COMPARATIVE STUDY OF THE EFFECTS OF THREE FOOD PROTEINS ON THE COOKING QUALITY OF PORK SAUSAGES

by

PURANJAY PRIYADARSHI

(Under the Direction of William L. Kerr)

ABSTRACT

Low-fat pork sausages were prepared with soy protein isolate (SPI), egg albumin (EA) and whey protein isolates (WPI) as binders at 1%, 2% and 3% w/w. The quality of cooked sausages was evaluated against control through cook loss, expressible moisture, cutting force, hardness, adhesiveness, cohesiveness, color and torsion test. Significantly ($p < 0.05$) lower cook loss and expressible moisture were observed with the addition of protein. Cutting force, hardness and cohesiveness were also increased with increase in added protein. L^* and b^* color values increased, while a* decreased with higher levels of all proteins, indicating a slightly lighter and less-red color. Sausage with added protein was juicier than control and overall acceptability was greatest for sausage with 2% SPI whereas least cook loss at 3% SPI among the treatments.

Dispersions of 15% SPI, WPI and egg albumin were microfluidized and gel strength, particle size, storage and loss moduli, and torsional strength were measured at 0%, 1%, 2% and 3% NaCl. With increase in NaCl concentration up to 2% particle size and gel strength increased significantly ($p<0.05$) followed by a decrease at 3% NaCl. The

gel point time and temperature shear stain and slope ratio decreased whereas shear stress increased with increasing NaCl concentration and microfluidization. A decrease in particle size and strain hardening and increase in gel strength was observed due to microfluidization. Confocal laser scanning microscopy showed more uniform distribution of the protein aggregates after microfluidization.

Microfluidized dispersions of SPI, WPI and EA were added as binders at 1%, 2%a and 3% levels to 80% lean pork trimming to formulate pork sausages. At the same level of protein microfluidization resulted in significantly ($p < 0.05$) lower cook loss and expressible moisture. No significant difference was observed in cutting force hardness, cohesiveness, adhesiveness, shear stress, shear strain, fracture modulus and color due to microfluidization. Microfluidization resulted in significant changes in the structure and gelation of all the protein dispersions in isolation, as well as in sausage emulsions, and can be used as a useful tool for manipulating the functionality of the proteins for different food processing conditions.

INDEX WORDS- Pork sausage, soy protein isolate, whey protein isolate, egg albumin, microfluidization.

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DEDICATION

To my parents.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

In order to survive we must eat, but poor dietary choices can lead to a decrease in wellness, or even lead to severe health problems. The amount and type of fat intake in our diet has been under scrutiny, and numerous research studies have associated increased saturated fat intake with major health problems such as coronary heart disease (Hu and others 1999) and type 2 diabetes (Salmeron and others 2001). Higher intake of fat has also been implicated in increased obesity in the population, which may further increase the risk of cardiovascular disease and diabetes (Haslam 2010).

Due to increased consumer awareness, food processors are now more focused on formulating foods with reduced fat. Meat sausages are a very popular food all around the world, but often contain more than 30% fat. Formulating reduced fat products is always a challenge, and in the case of sausage it is even more difficult as sausage is a “meat emulsion” that relies on fat to maintain emulsion stability. Reduction in fat level results in decreased emulsification and ultimately causes an undesirable product with very high cook loss and low retention of water and fat (Crehan and others 2000; Hughes and others 1997; Hughes and others 1998). The use of binders in sausage and related products has been shown to decrease cook loss by increasing the water holding capacity and improving emulsification. There are several works done on different types of binders but very few comparative studies have been reported (Ahamed and others 2007; Ensor and others 1987; Motzer and others 1998; Youssef and Barbut 2011). Globular proteins such

as soy protein isolate, whey protein isolate and egg albumin can act as good binders in comminuted meat products (Pietrasik 2003; Cassens and others 1975; Chin and others 2000; Marit Risberg EllekjÆR 1996).

A comparison among these proteins as binders in comminuted meat products will give good insight into their effectiveness at different levels, and also highlight any problems associated with their incorporation. High levels of binders sometime result in off-flavors due to inherent characteristics of the individual proteins (Das and others 2006). Microfluidization can help modify proteins through the high-shear conditions it creates. This can potentially increase the effectiveness of binders at lower level of incorporation (Cheng-Mei and others 2011; Shen and Tang 2012; Tang and Liu 2013). Microfluidization can also reduce the particle size and allow for greater interaction between muscle proteins and the added proteins, resulting in stronger network formation. This in turn allows for a further increase in water holding capacity and reduction of cook loss (Dissanayake and others 2010; Dissanayake and Vasiljevic 2009). Interactions between amino acid residues in different proteins at tertiary and quaternary levels are similar, so that comparable effects of pressure on different proteins are expected. Shearing at high pressure can cause changes in the tertiary and quaternary structure of most globular protein resulting in change of their functionality (Silva and Weber 1993). Several studies have reported on the effects of high-pressure processing of individual food ingredients such as proteins, fats and oil, and polysaccharides and how these may change their functionality. However, few studies have been done on the incorporation of high-pressure treated food ingredients in complex food systems such as sausages

(Iordache and Jelen 2003b; Shen and Tang 2012; Tang and Liu 2013). This work was conducted with the following objectives:

(1) The comparative evaluation of three common food proteins as binders in pork sausages and their effects on water holding capacity, cook yield and textural changes.

(2) The investigation of the effects of microfluidization on the rheological and gelling behavior of these proteins at different levels, and with the incorporation of different levels of NaCl.

(3) The examination of how different levels of microfluidized proteins effect the quality when incorporated into pork sausages.

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CHAPTER 2

LITERATURE REVIEW

The intake of excess dietary fat can lead to obesity and has been associated with several debilitating diseases such as cardiovascular disease and type-2 diabetes. The relationship between diet and disease has been of interest for several decades and numerous research studies have been conducted with conflicting results. Hu and others (1999) suggested that there is an increased risk of coronary heart disease with increased intake of saturated and trans fat, and a decreased risk with increased intake of monounsaturated and polyunsaturated fats. Salmeron and others (2001) found that trans fat is the main culprit, while saturated fats are not responsible for the increased cases of type-2 diabetes. Overall, research has shown that several types of fats may be associated with disease conditions; therefore the best approach would be to reduce the overall intake of fat in the diet.

The 2010 USDA and HHS Dietary Guidelines for Americans suggested that adults 19 and older should limit fat intake to within 20 and 35% of the total DRI, with an emphasis on reducing fat and increasing protein in the diet. In addition, they recommended that less than 10% of calories come from saturated fatty acids, and wherever possible replacing them with mono- and poly-unsaturated fatty acids while keeping consumption of trans fats as low as possible. The USDA and HHS expressed their concerns due to the high prevalence of obesity and overweight in the population, and noted that these populations are at increased risk of type-2 diabetes, heart disease and

certain types of cancer which can ultimately increase the risk of premature death (HHS 2013; USDA 2013). With increases in household income, societies tend to consume more meat and meat-based products (Jikun Huang and others 1999). However, many meat products have high amounts of fat, therefore there has been a push to develop ingredients or technologies that can help produce acceptable meat products with lower levels of fat in the formulation.

Sausages

Sausages are a type of comminuted meat product that form a meat or gel emulsion. The mixture contains various amounts of intact tissue, extracted meat proteins, fat, water and spices immobilized in a continuous gel network. Dickinson (2012) studied several types of meat emulsions and reported that most are heat-set, although some may be formed due to enzymatic action or acidification. Sausage emulsions are made after comminuting (chopping/grinding) of the meat, stuffing it in to natural hog or synthetic casings, and cooking it typically in the presence of smoke. A very good emulsification of the sausage batter is the first step in getting high yield with minimum water and fat loss. Thus, the initial formulation, chopping and mixing of sausage ingredients are as critical as the final cooking. The stability of the sausage emulsion is dependent on temperature, pH and ionic strength. De Ng and others (1981) reported a decrease in fat and water binding when meat is comminuted above 16°C. Brown and Toledo (1975) also observed this phenomena, and using wide-line NMR, found restricted mobility of water at the maximum bind during the chopping process. McClements (2005; 2007) extensively studied the mechanism of emulsion formation and stability and reported that stability of

the comminuted meat system can be achieved by enhancing protein-protein interactions, which tend to increase at higher temperature and lower pH.

Fat Reduction

The reduction of fat in sausage decreases greasiness, but at the same time reduces some of the desirable characteristics such as juiciness, meat flavor, firmness and color intensity (Tomaschunas and others 2013). Pork sausage traditionally contains 30% to 50% fat and interaction of muscle protein with water and fat in the sausage is a complex process that results in the characteristic juiciness, flavor and mouth feel of the sausage. Understanding the interaction of the muscle protein with the fat replacer is crucial in restoring the original flavor and taste in reduced fat sausages (Jiménez Colmenero 2000). Fat reduction in sausages can be achieved by using lean meat or replacing fat with replacers such as proteins or carbohydrates (Chin and others 2000; Keeton 1994; Weiss and others 2010; Colmenero 1996; Lurueña-Martínez and others 2004; Piñero and others 2008). Sausages have been formulated successfully with varying degree of fat reduction. Mora Gallego (2013) showed a satisfactory overall sensory quality in reduced fat non-acid fermented sausages with less than 12.5% final fat. In general, reduction in fat results in higher cook loss, undesirable texture and lower overall acceptability in comminuted meat products. Shai Barbut (1996) observed that reducing fat resulted in increased hardness, brittleness, gumminess and chewiness in pork and beef frankfurters. The addition of cellulose gum in the low-fat frankfurters reduced the amount of cook loss. In addition, high-fat frankfurters were lighter in color compared to low-fat versions. Tenderness was also lower in the low-fat frankfurters and the texture was improved by incorporating one of the two cellulose gums used. Pietrasik and Janz (2010) found that

when compared to full-fat bologna, low-fat bologna was chewy, elastic and had low water-binding ability. The loss of functionality as fat was replaced by water was ameliorated by the addition of pea starch and pea fibers. Emel Cengiz (2007) found an increase in moisture content and protein, with the decrease in fat level in low-fat frankfurters.

