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COMPOSITION AND FUNCTIONAL PROPERTIES OF MEAT BY-PRODUCT PROTEIN FRACTIONS

By

Rodney Owen Nuckles

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ABSTRACT

COMPOSITION AND FUNCTIONAL PROPERTIES OF MEAT BY-PRODUCTS

By

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Composition and functional properties of selected meat by-products were studied. Mechanically deboned chicken meat (MDCM) and meat by-products (pork lung lobes, pork liver, beef lung lobes, beef spleen, beef heart) varied in their proximate composition, amount of the three major protein fractions [low ionic strength soluble (LIS), high ionic strength soluble (HIS), insoluble] and collagen content. Least concentration endpoint of the high ionic strength soluble (HIS) protein gels were beef semitendinosus muscle (skeletal) 3%, heart 4%, lung 5%, and spleen 6%. The addition of low ionic strength soluble (LIS) proteins to high ionic strength protein multicomponent gels initially decreased expressible moisture (EM) but reduced gel strength. The addition of insoluble (IN) proteins in multicomponent gels increased expressible moisture and reduced gel strength. Variations in microstructure may help explain the physical properties of the gels.

Beef skeletal meat frankfurter formulations were modified by heart, lungs and spleen additions. By-product protein fractions [high ionic strength soluble (HIS), low ionic strength soluble (LIS), insoluble (IN)], percentage myosin and actin:myosin ratio in formulations varied by substitution of by-products to investigate functional

properties. Myosin percentages and actin:myosin ratios positively correlated to frankfurter water and fat-holding capacity, reheat yield and strength. Less than or equal to 36% protein as LIS proteins positively correlated to water-holding capacity and negatively to other tests. Addition of less than or equal to 38% of IN proteins were not highly correlated with functional tests. Protein gelation and frankfurter strength correlated highly. Results suggest protein gelation can predict processed meats protein functionality.

This dissertation is dedicated to my parents, Darlene and Leonard Nuckles. There ability to motivate, encourage and love me has helped me be what I am today.

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I. INTRODUCTION

A. Introduction to Research

The food industry must effectively use existing traditional protein sources and search for new sources of this valuable nutrient (Gorska et al., 1988). The wastage of animal by-products which occurs by failure to utilize them at their highest value plus pollution problems created by disposal of underutilized by-products are causes for worldwide concern.

In 1985 the University of Illinois in conjunction with the National Live Stock and Meat Board conducted a survey to update information on animal by-product usage. The results are summarized as follows (Berry, 1986):

1. all by-products saved by packers are being utilized, but the variety of uses is declining

2. many inedible by-products, especially from pork, are not being saved or utilized by packers

3. many packers are not saving by-products that are useful to the pharmaceutical industry, because they are difficult to collect and store

4. the packers that are saving by-products for the pet food industry and/or pharmaceutical industry allow these companies to be the "value adders" to the by-products

5. replacement of by-products by non-meat products such as bacterial produced pharmaceuticals (insulin, etc.) and non-meat binders in pet foods is increasing

6. exporting of by-products and/or products from by-products needs to be expanded

7. blood utilization needs to be expanded and

8. in 1984-85 the value of by-products maintained live steer prices as the wholesale prices declined.

Kenney (1986) outlined several areas to improve by-product utilization, including improved packaging and presentation of by-products, use of by-products in reformed meats, texturization of proteins extracted from by-products, maximizing food uses of by-products especially spleen, lungs and tripe, and increased use of blood fractions in food products.

Many processed meat products are currently formulated using least cost formulation based on bind constants. Least cost formulation necessitates that a meat product be formulated using the least expensive meat sources which exhibit a satisfactory bind value when combined into an overall formulation (Regenstein, 1988). Saffle (1968) described the methodology on which current bind values are based. Emulsifying capacity was measured based on the formation of a true emulsion in a model system. The bind values are e emulsifying capacity of the soluble protein fraction and no measure of emulsion stability is incorporated into the values. Processed meat product production failures (greasing out, fat caps and water loss) can result when bind constants are used because emulsifying capacity and ingredient functional performance are not

highly related (Comer, 1979). Swift et al. (1961) determined that meat proteins emulsify less fat in meat products than in emulsion capacity tests. Researchers recently have suggested the use of a model protein gelation system rather than emulsion capacity may better predict protein functionality in a meat product (Comer and Dempster, 1981).

The objectives of this research were:

Study 1. To develop a knowledge base of selected meat by-product protein fractions composition and functionality to allow for the practical and effective substitution of by-products into processed meat products.

Study 2. To test equivalent gelation capabilities of by-product high ionic strength proteins and determine how the addition of low ionic strength soluble and insoluble proteins influence the texture, water-holding capacity and microstructure of high ionic strength soluble proteins from skeletal meat and by-products.

Study 3. To determine how the by-product protein fraction composition (low ionic strength soluble, high ionic strength soluble and insoluble fractions) influence textural and water-holding properties when substituted for skeletal meat in a meat model system. To determine if the data obtained from protein fraction gels predict the attributes of a model meat system.

B. Dissertation Organization

The research in this dissertation has been divided into

three studies with common sections of text arranged around them. The first section is a common review of the literature for the entire dissertation. The next section is a detailed materials and methods section in which every method used in the research is described in detail along with the study in which it was used.

The next three sections are the three studies which are the dissertation research. It should be noted that the studies are organized using the format of the Journal of Food Science. Each study contains an introduction, literature review, materials and methods and results and discussion sections. The final three sections of the dissertation are overall conclusions of the research, future research and references for the entire dissertation.

II. REVIEW OF THE LITERATURE

A. Meat By-Products

A.1. Definition

Meat by-products are commonly classified as edible or inedible (Oliveros et al., 1982). Edible meat by-products are usually referred to as variety meats. As a general definition, by-products are "everything of economic value, except for the carcass, that are incidental to the slaughter of animals" (Booren and Weiss, 1988). Meat by-products are underutilized and low priced because they are regarded as an inferior protein source compared to skeletal muscle meat (Oliveros et al., 1982). The utilization of meat by-products has been avoided due to undesirable sensory quality, low biological value of the proteins and high microbial contamination (Kosiba, 1983). Mishandling, poor sanitation and slow chilling decrease quality and increase the microbial load of meat by-products (Booren and Weiss, 1988). The increasing price of meat and processed meat products is causing the food industry to evaluate the utilization of all protein sources, including by-products (Gorska et al., 1988).

A.2. Current Food Uses

In Southeast Asia meat by-products are as prized and as valuable as carcass meat (Spooncer, 1988). Europe and South Asia both have high demand for edible by-products. The export of edible by-products from the U.S. to Europe and Southeast Asia is limited because markets in those areas

prefer chilled edible by-products, not frozen by-products. The worldwide uses of by-products as human food are outlined in Table 1.

Table 1 - World human food uses of meat by-products[a]

Organ/gland	Species	Alternative name	Use or Cooking method
Brain	All		Blanch/Fry
Heart	All		Roasted/Saute
Kidney	All		Grill
Liver	All	Lamb's Fry	Fry
Lung	All	Lites	Stuffings, Fry
Pancreas	Bovine	Gutbread	Fry
Spleen	All	Melt	Stuffings, Pates
Stomach	Porcine	Maw (Tripe)	Soups, Braise
Rumen	Ovine, Bovine	Blank (Tripe)	Soup
Reticulum	Ovine, Bovine	Honeycomb (Tripe)	Soup
Omasum	Ovine, Bovine	Bible	Soup
Abomasum	Ovine, Bovine		Soup
Testes	Ovine, Porcine	Fries	Fry
Thymus	Ovine, Porcine	Sweetbread	Fry
Tongue	All		Boil
Udder	Bovine		Sausages
Uterus	Porcine		Boil

[a] adapted from Spooncer, 1988

The most common by-products used for human food in the U.S. are listed in Table 2. In the U.S. half of poultry by-products are bought by the pet food industry (Anon., 1988).

Table 2 - U.S. human food uses for meat by-products[a]

By-product	Use
Liver	Braunschweiger
Tripe	Potted meats
Blood	Blood sausage
Pork tongues	Sulze
Heart	Sausages, luncheon
Intestines	Casings, chitterlings
Pork skin	Fried pork skins

[a] adapted from Rust and Olson, 1987

A.3. Composition and Functionality

Information on the functional and physicochemical properties of by-product proteins is extremely limited. The protein content and composition of some beef by-products has been reported (Schaefer and Schierhorn 1974; Oliveros et al., 1982; Gorska et al., 1988), but the protein fraction (sarcoplasmic, myofibrillar and stromal) content of by-products from other species is not known. Gorska et al. (1988) studied the physiochemical and histological characteristics of beef gullet meat tissue. Beef gullet meat tissue contained a low fat and high moisture content. Compared to lean beef, gullet tissue contained a higher collagen content and exhibited better water holding capacity and thus was suitable for use as a raw material in comminuted meat products.

Oliveros et al. (1982) conducted a study to determine selected physical and chemical characteristics of beef tongue, esophagus, tripe, abomasum, small and large intestines, heart, lungs, spleen, liver and kidneys. Lean beef was used as control in this study. All meat by-products had significantly lower protein content than lean beef. Spleen and liver contained significantly higher protein than the other by-products. Liver and spleen were similar to lean in proximate composition. Schaefer and Schierhorn (1974) reported that the order of protein content of selected beef by-products is liver > heart > tongue. The majority of the by-products contained lower amounts of myofibrillar proteins

and more sarcoplasmic and stroma proteins than lean meat. Liver had the highest sarcoplasmic protein content and the lowest myofibrillar and stromal protein contents. Spleen had the highest myoglobin content, lowest percent free water (expressible moisture) and lowest emulsion capacity while tripe had the highest pH. The stability of emulsions made from different by-products, except spleen, expressed as percent cooked yield was similar to the stability of the emulsion made of lean beef. Spleen had a lower cooked yield than lean meat.

Hudspeth and May (1969), Maurer et al. (1969), Maurer and Baker (1966) and Lyon and Thomson (1981) reported on the physical and functional characteristics of poultry giblets and concluded that poultry giblets could be utilized in further-processed meat products. Jimeniz-Colmenero and Cassens (1987) studied the influence of a water extract of liver on color and shelf stability of sliced bologna. The liver extract reduced product color fading and residual nitrite but did not affect the oxidative rancidity of the product. Mittal and Laurie (1982, 1985) performed extrusion studies of soy grits containing 20% or 35% of bovine or porcine offal. They concluded that the various extruded products exhibited different textural properties and that the products could be used as fillers, binders and texturizers for other food products.

B. Animal Proteins

It is known that meat or meat by-products contain 55-78% (w/w) water, 15-22% (w/w) protein, 1-15% (w/w) lipid and less than 4% carbohydrates, minerals and other organic materials. Studies on meat quality have focused on proteins which comprise the majority of the solids in meat. Goll et al. (1977) classified meat proteins on the basis of their solubility in aqueous solvents into three categories: sarcoplasmic, myofibrillar and stromal.

B.1. Sarcoplasmic Proteins

Sarcoplasmic proteins are soluble at ionic strengths of 0.1 or less and at neutral pH (Goll et al., 1977). They constitute 30-35% of total proteins in skeletal muscle (slightly more in cardiac muscle). The sarcoplasmic fraction is comprised of at least 100-200 different proteins. Sarcoplasmic proteins are found in the muscle cell cytoplasm including lysozymal enzymes, mitochondrial enzymes and myoglobin.

B.2. Myofibrillar Proteins

The myofibrillar proteins are the largest fraction of proteins in muscle tissue. The myofibrillar proteins are often defined as insoluble in water and soluble in dilute salt solutions. This classic definition has been applied to myofibrillar proteins because high ionic strength (greater than 0.3 M) is necessary to disrupt the myofibril and extract myosin, although myosin is water soluble once extracted from the myofibril (Goll et al., 1977). Ishioroshi

et al. (1983) reported that in 0.6 M KCl myosin monomers are present, whereas in 0.2 M KCl myosin is present as filaments.

Myosin and actin are the major myofibrillar proteins and constitute approximately 75% of total myofibrillar proteins. Myosin is a fibrous protein (rod) with two hydrophobic globular heads and is 1660 Å in length (Fig. 1).

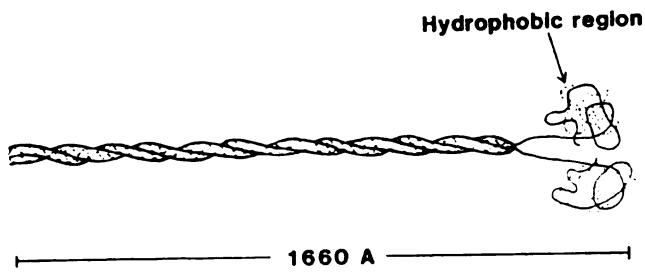


Figure 1. Schematic representation of the myosin molecule (Jones, 1984).

The myosin molecule contains six polypeptide chains (Smith et al., 1983) consisting of two heavy chains and four light chains. According to Bechtel (1986) the hydrolysis of myosin with trypsin yields light meromyosin (LMM) which is a 130 kilodalton fragment of the rod and heavy meromyosin (HMM) which is the remainder of the rod plus the two globular heads (290 kilodaltons). Heavy meromyosin contains one S1 (HMM-S1) and two S2 (HMM-S2) fragments. HMM-S1 has a molecular mass of 115 kilodaltons and each HMM-S2 fragment weighs 60 kilodaltons (Goll et al., 1977).

Actin is a monomeric globular shaped molecule (G-actin) that at monovalent salt concentrations of 50-500 mM or divalent cations (Ca^{+2} or Mg^{+2}) aggregates into a double-stranded, helical filament (F-actin).

B.3. Stromal Proteins

The stromal proteins are insoluble in water and salt solutions and are the proteins remaining after exhaustive extraction of all soluble muscle proteins. Stromal proteins are a very diverse group of proteins, but collagen and elastin constitute the majority of this fraction. Collagen is "a family of molecules" of which at least 11 types have been identified. Type III is present in muscle perimysium and plays a role in meat texture. Type III collagen is thought to be the protein formerly called reticulin. Elastin is a highly crosslinked, highly insoluble protein derived from proelastin (72,000-dalton monomer). Elastin is comparable to collagen in amino acid residues except that it does not contain hydroxylysine. The proportions of collagen and elastin vary widely among different muscles in animal species (Goll et al., 1977).

B.4. Functionality of Animal Proteins

Solubility, emulsifying capacity, gelation and water binding are the most important protein functional properties in processed meat products (Kinsella, 1982), although cooked product stability is currently not thought to rely upon protein emulsification (Regenstein, 1988). Characteristics of the soluble muscle proteins are in Table 3.

B.4.a. Water-binding

The functional water-binding property of foods has traditionally been called "water holding capacity." Jaurequi et al. (1981) proposed replacing the term "water holding capacity" with three more descriptive terms: (1) water-binding potential, (2) expressible moisture, and (3) free drip.

Water-binding potential (WBP) and expressible moisture (EM) are both defined as the ability of a protein system under the influence of an external force to hold added water. Water-binding potential is the moisture lost per unit weight of protein in the system, whereas EM is the moisture lost per unit weight of sample. Free drip is the amount of liquid lost by a protein system due to gravity.

Table 3 - Characteristics of soluble muscle proteins[a]

Property	Sarcoplasmic proteins	Myofibrillar proteins
Conformation	Globular	Fibrous
Molecular weight	Average of 80 kilodaltons	Range of 50 - 500 kilodaltons
Primary solubility	Water	0.3 M NaCl
Water-binding capacity	Very low	Very high
Viscosity	Low viscosity	High viscosity
Lipid emulsification	Slight	Extensive
Gel-forming ability	None, forms coagulum	Extensive, forms protein matrix

[a] adapted from Acton et al., (1983)

Acton and Dick (1985) defined water-binding capacity as the "ability of meat to retain its inherent tissue water as well as any water added during further processing".

Water in muscle tissues exists in three forms: bound water, immobilized water and free water (Acton and Dick, 1985). Bound water is tightly bound to the protein molecules as a surface layer about one molecule thick (monolayer). Bound water accounts for about four to five percent of water in muscle (Acton and Dick, 1985). Bound water can neither be added or removed from muscle. Immobilized water is located adjacent to the bound water and consists of two to five molecular layers. The amount of immobilized water is determined by "(1) the amount of ionization or the surface charge density of the protein's hydrophilic amino acid groups, (2) the physical forces exerted on the proteins and (3) the distance of the water location from the protein molecules" (Acton and Dick, 1985). Free water is held only by capillary action. Water added during processing is free water. Expressible moisture (EM) as measured according to Jauregui et al. (1981), using centrifugation for 15 min at 16,000 rpm, will remove some or all of the free and immobilized water and may be used to compare EM of different protein systems.

Regardless of the nomenclature, the ability of foods to retain moisture is important for several reasons: (1) economic (reduction of cooking losses), (2) organoleptic and to some extent (3) fat binding as proteins will unfold to bind moisture and fat both (Hamm 1981). Protein solubilization, as affected by moisture in the food, will enhance fat binding.

The amount of bound, immobilized and free water is affected by type of protein, salt addition (amount and type), ionic strength, pH, presence of phosphates, capillary action and product final internal temperature. Weinberg et al. (1984) tested nine salts (NaCl, NaI, $NaNO_3$, Na_2SO_4, KCl, LiCl, NH_4Cl, $MgCl_2$, $CaCl_2$) in comminuted cod muscle and concluded the EM was similar among the salts, except for $MgCl_2$ and $CaCl_2$ which had higher EM (less water binding ability). Acton et al. (1883) reported that sarcoplasmic proteins have very low water-binding potential (WBP), whereas myofibrillar proteins have very high WBP. Venegas et al. (1988) reported that meat by-products, especially spleen, had lower EM than skeletal muscle because the by-products used had a higher pH than the skeletal muscle. Oliveros et al. (1982) tested beef by-products (tongue, esophagus, tripe, abomasum, small intestine, large intestine, heart, lung, spleen, liver, kidney) and lean beef muscle for EM. They reported that spleen had lower EM than the other by-products or lean muscle. The order of EM from most to least was large intestine > tongue > small intestine > tripe > esphoagus > abomasum > lean muscle > heart > kidney > lung > liver > spleen.

Figure 2 shows the effect of pH and 2% NaCl on the water-retaining capacity (water binding capacity) of meat proteins. Meat has a higher water-retaining capacity at pH values above and below pH 5.0, the approximate isoelectric point of meat. The addition of 2% NaCl increased the water-

retaining capacity of meat as the meat pH increased. The NaCl provided ions that bound to the proteins. Proteins carrying a negative or positive electric charge will repel each other and interact with water and retain moisture (Cheftel et al., 1985).

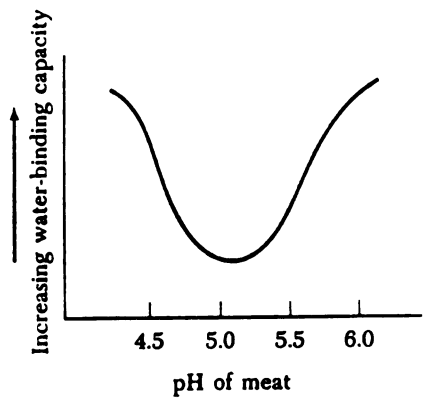


Figure 2 - Effect of muscle tissue pH on water-retaining capacity [a]

[a] adapted from J. Wismer-Pederson (1987).

Regenstein and Stamm (1979) reported that sodium pyrophosphate increased water holding capacity (WHC), whereas divalent cations decreased WHC in chicken breast muscle. Thomsen and Seuthen (1988) reported that final internal temperatures of 50-70^0 C increased WHC while temperatures greater than 70^0 C markedly decreased WHC in model pork sausages.

Several methods have been developed to determine EM or WHC in protein products. Hamm (1986) summarized many methods

used for evaluating water holding capacity or expressible moisture of meat and meat products and may be referred to for further methods. Jaurequi et al. (1981) developed a centrifugal method to measure moisture loss from protein gels or meat product samples. The method uses thimble shaped filter paper, a test tube, sample and high speed centrifugation at 31,000 x g for 15 min. After centrifugation, the moisture transferred from the sample to the filter paper is determined.

