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Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar gels

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Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar gels

by

Darin Richard Doerscher

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

Program of Study Committee:
Steven M. Lonergan (Major Professor)
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Joseph G. Sebranek
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Gits Prabhu

Iowa State University
Ames, Iowa
2002
Graduate College
Iowa State University

This is to certify that the master's thesis of

Darin Richard Doerscher

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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## EFFECTS OF PORK COLLAGEN ON THERMAL AND VISCOELASTIC PROPERTIES OF PURIFIED PORCINE MYOFIBRILLAR GELS

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GENERAL INTRODUCTION

Meat processors are constantly striving to improve product quality and reduce blend costs to remain competitive in a rapidly changing business environment. This has generated a need for the development of cost-effective ingredients that can add value to meat and processed meat products (Prabhu and Doerscher, 2000). The form of added value to such products includes improving product texture, increasing processing yields, or enhancing product flavor. Examples of products that can fulfill this growing demand of needs are collagen-based protein ingredients.

The effect of collagen on various quality attributes in processed meats has been well documented (Satterlee et al., 1973; Puolanne and Ruusunen, 1981; Webster et al., 1982; Jobling, 1984; Jones, 1984; Gillet, 1986; Whiting, 1989; Eilert et al., 1993; Osburn et al., 1997). These studies have shown that when processed meat products are formulated properly with collagons, some or all of the previously mentioned quality attributes can be obtained. They also indicated that collagens can have positive effects on other attributes like emulsion stability, nutritive value, and cost savings. To optimize the effect of collagen, its interaction with meat systems must be defined. Gel model systems can help achieve this goal.

Gel model systems have been used extensively for the purpose of testing various ingredients such as starches, soy proteins, whey proteins, plasma proteins, caseinates, and carrageenans (Hermansson, 1982a; Hermansson, 1982b; DeFreitas et al., 1997; McCord et al., 1998; Smyth et al., 1998; Kerry et al., 1999a; Kerry et al., 1999b; Kerry et al., 1999c). In addition, collagen’s functionality and growing popularity as an ingredient also warrants the need for it to be tested in such systems. Typically, these systems utilize meat proteins (i.e. extracted exudates, purified myofibrils, etc.) as a basis for the study so the effects (protein-
ingredient interaction) of the added ingredient are fully understood. Attributes like water-binding, protein loss, processing yields, thermal stability, and texture can be effectively measured and ultimately related to the ingredient performance in the finished processed meat application.

Thesis organization

This thesis is organized according to an alternate style format. It is arranged beginning with a general introduction, followed by a general review of literature, a publishable paper, and a concluding summary. References cited within each chapter are listed at the end of that chapter. The paper will be submitted to Meat Science.

Literature Cited


GENERAL REVIEW OF LITERATURE

Meat Proteins

Understanding the impact of the inclusion of various ingredients to a meat system is very important to processors in terms of finished product quality and blend cost. Moreover, understanding the native system that these ingredients are being applied to is of even greater importance. More specifically, knowing the chemistry of meat proteins yields the ability to effectively produce high quality processed meat products and to manipulate processing systems to your advantage. Meat proteins can be classified into discrete categories based on their cellular localization and chemistry. The three major categories are myofibrillar, sarcoplasmic, and stromal proteins.

The primary function of myofibrillar proteins in muscle is contraction. The hydrolysis of ATP to produce mechanical energy (contraction) by these proteins is one of the most basic yet important concepts of meat science. After the conversion from muscle to meat (rigor), this concept has important implications on water holding capacity (WHC) and tenderness in meat (Varnam and Sutherland, 1995; Xiong, 1994; Bandman, 1987). The major proteins of the myofibril are myosin, actin (acto-myosin in post rigor muscle), tropomyosin, troponin, the actinins, and titin. Through common processing practices of meat, these proteins can be extracted from the muscle structure with the use of salts (NaCl) and phosphates. Therefore, these NaCl soluble proteins are more readily available to interact with non-meat ingredients proposed in processed meat formulations (Lawrie, 1998; Xiong, 1994; Bandman, 1987).

Muscle pigment proteins and muscle proteases are two subdivisions of sarcoplasmic proteins that play a major role in the antemortem metabolism of muscle and its postmortem
transition into meat. Moreover, the ability of sarcoplasmic proteins to bind water is limited when compared to the functionality of myofibrillar protein. The major sarcoplasmic proteins are myoglobin, proteolytic enzymes, and glycolytic enzymes (van Laack, 1999; Lawrie, 1998; Varnam and Sutherland, 1995).

Stromal proteins are involved in many functions of living muscle. These structural proteins act as coverage of the body and to connect muscles and other organs to the skeleton and each other (Lawrie, 1998; Varnam and Sutherland, 1995; Bandman, 1987). Furthermore, this category of meat protein plays a major role in the background tenderness of meat products (Bailey, 1989; Bailey and Light, 1989). Although not a strong water binder, (as compared to myofibrillar and sarcoplasmic proteins) they play an intricate role in the WHC of meat and meat products. The two major proteins in this category are collagen and elastin. Collagen will be discussed in greater detail in the following section of this literature review.

**Collagen Types, Origin, and Use**

Collagen is a major structural component of all connective tissues including tendon, bone, cartilage, skin, vascular tissues, and basement membranes of any given mammalian species. It has been characterized biochemically, and is known that each kind of tissue contains a characteristic composition of collagen types (Lawrie, 1998; Varnam and Sutherland, 1995; Bailey, 1989; Bailey and Light, 1989; Bandman, 1987). Although there are many, most collagens fall into five types. Type I is the most prevalent and is found as the main collagenous component of bone, tendons, and skin. Type II is the major constituent of cartilage while Type III is mainly found in skin and vascular tissue. Lastly, Types IV and V are non-fibril forming and are components of basement membranes surrounding muscle cells (Lawrie, 1998; Varnam and Sutherland, 1995; Bandman, 1987; Sanes and Cheney, 1982).
The fore mentioned sources of collagen can be used in the manufacture of sausages and other meat products (Bailey, 1989; Bailey and Light, 1989). The addition of collagen to a processed meat system can influence the texture, WHC, among other quality attributes of the finished product. Furthermore, substituting portions of a meat block with rehydrated (typically 1 part collagen to 5 parts water; hydrated composition: 14.2% protein, 2% fat, and 80.3% moisture) collagen has the potential for providing cost savings in some processed meat formulations (Prabhu and Doerscher, 2000).

When collagen is utilized in processed meat products some unacceptable effects may be observed. At high levels, collagens can cause textural differences in hot dogs (Gillet, 1986). The product may have a more coarse or grainy texture. Also, upon heating, high collagen products may show signs of fattening out or gelatin on the surface (Gillet, 1986) which is aesthetically unappealing. Another area of concern is color. High levels of pork collagen can cause increased yellowing (increased Hunter b* Values) in ham products (unpublished data, Proliant Inc.). Further instances of the utilization of various types of collagens in processed meat applications will be discussed in the following section of this literature review.

**Collagen in Processed Meat Applications**

Collagen plays a major role in the texture of meat and meat products. The influence of collagen on meat product quality depends on the degree of comminution and extent of gelatinization during cooking (Whiting, 1989). Collagen also contributes to the nutritional value, flavor, and succulence of meats. Collagen from various sources has been used as an ingredient to improve water and fat retention in meat products (Webster et al., 1982; Jobling,
1984). The source of collagen and its physical form might also influence the amount of collagen that can be used in a comminuted meat product (Gillet, 1986).

Eilert et al. (1993) used modified connective tissue (MCT) from beef in meat batters to determine its effects on batter pH, emulsification temperature, collagen levels, and processing yields. As the level of MCT increased, batter pH, emulsification temperature, and collagen levels increased. Emulsification temperature did not affect batter stability as MCT was added to the system. Additionally, thermal processing yield losses were minimized (versus the control) as the addition of MCT increased. The authors concluded that MCT could be used in minimizing these losses in low-fat meat batters without changing batter stability.

Satterlee et al. (1973) added hydrolyzed beef and pork skin as a substitute for non-fat dry milk in sausage emulsions. Addition of hydrolyzed pork and beef skins resulted in greater emulsion stability and higher fat and water-binding than emulsions formulated with non-fat dry milk. Although it is not completely dissolved in the emulsion, the collagenous product, along with soluble myosin, coated fat particles during comminution resulting in a more viscous and stable emulsion (Whiting, 1989).

