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CARRAGEENAN EFFECT ON THE WATER RETENTION AND TEXTURE IN
PROCESSES TURKEY BREAST

by

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And approved by

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ABSTRACT OF THE THESIS

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A wide range of comminuted meat products are produced in the food industry for deli meats and sandwich products. A major problem in processing these meats, particularly low fat poultry products, is the loss of water (syneresis) and toughening of texture during cooking, accompanied by crumbling during slicing. To overcome these problems, carrageenan is often added to meats to bind water and entrap muscle tissue particles, providing a more cohesive product. Up to 1.5% carrageenan is permitted by law and early applications typically used these levels. However, high levels of carrageenan contribute distinctive off-flavors, textures uncharacteristic of meat, and decreased freeze-thaw stability. Consequently, the lowest levels feasible to maintain meat qualities should be used.

This study investigated stabilizing effects of low carrageenan levels (0.2, 0.4, and 0.6%) in processed turkey breast formulated with moisture: protein ratios of 4:1, 5:1, and 6:1. Ground turkey breast was tumbled with brine, packaged in bags, baked at 180 °F, and cooled. Traditional meat properties of cook yield, refrigerator purge, freeze thaw

stability, and textural characteristics were measured. Hydration and swelling vs. full solubilization and gelation of carrageenan were visualized microscopically.

At the lowest moisture level, protein and component salts controlled water binding; carrageenan added no extra stability and had little effect on cohesiveness or other textural attributes. In turkey breast formulations with higher moisture, carrageenan increased cook yields. Microscopy revealed hydrated, swollen, and intact carrageenan granules, as well as release and gelation of carrageenan polymers.

A mechanism to explain carrageenan action in meats was proposed. In low water systems, muscle proteins control water binding and carrageenan has no influence on product qualities. As added water increases, carrageenan binds excess water not bound by the proteins, and particles begin to swell, contributing to water retention and firmness in meat products. At the highest water levels, carrageenan binds sufficient water to burst some particles and release carrageenan polymers, which then gel in regions surrounding proteins. Some hydrated, swollen particles also remain intact and contribute to solidity. Carrageenan gelation contributes to softening of textures and freeze thaw stabilization in high moisture systems.

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INTRODUCTION

Carrageenan has played an increasingly important role in the formulation of processed meats over he past several decades. Carrageenan was first added to poultry and cured pork products in the early 1980's as a starch replacement to prevent moisture loss during the cooking process. Its use in meat expanded when benefits of improved sliceability and flavor release also were observed. Carrageenan applications in meat continued to grow with development of low fat meat products such as the McLean® Hamburger. Due in part to recent changes in the standard of identity in poultry and meat products (1). to allow carrageenan as an ingredient, carrageenan is now an accepted component of nearly all delicatessen meats (turkey, ham, chicken roll, and beef), in marinated products such as rotisserie chicken and case-ready raw marinated meats, and in whole smoked turkeys (2), and it is increasingly being used to develop products from lean meats with low myofibrillar protein contents (sometimes called textured meats) (3). Indeed, the current market for carrageenan in meats is one of the most profitable in the food industry: sales were approximately $300 million in the U.S. alone in 2001(4) and world-wide have grown exponentially since then (5).

Finding the correct levels of carrageenan for meat products has not been straightforward. Currently, carrageenan usage levels in meat products vary widely but cannot exceed the legal limit of 1.5% by weight (1). Most manufactures empirically rely on physical attributes of individual products to determine carrageenan levels that produce desired texture, moisture, and flavor. Application scientists too often work with a "black box" research plan to develop existing and new meat formulations that play critical roles in meat industry here in the US and in other countries, including poverty stricken areas.

Most meat applications to date have used carrageenan in the higher concentration range, but at these levels carrageenan gels tend to become brittle and show reduced freeze-thaw stability, and off-flavors and soft, slimy textures are a definite issue. These problems have generated interest in applications with lower carrageenan (0.5-1%), which also reduce production costs. Very few studies have investigated whether carrageenan can be effective at even lower levels, so there is almost no information to guide systematic development or to predict properties of meat products with various levels of carrageenan. Certainly, there is little information about changes of carrageenan and interactions with proteins on a molecular level. Consequently, despite extensive use of carrageenan in meats, our understanding of how carrageenan functions in meat products remains very limited, and there is not yet a clear understanding of how particular chemical properties of carrageenan are related to molecular interactions in meats.

This thesis explores the feasibility of using very low levels of carrageenan (0.2 – 0.6% by weight) to retain moisture, maintain a pliable cohesive texture, and provide freeze-thaw stability in roasted turkey breast deli meat products. As a first step in elucidating effects of carrageenan at the molecular level, microscopic analyses were included, along with standard functional analyses, to track changes that occur in carrageenan during cooking and contribute to its function and textural effects at different moisture levels. The focus remains necessarily on physical properties, but systematic studies reveal competition and interactions between meat proteins, carrageenan, and water. Results of this research are aimed at producing meat products with higher quality at lower carrageenan levels and lower cost.

BACKGROUND

I. Meats

Meat is considered to be edible muscle tissues from animals. Human butchering and consumption of meat dates back more than 12,000 years, beginning with hunting and progressing to domestication of animals and animal husbandry. The USDA reports that, of all countries, consumption of meat and meat product are highest in the US. In the United States, turkey consumption was 2,513 metric tons and 4,167 world wide in 1997 (6).

A. Composition.

Meat is composed mainly of water, protein, and lipids. The water content in meat of most land animals ranges as high as ~70 %, protein is ~20%, and fat varies from 4.7% in poultry to 4-8% in beef and 9-11% in pork (7,8).

1. Meat Proteins. The skeletal muscle found in meat is made up of long narrow multinucleated cells or fibers composed mostly of protein. There are three classes of proteins in meats:

Salt-soluble -- contractile or myofibrillar proteins

Water-soluble – sarcoplasmic proteins

Water-insoluble – connective tissue proteins or stroma proteins

Contractile proteins myosin and actin, the actomyosin complex, tropomyosin and troponin are functionally the most important physiologically and in meat as food.. Accounting for 45% of the myofibrillar meat proteins, myosin forms heavy filaments consisting of two heavy chains having globular portions and rod like regions (the head

and tail domains, respectively) and four lighter filaments (the neck) connecting these two regions. Actins comprise 20% of the myofibrillar proteins and are thin filaments to which myosin binds in contraction. Actomysin is the complex that forms reversibly between the myosin and actins, allowing muscle to contract.   Tropomyosin and Troponin each provide 5% of the myofibrillar protein. Tropomyosin is similar in structure to myosin. Troponin is made up of three subunits for calcium binding, tropomysin binding and ATPase inhibitory (7,9).

Soluble sarcoplasmic proteins makes up 25% of the muscle cell. Most of the soluble proteins are enzymes that, along with myoglobin, store oxygen in muscle tissue. The stroma proteins in connective tissue comprise the last class of muscle proteins, accounting for 10% of the total protein in mammalian muscle. Stroma proteins collagen and elastin contain high proportions of proline and hydroxyproline that contribute to their insolubility and tendency to form triple helixes and crosslinks. Despite being found almost exclusively as a triple helix, collagen is an amorphous protein with formed elements of fibrin and elastin proteins embedded in it. This complex is the "glue" that connects muscle the skeletal system (7,8).

B. Contributions of Proteins to Meat texture

1. Organization into muscle fibers and fibers into tissues. Structural meat muscle consists of bundles of long cylindrical fibers that vary in length and diameter. Each muscle fiber is surrounded by connective tissue called the endomysium. A connective tissue layer called the perimysium hold the bundles together and extends to the end of the muscle to provide an anchor between muscle and bone (7).

Muscle fibers consist of intracellular sarcoplasm and organelles, the contractile fibrils called myofibrils, and the sarcolemma surrounding the myofibrils Figure 1a. The sarcolemma consists of a tubular system call T tubules that meet the muscle fibrils at the Z line along with the sarcoplamic reticulum. This extension of the T-tubes in to the muscle fiber allows quick response time that result in the muscle moving as a unit.

The contractile unit of the myofibril is one sarcomere. The sarcomere consists of bands of thick or thin filaments majority consisting of the contractile proteins myosin and actin, respectively (Figure 1b). The A-band has thick and thin filaments that partially overlap. The thin filaments (lighter section) of the A band that do not overlap the thick filaments is called the H zone. The I-band contains thin filaments that are connected at the Z line to provide an anchor when the muscle contracts. During muscle contraction the thin filament of the I-band slide past the thick filaments of the A-band. The I band and the H zone shortens and the A-band remains the same length (7,8).

2. Postmortem changes in muscle structure. Immediately after the death of animal the pH in the muscle drops as glycogen is consumed and lactic acid accumulates. Failure to control the pH by rapid cooling, etc. during post-slaughter processing can lead to significant loss in meat quality. Low pH leads to denaturation first of the sarcoplasmic and eventually also the contractile protein, and this reduces water binding capacity. When the pH of meat is too high, prevention of postmortem glycolysis leaves meat dark and dry, and microbial growth is very rapid. All of these factors affect the quality of muscle tissue that eventually is cooked into a wide variety of food products (7,10).

Another important change that occurs during rigor mortis is that muscles contract and lock, leaving actin and myosin permanently overlapped. This change contributes to
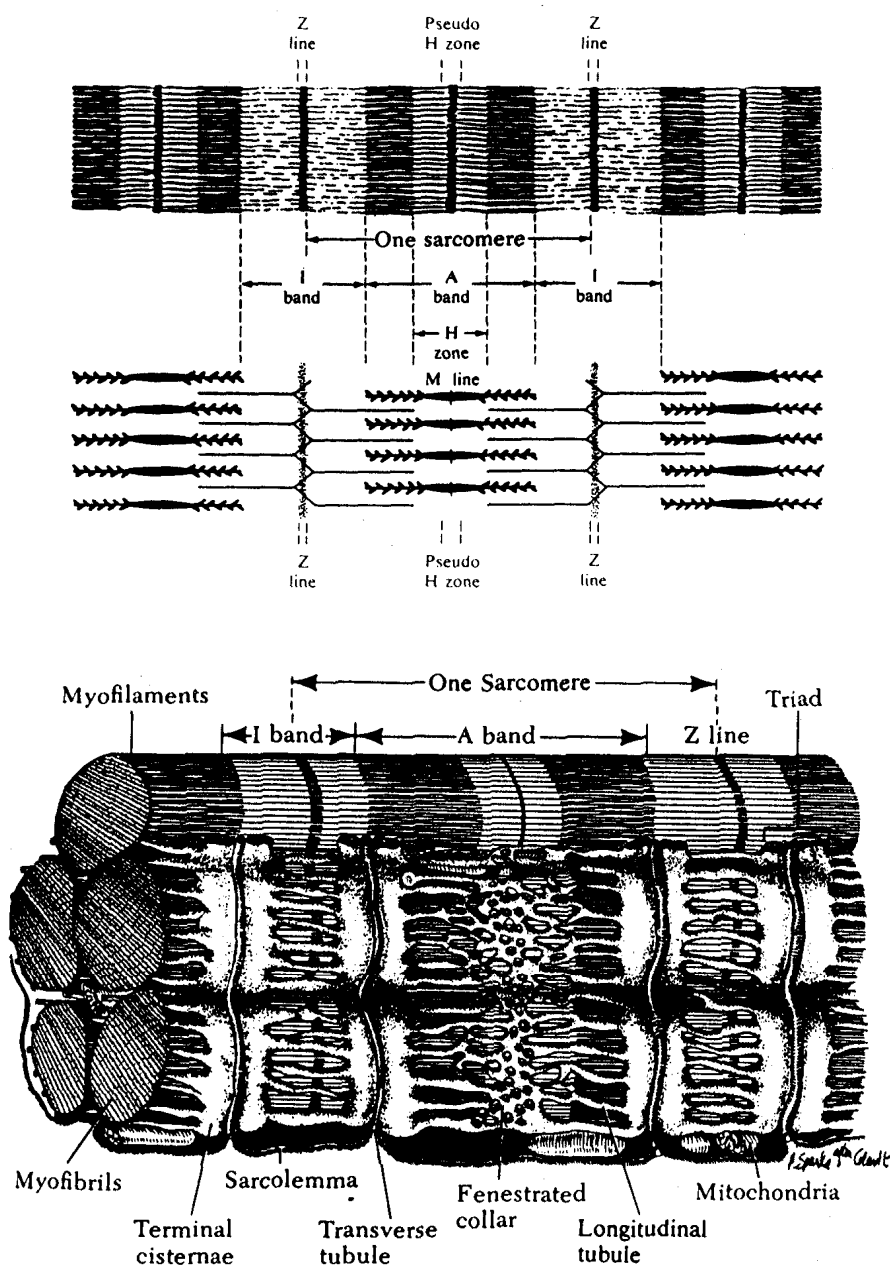
Figure 1. Protein structure and organization in muscle foods. A) Organization of actin and myosin into sarcomeres (muscle cells); B) organization of sarcomeres into myofibrils singly, and bundled into fibers connected by tubules and encased by the sarcolemma. Adapted from (11).

toughening of meats that cannot be overcome by standard cooking and processing. It has also led to meat tenderization practices such as use of proteases that cleave muscle cells at the Z-line (12), and addition of phosphates that release the actin-myosin binding (10). The latter is one reason why phosphates such as sodium tripolyphosphate (STP) are added to most processed meat products.

3. Changes in muscle structure during heating and consequences to quality. It is obvious to everyone who cooks meat that significant shrinkage of the meat product occurs during the cooking process. Heat processing of meats is used extensively because it combines cooking the meat with a highly effective method of destroying the growth activity of microorganisms in the products (7,8). However, meat proteins denature at relatively low temperatures (50°C for actinin, 55°C for myosin, and 70-80° for actin) (7), and this has several important consequences to meat quality:

a) Destruction of muscle enzyme activity

b) Increased water loss → shrinkage and drying

c) Muscle contraction → tissue shrinkage

d) Increased tendency for cross linking → toughening

e) Loss of connective tissue → disintegration of structure (crumbling

when cutting).

The major water loss that occurs in meats is during the cooking process. As proteins denature from heat, muscle tissues shrink due to contraction of both the perimysuim and endomysium in the encomium sheath, reducing both the diameter and length of the myofibril (8,10). As myofibrils begin to shrink, water trapped within the muscle fibers is forced out. Water loss is slow at low temperatures, but as increasing

temperatures accelerate denaturation and exposure of hydrophobic amino acid residues, water binding capacity decreases and more rapid fluid loss occurs from the myofibrils. At high temperatures, protein crosslinking decreases water holding capacity further (8), forcing more water from tissues; the molecular interactions and tissue dryness both contribute to development of meat toughness.

In addition to dehydration and shrinkage, there is partial conversion of collagen to gelatin during heating. Collagen hydrolyzes and the triple helix unwinds, converting collagen to gelatin. At high temperatures, gelatin is liquid and leaks out from between muscle fibers. This removes the protective layer between the muscle fibers and promotes the crosslinking between proteins in muscle fibers that contributes significantly to meat toughening during cooking (8,10), as noted above.

These changes are well known and accepted in home cooking, but in commercial products that must be stored for longer periods of time and need to be sliced in industrial meat slicers, the quality in not acceptable – it is too dry, too tough, and too crumbly for sandwich or other sliced meat applications. Consumers do not want to purchase sandwich meats or eat sandwiches in restraints where meat is dry and chewy, lacking tenderness and cohesiveness, and falling out of the sandwich. In addition, moisture loss equals loss of mass, and that reduces profits (e.g. 100 lbs of original meat product losing 10% moisture during cooking process yields only 90 lbs of meat to sell). Consequently, meats processed for commercial applications require modifications to retain critical qualities.

C. Water holding capacity.

One of the key characteristics of meat proteins contributing to overall quality of all processed meat products is water holding capacity (WHC). WHC refers to the ability of proteins to bind large amounts of water by hydrogen bonding to polar amino acids residues, by electrostatic interactions with charged amino acids, and by entrapment between peptide chains. Water binding is responsible for solubilization, moisture retention, and swelling properties of proteins. It is important in gel and emulsion formation and in providing viscosity. Typical amounts of water bound by various amino acids and proteins are shown in Table 1.

As can be seen in Table 1, the amount of water that can be bound by a protein depends on the proportion of polar amino acids that are located on the protein surface. Thus, water binding is strongly affected by protein charge. It also decreases as temperature increases (and drives molecules apart) and as salt concentrations increase (and compete with proteins for water). Water binding increases at low salt concentration which provides more sites for electrostatic interactions without competing for the water.

In meats, water holding capacity resides primarily in the structural proteins (~65%), in particular the myofibrils, partly because they are by far the dominant protein in terms of amount, and partly because they contain high concentrations of charged amino acids that bind water strongly (7). Water found in the muscle fiber is located in the spaces between the thick and thin filaments. This spatial water is both "bound" (associated with the negatively charge groups on the amino acid side chains) and " free" water trapped inside the spaces. Only about 5% of the water holding capacity is contributed by the water-soluble sarcoplasmic proteins and the remaining 30% is Table 1.

Table 1. Water binding (hydration) capacities of various amino acids and proteins.

Adapted from (13).

Amino acid	Hydration capacity mol H_2O / mol aa	Protein	Hydration capacity g H_2O / g protein
Polar			
Asn	2	Ribonuclease	0.53
Gln	2	Lysozyme	0.34
Pro	2	Myoglobin	0.44
Ser, Thr	2	β-Lactoglobulin	0.54
Trp	2	Chymotrypsinogen	0.23
Asp (COOH)	2	Serum albumin	0.33
Glu (COOH)	2	Hemoglobin	0.62
Tyr	3	Collagen	0.45
Arg (NH_2)	3	Casein	0.40
Lys (NH_2)	4	Ovalbumin	0.30
Ionic			
Asp^-	6	Whey protein	0.45-0.52
Glu^-	7	concentrate	
Tyr^-	7	Na^+ caseinate	0.38-0.92
Arg^+	3	Soy protein	0.33
His^+	4		
Lys^+	4		
Nonpolar			
Ala	1		
Gly	1		
Phe	0		
Val, Ile,	1		
Leu, Met	1		

soluble non-protein materials (7,8,10).