Eide et al. (1982) determined EM by placing samples in test tubes containing a platform of polyester mesh and centrifuging for 5 min at 1500 x g. After centrifugation, the moisture that flowed though the mesh to the bottom of the test tube was measured and EM determined. Hermansson (1975) used a "net test" to determine WHC. A meat plug was transferred to a net of stainless steel fastened in a steel ring. The plug, net and steel ring were placed in a test tube and centrifuged at 500 x g for 5 min. The moisture lost from the meat plug was determined and the moisture retained was calculated.

Jauregui et al. (1981) determined that EM is highly dependent upon both time and centrifugation speed with the latter being more important. An advantage to the Jauregui et al. (1981) method compared to the Eide et al. (1982) or Hermansson methods is that all the moisture is contained in a removable filter paper that can be analyzed for adhering fat or proteins. Moisture or meat residues trapped in the

polyester mesh or net is more difficult to remove by washing and may be lost. A disadvantage of high speed centrifugation is that the product being centrifuged may be torn apart or deformed and an artificially high EM may be calculated.

Wu et al. (1985) measured EM of cooked starch-fish protein gels using a Carver Laboratory press. Samples about 2 mm thick were cut from the gels, placed between Plexiglass sheets and pressed for 1 min at 1000 psi. The wet surface area and the meat film area on the Plexiglass sheets were measured and the following equation used:

$$\text{Percentage free water} = \frac{(\text{Total wet surface area} - \text{meat film area})(61.10)}{\text{Measured moisture (mg) of gel sample}}$$

The 61.10 is a constant derived from relating centrifugation and Carver Laboratory press methods.

An advantage of low speed centrifugation methods to measure EM or WHC compared to other physical methods is that it is easier to apply to weak products such as protein gels because the gel matrix will be less deformed (crushed) and the protein and moisture will be easier to separate. Other physical methods may destroy the protein gel beyond the point where protein and moisture separation are possible.

B.4.b. Emulsion capacity

Emulsion capacity (EC) is based on the weight of meat or protein in the model system:

$$\text{EC (meat basis)} = \frac{\text{ml of fat (oil) emulsified}}{\text{per unit weight of meat}}$$

EC (protein basis) = ml of fat (oil) emulsified per unit weight of protein

The methodology to measure EC is based on the original work of Swift et al. (1961). A protein solution or meat homogenate of known concentration or amount, and a specific amount of lard or oil are added to a blender. The blending process is started and more lard or oil is added until the viscosity of the mixture in the blender decreases. The amount of lard or oil added to the blender is measured and divided by the amount of protein in the blender and called emulsion capacity. Emulsion capacity is widely reported (Asghar et al., 1985; Maurer and Baker, 1966; Hudspeth and May, 1969; Galluzzo and Regenstein, 1978) even though it is currently not thought to play an important role in cooked product stability (Regenstein, 1988). Galluzzo and Regenstein (1978) developed a timed emulsification model system to define the role of specific muscle proteins in emulsification and emulsion stability, the amount of oil remaining in the emulsion after centrifugation.

The EC of a protein depends upon both its amount and solubility in a model system (Comer and Dempster, 1981). Protein solubility is one of the major factors causing differences in EC among ingredients. Gaska and Regenstein (1982) showed that myosin, actomyosin and insoluble myofibrillar proteins all were involved in emulsion formation. Emulsion capacity is currently one of the

parameters used to determine least cost formulation of meat products. Comer and Dempster (1981) found that EC did not predict bind values and proposed that "protein quality factors" be used to predict bind values. Protein quality factors may include protein gelation tests or other protein functional property tests that are representative of ingredient gelation properties. Research is improving EC data used in least cost formulation and/or will replace EC with more appropriate methods.

Myofibrillar proteins are capable of emulsifying fats, while sarcoplasmic proteins only have slight emulsification properties (Acton et al., 1983). Asghar et al. (1985) reported the EC of different proteins as: myosin > actomyosin > sarcoplasmic proteins > actin (in 0.3 M NaCl solution). Maurer and Baker (1966) reported that poultry meats with high levels of collagen had low EC. Skin and gizzard containing large amounts of connective tissue were inferior in EC compared to muscle tissue (Hudspeth and May, 1969). Venegas et al. (1988) reported that lean skeletal meat was better able to emulsify and retain fat compared to beef by-products. They reported that by-products low in collagen stabilized fat when cooked at low temperatures (70^0 C) and by-products high in collagen cooked to high temperatures (100^0 C) stabilized fat. Oliveros et al. (1982) measured EC (based on Swift's methodology) of lean beef and beef by-products and reported that by-products high in myofibrillar proteins and low in stromal proteins and fat,

emulsified the most corn oil.

Hydrophilic and lipophilic properties of proteins have been used to characterize properties of surfactants and emulsifiers. Based on the work by Griffin (1949), DeKanterewicz et al. (1987) developed an index to describe the relative hydrophilic-lipophilic character of proteins for use in predicting their emulsifying properties of corn oil. They found that the EC of a protein depends on both its hydrophilic and lipophilic characteristics rather than each independently. Myofibrillar proteins exhibit both fat emulsification (lipophilic interaction) and water-holding capacity (hydrophilic interactions).

B.4.c. Gelation

Van Kleef (1986) defined heat induced gelation of proteins as a denaturation and aggregation process. A change from the native protein conformation to a more random, unfolded structure is denaturation, whereas aggregation is the interaction between proteins that leads to the formation of very large crosslinked entities. When the protein concentration is sufficiently high and heat is applied, the large protein entities eventually precipitate.

Physical and chemical stabilization of fat and water in comminuted meat products is caused by heat-induced gelation of muscle proteins (Ziegler and Acton, 1984). Lee (1985) reasoned that fat is stabilized through physical entrapment in a protein matrix instead of via emulsification because: (1) products emulsified, stuffed into casings and cooked

(frankfurters) stabilized less fat than coarse ground, stuffed, cooked products (2) during emulsification in a bowl chopper a meat batter loses its stability when the fat reaches its melting temperature, whereas when cooked, actomyosin and melted fat produced a stable product, (3) after prolonged emulsification, meat batter instability due to fat loss is reversed by cooling the batter below the fat melting temperature, (4) a stable meat emulsion was produced with fat, but not with oil, (5) some proteins that exhibit good EC failed to produce a stable meat product matrix, and (6) synthetic emulsifiers added to meat batters reduced fat stabilization of the meat batter.

The gelation of muscle proteins chemically or physically entrap fat and moisture (Foegeding and Lanier, 1987). Gels can be formed from a single type of polypeptide (Foegeding, 1988) or from complex systems. Tolstoguzov and Braudo (1983) defined gelation of complex systems such as frankfurters or other comminuted meats as multicomponent gels. They defined three types of multicomponent gels; filled, mixed and complex. Filled gels are formed when one protein forms the gel matrix and another macromolecule fills the interstitial spaces. Mixed gels result when at least two macromolecules gel independently and form three dimensional networks without interacting. Interactions between different types of macromolecules lead to the formation of complex gels.

Acton and Ziegler (1983) reported that myofibrillar proteins have excellent gel forming abilities and form

extensive protein matricies, whereas sarcoplasmic proteins form coagulums and do not posses gel forming abilities. Foegeding and Lanier (1987) also reported that fish sarcoplasmic proteins exhibited very poor gelation capabilities (formed a coagulum) and interfered with the gelation of myofibrillar proteins.

The myofibrillar protein most responsible for gelation is myosin (Schmidt et al., 1981). Myosin forms a heat-induced gel that is irreversible, has high water-binding capacity and strong elastic properties. Siegel and Schmidt (1979) studied heat-induced gelation of myosin and found that gelation was dependent upon an intact myosin heavy chain (tail). Whiting (1988) described protein gelation as "disulfide bond formation between myosin heavy chains at 30^0 to 50^0 C and followed by hydrophobic interactions and aggregation at 50^0 to 70^0 C of the remaining parts of the molecule". Yasui et al. (1982) reported that F-actin enhances the gelation of myosin by forming F-actomyosin which acts as a crosslinker between the tail portion of bound and free myosin molecules. Maximum gel strength was in 0.6 M KCl, pH 6.0 and at a myosin to F-actin ratio of 2.7:1 which corresponds to a weight ratio of 15:1. Gelation between myosin molecules (monomers in 0.6 M KCL) was dependent upon the tail (rod) section (Ishioroshi et al., 1983). Myosin filaments in 0.2 M KCl gelled by head-head aggregations. Samejima et al. (1984) reported that myosin heavy chains (MHC) formed gels almost equal in strength to

intact myosin. They also reported that myosin light chains (MLC) do not contribute to MHC gelling and actin weakened the MHC gel. The MLC/actin complex as actomyosin may provide some gel stability at a pH other than 6.0.

Cardiac myosin, as reported by Samejima et al. (1985), formed a stronger gel than skeletal muscle myosin at pH 5.5, 0.1 M KCl and 60^0 C final temperature. Asghar et al. (1984) reported that under identical situations (pH, ionic strength, protein concentration, temperature) myosin from broiler white fiber muscles formed stronger gels than myosin from red fiber muscles. They related different gelling abilities of myosin from red and white muscles to myosin isoforms. Wagner and Anon (1985) studied bovine muscle depleted of sarcoplasmic proteins and connective tissue using differential scanning calorimetry (DSC). They reported that myosin was more sensitive to heat (totally denatured at 57^0 C) and low pH (5.4 - 5.6) than actin.

Sato and Nakayama (1970) found that myofibrillar protein content and gelation were related to binding quality of minced meats. Whiting (1988) stated that successful gelation requires muscle and fat cell disruption, fat particle suspension, actin and myosin disassociation and the prevention of actomyosin formation. Myosin also forms complex gels with fibrinogen (Foegeding et al., 1987) and filled gels with starch (Foegeding and Lanier, 1987).

Insoluble proteins have been reported to be both beneficial and a hinderance to myofibrillar protein gelation

in processed meat products. Puolanne and Ruusunen (1981) studied the effect of cooking temperature on gel water-holding capacity and strength of meat batters containing 5% and 15% connective tissue, respectively. They reported that at 60^0 C, connective tissue swelled, gelled and bound water, but cooking to higher temperatures resulted in greater water loss in the high connective tissue batter.

Schmidt (1987) reported an increase in emulsion stability when connective tissue proteins were added and the emulsion was heated to a sufficient temperature to gel the connect tissue proteins.

C. Gel Evaluation

Observation of gel microstructure can help interpret gel textural properties (Sone et al., 1983). Gel microstructure is determined by protein chemical composition and interactions, therefore understanding the microstructure will promote understanding of protein functionality. Microstructural characteristics have been shown to be related to soybean protein gel hardness (Furukawa, 1978) and milk gel high stress relaxation or hardness (Kalab and Harwalker, 1974).

C.1. Textural Properties

Several empirical methods exist for the testing of gel strength of meat batters or meat products. The methods include torsion failure tests (Montejano et al., 1983), texture profile analysis (TPA) (Bourne 1978), 70% compression (Lee et al., 1987), back extrusion (Hickson et

al., 1982), fold test (Kudo et al., (1973), tube viscometry (Toledo et al., 1977) and tensile strength (Trout and Schmidt, 1984). In this research, protein gel compression and frankfurter compression and tensile strengths were measured, thus they will be discussed.

Compression (70%) testing utilizes a compression cell which is composed of parallel plates between which protein gels or meat cores can be compressed. Apparent stress at failure and apparent strain at failure can be calculated (Diehl et al., 1979). True stress and strain cannot be calculated because a uniform cylinder is not maintained during compression. Strain is highly correlated to sensory and texture profile analysis (TPA) cohesiveness, while stress to failure (compressive force to failure) is correlated to TPA hardness and sensory firmness (Montejano et al., 1985).

McKeith et al. (1988) used compression to evaluate surimi produced from pork, beef, beef by-products and commercial fish surimi. They reported that the compressive force required to crush the surimi produced from pork, beef and beef heart was similar or stronger than the compressive force required to crush commercial fish surimi. They concluded that surimi-like gels could be successfully produced from pork, beef and beef heart. Van Kleef (1986) studied the fracture behavior of egg ovalbumin gels and soybean protein gels using compression. He reported that the stress at fracture (hardness) of the ovalbumin gels was

greater than the soybean protein gels. He explained the hardness of the ovalbumin gels was greater because of covalent crosslinkages in the ovalbumin gels and that the ovalbumin gels formed from dissolved proteins, whereas the soybean protein gels contained dispersed, not well dissolved proteins. Morris et al. (1980) tested iota and kappa carrageenan gels produced with the Hofmeister series of cations using compression to measure yield stress. They reported that the use of Rb^+ or K^+ helped form stronger gels which they related to the large size of the cations. Randall and Voisey (1977) used compression to measure firmness of meat and non-meat (soy protein isolate or whey protein concentrate) emulsions containing various concentrations of salt soluble proteins (SSP) and/or connective tissue proteins (CT). They reported that SSP and CT added firmness to the emulsions.

Tensile strength measures the force per cross-sectional area or force per unit weight of sample to tear apart cooked meat products. Tensile strength was used to measure the binding strength of chunked and formed products, but currently is also applied to comminuted meat products (Trout and Schmidt, 1987). Tensile strength can be measured using an Instron Universal Testing Machine or apparent tensile strength can be measured using machines such as the Food Technology Corporation's Texturerecorder. The advantage of the texturerecorder is that it is less costly to purchase than the Instron.

Gillett et al. (1978) used the Instron Universal Testing Machine to test tensile strength of ham slices containing various concentrations of salt, phosphate and/or nonfat dry milk. They concluded that tensile strength successfully measured the increase adhesion of the ham pieces due to the effect of increased concentrations of salt, phosphate and/or nonfat dry milk upon the meat proteins. Bouton et al. (1975) investigated the relationship between tensile strength and the percentage of myofibrillar or connective tissue proteins in processed meats. They reported that the myofibrillar proteins produced the initial tensile strength reading (increase in force to tear apart the meat) and that connective tissue proteins produced the peak force of the tensile strength reading. Trout and Schmidt (1987) used tensile strength to measure the effects of ionic strength, pH, pyrophosphate and cooking temperature on beef muscle homogenates (beef ground through 2.5, 1.5 and 0.5 cm plates). Maximum tensile strength was produced at 66^0 C for treatments with no cooking loss. Cooking loss from treatments caused tensile strength to increase linearly with increased temperature. Overall, tensile strength was maximum at 66^0 C, pH 6.0, ionic strength > 0.25 and 0.31% pyrophosphate.

C.2. Scanning Electron Microscopy of Gels

Lee (1985) studied fat stabilization in meat emulsions via light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). He found

that LM was good for evaluation of gross structure and fat dispersion patterns, whereas SEM and TEM were appropriate for assessing the interactions between the gel matrix and fat globules. Sone et al. (1983) reported that in most cases SEM is used to observe the protein gel microstructure and that freeze drying, glutaraldehyde fixation followed by critical point drying, and cryo-SEM are useful tools in food research. In some cases the use of osmium tetroxide may be added to help fix the fat in a meat emulsion (Ray et al., 1979).

Oelker (1988) used SEM to study the structure of dry sausage. He found that collagen fibril and microfibrils and myofibrillar and sarcoplasmic proteins are all essential to form the structure of a dry sausage. Bernal and Stanley (1986) examined a commercial restructured beef product using SEM and found that the distinctive elements of intact beef muscle (muscle fibers surrounded by connective tissue and grouped into fasciculi) were not apparent, showing that restructuring had a severe effect on the microstructure of the product. The surfaces of the restructured particles were covered by a protein film, due to protein being extracted during restructuring operations. Hermansson (1986) using SEM found that cooking of protein, fat and water mixtures above their gelation point caused random aggregation of proteins rather than an ordered three dimensional matrix being formed and increased fat and water loss. Comer and Allan-Wojtas (1988) studied the role of starch fillers in comminuted meat

products using LM and SEM. Light microscopy revealed that the "all-meat" wieners had a higher degree of fat agglomeration than did the more stable wieners containing added starch fillers. Electron microscopy revealed that the starch granules helped to physically entrap fat globules. Jones and Mandigo (1982) studied the effects of chopping temperature on the microstructure of meat emulsions. The use of SEM revealed that meat emulsion stability is related to two functions, the interfacial protein film thickness around the fat globules and the ability of the surrounding emulsion to maintain the stability of the interfacial protein film during cooking. Ray et al. (1979) developed methodology to identify the protein and fat component in scanning electron micrographs by fixing two different samples of a cooked meat emulsion (sample 1 and sample 2). For sample 1 they used glutaraldehyde to fix the protein and ether to extract the fat, for sample 2 they fixed the cooked meat emulsion using osmium tetroxide (fixes both protein and fat). They viewed and compared the sample to identify the protein and fat components. Smith and Brekke (1985) used SEM to view myofibril gels produced from enzymatically modified mechanically deboned fowl proteins. Gel ultrastructure and functional properties were related by assessing the scanning electron micrographs. The control gels were compact and globular and exhibited high gel strength and gel syneresis. Gels produced from myofibrils modified to 18% solubility were fibrous and exhibited high gel strength and water

holding ability. Clarke et al. (1988) studied the effect of algin/calcium, pH and muscle proteins on gelation and microstructure of gels and restructured beef. They studied microstructures of sarcoplasmic proteins, myofibrillar proteins and restructured beef with added algin and calcium. At the same pH, myofibrillar/algin gels were more fibrous in structure than sarcoplasmic/algin gels. Low pH (3.9) did not alter the sarcoplasmic/algin gel structures, whereas the myofibrillar/algin gels were more globular. The addition of algin to the restructured beef visibly added globular structures (algin) to the beef fibrils. Sone et al. (1983) reported that meat product microstructures viewed by SEM are related to gel hardness and firmness.

D. Applications

D.1. Formed Meat Chunks

The pet food industry in the U.S. and both the pet and human food industry in Canada use a technique to restructure meat. The meat products are called formed meat chunks (FMC). Formed meat chunks are manufactured using non-meat binders (mainly wheat gluten), because the meats do not contain enough myofibrillar proteins for binding. Formed meat chunks are cooked by high temperature water bath techniques or high temperature/pressure cooking.

Myofibrillar proteins, mainly myosin, are responsible for the binding of restructured meats and FMC (King and MacFarlane, 1987). As the sliced or flaked meats are mixed with salt and phosphates, myosin is extracted to the meat

surface and forms a sticky (tacky) layer. The meat is pressed together and upon heating the proteins are denatured and bind the meat pieces together.

Factors that can influence the extraction of myofibrillar proteins include the following: (1) the state of rigor development; (2) the ionic environment and the pH of the system; (3) the temperature history of the meat during rigor onset; (4) the temperature during extraction; (5) storage of meat; (6) application of high pressure; and (7) miscellaneous physical factors such as the duration of extraction, the degree of agitation of the system (e.g., by mixing or tumbling), the size of the meat particles, and application of vacuum (vacuum mixing).

A better understanding of protein functionality will allow the use of meat by-products and/or meat by-product proteins in traditional restructured meats or FMC.

III. Material and Methods

A. Sources of Meats and Meat By-Products

Frozen mechanically deboned chicken meat (MDCM) prepared from the whole carcass (Study 1) was purchased from Nottawa Gardens Corp., Athens, MI. Beef skeletal muscle used in Study 2 and 3 was purchased from Monfort of Colorado Inc., Greeley, CO. By-products [pork lung lobes, pork liver, beef lung lobes, beef spleen, beef heart (Study 1)] and [beef lung lobes, beef spleen, beef heart (Studies 2, 3)] were obtained from market weight hogs and steers slaughtered at the Michigan State University Meat Laboratory. Additional beef spleen and beef fat (Study 3) were obtained from Ada Beef Co., Ada, MI. Pork backfat (Study 1) was purchased from a local grocery store. All meats, by-products and fats were stored vacuum packaged (2.5 mil polyethylene) and frozen until used within three months.

B. Analytical Methods

B.1. Compositional Analysis (Studies 1, 2 and 3)

B.1.a. Protein

The protein content of the meats, meat by-products, protein fractions and raw and cooked meat batters was determined using AOAC Method 24.038 - 24.040 Crude Protein in Meat; Block Digestion Method (AOAC, 1984). The following modifications were made in the Block Digestion Method: a sample weight sufficient to require approximately 8 mL of HCl acid to titrate the boric acid/nitrogen mixture to its

original pH, 5 mL instead of 15 mL H_2SO_4 were used and no H_2O_2 (instead of 3 ml) was added to the sample. All protein determinations were performed in triplicate and the protein content was determined on a wet weight basis using a nitrogen conversion factor of 6.25.