Various types of ingredients have been tested to improve the texture in low-fat meat products, while at the same time reducing cook loss. Water (10 and 30%) along with several other ingredient (DuoFiber®, oat fiber, pea fiber, wheat starch, Firm-tex®, and isolated soy protein) were tested in low-fat bologna with mixed effects (Claus and Hunt 1991). Tomaschunas and others (2013) studied inulin and citrus fibers as fat replacers in reduced fat pork sausage and observed that decreasing fat resulted in less flavor and juiciness, whereas spiciness, coarseness and firmness were increased. However, the addition of inulin and citrus fiber helped make reduced fat sausage with similar sensory scores to the full fat variety. Several authors have studied various other ingredients such as carageenan (Modi and others 2009; Kumar and Sharma 2004), oat fiber (Pinero and others 2008), konjac, and gellan (Lin and Huang 2003) as fat replacers in comminuted meat products with encouraging results. Low-fat bologna with pea starch and pea fiber performed equally well as the high-fat bologna and a substantial increase in purchase potential was predicted for the products with pea starch and pea fiber (Pietrasik and Janz 2010). Replacing the fat with protein is an attractive option as consumers perceive protein as a healthy addition to comminuted meats.

Soy Protein

Soy protein can be found in several forms including soy grit, soy protein concentrate and soy protein isolate. Granata and Morr (1996) felt that the use of soy protein in foods is limited by its sensory attributes, namely that it exhibits beany flavor. Soy protein isolate (SPI) is a light yellow-colored powder with very little flavor and a rather bland taste. Most soy protein isolates are extracted from defatted soy flakes, grits or flour and contain at least 90% protein on a dry weight basis. SPI contains isoflavones and some other potential anti-carcinogens (Messina and Barnes 1991). It has very good solubility in water, relatively high water-holding capacity and good emulsifying properties. Thus, it can act as a very good binder and extender in comminuted meat products.

Most of the properties of soy protein is attributed to its two major protein fractions- glycinin and beta-conglycinin (Riaz 2006; Renkema and others 2001). Soy protein isolate exhibits several desirable characteristics that are of great interest to different food processors. These include water holding capability, foaming enhancement, emulsifying capacity, the ability to modify texture or be formed into fibers, dough promotion, the formation of gels with heating, and the ability increase the viscosity of suspensions. Different soy protein isolates vary in all these properties due to a number of factors, including the characteristics of the soy flakes, grits or powder from which SPI is extracted; the conditions during extraction; and other processing and storage conditions (Johnson and Kikuchi 1989).

Whey Protein

Whey protein can also be found in several forms of which whey protein concentrate and whey protein isolate are the most important. Whey protein isolate (WPI) is produced as a creamy white powder and has a very slight milk flavor. It is highly bioavailable and absorbed quickly in the body, and is often used by the body-building community as a food supplement. WPI is at least 90% protein and is almost free of cholesterol, lactose and carbohydrate (Morr and Ha 1993; Huffman 1996).

Yetim and others (2001) suggested the use of fluid whey to replace ice in comminuted meat products, such as sausages and frankfurters, in order to improve their emulsion stability. They found that there no significant differences in most technological, chemical and sensory parameters with the addition of whey.

Egg Albumin

Egg albumin is obtained after separating the whites from the yolk, and then dying them into a slightly yellowish powder which has the characteristic flavor of egg. Dried egg albumen typically contains ~80% protein (Stadelman and Cotterill 1995).

High Pressure Processing

Over the years, several non-meat ingredients have been studied for their use in comminuted meat products, with the hope that they may improve yield, physical properties or consumer acceptability of the products. Such ingredients may be incorporated in the formulation with or without modification. Modification of the

ingredients has been achieved in several ways in order to impart desired functionality in meat products. These include thermal treatments, pH alterations and enzymatic reactions. A promising approach has been the use of high-pressure processing, as it has the potential to change the surface properties of proteins. There are several types of high-pressure processing however. In static units, the food is packaged and introduced into a vessel, then subject to high pressures (300-600 MPa), usually transmitted by water. This process has been touted as a form of cold pasteurization. In dynamic, or continuous, units liquid foods are pumped at high-pressure through a valve or interaction chamber which causes very high shear and a sudden release in pressure.

Most of the initial work with high pressure processing has been done with the focus on food safety and microbial inactivation (Farkas and Hoover 2000). Recently, several studies have been done on the modification of proteins to enhance their functional properties. Microfluidization (MF) is a high pressure processing technique which utilizes a positive displacement intensifier pump, and can be used to process liquid and some semi-solid material up to a pressure of 30,000 psi (207 MPa). The high-pressure pump accelerates the fluidized material to a velocity of over 400 m/s into the interaction chamber or valve. The interaction chamber consists of bifurcated Y-shaped micro-channel that separates the fluid into two streams and then directs them into each other thus facilitating very high velocity impact, high frequency vibration and intense shear (Microfluidics 2008). High pressure processing of proteins through MF results in changes in several parameters including particle size and rheological properties of fluid suspensions, as well as the textural properties, color and sensory attributes of foods to which they are added.

Floury and others (2000; 2002) studied the effect of high-pressure homogenization on the oil-in-water emulsions made with modified soy protein. They found that MF caused significant modifications in the structure and the texture of emulsions with increasing pressure. Ultra high-pressure homogenizing conditions also allowed the formation of high oil-content emulsions (> 40%), as well as a change from shear-thinning behavior (at 20 MPa) to Newtonian behaviors (at 300 MPa). Emulsion droplet size was also reduced with increasing pressure. Dickinson and others (1997) observed no effects of high-pressure processing on β-casein, a major milk protein, even when treated with up to 800 MPa. In contrast, they observed that globular proteins such as bovine serum albumin display substantial changes in surface activity and functional properties when processed at 200 MPa and above. Dickinson and Pawlowsky (1996) studied high-pressure processing of mixed biopolymer emulsions, and reported significant changes in the nature of the protein-polysaccharide interactions. They suggested that high-pressure can be a great tool in controlling the viscoelasticity and stability of mixed macromolecule emulsions.

Lee and others (2009) found that there was compaction of the interfacial protein layer in whey protein stabilized emulsions due to high-pressure homogenization. High pressure processed emulsions were comparatively more stable due to small droplet size and an increase in protein-protein interactions which resulted in the formation of an interfacial protein layer. Ronkart and others (2010) found that that high-shear microfluidization at 30 MPa did not change the chemical composition of insuin dispersed in water, but increased the viscosity of the dispersion. Viscosity was observed to increase with the number of passes as well as with increase in the concentration of inulin.

Microfluidization at 120 Mpa enhanced the surface hydrophobicity and solubility in both unheated and preheated soy protein isolate. Due to the high-shear conditions, large insoluble aggregates were broken down in to small soluble particles. In addition, denaturation and protein unfolding was observed, which together with the high-shear, induced disruption of hydrophobic domains and led to changes in the tertiary conformation of SPI (Shen and Tang 2012). Subirade and others (1998) observed that dynamic high pressure at 140 MPa did not result in any changes in the secondary structure of betalactoglobulin (BLG). However, the treated BLG had greater thermostability compared to untreated BLG, and the treated protein showed different gelation behavior during the cooling regime of a thermal gelation process. Three stages of structural breakdown of soy protein dispersion were reported by Jong (2012) when subjected to high shear microfluidization. Viscosity as well as storage and loss modulus was reported to be decreased due to reduced interaction between small size aggregates.

Color

One measure of meat quality is in the appearance and color of both the raw and cooked product. Uncooked muscle foods typically have a red color due to the heme-containing muscle myoglobin. One measure of color is through the tristimulus CIELAB L*a*b* system. In this system, L* ranges from 0 (black) to 100 (white). The value a* ranges from -100 (green) to 100 (red), while b* values range from -100 (blue) to 100 (yellow). With decreasing levels of fat in low-fat frankfurter, L* values were lower and a* values were higher (Emel Cengiz 2007). With the addition of soy protein concentrate, a* and b* values did not change but L* values decreased.

Ferrini and others (2012) observed increases in L*, and decreases in a* and b*, after high-pressure processing of moist minced pork meat.

Texture and Sensory Properties

In general, texture is a sensory property sensed by the myriad mechanical sensations in the mouth when chewed, and sometimes supplemented by visual or auditory information. Textural properties are often assayed through mechanical tests that compress or slice through a product, while the forces or distance (or both) are monitored. Crehan and others (2000) reported increased cook loss and decreased emulsion stability in frankfurters when fat was reduced from 30% to 5%. Changes in texture profiles were also reported with decreases in fat with decreases in hardness, chewiness and gumminess and increases in springiness. Microfluidization of ice cream mixes with locust bean or xanthan gum at 220 -250 MPa resulted in an increase in hardness and adhesiveness of the finished ice cream (Cavender and Kerr 2013).

In the final analyses, the quality of foods is assessed through a variety of sensory properties that encompasses appearance, texture, flavor and aroma. These are determined by consumers through the use all senses including sight, touch, hearing taste and smell. Some sensory properties can be described as descriptive such as the degree of hardness or juiciness, and are related to how people assess the relative intensity of various attributes. Other sensory properties are categorized as affective and are related to how consumers like a product, or prefer one product to another. Crehan and others (2000) found that maltodextrins could be added to produce reduced-fat frankfurters without any adverse effects on the some several characteristics.

Emel Cengiz (2007) used citrus fiber and soy protein to produce frankfurters with 20, 10 and 5% fat. They found that while cook loss was reduced for frankfurters with citrus fiber, the addition of fat replacers increased sensory hardness and resulted in lower likeability scores. Few studies have examined how MF or other high-pressure processing or modification of ingredients affects the sensory properties of foods. In a consumer preference test, ice cream made from MF mix had greater acceptability, which related to the enhancement of desirable characteristics such as creaminess and a smooth texture (Cavender and Kerr 2013).

Cook Loss

The quality of muscle foods is also related to several properties involved with how the products retain moisture, and to a lesser extent fat. To the processors, yield is one such property. Thus, if liquids are added, injected are absorbed during marination, loss of fluids during handling will result in lower yield. Purge loss is related to how packaged products retain liquids. Thus, if liquids emerge from the product in the package, this is seen as a defect. Cook loss is related to the performance of the food as cooked by consumers or in retail operations. Thus, as the meat product is fired, baked or grilled juices may emerge. The weight of the product before and after cooking is used to describe the cook loss. In addition to reducing the weight of the product, excessive cook loss can cause a tough texture and loss of desirable juiciness.Crehan and others (2000) found that in reduced-fat frankfurters, the addition of maltodextrin resulted in decreased cook loss and emulsion stability. Das and others (2006) suggested that treating soy beans

with moist heat could reduce beany flavor in soy pastes and reduce cook loss in comminuted meat products that were formulated with the paste.