Water holding capacity, a critical factor for meat quality, is strongly affected by nearly every stage of postmortem handling of meat products – initial freezing, cooking, refrigerated storage, and secondary frozen storage after incorporation in meat products. Water holding capacity is particularly important in comminuted meats where destruction of the muscle tissue increases seepage of water. Freezing damage to muscle cells can begin water loss, but the majority of water loss occurs during cooking when heat penetrates meat muscle, forcing rupture of the muscle cells and denaturing actin and myosin. Loss of water at this stage reduces "cook yield", the % weight of product that is retained during cooking. Loss of water during storage ("refrigerator purge") decreases meat quality in undesirable appearance, loss of vital nutrients, and loss of juiciness of the product. The final stage of water binding is during freezing of cooked meat products, alone or with water-binding adjuncts. Protein aggregation or polysaccharide retrogradation due to salt concentration during freezing can lead to phase separation and release of bound water, a process called syneresis. This susceptibility to water loss and associated texture changes can be manipulated by additives, and is referred to as "freeze-thaw stability".

D. Meat Emulsions.

1. **Fundamental concepts**. Emulsions are blends of two immiscible components, with one phase (dispersed phase) dispersed in the other (continuous phase) in the form of tiny droplets (14). Meat emulsions are oil in water emulsions in which fat is dispersed in the continuous phase of water and water soluble components and is emulsified by the

phospholipids in water soluble/salt soluble proteins (Figure 2). Meat emulsions differ from normal emulsions (butter, milk etc) in that fat particles can be very large (as large as 50 microns), which classifies them technically as coarse or unstable emulsions (15). Typical meat emulsions such as frankfurters, sausages, and deli meats contain not only the meat (chopped or comminuted muscle, fat, and water) but also binders, salts, stabilizers and in most cases also spices and other flavoring ingredients. Each of these components of the meat emulsion plays a key roll in the final product quality.

Meat emulsions require specific manufacture to disperse the fat and other additives into the meat.   The first step mechanically grinds the meat muscle. Tearing the meat muscle apart disrupts the structure of the muscle proteins, leading to loss of water within the muscle fiber. Grinding reduces the fat particle size, but generates a wide range of particle sizes (15). Homogenizers are used when finer, more homogeneous products are desired. Tumbling under vacuum offers processors the opportunity to add specialty ingredients to the product, replace lost water and make a homogenous mixture. For sausages, a curing step is added before stuffing the meat emulsion into casings. Products are then cooked by various methods, e.g. oven, roasting smoking, etc.
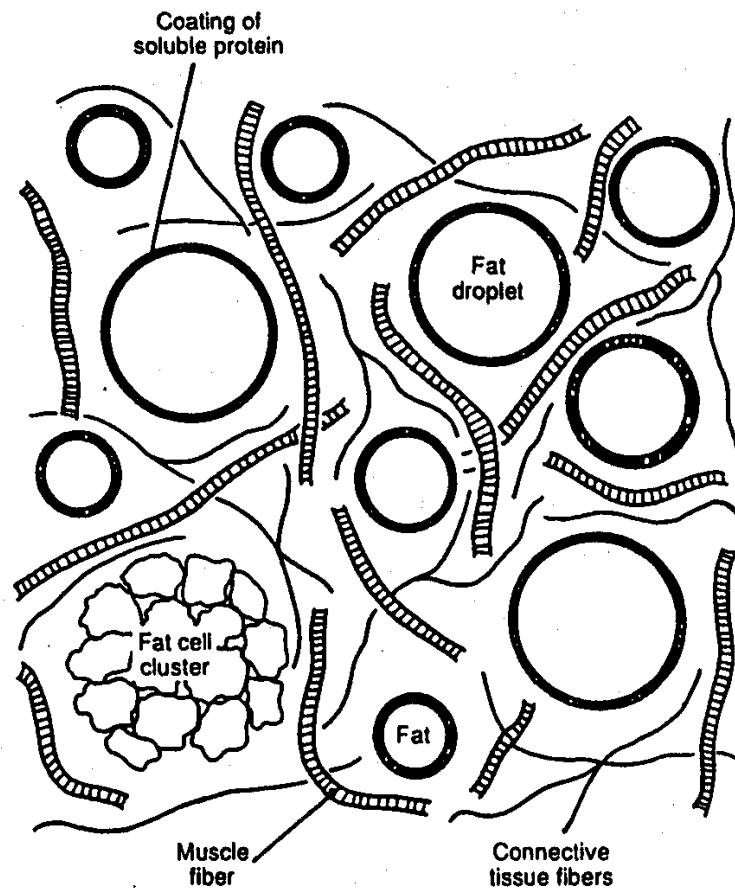
Figure 2 Diagram of component organization in meat emulsions. From (11).

2. Ingredients in meat emulsions. Water is added to replace processing losses, increase juiciness, and extend the meat product to maintain profitability. Sodium chloride is added for development of flavor and solubilization of the proteins, thus improving water holding capacity. 2.5% NaCl appears to optimize water holding capacity, regardless of the pH (16). Phosphates significantly increase the water holding capacity by binding water directly and by increasing the pH which increases the number of negative charges on the protein groups. The latter increases water binding to protein surfaces, and also increases repulsion between protein chains to enlarge spaces that may then fill with water. Phosphates show synergism with salt, allowing use of lower levels of NaCl (e.g. 1.5%) while maintaining water holding capacity over and above that of phosphate alone (16). Phosphates also dissociate actin and moysin in the thick and thin filaments; this induces a "relaxation effect" in the contracted muscle, thereby improving tenderness. However, all salts also increase interfacial tension and thus must be used at very low levels; high levels of salts disrupt emulsion stability (15).

Sodium tripolyphosphate (STPP or STP) lowered cooking loss in sausage model systems. (17) and frankfurters (18), and it reduced moisture loss in freeze-thaw stability testing in sausages (19). Both of these studies also observed that STPP improved texture hardness needed for sliceability. All salts increase interfacial tension and thus have the potential to disrupt emulsion stability when used at high levels (15). However, the low levels of STPP used in the frankfurters salt increased emulsion stability (18).

Deli Meats are considered fully cooked meat emulsions. During processing and cooking these meat products lose considerable moisture resulting in losses in weight and textural quality and consequently also profits. To overcome these moisture and textural

problems, the industry uses various water binding additives, including starch, alginates, and carrageenan, Amylose and amylopectin have been used in the deli meat industry for many years, but starch has many disadvantages in deli meat, including retrogradation, texture degradation, and development of off flavors. Addition of starch to oven roasted turkey breast increased cook yield but showed poor sliceability compared to carrageenan (20). These disadvantages from starch have led to increased use of carrageenan, which is now the dominant modifier used in deli meat formulations (FMC, internal communication).

II. Carrageenan

A. Origin and chemical composition. Carrageenan is a water soluble hydrocolloid derived from red seaweed. It is found in the cell wall of the seaweed from which it is derived and its content can vary from species to species and seasonally. It is harvested from various regions of the world including the northern part of the US, Philippines, Indonesia, Chile, Argentina, Morocco, and France. The most common species of carrageenan used commercially are *Chondrus Crispus, Eucheuma*, and *Gigartina*. (21)

Carrageenan is a polymer composed of repeating linear chains of galactans units with negative charge from numerous ionic sulfate half-ester groups. The repeat unit is a dimer of galactose and anhydogalactose linked by a beta 1, 4 glycosidic linkage (Figure 3). These dimers are then linked together through alpha 1, 3 glycosidic linkage (21-23). This secondary structure assumes the chair conformation to minimize steric repulsions caused by axial components (24). The charged sulfate groups are largely responsible for the water-binding, gelling properties, and high viscosity solutions stable over a wide pH
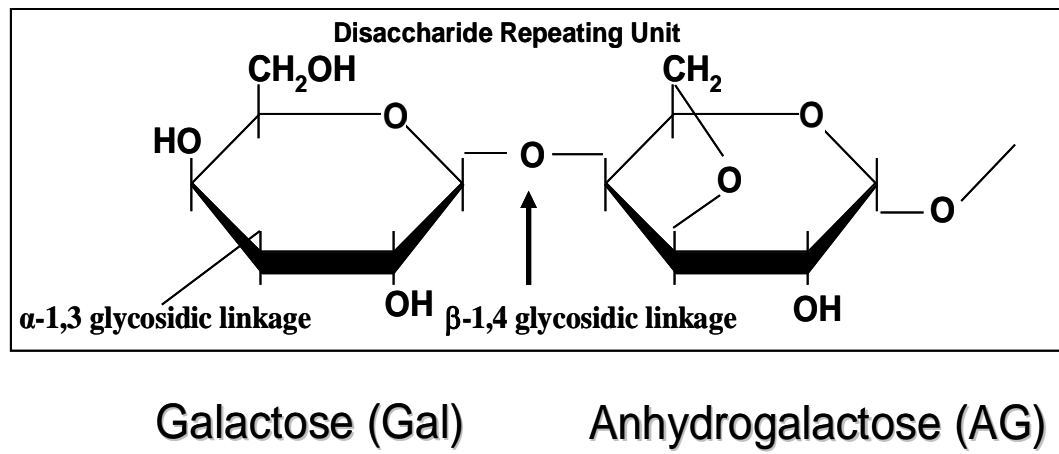
Figure 3. 1,4 glycosidic linkages between galactose and anhydrogalactose monomers in carrageenan.

range, for which carrageenan is widely used in the food industry (22).

B. Types of Carrageenan. There are three forms of carrageenan -- kappa, iota, and lambda -- determined by the number and position of the sulphate groups on each sugar and the presence or absence of the 3,6 anhydro group on the B monomer. The 3,6 anhydro group promotes α helix formation which is important for gelling. (21-23). This is a result of increased flexibility that promotes a random coil structure. The conformation of the glycosidic bond changes to equatorial (24).

Kappa carrageenan contains one sulfate group per repeat dimer, located on the O-3 galactose ring (Figure 4) (21-23). X-Ray fiber diffraction has shown that its structure is right-handed double helix of parallel chains (24). With this structure, kappa carrageenan forms durable thermoreversible gels by itself. In the presence of salts, particularly potassium, it forms even more strong and rigid gels, although these gels are very susceptible to syneresis. Kappa carrageenan can also react with milk proteins via charge complexes (22,23), as will be discussed in more detail below.

Iota carrageenan, also a right handed double helix of parallel chains, contains two sulphate groups per repeat dimer, located one on each of the sugar units (Figure 4). Iota carrageenan forms strong, elastic, thermoreversible gels with limited syneresis. Calcium forms ionic bridges between iota carrageenan chains, yielding gels with increased gelling and melting temperatures (22,23).

Non-gelling lambda carrageenan contains three sulphated groups with repeating dimer units of D-galatose-2-sulphate-D-galactose-2, 6-disulphate; it does not contain the 3, 6 anhydro group necessary to form the double helix (Figure 4). Lambda carrageenan does not form gels but is widely used as a viscosifier in many food applications (22,23).

Carrageenan 3 major types kappa, iota, & lambda
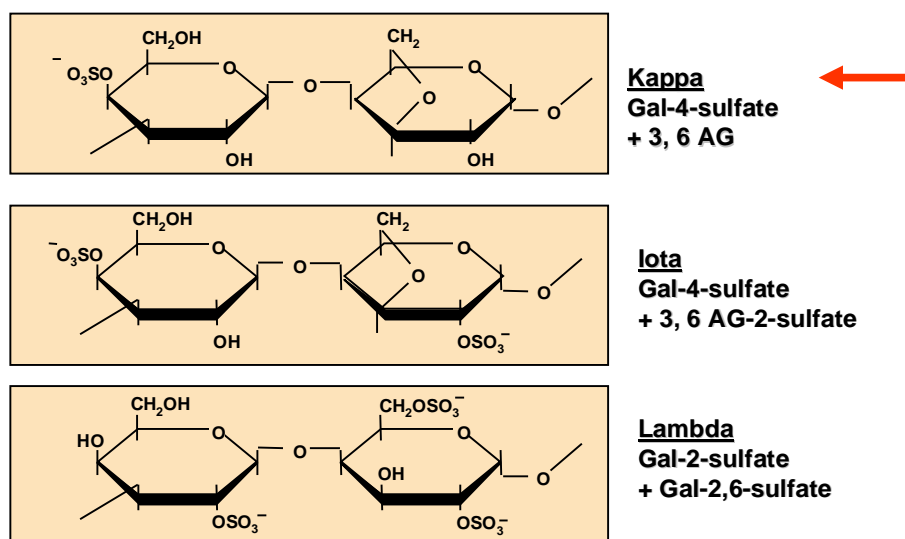
Figure 4. Molecular structures of kappa, iota, and lambda carrageenan. Kappa

carrageenan (with arrow) was used in this study. http://www.fmcbiopolymer.com.

C. Modes of molecular interaction in carrageenan. Carrageenan has three modes of interaction in foods: hydration and solubilization, gelation, and protein binding. Each of the interactions is critical to the properties carrageenan contributes to various food systems. Kappa and iota carrageenan interact via all three modes, but lambda only displays hydration and solubilization

    1. Hydration and solubilization. The carrageenan molecule has large potential for water binding and hydration; ~50g of water is bound for every gram of kappa carrageenan (25). When carrageenan is dispersed in a water solution it begins to hydrate through electrostatic interactions of water with negatively charged sulphate groups and through hydrogen bonding of water to the OH groups on the polymer chain. As hydration increases, carrageenan particles begin to solubilize and become colloidal. Kappa and iota generally require heat (~80°C) to completely solubilize. Lambda does not require heat and solubilizes easily, forming viscous solutions at ambient temperatures (22).

    2. Gelling. Carrageenan gelling is of great importance in food industry, particularly in meat, beverage, dairy, and confectionary applications. The gelling mechanism of kappa and iota carrageenan has been hotly debated and is still being investigated today. The tertiary structure of the carrageenan type is thought to dictate gelation (24) as local regions of ordered molecular associations aggregate to form a disordered polymer network (21).   Whether gelation can occur is highly dependent on the concentration and type of carrageenan (kappa or iota) and on the presence of cations.

    Hydration and solubilization of the carrageenan polymer is a critical prerequisite critical for gelation. All carrageenans bind water and swell at room temperature, but

kappa and iota forms generally require heat (~80 °C) to completely solubilize. Once solubilized, the polymer chains are released into a colloidal state (23) (Figure 5). Solubilized carrageenan has negative charged sulphate groups all along the polymer chain, which induces repulsions that prevent chain folding and intermolecular associations. Cation interactions with the sulphate groups neutralize the negative charges so that intermolecular interactions can occur (22). As the solubilized carrageenan solution cools, intramolecular hydrogen bonds stabilize the α-helix conformation in individual carrageenan chains and intermolecular hydrogen bonds stabilize the formation for the double and triple helices between carrageenan chains (23).

Several lines of evidence confirm that the locally-ordered double helices in carrageenan gels are organized into an amorphous matrix in which double helix regions are randomly associated rather than forming fixed structures. Coil to double helix transformation has been demonstrated by multi-angle laser scattering coupled to gel permeation chromatography; Zimm plots of this data that carrageenan molecular weight almost doubled with helix confirmation (26). That the gels are amorphous is confirmed by sigmoidal plots of optical rotation versus heating temperature for iota and kappa carrageenan solutions with added salts (24), and by transmission electron microscopy of gelled kappa carrageenan (27).

Early studies thought that formation of the double helix alone caused gelation. Traditional explanations described carrageenan gelling as having nested helical regions associated linearly in"egg-box" conformation. However, subsequent research has led to several alternative models, the most widely accepted of which is the "domain" model described above and shown in Figure 5 (24): rather than having surface interactions

- **Gelation of solubilized carrageenan polymers**
  - **K^+ promote α-helix associations and double helix formation**
  - **Ca^{++} form ionic bridges between ($-OSO_3^-$)**
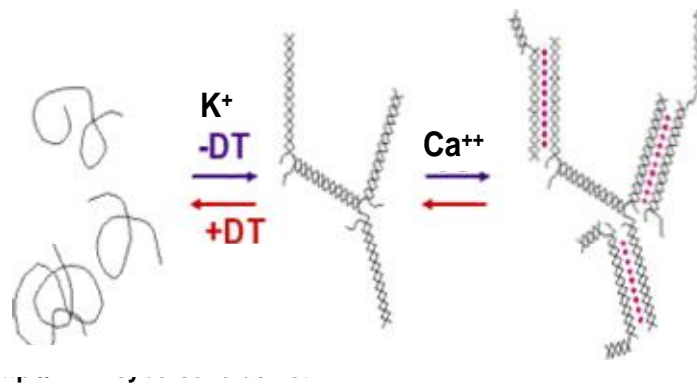
Figure 5. Molecular associations involved in the gelation of carrageenan. Random coils first associate in domains of α-helices. Entanglement in regions of single chains then brings helical domains into close proximity. Association of the helical domains into an amorphous matrix then forms the final gel. Association of helical domains is facilitated and mediated by potassium and calcium ions. Adapted from (24).

 between carrageenan helices, single carrageenan molecules randomly entangle in isolated regions, bringing double helix domains into proximity so they can form domain-domain aggregates by hydrogen bonding between chains or by ionic bridging involving shared ions.

The "domain model is supported by optical rotation dispersion, light-scattering, and rheological behavior of kappa carrageenan. As a K-carrageenan solution cools, the random coils form double helices before aggregation occurs. When the same gel is reheated, the process reverses, dissociating the aggregates first, followed by restoration of the random coils (28). Studies of kappa carrageenan gels at various concentrations and temperatures using small angle X-ray scattering found that kappa carrageenan formed two to three associated double helices during gelation (29). This results in tighter and more extensive molecular aggregation and yields somewhat rigid and brittle gels that exhibit a high rate of syneresis (23). In contrast, iota carrageenan forms a limited number of double helical aggregates, so the gels are more flexible and elastic and show little to no syneresis. Carrageenan gels are thermally reversible, with gels melting when heat is applied and reforming with cooling. However, the gel strength decreases with each melt-gel cycle, particularly with kappa carrageenan. Iota gels change less with heating and are stable at ambient temperatures, which is desirable in many food applications (23).