B.1.b. Moisture

Moisture content of the meats, meat by-products and raw and cooked meat batters was determined using AOAC Method 24.003, Moisture in Meat (AOAC, 1984). Moisture contents were determined in triplicate.

B.1.c. Fat

Fat content of the meats, meat by-products and raw and cooked meat batters was determined using AOAC Method 24.005. Fat contents were determined in triplicate.

C. Electrophoresis of Protein Fractions (Study 1)

Sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS-PAGE) of high ionic strength (HIS) proteins and insoluble (IN) proteins was performed on 12% (0.25% bis) acrylamide running gels with a 4% stacking gels using the system of Laemmli (1970).

The HIS proteins were prepared for electrophoresis by adding 9 ml of protein solution, 1 ml 10% sodium dodecyl sulfate (SDS) and 3 ml of beta mercaptoethanol (BME) to a screw top test tube. The capped test tube was heated in 95° C water for 10 min. After heating, the protein mixture was dialyzed for 18 hr with one change of dialysis buffer. The dialysis buffer was 25 mM Tris - HCl, 0.2% SDS, 0.2 mM

ethylenediamine tetraacetic acid (EDTA) and pH 7.25. After dialysis, 20% w/w glycerol and one grain (granule) of bromophenol blue were added to the protein solution. The insoluble proteins were solubilized for electrophoresis by adding a 9:1 (w/w) ratio of insoluble proteins and solubilization buffer to screw top test tubes. The solubilization buffer was 2.5% SDS, 4M urea, 0.5M Na phosphate and pH 7.2. Three drops of BME were added to each test tube, the tubes were capped and cooked for 2 hr in a 50° C water bath. After 2 hr, the test tubes were boiled in water for 10 min. The liquid in the test tubes was decanted into dialysis tubing and dialyzed as discussed for the HIS proteins.

Electrophoresis was performed with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Heathkit Model 1P-17, Benton Harbor, MI). One hundred micrograms of the HIS protein fraction and 200 ug of the IN protein solution were put on the stacking gel. A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased to 60 mA until the bromophenol blue tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 hr in 0.125% Coomassie Blue in 50/10/40 (v/v/v) methanol/acetic acid/water. The gels were destained in 7/5/88 (v/v/v) acetic acid/ methanol/ water until the protein bands were clearly visible.

Subunit molecular weights were estimated using a mixture of high molecular weight proteins MW-SDS-200 Kit; Sigma Chemical Corp., St. Louis, MO). The mixture consisted of the following proteins: carbonic anhydrase (29 kilodalton), egg albumin (45 kilodalton), bovine plasma albumin (66 kilodalton), phosphorylase B subunit (97.4 kilodalton), B-galactosidase subunit (116 kilodalton) and rabbit muscle myosin subunit (205 kilodalton).

The relative mobility (RM) of the protein standards was calculated using the formula:

$$RM = \frac{\text{Distance of Protein Migration (cm)}}{\text{Marker Dye Distance (cm)}}$$

and a plot of relative mobility vs log molecular weight was constructed. The relative mobility of each protein subunit was calculated and the molecular weight was estimated from the standard curve. The amount of protein present in each protein band on the gels was determined using a Shimadzu Dual Wavelength Thin-Layer Chromato Scanner (Model CS-930, Kyoto, Japan). The protein bands were identified by their subunit molecular weights. The percentage of myosin and actin was calculated by dividing the amount of myosin or actin by the amount of total protein present on the gel. Actin:myosin mole ratio was calculated (Goll 1977; Beas et al., 1988) by dividing the total amount of actin by its molecular weight (41.785 kilodaltons) and the total myosin

by its molecular weight (475 kilodaltons) and then calculating the ratio of the moles of actin to the moles of myosin.

D. Timed Emulsification (Study 1)

Timed emulsification was developed by Galluzzo and Regenstein (1978) to measure the ability of proteins, singly or in mixtures at 5 mg protein/ml in 0.6 M NaCl, to form an emulsion or cream phase when emulsified with 5 ml of corn oil. The emulsion or cream phase was formed using a Sorvall Omni-mixer (Ivan Sorvall, Inc., Norwalk, CT) equipped with an adapter ring for 50 ml clear polycarbonate tubes. A 3 ml protein sample was pipeted into the 50 ml clear polycarbonate tube, overlaid with 6 ml of corn oil and emulsified for 5 min. The emulsified samples were centrifuged at 30,000 x g for 15 min to determine stability (ability to hold the oil) of the emulsion.

In this study, timed emulsification was performed with modifications because an emulsion was not formed using the recommended protein concentration of 5 mg/ml. Mechanically deboned chicken meat and by-products were evaluated in triplicate at total protein concentrations of 10 to 150 mg/mL. The lowest concentrations of protein required to produce an emulsion after centrifugation at 30,000 x g for 15 min was recorded and used as an index of protein functionality.

E. Extraction (Studies 1 and 2) and Quantitation (Study 1) of Protein Fractions

All extraction procedures were performed in a 4° C cold room. A weighed portion of MDCM or by-product was blended (Waring Blendor, Model 1120, Winsted, CT) for 1 min with 4 volumes of 0.05M Na phosphate buffer, pH 7.4, stirred for 3 hr (Talboy T-Line Laboratory Stirrer, Model 107, Emerson, NJ) in a cold room and centrifuged at 23,000 x g for 15 min. The supernatant was saved and the residue re-extracted for 1 hr. Following centrifugation, the supernatants containing the 0.05M Na phosphate soluble proteins were combined, designated as low ionic strength soluble (LIS) proteins and quantitated. The precipitate was mixed with 4 volumes of 0.6M NaCl, 0.05M Na phosphate buffer, pH 7.4 and extracted twice as described previously. The supernatants, designated as high ionic strength soluble (HIS) proteins, were combined and quantitated. The precipitate was designated insoluble (IN) proteins and weighed. The protein content of each fraction was determined by Kjeldahl procedures (AOAC, 1984). Non-protein nitrogen was measured on the low ionic strength soluble proteins according to Helander (1957) by the addition of 10% (w/w) of trichloroacetic acid. Non-protein nitrogen content was subtracted from the low ionic strength soluble nitrogen before calculating protein (N x 6.25).

The protein fraction percentages were calculated by dividing the weight of total protein in each fraction by the weight of total protein in the meat. Percentage protein in a

weighed portion of each protein fraction multiplied by the total fraction weight equaled the total protein in each fraction.

F. Collagen Determination (Study 1)

Collagen was determined in triplicate on each by-product, MDCM and the HIS protein and IN protein fractions (Bergman and Loxley, 1963). Prior to collagen determination, MDCM, by-products and insoluble proteins were frozen with dry ice and ground in a IKH - Universal mill M20 (Jankel and Kunkel Co., West Germany).

F.1. Collagen Solubilization

a. Weigh out, in triplicate, 2 ± .030 g of powdered meat sample (liquid nitrogen ground) into 50 mL screw cap test tubes. Record the net weight of the sample.

b. Add 20 mL of 6 N HCl and secure the tubes with teflon lined screw caps.

c. Autoclave the tubes overnight at 121° C, 18-20 psi.

d. Cool tubes to room temperature.

e. Add 1.0 (± .10) g of charcoal to each tube.

f. Shake the tubes (by hand) 20 times and filter the sample through 15 cm Whatman #2 filter paper into 400 mL beakers.

g. Rinse the tubes and caps 3 times, then rinse the filter paper 5 times with H_2O.

h. Adjust the pH of the samples to 6.0 (± 0.1) using 12 N NaOH. Rinse the pH probe with a squeeze bottle into the

sample beaker.

i. Transfer the solutions to a 500 mL volumetric flask or graduated cylinder, rinsing the beaker 3 times.

j. Dilute to volume with H_2O (ie: to 500 mL)

k. Prior to sampling for color reaction, filter a representative portion through Whatman #1 (or Fisher no. 09-795) filter paper.

F.2. Color Reaction

a. Prepare the appropriate standards in 50 mL volumetric flasks using the 50 ug/mL stock solution of hydroxyproline.

b. Prepare Ehrlich's Reagent and Oxidant solution.

c. Preheat water bath to 60° C.

d. Pipet 1 mL of H_2O into 4 screw cap test tubes. Two are for zero standards, two are for sample references.

e. Pipet 1 mL of each standard into duplicate screw cap test tubes.

f. Pipet 1 mL of each sample into duplicate screw cap test tubes.

g. Add 2.0 mL of isopropanol to each tube, and vortex the tubes.

h. Start a timer and add 1 mL of oxidant solution to each tube and vortex.

i. Four minutes after adding the oxidant solution to the first tube, begin adding 4 mL of Ehrlich's reagent to each tube, in the same order. Vortex each after pipeting.

j. Screw the caps on to the tubes and vortex again.

k. Set the tubes into the 60° C water bath for 25 min.

l. Cool the tubes for 5 min in chilled water.

m. Vortex the tubes and measure the absorbance at 558 nm.

F.3. Chemicals, Reagents and Calculations

a. To produce 6 N HCl, dilute concentrated HCl 1:1 with H_2O.

b. Charcoal must be MCB CX 0655-1 or J.T. Baker E344-7.

c. Prepare stock hydroxyproline standard in .001 N HCl which is a concentration of 50 ug/mL. Weigh 0.0250 g of L-hydroxyproline (Sigma H-6002) into a 500 mL volumetric flask. Add 5 mL of 0.1 N HCl and dilute to volume with H_2O. Solution will be stable for 2-3 weeks when stored under refrigeration.

d. Prepare acetate citrate buffer by using 0.3 g sodium per 28.5 g H_2O (or sodium acetate anhydrous, 17.2 g), 0.2g trisodium citrate into 18.75 g H_2O and 0.1 g citric acid added to 2.75 g H_2O. Add chemicals to an 800 mL beaker. Add 192.5 mL of isopropanol and approximately 250 mL H_2O. Adjust to pH 6.0 with concentrated acetic acid. Transfer to a 500 mL volumetric and dilute to volume with H_2O. Mixture will be stable refrigerated for 2-3 weeks.

e. Prepare chloramine T solution by using 1.75 g of chloramine T diluted to 25 mL with H_2O. Prepare fresh daily.

f. To prepare oxidant solution, mix 1 volume chloramine T with 4 volumes acetate citrate buffer. This solution should be prepared immediately prior to performing the color reaction.

g. To make dimethylaminobenzaldehyde (DABA, Sigma D-2004), mix 10 g of DABA with 9.4 mL of 70% perchloric acid. Combine while beaker is surrounded by ice. Place covered beaker on stir plate to mix contents.

h. Prepare Ehrlich's Reagent by mixing 15 mL of DABA-$HClO_4$ solution with 80 mL of isopropanol in brown-glass jar. Prepare fresh daily.

i. Use the following equation to convert ug/mL of hydroxyproline to mg/g of collagen:

$$\text{mg/g of collagen} = \frac{(\text{ug/mL})\ (\text{dilution factor})\ (\text{constant})}{(\text{sample weight})\ (1000)}$$

The dilution factor is 500 and the constant is 7.25.

G. Heat Induced Protein Gels (Study 2)

G.1. Gel Manufacturing

Gels were produced in triplicate from protein solutions in stoppered glass tubing of 1.0 cm internal diameter and 11.5 cm length. The protein solutions (low ionic strength soluble, high ionic strength soluble and insoluble proteins homogenized with buffer) were in 0.05 M Na phosphate, 0.6 M NaCl and pH 7.0 buffer. For least concentration endpoint, HIS protein solutions (2% to 6% w/w) were used. Multicomponent gels (6% protein w/w) were manufactured using HIS proteins plus LIS proteins or HIS proteins plus IN proteins. LIS proteins or IN proteins were substituted for HIS proteins in the composite gels at the following percentages (8.33, 16.67, 25.00, 33.33 and 50.00). The HIS plus LIS protein fractions or HIS plus insoluble protein

fractions were combined by blending with a Polytron homogenizer (PTA 20TS, Brinkman Instruments, Inc., Westbury, NY.) for 30 sec on speed setting 6. The protein solutions were pipeted into the stoppered glass tubing. The glass tubing containing the protein solutions were centrifuged at 750 x g for 5 min (IEC Model K, Damon/IEC Division, Needham Hts., MA.) to remove air bubbles. After centrifugation, the protein solutions were cooked for 10 min at 70^0 C in an agitating water bath (Magne Whirl, Blue M Electric Co., Blue Island, IL.) and immediately cooled in an ice bath. Protein gels were manufactured in triplicate.

G.2. Gel Evaluation

G.2.a. Least Concentration Endpoint

The least protein concentration or least concentration endpoint (LCE) was performed on HIS protein gels in 0.05 M Na phosphate, 0.6 M NaCl, pH 7.0 as described by Trautman (1966) with modifications. The LCE value was defined as an apparent stress at failure of greater than or equal to 4 kPa (a kiloPascal is equal to rather than a subjective analysis of the gel remaining intact in an inverted tube.

G.2.b. Expressible Moisture

Expressible moisture (EM) was measured as modified from a high speed centrifugation method of Jaurequi et al. (1981). Three pieces of Whatman #2 filter paper, 9 cm diameter were folded into a cone shape and placed in a 50 ml polycarbonate centrifuge tube. A weighed 1.5 +/- 0.3 g

sample of protein gel was placed inside the filter paper and centrifuged at 755 x g for 5 min in a 2^0 C refrigerated centrifuge. The weight of the released fluid and weight gain of the filter papers were recorded. Expressible moisture of the protein gels was calculated by dividing the weight of the released fluid plus weight gain of the filter papers by the weight of the original weighed gel sample. All gel expressible moistures were determined in triplicate.

G.2.c. Apparent Shear Stress and Strain at Failure

Apparent shear stress and strain at failure of the heat-induced HIS and multicomponent gels were measured using an Instron (Model 4202, Canton, MA.) at crosshead speed of 10 mm/min with a 50 N compression cell. The gel cores were compressed between two lubricated (silicone spray lubricant) parallel plates. The cores compressed were 1.0 cm in diameter and 1.0 cm in length which were cut using a razor blade and template. Cores were equilibrated to 1^0 C prior to testing. Six to nine cores were evaluated per replicate. Strain was measured from the compression peaks measuring the millimeters (x-direction on recorder paper) the crosshead travels before the sample fractures divided by the original sample length. Apparent strain at failure is calculated using the following equation:

$$\delta_H = -\ln (1 - \delta_z)$$

δ_H = apparent strain at failure

ln = natural log

δ_z = strain calculated from recorder paper

Stress is measured by determining the height in Newtons force (N) (y-direction on recorder paper) the crosshead achieves before the sample fractures. Apparent stress is calculate using the following equation:

$$f_{apparent} = \frac{F}{ÐR^2 (1 + ó\delta_z)^2}$$

F = force (Newtons) calculated from recorder paper

Ð = Pi

R^2 = radius of sample (meters) squared

ó = 0.48 (a constant)

δ_z = strain calculated from recorder paper

G.2.d. Scanning Electron Microscopy

Small (5 mm diameter x 2 mm thick) samples of the heat induced gels (6% HIS only and multicomponent gels with 33.33% substitution of LIS or IN fractions) were cut with a razor blade and fixed for 6 hr in 4% glutaraldehyde solution. After fixation, the samples were dehydrated gradually using an ethanol series (25%, 50%, 75%, 95% and 100%) for 15 min/step. The 100% ethanol step was performed three times. Following critical point drying with CO_2 (Balzer FL 9496 Critical Point Dryer, Balzer's Union, Furstentum, Liechtenstein, West Germany), the fixed and dried samples were mounted using adhesive tabs (M.E. Taylor Engineering Inc., Brookeville, MD.) to aluminum mounts (Electron Microscopy Sciences, Ft. Washington PA.). The mounted samples were sputter coated with gold (Emscope SC

500, Emscope, Ashord, Kent, England) for 3 min at 20 mA. Examination of the samples at 10,000 X magnification was done in a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan) using an accelerating voltage of 15 kV.

H. Model System Meat Batters (Studies 1 and 3)

H.1. Formulations

H.1.a. Study 1

Formulations were prepared using a 50:50 blend (based on the protein content) of MDCM and meat by-product. Formulations were standardized to 56% moisture, 30% fat, 12% protein and 2% salt by the addition of pork backfat or ice.

H.1.b.Study 3

Meat batters were formulated to 56% moisture, 30% fat, 12% protein and 2% salt. Meat batters were formulated based on percentages of protein fractions [low ionic strength soluble (LIS), high ionic strength soluble (HIS), insoluble (IN)] contained in the meat and meat by-products based on Nuckles et al. (1990). During phase 1 the meat batters were formulated to vary the percentage of HIS proteins while maintaining the percentage of LIS or IN proteins. During phase 2 the meat batters were formulated to maintain a constant percentage of HIS proteins while varying the percentages of LIS or IN proteins.

H.2. Production

Meat batters were prepared in triplicate using a silent cutter placed in a cold room. Lean meats (MDCM or skeletal muscle) and by-products, salt and ice were added to the

silent cutter bowl and cut for 3 min. The fat (pork backfat or beef) was added to the mixture and cut for an additional 2 min. The final batter temperatures were not greater than 2° C. The batter was stuffed into 50 mL conical centrifuge tubes, capped and heated in a 75° C water bath to an internal temperature of 72° C.

H.3. Cooked Meat Batter Evaluation

H.3.a. Cook Yield

Cook yield of the model system frankfurters was calculated by dividing the drained, cooked frankfurter weight (drained of cook-out fat and moisture) by the original batter weight.

H.3.b. Severe Reheat Yield

Severe reheat yield was calculated after heating a 20 g sample in 100 mL of water at 95° C for 10 min and cooling at room temperature for 5 min. Reheat yield equals the reheated, cooled frankfurter weight divided by the original, frankfurter sample weight.

H.3.c. Apparent Shear Stress and Strain at Failure

Apparent shear stress and strain at failure of the cooked meat batters were measured and calculated as described for heat-induced gels (G.2.c.) except that the cores compressed were 1.5 cm in diameter and 1.5 cm in length.

H.3.d. Apparent Tensile Strength (Study 3)

Apparent tensile strength was measured on the whole frankfurters using a TR-5 Texturepress with a ST Thin Slice

Tensile Test Cell (50 pound cell) attachment and Texturerecorder (Food Technology Corp., Rockville, MD) at a speed setting of 4 and X1/10 range setting. Frankfurter samples (1^0 C) were weighed prior to apparent tensile strength testing and loaded on the cell perpendicular to the cell break-line. The force (N/g) to tear the frankfurters was calculated by reading the chart peak height and multiplying the result by 0.01 thus converting the chart reading to percentage. Multiplying the percentage by 50 (conversion for load cell pounds size) and 0.1 (conversion for the X1/10 setting) converts percentage to pounds force. Multiplying the pounds of force by 4.4482 converts pounds to Newtons (N) of force. Dividing the N of force by the frankfurter weight determines the N force per gram of frankfurter (N/g) to tear the frankfurter.

IV. Study 1: Meat By-Product Protein Composition and Functional Properties in Model Systems

A. Introduction

Meat by-products are commonly classified as edible or inedible (Oliveros et al., 1982). As a general definition, by-products are "everything of economic value, except for the carcass, that are incidental to the slaughter of animals (Booren and Weiss, 1988)." Meat by-products are underutilized and low priced because they are regarded as an inferior protein source compared to skeletal muscle meat (Oliveros et al., 1982). The utilization of meat by-products has been avoided due to undesirable sensory quality, low biological value of the proteins and high microbial contamination (Kosiba, 1983). Mishandling, poor sanitation and slow chilling decrease quality and increase the microbial load of meat by-products (Booren and Weiss, 1988). The increasing price of meat and processed meat products is causing the food industry to evaluate the utilization of all protein sources, including by-products (Gorska et al., 1988).

Restructuring techniques, such as formed meat chunk technology, allows meat by-products to be utilized in value-added products. Formed meat chunk technology is used to restructure meats that are difficult to incorporate into other further-processed products. Meat chunks are formed using non-meat binders. The process is used commercially in Canada for human foods and in the United States for premium

pet foods. Formed meat chunk technology for human consumption is limited in the United States due to consumer perceptions of undesirable sensory quality. Understanding the composition and function of by-product proteins may allow for the replacement of non-meat binders with combinations of by-products to achieve the desired bind, texture and mouthfeel.

