (0 and 0.5 %), two salt levels (NaCl and KCl), and four CGN levels (non-CGN, κ-, τ-, λ-CGN). The Statistical Analysis System (SAS, 1991) was used to determine means, standard errors and analysis of variance. Least significant difference was used to test differences between means (P<0.05).

**Experiment 2: Effect of pH and carrageenan on the freeze/thaw stability of cooked pork sausages**

PSE (pale, soft and exudative) and DFD (dark, firm and dry) pork loins were selected from a loin line at Monfort's packing plant (Marshalltown, IA). The loins were selected by color and separated by pH. pH measurements were done on site by means of a microprocessor pH meter model PHH-70 (Omega Engineering, INC, Stanford, CN),
equipped with a Xerolyt® electrode (Mettler-Toledo AG, Greifensee, Switzerland). Loins with pH values lower than 5.5 were categorized as low-pH and those with pH values higher than 6.0 as high-pH. The loins were brought to the Meat Laboratory at Iowa State University, where they were trimmed of excess fat and skinned with a skinner machine (Townsend, Des Moines, IA). The loins were vacuum packaged and frozen at -40 °C for no more than two weeks.

**Preparation of the Sausages**

The loins were held for 48 hr at 4 °C to thaw; ground through a 1/8 inch plate (Biro grinder, The Biro Mfg. Co., Marblehead, OH); and mixed for 30 sec. Protein measurements were determined with a nitrogen analyzer (model FP-428, LECO corporation, St. Joseph, MI). Moisture and fat analysis were performed using official analytical methods (AOAC, 1990).

Eight brine solutions were prepared by dissolving or dispersing dry ingredients in distilled water (30 % of meat weight). The dry ingredients used for the brine solutions included food-grade NaCl (Morton International, Chicago IL), and κ-, τ- and λ-CGN (Sanofi, Bio-Industries, Waukesha, WI). The mixing sequence was as follows: 2 % sodium chloride (0.34 μ) and 0.5 % carrageenan. Control treatments included sausages without CGN (Table 2). The brines were held overnight at 2 °C.

The treatments were randomly assigned to the experimental units (3.63 Kg ground meat) using a random permutation table of nine numbers (Cochran and Cox, 1950). The experiment was a factorial combination of two pH values (<5.5 and >6.0) and four CGN
treatments (non-CGN, κ-, κ- and λ-CGN). The sausages were prepared by mixing the meat and brine for a total of 3 minutes in a vacuum mixer (Higashimoto Kimai, Tokyo, Japan). The meat mixtures were stuffed with a piston stuffer (Hollymatic Corp., Countryside, IL) into presoaked 4 x 24 opaque CMVP fibrous casings (Teepak, Kansas City, MO), then the casings were closed and clipped. The sausages were steam cooked to an internal temperature of 70 °C and showered for 10 min in an Alkar thermal processing unit (Alkar, DEC International, Lodi, WI), equipped with a direct digital control system. The sausages were placed in a cooler (2 °C) overnight, boxed, frozen at -20 °C in a blast freezer (Vollrath Refrigeration, Inc., River Falls, WI) for 72 hr.

After frozen storage, slices of 2.5 cm thickness were cut using a Butcher-boy bandsaw (Lasar, Mfg. company Inc., Los Angeles, CA), placed in Ziploc® bags and held in a walk-in cooler for 48 hr at 2 °C to thaw. Four slices of sausage per treatment were used to measure thaw drip and texture measurements.

**Sausage Evaluation**

The thaw drip, texture and pH characteristics of these sausages were evaluated as described in experiment 1.

**Statistical Analyses**

The experiment was replicated twice and was arranged in a 2 x 4 factorial design with two pH (<5.5 and >6.0) and four CGN levels (no added CGN, kappa-, iota-, lambda-CGN). The Statistical Analysis System (SAS, 1991) was used to determine means, standard errors and analysis of variance. Least significant difference was used to test differences between means (P<0.05).
RESULTS AND DISCUSSION

Experiment 1: Effect of salt, phosphate and carrageenan on the freeze/thaw stability of cooked pork sausages

The raw meat had the following composition: Moisture 74.60 ± 0.67 %, protein 19.96 ± 0.07 %, fat 4.08 ± 0.32 % and pH 5.93 ± 0.15.