Much research as well as practical food applications show that random aggregation of double helix regions is facilitated by potassium and calcium ions (Figure 5) (22,30). This characteristic makes carrageenan particularly useful for dairy applications (31-34). Potassium ions are particularly important in gelation of kappa carrageenan, while calcium ions are more associated with iota gelling. Although

interactions of cations with carrageenan have been widely studied, there is still no complete understanding of how ionic bonding affects carrageenan helix during gelation (35).

Gel promotion is thought to occur through enhanced ionic interactions which increase intermolecular associations and, in turn, alter gel transition temperatures. Chen et al (36) found without the addition of potassium ions to kappa carrageenan (0.7 to 1.4%) solutions formed very weak gels. However, small amounts KCl (as low as 0.005M) and 1% carrageenan induced significantly stronger gelling, and the effect was even more pronounced at higher KCl levels (0.01M). Kappa carrageenan gelation in various salt solutions showed that $K^+ > Ca+ >> Na+$ in effectively increasing gelling rate, gel melting temperature, and gel strength (37). Similar results were seen using rheological and differential scanning colorimetry (38). Thermal phase transitions of kappa carrageenan solution with and without KCl also showed that the conversion temperature from coil to helix and from helix to coil (when reheated) was higher with the addition of KCl (39). A stoichiometric molar ratio of ~ 1 calcium per sulfate group of kappa carrageenan appears to be ideal for forming clear gels with maximum gel strength. However, higher calcium concentrations excessively neutralize charges and cause excessive associations and precipitation (40). Hence, the hardness of water used in carrageenan-meat applications must be very closely controlled. In general, hardness levels lower than 60 ppm calcium are recommended (2). In practice, this is frequently not followed, leading to considerable variability between plants.

Cations other than K^+ have also been studied. Cryo-TEM images showed formation of "superhelical" rods in kappa carrageenan gels formed in the presence of

cesium. The super helical rods are very strong aggregation of helices that are not thermally reversible as in normal kappa carrageenan gelling with K^+ (35) .

Although much focus has been on cations effects in carrageenan gelation, anion can also affect the conformational transitions of kappa carrageenan gels (35). Size Exclusion Chromatography using refractive index and multiangle light scattering detection extrapolated values of characteristic ratios for kappa carrageenan, and found that the characteristic ratio for kappa carrageenan required for gelling with NaI was twice that of NaCl. The data showed that I^- promoted formation of helical sequences, but these were limited to relatively short sections of the carrageenan polymer separated by a few monomeric units in different conformations. The monomeric regions act as flexible hinges or elastic joints, so overall the chain behaves more like a random coil with expanded molecular dimensions. In contrast, Cl^- tend to promote association of monomer units into rigid rod-like units(41).

However, in meat processing calcium ions creates problems and destroy carrageenan functionality by excessive associations. Hence, the hardness of water used in carrageenan-meat applications must be very closely controlled at each facility. In general, hardness levels lower than 60 ppm calcium are recommended (2). In practice, this is frequently not followed, leading to considerable variability between plants.

3. Associations with proteins. The third mode of carrageenan interactions in food is binding to proteins, particularly milk proteins. This mode of interaction is extremely import in the dairy industry, where carrageenan is used to stabilize milk proteins. Carrageenan is added to milk-based beverages to prevent protein precipitation and to suspend insoluble solids such as cocoa. Carrageenan interactions with caseins in

milk are unique and well documented (33,34,42). Milk contains 30-36 g/L of protein; approximately 80% of this is casein and the remainder is mostly whey protein (43).

Whey proteins are globular in nature and show little to no interaction with carrageenan, but some research has attempted to elucidate possible interactions that may occur. Kinetics of heat induced destabilization of whey protein emulsions showed oil droplet aggregation increased almost three times in the presence of kappa carrageenan. concluding that carrageenan can interact with denatured whey protein but not native whey proteins (44). Both whey protein and carrageenan gel during heating and have been determined to be a physical mixture of both at pH 6-7 (45). Whey proteins gel during heating while carrageenan gelation occurs during cooling. When a whey protein-carrageenan gel is reheated, the protein gel remains (no rupture) while the kappa carrageenan gel network melts (46).

In contrast, caseins have unique secondary, tertiary, and quarternary structure, being highly organized in micelles composed of micelle subunits, calcium phosphate, and water (43) . Caseins are ~80% of the milk proteins. One of the four gene products of casein is kappa casein (~12% of casein protein), which is represented as a protein chain that is on the surface of the micelle with its C-terminal sticking out to prevent association with other micelles through steric repulsions (31). This hairy like structure is where interactions occur between carrageenan and caseins. The negatively-charged sulfate groups in kappa carrageenan bond directly to patches of positively-charged amino acids on the surface of the casein micelles to form very strong milk gels.

Scanning electron microscopy showed that through light scattering data the diameter of the casein micelle increased with increasing kappa carrageenan

concentration. This experiment also demonstrated a prevention of phase separation with the addition of kappa carrageenan (33). Such gels are industrially very important in milk applications, increasing suspension and improving texture in chocolate milk and reducing ice crystals and slowing melting in ice cream.

Iota and lambda carrageenan show less interaction directly with casein sulfate groups due to steric hindrance. However, as also occurs with kappa, calcium ions form charge bridges between sulfate group on the carrageenan and negatively charged amino acids on caseins (Figure 6). This results in weak gelation (kappa and iota) and increased viscosity (all types) which aid in suspension and texture, respectively (7).

Vegetable protein-carrageenan interactions have also been investigated. Pea protein behaved very much like whey protein when heated with carrageenan. When samples were cooled slowly, pea protein aggregated extensively with itself, but with rapid cooling the kappa gel was able to form competitively and interrupt the protein aggregation (47). In mixed systems of canola protein and kappa carrageenan, rigid gels form via significant disulfide crosslinking with protein alone, but when carrageenan regions disrupt this matrix linkage, generating more elastic mixed gels. In addition, proteins were less sensitive to environmental conditions, e.g. pH changes and ionic strength, in the presence of carrageenan. Apparently, carrageenan binds to sites on the protein, preventing them from interacting with changing pH and salt additions (48,49).
.
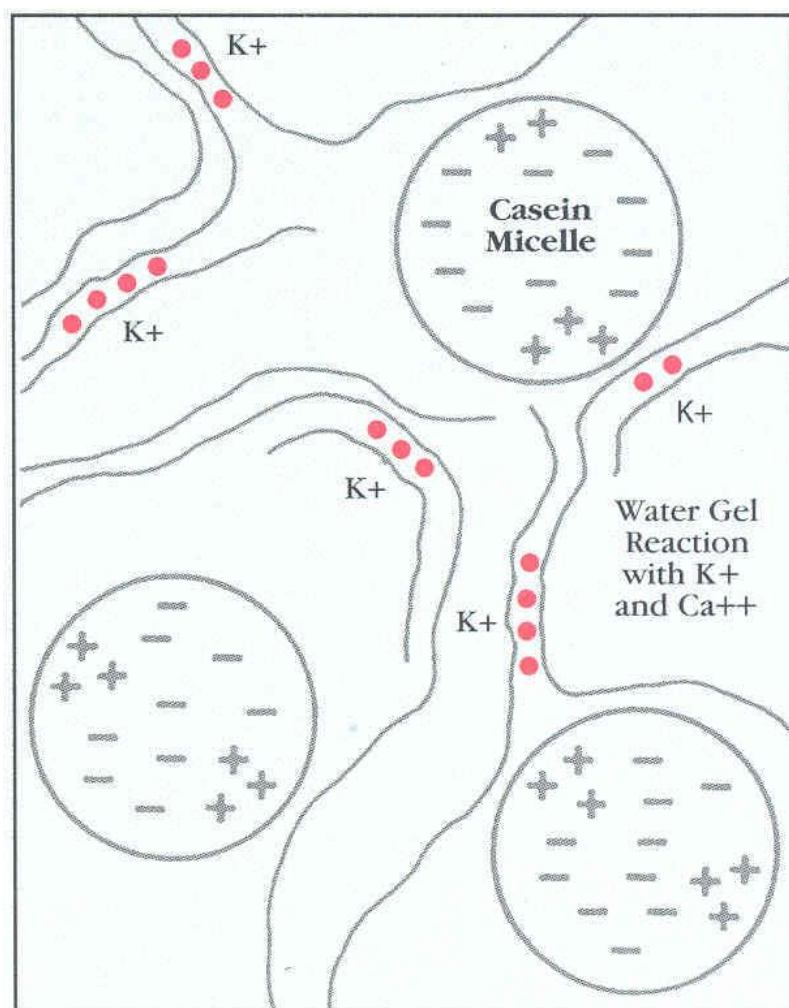
Figure 6. Diagram of casein interactions with carrageenan. (FMC Corp. used with permission.)

D. Carrageenan in Meats.

Carrageenan is now a standard industry additive in deli meats(5). By binding water, kappa carrageenan prevents moisture loss during heating, thereby improving cook yield in a wide range of meats and meat products (17-20,50-58). For example, addition of kappa carrageenan to beef gels with 1% and 3% sodium chloride concentration improved cook yields significantly, and the effect increased with carrageenan and salt concentrations (54). Similarly, kappa carrageenan with 1% sodium chloride significantly reduced refrigerator purge of vacuum packaged beef roll (54). Kappa carrageenan also improved water retention in a cooked low fat beaker sausage model system at a lower pH (~5.8) (17) In pork muscle gels, a combination of kappa carrageenan and sodium caseinate significantly increased water holding capacity and reduced cooking losses versus either one alone (32) Percent cooking loss in formulation turkey breast from carrageenan have been show to be less then additions of versus starch (20,57).   In formulated turkey breast, cooking losses with carrageenan were lower than with starch (20,57). In contrast, when kappa carrageenan was added to cooked beef patties at levels comparable to the studies cited above (0.5%), cook yields were significantly lower than the control without carrageenan (59).   In this case, beef carcasses were electrically stimulated prior to deboning, addition of carrageenan and cooking – a critical difference from standard slaughter and processing. Freeze thaw stability in pork sausages showed no advantages with added kappa carrageenan in combination with sodium tripolyphosphate. However, when the pH was lowered without sodium tripolyphosphate, carrageenan showed a significant reduction in freeze thaw losses (52).

Textural effects in meats containing carrageenan have also been studied. A study with beef gels rolls showed that kappa carrageenan improved or sustained texture in both raw and cooked meat, while other gum systems (xanthan gum, guar, gum, pectin, and carboxymethyl cellulose) induced unfavorable effects on texture (60). In gelled meat batters, kappa carrageenan effectively increased hardness; again xanthan gum had a negative effect on texture (60). Addition of carrageenan increased firmness in frankfurters with lean finely textured tissue (18). Hardness and fracturability were higher in beef gels with kappa carrageenan, but cohesiveness and springiness not affected (50). Combining carrageenan with sodium tripolyphosphate increased hardness in pork sausages; without sodium tripolyphosphate, carrageenan still significantly increased product hardness, although overall hardness reading were lower (50) and the magnitude of difference was much smaller (52).

There have been a few studies citing no effects of carrageenan in meats. Kumar (51) found no significant difference between controls (full fat) and the low fat ground pork patties with carrageenan. Evaluation of hardness measured as peak stress and peak energy (first and second compression) showed no significant difference between control samples with added kappa carrageenan (17), but this interpretation may be an error resulting from very high variation between sample measurements. Evaluation included hardness, cohesiveness, springiness, gumminess, and chewiness.

There is a small amount of research that includes evaluation of carrageenan in meats on a microscopic level. However, none of these included any in-depth evaluations or was the major focus of the research. Microscopic evaluations in cooked ham-based model systems used confocal scanning light microscopy, covalently labeling the kappa

carrageenan with fluorescein isothiocyanante. Kappa carrageenan was observed in discrete patched rather than in a continuous gel matrix (56). Methylene blue staining of beaker sausage samples similarly found carrageenan localized in defined locations regardless of the pH of the meat or addition of sodium tripolyphosphate (17). However, similar staining of ham cross-sections showed very distinct pattern of striations through out the meat (57). Again the carrageenan gelling was distinct and not continuous throughout. Light microscope evaluations of carrageenan in blue whitening mince using alcian blue stain located kappa carrageenan in large round cavities, while other hydrocolloids such as xanthan gum, locust bean gum, guar gum, carboxymethylcellulose, and alginate all formed filamentous structures throughout the protein gel (61). Another study with carrageenan in blue whiting mince using scanning electron microscopy found that kappa carrageenan formed small fine reticular structures (62).

Knowledge Gaps

Addition of carrageenan to meats has evolved based primarily on empirical product development research (mostly on cook yields) rather than systematic experimentation. Initial applications used carrageenan in the higher range of allowed concentrations (1.0-1.5% w/w) and this usage level is still standard. However, ingredient costs, off-flavors, and consumer concern over safety have forced development of meat products with lower carrageenan, so that now products can be found with carrageenan levels as low as 0.5%.

There are several areas in which critical information about carrageenan in meat products is still missing:

1) There is not yet information demonstrating whether very low levels of carrageenan (<0.5%) may be used while maintaining product quality.

2) Effects of carrageenan on storage properties such as refrigerator purge and freeze-thaw stability have not been documented.

3) There is little understanding of how carrageenan acts in meats at the molecular level.

This thesis this seeks to address these three issues.

HYPOTHESIS AND SPECIFIC OBJECTIVES

I. Hypothesis

Low concentrations (0.2-0.6%) of carrageenan can effectively maintain cook yields and textural properties of processed turkey breast without decreasing freeze thaw stability.

II. Specific objectives

A. Determine the effects of low carrageenan concentrations on physical properties (cook yield, freeze thaw stability, and textural characteristics) of turkey breast deli meat at four different moisture: protein ratios.

B. Investigate the role of hydration and swelling versus full solubilization and gelation of carrageenan in texturization of turkey breast deli meat. Carrageenan particles go through a sequence of hydration, swelling, solubilization, and gelation in meats, but the extent to which any of these dominate under different conditions and thus alter meat properties is not known.

EXPERIMENTAL METHODS

I. Experimental Design. A turkey breast deli product was prepared with four
concentrations of carrageenan (0, 0.2, 0.4, and 0.6% w/w) and three formulated moisture:
protein ratios (4:1. 5:1, 6:1 w/w) (Figure 7); these 12 treatments were run in duplicate
trials. To follow changes during cooking, each turkey breast product was baked at 180
°C and sampled at 80, 100, 120, 140, 160, and 180 °C. To determine effects of
carrageenan and carrageenan:protein interactions on stability parameters, half of each
batch was stored in the refrigerator and analyzed for moisture release (refrigerator purge);
the remaining samples were frozen and analyzed for freeze-thaw stability.

II. Materials

Turkey breast was chosen for this study because of the consistency in water,
protein, and especially fat content. Deboned meat was purchased from a local butcher
and processed within 24 hours.

Food-grade salt (NaCl) non-iodized, sodium tripolyphosphate (STPP), dextrose
were from standard industrial sources.

Kappa type carrageenan (FMC Gelcarin® ME 8121 carrageenan-1327) was
provided by FMC Biopolymer, Princeton, NJ.

| % Carrageenan

0
0.2
0.4
0.6 | **X** | Formulation
Moisture:Protein Ratio

4:1
5:1
6:1 | ⇒ | **12 treatments** |

#5 0% Cgn 4:1 MP	#11 0.2% Cgn 4:1 MP	#8 0.4% Cgn 4:1 MP	#4 0.6% Cgn 4:1 MP
#3 0% Cgn 5:1 MP	#1 0.2% Cgn 5:1 MP	#12 0.4% Cgn 5:1 MP	#9 0.6% Cgn 5:1 MP
#6 0% Cgn 6:1 MP	#10 0.2% Cgn 6:1 MP	#7 0.4% Cgn 6:1 MP	#2 0.6% Cgn 6:1 MP
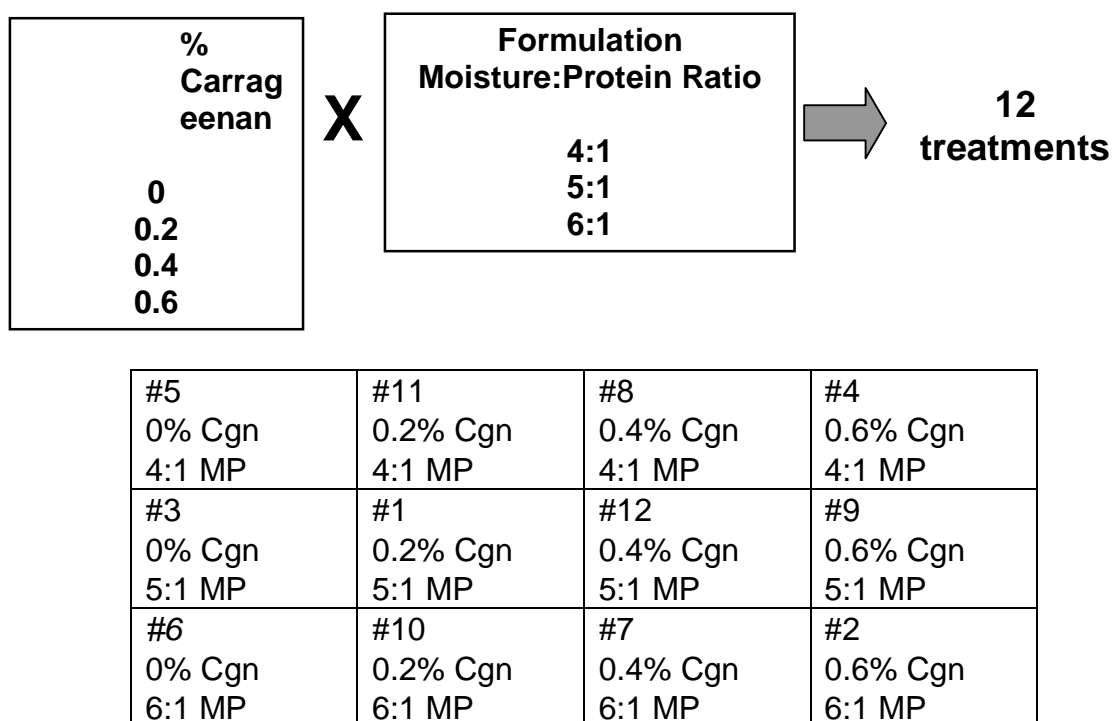
Figure 7. Experimental design for preparation and analysis of turkey breast deli meat with various levels of carrageenan added. Numbers refer to randomization sequence for preparation and analysis.