Further-processed meats utilize proteins as the principal functional and structural components. The proteins determine the characteristic texture, water-holding and appearance of these products (Hermansson, 1985). Protein gelation, fat binding and water-holding are the most important functional properties in further-processed products. Cooked product stability is currently not thought to rely upon protein emulsification (Regenstein, 1988). The individual product, processing method and stage of processing will determine the relative importance of each functional property. As the variety of meat products increases, the need to understand, modify and control protein functionality becomes more important (Smith, 1988).

Proteins are denatured and reaggregate into a cross-linked three dimensional gel matrix during cooking (Foegeding, 1988). Fat and water are physically or chemically trapped inside this matrix. The texture and cook yield of restructured products are directly related to this matrix formation. The characteristics of the gel formed are a function of the type and amount of proteins present and

are influenced by environmental (e.g., pH, salt) and processing factors during heating (Whiting, 1988).

Meat by-products exhibit large differences in protein content and distribution and consequently vary widely in protein functionality and bind values. Bind values are based on the emulsion capacity of the soluble protein fraction. The type and amount of meat by-products in a formulation influence the texture, fat emulsifying capacity and water-holding properties of the finished products. Each by-product contributes different, unique textural attributes to the finished product. Information on the functional and physicochemical properties of by-product proteins is extremely limited. The protein content and composition of some beef by-products has been reported (Schaefer and Schierhorn 1974; Oliveros et al., 1982; Gorska et al., 1988), but the salt soluble protein and stroma protein content of by-products from other species are not known. The objective of this study was to develop a knowledge base of selected meat by-product composition and function for use in restructured meat products.

B. Material and Methods

B.1. Materials

Frozen mechanically deboned chicken meat (MDCM) prepared from the whole carcass was purchased from Nottawa Gardens Corp., Athens, MI. By-products (pork lung lobes, pork liver, beef lung lobes, beef spleen, beef heart) obtained from 15 market weight hogs or 15 market weight

steers slaughtered on three different days at the Michigan State University Meat Laboratory were used in this study. Five animals were slaughtered each day and like by-products combined and designated as one replicate. By-products were removed within 1 hr after exsanguination. By-products were ground sequentially through the 10, 6, and 3 mm plate of a Hobart grinder (Model A-200, Troy, OH), vacuum packaged (2.5 mil polyethylene) and frozen until used within three months. Proximate composition (fat, protein and moisture) were determined by AOAC (1984) procedures. The pH was determined after blending 10g of MDCM or by-product with 100g of double distilled water on setting 3 for 1 min in a Sorvall Omni-Mixer (Model 17105, Newtown, CT) and was measured using a Corning 145 pH meter. All experiments were performed in triplicate.

B.2. Methods

B.2.a. Timed Emulsification

Timed emulsification was performed as described by Galluzzo and Regenstein (1978) with modifications because a cream layer was not formed using the recommended protein concentration of 5 mg/ml. MDCM and by-products were evaluated in triplicate at total protein concentrations of 10 to 150 mg/ml. The lowest concentration of protein required to produce a cream layer after centrifugation at 30,000 x g for 15 min was recorded.

B.2.b. Quantitation of Protein Fractions

All extraction procedures were performed in a 4^0 C cold room. A weighed portion of MDCM or by-product was blended (Waring Blendor, Model 1120, Winsted, CT) for 1 min with 4 volumes of 0.05M Na phosphate buffer, pH 7.4, stirred for 3 hr (Talboy T-Line Laboratory Stirrer, Model 107, Emerson, NJ) in a cold room and centrifuged at 23,000 x g for 15 min. The supernatant was saved and the residue re-extracted for 1 hr. Following centrifugation, the supernatants containing the 0.05M Na phosphate soluble proteins were combined, designated as low ionic strength soluble (LIS) proteins and quantitated. The precipitate was mixed with 4 volumes of 0.6M NaCl, 0.05M Na phosphate buffer, pH 7.4 and extracted twice as described previously. The supernatants, designated as high ionic strength soluble (HIS) proteins, were combined and quantitated. The precipitate was designated insoluble proteins and weighed. The protein content of each fraction was determined by Kjeldahl procedures (AOAC, 1984). Non-protein nitrogen content was measured on the low ionic strength soluble proteins according to Helander (1957). Non-protein nitrogen content was subtracted from the low ionic strength soluble nitrogen before calculating protein (N x 6.25).

The protein fraction percentages were calculated by dividing the weight of total protein in each fraction by the weight of total protein in the meat. Percentage protein in a weighed portion of each protein fraction multiplied by the

total fraction weight equaled the total protein in each fraction.

B.2.c. Collagen Determination

Collagen was determined in triplicate on each by-product, MDCM, the HIS protein fraction and the insoluble protein fraction according to Bergman and Loxley (1963). Prior to collagen determination, MDCM, by-products and insoluble proteins were frozen with dry ice and ground in a IKH-Universal mill M20 (Jankel and Kunkel Co., West Germany).

B.2.d. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the HIS and insoluble protein fractions was performed using 12% polyacrylamide gels according to Smith and Brekke (1985). The insoluble protein fractions were solubilized prior to electrophoresis according to Wu et al. (1982). One hundred micrograms of the HIS protein fraction and 200 ug of the insoluble protein extract were loaded on separate gels. Protein bands were stained with Coomassie Blue (Sigma Chemical Co., St. Louis, MO). Myosin, actin and other protein bands were identified by comparing relative retention times to molecular weight standards (MW-SDS-200, Sigma Chemical Co., St. Louis, MO). Myosin:actin molar ratios of MDCM and by-product protein fractions were calculated according to Beas et al. (1988). The protein bands were quantitated by densitometric scans at 580 nm (Schimadzu CS-930, Kyoto, Japan).

B.2.e. Model System Frankfurters

Batters were prepared in triplicate using a silent cutter as described by Smith and Brekke (1984), except the batter was stuffed into 50 mL conical centrifuge tubes, capped and heated in a 75^0 C water bath to an internal temperature of 72^0 C. Formulations were prepared using a 50:50 blend (based on the protein content) of MDCM and meat by-product. Formulations were standardized to 56% moisture, 30% fat, 12% protein and 2% salt by the addition of pork backfat or ice.

Cook yield of the model system frankfurters was calculated by dividing the drained, cooked frankfurter weight (drained of cook-out fat and moisture) by the original batter weight. Reheat yield was calculated after heating a 20g sample in 100 mL of water at 95^0 C for 10 min and cooling at room temperature for 5 min. Reheat yield equals the reheated, cooled frankfurter weight divided by the original, cooked frankfurter weight.

Apparent shear stress and strain at failure (Diehl et al., 1978) of model system frankfurters cores were measured using an Instron (Model 4202, Canton, MA) at a crosshead speed of 10 mm/min with a 50 N compression cell. The cores were compressed between two parallel plates. A 1.5 cm diameter central core was removed along the longitudinal axis of the model system frankfurters using a cork borer and cores were cut to 1.5 cm lengths using a razor blade and

template. Cores were equilibrated to 1^0C prior to testing. Six cores were evaluated per replicate.

C. Statistics

The statistical design was completely randomized. Basic statistics, analysis of variance (ANOVA) and correlations were performed using M-STAT (1988). Two way ANOVA was performed to test significance within replications and between treatments. Mean separations (significance) were tested using Tukey's test.

D. Results and Discussion

The proximate composition of MDCM and by-products is shown in Table 4.

Table 4. Proximate composition and collagen content of meat by-products.

Sample	Moisture (%)	Fat (%)	Protein (%)	Collagen (% of total protein)	Collagen (mg/g sample)
Mechanically Deboned Chicken Meat	65.6^{cd}	14.2^{b}	17.4^{b}	3.9^{c}	6.8^{c}
Beef Heart (cap on)	65.5^{cd}	17.5^{a}	15.4^{c}	5.5^{b}	8.5^{b}
Beef Lung (lobes only)	79.7^{ab}	1.9^{d}	17.7^{b}	5.5^{b}	9.7^{a}
Beef Spleen	79.9^{ab}	3.5^{c}	15.3^{c}	2.3^{d}	3.5^{d}
Pork Liver	71.7^{b}	1.8^{d}	22.1^{a}	1.5^{e}	3.4^{d}
Pork Lung (lobes only)	82.5^{a}	2.0^{d}	15.5^{c}	6.1^{a}	9.4^{a}
SEM	1.5	1.0	0.4	0.3	0.4

^{a,b,c,d,e} Means within columns followed by the same letter do not differ significantly ($P< 0.05$).

MDCM was used in this study because it has been extensively studied, only a small amount of mechanically separated red meats are produced in the U.S. and much of the production is used in pet foods (Field, 1988). Pork and beef lungs, beef spleen and pork liver generally contained higher moisture and lower fat than beef heart and MDCM. Pork liver contained significantly more ($P< 0.05$) total protein than the other by-products. Beef heart, beef spleen and pork lungs contained the lowest ($P< 0.05$) protein content. The proximate composition of beef heart (with cap) and pork liver are consistent with values published by Porteous (1979) and Wiley et al. (1979). The percentages of fat and moisture in beef heart published by Oliveros et al. (1982) are significantly lower because our analysis included the heart cap. Non-protein nitrogen (NPN) content of MDCM and by-products were not significantly different and contained an average of 2.38% NPN. Collagen content (mg/g meat) was significantly higher ($P< 0.05$) in beef heart and pork lung compared to MDCM and other by-products. The beef heart and liver collagen contents were comparable to the published values of Porteous (1981). Khalili and Zarkadas (1988) reported 1.96 to 3.08% collagen in chicken breast and 5.63 to 6.87% in leg muscles.

The protein distribution varied among the by-products examined (Table 5). Mechanically deboned chicken meat

Table 5. Protein fractions as a percentage of total protein and collagen content of the low ionic strength soluble (LIS), high ionic strength soluble (HIS) and insoluble protein fractions of by-products.

Sample	LIS Proteins (%)	HIS Proteins (%)	Collagen in HIS Fraction (%)	Insoluble Proteins	Collagen in Insoluble Fraction (%)
Mechanically Deboned Chicken Meat	28.6^{d}	40.4^{a}	1.9^{e}	31.0^{d}	12.0^{b}
Beef Heart (cap on)	30.1^{d}	21.2^{b}	5.1^{a}	48.7^{b}	10.1^{c}
Beef Lung (lobes only)	36.3^{c}	9.6^{d}	3.3^{d}	54.1^{a}	10.1^{c}
Beef Spleen	53.6^{b}	21.7^{b}	3.1^{d}	24.7^{e}	8.0^{d}
Pork Liver	76.1^{a}	14.8^{c}	3.8^{c}	9.1^{f}	13.2^{a}
Pork Lung (lobes only)	51.6^{b}	10.2^{d}	4.3^{b}	38.2^{c}	13.3^{a}
SEM	2.7	1.6	0.2	2.4	0.3

a,b,c,d,e,f Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

contained nearly twice as much HIS protein as meat by-products. The HIS percentages for beef heart, beef lung and beef spleen are lower than percentages reported by Oliveros et al. (1982). The differences may be explained by different extraction times (7 days versus 4 hr). Beef heart and beef lung contained the highest percentage of insoluble proteins, while pork liver contained the highest quantity of LIS proteins. Beef heart contained the highest percentage of collagen in the HIS fraction. The sum of the collagen in the HIS fraction and insoluble protein fractions is consistent with the amount of total collagen in the samples. Soluble collagen was measured on the HIS proteins because large amounts of collagen within this fraction may adversely affect functionality.

A representative SDS-PAGE eletrophoretogram of the by-product HIS proteins is shown in Fig. 3. Myosin or actin were not present on electrophoretograms of the insoluble proteins. MDCM and beef heart had significantly higher ($P < 0.05$) percentages of myosin and actin and a lower percentage of other proteins compared to the other by-products (Table 6).

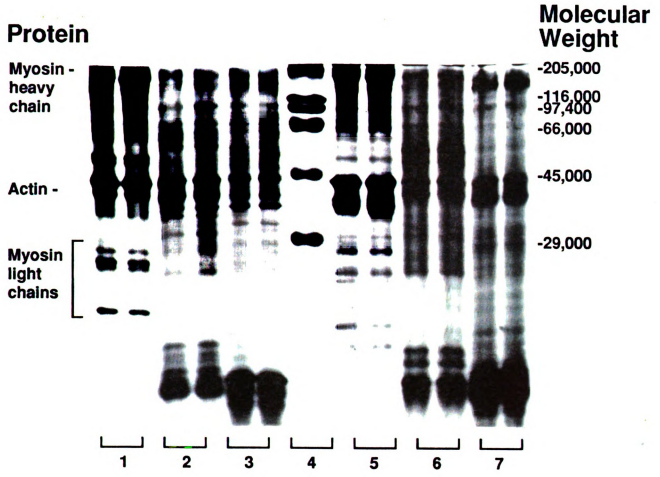


Figure 3. Representative SDS-PAGE electrophoretogram of by-product salt soluble proteins (1 = beef heart, 2 = beef lung, 3 = pork lung, 4 = molecular weight standards, 5 = MDCM, 6 = pork liver, 7 = beef spleen).

Table 6. The percentage of myosin and actin and the myosin/actin ratio of the by-product high ionic strength soluble protein fraction.

Sample	Myosin (%)	Actin (%)	Actin:myosin Mole:Ratio
Mechanically Deboned Chicken Meat	50.3[a]	22.3[a]	5.0
Beef Heart (cap on)	47.9[b]	20.6[b]	5.0
Beef Lung (lobes only)	37.6[c]	10.5[c]	3.2
Beef Spleen	22.6[d]	10.1[c]	4.5
Pork Liver	24.4[d]	9.2[d]	4.3
Pork Lung (lobes only)	38.6[c]	10.6[c]	3.0
SEM	2.7	1.3	

[a,b,c,d] Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

MDCM and beef heart contained twice as much myosin and actin compared to beef spleen and pork liver. The significant differences in the percentages of myosin, actin and other proteins may be related to differences between skeletal, cardiac and smooth muscle types. Myosin and actin are present in cardiac and smooth muscle and are extractable at high ionic strength (Garrels and Gibson, 1976; Cross et al., 1988). MDCM and beef heart had lower myosin/actin molar ratios (1.0:5.0, Table 3) when compared to the other by-products (1.0:3.0 to 1.0:4.5). Potter (1974) and Murakami and Uchida (1985) reported that the molar ratio of myosin to actin in skeletal muscle was 1.0:6.0. The myosin:actin ratio

in rabbit skeletal muscle was 1:6.6 based on amino acid analysis rather than densitometry (Yates and Greaser, 1983). Murakami and Uchida (1985) also reported a molar ratio of myosin to actin of 1.0:4.1 for cardiac muscle and 1.0:16.5 for smooth muscle (chicken gizzard). Yasui and Ishioroshi (1980) found that a weight ratio myosin:actin of 15 formed more rigid gels. Asghar et al. (1985) stated that the myosin:actin ratio rather than the actomyosin:actin ratio may be related to gel strength.

The timed emulsification procedure of Galluzzo and Regenstein (1978), developed to study the emulsifying properties of skeletal meat proteins was modified to evaluate meat by-products. The minimum protein concentration necessary to produce a cream layer after centrifugation was used as an index of protein functionality among the by-products. MCDM, beef spleen and pork liver formed cream layers (10, 20 and 40 mg protein/ml, respectively) but beef heart, beef lung and pork lung did not form cream layers at protein concentrations up to 150 mg/ml. Oliveros et al. (1982) reported that beef spleen emulsified more oil than beef meat or beef lung.

Cooked yield, reheat yield, apparent shear stress and apparent strain at failure of model system frankfurters (pH 6.3) were influenced by meat by-product protein composition (Table 7).

Table 7. Yield and textural characteristics of model system frankfurters prepared from mechanically deboned chicken meat and meat by-products (1:1 based on total protein content)[a]

Sample	Cook Yield (%)	Reheat Yield (%)	Apparent stress at failure (KPa)	Apparent strain at failure
Mechanically Deboned Chicken Meat	91.3[d]	82.6[b]	42.80[b]	0.76[b]
Beef Heart (cap on)	92.5[c]	80.1[c]	42.22[c]	0.76[b]
Beef Lung (lobes only)	93.0[b]	78.6[d]	20.35[d]	0.69[c]
Beef Spleen	93.7[b]	78.6[d]	20.35[d]	0.69[c]
Pork Liver	91.2[d]	74.4[e]	10.54[g]	0.56[d]
Pork Lung (lobes only)	92.3[c]	69.8[f]	17.37[e]	0.69[c]
SEM	0.2	0.7	0.59	0.01

[a]Batter formulation: 12% protein, 30% fat, 56% moisture and 2% salt.

b,c,d,e,f,g Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

Apparent strain is highly correlated to sensory and texture profile analysis (TPA) cohesiveness while apparent stress at failure is correlated to TPA hardness and sensory firmness (Montejano et al., 1985). Model system frankfurter percentage cooked yield ranged from 91.2 to 93.7% while reheat yield ranged from 69.8 to 82.6%. The model system frankfurters produced with by-products had significantly lower ($P< 0.05$) percentage reheat yield and compressive force at failure compared to MDCM frankfurters. Among the by-product frankfurters, beef heart had the highest reheat yield and pork lung exhibited the lowest reheat yield. The pH values of the frankfurter batters were not significantly different.

Changes in the modified timed emulsification test were positively correlated with the quantity of by-product insoluble proteins and the percentage collagen contained within the HIS proteins and negatively correlated with the quantity of HIS proteins (as HIS proteins increased, less protein was needed to form a stable cream layer, Table 8). The quantity of protein required to form a stable layer in the modified timed emulsification test was not highly correlated with the percentage of by-products LIS proteins, myosin or actin within the HIS proteins, collagen in the insoluble proteins or any parameters measured in the model system frankfurter test.

Reheat yield was positively correlated with the quantity of HIS proteins and the percentage of myosin and actin within the HIS proteins fractions (Table 5). Reheat yield was inversely correlated with LIS proteins. Apparent shear stress and strain at failure of the model system frankfurters were negatively correlated with LIS proteins and positively correlated with HIS proteins and the percentage of myosin and actin within the HIS fractions.

The HIS proteins are generally considered to impart the most functionality to processed meats. In this study, larger quantities of HIS proteins were related to larger reheat yields and better textural properties in the model system frankfurters. The quantity of insoluble proteins in the meat by-products was not strongly correlated with the parameters measured in the frankfurters. Timed emulsification was not an adequate tool to evaluate by-products.

These results indicate that the protein distribution of meat by-products was related to the yield and texture imparted to comminuted meat products. Results suggest it is possible that a certain percentage of HIS proteins or ratio of protein fractions are necessary to produce the desired bind in frankfurter formulations.

Table 8. Correlation coefficients between meat by-products protein composition and model system properties.

	Modified Timed Emulsification	Reheat Yield	Apparent stress at failure	Apparent strain at failure
LIS Proteins	-.31	-.71	-.85	-.82
HIS Proteins	-.71	-.78	-.80	-.61
Myosin in HIS fraction	.36	.64	.93	.98
Actin in HIS fraction	-.07	.79	.98	.74
Collagen in HIS fraction	.72	-.37	-.07	.08
Insoluble Proteins	.79	.16	.14	.30
Insoluble Proteins % Collagen	.04	-.24	-.04	.24
Apparent strain at failure	-.09	.63	.83	
Apparent stress at failure	-.35	.84		
Reheat Yield	-.41			

V. Study 2: Properties of heat induced gels prepared from beef skeletal, cardiac and smooth muscle protein fractions.

A. INTRODUCTION

Protein gelation is one of the most important functional properties in processed meat products. Gelation of muscle proteins, a transformation from viscous sols to elastic gels, contributes to desirable texture and the stabilization of fat and water in processed meat products. Ferry (1948) defined heat-induced protein gelation as a two step process where partial unfolding of protein domains is followed by reaggregation into a crosslinked, three dimensional network of protein fibers. The simplest type of protein gel is one formed from a single type of polypeptide (Foegeding, 1988).