At low levels, collagen is effective in stabilizing shrinkage and manipulating texture. Added collagen generally increases the firmness and juiciness of frankfurters (Jones, 1984). Another beneficial use of collagen is to reduce the drip in cold cuts where the absence of reheating avoids the melting and separating of gelatin (Puolanne and Ruusunsen, 1981).

In a study by Osburn et al. (1997), water-binding potential of pork skin connective tissue (PCT) was evaluated. Heating PCT to 70°C yielded gels with increased water binding
as well as hardness. Conversely, PCT gels in bologna (10-15%) decreased hardness and increased the juiciness of the finished product.

One such commercially available pork collagen product that has these capabilities in processed meat applications is called MyoGel™ Plus (MGP). MGP is produced by Proliant Inc., Ames, Iowa (formerly AMPC, Inc.). It is a light tan coarse powder (Figure 1) that is 88.3% protein, 12.3% fat, and 1.3% moisture. This commercialized product is produced from rendered pork trimmings that are extruded, dehydrated, and milled into its key functional collagenous components (AMPC, Inc., 1998). A general schematic of the process is seen in Figure 2. Given the source of the raw material, Type I collagen is the main constituent of MGP.

Figure 1. MGP

Generally Recognized as Safe (GRAS) status has allowed approvals through USDA for MGP to be labeled as “pork collagen” as a binder and extender in non-standardized meat and poultry products where binders are permitted. Based on USDA Policy Memo 123, MGP
can be used in modified breakfast sausage, cooked sausage, and fermented sausage identified by a nutrient claim (Figure 3) and a standard or traditional name (USDA, 1995a). For example, “reduced fat frankfurter”, “low fat pepperoni”, “lean sausage” or 97% fat free kielbasa. According to USDA Policy Memo 121B, labeling of modified, substitute versions of fresh (species) sausage, hamburger, or ground beef products with added ingredients to replace fat that qualify for use of certain nutrient claims associated with fat reduction (USDA, 1995b). The nutrient claim content may be used in conjunction with the standardized name provided the consumer is informed of the actual components of the product through labeling (Figure 4). The use of MGP in a standard product without a nutrient content claim would result in a product that could no longer be called a standard or traditional name such as “hotdog” or “sausage link” (Figure 5).

Figure 2. Manufacturing Schematic of MGP
Figure 3. Labeling example of a standardized fully cooked product with a nutrient claim utilizing MGP in the formulation.

PM121B Label – For informational purposes only!

Newco Foods
Lean Beef Patties

Citic Acid, BHT, and TBHQ
Added To Protect Flavor & Freshness

Lean Ground Beef, Water, and Pork Collagen Product

Nutrition Facts
Serv. Size: 6 oz. Patty Portion (168g)
Servings: 7
Calories 160
Fat Cal. 8

*Percent Daily Values (DV) are based on a 2,000 calorie diet

Newco Foods
Lean Beef Patties

PM123 Label – For informational purposes only!

Newco Foods
97% Fat Free

BREAKFAST SAUSAGE

InGREDIENTS: BEEF, AUTOLYZED YEAST, WATER, PORK COLLAGEN*, CITRIC ACID, BHT, TBHQ. *INGREDIENT NOT IN REGULAR GROUND BEEF PATTIES.

InGREDIENTS: PORK, WATER*, CORN SYRUP SOLIDS, MAY CONTAIN LESS THAN 2% OF THE FOLLOWING: SALT, DRY MALT, SPICES, PORK COLLAGEN*, FLAVORING. *INGREDIENT IN EXCESS OF AMOUNT PERMITTED IN REGULAR BREAKFAST SAUSAGE. **INGREDIENT NOT IN REGULAR BREAKFAST SAUSAGE.

Figure 4. Labeling example of a standardized fresh product with a nutrient claim utilizing MGP in the formulation.
Figure 5. Labeling example of a standardized product without a nutrient claim utilizing MGP in the formulation

Example of proposed label using MyoGel Plus in “Hot Dog” product without a nutrient content claim. For informational purposes only!

Newco Foods
Juicy Giants
Made with Pork, Beef, and Pork Collagen.

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
<th>Amount/Serving</th>
<th>% DV* Amount/Serving</th>
<th>% DV*</th>
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<tr>
<td>Serv. Size 1 Link Portion (75g)</td>
<td>Total Fat 23g</td>
<td>35%</td>
<td>Total Carb. 1g</td>
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<tr>
<td>Servings: 6</td>
<td>Sat. Fat 5g</td>
<td>4%</td>
<td>Polyunsaturated Fat 0g</td>
</tr>
<tr>
<td>Calories 240</td>
<td>Cholesterol 11mg</td>
<td>1%</td>
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<td>Fat Cal. 200</td>
<td>Iron 4%</td>
<td></td>
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<tr>
<td>*Percent Daily Values (PDVs) are based on a 2,000 calorie diet</td>
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INGREDIENTS: PORK, WATER, BEEF, SALT, CONTAINS LESS THAN 2% OF FLAVOR, PORK COLLAGEN, CORN SYRUP, DEXTROSE, SODIUM PHOSPHATES, SODIUM NITRITE.

Amendment to Pork Collagen Labeling

The USDA has determined that pork collagen is effective in reducing purge and improving cooked yields in those meat sausages whose standards permit binders, in certain standardized cured pork products and in non-standardized meat and poultry products. USDA has also agreed that pork collagen demonstrates efficacy up to 3.5% usage in these product formulations. Thus, USDA will permit the use of pork collagen as a binder, at the specified levels, in non-standardized meat and poultry products provided the term pork collagen is in the ingredient statement in its proper order of predominance (USDA, 2001).

In order for the USDA to permit the use of pork collagen in standardized meat and poultry products, they must conduct rulemaking to amend the individual product standards. Therefore, the USDA is amending only the Standards of Identity for certain meat sausages and certain standardized cured pork products (USDA, 2001).
Pork collagen can be used in the following two categories:

1. **Non-Standardized Meat and Poultry Products** such as:

   If there is no Standard of Identity and those products allow binders, they can use.
   - Meat links or patties
   - Modified versions of traditional products such as:
     - Low-fat pork sausages, water and pork collagen product

2. **Standard of Identity Products**:

   9 CFR 319.104 Cured pork products — **cannot use in**: cooked or uncooked hams, shoulders, picnic, butts, and loins; **can use in**: water added hams only, example: ham with natural juices, ham and water product, ham-water added

   9 CFR 319.140 Sausage

   9 CFR 319.143 Breakfast sausage

   9 CFR 319.180 Frankfurter, frank, furter, hotdog, weiner, vienna, bologna, garlic bologna, knockwurst, and similar products

   9 CFR 319.181 Cheesefurters and similar products

   9 CFR 319.182 Braunschweiger and liver sausage or liverwurst

   9 CFR 319.281 Bockwurst

Pork collagen may not be used in these Standard of Identity products:

   9 CFR 319.80 Barbecued meats

   9 CFR 319.81 Roast beef parboiled and steam roasted
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<td>Canned beef</td>
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<tr>
<td>319.101</td>
<td>Corned beef brisket</td>
</tr>
<tr>
<td>319.102</td>
<td>Corned beef round and other corned beef cuts</td>
</tr>
<tr>
<td>319.103</td>
<td>Cured beef tongue</td>
</tr>
<tr>
<td>319.105</td>
<td>Ham patties, chopped ham, pressed ham, spiced ham and similar products</td>
</tr>
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<td>319.106</td>
<td>Country ham, country-style ham, dry cured ham, country pork shoulder, country-style pork shoulder, and dry cured pork shoulder</td>
</tr>
<tr>
<td>319.141</td>
<td>Fresh pork sausage</td>
</tr>
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<td>319.142</td>
<td>Fresh beef sausage</td>
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<tr>
<td>319.160</td>
<td>Uncooked smoked pork sausage</td>
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<tr>
<td>319.260</td>
<td>Luncheon meat</td>
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<td>319.261</td>
<td>Meat loaf</td>
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Measurements of Protein Functionality

Various quality attributes of meat and processed meat products rely on the functionality of protein components to interact with each other and subsequently, their ability to form gels during heating. Measurements of protein functionality can be obtained with many different types of instrumentation and analysis. In this section, the utilization of Differential Scanning Calorimetry, SDS-PAGE, Texture Profile Analysis, Rheology, Moisture and Protein Loss, and Electron Microscopy will be discussed.