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CHAPTER 3

COMPARATIVE STUDY OF THE EFFECTS OF THREE FOOD PROTEINS ON THE COOKING QUALITY OF PORK SAUSAGE

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Abstract

Sausages were prepared from 80% lean pork trimmings using soy protein isolate (SPI), egg albumin (EA) or whey protein isolates (WPI) as binders in the formulation at 1%, 2% and 3% w/w. The cooking quality of the sausages was assessed against control that contained no added protein. Sausages with any of the added proteins had significantly lower cook loss and less expressible moisture when compared with control. Higher cutting force, hardness and cohesiveness were observed for sausage formulated with added protein and with increasing levels of protein. Color values L^* and b^* values increased, while a* decreased with higher levels of protein, indicating a lighter sausage with a slightly less red hue. Descriptive sensory analysis showed that sausage with added protein was juicier than control, but had no difference in springiness or cohesiveness. Overall acceptability was greatest for sausage with SPI. While samples with 3% SPI had the least cook loss, samples with 2% SPI had the greatest acceptability.

Key words: Pork sausage, soy protein, whey protein, egg albumin

Introduction

Our health is affected by the food we eat on a regular basis. Excessive consumption or consumption of high-fat foods is an important factor in the development of obesity, which in turn is implicated in the development of type II diabetes, cardiovascular and other diseases (Haslam 2010; Sowers 1998). Diabetes affects approximately 350 million people worldwide, and in the United States diabetes and cardiovascular diseases together result in over $400 billion a year in health costs (WHO 2012). Consumer awareness of diet-related health issues has increased tremendously resulting in demand for "healthier" food alternatives (Grunert and others 2004).

Meat products, especially non-lean varieties, are sometimes seen as a poor choice for a healthy diet. Bacon, sausages, highly marbled meats and several luncheon meats may all contain substantial fat content. Researchers and product developers have sought to develop meat products with the functional and nutritional properties afforded by muscle foods, while reducing the level of fat, calories and cholesterol (Chizzolini and others 1999; Jiménez-Colmenero and others 2001; Decker and Park 2010; Decker and others 1986). Fat reduction in meat products can be achieved by physically removing fat from the meat or by using non-fat additives to replace some of the total fat.

Development of alternative sausage formulations has been of considerable interest. Reformulation of sausage batter is generally aimed at reducing fat and cholesterol content, modifying fatty acid profile, and reducing sodium and total calories. Unfortunately, reduction of fat in meat products usually has a profound effect on the flavor, texture, color and cooking qualities of the finished product. For many sausages, a low percentage of fat results in undesirable texture and increased cook loss (Cengiz and

Gokoglu 2007; Hughes and others 1997; Hughes and others 1998). Researchers have developed low-fat sausage products utilizing several non-fat additives that mitigate some of the undesirable characteristics developed due to fat reduction. Some of the ingredients extensively used for this purpose are soy protein (Noda and others 1977; Sofos and Allen 1977; Sofos and others 1977; Chin and others 2000), whey protein (Barbut 2006; El-Magoli and others 1995; Hayes and others 2005; Ellekjaer and others 1996; Youssef and Barbut 2011), carrageenan (Trius 1994a; Trius 1994b), and cereal and fruit fibers (Chang and Carpenter 1997; Modi and others 2009; Kerr and others 2005; Yang and others 2007). In general, different non-meat proteins had little effect on cook loss in sausages with 30% or more fat in the formulation (Sofos and others 1977; Ahmed and others 1990). However, added proteins can help improve cook loss in sausages with less than 20% fat (Miles 1996).

Currently, many commercial sausages are produced from meat with 20% fat to provide a modestly lean product while keeping the cook and purge loss to a minimum. Cook loss is affected by several other factors other than fat percent. These include processing temperature (Murphy and Marks 2000), ionic strength (Chin and others 2009), processing conditions and type of binders and emulsifiers. Leaner sausage formulations may also have unacceptable moistness or texture attributes, or be lacking in the lubricity or mouthfeel afforded by fat. Most proteins from vegetable and non-meat sources can act as effective binders and emulsifiers. Various proteins have different swelling and gelling properties and therefore are expected to behave differently in the comminuted meat system. However, there has been little work on the comparative effects of these proteins in a given system. In this study, reduced-fat smoked sausage (20% fat) was formulated

with various levels of soy protein isolate (SPI), egg albumin (EA) or whey protein isolate (WPI) to compare their ability to contribute to acceptable flavor, color, texture and water binding capacity. The specific objectives were to compare the effectiveness of SPI, WPI and egg albumin in retaining moisture in the pork sausages during cooking; analyze texture and color of the sausages resulting after addition of SPI, WPI and egg albumin as binders; and to analyze sensory attributes as well as consumer likeability of sausage products with added non-meat proteins.

Materials and methods

Preparation of sausage

Pork meat (80% lean) was attained fresh from the UGA Meat Science Technology Center (Athens, GA) and coarse ground through a ½" plate using a model 4046 5-HP meat grinder (Hobart, Troy, OH). Other ingredients were added and mixed for 5 min in a Koch A-80 rotary blender (UltraSource, Kansas City, MO). A smoked sausage seasoning mix was used (Blend 2525-W, A. C. Legg Inc.) that contained salt, red pepper, sage, sugar, dextrose and black pepper. Once mixed, the batter was reground through a 3/16 inch plate to create a more uniform particle size. The batter was stuffed into 30-32 mm hand-pulled hog casings (International Casings Group, Inc, Chicago, IL) using a double-screw vacuum filler (Vemag Robot 500, Machinebau, Germany). All processing was done in a cold room at 4°C.

Three different non-meat proteins were added at three levels to create nine sausage formulations. These included soy protein isolate (Pro-Fam 932, ADM, Decatur, IL), whey protein isolate (Protient, St. Paul, MN), and egg albumin (P-39, Henningsen Foods Inc., Omaha, NE). Proteins were added as part of a mixture design at levels of 1, 2 and 3% by weight. A control sample was also prepared without any added non-meat

protein. The sausages in casings were cooked and smoked in an Model 1000 smokehouse (Alkar Inc., Lodi, WI) according to the following schedule: 40 min at 43°C to dry the casings; 2 h at 54°C/20%RH for initial smoking, followed by 2 h at 60°C/30%RH; cooking to an internal temperature of 75°C (165°F) at 88%RH, with a 1 min hold time to ensure product safety; 10 min cold shower at 10°C. The cooked sausages were refrigerated at 4°C overnight and tested the next day.

Cooking losses

Cooking loss was measured by subtracting the weight after cooking (w_{after}) from the weight before cooking (w_{before}) and expressed as a percentage:

$$\%\,Cook\ Loss = 100 \times \frac{w_{before} - w_{after}}{w_{before}} \tag{1}$$

Expressible moisture

Cylindrical samples for expressible moisture were prepared by cutting cooked sausages into 15 mm thick slices and coring into cylinders with a 20 mm brass cork borer. A TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY) fitted with 3-inch diameter aluminum cylinder plate (TA-30) was used to compress the sample between two Whatman No-1 filter papers. Samples were compressed to 50% deformation at 2 mm/s and then held for 30 s in this position. After removing the wet filter papers, the difference in weight before and after compression gave the expressible moisture:

$$\%\,Expressible\ Moisture = 100 \times \frac{w_i - w_f}{w_i} \tag{2}$$

Where w_i is the weight before and w_f the weight after compression.

Texture analysis

Shear test

To determine the force needed to cut through samples, sausage pieces were first cored to obtain 25.4 mm long and 16 mm diameter samples. Cylindrical samples were placed on the texture analyzer stage, and a Warner-Bratzler blade (TA-7) used to shear through the sample from above at a rate of 4 mm/s. The measure of tenderness was taken as the maximum force attained during shearing.

Texture profile analysis

Texture profile analysis (TPA) was conducted to determine other texture related attributes of the sausages. A 16 mm diameter and 15 mm thick sample was prepared by slicing and coring cooked sausage pieces. The TA-XT2i, fitted with a 3-inch diameter aluminum cylinder plate (TA-30), was used to compress the sample twice to 50% deformation at 2 mm/s. The test speed and post-test speed was kept the same so that area under the force-deformation curves would be comparable for both compression and withdrawal cycles. The maximum force attained during the first compression cycle was reported as hardness, the ratio of areas of the second and first compression cycles as cohesiveness, and negative area in the first withdrawal cycle as adhesiveness.

Torsion test

Measurements of torsional strength have been found to be an effective means of assessing gel strength in meat and other products, particularly those whose structure depends on a networked structure of proteins (Kim and others, 1986). Samples (20 mm in diameter and 25.4 mm thick) were prepared by slicing and coring the sausages. Each sample was mounted on two notched styrene discs, using a cyanoacrylate adhesive. Samples were ground into an hour glass shape using a gel grinder (Gel Consultants,

Raleigh,N.C., U.S.A) as described by Lanier and others (1985). A torsion Gelometer built on a DV-E viscometer (Brookfield Engineering, Middleboro, MA) was used to twist the sample at 2.5 rpm, and the generated shear stress and strain were measured up to the point of material fracture. The failure stress was used to measure the gel strength, while the failure strain measured the gel deformability. The ratio of shear stress and shear stain at failure was recorded as the failure modulus G. The slope ratio was calculated as the failure modulus G divided by the modulus (G_{30}) at 30% stain.

Color analysis

Sample color after cooking was analyzed using a Minolta Chroma-Meter series CR-200 (Minolta, Japan). The samples were split from the middle and placed on a flat surface to measure the internal color of the cooked sausage. The meter was contacted immediately above the sample and color values recorded in the CIE-LAB (L*, a*, b*) color space.

Sensory Analysis

Experienced panel testing

A total of 12 people were screened and recruited for the sensory evaluation of pork sausages from a pool of students and staff at the University of Georgia. Panelist ages ranged from 18 to 60 years old. Participants were trained to familiarize them with the different sensory characteristics applied to comminuted meat products. The six most widely used sausage sensory characteristics were selected and panelists were asked to judge each sample for the following characteristics: juiciness, springiness, cohesiveness, texture, flavor and overall acceptability. Panelists used a 9-point scale to record the intensity of an attribute. The scale ranged from 1 (extremely dry, non-springy, non-

cohesive, fine textured, flavorless or unacceptable) to 9 (extremely juicy, springy, tender, cohesive, coarse textured, flavorful and acceptable).