Salt soluble proteins (myofibrillar) contribute the most functionality to gels (Knight, 1988). The contribution of water soluble proteins (sarcoplasmic) and/or insoluble proteins (stroma and some myofibrillar) to processed meat products is not well understood (Knight, 1988). The gel properties of myosin and fibrinogen or albumin have been studied (Foegeding, 1983). Myosin and fibrinogen interacted to form a gel at 55^0 C and 70^0 C that was stronger than gels made from the individual proteins. Myosin and albumin did not form a gel until 80^0 C and formed a weaker gel than the myosin-fibrinogen gel. Mixtures of salt soluble proteins

plus sarcoplasmic or insoluble proteins have not been studied. Information from such studies should help explain the effects of protein interactions on product textures, fat and water-holding and help meat processors select meats to achieve desired product attributes. Knight (1988) reported that three protein fractions (salt soluble, insoluble myofibrillar and connective tissues) from pork shoulder meat had different functional properties. He suggested that manufacturers could use information on individual protein fractions to improve or create new products.

Venegas et al. (1988) studied whole offal homogenates (cattle lungs, spleen, kidneys, testicles and pig stomach) and beef skeletal muscle for water holding capacity (WHC), emulsion stability and gel strength. Their results showed that skeletal muscle exhibited significantly higher gel strength, but the offal showed better WHC probably due to its higher pH. Among the offal, lungs showed the best overall functionality, while spleen had good WHC but low gel strength.

The gel-forming abilities of proteins from various sources can be compared. Tests include 1) least concentration endpoint (LCE) (Trautman, 1966) to compare the minimum protein concentration required to form a gel that remains intact within an inverted tube, 2) expressible moisture (EM) (Jaurequi et al.,1981) to measure the water-holding capacity of the gels, 3) apparent shear stress and strain (Diehl et al., 1979) to ascertain gel strength and 4)

scanning electron microscopy (SEM) to visually compare gel attributes. The objectives of this research were to evaluate gels produced from high ionic strength soluble (HIS) proteins and multicomponent gels produced from combinations of HIS and low ionic strength soluble (LIS) or insoluble (IN) proteins from beef skeletal, cardiac and smooth muscles.

B. MATERIALS AND METHODS

B.1. MATERIALS

Beef semitendinosus muscle (beef skeletal muscle) and beef by-products (lung lobes, spleen and heart) were obtained from 9 market weight steers slaughtered on three different days at the Michigan State University Meat Laboratory. Three steers were slaughtered each day and like by-products combined and designated as one replicate. By-products were removed within 1 hr after exsanguination, vacuum packaged in 500 g aliquots (2.5 ml polyethylene) and frozen until used within three months.

B.2. METHODS

B.2.a. Preparation of Protein Fractions

Three protein fractions were extracted in triplicate as described by Nuckles et al. (1990). The fractions were low ionic strength soluble (LIS) proteins extracted with 0.05 M Na phosphate buffer, pH 7.4, high ionic strength soluble (HIS) proteins extracted with 0.6 M NaCl, 0.05 M Na phosphate buffer, pH 7.0 and insoluble (IN) proteins. The protein extraction procedures were modified so the first

extraction used only 2 volumes (rather than 4 volumes) of buffer. The modification eliminated additional protein concentration steps when producing the gels. Prior to gel production, the LIS proteins were dialyzed for 24 hr (two buffer changes) with 0.05 M Na phosphate, 0.6 M NaCl, pH 7.0 buffer. The nitrogen content of the LIS and IN proteins was measured and percentage protein calculated (AOAC, 1984). Non-protein nitrogen (NPN) was determined according to Helander (1957) and subtracted from the LIS nitrogen before calculating protein. The LIS, HIS and IN proteins were adjusted to 6% (w/w) protein with 0.05M Na phosphate, 0.6 M NaCl, pH 7.0 buffer.

B.2.b. Gel Manufacturing

Gels were produced in triplicate from protein solutions in 0.05M Na phosphate, 0.6M NaCl, pH 7.0 buffer by pipeting into stoppered glass tubing of 1.0 cm internal diameter and 11.5 cm length. For least concentration endpoint, HIS protein solutions ranging from 2% to 6% (w/w) were prepared. Multicomponent gels (6% protein w/w) were manufactured using HIS proteins plus LIS proteins or IN proteins. Low ionic strength soluble proteins or IN proteins were substituted for HIS proteins in the multicomponent gels at the following percentages (8.33, 16.67, 25.00, 33.33, 41.67 and 50.00). Fractions were combined by blending with a Polytron homogenizer (PTA 20TS, Brinkman Instruments, Inc., Westbury, NY.) for 30 sec on speed setting 6. Protein solutions were centrifuged at 750 x g for 5 min (IEC Model K, Damon/IEC

Division, Needham Hts., MA.) to remove air bubbles. After centrifugation, the protein solutions were heated for 10 min at 70^0 C in an agitating water bath (Magne Whirl, Blue M Electric Company, Blue Island, IL.) and immediately cooled in an ice bath. Three separate heatings of each treatment were performed.

B.2.c. Least Concentration Endpoint

The least protein concentration or least concentration endpoint (LCE) was performed on HIS protein gels in 0.05 M Na phosphate, 0.6 M NaCl, pH 7.0 as described by Trautman (1966) with modifications. The LCE value was defined in this study as an apparent stress at failure of greater than or equal to 4 kPa rather than a subjective analysis of the gel remaining intact in an inverted tube.

B.2.d. Gel Evaluation

Expressible moisture (EM) was measured as modified from a high speed centrifugation method of Jauregui et al. (1981). Three pieces of Whatman #2 filter paper, 9 cm diameter were folded into a cone shape and placed in a 50 ml polycarbonate centrifuge tube. A weighed 1.5 +/- 0.3 g sample of protein gel was placed inside the filter paper and centrifuged at 755 x g for 5 min at 2^0 C. The weight of the released fluid and weight gain of the filter papers were recorded. Expressible moisture of the protein gels was calculated by dividing the weight of the released fluid plus weight gain of the filter papers by the weight of the original weighed gel sample. Expressible moistures were

determined in triplicate.

Apparent shear stress and strain at failure (Hamann, 1983) of the heat-induced HIS and multicomponent gels were measured using an Instron (Model 4202, Canton, MA.) at crosshead speed of 10 mm/min with a 50 N compression cell. The gel cores were compressed between two lubricated (silicone spray lubricant) parallel plates. The cores were 1.0 cm diameter and 1.0 cm length cut using a razor blade and template. Cores were equilibrated to 1^0 C prior to testing. Six to nine cores were evaluated per replicate. Gels with an apparent stress at failure of < 4 kPa are not presented because they were too weak for compression testing.

B.2.e. Scanning Electron Microscopy

Small (5 mm diameter x 2 mm thick) samples of the heat-induced gels (6% HIS only and multicomponent gels with 33.33% substitution of LIS or IN fractions) were fixed for 6 hr in 4% glutaraldehyde solution. After fixation, the samples were dehydrated in a graded ethanol series (25%, 50%, 75%, 95%, and 100%) for 15 min/step. The 100% ethanol dehydration step was performed three times on the fixed samples. Following critical point drying with CO_2 (Balzer FL 9496 Critical Point Dryer, Balzer's Union, Furstentum, Liechtenstein, Germany), the samples were mounted using adhesive tabs (M.E. Taylor Engineering, Inc., Brookeville, MD.) to aluminum stubs (Electron Microscopy Sciences, Ft. Washington, PA.). The mounted samples were sputter coated

with gold (Emscope SC 500, Emscope, Ashord, Kent, England) for 3 min at 20 mA. Samples were examined at 10,000 X magnification using a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV.

C. STATISTICS

The statistical design was a 2 x 2 factorial to study the influence of protein source and type on functional tests. Basic statistics and analysis of variance (ANOVA) were performed to test significance within replications and between treatments. Mean separations (significance) were tested using Tukey's test (MSTATC, 1989).

D. RESULTS AND DISCUSSION

D.1. HIS Protein Gels Properties

The minimum concentrations of HIS protein required to form a heat-induced gel with an apparent stress at failure of greater than or equal to 4 kPa in beef skeletal muscle, heart, lung and spleen were 3, 4, 5 and 6%, respectively. Twice the beef spleen HIS protein concentration was required to form a stable (apparent stress at failure greater than or equal to 4 kPa) gel compared to beef semitendinosus muscle (skeletal) HIS proteins. Spleen contains a higher ratio of actin to myosin compared to skeletal muscle (Nuckles et al., 1990) and may explain the lower gel strength of spleen HIS proteins. Schmidt et al. (1981) reported that myosin is the protein mainly responsible for gelation. Yasui et al. (1980, 1982) reported that F-actin enhanced myosin gelation, but F-

actin alone has poor gelation capabilities. Venegas et al. (1988) reported that cooked homogenates of cattle spleen or cattle lungs both exhibited high water-holding capacity, but the gel strength of the spleen homogenate was low. The HIS proteins from skeletal, cardiac and smooth muscles do not exhibit equivalent functional properties. Further studies relating the HIS protein fraction composition to functionality are required.

The HIS protein gels produced from beef skeletal muscle expressed approximately 15% less moisture than beef spleen HIS gels (Fig. 4). The order of expressed moisture of the HIS gels from most to least was spleen > lung > heart > skeletal muscle. Less expressed moisture may be related to a higher amount of myosin in the HIS protein fraction (Nuckles, 1990), as higher amounts of myosin may enhance the production of a gel network that is better able to chemically and/or physically entrap moisture (Schmidt et al., 1981).

Apparent stress at failure of the HIS protein gels showed that skeletal muscle proteins produced significantly harder gels than the by-product proteins (Fig 5). Apparent stress at failure has been correlated to texture profile analysis (TPA) hardness and sensory firmness (Montejano et al., 1985). The HIS gels produced from skeletal muscle

Figure 4. Expressible moisture of high ionic plus low ionic strength soluble protein gels.

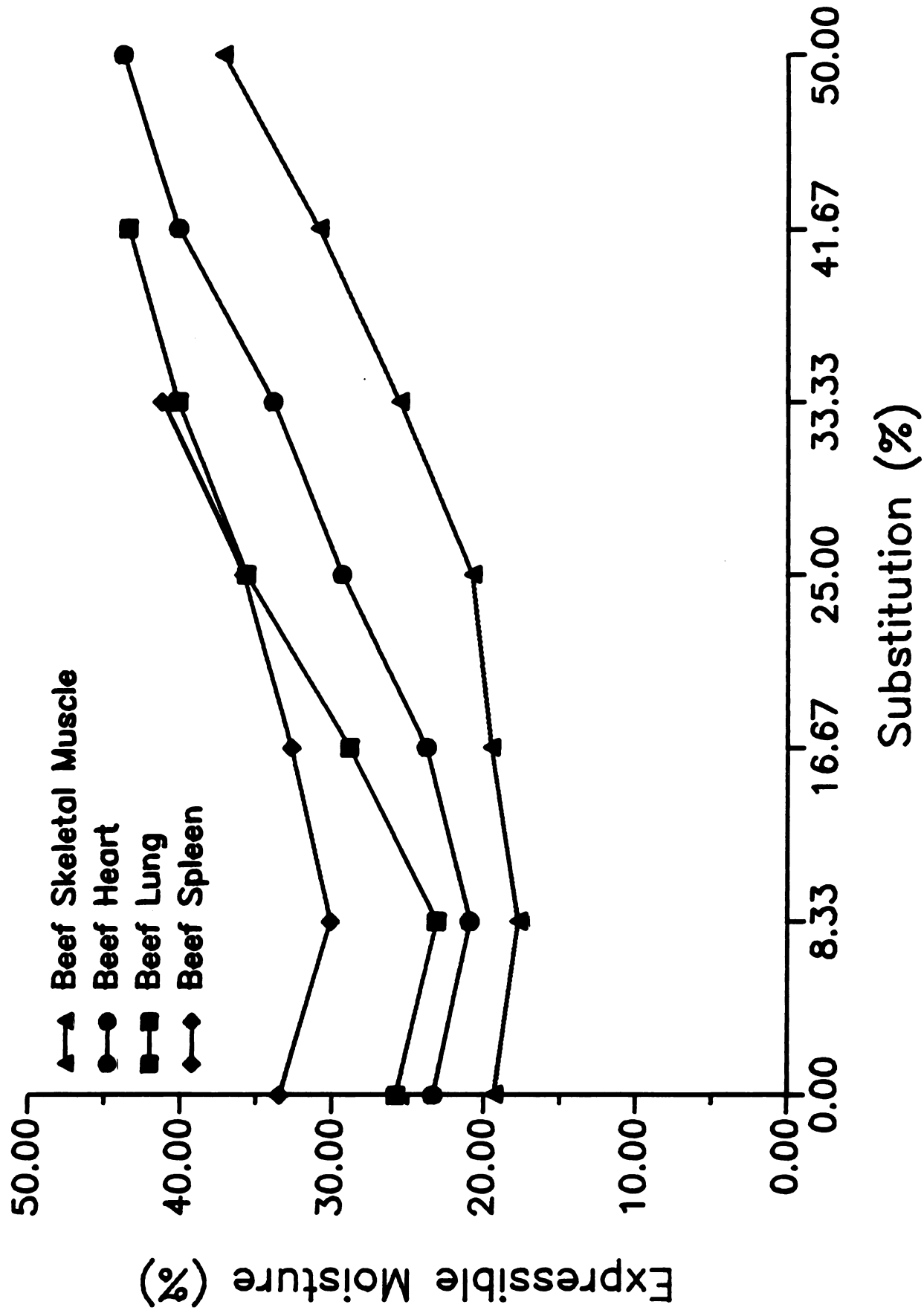
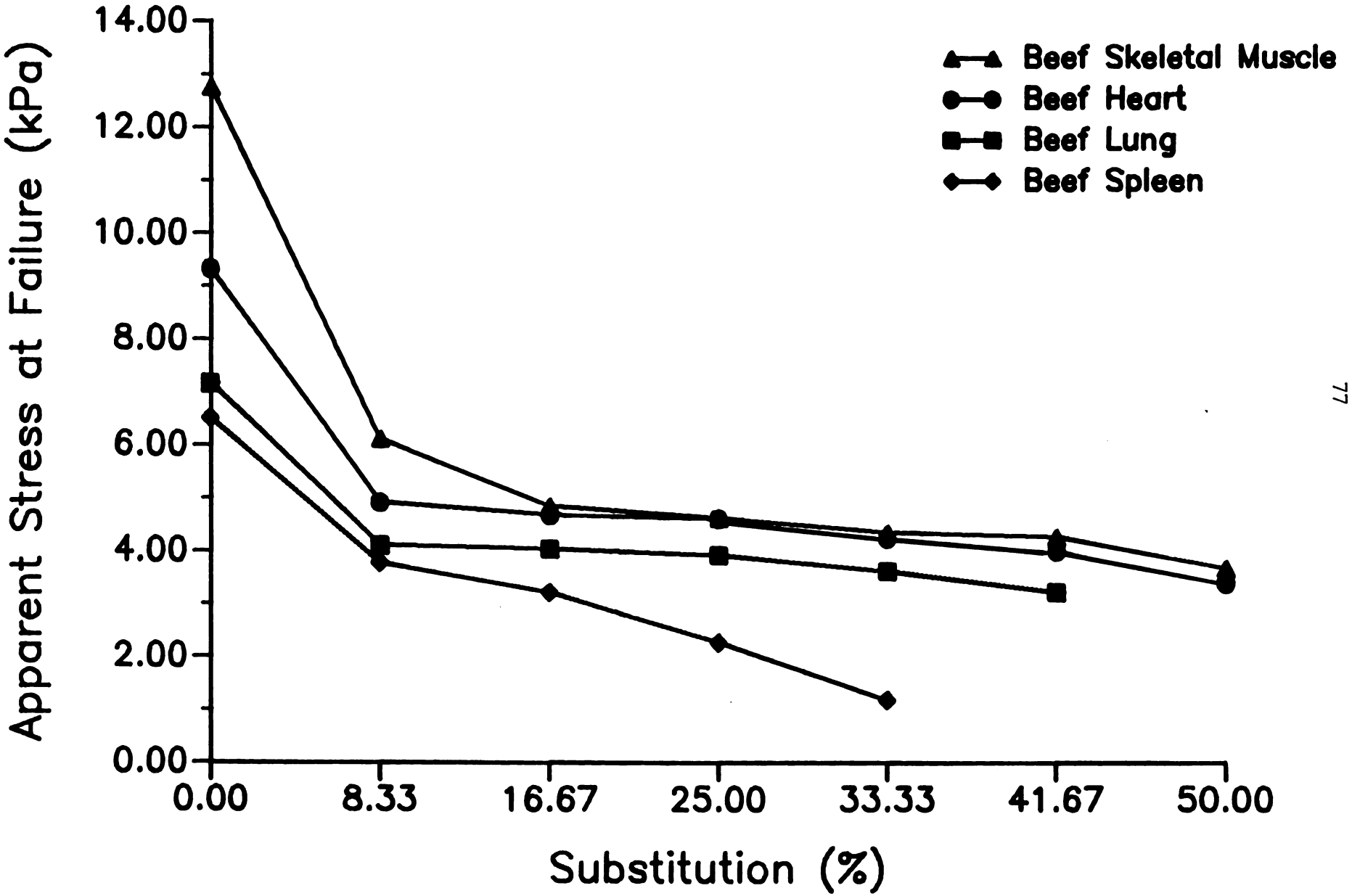

Expressible Moisture (%)
50.00
40.00
30.00
20.00
10.00
0.00
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)
Beef Skeletal Muscle
Beef Heart
Beef Lung
Beef Spleen

Figure 5. Apparent stress at failure of high ionic strength plus low ionic strength soluble protein gels.


Apparent Stress at Failure (kPa)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)
Beef Skeletal Muscle
Beef Heart
Beef Lung
Beef Spleen

exhibited nearly twice the apparent stress at failure compared to HIS gels from spleen and lung. The weaker gels from spleen and lung may be related to a lower percentage of myosin in those HIS protein fractions (Nuckles et al., 1990). Heart does not contain a significantly lower percentage of myosin in the HIS protein fraction (Nuckles et al., 1990), therefore further studies need to be performed to explain the lower apparent stress at failure for the heart HIS protein gels.

Beef skeletal muscle HIS protein gels had significantly greater apparent strain at failure (0.78) compared to the spleen HIS proteins gels (0.57) (Fig.4). Apparent strain at failure has been highly correlated to sensory and TPA cohesiveness and deformability (Montejano et al., 1985). Skeletal muscle HIS protein gels were the most cohesive followed by heart > lung > spleen.

D.2. Scanning Electron Micrographs of HIS Protein Gels

The HIS protein gels of beef semitendinosus muscle (Fig 6a) and heart (Fig 6b) appeared fibrous (thick fibers) and honeycomb-like, whereas the lung HIS protein gel was globular (Fig 6c) and the spleen HIS protein gel (Fig 6d) appeared filamentous (thin fibers). The fibrous gels of the semitendinosus muscle and heart HIS proteins may explain the increased strength and decreased expressible moisture as compared to the lung and spleen HIS protein gel. The fibrous honeycomb-like network may help entrap or chemically stabilize the moisture, whereas the globular lung HIS

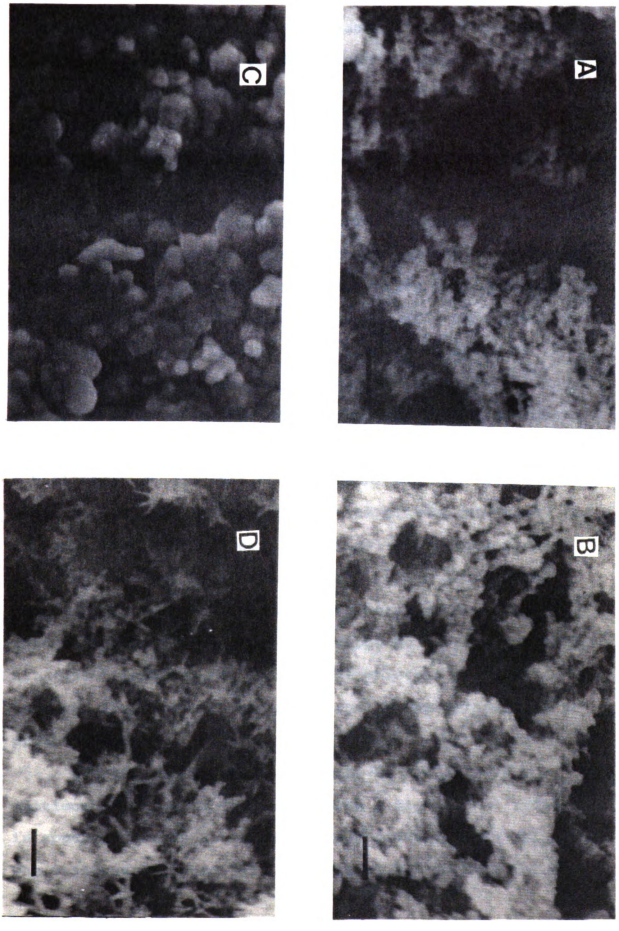
Figure 6. Scanning electron micrographs of high ionic strength soluble protein gels.