III. Procedures

A. Turkey loaf preparation

1. Meat preparation.   The turkey was ground into 2.5 cm pieces using the largest plate of a Hobart Model 4732A meat grinder. To slow microbial growth and lipid oxidation, the entire process was performed in a refrigerated lab set at approx 50°F and meat was processed immediately.

2. Brine Solution Preparation. The brine solution was prepared in cold water, with 1.5% salt (NaCl),0.46% sodium tripolyphosphate (STPP), 0.75% dextrose, and kappa type carrageenan at the four different concentration levels (0, 0.2, 0.4, and 0.6% w/w). The STPP was added first because it has very low solubility. It was dissolved in ~2-5 °F water using a Baldor XP-02 mixer in an ice bath for approximately 5 min. The NaCl was then added to the phosphate solution and mixed for 1 minute, then the dextrose was added and mixed for 1 minute. Carrageenan was added last to limit hydration during preparation.

3. Loaf formulation and mixing (Figure 8). Ground turkey breast meat was placed in a three chamber vacuum tumbler model VT375 by Leland Southwest, and the brine solution was added in volumes and proportions specified in Appendix A to make 25 lb batches. Vacuum was pulled and the meat-brine mixture was then tumbled at the highest speed for 90 minutes. The meat was removed from tumbler, and samples of approximately 1400 g were weighed into impermeable bags. The bags were vacuum-sealed (Koch Model X-200 Sealing System , Kansas City, MO) and labeled. All of the preparation was completed in a refrigerated lab set at approximately 50°F.

Figure 8. Flow chart for production and analysis of turkey breast deli meat with various levels of carrageenan added.

Water
STPP
Salt (NaCl)
Carrageenan

Brine Solution

Ground Turkey (2.5 cm pieces)

Tumbling Under vacuum (90 min)

Weighed Turkey Loaves Impermeable bags and Sealed (~1400 g)

Sample at 80°F 100°F 120°F 140°F 160°F

Cool in ice bath

160°F

All temps

Microscopy

Meat Properties Cook yield (drip loss) Freeze-thaw stability Texture Profile Analysis
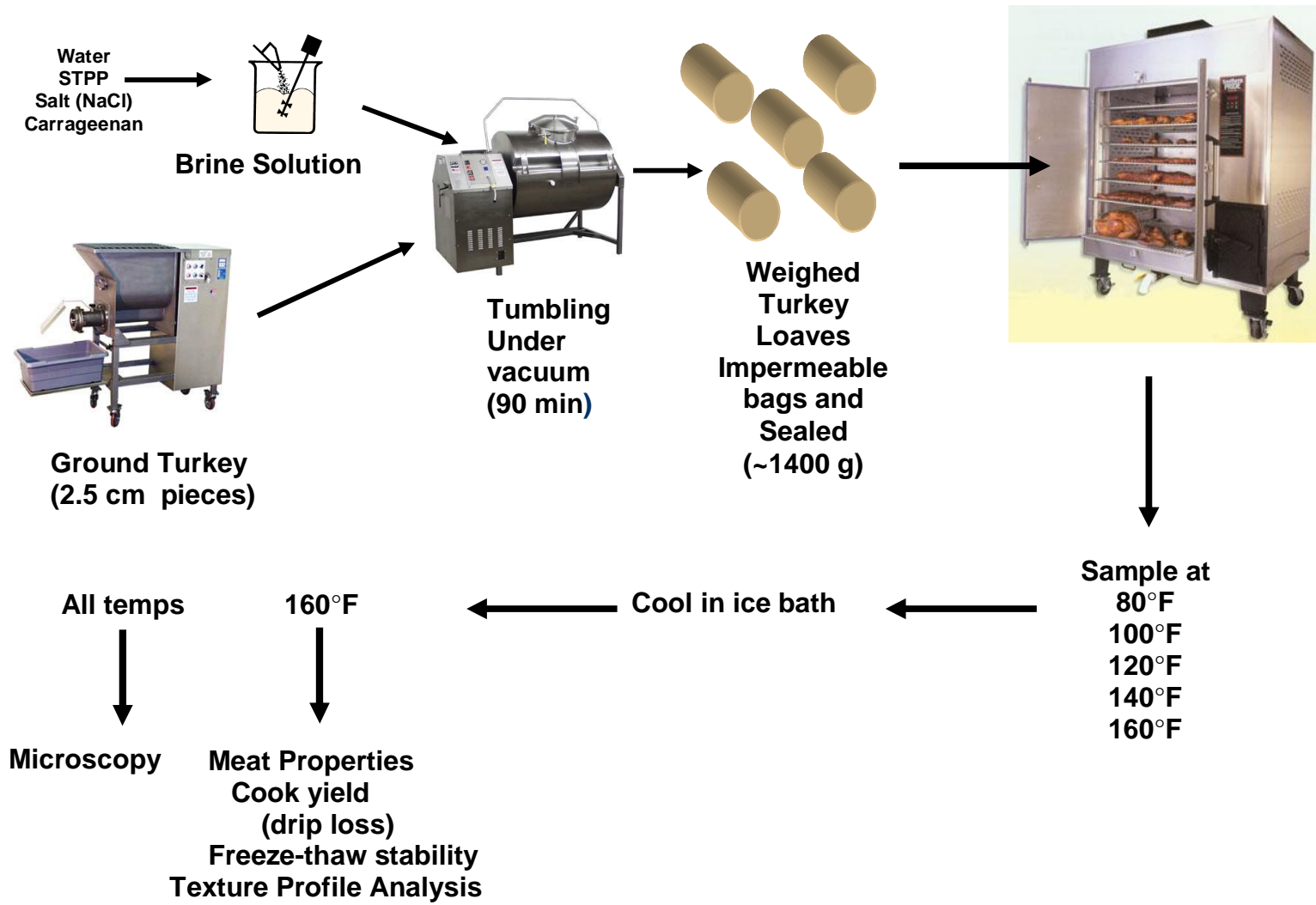
4. Cooking (Figure 8). The packaged meat was placed randomly in an Alkar Model 450-UA oven set at 180 °F. A thermometer probe was inserted into the center of one loaf to monitor the temperature during cooking. Samples were removed when the center of the packs reached five different temperatures (40°F uncooked, 80°F, 100°F, 120°F, and 140°F), immediately cooled to refrigeration temperatures in an ice bath, and set aside for microscopic analysis to evaluate changes in the carrageenan during heating. The remaining loaves were cooked 160°F, cooled to refrigeration temperatures, weighed, and analyzed for changes in physical properties (cook yield, refrigerator purge, freeze thaw stability, texture analysis) and molecular organization (microscopy).

B. Analyses

1. Cook Yield. Cook Yield is a measure of how much meat remains after the cooking process, based on the amount of water lost from the original mass. Three refrigerated loaves in bags fully cooked to 160°F were weighed, then removed from their bags and drained. Excess water was removed from the meat by blotting with a paper towel. The dried loaf was weighed and cook yields were calculated as a % of the original weight:

$$\frac{\text{Weight of the dry meat loaf}}{\text{Weight of the bagged meat} - \text{weight of the bag*}} \quad X \quad 100 = \text{Cook yield (\%)}$$

(*bags are preweighed and an average is used).

2. Refrigerator Purge. Refrigerator Purge is a measure of the water lost from meat during refrigerated storage. Three 1.5 inch slices were cut from the center of each of the three turkey loaves used for Cook Yield analysis (Figure 9). One slice from each loaf
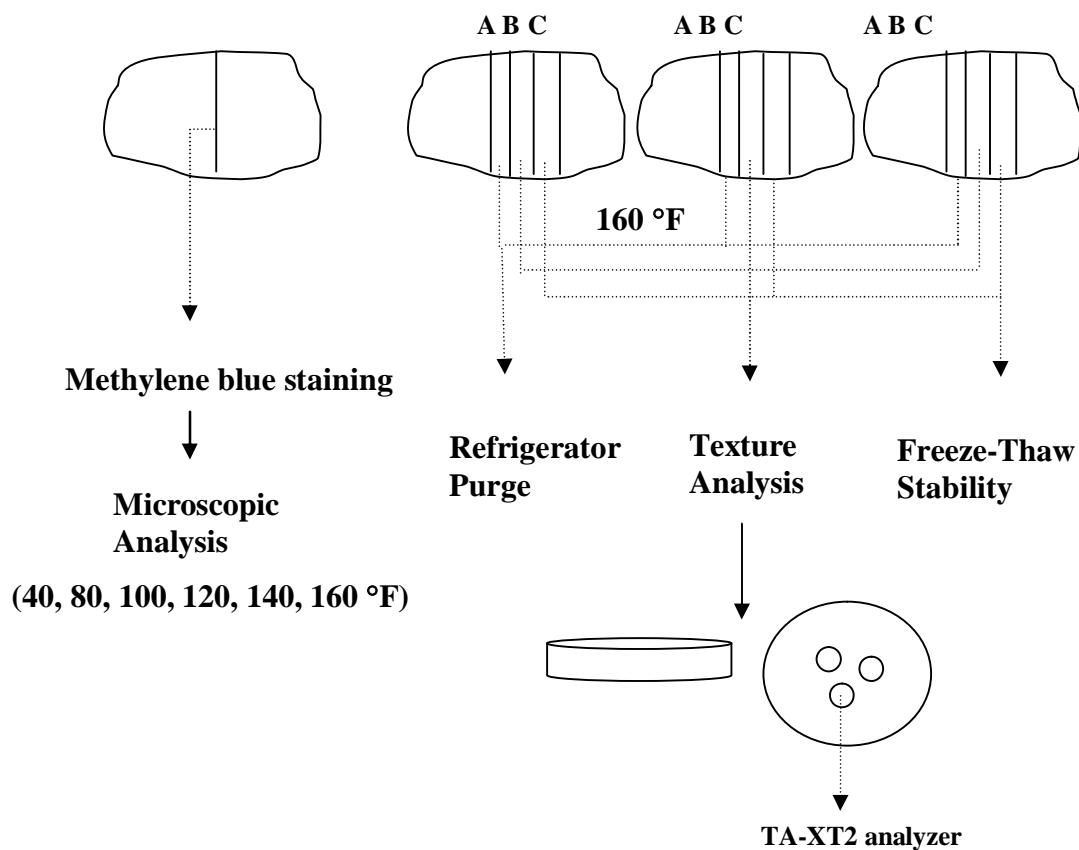
Figure 9. Schematic diagram for sampling and analysis of baked turkey breast loaves with varying levels of carrageenan. Four loaves were baked for each treatment. One loaf was sliced vertically through the center and thin slices were taken from the center for microscopic analysis. After cook yield analyses, three 1.5.inch slices were cut vertically from the center of each of the remaining three loaves. Slice A was used for Refrigerator purge determinations, Slice C was used for freeze-thaw stability analyses, and Slice B from the very center was used for texture profile determinations. For this, slice B was laid flat and three plugs were cut from close to the center for TA-XT2 texture analyses.

was vacuum sealed in pre-weighed bags, placed individually flat on trays without stacking, and refrigerated for one week. The meat packages were then removed from the refrigerator and immediately weighed. The meat slices were removed from the bags, drained, and excess water was removed by blotting with a paper towel. The dried samples were weighed and % refrigerator purge or moisture loss was calculated as

$$\frac{\text{Weight of the bagged meat – weight of the dried meat – weight of bag*}}{\text{Weight of the bagged meat}} \times 100$$

(*bags are preweighed and an average is used).

3. Freeze Thaw Stability. Freeze thaw stability is reflected in the amount of water retained after meat has been frozen and completely thawed. A second of the three 1.5 inch slices cut from each fully cooked loaf (Figure 10) was vacuum sealed in preweighed bags, placed flat on trays without stacking, and stored frozen (-20 °C) for at least one week. Samples were removed from the freezer and thawed at refrigerated temperature for 48 hours. Each bag of meat was weighed, and the meat slices in each bag were removed, drained, and excess water was removed by blotting with a paper towel. The dried meat sample was weighed and % moisture loss during freezing was calculated as

$$\frac{\text{Weight of the bagged meat – weight of the dried meat – weight of bag*}}{\text{Weight of the bagged meat}} \times 100$$

(*bags are preweighed and an average is used).

4. Texture analysis. From the center 1½ inch slice cut from each fully cooked loaf, three 1 inch diameter plugs of meat were cored from the center of each slice for a total of nine samples per treatment (Figure 9). Texture profiles of samples were recorded with a TA.XT2i Texture Analyzer (50 kg capacity, Texture Technologies Corp., Scarsdale, NY)

using the following TPA Test Mode Parameters and Texture Expert for Windows
Version 1.20 software:

Pre Test Speed	0.50 mm/s
Test Speed	0.50 mm/s
Post Test Speed	10.0 mm/s
Rupture Test Distance	1.0 mm
Distance	50 mm
Force	60 g
Time	0.10 sec
Count	5
Trigger	Button
Force	5 g
Units	Force/Grams, Distance/millimeters
Probe	TA 10 ½ inch dial. AOAC cylinder, acrylic.

A full texture analysis was recorded for each sample. Hardness and cohesiveness
were of particular interest relative to turkey breast sensory qualities. Hardness is reflected
in the peak force of the first compression. Cohesiveness is a measure of how the product
withstands the second compression relative to the first compression; it is calculated as the
area of work of the second compression divided b the area of work of the first
compression (Figure 10). Gumminess and chewiness are derived values. Gumminess is
calculated as hardness * cohesiveness (Area 2 / Area 1), and chewiness if gumminess *
springiness (Length 2 / Length 1), as designated in Figure 10.
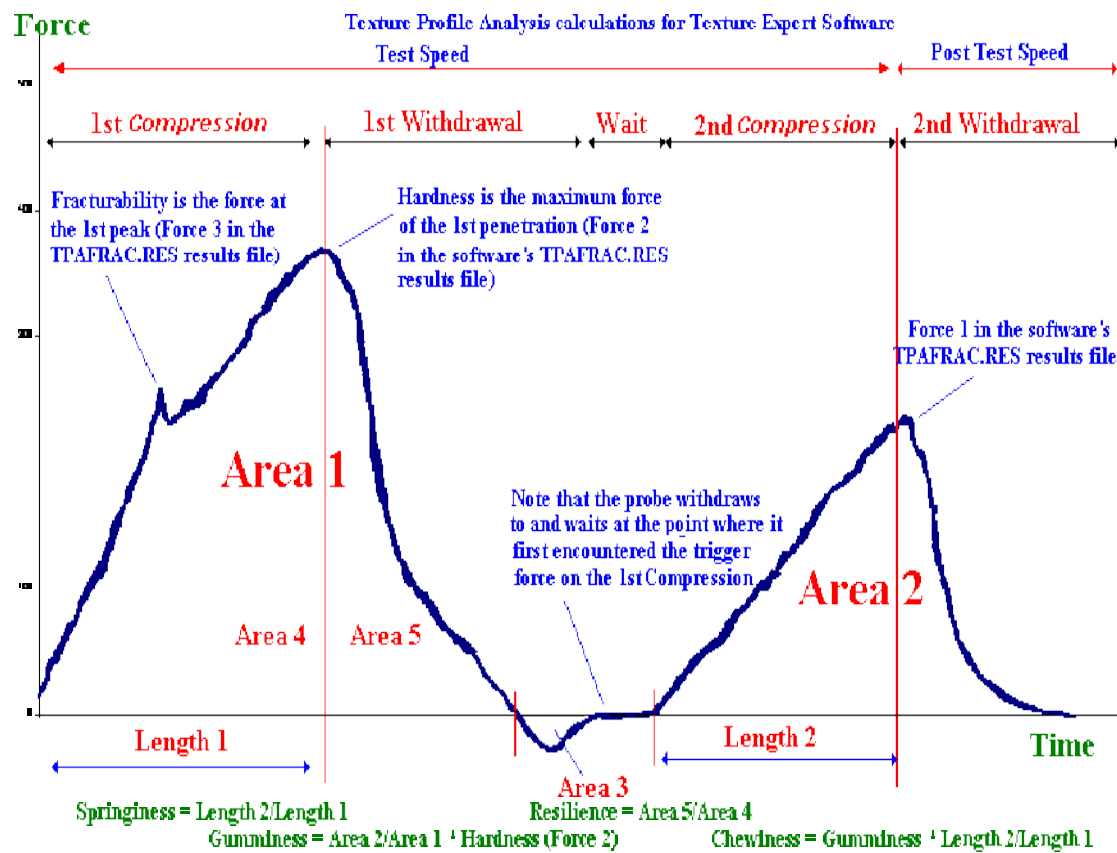
Figure 10. Graph generated inTA-XT2 texture profile analysis showing how each of the textural parameters are measured (63).

5. Microscopic analysis with Methylene Blue Stain. One refrigerated loaf from each of the various cooking temperatures (40°F uncooked, 80°F, 100°F, 120°F, 140°F, and fully cooked 160°F) was cut vertically down the middle. Thin slices were taken from the middle of each half and vacuum sealed in small bags for analysis (Figure 10).

Methylene blue stain was prepared by dissolving 0.1 gram of methylene blue in 49.9 grams of deionized water. The solution is diluted to 100 gms with isopropyl alcohol (99%) and filtered.

For analysis, the sample was placed on a slide, stained with several drops of methylene blue stain solution, and immediately visualized with a Nikon Optiphot light biological microscopic under 160X magnification. Micrographs were recorded digitally with a Nikon DXM 1200 Hi Resolution Color Digital Camera using Graphics/Imaging Workstation w/ Camera Control Program ACT-1 for DXM 1200 Version 2.20 software. Triplicate samples were prepared and analyzed for all 12 treatments.