Two sets of sensory tests were performed to limit panelist fatigue. In the first, sausages with three different proteins were evaluated against the control. In the second, three levels of soy protein in the sausage were evaluated against control. Sausages were cut into 1 cm slices and served warm (~40°C). Water and unsalted cracker was provided for rinsing the palate before each sample was tested. All tests were conducted in isolated sensory booths at the Food Product Research and Development Laboratory at the University of Georgia, under ambient conditions.

Consumer panel testing

Untrained panelists (50), consisting primarily of students and staff at the University of Georgia were recruited for the consumer evaluation of pork sausages. Panelists were asked to rate attribute (texture, flavor, and overall acceptability) on a 9-point hedonic scale, with 1 being “disliked extremely” and 9 being “liked extremely”. As above, two sets of panels were conducted. In the first, sausages with three different proteins were evaluated with control, whereas in the second sausages with three levels of soy proteins were evaluated with control. The sausages were cut into 1 cm slices and served warm. Water and unsalted crackers were provided for rinsing the palate before each sample was tested.

Statistic analysis

All data was statistically analyzed using SAS statistical software (Version 9.1, 2008). Analysis of variance (ANOVA) was performed, and when the F-test showed significant treatment effect, Fisher’s protected LSD test was performed to determine

differences amongst the treatment groups. Statistical significance was expressed at the $p < 0.05$ level.

Results and discussions

Cook loss and expressible moisture

While uncooked sausage batter retains ample moisture, during cooking some of the moisture and juices are lost. This results in lower yield and a less juicy tasting product (Kerr and others 2005). Less cook loss was observed in samples containing added protein than in the control (Table 3.1). While the cook loss in control sausage was 27.7%, those with added SPI ranged from 14.1-18.7%, those with egg albumen 19.0-22.6%, and those with WPI 19.9-24.5%. At a given level of addition, SPI was more effective at preventing cook loss than egg albumen, which was more effective than WPI.

Increasing the level of added protein in the sausage from 1to 3% also resulted in less cook loss. SPI added at 3% resulted in the lowest cook loss. This is likely due to the relatively high water absorbing ability of soy protein and its ability to form a network with the muscle protein, and to improve emulsification (Muguruma and others 2003; Youssef and Barbut 2011).

Others have found that higher levels of protein resulted in lower cook loss and juicier products made from meat batters, as the moisture is well-retained in the protein matrix (Youssef and Barbut 2009; Bejosano and Corke 1998). In several studies, binders and extenders such as hydrated oatmeal and tofu (Yang and others 2007) helped reduce cook loss and, to a point, increased levels resulted in successively less cook loss. Bejosano and Corke (1998) found that buckwheat and amaranth proteins could reduce cook loss in emulsion-type meat products. One study (Ensor and others 1987) found that

different protein types and levels of protein did not improve yield as compared to control sausage. This was possibly due to the fact that each treatment had relatively high fat (24%) in the formulation. Similarly, Sofos and others (1977) found that at 30% fat level added soy protein did not effect cook loss in wiener-type products. At higher fat levels, emulsion stability is not a problem and most of the cook loss is related to emulsion breakdown. Carballo and others (1995) found that starch and egg white did not improve binding properties in bologna sausage. Another consideration is that protein functionality is sensitive to pH and ionic strength; therefore different formulations and cooking conditions can alter their effect in comminuted meats (Pietrasik and Li-Chan 2002).

Expressible moisture was also lower in sausage with added proteins (Table 3.1). As with cook loss, the lowest expressible moisture was observed in SPI-sausage (3.22-3.70%), followed by EA-sausage (3.85-4.72%) and WPI-sausage (4.50-5.02%). In comparison, control sausage had expressible moisture of 5.96%. The level of added protein was also a significant factor, with higher levels of added protein resulting in less expressible moisture. Several non-meat proteins and carbohydrates have been shown to reduce expressible moisture, cook loss and purge, while improving hydration properties. These adjuvants include SPI, gelatin, sodium caseinate, egg albumin, *k*-carrageenan and modified starch (Pietrasik 1999; Pietrasik 2003; Pietrasik and Jarmoluk 2003; Pietrasik and others 2007; Pietrasik and Li-Chan 2002). Even some grains such as oat bran added to frankfurter or whole oats in sausage have resulted in less expressible moisture (Chang and Carpenter 1997, Kerr and others 2005). While easily expressed moisture, after cooking, does not lead to lower yield per se, it can cause drip loss in package or a decreased sensation of juiciness in the finished product.

Cutting force

One of the initial sensations of product texture is seen when cut with a knife or sheared by the incisors in the mouth. Somewhat higher cutting forces were observed for sausages formulated with added protein (Table 3.2). While control sausage had a cutting force of 446 g, those with SPI ranged from 769-1087 g, with EA 522-749 g, and with WPI 584-750 g. While addition of SPI necessitated higher shear forces, there were no differences in sausages with EA or WPI. Increased levels of added protein resulted in higher cutting force, and formulations with 3% soy protein had the highest cutting force amongst all treatments. In general, it is expected that higher protein levels would result in a stronger, more continuous gel network that provides firmer texture through resistance to compression and requires greater shear force to break through the network.

The effects of added proteins or polysaccharides on the texture of comminuted meat products depend on how it is added. When hydrated preformed whey protein-carageenan gels were added to low fat pork sausage, there was a decrease in measured shear force (Lyons and others 1999). They felt this was due to the formation of a less homogeneous structure, and a coarser textured sausage from the relatively brittle gels. In contrast, the addition of oat bran resulted in higher shear stress in frankfurters (Chang and Carpenter 1997). Again, these discrepancies in the results are likely due to the differences in the method of protein incorporation in the sausage. When protein was added as dry powder it resulted in higher shear with increased incorporation, but when protein was hydrated before addition the shear force was decreased with increased incorporation (Ensor and others 1987; Lyons and others 1999; Baardseth and others 1992).

Texture profile analysis

Texture profile analysis (TPA) is a method for measuring properties related to compression rather than shearing, and is an attempt to simulate attributes detected during chewing. TPA is often used to compare the cooking quality of meat and meat products, and especially sausage (Kerr and others 2005; Herrero and others 2008b). Hardness, cohesiveness and adhesiveness were calculated from the TPA data and are shown in Table 3.2.

Samples with added non-meat proteins were harder and more cohesive than control, and both hardness and cohesiveness increased with increase in level of protein. While control sausage had a hardness of 1296 g, values in SPI-sausage ranged from 2617-3179 g, in EA-sausage from 1844-2427 g, and in WPI-sausage 2037-2344 g. Likewise, control sausage had a cohesiveness of 0.29, while values in SPI-sausage ranged from 0.44-0.53, in EA-sausage from 0.33-0.40, and in WPI-sausage from 0.31-0.37. Sausages with added protein were also less adhesive than control. Control sausage had adhesiveness values of 83.7 g·s. SPI-sausage had values ranging from 40.4-26.3 g·s, EA-sausage values from 53.9-31.5 g·s, and WPI-sausage from 45.2-28.4 g·s. In general, higher levels of protein resulted in less adhesiveness in the sausages.

At given level soy protein resulted in firmer, more cohesive sausage and with less adhesiveness than sausages formulated with EA or WPI. The increased hardness would be expected to be associated with higher perceived firmness and an increase in the work of chewing. Higher cohesiveness translates to a product that is harder to break apart, again leading to greater work of chewing. Lower adhesiveness would be associated with a product that sticks less to surfaces such as the palate. Just because greater or lesser

forces are measured, however, does not necessarily mean that consumers will detect differences, nor does it mean the product will be more or less liked. These results will be discussed further in the section on sensory results.

Non-meat proteins have been used to improve the texture and appearance of comminuted meat products (Pietrasik 2003; Pietrasik and others 2007; Pietrasik and Li-Chan 2002). A certain degree of firmness and springiness is desirable in sausages and frankfurters (Roberts 1974). Hardness was shown to increase with soy protein addition in sausage batter cooked at 70°C (Herrero and others 2008a). Similar results were observed by Carballo and others (1995) when egg white was added to bologna. Pietrasik and Li-Chan (2002) observed a synergistic effect between egg albumin and microbial transglutaminase when added to beef gels, which resulted in greater hardness, cohesiveness and springiness. Others (Sofos and Allen 1977; Decker and others 1986) have observed decreased hardness in the comminuted meat when hydrated soy protein was added. Again these conflicting results are possibly due to different methods of protein addition and different processing conditions. Working with model systems, Bernal and others (1987) showed that combinations of myofibrillar proteins and whey protein concentrate formed stronger gels than myofibrillar protein extract alone. The amount of fat in the comminuted meat also affects the cohesiveness of the product, as the fat is a major component of the emulsion. For example, Matulis and others (1995) observed reduced cohesiveness in frankfurters at lower fat percentage (< 14%).

Torsion test

Torsion geometry has been found to be a useful means to measure gel strength and fracture. In a sense, it produces a “pure shear-stress”, in that the sample shape and

volume remain constant through the test. However, the test does not specifically detect whether the sample fails in compression, shear or tension. Data measured by the torsion gelometer are shown in Table 3.3. As with the shear and compressive tests shown in Table 3.2, the addition of protein increases the stress at failure. While control sausage had a shear stress at failure of 26.5 kPa, SPI-sausage had values of 44.3-52.9 kPa and EA-sausage had values of 33.3-39.7 kPa. Sausages with 1 or 2% WPI had values (27.0-30.7 kPa) similar to control, while samples with 3% had slightly higher shear stress at failure (30.7 kPa). Protein level was a factor for shear stress at failure, but differences were not always discernible from one level to the next. The shear strain at failure was 1.13 for control and in the range 0.98 to 1.10 for all other treatments. While control sausage had the highest value for shear strain at failure, only in a few cases was this significantly different than other treatment groups. Thus, addition of non-meat proteins tended to increase the force required straining the meat gel, particularly for sausage with SPI, and to a lesser extent with EA. While control sausage could be twisted a greater distance than sausage with added proteins, the difference was only slight if any.