A = skeletal

B = cardiac

C = lung

D = spleen


A
B
C
D

proteins may not provide the honeycomb-like protein network. The filamentous strands of the spleen HIS protein network may not provide the needed structural strength to entrap or chemically retain fat and water.

D.3. High Ionic Strength/Low Ionic Strength Multicomponent Gels

Multicomponent gels (HIS proteins plus LIS proteins or HIS proteins plus IN proteins) were prepared with 6% protein because this was the minimum concentration required for gelation of spleen proteins.

Data presented in Fig. 4 shows the percentage of moisture expressed from the HIS plus LIS protein multicomponent gels. The addition of 8.33% LIS proteins to HIS proteins reduced EM of the gels. At higher LIS substitutions the EM was equivalent or significantly higher than the HIS gels. Acton and Dick (1976) reported that sarcoplasmic (LIS) proteins do not gel but rather form a coagulum that may have deposited on the HIS protein gel matrix, increasing surface area for moisture/protein interaction and decreasing expressible moisture. Venegas et al. (1988) reported low expressible moisture for gels produced from spleen homogenates due to sarcoplasmic (LIS) proteins and high pH.

Addition of 8.33% LIS proteins to all HIS/LIS multicomponent gel treatments caused a significant reduction in apparent stress at failure of approximately 50% (Fig. 5). The 8.33% substitution of LIS proteins to the HIS protein

gels had an immediate, weakening effect while 16.67 to 50.00% LIS substitution caused more gradual gel weakening.

Generally, as the percentage of LIS proteins substituted into the HIS protein gels increased, the apparent strain at failure decreased (Fig. 7). A 16.67% LIS protein substitution caused a 12.5% reduction of apparent strain at failure. The higher substituted gels (>8.33% substitution) became less deformable possibly because the LIS proteins do not form a gel, but rather a coagulum (Acton and Dick, 1976) that may interrupt or modify the gelation of the HIS proteins.

D.4. Scanning Electron Micrographs of High Ionic Strength/Low Ionic Strength Protein Gels

In the scanning electron micrographs of the HIS/LIS multicomponent gels (Fig. 8a-d) it appeared that the HIS proteins formed a gel network (as seen in Fig 6a-d) and the LIS proteins formed a coagulum that deposited on the surface and in the interstitial spaces of the HIS protein gel network. The deposited LIS proteins may have increased the surface area for hydrophilic interactions between the proteins and moisture, thus the addition of the 8.33% LIS proteins decreased expressible moisture. Once the sites for HIS/LIS protein and LIS protein/moisture interactions were filled the excess moisture was not held by the gel and expressible moisture (EM) increased.

The addition of LIS proteins to the HIS proteins may have created a filled gel. Three types of multicomponent

Figure 7. Apparent strain at failure of high ionic strength plus low ionic strength soluble protein gels.

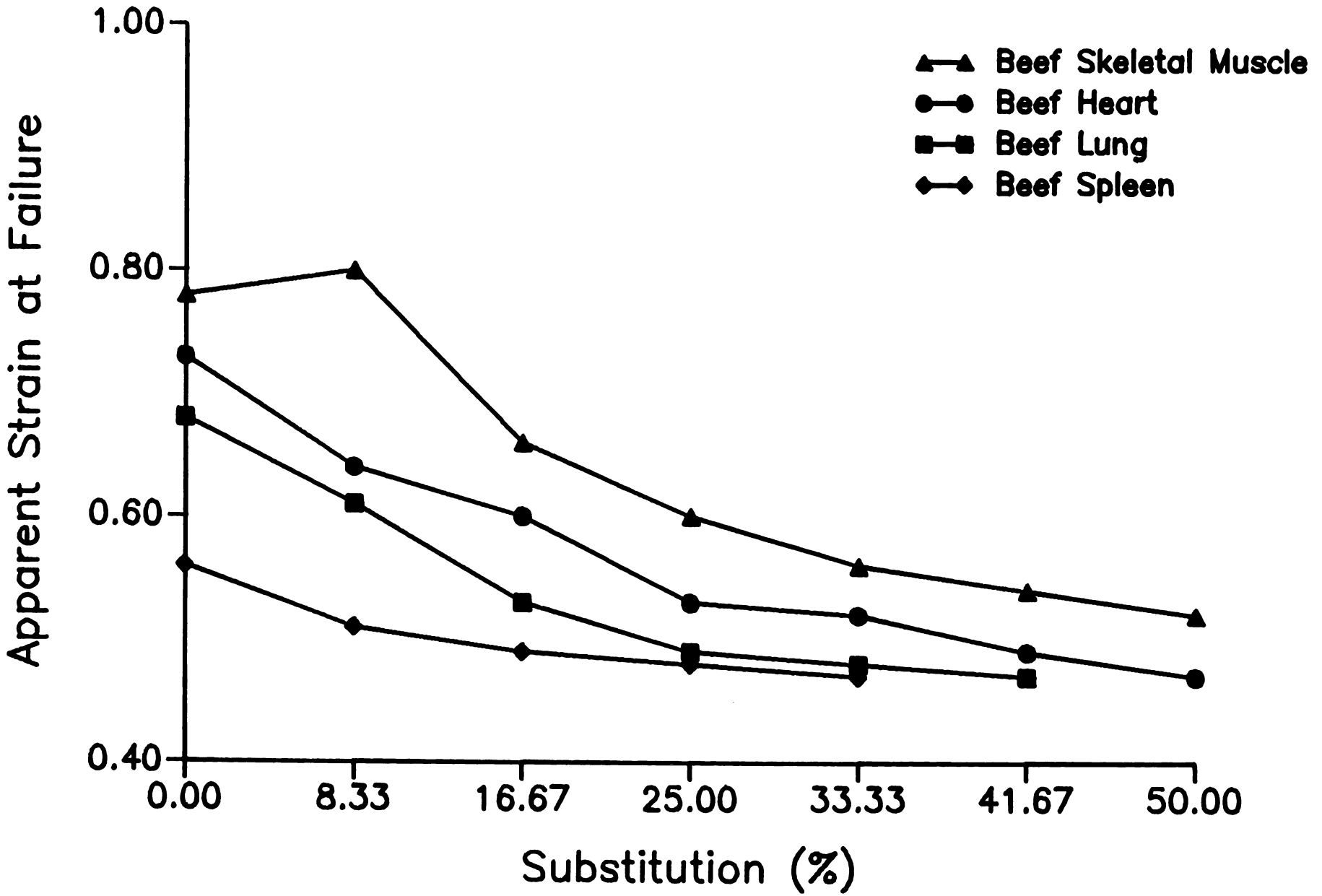

Beef Skeletal Muscle
Beef Heart
Beef Lung
Beef Spleen
Apparent Strain at Failure
1.00
0.80
0.60
0.40
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)

Figure 8. Scanning electron micrographs of high ionic strength soluble plus low ionic strength soluble protein gels.

A = skeletal

B = cardiac

C = lung

D = spleen

A
B
C
D

gels have been defined by Tolstoguzov and Braudo (1983); filled, mixed and complex. Filled gels are formed when the protein gel network is formed by one macromolecule and other components of the system fill the interstitial spaces. Mixed gels are formed when two or more different macromolecules gel independently and do not interact. Complex gels are produced when the components of the system interact to form the gel.

The HIS/LIS proteins appeared not to have formed mixed or complex gels because the LIS proteins did not gel, singly or in conjunction with the HIS proteins, but rather formed a coagulum as described by Acton and Dick (1985). Morris (1985) described a filled gel as a gel matrix with particulate inclusions such as gas bubbles, liquid droplets, ice or fat crystals and/or starch granules. Ma et al., (1990) reported on co-gels of egg white and oat globulin. They found that heating the proteins resulted in the egg white forming a continuous network and the oat globulin was distributed randomly throughout the matrix. Foegeding and Ramsey (1987) increased the lipid content in a gelled meat batter and claimed that a filled gel was formed because of an increase in shear stress to failure and no significant change in strain to failure. Lee and Abdollahi (1981) reported that the firmer the fat used in gelled meat batter, the larger the increase in force to fracture. Morris (1985) reported that "inactive" polysaccharides, as defined as

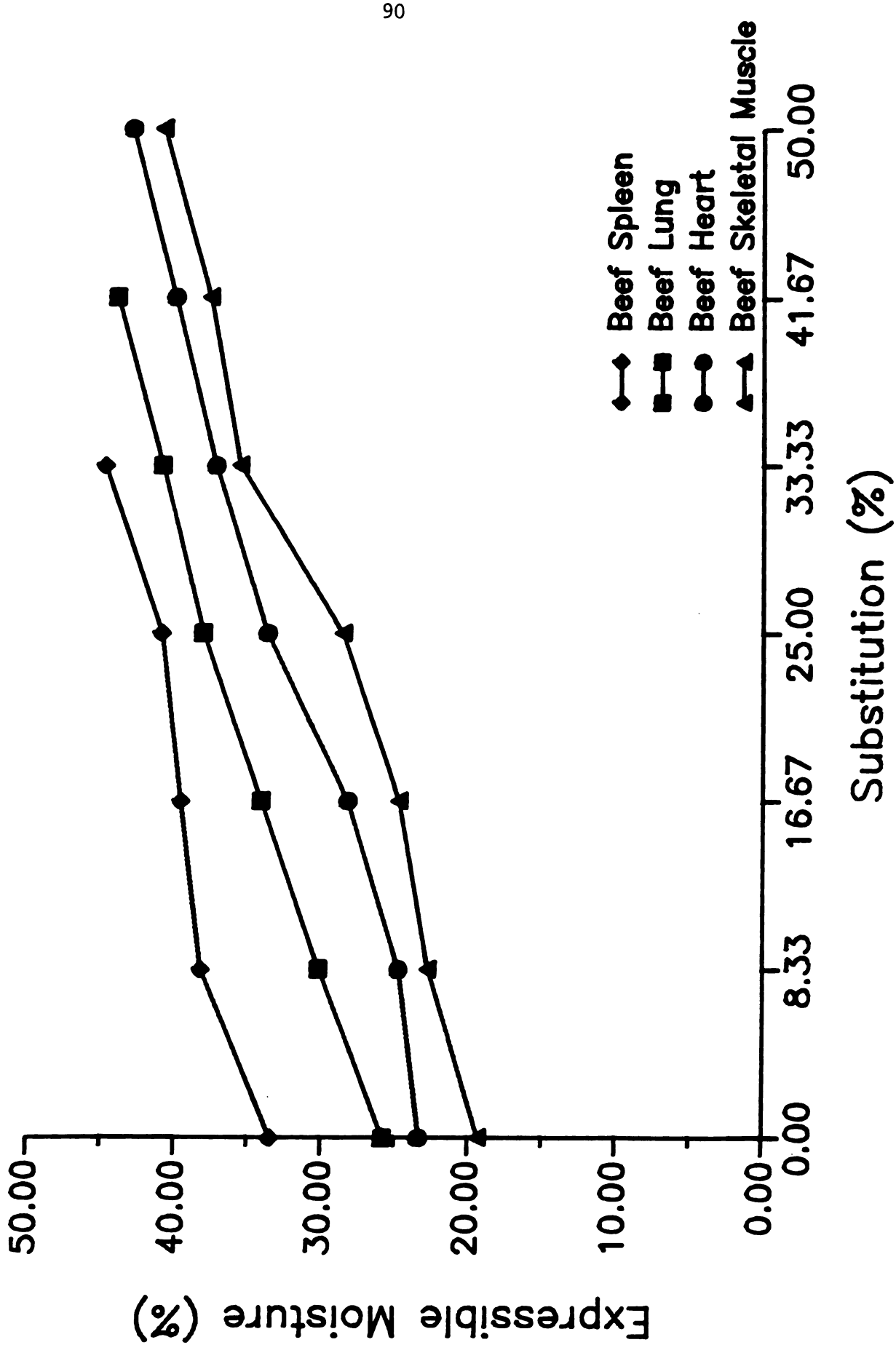
polysaccharides that do not form a gel network, enhance osmotic swelling of gels and reduce water loss. The LIS/HIS gels are probably filled gels with LIS proteins as inactive fillers as no increase apparent stress at failure was measured, rather added LIS proteins formed a soft coagulum that reduced apparent stress at failure.

D.5. High Ionic Strength/Insoluble Protein Multicomponent Gels

The substitution of 16.67% or more of IN proteins into HIS protein gels significantly ($P<0.05$) increased EM (Fig 9). The addition of 16.67% insoluble proteins increased expressible moisture approximately 5%, higher amounts of substituted IN proteins increased EM. The substitution of <16.67% IN proteins may have not been enough to affect the HIS protein network and therefore the IN proteins acted as "inactive" proteins in the HIS gel network and did not increase expressed moisture.

Poulanne and Ruusunen (1981) added isolated thick epimysial tissue (IN protein) from young bull skeletal muscle to comminuted meat products and found that at 60^0 C the tissue swelled and bound water but increasing cooking temperatures decreased the amount of water bound in the protein matrix. Sadowska et al. (1980) increased the amount of connective tissue added to an emulsified product by 100% and 200% (from 5% of total protein to 10% and 15%) and reported an increase in cookout loss. Knight (1988) reported that the role of connective tissue proteins (CTP) in

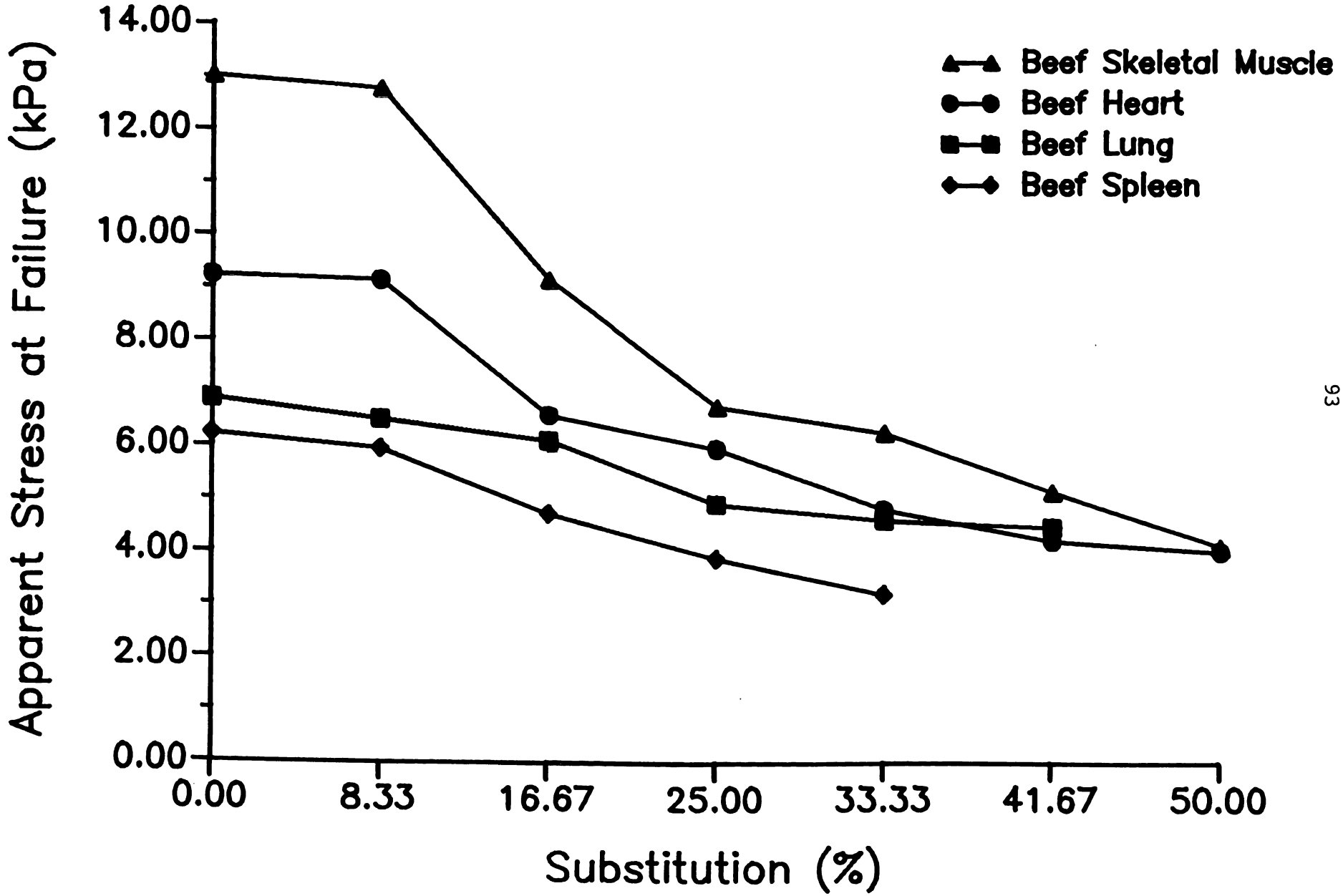
Figure 9. Expressible moisture of high ionic strength plus insoluble protein gels.


Expressible Moisture (%)
50.00
40.00
30.00
20.00
10.00
0.00
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)
Beef Spleen
Beef Lung
Beef Heart
Beef Skeletal Muscle

processed meat products is unclear, but that CTP would bind water if gelatin was produced upon cooking. Marshall and Petrie (1980) using differential scanning calorimetry (DSC) reported that the gelation temperature (T_g) of collagen (IN) was 82.5^0 C - 87.5^0 C. Since processed meat products are currently cooked at temperatures less than T_g, the role of collagen and other CTP for binding water are probably minimal. The substitution of IN proteins in multicomponent gels may have formed filled gels. The IN soluble proteins had not gelled at the final internal cooking temperature of 72^0 C, thus mixed or composite gels could not be formed. The substitution of IN proteins in multicomponent gels may have formed filled gels.