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is useful for studying the thermal behavior of meat proteins and protein mixtures (Starbursvik and Martens, 1980; Findlay and Stanley, 1984). This technology offers researchers the capabilities of measuring the thermal changes and denaturation of proteins in solutions, insoluble suspensions, and pastes (Shand et al., 1994). Measuring denaturation temperatures through the use of DSC allows for the manipulation of the heat-set gelation of proteins, which is useful in the formation of gels of models systems or within processed meat products. DSC thermographs denote the onset ($T_o$), peak ($T_d$), and end ($T_f$) point temperatures of protein denaturation. These data points not only depict denaturation, but can give insight on the properties of the protein gels as well. For example, to obtain a firm gel, the temperature should be set in the region from $T_d$ to $T_f$ (Hall, 1996). Concepts of DSC can be further applied to processed meat systems. Attributes such as cook yield, texture, and purge depend heavily on the properties of the protein mixture and the thermal processing approach used for that product. Moreover, knowing what effect non-meat ingredients have on the denaturation of meat protein is important to the integrity of these attributes.
Ensor et al. (1991) utilized DSC to determine the variation in thermal properties of meat protein when an algin/calcium binder or 5-10% connective tissue was added to the mixture. DSC thermal curves of beef semimembranosus showed peaks at 58, 67, and 78°C. The inclusion of either of these products to the system increased the extent of the 58°C peak. Furthermore, the addition of increasing amounts of native or denatured connective tissues along with the algin/calcium binder resulted in an even larger extension of the 58°C peak. These shifts indicated reduced thermal destabilization of myofibrillar, sarcoplasmic, and connective tissue proteins that could influence the texture of algin/calcium restructured meat products.

Thermal denaturation of ground chicken muscle (CM), soybean protein (SP), and a mixture of both were examined with DSC while subjected to different NaCl concentrations (0-3%) (Shiga et al., 1988). Thermograph peaks of the CM decreased as the salt (NaCl) concentration increased. Conversely, the opposite was seen when increasing NaCl concentrations were introduced to the SP; the endothermic peaks increased in magnitude. When a mixture of CM and SP was subjected to increased NaCl concentrations, a similar trend was seen from each individual protein source as was seen in earlier evaluations.

Beef protein heat stability during processing of meat into a sausage batter was investigated with DSC (Quinn et al., 1980). It was found that the mechanical processing or the addition of fat meats had no effect on the $T_d$ of the muscle proteins in the system. When NaCl (0.23 and 0.67M) was added to the system, heat stability of the muscle protein was decreased. This phenomenon produces the opportunity for the utilization of lower processing temperatures to denature and coagulate muscle proteins. Ultimately, this could have positive implications on cook yields in processed meat systems. However, reaching appropriate end
point temperatures is still necessary to ensure that a safe, wholesome product is manufactured.

The extent of muscle protein denaturation of cod and herring subjected to different processing conditions were determined qualitatively through the use of DSC (Hastings et al., 1985). The simulated processing conditions were freezing and frozen storage, salting/marinading (8% salt, 4% acetic acid), and drying. Freezing followed by immediate thawing did not alter the thermal properties of the fish muscle. Nonetheless, after two weeks of frozen storage and then thawing, partial denaturation of myosin was observed. The marinade (14% NaCl solution) decreased transition temperatures by 5-10°C and $T_d$ decreased as well. This trend was observed in previous citations. Lastly, as drying (45°C for 4 hours) of the muscle protein proceeded, transition temperatures increased. This trend became more evident as drying was continued overnight. The authors concluded that this observation was due to irreversible heat denaturation during drying or there was a lack of water as a medium for thermal unfolding (Hagerdal and Martens, 1976). Therefore, water (as a medium) can aid in optimizing protein functionality during the thermal processing of processed meat products.

Changes in thermal transitions and peaks of meat myofibrillar gels with the addition of carrageenans (CGN) were observed in a study by DeFreitas et al. (1997). It was determined that the addition of up to 2% of κ, λ, or ω CGN caused minimal changes in the thermal denaturation of the meat proteins. These data suggest that no interactions occurred between the meat proteins and CGN on a molecular level.

DSC can be used to define changes of protein and protein mixtures due to the processing conditions they are subjected to. Further identification of these specific changes
can offer insight regarding how thermal processing can be customized for a particular product or ingredient.

**SDS-PAGE**

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a relatively rapid, reproducible, cost-effective method, which quantifies, compares, and characterizes proteins. Electrophoresis relies on an electrical field to separate proteins by their molecular weight. Sodium dodecyl sulfate (SDS) binds to the hydrophilic portions of the protein, thereby disrupting its folded structure so it can exist in solution in an extended conformation (Bollag et al., 1996, Laemmli, 1970). Therefore, SDS-PAGE can be used to determine changes in protein profile due to solubility, denaturation, or degradation.

Electrophoresis is normally carried out by applying a thin layer of sample to a solution stabilized by a porous matrix. Polyacrylamide gels are the most common stabilizing media used in this evaluation of proteins. The gels are usually formed as flat slabs or sheets. The choice of matrix and its concentration determine the clarity of separation in terms of the molecular weight of the proteins and their ability to move through the matrix (Hoefer Scientific Instruments, 1994). For example, a 15% acrylamide gel would have a separating resolution of 15 to 45 kd whereas a 5% acrylamide gel would have a separating resolution of 60 to 212 kd. As the acrylamide concentration increases, the ability to distinguish smaller proteins increases as well (Bollag et al., 1996).

The separated proteins can be seen upon staining. There are two common methods to achieve this. The first is a direct staining technique (of the protein bands) with Coomassie Blue R-250. The other is a negative staining system (silver staining) which stains the gel matrix surrounding the protein bands. When using either of these staining techniques final
effects are permanent; the stained protein bands cannot be further evaluated. If further research is to be conducted, other staining regimens need to be incorporated (Huff-Lonergan, 1997).

Shiga et al. (1988) used SDS-PAGE to verify protein denaturation on DSC thermograph readings. Recovered protein from heat-induced gels (70°C) prepared from ground chicken muscle were prepared for electrophoretic evaluation. The SDS-PAGE gels showed the disappearance of myosin and decreased levels of actin and tropomyosin bands. SDS-PAGE further visualized that the actin band completely disappeared as 3% NaCl was added to similarly made gels. These results accurately reflected the protein denaturation temperatures depicted by the DSC thermographs.

In another study, SDS-PAGE was used to determine if purified myofibril (MF) or homogenized whole muscle (WM) was a more effective approach for analyzing the rate of degradation of titin and nebulin (Huff-Lonergan et al., 1996). Gels from samples removed from A maturity market steers at days 0, 1, 3, 7, 14, and 28 were run to determine trends in the specified protein degradation given sample type. The results indicated that there was no difference in postmortem degradation of either titin or nebulin regardless of sample preparation. The authors concluded that both MF and WM samples can be used to effectively evaluate to rate of degradation of titin and nebulin.

Beaulieu et al. (1999) utilized SDS-PAGE to determine which protein components were contributing to aggregate formation in casein:whey (C:W) solutions heated at 95°C for five minutes. Model C:W solutions ratios of 80:20, 60:40, 40:60, 20:80, and 0:100 were evaluated. Electrophoretic gels revealed that there was extensive denaturation of whey proteins. Moreover, as the concentration of whey protein increased the insoluble portion in
the well increased also. This suggested that the whey protein was present in the heated solution as an insoluble aggregate. The SDS-PAGE observations in this study were also used to confirm reversed-phase chromatography separation results as well.

SDS-PAGE can be used to determine the protein profile of the product or mixture being evaluated. Viewing specific protein profiles in regard to various treatment effects allows determination of which proteins have been degraded or partially degraded. Moreover, this visualizes the functionality of ingredients and how they may contribute to quality attributes such as WHC or texture. SDS-PAGE may also be used to verify the results of other analytical methods (e.g. DSC).