Incorporation of soy leads to a more extensively interconnected protein network in the sausage than in control, which results in greater force developed during torsion. This also likely explains the greater shear and compressive forces developed in sausages with SPI. Addition of EA also contributes to building the network, while WPI had little if any effect. Shear stress at failure is more sensitive than shear strain to protein concentration and processing conditions (Lanier and others 1985; Hamann 1988). Farouk and others (2002) reported that reduction of sarcoplasmic protein in the cooked sausage batter resulted in significantly lower shear strain at failure, but did not affect shear stress

at failure. Farouk and Swan (1997) observed lower shear stress in meat products that also had higher water retention.

Color

*L** values were somewhat higher for sausage with added protein as compared to control (Table 3.4). L* values for control were 42.6 as compared to 43.6-45.4 for SPI-sausage, 43.4-44.3 for EA-sausage and 43.8-44.7 for WPI-sausage. Thus samples with added non-meat protein were slightly lighter in color than control. In general, sausages with added non-meat protein had lower a* values and higher b* values than control, and these differences were increased with higher levels of added protein. Values for control sausage (L*=42.6, a*=25.8, b*=14.6) are associated with a moderately brown color typical of meat. Changes due to added non-meat protein resulted in slightly lighter color, slightly less saturation of color, and a small shift away from red and more towards the yellow spectrum. Calculation of ΔE values showed the greatest difference in overall color occurred with SPI sausages as compared to control.

Similar changes in color were observed when SPI was added to buffalo meat emulsion (Ahmad and others 2010) or when protein and k-carageenan were added to beef gels (Pietrasik and Li-Chan 2002). Andrès and others (2006) observed that increased whey protein or hydrocolloids produced lighter colored chicken sausage. Some studies have shown that the product color is affected by the color of the ingredients. For example, Youssef and Barbut (2009) observed that yellow colored canola oil increased the yellowness of frankfurters to which it was added. In addition, higher levels of lean meat resulted in higher values of redness due to high myoglobin pigment content. In this study, the SPI and egg albumin had a slight yellow color whereas WPI had creamy white color, both of which contribute to a less red color in the sausage to which they were

added. In addition, the inclusion of non-meat protein results in a slight reduction in the total amount of meat pigments, and this would also result in a slightly less red product.

Sensory tests

Results for descriptive and consumer sensory tests are shown in Tables 3.5 and 6. Addition of 2% SPI, EA or WPI did not change the perceived springiness or cohesiveness of the sausages as compared to control. Likewise, addition of 1, 2 or 3% SPI did not affect springiness or cohesiveness. Thus, while differences in shear or compressive forces, as well as cohesiveness, could be measured by physical tests, these differences did not significantly influence the panelist's perception of these attributes. In addition, this shows that 20% fat sausage with non-meat proteins could still form stable emulsions and have good cohesiveness. The addition of SPI at all levels resulted in a slightly juicier product. Chang and Carpenter (1997) found that different levels of oat bran added to sausage did not change the springiness, but higher levels of oat bran did result in a less juicy and harder product. In general, non-meat proteins added to comminuted meat products have been shown to increase firmness (Bloukas and Paneras 1993; Simon and others 1965; Baker and others 1969).

In terms of likeability attributes, all samples were reasonably well-liked in terms of texture, flavor and overall acceptability. Samples with 2% SPI had slightly higher overall acceptability (8.40) than control (7.70), while samples with EA or WPI had slightly lower overall acceptability (6.61 and 6.99). The texture of 2% WPI-sausage was liked somewhat less than 2% SPI-sausage (7.77 versus 8.27). In addition, the flavor of the WPI-sausage was liked less than SPI-sausage or control. The flavor of 2% EA-Sausage was rated particularly low (5.12). When samples with 1, 2 or 3% SPI were compared with

control, those with 2% SPI had the highest scores for texture (8.27), flavor (8.21) and overall acceptability (8.42).

Thus, addition of soy protein at 2% resulted in a juicier sausage with improved texture and significantly higher overall acceptability. Egg and whey proteins, likely due to their characteristic flavor profiles, resulted in significantly lower flavor scores and overall acceptability than control and soy formulations. Foegeding and Lanier (1987) noted that adding egg and collagen proteins can have some negative effect on texture of gelled muscle protein foods. We found no significant difference in flavor acceptability between control and SPI formulations. Hughes and others (1998) noted that many non-fat flavor notes are accentuated at lower fat levels. This suggests that SPI provides milder or lower intensity flavors than EA or WPI when incorporated into sausage. All levels of SPI in the sausage resulted in increased consumer acceptability although sausage with 2% SPI had the greatest acceptability. Lower cook loss at higher protein levels resulted in juicier product, as protein traps fat and water in the matrix. However, at a specific protein level any further increases result in drier product as there is comparatively less water and fat in the formulation. Matulis and others (1995) suggested less than 3% soy protein incorporation in the comminuted meat to prevent a loss of juiciness, increased hardness and off flavor development.

Conclusions

SPI, WPI and EA are common food proteins that can be utilized in comminuted meat products such as sausages and frankfurters. Their addition in sausage formulated with 20% fat resulted in lower cook loss and expressible moisture, resulting in higher sausage yield when compared with control. Egg protein and whey protein imparted a

characteristic flavor to the sausage resulting in diminished overall acceptability. Sausage with 2% SPI had the highest overall acceptability and significantly lower cook loss when compared to control. At 3% SPI, juiciness was diminished and consequently samples had lower overall acceptability. Thus, while higher levels of SPI may result in lower cook loss, processors cannot add too high a level without incurring unacceptable changes in flavor or texture. Therefore an optimum protein incorporation level must be calculated that can give highest overall acceptability without compromising cook loss.

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Table 3.1. Cook loss and expressible moisture in pork sausage with added soy protein isolate (SPI), egg albumen (EA) or whey protein isolate (WPI).

	Cook Loss (%)		Expressible Moisture (%)	
Treatments	Mean	SD	Mean	SD
Control	27.7[a]	1.44	5.96[a]	0.10
SPI 1%	18.7[e]	0.47	3.70[g]	0.12
SPI 2%	17.3[f]	0.52	3.60[g]	0.10
SPI 3%	14.1[g]	0.97	3.22[h]	0.12
EA 1%	22.6[c]	0.74	4.72[c]	0.07
EA 2%	20.7[d]	0.24	4.04[e]	0.08
EA 3%	19.0[e]	0.25	3.85[f]	0.06
WPI 1%	24.5[b]	0.51	5.02[b]	0.14
WPI 2%	22.1[c]	0.64	4.62[c]	0.04
WPI 3%	19.9[de]	0.69	4.50[d]	0.08

[a-h] Means in the same column with different superscripts are significantly different ($p < 0.05$). n=3

Table 3.2. Cutting force, hardness, adhesiveness and cohesiveness in pork sausage with added soy protein isolate (SPI), egg albumen (EA) or whey protein isolate (WPI).

	Cutting Force (g)		Hardness (g)		Adhesiveness (g.s)		Cohesiveness	
Treatments	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	446^{f}	16.2	1296^{i}	155.8	83.7^{a}	5.96	0.29^{f}	0.02
SPI 1%	769^{c}	34.2	2617^{c}	76.4	40.4^{c}	3.31	0.44^{b}	0.03
SPI 2%	936^{b}	44.5	2884^{b}	24.2	27.9^{d}	5.56	0.45^{b}	0.02
SPI 3%	1087^{a}	33.6	3179^{a}	33.7	26.3^{d}	4.41	0.53^{a}	0.01
EA 1%	522^{e}	17.7	1844^{h}	84.4	53.9^{b}	9.34	0.33^{e}	0.02
EA 2%	601^{d}	15.3	2217^{f}	67.5	43.4^{c}	1.93	0.37^{d}	0.01
EA 3%	749^{c}	34.1	2427^{d}	123.2	31.5^{d}	2.28	0.40^{c}	0.01
WPI 1%	584^{d}	11.8	2037^{g}	41.7	45.2^{c}	10.40	0.31^{e}	0.01
WPI 2%	602^{d}	22.3	2256^{ef}	54.2	40.1^{c}	7.06	0.36^{d}	0.01
WPI 3%	750^{c}	26.9	2344de	30.0	28.4^{d}	5.80	0.37^{d}	0.01

[a-i] Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

Table 3.3. Shear stress, shear strain, fracture modulus (G) and slope ratio at 30 % strain in pork sausage with added soy protein isolate (SPI), egg albumen (EA) or whey protein isolate (WPI).

	Shear Stress at Failure (kPa)		Shear Strain at Failure		G at Failure (kPa)		Slope Ratio (at 0.30 strain)	
Treatments	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	26.5^{f}	4.6	1.13^{a}	0.12	23.38^{f}	2.36	1.32^{cd}	0.26
SPI 1%	44.3^{b}	3.1	1.00^{b}	0.08	44.72^{b}	6.08	1.55^{ab}	0.13
SPI 2%	51.7^{a}	4.7	1.05^{ab}	0.06	49.25^{ab}	4.45	1.46^{abc}	0.21
SPI 3%	52.9^{a}	2.5	1.03^{ab}	0.08	51.67^{a}	5.36	1.57^{ab}	0.14
EA 1%	33.3^{d}	1.7	1.10^{ab}	0.11	30.37^{de}	2.52	1.32^{cd}	0.23
EA 2%	38.6^{c}	1.5	1.01^{b}	0.11	38.69^{c}	4.22	1.60^{a}	0.07
EA 3%	39.7^{c}	2.0	1.03^{ab}	0.07	38.87^{c}	4.44	1.58^{ab}	0.08
WPI 1%	27.0^{f}	1.4	1.08^{ab}	0.14	25.37^{ef}	3.01	1.23^{d}	0.17
WPI 2%	28.5^{ef}	1.1	1.02^{ab}	0.10	28.16^{def}	3.41	1.38^{bcd}	0.12
WPI 3%	30.7^{de}	1.7	0.98^{b}	0.08	31.50^{d}	3.34	1.45^{abc}	0.16

[a-f] Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

Table 3.4. Color values in pork sausage with added soy protein isolate (SPI), egg albumen (EA) or whey protein isolate (WPI).