The addition of 16.67% of IN proteins significantly reduced the apparent stress at failure of the skeletal, heart and spleen gels, whereas a 25% substitution was required to significantly reduce the hardness of the lung gels (Fig. 10). The HIS proteins from the various meats do not function equivalently in least concentration endpoint gelation, therefore the structure of the lung HIS protein gels may be less disrupted by IN proteins compared to the other HIS protein gels, although further investigations are required. Apparent stress at failure was reduced approximately 30% by the substitution of IN proteins at the 25% level to the HIS/IN multicomponent gels. Greater

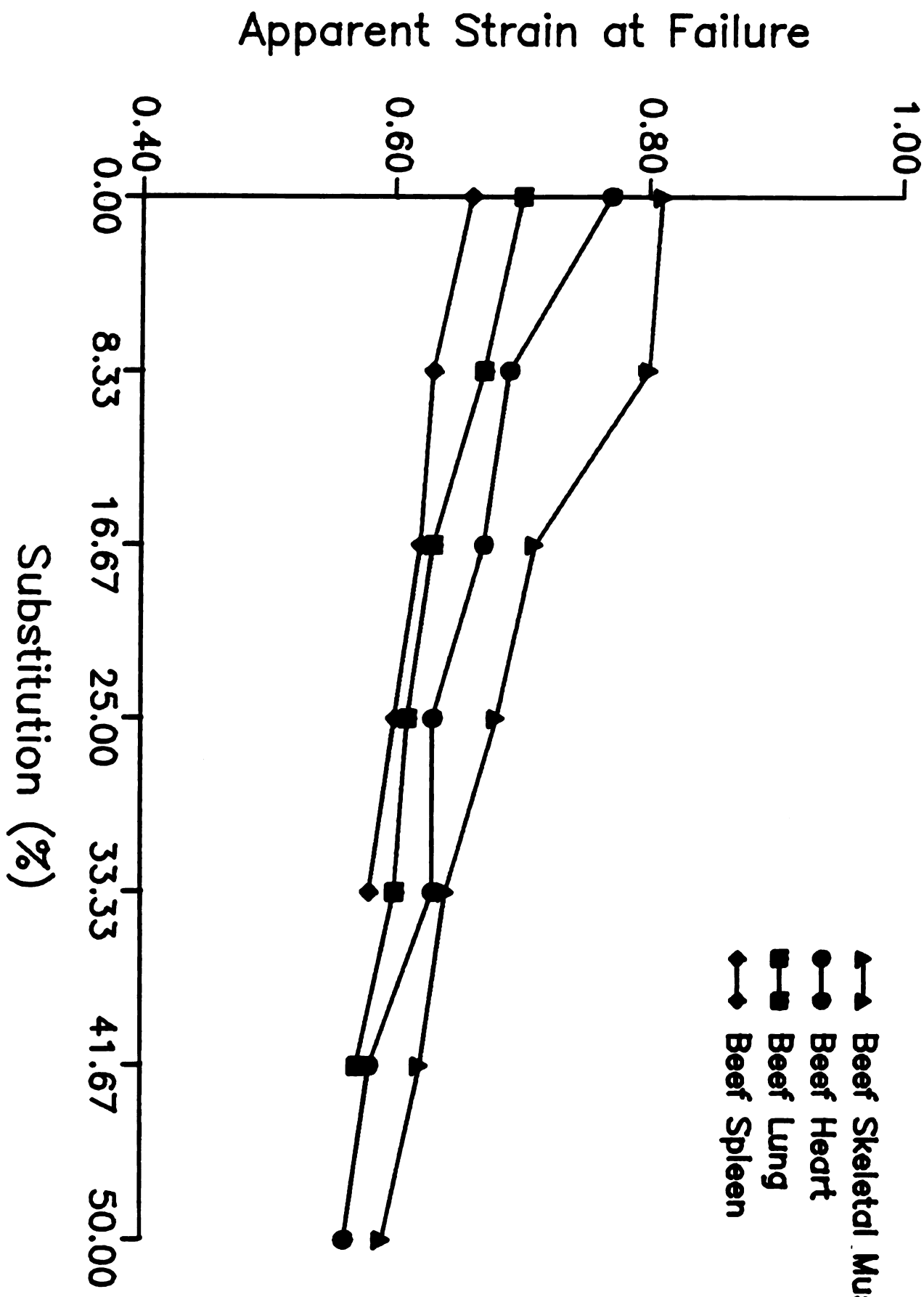
Figure 10. Apparent stress at failure of high ionic strength plus insoluble protein gels.


Apparent Stress at Failure (kPa)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)
Beef Skeletal Muscle
Beef Heart
Beef Lung
Beef Spleen

substitution of IN proteins into the HIS/IN protein gels resulted in weaker gels. The addition of the IN proteins within the HIS/IN gel matrix were less detrimental to gel apparent stress at failure compared to the addition of LIS proteins to HIS/LIS multicomponent gels. The addition of IN proteins may have filled the interstitial spaces and were "harder" than the LIS proteins and thus decreased apparent stress at failure less. Lee and Abdollahi (1981) reported that the addition of harder fats acting as fillers in processed meat products increased their force to fracture. Schmidt (1987) reported an increase in firmness when connective tissue was added to emulsion products, but Sadowska et al. (1980) reported that added connective tissue at greater than 5% of the total protein reduced firmness by 10-23%. Addition of stroma proteins will increase hardness and decrease rubbery texture common in low fat matrices such as frankfurters and bologna (Jones, 1984).

In contrast to the apparent stress at failure, the apparent strain at failure (Fig. 11) was significantly lower than the control for the lung multicomponent gel at 8.33% substitution and significantly lower for the skeletal, heart and spleen multicomponent gels at 16.67% substitution. Apparent strain at failure (cohesiveness and/or deformability) was lower for the 8.33% substituted lung gel while the apparent stress at failure (hardness) was not significantly reduced. Therefore it appears that IN proteins have a more negative effect on gel cohesiveness than gel

Figure 11. Apparent strain at failure of high ionic strength plus insoluble protein gels.


Apparent Strain at Failure
0.40
0.60
0.80
1.00
0.00
8.33
16.67
25.00
33.33
41.67
50.00
Substitution (%)
Beef Skeletal Muscle
Beef Heart
Beef Lung
Beef Spleen

hardness. Insoluble proteins may disrupt the HIS heat-induced protein matrix thus reducing gel cohesiveness.

D.6. Scanning Electron Micrographs of High Ionic Strength/Insoluble Multicomponent Gels

The addition of IN proteins (Fig 12a - d) visibly added more coarse strands or were deposited as globular masses to the HIS protein gel network. The coarse strands may interrupt the heat-induced HIS protein gel network and increase the moisture expressed and decrease the gel strength. As the coarse insoluble protein strands interrupted the HIS protein gel structure, at the HIS/IN protein junctions, moisture trapped during cooking could have leaked during centrifugation. The IN proteins disrupting the HIS protein matrix could have created a non-continuous HIS protein matrix thus reducing the gels' strength. Smith and Brekke (1985) related structurally compact and globular chicken myofibrillar protein gels to high gel strength and increased gel syneresis.

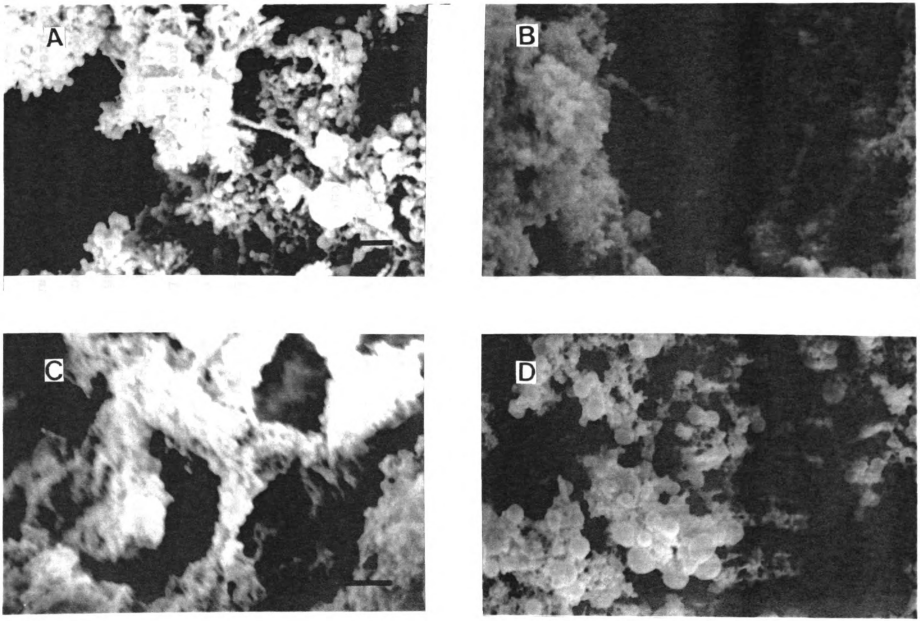
Figure 12. Scanning electron micrographs of high ionic strength plus insoluble protein gels.

A = skeletal

B = cardiac

C = lung

D = spleen


A
B
C
D

D.7. Correlations of Percentage Myosin and Gel Test Parameters

Results indicated that HIS protein fractions from various sources do not function equivalently. Myosin has been reported to be the protein most responsible for protein gelation (Schmidt et al., 1981). The percentage myosin in the HIS protein fraction for semitendinosus muscle was 50.7% (Lowey and Risby, 1971) and 47.9, 37.6 and 22.6% for beef heart, lung and spleen, respectively (Nuckles et al., 1990). Therefore correlations between gel functional tests and percentage myosin in the HIS protein fraction were calculated.

The quantity of myosin in the HIS protein fraction was strongly correlated to gel apparent stress at failure ($r=0.86$), apparent strain at failure ($r=0.80$) and expressible moisture ($r=-.82$). Therefore the percentage myosin in the HIS protein fraction may help explain gel functionality. Further work in model gel and meat systems needs to be performed.

E. CONCLUSIONS

Results indicate that the gel forming abilities of HIS protein fractions from skeletal, cardiac and smooth muscles are not equivalent and thus functionality of muscle tissues cannot be compared solely on the basis of the size of the HIS protein fraction. The addition of LIS and IN proteins affected HIS protein gel functionality. It may be possible to predict the gel strength and other functionalities of

muscle tissues based on the amount of the LIS, HIS and IN protein fractions and the amount of myosin in the HIS protein fraction. These results suggest a new method is needed for measuring bind constants or relative functionalities of meat ingredients used in least cost formulation calculations by meat processors.

VI. Study 3: Effect of Meat and Meat By-product Protein Fractions on Functional Properties in Model System Frankfurters

A. Introduction

Reformulation or designing of meat products to obtain high cooking yields and desired textures is an empirical process that often leads to many product failures (Knight, 1988). Product failures are caused in part by the different types and amounts of protein and fat in the raw meat ingredients and the lack of understanding of meat protein functionality. Meat by-product protein functionality is not well understood. Venegas et al. (1988) and Oliveros et al. (1982) reported on emulsion and water-holding capacities of by-products in model systems. They suggested each by-product would individually contribute different properties to a meat product, therefore utilization of by-product mixtures could be beneficial to water and fat-holding properties and textures of meat products.

Randall and Voisey (1977) studied the effects of meat protein fractions on textural characteristics of meat emulsions. Their results suggested that salt soluble proteins formed the cooked emulsion matrix and connective tissue proteins added hardness. Knight (1988) studied three protein fractions from minced pork shoulder and found that the salt soluble protein (SSP) fraction formed gels, the insoluble myofibrillar protein fraction acted as a filler in the SSP gel. The role of the connective tissue proteins was

unclear, but appeared to contribute to gelation. McKeith et al. (1988) reported on characteristics of beef, pork and beef by-product surimi. Results showed that beef, pork and beef heart SSP formed gels that were comparable to a commercial fish surimi. The effects of sarcoplasmic proteins on protein gelation or meat product texture are not well understood.

Nuckles et al. (1990) quantitated meat by-product protein, percentage of three protein fractions [low ionic strength soluble (LIS) high ionic strength soluble (HIS) and insoluble (IN)], percentage myosin and the actin:myosin ratio of several by-products (pork liver, pork lung lobes, beef heart, beef lung lobes, beef spleen). By-products were generally higher in moisture and lower in fat than skeletal muscle, varied in their percentage of the three protein fractions and contained less myosin than skeletal muscle. Substitution of LIS and IN protein fractions from meat by-products caused negative effects on functional properties (increased expressible moisture, lower apparent stress and strain at failure) of heat-induced HIS gels (Nuckles et al., 1990b). The effects of substituting by-product proteins for skeletal HIS proteins in processed meats are not understood.

The objectives of this study were to determine the effects of beef by-product (heart, lung, spleen) protein fractions [low ionic strength soluble (LIS), high ionic strength soluble (HIS), insoluble (IN)], the percentage of myosin in the formulation and the myosin:actin ratio of the

HIS protein fraction on beef skeletal muscle frankfurters. The frankfurters were evaluated using functional tests (cook yield, severe reheat yield, apparent stress and strain at failure, apparent tensile strength).

In the first experiment, the percentages of high ionic strength (HIS) proteins were varied in the frankfurter formulations by addition of by-products to determine a minimum percentage of HIS proteins required so that the results of a functional test (severe reheat yield) would not be significantly lower when the formulation contained a different percentage HIS proteins. Experiment 2 formulations contained a constant percentage of HIS proteins to determine the effect of substituting LIS and IN proteins for HIS protein on model system frankfurter functionality. Nuckles et al., 1990b tested beef skeletal muscle and by-products protein fractions (LIS, HIS, IN) in a model gelation system. Correlations were performed to test if results of the gelation model system predicted results in the frankfurters.

B. Materials and Methods

B.1. Materials

Beef semimembranosus muscle (beef skeletal muscle), beef by-products (lung lobes, spleen and heart) and beef fat were used in this study. The beef lung lobes and hearts were obtained from 9 market weight steers slaughtered at the Michigan State University Meat Laboratory. Beef spleen and fat were purchased from Ada Beef Co., Ada, MI and the beef

semimembranosus muscle was purchased frozen from Monfort of Colorado, Greeley, CO. By-products were removed within 1 hr after exsanguination, vacuum packaged (2.5 mil polyethylene) in 500 g aliquots and frozen until used within 1 month.

B.2. Methods

B.2.a. Meat Batter Formulations

Meat batters were prepared using a silent cutter as described by Smith and Brekke (1984), except that the batter was stuffed into 50 ml conical centrifuge tubes, capped and heated in a 75^0 C water bath to an internal temperature of 72^0 C.

Meat batters were formulated to 56% moisture, 30% fat, 12% protein and 2% salt. Meat batters were formulated to contain different percentages of protein fractions [low ionic strength soluble (LIS), high ionic strength soluble (HIS) and insoluble (IN)]. Calculation of percentage protein fractions, myosin and actin:myosin ratio in the formulations was based on results reported by Nuckles et al. (1990a) and Lowey and Risby (1971). In the first experiment, meat batters were formulated to contain HIS proteins at 25 - 45% of the total protein by substituting spleen or lung for beef skeletal muscle. In the second experiment, meat batters were formulated to a constant percentage of HIS proteins while the percentages of LIS or IN proteins were varied (27 - 38%) by substituting beef heart, spleen or lungs singly or in combination for beef skeletal muscle.

B.2.b. Meat Batter Evaluation

Proximate composition (fat, protein, and moisture) of the raw batter and frankfurters were determined in triplicate by AOAC (1984) procedures. Raw batter and frankfurter pH was determined in triplicate by blending 10 g of batter with 100 g of double distilled water on setting 6 for 30 sec using a Polytron homogenizer (Brinkman Instruments Co., Westbury, N.Y.). The pH was measured using a Corning 145 pH meter.

Cook yield and severe reheat yield were determined as described by Nuckles et al. (1990a). Apparent shear stress and strain at failure (Diehl et al., 1979) of the model system frankfurters were measured at 1^0 C on 1.5 cm x 1.5 cm central cores of the frankfurter using an Instron (Model 4202, Canton, MA) at a crosshead speed of 10 mm/min with a 50 N compression cell as described by Nuckles et al. (1990).

Apparent tensile strength was measured on whole frankfurters using a TR-5 Texturepress with a ST Thin Slice Tensile Test Cell attachment and Texturerecorder (Food Technology Corp., Rockville, MD) at a speed of 4 and range X 1/10. Frankfurter (1^0 C) weights were recorded prior to tensile strength testing and loaded on the cell perpendicular to the cell break-line. The force (N/g) to break the frankfurters was calculated. Twelve frankfurters per formulation (4 from each replicated formula) were tested.

C. Replications and Statistics

The statistical design was completely randomized. All data represent a mean value of at least triplicate analyses. Basic statistics, 2-way analysis of variance (ANOVA), treatments X replications, and correlations were performed using M-STAT (1988). Correlations were determined using amount of LIS, HIS or IN proteins, myosin percentage and actin:myosin ratio (Nuckles et al., 1990a), gel apparent stress and strain at failure (Nuckles et al., 1990b) and the frankfurter results. Mean separations were performed using Tukey's test.

D. Results and Discussion

D.1. Formulations containing different percentages of HIS proteins

The proximate composition (fat, moisture and protein) of the raw meat batters formulated with different amounts of HIS proteins from beef skeletal muscle and by-products were not significantly different. The pH of the raw meat batters and the frankfurters were significantly different from each other (5.8 and 6.2, respectively), but the pH within the treatments was not significantly different ($P < 0.05$).

As the protein fraction composition of the formulation was varied, frankfurter proximate composition (Table 9) changed due to differences in fat and moisture loss during cooking (Table 10). As the percentage of low ionic strength soluble (LIS) protein increased and the percentage myosin decreased due to substitution of spleen for skeletal meat in

Table 9 - Cooked proximate analysis of frankfurters formulated with different by-products to alter the percentage high ionic strength (HIS), low ionic strength (LIS) and insoluble (IN) protein fractions.

Treatment	Protein[1] Fraction (%) LIS	HIS	IN	Myosin[1] in Formula (%)	Cooked Proximate Analysis Protein (%)	Fat (%)	Moisture (%)
Control, 100% skeletal	26	45	29	50.7[a]	12.3[a]	30.6[a]	55.1[c]
Skeletal 81%, spleen 19%	31	41	28	45.4[b]	12.6a	23.2[c]	62.2[b]
Skeletal 56%, spleen 44%	38	35	27	38.3[d]	12.5[a]	22.9[c]	62.4[b]
Skeletal 35%, spleen 65%	44	30	26	32.4[e]	12.3[a]	18.8[d]	66.5[a]
Skeletal 14% spleen 86%	50	25	25	26.5[f]	12.0[a]	18.7[d]	66.7[a]
Skeletal 85% lung 15%	27	40	33	48.7[a]	12.5a	31.3[a]	53.8[e]
Skeletal 70% lung 30%	29	35	37	46.8[b]	12.3[a]	31.9[a]	53.6[e]
Skeletal 57% lung 43%	30	30	40	45.1[b]	12.5[a]	30.9[a]	54.4[d]
Skeletal 43% lung 57%	32	25	43	43.2[c]	12.2[a]	28.9[b]	56.7[c]
SEM				1.1	0.3	1.0	2.1

[1]calculated from Nuckles et al. (1990)

[a,b,c,d,e,f]Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

the formulation, the percentage fat retained in the frankfurter decreased (fat cook-out increased), and the percentage moisture in the frankfurter increased compared to the raw batter. Venegas et al. (1988) and Oliveros et al. (1982) reported that meat by-products, especially spleen, containing large amounts of LIS proteins had good water holding capacity.

Cook yield was significantly lower ($P < 0.05$) as spleen was substituted for beef skeletal muscle at percentages greater than or equal to 44% of the formula. The frankfurters contained significantly less fat than the meat batters (Table 9), thus as the percentage of LIS proteins increased and myosin decreased, fat cooked out of the frankfurters and reduced cook yield.

Cook yield decreased as the concentration of IN proteins increased and the percentage of total protein as myosin decreased (Table 10). A 15% substitution of beef lung (an increase of 4% insoluble proteins) caused approximately a 20% decrease in cooked yield. Higher amounts of substitution of skeletal muscle with lung did not significantly reduce cooked yield. When the percentages of HIS proteins were equivalent, the formulas containing 44% spleen (higher LIS protein concentration) had lower cook yields and myosin contents than the lung (high IN protein concentration) formulas.

Severe reheat yield (Table 10) was adversely affected by the addition of by-products. As the percentage of HIS

Table 10 - Cook yield, severe reheat yield and textural characteristics of frankfurters formulated with different by-products to alter the high ionic strength (HIS), low ionic strength (LIS) and insoluble (IN) protein fractions.

Treatment	Cook Yield (%)	Severe Reheat Yield (%)	Apparent Stress at Failure (kPa)	Apparent Strain at Failure	Tensile Strength (N/g)
Control, 100% skeletal	98.15[a]	80.89[a]	104.8[a]	0.98[a]	0.32[a]
Skeletal 81%, spleen 19%	95.77[a]	59.33[b]	42.8[c]	0.77[c]	0.19[c]
Skeletal 56%, spleen 44%	86.58[b]	48.78[d]	19.3[e]	0.61[d]	0.13[d]
Skeletal 35%, spleen 65%	76.35[c]	48.65[d]	15.8[e]	0.60[d]	<0.10[e]
Skeletal 14% spleen 86%	70.07[d]	48.44[d]	10.4[f]	0.55[e]	<0.10[e]
Skeletal 85% lung 15%	78.65[c]	59.88[b]	79.8[b]	0.96[a]	0.34[a]
Skeletal 70% lung 30%	77.77[c]	59.78[b]	48.4[c]	0.88[b]	0.23[b]
Skeletal 57% lung 43%	77.64[c]	59.77[b]	25.7[d]	0.77[c]	0.25[b]
Skeletal 43% lung 57%	76.93[c]	52.88[c]	23.4[d]	0.77[c]	0.24[b]
SEM	1.02	2.31	0.2	0.07	0.04

[a,b,c,d,e,f] Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

proteins and percentage myosin decreased and the percentage of LIS protein increased due to the addition of spleen the severe reheat yield was significantly reduced approximately 30% compared to the control frankfurters. HIS protein concentration of less than 35% in the skeletal meat/spleen formulas did not cause a significant reduction in severe reheat yield compared to formulas with 35% HIS proteins.