Methylene blue is a selective method for staining carrageenan without interference from the meat proteins. The reagent is blue in solution, but changes color to purple when it electrostatically binds to the sulfate groups on carrageenan (Figure 11). This provides a means of tracking changes in molecular organization of carrageenan in the meat emulsions at different temperatures and in different formulations. Figure 12 shows an example of the methylene blue visualization of the progression through carrageenan hydration, swelling, gelation, and solubilization in solution.
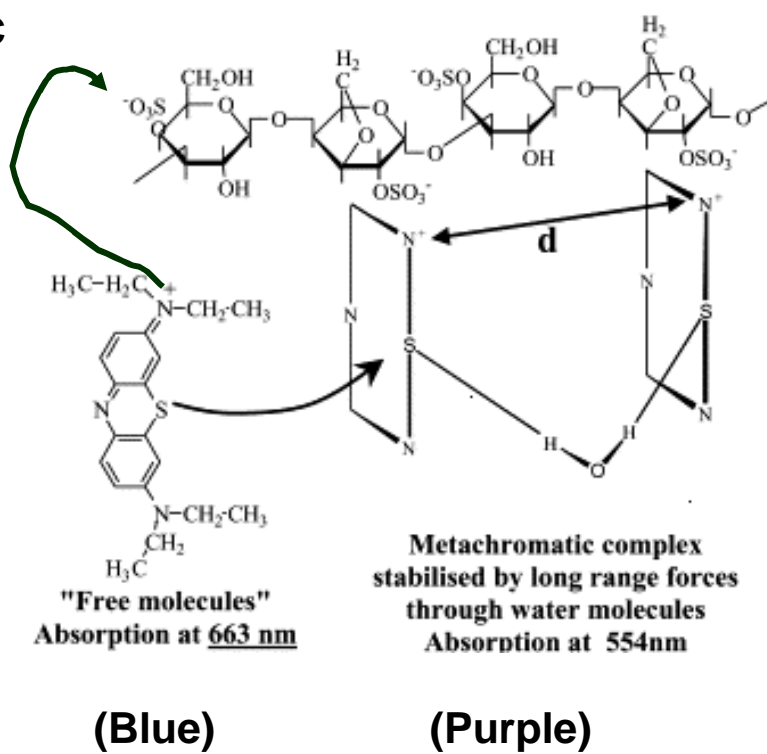
Figure 11. Mechanism of methylene blue binding to carrageenan. Adapted from (64).
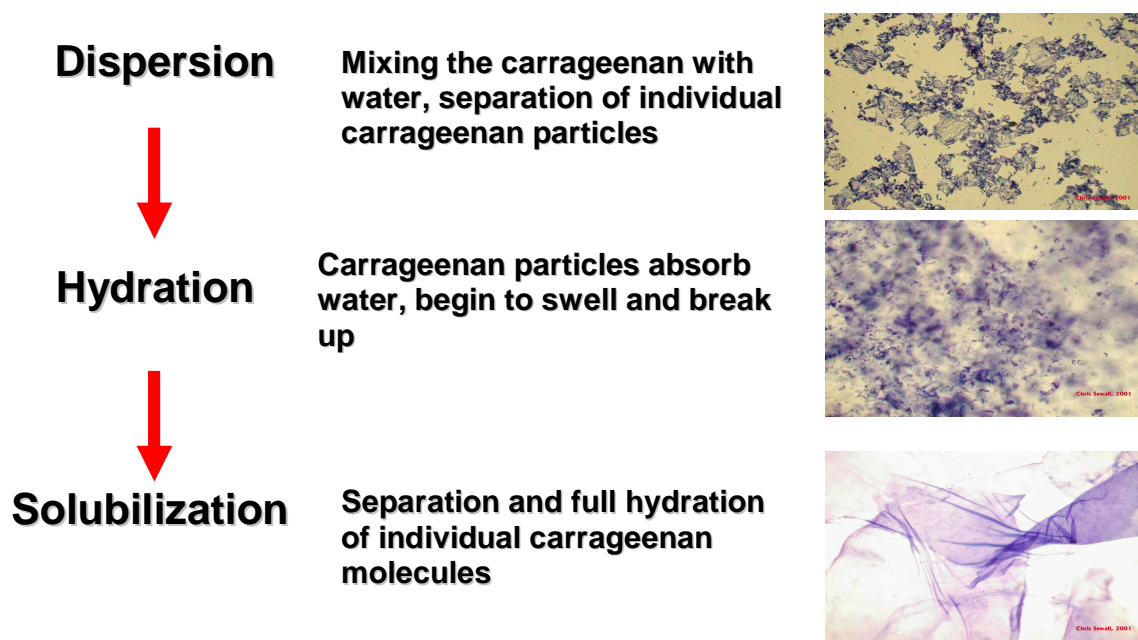
Figure 12. Visualization of progressive changes in carrageenan (in solution) with

methylene blue. Courtesy of FMC Biopolymer – Training.

RESULTS

I. Cook Yield

Averages of two cook yield analyses for all treatments are presented in Figure 13. At the lowest moisture protein ratio 4:1, minimal moisture loss occurred during cooking. The average cook yield was 97.7% for both 0% and 0.6% carrageenan, 98.0% for 0.2% carrageenan, and 97.9% for 0.4% carrageenan. Moisture loss was slightly higher in the 5:1 moisture:protein samples with no or low carrageenan (95.9% and 96.6% cook yields, respectively); water binding increased with carrageenan to the same levels as 4:1 samples. At the highest moisture:protein ratio (6:1), samples with no added carrageenan showed notable moisture loss (7.8 % loss), while cook yields increased linearly with added carrageenan. The highest carrageenan levels (0.6%) effectively maintained high cook yields (97.6 - 98.0, 97.7%) at all moisture:protein ratios.

II. Refrigerator Purge.

Averages of two refrigerator purge measurements for all treatments are presented in Figure 14. Moisture loss during refrigerated storage increased with moisture:protein ratio, suggesting that available protein levels were insufficient to bind the water added. At the lowest moisture levels (4:1 and 5:1 M:P), adding carrageenan did not improve water retention. At the highest moisture level (6:1 M:P), 0.2% carrageenan increased moisture loss and 0.4% carrageenan had no effect, but 0.6% carrageenan bound the water and reduced water loss to the same level as the lower moisture systems. Moisture loss with 0.6% carrageenan was maintained at a low 3.5% at all M:P ratios tested.
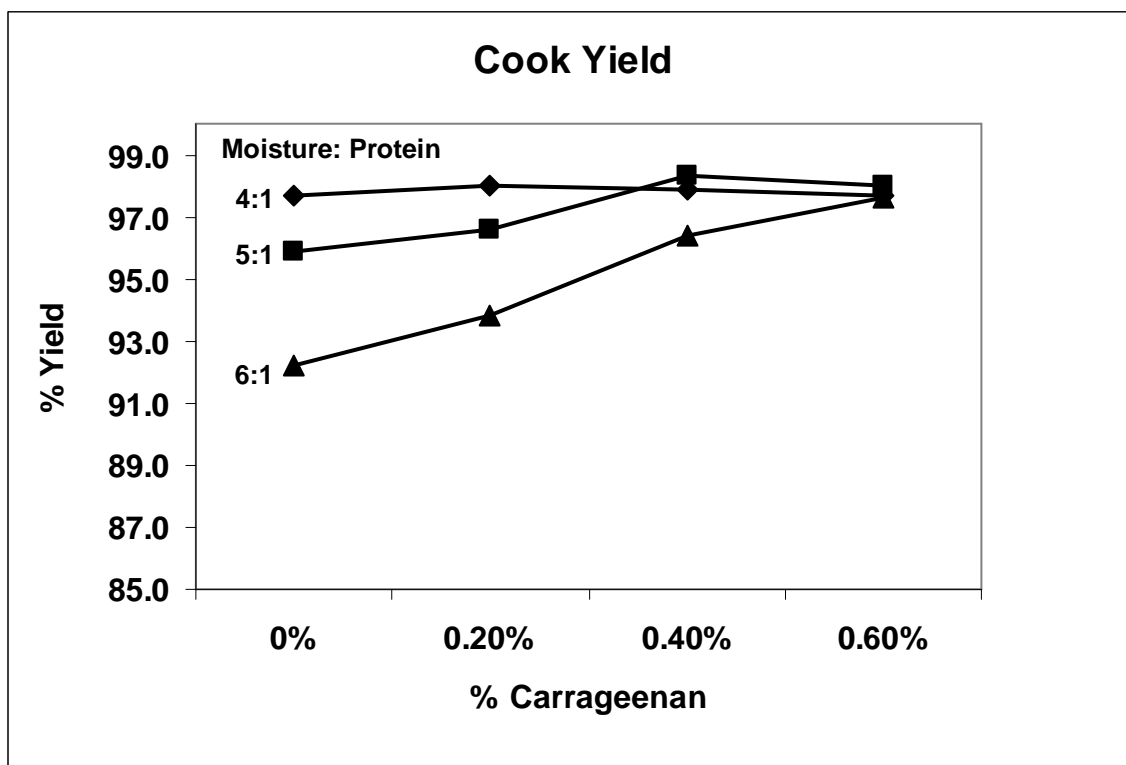
Figure 13. Effects of carrageenan concentration and moisture:protein ratios on cook

yields from roasted turkey breast deli meat.
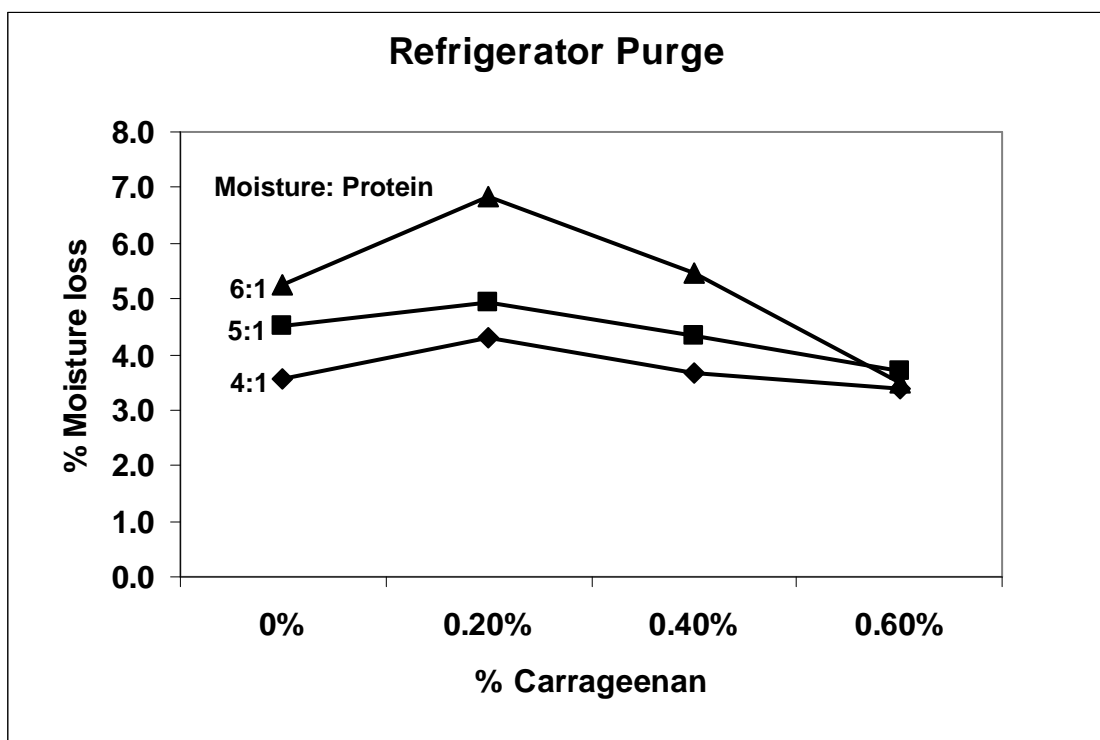
Figure 14. Effects of carrageenan concentration and moisture:protein ratios on refrigerator purge from roasted turkey breast deli meat.

III. Freeze Thaw Stability.

Averages of two freeze-thaw stability measurements for all treatments are presented in Figure 15. As with cook yield and refrigerator purge, moisture loss increased linearly with M:P ratio, from ~7% at the 4:1 moisture:protein ratio to ~12% at 6:1 M:P. This pattern again suggests that the available protein alone was insufficient to bind the amount of water added in these three formulations. In the lower moisture systems, adding 0.2% carrageenan increased moisture loss by about 2%, but higher levels of carrageenan had little effect on moisture retention relative to controls. In 6:1 M:P high moisture samples, 0.2% and 0.4% carrageenan increased moisture loss by 2% and 1% respectively, while 0.6% carrageenan bound water more effectively, reducing freezing losses to 9.13 %, the same level as 5:1 M:P (9.19 %) samples.

IV. Texture Analysis

A. Texture profile analysis. Representative data from texture profile analysis is presented in Table 2. Carrageenan and moisture:protein ratios induced differences in hardness, cohesiveness, gumminess, chewiness, and fracturability.

B. Hardness. Hardness values (average of three analyses) of turkey breast with varying levels of carrageenan are shown in Figure 16. Hardness decreased with increasing moisture levels. Although presenting the data as M:P ratios makes the drop in hardness appear non-proportional with carrageenan concentration, in fact, when plotted as % moisture this relationship is linear. In control systems, decreasing hardness translates practically as "squishiness" and greater difficulty in slicing. Indeed, the 6:1 M:P control was almost too soft to handle. Addition of carrageenan increased firmness in
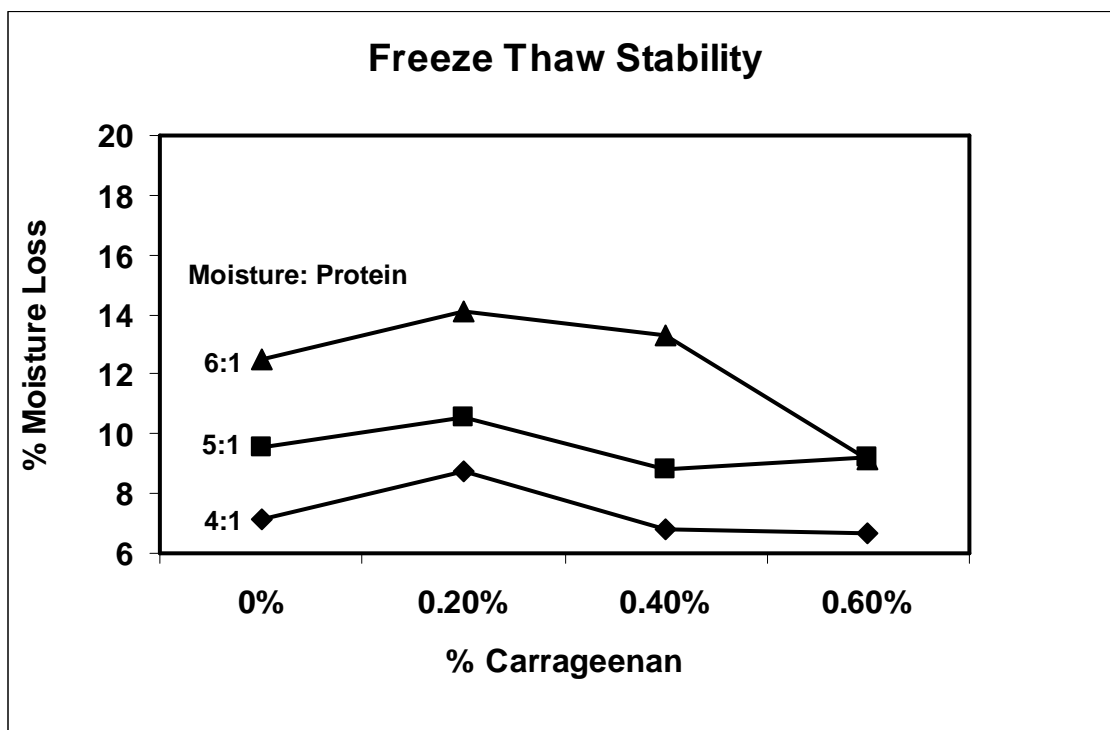
Figure 15. Effects of carrageenan concentration and moisture:protein ratios on freeze-

thaw stability of roasted turkey breast deli meat.

Table 2. Summary of TA-XT2 texture profile analysis of roasted turkey breast deli meats.

Run #	M:P	%CGN	Hardness	Fracturability	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
5	4:1	0	7170	10.7	-2.844	0.679	0.334	2396	1622	0.113
11	4:1	0.2	7460	11.7	-0.888	0.642	0.320	2401	1541	0.102
8	4:1	0.4	8727	9.3	-1.334	0.622	0.320	2794	1740	0.102
4	4:1	0.6	8699	12.2	-0.541	0.665	0.342	2983	1986	0.115
3	5:1	0	3468	10.3	-5.745	0.680	0.267	819	300	0.091
1	5:1	0.2	4733	11.1	-0.995	0.711	0.308	1462	965	0.104
12	5:1	0.4	4821	10.2	-2.548	0.641	0.295	1414	900	0.099
9	5:1	0.6	6065	**	-2.656	0.632	0.305	1863	1179	0.102
6	6:1	0	2748	**	1.143	0.622	0.247	692	430	0.089
10	6:1	0.2	3780	**	-4.025	0.587	0.283	1084	632	0.098
7	6:1	0.4	3558	**	-2.521	0.557	0.273	987	548	0.092
2	6:1	0.6	3468	**	-5.745	0.680	0.267	819	300	0.091

All data are averages of duplicate analyses.
** TPA measurements were highly variable. See Table 3.

Figure 16. Effects of carrageenan concentration and moisture:protein ratios on hardness

values in texture profile analyses of roasted turkey breast deli meat.