	L*		a*		b*		
Treatments	Mean	SD	Mean	SD	Mean	SD	ΔE
Control	42.6^{g}	0.30	25.8^{a}	0.42	14.6^{h}	0.35	0.00
SPI 1%	43.6^{def}	0.28	23.3^{cd}	0.57	15.6^{e}	0.14	2.87
SPI 2%	43.7^{de}	0.27	22.3^{e}	0.44	15.9^{d}	0.18	3.89
SPI 3%	45.4^{a}	0.31	21.6^{f}	0.25	17.1^{a}	0.30	5.63
EA 1%	43.5^{f}	0.12	24.3^{b}	0.36	15.1^{g}	0.14	1.82
EA 2%	43.5^{ef}	0.19	23.4^{cd}	0.25	15.5^{ef}	0.08	2.72
EA 3%	44.3^{c}	0.20	23.5^{cd}	0.40	15.8^{de}	0.12	3.10
WPI 1%	43.8^{d}	0.27	24.2^{b}	0.08	15.3^{fg}	0.24	2.12
WPI 2%	44.4^{c}	0.16	23.6^{c}	0.17	16.2^{c}	0.17	3.26
WPI 3%	44.7^{b}	0.22	23.2^{d}	0.36	16.7^{b}	0.20	3.95

$^{a-h}$ Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

$$\Delta E = \sqrt{(L^* - L^*_c)^2 + (a^* - a^*_c)^2 + (b^* - b^*_c)^2}$$

Table 3.5. Effect of SPI, WPI and EA on textural attributes and consumer acceptability of cooked pork sausage.

	Descriptive Attribute						Consumer Acceptability					
	Juiciness		Springiness		Cohesiveness		Texture		Flavor		Overall	
Treatments	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	6.83[b]	0.41	7.61[a]	0.30	8.33[a]	0.13	7.99[ab]	0.10	8.19[a]	0.06	7.70[b]	0.03
Soy 2%	8.28[a]	0.20	7.69[a]	0.13	8.50[a]	0.21	8.27[a]	0.26	8.19[a]	0.03	8.40[a]	0.16
Egg 2%	7.52[b]	0.18	7.49[a]	0.05	8.10[a]	0.04	7.82[ab]	0.14	5.12[c]	0.23	6.61[c]	0.20
Whey 2%	7.41[b]	0.18	7.45[a]	0.18	8.07[a]	0.20	7.77[b]	0.11	7.29[b]	0.18	6.99[c]	0.21

[a-d] Means in the same column with different superscript are significantly different ($p < 0.05$). n=12

Table 3.6. Effect of SPI levels on textural attributes and consumer acceptability of cooked pork sausage.

	Descriptive Attribute						Consumer Acceptability					
	Juiciness		Springiness		Cohesiveness		Texture		Flavor		Overall	
Treatments	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	7.41[c]	0.04	7.50[a]	0.05	8.06[a]	0.14	7.69[b]	0.05	7.91[ab]	0.04	7.35[c]	0.08
Soy 1%	7.92[b]	0.15	7.53[a]	0.06	8.13[a]	0.01	7.96[b]	0.09	7.98[ab]	0.04	7.89[b]	0.06
Soy 2%	8.29[a]	0.05	7.57[a]	0.18	8.27[a]	0.03	8.27[a]	0.18	8.21[a]	0.03	8.42[a]	0.06
Soy 3%	7.75[b]	0.02	7.66[a]	0.03	8.22[a]	0.06	7.87[b]	0.04	7.10[b]	0.78	7.54[c]	0.16

[a-d] Means in the same column with different superscript are significantly different ($p < 0.05$). n=12

CHAPTER 4

EFFECT OF HIGH PRESSURE MICROFLUIDIZATION AND NACL LEVEL ON THE RHEOLOGICAL PROPERTIES AND GELATION OF THREE FOOD PROTEINS

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Abstract

Dispersions (15% w/w) of soy protein isolate (SPI), whey protein isolate (WPI) and egg albumin (EA) were microfluidized at 30,000 PSI in solutions with 0%, 1%, 2% and 3% NaCl. The resulting homogenates and their gel-forming abilities were evaluated through particle size analysis, compressive gel strength, torsion gelometry and dynamic rheology. Microstructural features were examined by confocal laser scanning microscopy (CLSM). Microfluidization resulted in a decrease in particle size and increase in gel strength for all the protein dispersions. The particle size of each protein dispersion increased with NaCl concentration up to 2%. Compressive gel strength increased substantially for gels made from microfluidized proteins. Torsional tests showed an increase in shear stress at fracture, and decrease in shear strain, with both microfluidization and increasing NaCl levels. The storage modulus (G') also increased for gels formed from microfluidized protein, although gelling times were not greatly affected. CLSM showed a more uniform distribution of the protein aggregates after microfluidization. Microfluidization resulted in significant change in structure and gelation of all the protein dispersion and can be used as a useful tool for manipulating the functionality of the proteins for different food processing conditions.

Key words: Microfluidization, SPI, WPI, Egg albumin.

Introduction

Soy, whey and egg albumin proteins are common ingredients used in processed foods. They may be used to increase protein content, as a replacement for other proteins, to bind water, to form continuous macrostructures such as gels, or to provide other functionality. Thus, for example, egg white protein may be used in some cakes to improve volume or provide a more foam-like structure. Soy proteins are often used in meat analogues and can even be caused to have a meat-like stranded texture. One of the biggest sectors for these proteins are as binders in comminuted meats such as sausages and frankfurters, where one of the primary goals is to formulate low fat, high protein sausages without adversely affecting the palatability and flavor. Some of the inherent limitations for adding these proteins to foods are low solubility, the need for high concentrations to allow for gelling, and the possibility of developing off-flavors in the final product.

It has been of considerable interest to find ways to improve or modify protein functionality, or to allow these functions to occur at lower protein concentration. There are many ways proteins can be modified to cater to particular needs, as by altering pH or ionic strength, adding enzymes to cleave or modify specific groups, interacting proteins with polysaccharides or extruding the protein at relatively low moisture levels (de Jongh and Broersen 2012). A fairly new development is the potential use of high-pressure processing to change the functionality of the proteins in a desirable way. For example, O'Flynn and others (2014) investigated the use of high hydrostatic pressure (HP) to reduce the amount of phosphate needed in breakfast sausages. They found that, up to a point, HP could help improve perceived saltiness, juiciness and flavor in sausages

prepared without phosphate. HP processing also helped improve oxidative stability during long term storage (Chun and others 2012), allow reduced salt levels (Grossi and others 2012), and in combination with *enterococci* starter cultures, reduced the ability of *Listeria monocytogenes* to grow on sausages (Rubio and others 2013). Thus, HP could help enhance protein functionality, such as binding water to improve juiciness that is typically caused by the incorporation of salt or phosphates. Very little work has been done, however, on the use of static high pressure processing on individual ingredients.

Another version of high pressure processing allows for the continuous flow of foods or ingredients in a liquid state. These continuous high-pressure processing (CHP) systems pump the fluid through a metering valve, interaction chamber or other system that allows the sudden release of pressure (Cavender and Kerr 2011), and which produce tremendous shear forces or cavitation effects on the product. One type of CHP uses microfluidization. In this system, fluid is forced through an interaction chamber at 150-250 MPa, causing the fluid to split into two streams at 180°, then rejoining them. Microfluidization (MF) is a high shear fluid processing technique aimed at achieving uniformity in particle size reduction, and has been used most extensively in the pharmaceutical, biotechnological, cosmetic and chemical industries. In the food industry it has been mostly limited to small volume operations such as the preparation of nutraceuticals. For example, it has been used for creating submicron particles for nanoencapsulation to mask the foul smell and taste of some nutrients (Jafari and others 2008; Jafari and others 2007). It has also been useful for limiting phase separation and improving the bioavailability of lutein and other bioactive materials (Vishwanathan and others 2009; Acosta 2009).

There have been a few studies on the use of MF and other CHP equipment to change the properties of proteins and other food ingredients. Shen and Tang (2012) showed that MF could change several physicochemical properties of soy protein isolate including protein solubility, surface hydrophobicity and disulfide content. It also enhanced the emulsifying ability of the proteins and help limit creaming in oil-water emulsions. Tang and Liu (2013) used MF to produce gel-like emulsions from soy protein isolate without the need for heating. The continuous network was formed by bridging flocculation of oil droplets, made possible by proteins adsorbed at the interface. Iordache and Jelen (2003) used MF to improve the solubility of whey protein concentrate, and reduce sedimentation during storage. Sanchez et al (1999) found that MF could reduce protein aggregation and improve its ability to form gels. Hu and others (2011) used MF and a cross-linking agent (transglutimase) to alter the properties of peanut protein isolate. They found that MF at 120 MPa could improve the solubility, emulsifying properties and surface hydrophobicity of the peanut protein. Combinations of MF followed by treatment with transglutimase produced the greatest changes in functionality.

As previously mentioned, non-meat proteins may be used in comminuted meat products. Products such as sausage have much of their myofibrillar proteins extracted, which is denatured and forms a continuous network know as a meat emulsion (Doi 1993). In essence, this is a semi-solid gel system that holds water and entraps fat, bits of muscle tissue, spices and other ingredients. The protein also includes denatured collagen derived from the connective tissues.

When non-meat proteins are added, their interaction with the muscle proteins and their gelling behavior individually and within the mixed gel system is of prime

importance. Soy protein isolate (SPI), whey protein isolate (WPI) and egg albumin (EA) are all globular proteins commonly used with comminuted meats, and their mechanism of forming aggregates and gels are similar (Clark and others 2001; Kavanagh and others 2000a; Kavanagh and others 2000b). Their functionality is also often dependent on the pH and ionic strength of the system.

Studying the rheological and textural behavior of non-meat proteins during and after gelation is beneficial for predicting their behavior in a complex system such as sausage. The present study was aimed at evaluating the effects of high-pressure microfluidization (MF) and NaCl levels on the structure, rheological properties and gelation properties of SPI, WPI and EA dispersions. Particle size analysis was used to study the effects of MF on protein aggregates in an aqueous system. Properties of the gelled proteins were assessed through compression and torsional rheometry under conditions designed to cause fracture. In addition, dyanamic rheometry was used to study the fundamental viscoelatic properties of the proteins, and how these change during the course of gel formation initiated by heating.

Materials and methods

Sample preparation

Dry powders were obtained of soy protein isolate (Pro-Fam, ADM, Decatur, IL), whey protein isolate (Prolian 8600, Proliant Meat Ingredients, Ames, IA) and egg albumen (Type D-39, Hennigen Foods, Omaha, NE). Each protein was added to distilled water with constant stirring at 20°C, until a uniform dispersion of 15g protein per 100 ml was attained. An aspirator was used to remove any air incorporated during the mixing process. Protein dispersions with 0, 1, 2 and 3% NaCl (w/v) were prepared. The mixed

samples were subsequently passed through a MK 140 microfluidizer (Microfluidics, Newton, MA, USA) at 30,000 PSI in a single pass.