Effect of Sodium Tripolyphosphate

Among all the factors studied, STPP was the most important main effect responsible for changes in thaw drip (Table 3) hardness (Table 3) and pH (Table 4). The addition of STPP to pork sausages significantly (P<0.05) decreased thaw drip (TD) (Fig. 1) and increased hardness (Fig. 2) and pH (Fig. 3). Woyewoda and Bligh (1986) observed a decrease in TD and cooked drip in frozen fish fillets treated with phosphates, regardless of storage temperature. The statistical interaction among phosphate-, carrageenan-, and salt-main effects (Table 3), indicated that phosphate affected the stability of freeze-thaw sausages in different ways at the two salt and four CGN levels.

The ability of phosphates to increase pH, enhance water holding capacity (WHC) and improve texture has been extensively documented (Molins, 1991). Some researchers have attributed the increase of WHC in meats treated with phosphates to changes in pH and ionic strength (Trout and Smith 1986; Swift and Ellis, 1956). Others have suggested that phosphates induce solubilization of actomyosin that increases with increasing pH (Molins, 1991). Trius (1994) reported that the addition of 0.5 % STPP to pork sausages formulated with or without CGN (κ, τ and λ) significantly increased hardness and reduced cooking losses.
Similar improvement in texture upon addition of phosphates was observed by Barbut et al. (1988). These authors reported an increase in firmness of turkey frankfurters that contained 0.4 % phosphate at either 1.5 % or 2.0 % salt concentrations.

When STPP and NaCl were added to the sausages, TD and hardness were significantly (P<0.05) decreased and increased (Fig. 1-2), respectively. This effect was also observed in the presence of KCl. However, sausages containing KCl had higher TD and lower hardness values than those with NaCl. STPP has been reported to react with NaCl-free myosin similar to salts and exhibit increased affinity for myosin in the presence of high sodium chloride concentration (Molins, 1991).

A study conducted by Moirano (cited by Molins, 1991) in which κ-CGN, sodium salts of phosphates and potassium phosphate were used to improve the textural properties of dessert gels, indicated that κ-CGN gels were enhanced by potassium ions and that phosphate was a very effective Ca++ chelating agent. However, the addition of KCl to our sausages did not enhance the gelling properties of carrageenans in relation to controls. Control treatments (non-CGN added) had the highest TD of all CGN treatments at the two levels of phosphate and salt (Fig. 1). κ-CGN and control treatments were not significantly (P>0.05) different in hardness when KCl was added to the sausages (Fig. 2), which may suggest that the presence of KCl affected the functionality of κ-CGN at the two phosphate levels. This was not observed when NaCl was used. The addition of KCl to the sausage also had a negative effect on the ability of τ- and λ-CGN to retain water, with or without STPP added (Fig. 1). The ability of CGN to retain water upon freezing and thawing was better observed when no STPP
was added (Fig. 1). However, the effect on hardness was more evident in the presence of STPP (Fig. 2). As expected, phosphates increased the pH of the batter, regardless of the type of salt present (Fig. 3).

**Effect of Salt**

To study how thaw drip, hardness and pH changed at the two salt levels used, the data was replotted using salt levels as the independent variable (Fig. 4-6). Fig. 4-5 shows the effect of salt and CGN types on the TD and hardness of freeze-thaw cooked sausages at two levels of phosphate.

The addition of KCl to the sausages increased significantly (P<0.05) TD and decreased hardness when compared to NaCl (Fig. 4-5). Control sausages were significantly (P<0.05) affected by the addition of KCl. In the presence of this salt, control sausages had the highest (P<0.05) thaw drip values of all treatments at the two levels of phosphate. Similar observations have been reported by Whiting (1987), who evaluated the effect of various salts on the functionality of beef batters. He reported an increased in water and fat exudate in the batters treated with KCl. When STPP was present, thaw drip was decreased and the differences among CGN and control means were diminished (Fig. 4). As it has been previously reported, phosphate improves gelation of meat batters by either increasing the pH (Fig. 3) or the solubilization of actomyosin.

Texture of the sausages was also affected by the addition of KCl. t- and κ-CGN sausages became softer in the presence of KCl. It has been suggested that K⁺ could interact with κ-CGN, resulting in helix aggregation, which led to softer texture and water expulsion (Trius, 1994). This effect was greater when phosphate was added. Larger differences in
hardness among treatments were obtained in STPP treated sausages.