Texture Profile Analysis

Texture Profile Analysis (TPA) was originally pioneered at the General Foods Corporation Technical Center. The technique measures (by imitating mastication) different attributes of the bite-size food piece being analyzed. These attributes are hardness, fracturability, cohesiveness, adhesiveness, springiness, gumminess, and chewiness. These attributes are visualized graphically (usually two peaks) and are defined by the variable that is being measured (force, distance, time) or is a result of mathematically combining other attributes measured (Bourne, 1982).

The following are definitions of these attributes as discussed by Bourne (1978).

- Hardness is defined as the peak force during the first compression cycle ("first bite").
- Fracturability (brittleness) is defined as the force at the first significant break in the curve.
- Cohesiveness is defined as the ratio of the positive force area during the second compression to that during the first compression.
• Adhesiveness is defined as the negative force area for the first bite, representing the work necessary to pull the compressing plunger away from the sample.

• Springiness (elasticity) is defined as the height that the sample recovers during the time that elapses between the end of the first bite and the end of the second bite.

• Gumminess is defined as the product of hardness x cohesiveness.

• Chewiness is defined as the product of gumminess x springiness (which equals hardness x cohesiveness x springiness).

Summarizing these attributes or evaluating them singly yield objective parameters for comparing food systems and their treatment effects. In heat-induced gel model systems, hardness is the main TPA attribute of interest. Hardness of a gel is indicative of its strength and how it or its ingredients can contribute to the WHC and texture of a meat application.

In a study by Hermansson (1982a), compression (hardness) and penetration (fracturability) of blood plasma gels (BPG) were evaluated. The measurements were collected using an Instron Universal Testing Machine (IUTM) compressing the gel to 40% of their original height. The hardness and fracturability of the BPG increased as the gel preparation heating temperature of increased. The optimal temperature range for these readings was 82-87°C at pH 9.0. It was also concluded that increased protein and NaCl concentrations had positive effects on texture measurements as well.

Boye et al. (1997) measured the gel strength of whey protein gels with variations in protein concentration, heating temperature, heating time, NaCl concentration, and sucrose concentration. Compressions to 50% deformation with an IUTM were implemented. Gel strength increased as protein concentration, heating temperature and time increased.
Increased NaCl concentration had positive effects on gel strength, but only below pH 5.0. Sucrose concentration had no effect on texture measurements.

Camou and Sebranek (1991), measured gel strength on thermally induced protein gels made from NaCl soluble proteins of normal and PSE pork muscle by the compression-extrusion method (Bourne, 1982). This method was incorporated with a IUTM equipped with a 35mm plunger to compress gels formed in a 45mm-diameter beaker. The hardness was recorded as the force needed to compress the gel to 20% of its original height. Gels formed from the PSE muscle were 55% weaker than control gels produced within the same parameters. The authors concluded that the decreased gel strength was due to partial protein denaturation from the PSE condition prior to protein preparation for this study. Therefore, the early post mortem denaturation of PSE pork (due to high muscle temperature and lactic acid production) can result in poor gelling characteristics of its NaCl soluble proteins components.

In heat-induced gel model systems, TPA can be used to determine the gel strength of samples being evaluated given their various treatment effects. Furthermore, this objective measurement can accurately compare how specific ingredients contribute to the texture in meat products.

*Rheology*

Rheology is the study of the manner in which materials respond (deformation) to applied stress or strain. All materials have rheological properties and it is relevant in many fields of study, including food/meat science. Understanding rheological properties of food and meat products enable researchers to optimize product development efforts, processing
methodology, and final product quality (Steffe, 1996). One specific area where rheology can play a key role is determining ingredient functionality within a food system.

Instruments for measuring rheological properties can be classified into two types: Rotational Type and Tube Type (Figure 6). Rotational Type instruments are more suitable for understanding the internal structure of materials due to their ability to operate in a controlled stress mode. This mode allows many data points to be collected over time thereby measuring yield stresses and providing insight in process improvement. Tube Type instruments are limited in this capacity (time dependant behavior) as materials being tested only pass through the apparatus once (Steffe, 1996).

Figure 6. Common rheological instruments (Steffe, 1996).

Smyth et al. (1998a) studied the gelation properties of mixed protein systems containing chicken breast salt soluble proteins (SSP) and β-lactoglobulin (β-lg) or SSP and α-
lactalbumin (α-la) using dynamic rheology. At 70°C, SSP had a greater storage modulus (G') values than mixtures containing SSP/β-lg. However, at 90°C, 80:20 and 60:40 mixtures of SSP: β-lg had higher G' values than SSP alone. This indicated that the denaturation of β-lg contributed to the formation of a firmer gel. Upon cooling to 20°C the G' values were lower for SSP/β-lg mixtures indicating that the denatured β-lg was unable to interact with SSP during cooling. Mixtures containing SSP and α-la revealed lower G' values than SSP during heating and cooling reflecting on the poor gelling capabilities of α-la.

Heat-induced gelation of myofibrillar proteins from various fast- and slow-twitch rabbit muscles was evaluated using thermal scanning rheometry (Boyer et al., 1996). Proteins from slow-twitch muscle exhibited higher thermostability and lower gel strength than those from fast-twitch muscle. At 80°C, purified myosin changed heat-gelation profiles and generally increased G', however this trend was muscle-dependant. Moreover, the heat gelling ability of slow myosin was decreased when compared to fast myosin when actin was completely eliminated from the system. The author concluded that heat-behavior of different protein fractions of various muscle types are related to the type and degree of aggregation in the gel.

Atughonu et al. (1998) evaluated the rheological characteristics of frankfurter batters formulated with non-meat proteins in place of meat protein portions. Batters formulated with either 2% sodium caseinate (SC), 2% soy protein isolate (SPI), 3.5% whey protein concentrate (WPC), or 3.5% wheat germ flour decreased G' at 80°C when compared to all-meat frankfurter batters. Despite decreases in G', the authors concluded that the selected plant and milk additives can be included in comminuted meat products without adversely affecting finished product quality.
Small strain oscillatory rheology was implemented in a series of studies by Kerry et al. (1999a, 1999b, and 1999c) to evaluate the ability of non-meat proteins and polysaccharides to influence gelation profiles of meat exudates collected from tumbled ham manufacture. Exudates were heated from 20 to 80°C at 1°C/minute, held for 30 minutes, and cooled back down to 20°C at 1°C/minute. Exudates containing WPC at 1, 2, and 3% residual powder levels, SPI at 1% residual powder levels, combinations of high gelling WPC and modified starches, pectins, or carrageenans were found to increase G’ values when compared to control values. Exudates with residual powder levels of sodium caseinate and sodium alginate decreased G’ values when compared to control values. The authors concluded that the differences in G’ profiles between control and various test exudates demonstrate how various ingredients can influence the gelling properties of reformed meats.

Rheology can be used to define how proteins and protein mixtures interact with one another in terms of heat-set gelation and gel firmness. Further identification of these gelling properties can give insight to the functionality of an ingredient as it pertains to a food or meat application.

Moisture and Protein Losses

Determination of water-binding properties of foods is usually based on the application of external forces. Application of pressure in the form of compressions, centrifugation, or suction will release the water for measurement (Oshiai-Yanagi et al., 1978). When the amount of released water in measured, water-binding is either represented as WHC, which is the amount of water bound per gram of protein (expressed gravimetrically), or as moisture loss (ML), which is the amount of juice released per gram of sample. ML can be expressed using this calculation:
ML (%) = \frac{\text{Weight of juice released}}{\text{Weight of sample}} \times 100

Within ML, there is denatured protein that is lost as well. Protein loss (PL) can be captured and further analyzed for quantity and profile. SDS-PAGE is one such form of analysis to determine which proteins were lost. The amount and type of protein lost is an indicator of how different meat blocks (e.g. PSE pork) will contribute from a functionality standpoint in a processed meat system (Camou and Sebranek, 1991). This concept is especially important when concerning available extractable protein for interaction with other ingredients or for water binding purposes.