Particle size analysis

The particle size distribution of the protein suspensions was measured before and after MF processing with a Malvern Mastersizer S laser particle size analyzer (Malvern instruments Inc. Westborough, MA USA.) equipped with a 300 mm lens (for size range 0.5 – 880 µm). The impeller speed for all samples was kept at 2500 rpm while the sample was dispersed in deionized water in the diffractometer cell. The sample was added to the system until an obscuration point of 15 ± 2 % was obtained. The Malvern software was used to calculate the mean volume weighted diameter, $D_{(4,3)} = \sum n_i d_i^4 / \sum n_i d_i^3$ (where n_i is the number of particles in the size range of diameter d_i)

Gel compressive strength

Gel strength was tested under compression using bloom jar method as described by Gelatin Manufacturers of Europe (2000), with some modifications. From each protein sample, 80 ml was transferred to the bloom jar and heated at 80° C for 30 min in a constant temperature water bath. The samples were allowed to cool at 20°C for several hours until gel formation was complete. After cooling, the gels were stored at 4°C. Gel samples were tested the next day after equilibrating at 20°C for one hour.

The gel strength was measured using a TA XT2i texture analyzer (Stable Micro Systems, Ltd, Surrey, England) fitted with a 5 kg load cell, and using a cross-head speed of 1 mm/s. Maximum force was measured after a 0.5 inch cylindrical probe penetrated the gel to a depth of 4 mm. Measurements were done in triplicate for each sample.

Gel torsion tests

Cylindrical gel samples (20mm diameter X 25 mm length) were prepared by heating the protein dispersion in a glass cylinder held in a water bath at 80°C for 30 minutes. Each sample was mounted on two notched styrene discs with the help of cyanoacrylate adhesive and made into hour glass shape using a gel grinder (Gel Consultants, Raleigh, N.C.). A Torsion Gelometer (Brookfield Engineering, Middleboro MA) was used to twist the samples at 2.5 rpm, and the generated shear stress and shear strain were measured up to the point of sample fracture. The failure shear stress was measured to determine the gel strength whereas the failure shear strain was measured to assess deformability of the gels. The ratio of shear stress and shear stain at failure was recorded as the failure modulus (G). The 'slope ratio' was calculated as the failure modulus (G) divided by the modulus at 30% failure stain (G_{30}).

Dynamic Rheology

A SR 5000 dynamic stress rheometer (Rheometric Scientific, Piscataway, NJ) was used to measure viscoelastic properties. Liquid samples were applied to the base plate which rested on a Peltier heating/cooling unit. A 40 mm parallel plate probe was used for all measurements and brought to a gap distance of 1mm above the plate. The linear viscoelastic regions were first established for each sample by performing a shear-stress sweep test at a frequency of 1 Hz. To study gel-forming properties, a cure experiment was performed based on a method suggested by Cordobés and others (2004) with some modifications. The protein dispersions were subjected to two different temperature ramp rates, 1 and 3°C/ minute. Temperatures were increased from 25 to 80°C, at which point the dispersion was held at 80°C for 25 minutes before cooling. Gelation was observed

during the thermal cycle and the gel point for each sample was recorded from the crossover point of the elastic modulus (G') and loss modulus (G") curves.

Confocal microscopy

Gel samples for microscopy studies were made as described for the torsion tests. Thin slices of the gel (~200 um) were taken then immersed in 0.1% (w/w) aqueous solution of Florescein dye. The stained gels were quickly washed with de-ionized water before mounting the sample on the stage of an inverted microscope attached to Leica TCS FP5 multiphoton confocal laser scanning microscope (Leica Microsystems Inc., Eagleview Blvd., Exton PA). An oil immersion objective (UV lens 40X) was used to image the samples. The CLSM was operated in fluorescence mode using an argon laser at an excitation wavelength of 488 nm. Fluorescence from the sample was imaged and was processed using Leica Application Suite Advanced Fluorescence (LAS AF) software. All images are a single scan of a focal place.

Statistical analysis

All data was analyzed using SAS statistical software version 9.1, 2008 (SAS Institute Inc., Cary, NC). An analysis of variance (ANOVA) was first performed. Tests for significant differences amongst the treatment groups were done with the Fisher protected LSD test, at 95% level of confidence.

Results and discussions

Particle size analysis

The mean particle size and standard deviation for the 15% protein dispersions, with and without microfludization, is shown in Table 4.1. Without MF, particle sizes

were greatest for the SPI (163-193 um), intermediate for EA (140-177 um) and least for the WPI (103-143 um) suspensions. In general, particle sizes increased somewhat with NaCl concentration in the range of 0-2%, with no differences between samples at 2 and 3% NaCl. The proteins in this study carry a net negative surface charge, providing some degree of electrostatic repulsion. Addition of NaCl would be expected to decrease the thickness of the electric double layer, characterized by the Debye length, allowing the protein surfaces to approach more closely and eventually aggregate. Kaewmanee and others (2011) observed an increase in particle size for duck egg white albumen with increases in NaCl concentration. Increases in NaCl led to higher turbidity, increases in surface hydrophobicity and the appearance of larger clusters when examined by CSLM. Barbut (1995) observed that at low sodium levels, fine protein strands formed, allowing for the formation of clear gels. As sodium levels increased, the strands became thicker and led to decreased water hold capacity and the formation of opaque gels. Li and others (2007) found that at zero ionic strength, soy protein existed as aggregates, intermediate fractions and non-aggregated molecules. At higher ionic strength, the amount of aggregates increased substantially.

The high shear conditions of the MF at 207 MPa (30,000 Psi) resulted in significant decreases in particle size of all the protein dispersions, at all NaCl levels. For example, at 2% NaCl, EA particle size decreased from 176 to 52.5 um, SPI from 193 to 62.1 um, and WPI from 144 to 54.7 um. Cheng-Mei and others (2011) found that average particle size of whey protein concentrate decreased gradually as the MF pressure was increased from 40 MPa to 160 MPa. In addition, higher pressures resulted in a more narrow distribution of particle sizes. Similar results have been obtained by others

(Sanchez and others 1997; Sanchez and others 1999) working with micro-particulation of a blend of whey protein isolate and heat denatured whey protein isolate. Shen and Tang (2012) observed a decrease in mean volume diameter ($d_{4,3}$) of unheated SPI due to microfluidization at 120 MPa; however, heat pretreatment at 75 to 95°C progressively increased $d_{4,3}$ due to increased flocculation of protein covered oil droplets. Apichartsrangkoon (2003) observed an increase in storage and loss moduli with high-pressure treatment of hydrated soy protein concentrate and gluten. Maa and Hsu (1996) used a closed concentric cylinder shear device and a homogenizer with a rotor/stator assembly to study two different proteins (human growth hormone and deoxyribonuclease). They found that the high shear (up to 10^5 s^{-1}) in that device did not lead to changes in protein aggregate size, but had a small effect on melting temperature and did create fragments indicative of some peptide bond cleavage. It should be noted that shear rates in the microfluidizer can be substantially greater (up to 10^7 s^{-1}).

Gel compressive strength

The various protein dispersions had different hardness when formed into gels and subject to compressive stress (Table 4.2). EA gels were the firmest (105.0 – 272.3 g), followed by WPI gels (84.4 – 167.0 g) and SPI gels (14.5 – 18.1 g). Maximum hardness was found at 2% NaCl for EA and SPI, and at 1% NaCl for WPI gels. The inclusion of 3% NaCl resulted in less firm gels for EA and WPI. Guo and others (2013) found that whey protein emulsion gels became firmer under uniaxial compression as NaCl was increased from 0 to 200 mM. In addition, when subject to mastication the gels with lower levels of NaCl were characterized as softer and broke down with fewer chewing cycles as compared to gels with higher levels of NaCl. Gels formed at higher levels of

NaCl were not only harder, but fragmented into a greater number of pieces in the mouth. These researchers also observed a decrease in hardness after a threshold level of 100 mM. Similarly, Barbut (1995) reported a significant decrease in water holding capacity and gel strength due to formation of aggregation above a threshold level of NaCl.

Microfluidization of the protein dispersions resulted in greater hardness in the formed gels. For example, at 2% NaCl hardness increased from 271.1 to 390.3 g for EA gels, 16.2 to 112.2 g for SPI gels, and 110.8 to 353.3 g for the WPI gels. Similar results have been reported for WPI gels. Sanchez and others (1999) observed that increasing MF pressure and number of passes resulted in an increase in WPI gel hardness, due to smaller size of aggregates which in turn result in an increase in homogeneity of the heat-set gel.

Cheng-Mei and others (2011) found that MF resulted in de-aggregation and re-aggregation of whey protein concentrate and increased protein solubility, foaming capability and foaming stability. In addition, there was a reduction of emulsifying capacity of the protein as the MF pressure was increased.

Gel torsion strength

The gels were also tested under torsional forces, which subject the samples to twisting under an applied torque. Some studies have suggested this gives a more accurate appraisal of gel strength, particularly for soft hydrogels, as it gives a more uniform application of stress during the course of testing. Results from the torsion gelometer tests are shown in Table 4.3. Only WPI and EA produced gels strong enough to be ground into the hour-glass shape required for testing. All gels formed with the addition of NaCl were opaque suggesting formation of a particulate gel network formed through aggregation. In contrast, stranded gels (such as gelatin) are formed of fine stands and are transparent

(Stading and Hermansson 1990; Stading and Hermansson 1991). Gels formed without NaCl were translucent.

A progressive increase in shear stress at failure was observed in both WPI and EA gels as NaCl was increased up to 2% (Table 4.3). EA gels formed with 3% NaCl had no difference in shear stress compared to 2% NaCl gels, while WPI gels with 3% NaCl had lower shear stress. Gill and others (1992) observed no effect of NaCl on the shear stress in fish myosin surimi gel. We can reasonably expect different behaviors from different proteins due to different size of aggregates they form under the same conditions of concentration, ionic strength and pH. Microfluidization resulted in an increase in shear stress at fracture for each of the samples tested, although the differences were not large. Thus, shear stresses for the non-MF EA gels ranged from 31.7 to 61.0 kPa (at 0-3% NaCl), while the MF EA gels ranged from 45.1 to 72.2 kPa. For WPI gels, shear stresses ranged from 31.4 to 55.4 kPa for the non-MF gels, and from 45.4 to 69.7 kPa for the MF gels.