The effect of NaCl, KCl and CaCl₂ on the hardness and cooking loss of meat batters was studied by Trius (1994). No differences between NaCl and KCl salts in back-extrusion forces of cooked batters were observed. However, cooking losses were affected by salt and CGN type. Addition of KCl resulted in higher and lower cooking losses for κ- and λ-CGN sausages, respectively.

No important changes in pH on freeze/thaw pork mixtures were observed upon addition of salt, particularly in sausages made with high pH meats (Fig. 6).

**Effect of Carrageenans**

Fig. 7-9 show the effect of carrageenans and STPP on the TD and hardness of freeze-thaw cooked sausages and pH of uncooked sausages at two levels of phosphate. The data was reploted using CGN levels as the independent variable. Addition of CGN to sausages treated with NaCl significantly decreased the thaw drip of freeze/thaw cooked sausages at the two levels of phosphate and salt (Fig. 7). Similar results were observed by Bater et al. (1993), who reported a decrease in freeze and thaw purge of cured turkey meat with κ-CGN added. Differences in TD values among CGN treatments were very low in the presence of NaCl. When STPP and NaCl were added to the sausage, no significant (P>0.05) differences in TD were found among CGN (κ, 𝜏 and λ). A similar effect was observed in sausages without phosphate added in the presence of NaCl. However, significant (P<0.05) differences in TD among all CGN including control were observed when KCl was added to the system. Particularly, control, κ- and 𝜏-CGN sausages were significantly affected by the presence of this
salt. This effect was less pronounced when phosphate was present. Trius (1994) observed an improvement in cooking loss in meat batters prepared with λ-CGN and KCl. This researcher suggested that the presence of KCl induced the formation of a structure capable of holding water. However, our data suggested that the TD values for κ-, τ-, and λ-CGN in the presence of KCl resulted from a negative effect of this cation toward κ- and τ-CGN, rather than to an improvement in λ-CGN water holding capacity (Fig. 7).

The addition of κ-CGN improved the texture of freeze-thaw sausages at the two phosphate levels when NaCl was added (Fig. 8). When KCl was present, κ-CGN sausages were as hard as the control, with or without phosphate added. The ability of τ-CGN to increase hardness was only observed in the presence of NaCl with no-STPP added. The addition of KCl affected the performance of τ-CGN, resulting in softer sausages. Sausages containing λ-CGN were significantly softer than the other treatment at both of the phosphate and salt levels (Fig. 8). This results are in agreement with some of the data reported by Trius (1994) and with observations made by DeFreitas et al (1994a) using salt-soluble meat proteins as model systems.

pH values were significantly changed with the addition of carrageenan (Fig. 9). The effect was more pronounced in the absence of phosphate. Control and λ-CGN sausages had the lowest and highest pH values, respectively (Fig. 9). Similar increase in pH values upon addition of carrageenan was observed by Trius (1994).
Experiment 2: Effect of pH and carrageenan on the freeze/thaw stability of cooked pork sausages

The raw meat had the following composition: 1) Low-pH meat; moisture 74.14 ± 0.37 %, protein 21.97 ± 0.17 %, fat 3.04 ± 0.03 % and pH 5.47 ± 0.05; 2) High-pH meat; moisture 73.92 ± 0.15 %, protein 22.93 ± 0.39 %, fat 2.56 ± 0.11 % and pH 6.18 ± 0.03.

Effect of Meat pH

Significant (P<0.01) interaction between meat pH and CGN levels were obtained for hardness and TD values. This indicates that pH affected these variables differently at the four levels of CGN. Fig. 10 shows the effect of pH and CGN on the TD and hardness of pork sausages. pH was the most important factor affecting TD and hardness of cooked pork sausages. Sausages prepared with high-pH meats were harder to compress than those made with low-pH meats. Low-pH (5.3-5.4) meats result from a condition called pale, soft and exudative (PSE) pork, in which muscle pH decreases abnormally, due to rapid rate of glycolysis. The muscle is identify by having light color, soft and watery lean with open structure (Enfält et al., 1993). As a consequence of the rapid decline of pH, denaturation of muscle protein occurs, due to the increase concentration of lactic acid and high temperature. Therefore, the ability of the proteins to form gel and hold water is greatly impaired. Park et al (1975) reported lower hardness values of sausages made with PSE meat in comparison with normal muscle.