The substitution of 30% or greater lung into the beef skeletal muscle formulas which resulted in an increase in IN proteins and a decrease in HIS proteins and myosin, caused a reduction in severe reheat yield compared to the control. As with the spleen formulas, 35% HIS proteins in the formulation did not give a significantly different severe reheat yield compared to 25 or 40% HIS proteins.

An increase in LIS proteins in the formulation had a more severe effect on reheat than an increase in IN protein concentration. LIS proteins at 38% of the total 12% protein had significantly lower cook yields than IN proteins at 43% of the total protein.

The addition of 19% spleen (increase of 4% LIS proteins and a 5% reduction of percentage myosin in formulation) or addition of 15% lung (increase of 4% IN proteins but no significant change in percentage myosin in the formulation) reduced apparent stress at failure of the frankfurters by more than 50% compared to the 100% skeletal meat frankfurter (Table 10). The substitution of higher amounts of spleen or lung, thus increasing the LIS or IN protein

fraction and reducing percentage myosin of the total protein in the meat batter, significantly reduced ($P < 0.05$) apparent stress at failure. The addition of lung to the formulations was less detrimental to the apparent stress at failure compared to the addition of spleen. Substitution of 35% lung or 19% spleen resulted in comparable apparent stress at failure of the frankfurters.

Apparent strain at failure or deformability of the frankfurters (Table 10) was significantly reduced by the addition of spleen (increase in amount of LIS protein fraction) to the formulation. The addition of lung to the frankfurters had a less pronounced effect upon apparent strain at failure compared to the addition of spleen. Substitution of 19% spleen into the formulation had the same effect as a 43% lung substitution. Greater than 15% lung had to be substituted into the skeletal meat formulas before apparent strain at failure was significantly ($P<0.05$) reduced.

Apparent tensile strength of the cooked meat batters was reduced by both the addition of spleen or greater than 15% lung to the formulation (Table 10). A 19% substitution of spleen into the beef skeletal muscle formulation (a 4% increase of the LIS protein fraction and a 5% decrease in percentage myosin) caused approximately a 40% reduction in tensile strength. The substitution of 30% lung (an 8% increase in the IN protein fraction and 4% decrease in percentage myosin) compared to the control caused

approximately a 30% reduction in tensile strength. The tensile strength for the skeletal/lung formula containing 35% HIS proteins was approximately 75% stronger than the skeletal/spleen formula containing the same amount of HIS proteins. Substitution of 65% or greater of spleen into the formulation resulted in frankfurter tensile strength < 0.10 N/g.

Nuckles et al. (1990b) reported that HIS proteins from various meat sources do not function equivalently in gelation tests and that the addition of LIS or IN proteins to HIS protein gels increased gel expressible moisture and reduced gel strength. They proposed that gel functionality was related to HIS protein fraction size and composition (% myosin or actin:myosin ratios) and the influence of LIS and IN proteins. The present research further suggests that substitution of LIS and IN proteins for HIS proteins by the addition of by-products modify frankfurter functionality by decreasing severe reheat yield and frankfurter strength as measured by apparent stress and strain at failure and apparent tensile strength.

D.2. Formulations with 35% HIS proteins and different amounts of LIS and IN proteins

Formulations substituted with spleen or lung for skeletal meat containing 35% HIS proteins of the total 12% protein did not have significantly different severe reheat yields when compared to formulating to a lower percentage (25 or 30%) HIS proteins. Therefore formulations with 35%

HIS proteins were tested to determine the effects of LIS and IN proteins on frankfurter functionality.

The proximate composition of the raw meat batters formulated with 35% of the total protein as HIS proteins from by-products were not significantly different. The frankfurter proximate compositions (Table 11) and cook yield (Table 12) varied with the amounts of LIS and IN proteins and the percentage myosin in the meat batters. The substitution of 44% beef spleen increased LIS proteins by 10% and increased fat loss as indicated by lower cook yield and higher moisture in the frankfurters compared to the raw meat batters. A 30% lung substitution, an increase in IN proteins by 8%, increased moisture loss as indicated by lower cook yield and higher fat in frankfurters compared to raw meat batters. A substitution of 44% heart, which was a 12% increase in LIS proteins, lowered cook yield but did not cause a significant change in frankfurter proximate composition compared to the control. This result suggests that both fat and moisture were lost during cooking.

The skeletal/heart formula contained a higher percentage LIS proteins but myosin percentage did not significantly differ from the control. These results suggested that not only percentage of the three protein fractions influenced the frankfurters, but that the percentage myosin and myosin:actin ratios also affected cook yield. Both beef heart and beef semimembranosus muscle contain an actin:myosin ratio of 5.0 (Nuckles et al., 1990;

Table 11 - Cooked proximate analysis of frankfurters formulated to contain 45% or 35% of high ionic strength soluble (HIS) proteins by substitution of by-products.

Treatment	Protein[1] Fraction (%) LIS	HIS	IN	Myosin[1] in Formula (%)	Cooked Proximate Analysis Protein (%)	Fat (%)	Moisture (%)
Control, 100% skeletal	26	45	29	50.7[a]	12.3[a]	30.6[b]	55.1[d]
Skeletal 56%, heart 44%	38	35	27	49.5[a]	12.2[a]	30.2[b]	55.2[d]
Skeletal 70%, lung 30%	28	35	37	46.8[b]	12.3[a]	31.9[a]	53.6[f]
Skeletal 56%, spleen 44%	36	35	29	38.3[e]	12.3[a]	23.9[e]	61.4[a]
Skeletal 60, heart 30%, lung 10%	27	35	38	48.6[a]	12.2[a]	30.9[b]	54.7[e]
Skeletal 55%, heart 25%, spleen 20%	31	35	34	44.4[c]	12.5[a]	30.8[b]	54.4[e]
Skeletal 60%, spleen 30%, lung 10%	36	35	29	41.0[d]	12.5[a]	25.8[d]	59.4[b]
Skeletal 60%, heart 20%, spleen 10%, lung 10%	30	35	35	46.0[b]	12.3[a]	31.8[a]	53.6[f]
Skeletal 60%, spleen 20%, heart 10%, lung 10%	32	35	33	43.5[c]	12.3[a]	27.8[c]	57.5[c]
SEM				1.1	0.1	1.3	0.7

[1]calculations based on Nuckles et al. 1990

[a,b,c,d,e,f]Means within columns followed by the same letter do not differ significantly ($P<0.05$).

Risby and Lowey, 1971, respectively), whereas spleen and lung contain a actin:myosin ratio of 4.5 and 3.2, respectively (Nuckles et al., 1990). The percentage myosin in the spleen and lung formulations was significantly ($P<0.05$) lower than the control, skeletal/heart and skeletal/heart/lung formulas (Table 11). The frankfurters produced from skeletal meat plus 2 or 3 by-products showed that increasing amounts of LIS proteins and decreased percentage myosin in the formulation caused fat loss. Increasing amounts of IN proteins and decreasing myosin percentage in the formulations favored moisture loss.

Severe reheat yield (Table 12) was reduced by the addition of spleen or lung, whereas the addition of heart did not significantly reduce severe reheat yield. Severe reheat yield appeared to be influenced by percentage myosin, actin:myosin ratio and percentage of LIS or IN proteins in a formulation. Low ionic strength proteins exerted a more negative effect upon severe reheat yield than IN proteins. Skeletal 60/spleen 44% and skeletal 60/spleen 30/lung 10% formulas contain 10% more LIS proteins but no more IN proteins compared to the control and exhibited approximately 30% lower severe reheat yields. Formulas containing 6 -9% more IN proteins had 5 - 14% lower severe reheat yields.

Apparent stress at failure (Table 12) decreased upon the addition of by-products to the formulations. Substitution of heart affected frankfurter strength the least compared to substitution of spleen and lungs. Heart

Table 12 - Cook yield, severe reheat yield and textural characteristics of frankfurters formulated to contain 45% or 35% of high ionic strength soluble (HIS) proteins by substitution of by-products.

Treatment	Cooked Yield (%)	Severe Reheat Yield (%)	Apparent Stress at Failure (kPa)	Apparent Strain at Failure	Tensile Strength (N/g)
Control, 100% skeletal	98.15[a]	82.66[a]	103.6[a]	0.94[a]	0.37[b]
Skeletal 56%, heart 44%	85.33[b]	80.89[a]	80.2[b]	0.92[a]	0.41[a]
Skeletal 70% lung 30%	87.13[b]	78.22[b]	50.8[d]	0.88[b]	0.29[d]
Skeletal 56% spleen 44%	86.22[b]	50.32[e]	20.0[f]	0.61[d]	0.13[g]
Skeletal 60, heart 30%, lung 10%	86.55[b]	78.83[b]	82.0[b]	0.93[a]	0.42[a]
Skeletal 55%, heart 25%, spleen 20%	85.97[b]	75.87[b]	44.3[e]	0.62[d]	0.32[c]
Skeletal 60% spleen 30%, lung 10%	85.12[b]	55.34[d]	42.6[e]	0.64[d]	0.19[f]
Skeletal 60% heart 20%, spleen 10%, lung 10%	85.67[b]	68.93[c]	55.1[c]	0.70[c]	0.32[c]
Skeletal 60% spleen 20%, heart 10%, lung 10%	84.88[b]	58.22[d]	41.7[e]	0.62[d]	0.24[e]
SEM	0.33	1.23	1.1	0.04	0.02

[a,b,c,d,e]Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

increased the strength of formulas containing 2 or more by-products compared to the comparable formulas without heart. Frankfurter apparent strain at failure did not significantly differ from the control when 44% heart was substituted into the formula even though the percentage LIS proteins increased. The percentage myosin in the formula did not change significantly. The addition of lung or spleen reduced frankfurter cohesiveness (apparent strain at failure) by 6% and 35%, respectively (Table 12). Spleen increased the percentage LIS proteins and lung increased the percentage IN proteins in the formula. Both by-products contain less myosin and a lower myosin:actin ratio in the HIS protein fraction compared to heart and beef skeletal muscle. The addition of heart to the frankfurters increased tensile strength compared to the control. The negative effect, lower apparent tensile strength, from the addition of spleen and/or lung to the formulation was less when heart was also added (Table 12). The skeletal/heart/spleen formula which contains 5% less LIS proteins and 6% more myosin than the skeletal/spleen formula exhibited 2.5 times the tensile strength of the skeletal/spleen formula. The skeletal/heart/lung formula which contains 11% less LIS and 11% more IN proteins and 2% more myosin than the skeletal/lung formula, exhibited a tensile strength 1.4 times stronger than the skeletal/lung formula. Addition of lung was less detrimental to frankfurter tensile strength than spleen.

D.3. Effect of HIS proteins on model system frankfurters

Frankfurter water- and fat holding capacity, severe reheat yield, apparent stress and strain at failure and apparent tensile strength were all correlated 0.70 or greater to the amount of high ionic strength (HIS) proteins, percentage myosin in the HIS proteins and actin:myosin ratios in the HIS protein (Table 13). Schmidt et al. (1981) reported that myosin is the myofibrillar protein (HIS) most responsible for gelation. Myosin forms a heat-induced gel that is irreversible, has high water-binding capacity and strong elastic properties. Yasui and Ishioroshi (1980) reported that F-actin enhanced the gelation of myosin.

Cook yield was not highly correlated to HIS protein concentration, myosin percentage or the actin:myosin ratio. HIS proteins from various sources do not function equivalently (Nuckles et al., 1990b) therefore equal percentages of HIS proteins in the frankfurter formulations are not capable of equivalent water- and fat holding. Also the centrifuge tubes used to cook the frankfurters are impervious to air and moisture, unlike frankfurter casings, therefore moisture and fat are more readily retained as part of the frankfurter.

D.4. Effect of LIS proteins on model system frankfurters

Cook yield of the frankfurters was not highly correlated (-0.60) to amount of LIS proteins in the meat batters. Cook yields were not significantly different even though the percentage of LIS proteins in experiment 2

Table 13 - Correlation coefficients between meat by-product (LIS), insoluble (IN) and high ionic strength (HIS) protein fractio and functionality tests of model system frankfurters.

	Water-holding cap.[2]	Fat hold. cap.[3]	Cook yield	Severe reheat yield	Frank-furter apparent stress at failure	Frank-furter apparent strain at failure	Tensile strength
Amt. of LIS proteins[4]	0.86	-0.92	-0.60	-0.96	-0.93	-0.77	-0.96
Amt.[4] of IN proteins	-0.40	-0.45	-0.31	-0.40	0.64	0.30	0.30
Amt.[4] of HIS proteins	0.92	0.93	0.24	0.79	0.80	0.78	0.90
Myosin[5] %	0.90	0.88	0.22	0.80	0.70	0.82	0.84
Actin:myosin ratio[6]	0.76	0.74	0.31	0.71	0.71	0.71	0.78

[1]Results from Nuckles et al. (199X)
[2]Water-holding capacity
[3]Fat holding capacity
[4]Amount of proteins
[5]From Lowey and Risby (1971); Nuckles et al. (1990)
[6]Calculated from Nuckles et al. (1990)

formulas varied by 10%. This result may be attributed to the moisture retention (but fat loss) of the frankfurters during cooking, as shown by high moisture and lower fat percentages of experiment 1 spleen containing formulas. This result may also explain why LIS proteins were positively correlated to water-holding capacity (0.86) and negatively correlated to fat holding capacity (-0.92). Severe reheat yield was highly negatively correlated (-0.96), which indicated that as amount of LIS proteins in the frankfurters increased, severe reheat yield decreased. Frankfurter apparent stress and strain at failure and tensile strength were highly negatively correlated with the quantity of LIS proteins in the formulation. The strength of the frankfurters decreased as the amount of LIS proteins in the meat batter increased. These results agree with results reported by Nuckles et al. (1990).

D.5. Effect of IN proteins on model system frankfurters

The amount of insoluble (IN) proteins was not highly correlated with the parameters measured on the frankfurters (Table 13). The amount of IN proteins in the formulations, which was not highly correlated to any parameter measured, was most highly correlated (0.64) to frankfurter apparent strain at failure. The low correlations suggest that IN protein amounts may act as fillers (rather than binders) in the meat batters and do not stabilize or destabilize the raw batters. Nuckles et al. (1990b) reported that IN proteins act as fillers in HIS protein gels. It may be necessary for

connective tissue proteins (IN) to gel to influence the functional properties to frankfurters. Currently processed meat products are not cooked to temperatures sufficient to gel the connective tissue proteins (Knight, 1988).

D.6. Correlations of model system gelation and frankfurters

The apparent stress and strain at failure of heat-induced multicomponent gel prepared with HIS and LIS proteins (Nuckles et al., 1990b) were highly correlated to frankfurter apparent stress and strain at failure (r=0.90 and 0.91, respectively). Results indicate that the LIS multicomponent gelation system used by Nuckles et al. (1990) predicted the functionality of LIS proteins in model system frankfurters.

The apparent stress and strain at failures of heat-induced gels prepared with HIS and IN proteins measured by Nuckles et al. (1990b) were highly positively correlated to the apparent stress (0.88) and strain (0.91) at failure for the frankfurters. The HIS gel apparent stress and strain at failure (Nuckles et al., 199X) were highly correlated to the measured apparent stress (0.95) and strain (0.93) of the frankfurters. Expressible moisture (EM) of the protein gels (Nuckles et al, 1990b) and frankfurter reheat yields were negatively correlated (-0.73). Gel EM increased and frankfurter reheat yield decreased by losing moisture or fat during reheating. The results suggest that the model gel system adequately predicted the frankfurters results.

E. Conclusions

The results indicate that the proportions of the protein fractions (LIS:HIS:IN) and the percentage myosin and/or myosin:actin ratio in the meat batters were related to the cook and reheat yields, fat and water-holding capacity, apparent stress and strain at failure and tensile strength of the frankfurters.

The quantity of the HIS proteins was the most important factor to provide the most functionality to the model system frankfurters. The LIS and IN proteins were detrimental to the model system frankfurters. Low ionic strength (LIS) and IN proteins acted like fillers in cooked frankfurters. The LIS proteins decreased fat-holding of the raw meat batters during cooking, as exhibited by the cooked frankfurters being higher in moisture and lower in fat than the raw batters. Both the LIS and IN proteins were detrimental to severe reheat yield, with IN proteins being less detrimental to frankfurter production.

The protein gelation system described by Nuckles et al. (199b) predicted the protein functionality in the by-product substituted model system frankfurters. These results suggest that protein gelation in model systems can be used by processors to predict protein functionality in meat products. Results also suggest that bind values currently used by meat processors to formulate meat products may be improved by incorporating additional information about raw materials such as the percentage LIS, HIS and IN protein

fractions of the total protein, percentage myosin in the HIS protein fraction and the HIS protein fraction actin:myosin ratio.

VII. Summary and Conclusions

A. Study I.

Mechanically deboned chicken meat (MDCM), and meat by-products (pork lung lobes, pork liver, beef lung lobes, beef spleen, beef heart) varied in their proximate composition, amount of three major protein fractions [low ionic strength (LIS), high ionic strength soluble (HIS), insoluble (IN)] and collagen content. Frankfurters produced with by-products exhibited lower severe reheat yield and apparent stress and strain at failure compared to frankfurters manufactured from mechanically deboned chicken meat.

B. Study II.

Results indicate that the gel forming abilities of HIS protein fractions from skeletal, cardiac and smooth muscles were not equivalent and thus functionality of muscle tissues cannot be compared solely on the basis of the size of the HIS protein fraction. It may be possible to predict the gel strength and other functionalities of all muscle tissues based on the size of salt soluble protein fraction and the amount of myosin in the salt soluble protein fraction. Results suggested a new method is needed for measuring bind constants or relative functionalities of meat ingredients used in least cost formulation calculations of meat processors.

C. Study III.

The gelation of the HIS proteins provide the most functionality to the model system frankfurters. The amount

of HIS, LIS and IN protein fractions in a formulation, the percentage of myosin in the HIS protein fraction and of the total protein and the actin:myosin ratio in the HIS protein fraction were all important factors in HIS protein fraction functionality.

The LIS proteins were detrimental to production of the model system frankfurters. Insoluble (IN) protein were less detrimental that LIS proteins and tended to act like fillers in cooked model system meat products.

The protein gelation system and results reported by Nuckles et al. (1990b) predicted the protein functionality in model system frankfurters. These results suggest that protein gelation in model systems can be used by processors to predict protein functionality in meat products. Results also suggest that bind values currently used by meat processors to formulate meat products could be improved by incorporating additional information about raw materials such as the percentage LIS, HIS and IN protein fractions of the total protein, percentage myosin in the HIS protein fraction, % myosin in the formulation and the HIS protein fraction actin:myosin in ratio.

VIII. Recommendations for Future Research

A. Test isolated protein fractions in current or novel food products.

   1. Low ionic strength (LIS) proteins to replace added water in meat products. Various amounts of LIS proteins substituted for water in meat products should be tested to determine substitution effects on color, oxidative rancidity, sensory and other parameters.

   2. High ionic strength (HIS) proteins singly or in combinations with insoluble proteins (IN) to replace binders in formed meat chunks (FMC) or restructured meats (traditional or extruded).

   3. Conduct further tests of the use of high ionic strength soluble (HIS) proteins in surimi-type products such as human and cat foods.

   4. Determine the gelation temperatures of high ionic strength soluble (HIS) proteins to determine the optimal temperature to utilize the protein fraction in processed meats.

B. Test model meat and/or by-product formulations based on % myosin and/or myosin to actin ratios. Determine if the % myosin or myosin/actin ratio within the high ionic strength soluble proteins determines the functionality of the meat or by-products in the formulations.

C. Work in conjunction with engineers to develop and test methodology to extract protein fractions on a commercially feasible basis. Engineers could develop the extraction machinery and protein scientists could test the functionality of the extracted proteins.

D. Extract red meat muscles (or meat cuts) into the protein fractions (HIS, LIS, and IN) and determine the % myosin and myosin to actin ratio in the HIS protein fraction. The information could be used to develop a computer knowledge base to replace the current bind constants used in least cost formulation.

E. Expand the extraction of proteins fractions to include all red meat by-products and poultry meats and by-products to develop a complete knowledge base to use for computer program least cost formulation.

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