The WHC of whey protein gels were evaluated with regard to protein concentration (10-30%), heating temperature (65-90°C), heating time (15-120 minutes), NaCl concentration (0-2M), and sucrose concentration (10-30%) (Boye et al., 1997). The gels were centrifuged and WHC was measured gravimetrically. WHC increased as protein, NaCl, and sucrose concentrations increased. The same trend was seen as heating time and temperature of the gels increased as well. Hermansson and Lucisano (1982) studied the water-binding properties of blood plasma gels (BPG) as a function of heating temperature (72-92°C), pH (7.0 and 9.0), protein concentration (0-6%), and NaCl concentration (0-5%). A net test was implemented to capture the ML from the BPG. The net test involves the gel being made in a plexiglass tube, which has means for catching the ML below. The percent ML was the lowest as the gels were heated at 75-77°C. Increasing gel pH and NaCl concentration also had positive results for ML. Protein concentrations had minimal effect on ML at various pH
points when evaluated in the BPG system. These studies give insight to ingredient functionality (whey proteins and BPG) and how they can be incorporated into a processed meat application to optimize desired finished product attributes.

Camou and Sebranek (1991) measured ML and PL in thermally induced protein gels made from NaCl soluble proteins of normal and PSE pork muscle. Low speed centrifugation was utilized to maintain gel structure while separating the moisture. ML was expressed as a percent loss from the original gel weight. ML was higher in the PSE gels (47%) versus the control gels (36%) at the same protein concentration (54mg/ml). The recovered liquid (ML) was centrifuged and evaluated for protein content (losses) by the Biuret method. SDS-PAGE was also used to characterize the proteins in the expelled liquid. At any protein concentration or heating rate evaluated, the PL was lower in the control versus PSE samples indicating that there was less protein-protein interaction in PSE samples. This observation is due to the early post mortem denaturation of PSE pork where NaCl-soluble proteins are damaged and their functionality hindered.

ML and PL are important attributes to measure in gel model systems because they are indicative of gel structure and integrity. These losses can give insight to what gel components (e.g. proteins; meat-originated or ingredient-originated) and their quantities that contribute to the gel infrastructure in terms of WHC and texture.

**Electron Microscopy**

Data compiled from the use of electron microscopy (EM) is useful in determining the relationship between the physical properties and the microstructure of protein gels. The two main types of EM used by meat scientists in research are Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) (Hall, 1996). Of these, SEM is preferred
due to easier sample preparation and more realistic imaging. Additionally, thickness of network structures are more readily determined using SEM versus TEM due to improved resolution (Yuno-Ohta et al., 1992).

McCord et al. (1998) utilized SEM to determine structural interactions between whey protein concentrate (WPC), whey protein isolate (WPI), or soy protein isolate (SPI) with NaCl-soluble muscle proteins (SSP). Micrographs showed that the SSP was the main contributor to the heat-induced microstructure of the SSP: WPC and SSP: WPI gels. The SSP: SPI combination yielded a more aggregated appearance due to the occurrence of SPI clusters throughout the gel matrix.

SEM micrographs were used to depict the functions of disulfide bonds (SS) of chicken breast muscle in terms of denaturation, aggregation, and gelation (Smyth et al., 1998b). It was determined that SS bond formation was not necessary for the gelation of myosin in this system. However, thiol groups, from protein subfragment-1, were factors in the formation of the gel network. Furthermore, the addition of non-meat ingredients may contribute similarly, thus aiding in the formation of the gel network.

In another study (Hermansson, 1982b), SEM was used to evaluate the structure of BPG and how it relates to texture and water binding. BPG were subjected to various heating temperatures (77°C, 92°C), pH levels (7.0-10.0), and NaCl (0%-5%) and protein concentrations (4%-6%). Micrographs showed that increases in protein and NaCl concentrations or decreases in pH increased the degree of aggregation of the protein gel network. This ultimately had negative effects on the texture and WHC of the BPG.

EM is ideal for viewing the infrastructure of gel model systems. EM will show what gel components (e.g. meat, ingredients, etc.) are contributing to the gel network and if there
are any specific interactions among these components. For example, an added ingredient could contributing (interacting with other proteins) to the network or it could merely be trapped with in the network. In this respect, EM will help visualize ingredient functionality.

As previously stated, protein functionality plays a major role with regard to finished product quality in meat and processed meat systems. Whether it be native meat proteins (from muscle) or added protein (from ingredients), they can affect texture, processing yields, emulsion stability, pH, purge, and appearance. Therefore, measuring their functionality gives the processor the knowledge base on how to effectively manipulate them in a meat system.

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EFFECTS OF PORK COLLAGEN ON THERMAL AND VISCOELASTIC PROPERTIES OF PURIFIED PORCINE MYOFIBRILLAR GELS

A paper to be submitted to Meat Science

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Abstract

The objective of this study was to examine the thermal and viscoelastic properties of mixed protein systems containing purified myofibrils from porcine semimembranosus (MP) and commercially available pork collagen (PC) using differential scanning calorimetry and oscillatory rheology. MP:PC mixtures (100:0, 90:10, 80:20, 70:30, 60:40, 50:50) were standardized in a 0.6M NaCl, 50mM Na phosphate buffer, pH 6.0 at a total protein concentration of 4% (w/v). One primary endothermic transition peak with a denaturation temperature of 66°C was observed for all treatments. There were no significant differences (P>0.05) observed for the temperature of onset ($T_o$), temperature of denaturation ($T_d$), and the enthalpy of transition ($\Delta H$). Storage modulus ($G'$) increased upon heating for all treatments, but the rate of gel formation and the 85°C $G'$ value were significantly lower (P<0.05) as PC was added to the mixed protein system. Upon cooling, gels revealed a significantly lower (P<0.05) rate of gel formation and significantly lower (P<0.05) $G'$ value at 5°C in samples with 20% inclusion of PC and higher. The elasticity of all gels were comparable at 85°C (~3°) and 5°C (~7°) despite the formation of a less rigid gel structure in samples containing PC. Addition of PC yielded a significant linear ($R^2=0.65; P<0.01$) increase in the water holding capacity (WHC) of the gels indicating that the matrix formed in MP:PC gels had a
greater ability to entrap water than that of the control gels. The 10% inclusion of PC resulted in gels with significantly higher (P<0.05) WHC and similar structural rigidity when compared to gels comprised of all MP.

Keywords: Pork collagen; myofibrillar protein; DSC; rheology; water holding capacity

Introduction

Myofibrillar proteins are the primary component responsible for the heat-set gel matrix formed in thermally processed meats (Siegel, Church, and Schmidt, 1979). Functional ingredients may also contribute to the gel structure, but are typically included in formulations for improving water-binding, purge, processing yield, juiciness, or even blend cost. Examples of such ingredients are soy and whey proteins (Hoogenkamp, 2001). Additionally, prior studies have shown that collagens can contribute to these quality attributes in processed meats (Satterlee, Zachariah, and Levin, 1973; Puolanne and Ruusunen, 1981; Webster, Ledward, and Lawrie, 1982; Jobling, 1984; Jones, 1984; Gillet, 1986; Whiting, 1989; Eilert, Blackmer, Mandigo, and Calkins, 1993; Osburn, Mandigo, and Eskridge, 1997). Even though past research has indicated the usefulness of these ingredients in finished processed meat systems, their specific function is better measured in model systems where experimental parameters are easier to control.

The objective of this study was to evaluate the thermal, water-binding, and viscoelastic properties of mixed gels containing different ratios of highly purified myofibrils from porcine semimembranosus and commercially available pork collagen.
Materials and Methods

Myofibril Isolation and Preparation

Myofibrils from porcine semimembranosus muscle (frozen) were purified at 4°C using differential centrifugation according to Goll, Young, and Stromer (1974). Trimmed, ground muscle (600 grams) was homogenized and washed using four volumes of a post rigor extraction buffer (100 mM Tris, 10 mM EDTA, pH 8.3) in a Waring Blender, Model No. 31BL92 (Waring Commercial, New Hartford, CT) and a Kinematica Homogenizer, Model No. 5442 (Brinkman Instruments, Inc., Westbury, NY). Samples were then centrifuged (Sorvall Legend RT, 6441 Rotor, Kendro Laboratory Products, Hanau, Germany) 1000 x g for ten minutes. The myofibrillar pellet was then re-suspended and washed three times in four volumes of a standard salt solution (SSS) (100 mM KCl, 20 mM K₂HPO₄/KH₂PO₄, 2 mM MgCl₂, 1 mM EGTA, 1 mM NaN₃, pH 7.0). Centrifugation (1000 x g) was used to collect the pellet between washes. The pellet was re-suspended and washed two times in four volumes of SSS + 1% Triton X-100 and centrifuged 1500 x g for ten minutes. The pellet was then resuspended and washed two additional times in four volumes of SSS and centrifuged 1500 x g for ten minutes. The washed myofibril pellet was then resuspended in 150 ml of 100 mM KCl, 5 mM Tris, pH 7.0. Glycerol (150 ml) was added to sustain the myofibrillar mixture at a storage temperature of −20°C before further preparation.