Meat pH greatly (P<0.01) affected the water holding capacity of cooked pork sausages. The lower the pH, the higher the TD observed in all treatments. The pH value of the low-pH meats used for this study was 5.47 ± 0.05, which is close to the isoelectric point
myofibrillar proteins (pH 5.5) (Hamm and Deatherage, 1960; Lewis et al., 1986). The pH at which water holding capacity is minimum corresponds to the isoelectric point of meat proteins. Therefore, it is expected that low-pH meats would exhibit higher water losses. Trius (1994) observed greater cooking losses of batter prepared with low-pH meats. Similar results were observed in this study in terms of TD. TD was significantly (P<0.01) lower at pH greater than 6.18. High pH-meats have been attributed to a condition present in pigs before slaughter. Highly stressed pigs, just prior slaughter, may exhibit depletion of glycogen, resulting in slow decline of pH postmortem. This condition is called dark, firm and dry (DFD) pork, which is also characterized by having high pH and high water holding capacity. At high pH, proteins are highly negative charged, which causes the proteins to repel each other, creating large spaces were water is trapped, therefore increasing water holding capacity.

**Effect of Carrageenans**

Fig. 11 show the effect of carrageenans and meat pH on the TD and hardness of freeze-thaw cooked sausages. The data was replotted using CGN levels as the independent variable. The addition of various types of CGN to low- and high-pH meats significantly (P<0.01) changed the texture and WHC characteristics of cooked pork sausages. CGN affected (P<0.01) these variables in different ways depending upon the meat pH. A slight improvement in hardness was observed when κ- and τ-CGN were added to low-pH meats. A greater increase in hardness was obtained in high-pH sausages containing κ- and τ-CGN in relation to control and λ-CGN. Addition of λ-CGN resulted in softer sausages than control (non-CGN added).
Carrageenan also affected the TD of cooked sausages at a low pH. All CGN seemed to have contributed to WHC, indicating that they were able to trap water at a low pH. No effect at high pH on TD was observed (Fig. 11). These results are particularly important for the meat industry, since pork muscle quality affects pork processing revenues. In the United States the incidence of PSE and DFD was reported around 16% and 10% (Kauffman et al., 1992). Control sausages had the highest TD of all treatments when low-pH meats were used to make the batter.

Carrageenan and meat pH significantly (P<0.01) affected the final pH of the meat batters (Fig. 12). However, non-statistical (P>0.05) interaction was observed between these two main effects. As it was observed in experiment 1, control and λ-CGN batters had the lowest and the highest pH of all treatments, respectively. Because the amount of ester sulfate groups and of 3,6 anhydro-D-galactose units can be used to distinguish among CGN, λ-CGN has the highest number of sulfate groups, followed by ι- and κ-CGN. This may explain the increase in pH observed upon addition of CGN.

CONCLUSIONS

The effect of phosphate, salt and CGN on the stability of freeze/thaw cooked pork sausage was studied. The addition of sodium tripolyphosphate was the most important factor in determining changes in thaw drip, texture and pH of the sausages. Sausages that contained STPP always had the lowest thaw drip and the highest hardness values, regardless of salt and
Salt and CGN also significantly (P<0.05) affected the texture and water retention ability of the sausages. The use of KCl did not affect the hardness of control samples in the presence of STPP, but decreased the functionality of all CGN. Control samples had the highest TD of all treatments at the two levels of salt and phosphate.

Thaw drip was significantly reduced by the addition of CGN at the phosphate and salt levels studied. However, the addition of KCl affected CGN functionality and reduced CGN effectiveness. Of all CGN treatments, κ-CGN was the most effective in improving hardness when NaCl and STPP were added. In the absence of STPP, κ- and λ-CGN behaved similarly, resulting in the highest hardness values.