4:1 and 5:1 M:P (low moisture) turkey breast products, but had only very small effects in the highest moisture systems (6:1 M:P). These results suggest that gels formed by 0.6% carrageenan in the 6:1 M:P systems are too dilute to counteract fluidizing effects of higher moisture. Nevertheless, all samples with added carrageenan were judged to have acceptable firmness for commercial slicing.

C. Cohesiveness. Cohesiveness values (average of three analyses) of turkey breast with varying levels of carrageenan are shown in Figure 16. Cohesiveness values decreased 0.05-0.1 unit as moisture increased. In the lowest moisture (4:1and 5:1 M:P) systems, adding carrageenan changed cohesiveness very little. However, in the higher moisture (6:1 M:P) systems, cohesiveness increased slightly with addition of 0.2% carrageenan (0.4 unit), but higher levels of carrageenan did not improve cohesiveness further. This suggests that carrageenan acts independently of protein in these deli meats, i.e. carrageenan binds water but, at the levels used in this study, do not interact with proteins and do not change molecular intercconnections that create the molecular matrices in these systems.

D. Gumminess and chewiness. Gumminess and chewiness showed similar patterns (Figure 17 and 18, respectively). The dominant effect was a decrease in these values with moisture, with or without carrageenan. In the low moisture products, gumminess and chewiness progressively increased with carrageenan concentration, but in the high moisture products (6:1 M:P), adding carrageenan had no effect.
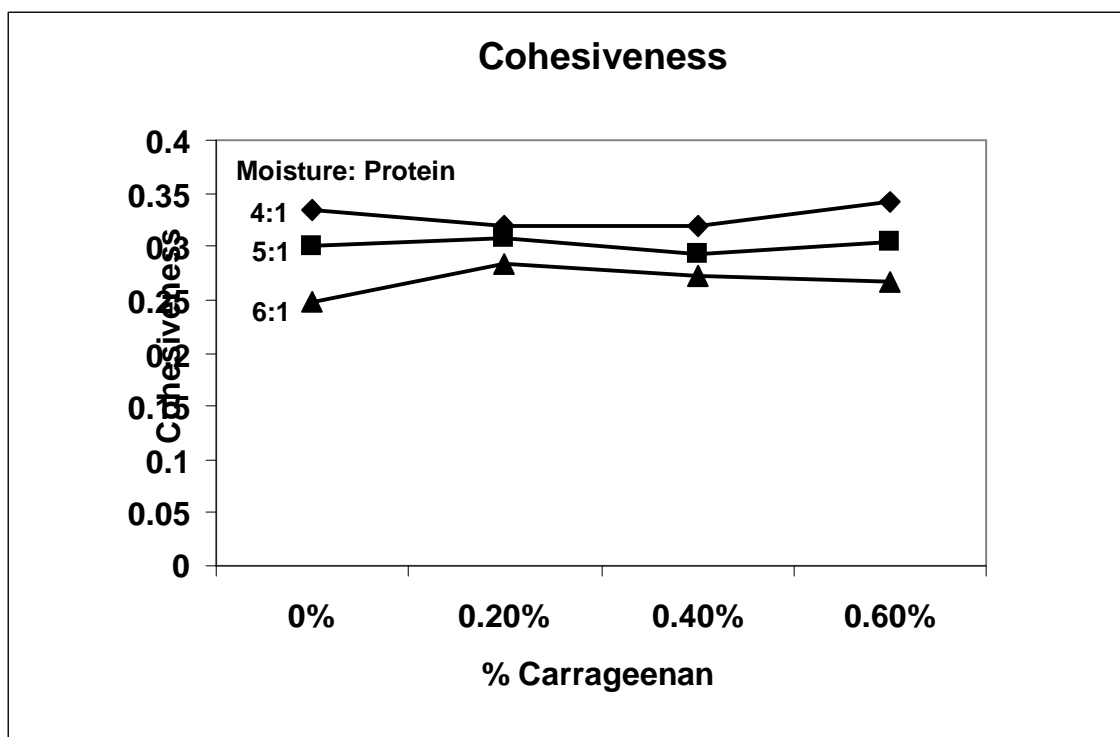
Figure 16. Effects of carrageenan concentration and moisture:protein ratios on

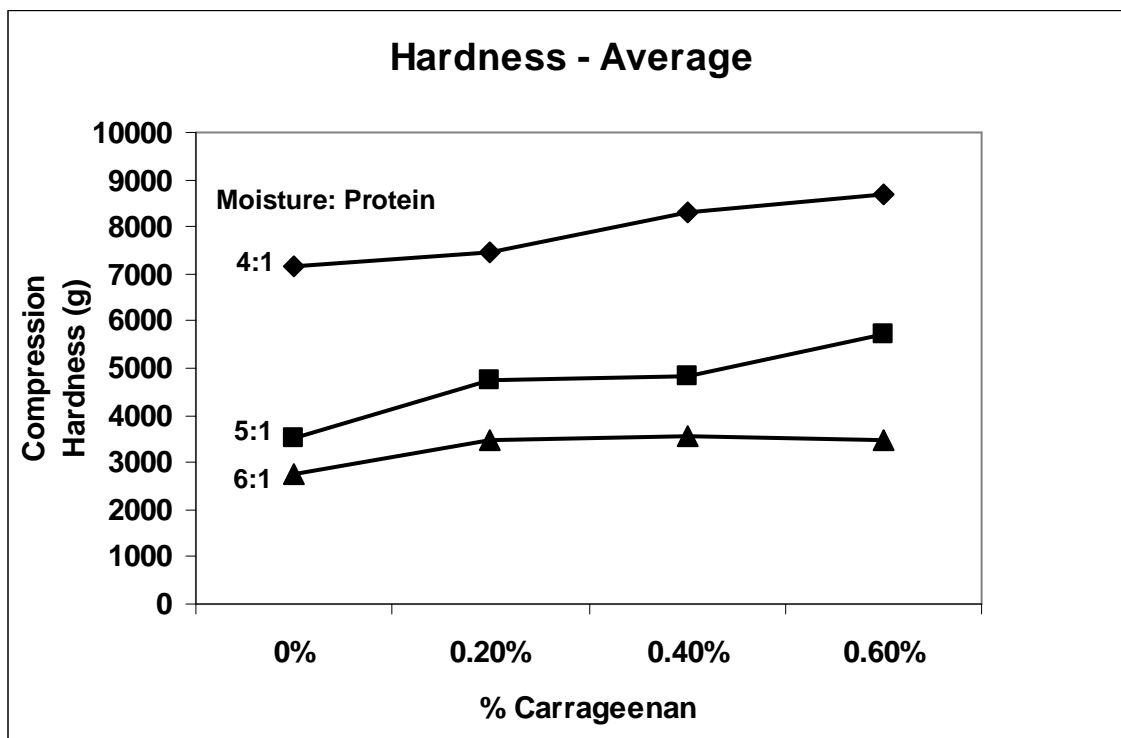cohesiveness values in texture profile analyses of roasted turkey breast deli meat.
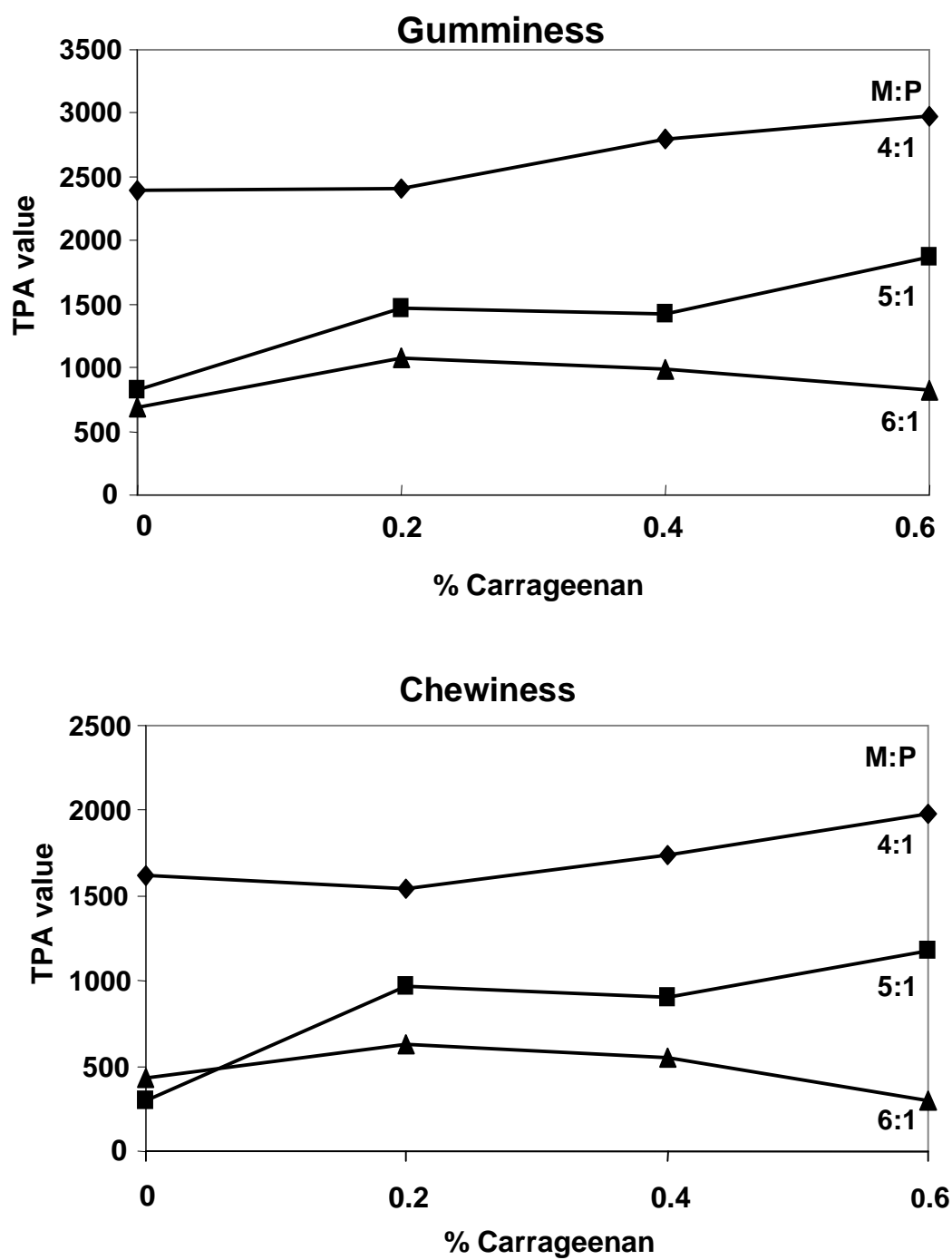
Figure 16. Effects of carrageenan concentration and moisture:protein ratios on TPA

gumminess and chewiness values of roasted turkey breast deli meat.

E. Fracturability. Fracturability values in texture profile analysis were low (10-12) in the 4:1 M:P products and the 5:1 M:P products with no or low carrageenan. However, in the remaining samples, aberrations were observed in which very high fracturability scores (e.g. ~3000) were mixed with the low scores (Table 3). Only three aberrant scores were present in the 5:1 M:P 0.6% carageenan samples, but in the 6:1 M:P samples, the majority of the scores were high. This effect was observed primarily in samples from the first run; the high fracturability scores were less frequent in Run 2, except in high moisture – high carrageenan samples.

The possibility of TA-XT2 malfunction can be eliminated since samples were analyzed randomly. The appearance of the aberrant values in both production runs excludes problems with a single run, but also indicates a large inhomogeneity within the samples. A likely explanation may be that in the high moisture samples, not all water is uniformly bound to the various macromolecules but a part of it remains pooled and relatively free in some regions of meat mix. This compartmentalization could originate in mixing or may result from reorganization and reassociation of the polymer matrix after cooking. When the texture analyzer plunger encountered a more fluid region, aberrantly high fracturability was registered.

Table 3. Aberrant and variable fracturability values for high moisture and high

carrageenan samples.

9	6	10	7	2
0.6% Cgn 5:1 M:P	0% Cgn 6:1 M:P	0.2% Cgn 6:1 M:P	0.4% Cgn 6:1 M:P	0.6% Cgn 6:1 M:P
		Run 1		
2411	1330	2603	1483	2250
12	1897	2416	2222	2904
14	11	9	2238	3370
3111	7	7	2370	1496
13	1210	2090	11	8
11	1562	2372	11	2654
6	1120	9	2617	10
3832	2069	2245	2644	2467
10	14	12	2087	10
		Run 2		
	11	10	10	2852
	14	12	10	2915
	7	8	11	2533
	1887	12	6	7
	2322	15	10	12
	6	11	2343	2110
	10	9	8	2714
	11	7	14	11
	10	11	11	1795

V. Microscopic Analysis with Methylene Blue Staining.

Results from cook yield, refrigerator purge, freeze-thaw stability, and texture analysis suggest that effects of carrageenan are strongly modulated by % moisture in the system as well as competition between muscle proteins and carrageenan for water binding. There is little evidence implicating protein-carrageenan interactions.

To pursue these explanations further and visualize the protein-carrageenan molecular organization in the deli meats and the changes in carrageenan at different temperatures and different environments, thin slices of turkey breast were taken from the center of baked loaves and stained with methylene blue to selectively locate carrageenan. Using this technique, carrageenan water binding can be followed by initial swelling and disruption of the gum granules, then dispersion and gelation of released molecules, and finally full gelation by loss of intact particles and total staining of the slide as pink to purple.

Controls without carrageenan do not bind methylene blue so no purple staining is visible (Figure 19). Composite micrographs of samples from all processing conditions are presented in Figures 20 (0.2% carrageenan), 21 (0.4% carrageenan), and 22 (0.6% carrageenan).

A. Effects of temperature. Carrageenan particles generally showed limited hydration and swelling at low temperatures, but particle swelling and bloating to irregular shape increased noticeably with temperature (Figures 20-22). Carrageenan particles began to rupture at about 140 °F. The transition of blue or pink to purple in the background of 140 and 160 °F samples signals that some carrageenan polymer has been released and gelled in the supporting matrix. However, particle hydration was

0 % Carrageenan M:P 4:1 (Run 2)
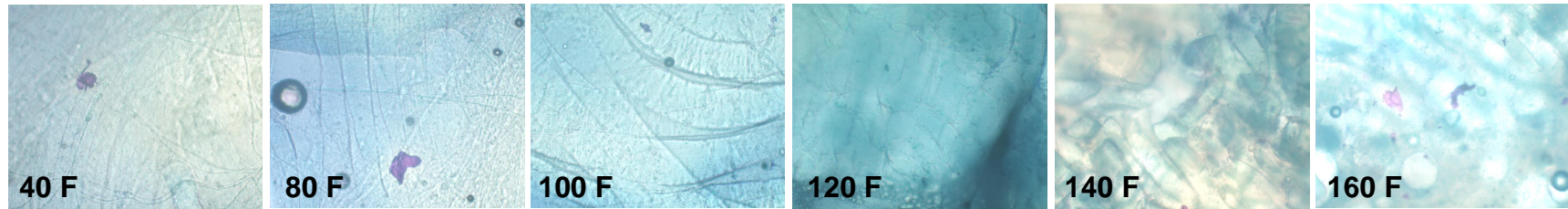
0 % Carrageenan M:P 5:1 (Run 2)
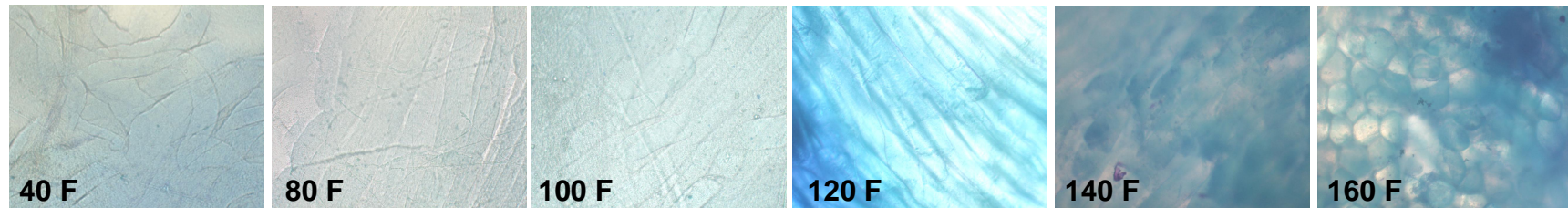
0 % Carrageenan M:P 6:1 (Run 2)
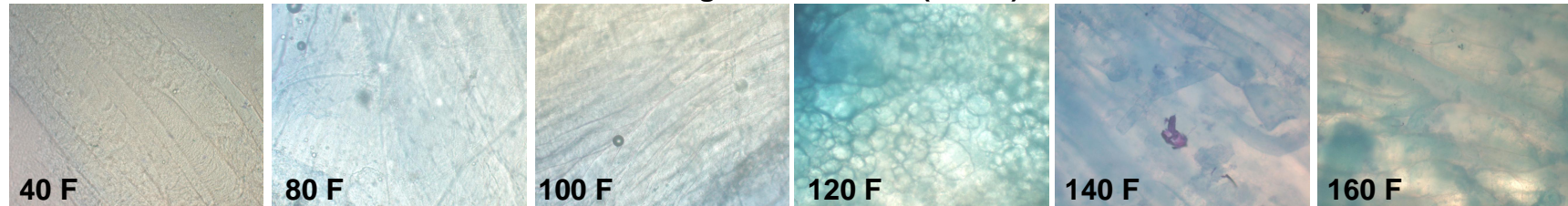
Figure 19. Methylene blue staining of turkey breast controls showing no specific staining. Run 2. Magnification for all samples was 160X.