Myofibrils in glycerol were collected at 4°C by centrifugation (3000 x g for ten minutes). The mixture was re-suspended and washed in four volumes of 50mM sodium phosphate buffer, pH 6.0 and centrifuged 3000 x g for ten minutes. One and a half volumes of 50mM sodium phosphate, 1.2M NaCl, pH 6.0 buffer was added to the pellet and thoroughly mixed with a plastic stir rod. The ionic strength of the final solution was
equivalent to 0.6 M NaCl. Protein content (Leco, Model No. FP528, St. Joseph, MI) was then determined for sample preparation (AOAC, 2000).

Sample Preparation

Samples were prepared using the MP and pork collagen (PC) (Proliant™ P5601 MyoGel™ Plus, Proliant Inc., Ames, IA). Protein content of the PC was determined using the same methodology as the MP. Six treatments were mixed in the following ratios of MP to PC: 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50 (Figure 1). A 50mM sodium phosphate, 0.6 M NaCl, pH 6.0 buffer was used to standardize the treatments at a total protein content of 4.0%. Sodium azide (1 mM) was added to the samples as an antimicrobial agent. Treatments were stored at 4°C before further analysis.

Differential Scanning Calorimetry

Variations in thermal properties of six MP:PC treatments (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50) were measured using differential scanning calorimetry (DSC). Samples were weighed and sealed in aluminum pans. DSC was performed on an Exstar 6000, Model No. 6200 (Seiko Instruments, Inc., Japan) (Figure 2). Samples were thermally scanned from 5°C to 95°C at 10°C/minute. Samples were referenced with an empty, hermetically sealed aluminum pan.

Onset ($T_o$), peak ($T_d$), and end ($T_f$) point temperatures of significant endothermic peaks were determined and enthalpy of thermal denaturation ($\Delta H$) was calculated as the peak area. $\Delta H$ was expressed as millijoules/milligram (mj/mg) of protein. Data reported represent mean values from three replicates.
**Water Holding Capacity**

Water Holding Capacity (WHC) was measured gravimetrically (as described by Kocher and Foegeding, 1993) using thermally induced gels prepared from six MP:PC treatments (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50). Before thermal processing, weights of the filters, receivers (0.2 µm, cellulose microspin filters with 1.9-ml receivers, Lida Manufacturing Corp., Rochester, NY) (Figure 3), and sample were recorded. Samples were pipetted into the filters, placed in the receivers, and heated in a 20°C waterbath (Isotemp® Waterbath Model No. 20L-M, Fisher Scientific, Pittsburgh, PA) at 1°C/minute to a final bath temperature of 70°C. After thermal processing, cooked gel weights (in filter and receiver) (in grams) were recorded. Samples were stored at 4°C for 24 hours.

Gels were centrifuged (Sorvall Legend RT, 6441 Rotor, Kendro Laboratory Products, Hanau, Germany) at 1000 x g for ten minutes. Weight of the moisture loss captured in the receivers was recorded. WHC (%) was expressed using the following formula:

\[
\% \text{WHC} = 1 - \frac{\{(R + ML) - R\}}{\{(F + CG) - F\}} \times 100
\]

Where R was the weight of the empty receiver, ML was the weight of the moisture loss from the gel after centrifugation, F was the weight of the empty filter, and CG was the weight of the cooked gel. Data reported represent mean values from three replicates. One replicate consisted of three observations per treatment.

**Oscillatory Rheology**

The viscoelastic properties of six MP:PC treatments (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50) were measured using a controlled stress rheometer (RS150 Rheo Stress Rheometer, Thermo Haake, Madison, WI)(Figure 4) equipped with a 35-mm titanium parallel plate. Using a Peltier temperature controller, samples were heated between the two
plates (1.5mm gap, Strain 0.0025, Frequency 1.00 Hz) from 20°C to 85°C at a rate of 1°C/minute (Figure 5). Samples were held at 85°C for three minutes and then chilled to 5°C at rate of 5°C/minute. Exposed surfaces of the samples were coated with liquid paraffin (Dow Corning, Midland, MI) to prevent evaporation.

The storage modulus (G') measured in Pascals (Pa), and the phase angle (δ) measured in degrees were monitored during the heating, holding, and cooling cycles. Inflection points for the heat and cooling segments were derived by plotting the linearity of groups of individual data points (series) and noting the point at which the series intersect. Temperature at each inflection point was recorded for further analysis. Data reported represent mean values from three replicates.

Statistical Analysis

The experiment was replicated three times and results, when necessary, were verified for replication effect. Each DSC and rheology run served as a replication, while each WHC run consisted of three observations per replication in the experiment. Statistical analysis of results was performed using StatView Software Package, Version 5.0.1 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was employed to determine the significance of main effects (pork collagen). Significant differences (P<0.05) between means were identified using Fisher's PLSD. Simple linear regressions (P<0.01) were also conducted to determine additive effects of pork collagen on various attributes.
Results and Discussion

Model System Components

MP was used as the basis for the mixed protein model system because of their relatively homogeneous nature. MP is free of water soluble, sarcoplasmic proteins and water insoluble, stromal proteins that could contribute to the system from a functionality standpoint (i.e. water-binding, gelation). Using a homogeneous system is ideal for testing the efficacy of PC because experimental parameters are easier to control. Subsequently, treatment effects can also provide insight to potential protein-protein interactions.

Differential Scanning Calorimetry

One primary endothermic transition peak with a denaturation temperature \((T_d)\) of 66°C was observed for all treatments (Figure 6). In typical muscle homogenate, this peak (65-67°C) is indicative of sarcoplasmic proteins and collagen (Wright, Leach, and Wilding, 1977; Starbursvik and Martens, 1980; Findlay, Stanley, and Gullett, 1986). Since our treatment preparations were comprised of purified MP and PC, this thermal transition is attributed to the presence of PC or irrecoverable forms of connective tissue remaining after MP isolation. There were no significant differences \((P>0.05)\) for the temperature of onset \((T_o)\), \(T_d\), and enthalpy of transition \((\Delta H)\). Temperature of finish \((T_f)\) measurements yielded significant differences \((P<0.05)\), but no discernible trends were evident (Table 1).

Furthermore, there were indications of potential thermal transitions ranging from 45°C to 58°C, but the magnitude of the peak(s) appeared small and overlapping, which limited the ability to effectively calculate an \(\Delta H\).

Changes in thermal stability are good indicators of protein-polysaccharide interactions (Ensor, Sofos, and Schmidt, 1991; DeFreitas, Sebranek, Olson, and Carr, 1997).
The same concept can be suggested for protein-protein interactions (Starbursvik and Martens, 1980; Findlay and Stanley, 1984). Therefore, any changes in MP that may occur due to the addition of PC would indicate that there is an interaction. However, data collected suggest that no protein-protein interactions were evident between the MP and PC. DeFreitas et al. (1997) reported similar findings when adding carrageenans to meat protein extracts (MPE). Minimal changes in the thermal denaturation of the MPE were observed, suggesting that no interactions occurred when carrageenans were incorporated into the system.

Water Holding Capacity

A summary of WHC results is listed in Table 2. The MP:PC mixed gels had a greater WHC (P<0.05) than the control gels, comprised of MP alone. Additionally, as the ratio of PC increased in the mixed gel system, the WHC significantly increased (P<0.05) (Table 2). To further illustrate, a significantly linear effect of PC on WHC was observed (R²=0.65; P<0.01). These results indicate that the matrix formed in the MP:PC gels had a greater ability to entrap water than that of the control gels. These results confirmed reports of McCord, Smyth, and O’Neill (1998) when reviewing a similar mixed protein gel system composed of salt soluble protein from porcine semimembranosus and whey protein isolate.