The effect of meat pH and carrageenans on the freeze/thaw stability of cooked sausages was also evaluated. Meat pH was the most important factor determining the changes in texture and water retention. Meat pH was the most important factor determining the changes in texture and water retention of cooked pork sausages. The functionality of CGN was affected by pH conditions. At low pH (5.47 ± 0.05), κ-, and λ-CGN significantly improved the thaw drip loss in the cooked sausages. λ-CGN, however, did not improve the stability of the sausages. Differences in thaw drip values at high pH (6.18 ± 0.03) were not that evident as at low pH. Hardness was significantly affected by pH, having greater values at higher pH. κ-CGN-containing sausages were the hardest among all treatments, followed by λ-CGN, control (non-CGN added) and λ-CGN, the latter was the softest of all sausages.

The results in these experiments showed that kappa- and iota- and lambda-
carrageenans were very effective in decreasing thaw drip of cooked sausages made under normal processing conditions. Furthermore, kappa and iota-carrageenan improved the functionality of low pH meats. These results suggested that carrageenans may be used to improve overall characteristics of cooked products that have been subjected to freezing and thawing.

REFERENCES


Whiting, R. C. 1987. Influence of various salts and water soluble compounds on the
water and fat exudation and gel strength of meat batters. J. Food Sci. 52:1130-1132,
1158.

Woyewoda, A. D., and Bligh, E. G. 1986. Effect of phosphate blends on stability of cod
fillets in frozen storage. J. Food Sci. 51:932-935.
Table 1. Treatment combinations used to study the effect of CGN, SSTP and salts on freeze/thaw stability of cooked pork sausages.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CGN(^1)</th>
<th>STPP(^2)</th>
<th>Salt(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>NaCl</td>
</tr>
<tr>
<td>2</td>
<td>kappa</td>
<td>0</td>
<td>NaCl</td>
</tr>
<tr>
<td>3</td>
<td>iota</td>
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<td>NaCl</td>
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<tr>
<td>4</td>
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<td>NaCl</td>
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<tr>
<td>16</td>
<td>lambda</td>
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<td>KCl</td>
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</table>

\(^1\) 0.5 % carrageenan.
\(^2\) Sodium tripolyphosphate.
\(^3\) 0.34 ionic strength.
Table 2. Treatment combinations used to study the effect of CGN and pH on freeze/thaw stability of cooked pork sausages.

<table>
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<tr>
<th>Treatments</th>
<th>CGN(^1)</th>
<th>pH(^2)</th>
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<tr>
<td>8</td>
<td>lambda</td>
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\(^1\) 0.5 % carrageenan.
\(^2\) low-pH: 5.47 ± 0.05.
high-pH: 6.18 ± 0.03.
Table 3. F values for selected source of variation from the factorial analysis of variance of thaw drip and hardness.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Thaw drip</th>
<th>Hardness</th>
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<td>0.59</td>
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<td>77.37***</td>
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</tr>
<tr>
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<td>1351.44***</td>
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<tr>
<td>SALT</td>
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<td>80.52***</td>
<td>49.16***</td>
</tr>
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<td>22.15***</td>
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<td>CGN*SALT</td>
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<td>6.48**</td>
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<tr>
<td>Corrected Total</td>
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*, **, *** Significant at P<0.05, P<0.01, P<0.001, respectively.
Table 4. F values for selected source of variation from the factorial analysis of variance of pH.

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<td>CGN*SALT</td>
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<td>CGN<em>STPP</em>SALT</td>
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<td>5.48</td>
</tr>
<tr>
<td>Error</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