0.2% Carrageenan M:P 4:1

40 F 80 F 100 F 120 F 140 F 160 F

0.2% Carrageenan M:P 5:1

40 F 80 F 100 F 120 F 140 F 160 F

0.2% Carrageenan M:P 6:1

40 F 80 F 100 F 120 F 140 F 160 F
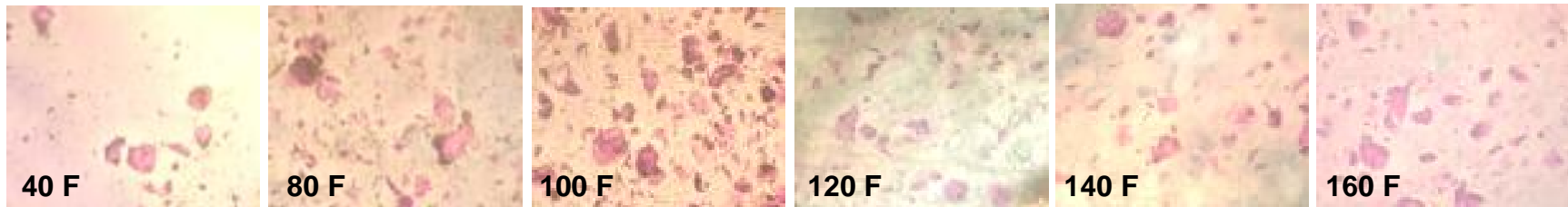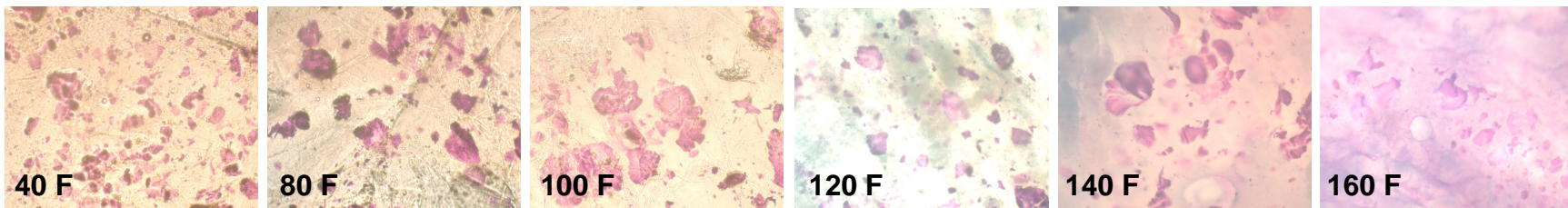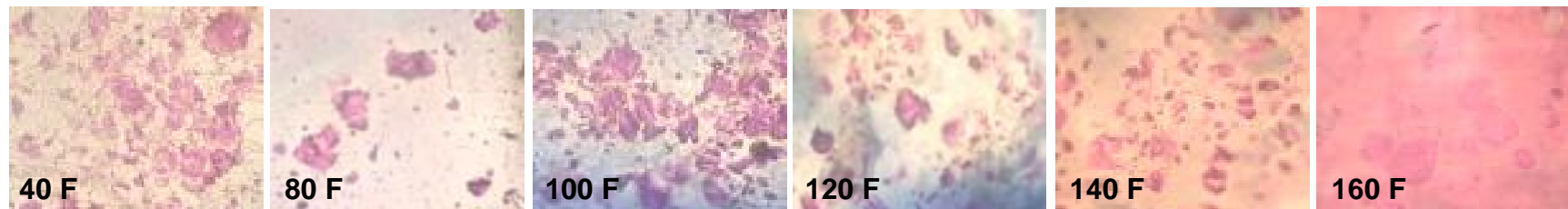
Figure 20. Methylene blue staining of turkey breast deli meats showing progressive hydration, swelling, and release of carrageenan molecules (increasing pink background) with 0.2% carrageenan added. Magnification for all samples was 160X.

0.4% Carrageenan M:P 4:1
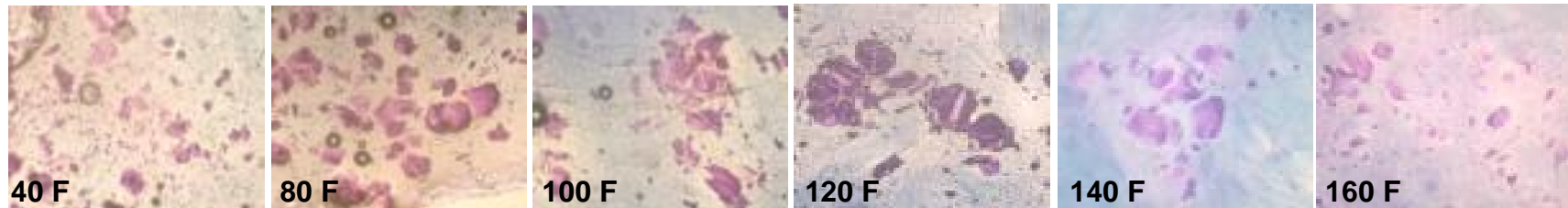
0.4% Carrageenan M:P 5:1
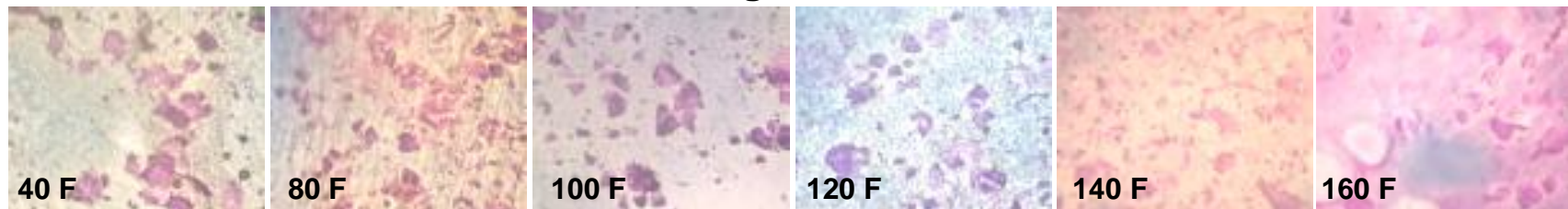
0.4% Carrageenan M:P 6:1
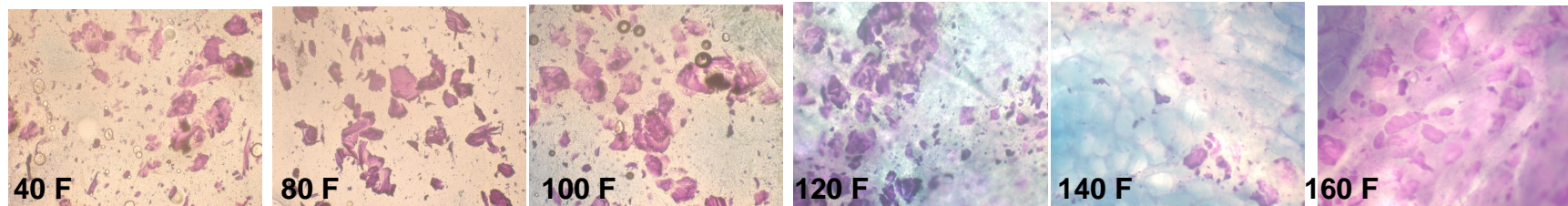
Figure 21. Methylene blue staining of turkey breast deli meats showing progressive hydration, swelling, and release of carrageenan molecules (increasing pink background) with 0.4% carrageenan added. Magnification for all samples was 160X.

0.6% Carrageenan M:P 4:1
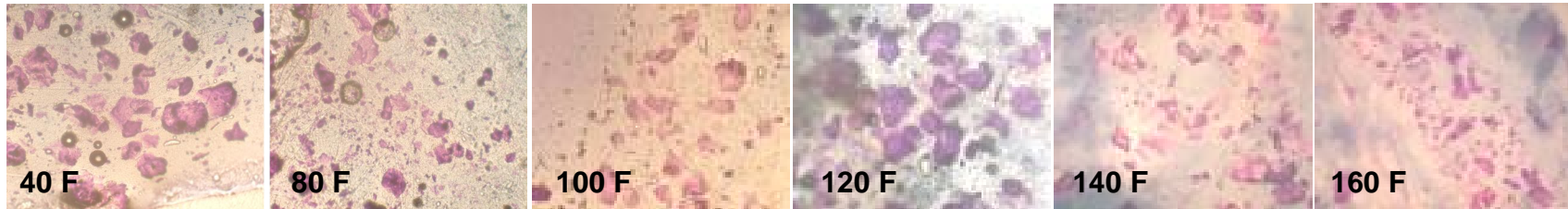
0.6% Carrageenan M:P 5:1
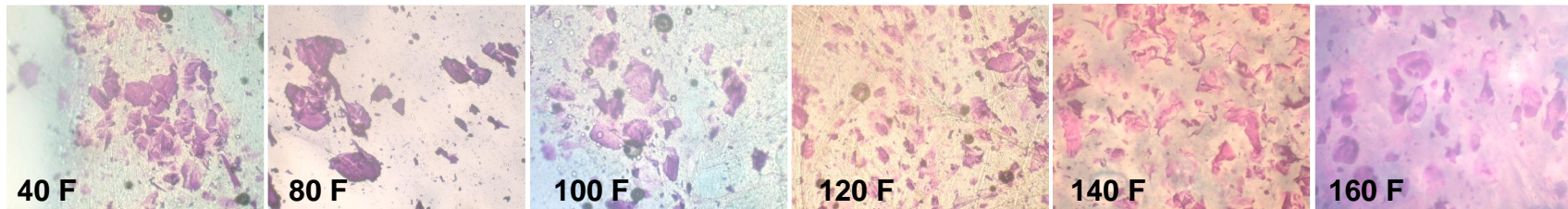
0.6% Carrageenan M:P 6:1
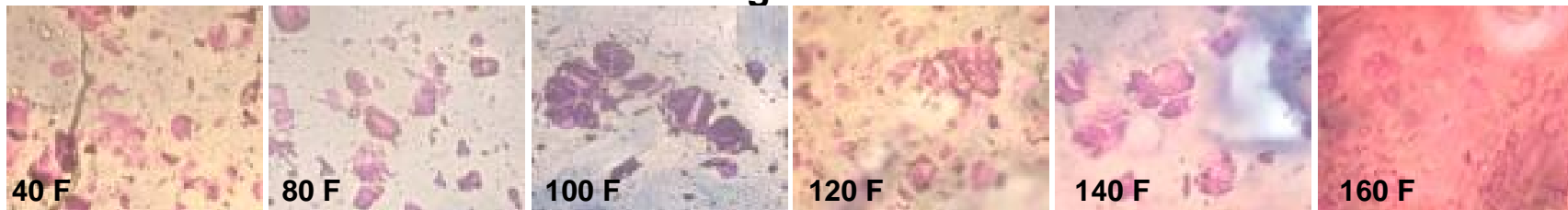
Figure 22. Methylene blue staining of turkey breast deli meats showing progressive hydration, swelling, and release of carrageenan molecules (increasing pink background) with 0.4% carrageenan added. Magnification for all samples was 160X.

incomplete, even in the 6:1 M:P samples because swollen intact carrageenan particles also remained evident in all fully cooked samples.

B. Effects of moisture. As expected, carrageenan hydration, swelling, granule rupture, and gelation increased with moisture level (Figures 20-22). Micrographs showed that the carrageenan state depended on the available water, which is determined in part by the carrageenan concentration, in part by the amount of added water, and in part by competition with muscle proteins. Swelling was lowest in the 4:1 M:P samples. At this limited moisture level, carrageenan granules began to swell, but little rupture occurred and little gelation was observed. Carrageenan swelling increased with moisture levels added to the turkey products; notable rupture and gelation (recognized by the darkening of the pink background, disappearance of intact granules, and appearance of new matrix structures) occurred only in the high moisture (6:1 M:P) systems. Even in samples with visible gelation, carrageenan appeared to be located in discrete patches between meat muscles.

C. Effects of carrageenan concentration. Gelation and development of visible structure increased with carrageenan concentration (Figures 20-22), as well as with moisture. Indeed, there seemed to be an interaction between carrageenan concentration and moisture levels: at higher levels and with more available water, more water appeared to be associated with the carrageenan, leading to increased swelling and rupture of the granules.
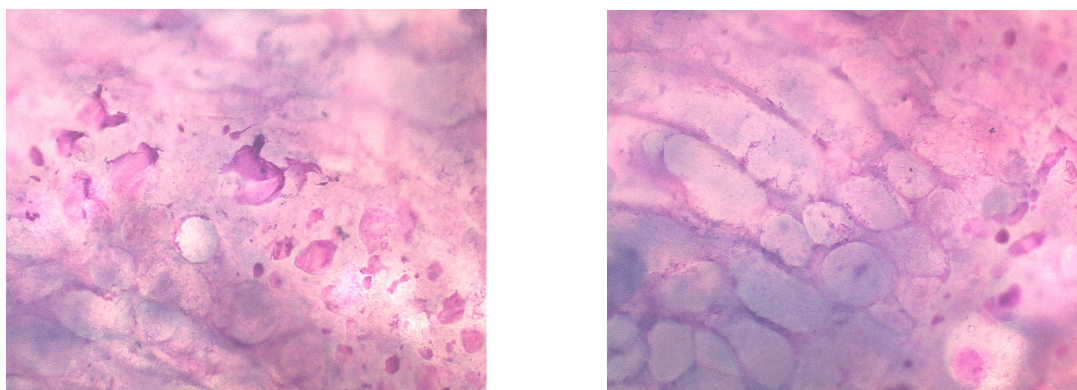
Figure 23. Carrageenan gel structures evident in turkey breast formulated with high moisture (6:1 M:P) and high carrageenan (0.6%). Both samples show hydrated and swollen carrageenan particles dispersed in a carrageenan gel matrix.

DISCUSSION

Cook yield, refrigerator purge, freeze-thaw stability, and texture characteristics all reflect a dynamic process of water binding in the roasted turkey breast deli meats. Major questions are:

1) Which biopolymer controls or dominates the water binding?

2) Do the proteins and carrageenan act in concert, either by interacting or by synergism, or do they contribute independently.to water-binding and texturization?

3) What levels of carrageenan are needed for positive effects?

4) How does carrageenan act in deli meat systems?

Cook yield, refrigerator purge, and freeze-thaw stability all showed comparable behavior relative to added water and carrageenan. Water loss increased with the amount of water added.

At the lowest moisture level (4:1 moisture:protein), moisture losses in controls were only 2% during cooking and 3.5% during refrigeration, indicating that protein alone, together with the added salts, were able to bind most of the water. Adding carrageenan had no effect on the ability of the system to hold onto water at any stage -- cooking, refrigeration, or freezing.

At the 5:1 moisture:protein level, drip loss increased at all stages; once again, adding carrageenan was unable to prevent this loss during refrigeration and frozen storage, although 0.4 and 0.6 % carrageenan did restore cook yields.

Moisture loss was greatest at the 6:1 moisture:protein level, indicating the protein and salts were no longer able to bind all the water so cooking losses increased. Under

these conditions, water binding by carrageenan became increasingly important: the more water was added, the more carrageenan was needed to bind it. Surprisingly, though, effects of carrageenan were not linear. The lowest levels of carrageenan (0.2%) were only marginally effective in reducing cooking losses, and were actually detrimental after cooking, increasing both refrigerator purge and freeze-thaw syneresis. 0.4% carrageenan maintained cook yields at control levels, but did not improve them, and this level of carrageenan had no effect on storage losses. 0.6% carrageenan was needed for noticeable improvements in water-binding.

Altogether, these results were not what we expected. Carrageenan at concentrations less than 0.5% does not seem to be effective in binding water added to meats or in limiting moisture expulsion during processing and refrigerated or frozen storage. In fact, 0.2% carrageenan may even be detrimental to water retention. Only 0.6% carrageenan was able to reduce moisture loss in any of the turkey breast formulations, and it was more effective in the high moisture systems.

What can be happening on the molecular level to explain this behavior? There is a competition for water binding in the turkey breast loaves. Without carrageenan, muscle protein is the sole polymer for water binding. Apparently, the amount of protein naturally present in turkey breast, together with added salts and phosphates, is nearly sufficient to hold all the water in the lowest moisture systems (4:1 M:P) since cook yields are 98%. However, additional water cannot be fully bound and moisture loss increases, during baking via evaporation and during storage due to syneresis. Our thinking in establishing these formulations was that low levels of added carrageenan would bind the excess water

and prevent release during cooking and storage, but at the 4:1 and 5:1 M:P levels carrageenan surprisingly had little effect.

Several explanations seem possible. At low moisture levels, the carrageenan particles may not be able to hydrate sufficiently to expand and expose water binding sites along the carrageenan molecular surface. Thus, it cannot compete effectively with muscle proteins for the water. Alternatively, the carrageenan may bind water but at low concentrations the polymer structure may be insufficient to bind and entrap the available water more than temporarily. During heating, hydrated carrageenan molecules become random coils and stretch out, binding water molecules along the molecular surface. As the product cools, carrageenan polymers hydrogen bond and form double helices that link in monomer regions to generate a gel matrix that both binds and entraps water . This explains the improvements in cook yields. During refrigerated or frozen storage, aggregation of helical regions collapses the gel structure and bound water is forced out as drip loss. When higher levels of carrageenan reduce the moisture losses, it is because carrageenan concentrations exceeding the C* value of about 0.5% (25) allow the double helices associate to form an extended gel network, as shown in Figure 5, and released water and well as unbound water becomes entrapped in spaces between linkage points (Figure 24). Even when helix aggregation occurs during chilled storage and dehydration, the gel matrix with entrapped water is able to retain more moisture. These explanations will be considered again in more detail later in this discussion.

If low concentrations of carrageenan did not markedly increase moisture retention, did they at least improve the texture characteristics, making the loaves softer (perceived as more tender) yet firmer and more cohesive, thus easier to slice?

How Does Carrageenan Act in Meat?