Oscillatory Rheology

Changes in G’ of MP:PC mixed gels during heating from 20°C to 85°C can be divided into three distinct series by two inflection points (IP1 and IP2) (Figure 7). Initial phase shift angle (δ, in degrees) values of the mixtures were recorded (Table 3). The G’ in series 1 (20°C-IP1) remained constant, but increased markedly between IP1-IP2 (series 2), and again in series 3 (IP2-85°C) for all treatments. Despite increases in G’, significant decreases (P<0.05) in the slope of series 2 and 3 were observed as the ratio of PC increased.
in the mixed protein system (Table 4). To further illustrate, PC had a significant linear effect on the decrease of these series slopes. (Series 2 $R^2=0.99$ and Series 3 $R^2=0.92$; $P<0.01$). In addition, the temperature at IP1 significantly increased ($P<0.05$) as PC increased. Further analysis also indicated a significant linear relationship between PC and IP1 ($R^2=0.77$; $P<0.01$). Significant differences ($P<0.05$) in temperature were observed for the IP2 values, but no consistent trends were evident (Table 4). Increase in $G'$ during the heating segment, particularly series 2 and 3, was attributed to the development of the heat-set gel matrix produced by the MP (Wang, Smith, and Steffe, 1990). This was further reinforced as recorded IP2 values (~57°C) reflect typical $T_d$ for myosin (Findlay and Stanley, 1984; Ensor et al., 1991). Therefore, the reduced rate of gel formation, as evident by the slope, and lower $G'$ values of the PC added gels can be explained by the reductions (i.e. 50:50 has less MP than 90:10) of MP in the mixed protein system.

$G'$ values were recorded to denote the firmness of the gels at a typical thermal processing end point temperature for processed meats (72°C) and at the end of heating (85°C). $G'$ values were significantly lower ($P<0.05$) at these temperatures when the ratio of PC was increased in the mixed gel system (Table 5). In addition, significant linear effects of PC on $G'$ at 72°C and 85°C were recorded ($R^2=0.97$ and 0.96; $P<0.01$). Upon holding (85°C/3 minutes), no changes in $G'$ were observed, indicating that thermally induced denaturations and aggregations of the protein-protein mixture were complete (Smyth, McCord, and O'Neill, 1998). Delta ($\delta$) values at 85°C were low (~3°) for all treatments which is characteristic of a very elastic gel structure (Table 3).

On cooling from 85°C to 5°C, $G'$ increased continually in series 4 and 5 (Figure 8). The slope of series 5 significantly decreased ($P<0.05$) as the ratio of PC increased to 20%
and higher in the mixed gel system (Table 4). Moreover, a significant linear effect of PC on series 5 slope was observed ($R^2=0.88; P<0.01$). The plotting of an inflection point (IP3) derived the two series in the cooling segment for each treatment. The temperature of IP3 significantly decreased ($P<0.05$) as PC increased (Table 4). This effect also revealed a significant linear trend ($R^2=0.81; P<0.01$). $G'$ values at the end of cooling (5°C) also significantly decreased ($P<0.05$) resulting in a less rigid gel as PC (20%-50%) was incorporated into the system (Table 5). This also resulted in a significant linear effect ($R^2=0.89; P<0.01$). However, at 5°C $G'$ values and series 5 slopes of the control and 10% PC (90:10) gels were not significantly different ($P>0.05$). The elasticity of the gels were comparable (~7°) for all treatments at the end of cooling (Table 3).

As previously discussed, $G'$ values continually increased throughout the cooling segment (85°C-5°C), regardless of treatment effect. However, changes (difference and % increase) in $G'$ during this segment produced mixed results (Table 5). The 90:10 treatment revealed a significantly greater ($P<0.05$) increase in $G'$ (4418.67 Pa, 150.61%) than the control (3409.33 Pa, 102.62%) while the remaining treatments were either not significantly different ($P>0.05$) or significantly lower ($P<0.05$) than the control. The inclusion of 10% PC increased rate of gel formation ($P<0.05$) and the similar $G'$ value at the end of cooling versus the control ($P>0.05$) suggest that there may have been slight interactions between the two protein sources in the system. Similar results were reported by Kerry, Morrissey, and Buckley (1999a and 1999c) and Kerry, Stack, and Buckley (1999b). In these studies, the authors demonstrated that the inclusion of WPC, SPI, and combinations of high gelling whey protein concentrate and modified starches, pectins, or carrageenans to meat exudates increased $G'$, suggesting how non-meat ingredients can affect the gelling properties of
reformed meats. Upon further analysis of our data, the inclusion of PC (20%-50%) to the system lowered $G'$ and change in $G'$ values suggesting that no interactions occurred between the MP and PC. Smyth et al. (1998) and Atughonu, Zayas, Herald, and Harbers (1998) reported similar results in SSP/β-lg mixtures and meat batters with plant and milk additives, respectively. The authors in these studies suggested that the ingredients incorporated had no effect on the gelling properties of meat proteins.

Conclusions

Despite using highly purified MP as the basis for this model system, specific protein-protein interactions between it and PC were inconclusive when mixed MP:PC gels were evaluated with DSC and oscillatory rheology. DSC analysis revealed that thermal stability of all mixed protein treatments were comparable indicating that no interactions occurred between the two protein components. Rheological measurements revealed similar conclusions. The rate of gel formation decreased as the ratio of PC increased to 20% and higher in the system suggesting that PC did not interact with the MP and possibly that PC interrupted the heat-set gel matrix formation by the MP. However, the 90:10 treatment had a higher rate of gel formation upon cooling suggesting that an interaction may have occurred. Furthermore, gel firmness ($G'$) decreased at the end of heating and cooling segments as PC was increased in the system. This phenomenon can be attributed to the dilution effect of MP and the increased WHC in the PC added gels when compared to control gels. Ultimately, the optimum system in this study with regard to rate of gel formation, gel firmness (at 5°C), and WHC was the 90:10 treatment. The inclusion of 10% PC improved WHC while maintaining gel firmness when compared to the control gels. These results give insight to how PC may
react in a meat application. In such applications, PC could be used to manipulate the texture, control purge, or improve cook yields, all of which are beneficial in the development and continuing improvement of processed meat products.

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**Literature Cited**


Table 1. Temperature of onset ($T_o$), denaturation ($T_d$), finish ($T_f$), and enthalpy ($\Delta H$) of thermal transition of MP:PC gels.

<table>
<thead>
<tr>
<th>% Pork Collagen</th>
<th>$\Delta H$ (mJ/mg)</th>
<th>SEM</th>
<th>$T_o$</th>
<th>SEM</th>
<th>$T_d$</th>
<th>SEM</th>
<th>$T_f$</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>2.457</td>
<td>0.512</td>
<td>62.47</td>
<td>1.01</td>
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<td>71.53$^{ab}$</td>
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<tr>
<td>10</td>
<td>1.429</td>
<td>0.544</td>
<td>63.27</td>
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<td>66.37</td>
<td>0.17</td>
<td>69.30$^a$</td>
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<td>0.19</td>
<td>70.30$^a$</td>
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<tr>
<td>30</td>
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<td>0.377</td>
<td>63.43</td>
<td>0.49</td>
<td>66.47</td>
<td>0.44</td>
<td>70.17$^{a}$</td>
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</tr>
<tr>
<td>40</td>
<td>0.813</td>
<td>0.417</td>
<td>64.10</td>
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<tr>
<td>50</td>
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<td>65.77</td>
<td>0.98</td>
<td>74.20$^b$</td>
<td>1.99</td>
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Means with different letters in the same column are significantly different ($P<0.05$).
<table>
<thead>
<tr>
<th>% Pork Collagen</th>
<th>W/HC (%)</th>
<th>SEM</th>
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</thead>
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<tr>
<td>0</td>
<td>83.23d</td>
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</tr>
<tr>
<td>10</td>
<td>85.05c</td>
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<tr>
<td>20</td>
<td>94.76c</td>
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</tr>
<tr>
<td>30</td>
<td>94.94bc</td>
<td>0.16</td>
</tr>
<tr>
<td>40</td>
<td>96.61ab</td>
<td>0.28</td>
</tr>
<tr>
<td>50</td>
<td>95.77a</td>
<td>0.13</td>
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</table>

Means with different letters in the same column are significantly different (P<0.05).
Table 3. Initial, 85°C, and 5°C phase shift angle (δ) values of MP:PC gels.