*, **, *** Significant at P<0.05, P<0.01, P<0.001, respectively.
Fig. 1. Effect of 0.5 % sodium tripolyphosphate and 0.5 % carrageenan on the thaw drip of pork sausages at two salt types (NaCl and KCl).
Fig. 2. Effect of 0.5 % sodium tripolyphosphate and 0.5 % carrageenan on the hardness of pork sausages at two salt types (NaCl and KCl).
Fig. 3. Effect of 0.5 % sodium tripolyphosphate and 0.5 % carrageenan on the pH of meat batters at two salt types (NaCl and KCl).
Fig. 4. Effect of salt (NaCl and KCl, 0.34 μ) and 0.5 % carrageenan on the thaw drip of pork sausages at two sodium tripolyphosphate levels (0 % and 0.5 %).
Fig. 5. Effect of salt (NaCl and KCl, 0.34 μ) and 0.5 % carrageenan on the hardness of pork sausages at two sodium tripolyphosphate levels (0 % and 0.5 %).
Fig. 6. Effect of salt (NaCl and KCl, 0.34 μ) and 0.5 % carrageenan on the pH of meat batters at two sodium tripolyphosphate levels (0 % and 0.5 %).
Fig. 7. Effect of 0.5% carrageenan and 0.5% sodium tripolyphosphate on the thaw drip of pork cooked sausages at two salt types (NaCl and KCl).
Fig. 8. Effect of 0.5 % carrageenan and 0.5 % sodium tripolyphosphate on the hardness of pork cooked sausages at two salt types (NaCl and KCl).
Fig. 9. Effect of 0.5% carrageenan and 0.5% sodium tripolyphosphate on the pH of meat batters at two salt types (NaCl and KCl).
Fig. 10. Effect of meat pH and 0.5 % carrageenan on the hardness and thaw drip of pork sausages. Low-pH = 5.47 ± 0.05; high-pH = 6.18 ± 0.02.
Fig. 11. Effect of 0.5 % carrageenan and meat pH on the hardness and thaw drip of pork sausages. Low-pH = 5.47 ± 0.05; high-pH = 6.18 ± 0.02.
Fig. 12. Effect of 0.5 % carrageenan and meat pH on the pH of meat batters
Low-pH = 5.47 ± 0.05; high-pH = 6.18 ± 0.02.
GENERAL SUMMARY

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. Therefore, one of the objectives of this study was to evaluate the interactions between CGN and salt-soluble meat proteins (SSMP) and the forces involved during such interactions.

Differential scanning calorimetry has been extensively used to study thermal denaturation of meat proteins. Changes in the thermal denaturation characteristics of proteins can be induced by the addition of non-meat ingredients. These changes may indicate shifts in the physical state of the proteins or interactions between proteins and non-meat ingredients. Thus, the second objective of this study was to examine the effect of CGN on the thermal denaturation characteristics of SSMP and ground muscle using differential scanning calorimetry.

The addition of CGN to low-fat meats to improve water holding capacity and texture is well documented. However, not much literature is available regarding the behavior of CGN in meat products that have been subjected to freezing. Therefore, the last objective of this study was to evaluate the effect of CGN (κ, τ and λ) on the freeze/thaw stability of cooked pork sausages under various salt, pH and phosphate conditions.

This study was divided in three major parts, each part containing at least two separate experiments. The first part studied the functionality of CGN and SSMP in model systems.
The first experiment in this part evaluated the effect of dry κ-, τ-, and λ-CGN on the gel strength, water holding ability and rheological characteristics of heat-induced SSMP gels. It also examined the forces involved during the formation of CGN/SSMP gels. The gels were analyzed for texture and water losses and the proteins expelled from the gels after centrifugation were characterized by using sodium dodecyl sulfate polyacrilamide gel electrophoresis (SDS-PAGE). The addition of CGN to SSMP extracts significantly affected the physical properties of SSMP gels. κ and τ-CGN significantly (P<0.05) increased the gel strength of SSMP, while λ-CGN was detrimental to gelation. The force needed to compress κ-CGN and SSMP gels independently did not add up to the total force required to compress combined SSMP/κ-CGN gels, suggesting that a protein/hydrocolloid interaction between SSMP and κ-CGN may have occurred. All CGN improved water retention of SSMP gels.

The addition of destabilizing reagents such as, sodium chloride, sodium thiocyanate (NaSCN), urea, 2-mercaptoethanol (MeSH) and propyl glycol (PG), to SSMP, κ-CGN and SSMP/κ-CGN mixtures was done to study the forces involved during gel formation. κ-CGN increased the gel strength and water retention of all SSMP/κ-CGN gels, independently of protein concentration. No significant (P>0.05) differences in gel strength and water loss were observed among NaCl, NaSCN, urea, MeSH and control gels containing 5% SSMP. However, greater differences among reagents were observed when 1 % SSMP was used to prepare the gels. NaCl, urea and PG gels had significantly lower gel strength than control and MeSH gels. κ-CGN gels showed similar behavior as SSMP/κ-CGN gels in the presence of these reagents, suggesting that the changes in gel strength were due to CGN instability rather
than to the weakening of SSMP and CGN interactions. The amount of protein expelled after centrifugation was not affected by the addition of κ-CGN, which suggested that carrageenan was not involved in stabilizing the proteins. Electrophoresis studies indicated no differences in the protein profiles of SSMP and SSMP/κ-CGN gels.