Low Temp ──────────────→ **Increasing Temp**

Swelling of carrageenan particles without disruption

water

Gel formation

Release of carrageenan polymers
↓
random coils
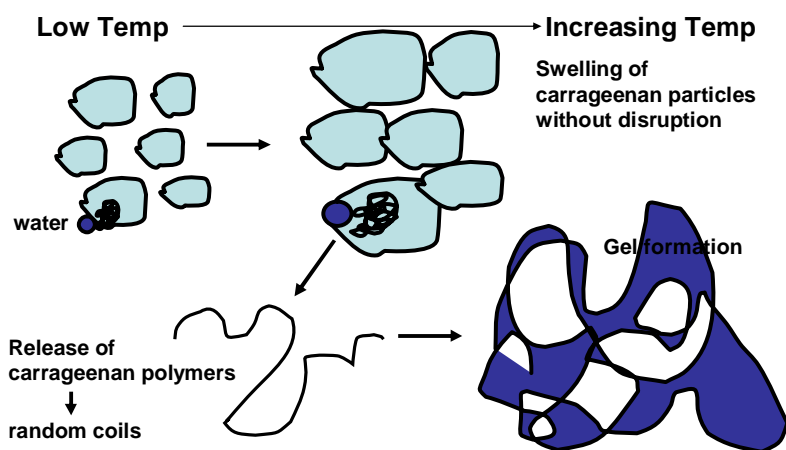
Figure 24. Schematic diagram of molecular changes in carrageenan as it is heated in moist systems.

Carrageenan could contribute to such textural changes as hydrated and swollen particles even if full solubilization and gelation does not occur (Figure 24). Total profile analysis was performed to answer this question. Since hardness and cohesiveness are properties that contribute importantly to sliceability of deli meats, these attributes were examined first.

Hardness (or firmness) values decreased with increasing moisture, as may be expected since the trapped water should soften the meat matrix. Adding carrageenan only slightly increased the hardness in dose-responsive manner. This is appropriate considering the low carrageenan concentrations. Hardness increased most in the 4:1 M:P systems and very little in the 6:1 M:P systems. This behavior could result from two effects of low moisture: 1) With limited moisture, carrageenan particles hydrate and swell forming physical blockades, but do not disrupt and form gels; 2) carrageenan polymers do fully hydrate and gel since the effective concentration is increased (same amount of carrageenan in less water) and may approach or exceed the C* value for entanglement of molecular chains (Figure 24).

Cohesiveness values also decreased with increasing moisture, but were unaffected by added carrageenan. This would suggest that carrageenan and muscle proteins are acting independently, for the most part, and that there are no carrageenan-protein interactions that would increase the coherence between phases.

Gumminess and chewiness showed a pattern of effects similar to that of hardness: decreasing with added moisture and increasing slightly in 4:1 and 5:1 M:P samples, but no effect in the high moisture (6:1 M:P samples). This likely reflects perception of

incompletely hydrated carrageenan particles and polymer matrices, since the effect disappears altogether in the high moisture products.

To learn more about how carrageenan changes in turkey breast deli meats and determine whether there are differences between hydration, swelling, rupture, and gelation of carrageenan in different formulations can account for the stability and textural characteristics of the meat products, light microscopy with methylene blue staining was used to visualize carrageenan selectively in meat matrices. This was low resolution microscopy and observations will need to be confirmed with more definitive methods. Nevertheless, clear difference in patterns of carrageenan changes as a function of moisture and carrageenan concentration were observed. These changes were consistent with fundamental gelation sequences in gums and, further, suggested that carrageenan and proteins act independently in deli meat products rather than interacting.

In Figures 20-22, following samples horizontally from left to right shows the process of carrageenan hydration, swelling, rupture, and gelation as the temperature is raised at different moisture levels. Following from top to bottom ast a given temperature shows effects of moisture. Following the same position through Figures 20, 21, and 22 shows changes associated with carrageenan concentration. At low moisture (4:1 M:P), carrageenan particles swell but little rupture and release of carrageenan monomers is seen, even at cooking temperatures. At 5:1 M:P, carrageenan particle swelling increases and at least some gelation occurs at baking temperatures (note the increased pink in the background). At 6:1 M:P the swelling and gelation is even more pronounced. These observations suggest that in the lower moisture systems, carrageenan is unable to fully hydrate and its effects therefore must be mediated promarily by its hydrated particles. In

contrast, the high moisture systems do indeed gel and some hydrated particles also remain, so carrageenan effects under these conditions involve both particles which increase solidity and full gelation which binds and entraps more water.

Although there is variation between samples, the micrographs show increasing density of carrageenan particles and also tendency to rupture and gel with concentration.

There was no evidence in any of the micrographs for carrageenan interaction or association with muscle proteins. However, high concentration regions of carrageenan may align with concentrated regions of structural proteins in muscle tissue, as shown in Figure 23. This is not surprising since there is no real basis for molecular interaction. Unless the negative charges on carrageenan are neutralized by salts, carrageenan charges would actively repel neutral and negatively charged regions on the protein, preventing interaction. In addition, muscle proteins are highly structured and compartmentally organized, even after grinding and tumbling. This, they are not very accessible for interaction with other molecules, especially other polymers.
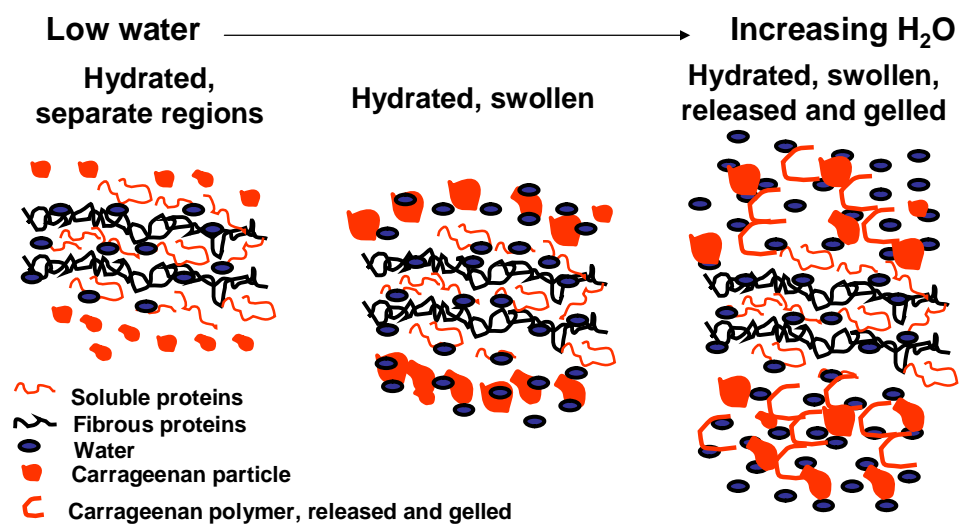
Figure 20. Proposed mechanism to explain carrageenan behavior in deli meats.

SIGNIFICANCE AND PRACTICAL APPLICATIONS

- This research confirmed the benefits kappa carrageenan in a practical application of comminuted turkey products and possibly other meat products.

- Industry standard is ~0.5% kappa carrageenan and this was confirmed by our physical observations.

- In the overall picture our research showed that our 0.6% usage rate was needed to have the most benefit with little negative effects. However, in food industry maybe a more practical approach in some instances to neglect all the freeze thaw results (many deli meat are never frozen). The benefit of adding 0.4% may be practical at high meat extensions (higher moisture protein levels) from a cost in use standpoint.

- This researched allowed us to confirm and explain the usage rate of 0.5% industry on a more molecular level something that has not been previously reported.

- This research showed the significance in method development and research with methylene blue for carrageenan applications leading to better tailoring of carrageenan products to show improvements and why.

SUMMARY AND CONCLUSIONS

To explain the physical results we need to know more about how carrageenan is acting in meats such as turkey breast. Fundamental carrageenan chemistry suggests several possibilities which we depict in figure? Carrageenan starts out as particles, aggregates with molecules of carrageenan inside. Water binds to the carrageenan molecules on the surface of the particles, and with increasing moisture and increasing temperatures the particles begin to swell. When enough water is absorbed or a high enough temperature is reached, the particles swell to the point of disruption and release individual carrageenan polymers which can then interact to form expanded gels with water trapped within the matrix when the mixture is cooled. To combine all of our findings we propose the following mechanism (figure??) to show what we think is happening to the carrageenan in meats during the cooking process. In low moisture systems, muscle proteins bind most or all of the water. Any added carrageenan has restricted hydration and shows minimal effects on system behaviors. As moisture increases the protein can no longer bind all the water so carrageenan absorbs the excess water and begins to swell. At higher moisture levels, some carrageenan particles swell to the point of bursting and release polymers that can then gel to form an extended matrix that alters textures and other properties. However, highly swollen hydrated particles are still present, and these also contribute to observed behaviors in cohesiveness and water holding capacity in cook yields.

Findings Supported Hypothesis. Our results supported my hypothesis that low concentrations of carrageenan , 0.4 and 0.6%, effectively improves water binding cook yield, and in low moisture formulations, also firmness of turkey. At low moisture,

carrageenan does not alter freeze thaw stability. In high moisture systems, 0.2 and 0.4% increase freeze that stability but 0.6% carrageenan improves freeze thaw stability. Both intact and swollen granules and released gelled carrageenan molecules contribute to the effects we observed.

FUTURE RESEARCH

To move applications of carrageenan in meats from the realm of art to solidly supported science, much research is still needed to fully understand carrageenan behavior in meat products such as turkey breast. The light microscopy used in this study provided a first look at changes in carrageenan when incorporated in processed turkey breast products, but is not definitive enough for guiding industry formulations and practices. To fully track what is happening to carrageenan on the molecular rather than tissue level in order to elucidate why carrageenan is ineffective under some conditions, high resolution microscopic methods such as scanning electron microscopy, confocal microscopy, or atomic force microscopy will be needed. Computer image analysis will be necessary to quantitate carrageenan particle size under different conditions. Because kappa carrageenan has problems with freeze-that stability, it will be important to evaluate the effect of carrageenan types i.e., iota, lambda, and combinations, as well as carrageenan particle size on water binding and stabilization in meats. Results should provide important guidance for optimizing carrageenan source materials and specifically tailoring individual meat products. Investigation of optimum moisture: carrageenan ratios for meat product would also add formulation benefits. And lastly, since protein is the major driver of product characteristics, we also need to investigate how the presence of carrageenan alters protein functional properties such as protein denaturation, elastic modulus, as well as matrix structure formed and responses to heat independently and in possible association with carrageenan.

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APPENDIX A. Formulations for Deli Turkey Breast Products

SYSTEM #1

Turkey Breast	17.64 lb				
Brine Addition	7.36 lb				
Total	25.00 lb				
Level of Extension	141.70%				
Added Ingredients	29.4%				
		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	70.57%	-		17.64	8009.88
Water	27.27%	90.37%	32822.6	6.82	3095.41
Salt	1.50%	4.97%	1805.3	0.38	170.25
STPP	0.46%	1.51%	548.8	0.11	51.76
Dextrose	0.75%	2.49%	902.6	0.19	85.13
Carrageenan	**0.20%**	**0.66%**	240.7	**0.05**	**22.70**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**5 to 1**				

SYSTEM # 2

Turkey Breast	15.20 lb				
Brine Addition	9.80 lb				
Total	25.00 lb				
Level of Extension	**164.50%**				
Added Ingredients	**39.2%**				
		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	60.79%	-		15.20	6899.70
Water	36.65%	91.73%	33315.1	9.16	4160.20
Salt	1.50%	3.75%	1363.4	0.38	170.25
STPP	0.46%	1.14%	414.5	0.11	51.76
Dextrose	0.75%	1.88%	681.7	0.19	85.13
Carrageenan	**0.60%**	**1.50%**	545.3	**0.15**	**68.10**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**6 to 1**				

SYSTEM # 3

Turkey Breast	17.64 lb				
Brine Addition	7.36 lb				
Total	25.00 lb				
Level of					
Extension	**141.70%**				
Added Ingredients	**29.4%**				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	70.57%			17.64	8009.88
Water	27.47%	91.03%	33063.3	6.87	3118.11
Salt	1.50%	4.97%	1805.3	0.38	170.25
STPP	0.46%	1.51%	548.8	0.11	51.76
Dextrose	0.75%	2.49%	902.6	0.19	85.13
Carrageenan	**0.00%**	**0.00%**	**0.0**	**0.00**	**0.00**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**5 to 1**				

SYSTEM # 4

Turkey Breast	20.83 lb				
Brine Addition	4.17 lb				
Total	25.00 lb				
Level of					
Extension	**120.00%**				
Added Ingredients	**16.7%**				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	83.33%	-		20.83	9458.33
Water	13.36%	80.16%	29115.6	3.34	1516.44
Salt	1.50%	9.00%	3268.8	0.38	170.25
STPP	0.46%	2.74%	993.7	0.11	51.76
Dextrose	0.75%	4.50%	1634.4	0.19	85.13
Carrageenan	**0.60%**	**3.60%**	1307.5	**0.15**	**68.10**
Total	100.00%	100.00%	36320.0	25.00	11350.00
M:P	**4 to 1**				

SYSTEM # 5

Turkey Breast	20.83 lb				
Brine Addition	4.17 lb				
Total	25.00 lb				
Level of Extension	120.00%				
Added Ingredients	16.7%				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	83.33%	-		20.83	9458.33
Water	13.96%	83.76%	30423.1	3.49	1584.54
Salt	1.50%	9.00%	3268.8	0.38	170.25
STPP	0.46%	2.74%	993.7	0.11	51.76
Dextrose	0.75%	4.50%	1634.4	0.19	85.13
Carrageenan	**0.00%**	0.00%	0.0	0.00	0.00
Total	100.00%	100.00%	36320.0	25.00	11350.00
M:P	**4 to 1**				

SYSTEM # 6

Turkey Breast	15.20 lb				
Brine Addition	9.80 lb				
Total	25.00 lb				
Level of Extension	**164.50%**				
Added Ingredients	**39.2%**				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	60.79%	-		15.20	6899.70
Water	37.25%	93.23%	33860.5	9.31	4228.30
Salt	1.50%	3.75%	1363.4	0.38	170.25
STPP	0.46%	1.14%	414.5	0.11	51.76
Dextrose	0.75%	1.88%	681.7	0.19	85.13
Carrageenan	**0.00%**	**0.00%**	**0.0**	**0.00**	**0.00**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**6 to 1**				

SYSTEM # 7

Turkey Breast	15.20 lb				
Brine Addition	9.80 lb				
Total	25.00 lb				
Level of Extension	**164.50%**				
Added Ingredients	**39.2%**				

			80 lbs Brine		
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	60.79%			15.20	6899.70
Water	36.85%	92.23%	33496.9	9.21	4182.90
Salt	1.50%	3.75%	1363.4	0.38	170.25
STPP	0.46%	1.14%	414.5	0.11	51.76
Dextrose	0.75%	1.88%	681.7	0.19	85.13
Carrageenan	**0.40%**	**1.00%**	363.6	**0.10**	**45.40**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**6 to 1**				

SYSTEM #8

Turkey Breast	20.83 lb				
Brine Addition	4.17 lb				
Total	25.00 lb				
Level of Extension	**120.00%**				
Added Ingredients	**16.7%**				

			80 lbs Brine		
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	83.33%	-		20.83	9458.33
Water	13.56%	81.36%	29551.4	3.39	1539.14
Salt	1.50%	9.00%	3268.8	0.38	170.25
STPP	0.46%	2.74%	993.7	0.11	51.76
Dextrose	0.75%	4.50%	1634.4	0.19	85.13
Carrageenan	**0.40%**	**2.40%**	**871.7**	**0.10**	**45.40**
Total	100.00%	100.00%	36320.0	25.00	11350.00
M:P	**4 to 1**				

SYSTEM #9

Turkey Breast	17.64 lb
Brine Addition	7.36 lb
Total	25.00 lb
Level of Extension	**141.70%**
Added Ingredients	**29.4%**

80 lbs Brine

Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	70.57%			17.64	8009.88
Water	26.87%	89.05%	32341.2	6.72	3050.01
Salt	1.50%	4.97%	1805.3	0.38	170.25
STPP	0.46%	1.51%	548.8	0.11	51.76
Dextrose	0.75%	2.49%	902.6	0.19	85.13
Carrageenan	**0.60%**	**1.99%**	**722.1**	**0.15**	**68.10**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**5 to 1**				

SYSTEM # 10

Turkey Breast	15.20 lb
Brine Addition	9.80 lb
Total	25.00 lb
Level of Extension	**164.50%**
Added Ingredients	**39.2%**

80 lbs Brine

Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	60.79%			15.20	6899.70
Water	37.05%	92.73%	33678.7	9.26	4205.60
Salt	1.50%	3.75%	1363.4	0.38	170.25
STPP	0.46%	1.14%	414.5	0.11	51.76
Dextrose	0.75%	1.88%	681.7	0.19	85.13
Carrageenan	**0.20%**	**0.50%**	**181.8**	**0.05**	**22.70**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**6 to 1**				

SYSTEM # 11

Turkey Breast	20.83 lb				
Brine Addition	4.17 lb				
Total	25.00 lb				
Level of Extension	**120.00%**				
Added Ingredients	**16.7%**				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	83.33%			20.83	9458.33
Water	13.76%	82.56%	29987.2	3.44	1561.84
Salt	1.50%	9.00%	3268.8	0.38	170.25
STPP	0.46%	2.74%	993.7	0.11	51.76
Dextrose	0.75%	4.50%	1634.4	0.19	85.13
Carrageenan	**0.20%**	**1.20%**	**435.8**	**0.05**	**22.70**
Total	100.00%	100.00%	36320.0	25.00	11350.00
M:P	**4 to 1**				

SYSTEM # 12

Turkey Breast	17.64 lb				
Brine Addition	7.36 lb				
Total	25.00 lb				
Level of Extension	**141.70%**				
Added Ingredients	**29.4%**				

		80 lbs Brine			
Ingredients	%	Percentage	Grams	Lbs	Grams
Turkey Breast	70.57%			17.64	8009.88
Water	27.07%	89.71%	32581.9	6.77	3072.71
Salt	1.50%	4.97%	1805.3	0.38	170.25
STPP	0.46%	1.51%	548.8	0.11	51.76
Dextrose	0.75%	2.49%	902.6	0.19	85.13
Carrageenan	**0.40%**	**1.33%**	**481.4**	**0.10**	**45.40**
Total	100.75%	100.00%	36320.0	25.19	11435.13
M:P	**5 to 1**				

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