<table>
<thead>
<tr>
<th>% Pork Collagen</th>
<th>δ - Initial (°)</th>
<th>SEM</th>
<th>δ - 85°C (°)</th>
<th>SEM</th>
<th>δ - 5°C (°)</th>
<th>SEM</th>
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<tr>
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<td>7.70&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
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<td>3.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
<td>7.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.09</td>
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<tr>
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<td>0.06</td>
<td>7.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15</td>
</tr>
<tr>
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<td>3.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
<td>7.28&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>5.40</td>
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<td>7.53&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
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<td>3.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
<td>7.83&lt;sup&gt;c&lt;/sup&gt;</td>
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Means with different letters in the same column are significantly different (P<0.05).
Table 4. Inflection points and slopes of heating and cooling segments of MP·PC gels.

<table>
<thead>
<tr>
<th>%Pork Collagen</th>
<th>IP1</th>
<th>SEM</th>
<th>IP2</th>
<th>SEM</th>
<th>IP3</th>
<th>SEM</th>
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<tr>
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<td>0.02</td>
<td>57.14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.48</td>
<td>43.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>43.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14</td>
<td>57.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.29</td>
<td>45.30&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>42.21&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
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<td>55.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35</td>
<td>38.07&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>57.11&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>36.66&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
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<td>0.09</td>
<td>57.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
<td>32.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.33</td>
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<table>
<thead>
<tr>
<th>%Pork Collagen</th>
<th>Series 2</th>
<th>SEM</th>
<th>Series 3</th>
<th>SEM</th>
<th>Series 5</th>
<th>SEM</th>
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<tbody>
<tr>
<td>0</td>
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<td>174.43&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>141.79&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>83.33&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>20</td>
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<tr>
<td>30</td>
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<td>17.30&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.19</td>
<td>70.67&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.61</td>
<td>33.82&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>50</td>
<td>10.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.40</td>
<td>54.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.61</td>
<td>19.69&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.26</td>
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Means with different letters in the same column are significantly different (P<0.05).
Table 5. Storage modulus (G') at 72°C, 85°C, 5°C, and change in G' from 85°C to 5°C of MP:PC gels.

<table>
<thead>
<tr>
<th>% Pork Collagen</th>
<th>Storage Modulus (Pa)</th>
<th>Change In Storage Modulus (85°C - 5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72°C</td>
<td>SEM</td>
</tr>
<tr>
<td>0</td>
<td>3273.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177.52</td>
</tr>
<tr>
<td>10</td>
<td>2702.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125.79</td>
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<tr>
<td>20</td>
<td>2189.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>88.09</td>
</tr>
<tr>
<td>30</td>
<td>1643.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>93.61</td>
</tr>
<tr>
<td>40</td>
<td>1239.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>78.32</td>
</tr>
<tr>
<td>50</td>
<td>824.40&lt;sup&gt;f&lt;/sup&gt;</td>
<td>44.37</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different (P<0.05).
Figure 1. Sample preparation of MP:PC treatments. Six treatments were mixed in the following ratios of MP to PC: 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50. A 50mM Na phosphate, 0.6 M NaCl, pH 6.0 buffer was used to standardize the treatments at a total protein content of 4.0%. Sodium azide (1 mM) was added to the treatments as an antimicrobial agent.
Figure 2. DSC was performed on an Exstar 6000, Model No. 6200 (Seiko Instruments, Inc., Japan). Samples were thermally scanned from 5°C to 95°C at 10°C/minute. Samples were referenced with an empty, hermetically sealed aluminum pan.
Figure 3. 0.2 µm, cellulose microspin filters with 1.9-ml receivers (Lida Manufacturing Corp., Rochester, NY). MP:PC treatments were pipetted into the filters, placed in the receivers, and heated in a 20°C waterbath at 1°C/minute to a final bath temperature of 70°C. Gels were centrifuged at 1000 x g for ten minutes. Weight (in grams) of the moisture loss captured in the receivers was recorded for further analysis.
Samples were heated from 20°C to 85°C at a rate of 1°C/minute. Samples were then held at 85°C for three minutes and then chilled to 5°C at rate of 5°C/minute.
Figure 5. During rheological measurement, samples were heated between the two 35mm parallel plates (1.5mm gap, Strain 0.0025, Frequency 1.00 Hz). Exposed surfaces of the samples were coated with liquid paraffin to prevent evaporation.
Figure 6. Representative DSC endotherm of MP:PC gels in 0.6M NaCl, 50mM Na phosphate buffer, pH 6.0 at a total protein concentration of 4% (w/v). Samples were heated from 5°C to 95°C at 10°C/min. Treatment plots from bottom to top are: 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50. One endothermic transition peak at 66°C is noted with the dotted line for all treatments.
Endothermic Heat Flow
Figure 7. Storage modulus (G') of MP:PC gels in 0.6M NaCl, 50mM Na phosphate buffer, pH 6.0 at a total protein concentration of 4% (w/v). Samples were heated from 20°C to 85°C at 1°C/min. Treatment plots from top to bottom are: 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50. Three series (series 1, series 2, and series 3) are denoted and differentiated by two inflection points (IP1 and IP2).
Figure 8. Storage modulus (G') of MP:PC gels in 0.6M NaCl, 50mM Na phosphate buffer, pH 6.0 at a total protein concentration of 4% (w/v). Samples were cooled from 85°C to 5°C at 5°C/min. Treatment plots from top to bottom are: 90:10, 100:0, 80:20, 70:30, 60:40, and 50:50. Two series (series 4 and series 5) are denoted and differentiated by one inflection point (IP3).
GENERAL CONCLUSIONS

In this study, observations of thermal stability, rate of gel formation, gel firmness, and WHC were recorded for gels formulated with different ratios of MP and PC. The thermal stability of all treatments was similar in profile as indicated by DSC thermographs. Only one discernable thermal transition peak (~66°C) was observed for all MP:PC gel ratios. The peak indicated the thermal denaturation of collagen protein in the system. Furthermore, consistencies in its magnitude or shifts of this peak itself could not be determined by $T_o$, $T_d$, $T_f$, and $\Delta H$ measurements. This indicated that no specific protein-protein interactions occurred between the two protein components in the system (Starbursvik and Martens, 1980; Findlay and Stanley, 1984).

Oscillatory rheology data also suggested that specific interactions were not apparent between MP and PC. The rate of gel formation upon heating and again during cooling was reduced as PC was increased in the system. Only the addition of 10% PC resulted in a higher rate of gel formation during cooling to 5°C. The decrease in rate would indicate that no interactions occurred while an increased rate would suggest that there might have been a protein-protein interaction (Kerry et al, 1999a; Kerry et al, 1999b; Kerry et al., 1999c).

Gel firmness decreased as PC was added to the mixed protein system. This was observed at the end of heating (85°C) and at the end of cooling (5°C). It was concluded the lower $G'$ values were due to the decreasing amounts of MP in the system and the increased WHC of the gels formulated with PC. The reduction of MP resulted in a lower percentage (in the system) of the protein component responsible for the formation of the heat-set gel matrix. Meanwhile, the additional water in the system (bound by PC) introduced additional stress to the heat-set gel structure produced by the MP. The combination of these two
observations suggest that an interruption may have occurred during the formation of the gel structure resulting in a less firm gel.

These observations can give tremendous insight on how PC can be effectively used in processed meat systems to improve finished product quality. In such applications, PC could be used to manipulate the texture, control purge, or improve cook yields. Jones (1984) reported that when used at low levels, collagen reduces shrinkage and improves texture. Other studies have show that collagens improve water-binding during processing (Satterlee et al, 1973; Webster et al, 1982, Jobling, 1984; and Osburn, et al., 1997) and reduce drip in cold cuts during storage (Puolanne and Ruusunsen, 1981). Additionally, processing yield losses were minimized with the addition of modified connective tissue from beef in low fat, emulsified meat products (Eilert et al., 1993). These findings, coupled with the results in our gel model system, indicate that collagens (including PC) are effective ingredients for aiding in the improvement of existing and developmental processed meat products.

**Literature Cited**


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A special thanks is also given to my wife, Cate, for her never ending love and support. My family also deserves recognition for the love and encouragement that they have extended to me throughout my academic and professional careers.