The second experiment of this part examined the effect of CGN in solution on the heat-induced gelation of SSMP. Since κ-CGN gelled at temperatures lower than 40 °C, a temperature control system was designed to solubilize the CGN into the SSMP extract. Rheological, water loss and scanning electron microscopy analyses were performed in κ-CGN/SSMP and SSMP gels. Also, the forces involved in maintaining the gels were evaluated. Addition of κ-CGN to various SSMP concentrations (1, 1.5 and 2.0 %), increased the gel strength and water retention of all treatments. Protein concentration did not have an effect in gelation of SSMP/κ-CGN gels, however, an increase in water retention was observed when 2% protein was present. Scanning electron microscopic studies showed distinct differences in gel structures among SSMP, κ-CGN and SSMP/κ-CGN gels. 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. The SSMP gels were discontinuous with poor linkage between protein strands and large open spaces within the matrix, which may explain the poor water retention and texture of these gels. Although the κ-CGN gels showed large open spaces, the gels seemed to be well interconnected and smooth. The addition of destabilizing agents (NaCl, 2-MeSH, and PG) to SSMP and SSMP/κ-CGN gels to study the forces involved in maintaining the gel together did
not alter the protein concentration release from the gels upon centrifugation.

The second part of this study evaluated the thermal denaturation characteristics of meat proteins/CGN mixtures using differential scanning calorimetric techniques. In this study, SSMP, myofibrillar proteins (MP) and ground meat were used as model systems. In the first experiment, the effect of CGN in the thermal transition of SSMP and MP was studied. The protein concentration obtained during the extraction of SSMP was in the order of 5%. At this concentration, the typical myosin and actin peaks could not be distinguished. MP extractions yield concentrations as high as 10% protein concentrations. DSC measurements of MP showed the presence of three transition temperatures, which were characteristic of meat proteins. However, no changes in thermal denaturation of the proteins were observed at concentrations of CGN as high as 2%. The effect of carrageenan in ground pork under two ionic strength conditions was investigated. Addition of 2% NaCl to ground pork, decreased the temperature of transition of the first and the third peaks by 4°C and 9°C, respectively. The second transition temperature was not significantly affected by ionic strength. Ionic strength also affected the temperature of transition of CGN. Higher transition points were observed at high ionic strength. However, CGN did not significantly change the thermal transition temperatures of myosin and actin. This further supports the conclusion that no direct molecular interaction occurs.

The third part of this study examined the effect of salt, phosphate, pH and CGN on the freeze/thaw stability of cooked pork sausages. One of the experiments in this part included the evaluation of salt types (NaCl and KCl), phosphate and CGN (κ, ι, and λ) on the freeze/thaw stability of cooked pork sausages. The second experiment examined the effect of
pH (<5.6 and >6.0) and CGN (κ, τ, and λ) on the freeze/thaw stability of cooked pork sausages. Phosphate was the most important factor in improving the overall stability of the sausages among the factors studied during the first experiment. It increased hardness and decreased thaw drip, regardless of the addition of CGN. Salt type also affected the texture and the water retention ability of the sausages. Particularly, KCl greatly decreased the hardness and the water retention of all treatments. This effect was more pronounced in the absence of phosphate. Among all the carrageenan (κ, τ, and λ) studied, κ-CGN was the most effective treatment in improving hardness. κ- and τ-CGN were equally effective in decreasing thaw drip in the presence of phosphate and NaCl. When KCl was added, the effectiveness of CGN was greatly compromised. In the second experiment, pH was the most important factor determining the changes in texture and water retention. The functionality of CGN was affected by pH conditions. At low pH (5.47 ± 0.05), κ-, and τ-CGN significantly improved the thaw drip loss in the cooked sausages. λ-CGN, however, did not improve the stability of the sausages. Differences in thaw drip values at high pH (6.18 ± 0.03) were not that evident as at low pH. Hardness was significantly affected by pH, having greater values at higher pH. κ-CGN-containing sausages were the hardness among all treatments, followed by τ-CGN, control (non-CGN added) and λ-CGN, the latter was the softest of all sausages.
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