Shear strain and slope ratio decreased with increasing NaCl concentration. Thus, the higher levels of NaCl tended to make for firmer gels that fractured over a shorter distance.

All the samples showed strain hardening as the slope ratio was more than 1 in all cases, but the magnitude of stain hardening decreased with increasing NaCl concentration. Microfluidization resulted in a decrease in the shear strain at fracture, again indicative of somewhat firmer gels that fracture at smaller deformations. The slope ratio was also smaller for gels made from MF protein. Gwartney and others (2004) reported similar strain hardening for both particulate and stranded emulsion gels prepared

from whey protein isolate. Particulate gels displayed lower strain hardening compared to stranded gels. Most food gels have a pH and ionic strength which support the formation of particulate gels (McGuffey and Foegeding 2001).

Dynamic rheology at small deformation

Microfluidization significantly increased the shear storage modulus (G') of all the protein dispersions at each level of NaCl, after subject to controlled heating (Tables 4.4-4.5). When temperature was increased at 3°C/min, the final G' ranged from 6768 to 17,995 Pa for EA gels, 18,915 to 28,311 Pa for SPI gels, and 3231 to 12,614 Pa for WPI gels. For proteins first subject to MF, these values ranged from 9831 to 20,529 PA for EA gels, 24,137 to 31,269 Pa for SPI gels, and 534 to 13,729 Pa for WPI gels. Interestingly, SPI gels had the highest G' values yet were relatively soft when tested by large-deformation compressive and torsional tests. This may be related to the testing conditions. In general, SPI requires higher temperatures and longer times to completely unfold (>90°C). WPI and EA proteins can denature and aggregate at relatively lower temperatures (>65°C) (Morr and Ha 1993; Jiang and Xiong 2013; Jiang and others 2010; Donovan and others 1975). Others (Apichartsrangkoon 2003; Apichartsrangkoon and others 1999) have also observed increases in storage and loss moduli for gels formed from high-pressure treated soy protein concentrate and wheat gluten.

Surface hydrophobicity of soy protein isolate has been shown to increase due to high-pressure treatment at 200 MPa, as the hydrophobic groups become exposed due to partial denaturation of the protein (Molina and others 2001; Galazka and others 1999). Increases in G' in all the MF proteins during the cure test can be attributed to increases in hydrophobic interactions. Increased intermolecular associations of the globular proteins

due to high-pressure homogenization has been reported by several researchers, and attributed to changes of the secondary structure. These can lead to significant changes in both the viscous properties of the protein dispersion and the solid-like behavior of the gels they form. Lee and others (2007; 2009) suggested that there was a decrease in α-helix and unordered structure after high-pressure homogenization of soy and soy oil emulsions. This leads to enhanced protein-oil and protein–protein interactions and consequently formation of irreversible protein membranes at the oil-water interface due to protein aggregation.

Significant increases in elastic modulus were observed with increasing NaCl for both MF and non-MF protein dispersions up to 2% NaCl. Higher levels (3%) of NaCl resulted in a decrease in elastic modulus in all the samples except for EA gels formed at 1°C/min ramp rate (Tables 4.4 and 4.5). Other researchers (Liu and Tang 2011; Tang and Liu 2013) have also observed increases in elastic modulus and apparent viscosity at higher levels of NaCl in soy protein and whey protein cold gel-like emulsions. Floury and others (2002b) found that ultra high-pressure homogenization caused some denaturation of soy proteins due to strong mechanical forces and high temperatures encountered in the process. When oil-water emulsions were formed with soy protein, the high pressure was able to produce much finer emulsions. Studies on methylcellulose (Floury and others 2003; Floury and others 2002a) showed that the HP process could reduce molecular size, alter solution viscosity and improve the emulsification properties of the polysaccharide.

Gel points during controlled heating were determined based on the cross-over point, that is the time (or temperature) at which G'=G" (Tables 4.6 and 4.7). Definitive gel points were observed for EA and WPI gels. For SPI, both the G' and G" increased in

a parallel manner as temperature increased, so that a crossover point could not be detected. Gel points decreased slightly in both EA and WPI as NaCl levels increased, regardless of the temperature ramp rate (that is, 3°C/min (Table 4.6) or 1°C/min (Table 4.7)). For example, for EA the gel time decreased from 15.6 to 14.7 min as NaCl levels were increased from 0 to 3%, while the gel temperature decreased from 66.4 to 63.7°C. MF proteins also had reduced gelling times and temperatures. Thus, for non-MF EA gel times ranged from 14.7 to 15.6 mins, while gel temperatures ranged from 63.7 to 66.4°C when heated at 3°C/min. For the MF EA, gel times ranged from 13.3 to 14.6 min and gel temperatures from 59.5 to 63.3°C. Forming gels at the slower ramp rate (1°C/min) resulted in longer gel times, but the overall effects of NaCl and MF were similar. As gel formation requires denaturation of protein, it would take longer to reach a critical temperature at the slower heating rate. Gel temperatures were also somewhat lower at the slower ramp rate. For example, for MF EA protein at 2% NaCl the gelling temperature was 60.2°C when formed at 3°C/min and 58.3°C when formed at 1°C/min. In general, the gel onset temperatures were 2-5°C lower when gels were formed at 1°C/min versus 3°C/min. This is not unexpected as gel formation is a time-dependent phenomenon. The movement of macromolecules and development of mesoscale interactions amongst these molecules takes time. Thus, once a denaturation temperature is achieved, it still takes time for associations to be formed and this is more easily realized at 1°C/min as compared to 3°C/min.

Confocal microscopy

Images obtained from multiphoton CSLM for WPI gels are presented in Figure1. WPI gels produced the best contrast between protein molecules and void space compared

to EA and SPI gels. In general, larger aggregates were observed with incorporation of NaCl when compared to the gels without NaCl. In addition, microfluidization resulted in a more uniform distribution of the aggregated proteins. Results were found to be consistent with the compressive and torsion-based rheological tests.

Conformational changes of protein due to pH, ionic strength, concentration and combinations with other food ingredients have been extensively studied using confocal microscope. For example, Gonçalves and others (2004) studied the effects of galactomannans on the heat-set gelation of WPI. They found that at low concentrations, the presence of galactomannans enhanced the aggregation and strength of WPI gels, but did not modify the basic structure of the system. At higher galactomannan concentration, the gel strength was lower, which was linked to depletion-induced liquid-liquid phase separation occuring during the aggregation process. Sittikijyothin and others (2007) studied heat-induced gelation of β-lactoglobulin at varying pH, and with incorporation of non-gelling tara gum. CSLM showed that the gels were mixed two-phase systems, and that the degree of aggregation depended on the concentration of tara gum and pH.

Conclusions

Microfluidization resulted in a decrease in particle size and changes in the surface properties of the protein dispersions, which manifested in an increase in storage modulus and gel hardness of SPI, WPI and EA gels. Protein aggregates in the gels showed more uniform distribution and less strain hardening due to microfluidization. Microfluidization resulted in significant changes in rheology and gelation properties for all the proteins and can be used as a useful tool in changing the functionality of protein, thus making each more suitable for particular food applications.

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Table 4.1 Effects of microfluidization and Nacl levels on the $D_{(4, 3)}$ particle size (μm) of egg albumen (EA), soy protein isolate (SPI) and whey protein isolate (WPI) dispersions.

		Non-Microfluidized		Microfluidized	
	Nacl g/100g	Mean	SD	Mean	SD
EA	0	140^{ef}	14.5	20.5^{m}	2.6
EA	1	168^{cd}	3.9	45.4^{jkl}	2.6
EA	2	177^{bc}	6.6	52.5^{hijk}	2.9
EA	3	174^{bc}	8.5	52.5^{hijk}	4.0
SPI	0	163^{d}	11.4	36.2^{l}	1.3
SPI	1	184^{ab}	6.5	51.4^{ijk}	4.3
SPI	2	193^{a}	3.4	62.1^{h}	3.4
SPI	3	187^{a}	7.5	59.1^{hi}	3.2
WPI	0	103^{g}	7.4	24.6^{m}	3.4
WPI	1	131^{f}	6.0	42.7^{kl}	3.0
WPI	2	143^{e}	4.8	54.7^{hij}	4.5
WPI	3	138^{ef}	9.0	53.5^{hij}	4.5

$^{a-m}$ Means in both the column with different superscript are significantly different ($p < 0.05$). n=3

Table 4.2 Effects of microfluidization and Nacl levels on protein gel hardness measured at 4 mm deformation.

		Non-Microfluidized Force (g)		Microfluidized Force (g)	
	Nacl g/100g	Mean	SD	Mean	SD
EA	0	105.0^{hg}	3.3	239.6^{e}	6.4
EA	1	272.3^{d}	7.2	426.7^{a}	30.8
EA	2	271.1^{d}	12.9	390.3^{b}	12.0
EA	3	241.7^{e}	11.0	371.3^{c}	19.0
SPI	0	14.5^{k}	2.5	11.1^{k}	2.5
SPI	1	18.1^{k}	4.2	64.1^{j}	12.9
SPI	2	16.2^{k}	3.8	112.2^{g}	6.8
SPI	3	16.4^{k}	3.7	89.9^{hi}	4.0
WPI	0	84.4^{i}	5.6	115.2^{g}	14.1
WPI	1	167.0^{f}	10.1	274.7^{d}	15.3
WPI	2	110.7^{g}	10.5	353.3^{c}	3.5
WPI	3	92.5^{hi}	5.7	279.7^{d}	7.7

$^{a-k}$ Means with the same superscript letter are not significantly different ($p < 0.05$). n=3

Table 4.3 Effects of microfluidization (MF) and Nacl levels on torsion shear stress and shear strain at failure, fracture modulus and slope ratio (measured at 30% strain).

	NaCl	Shear Stress (kPa)		Shear Strain		Fracture Modulus (kPa)		Slope Ratio	
	g/100g	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Non-MF									
EA	0	31.7^{i}	2.4	1.53^{d}	0.04	20.7^{h}	1.5	1.62^{a}	0.03
EA	1	43.9^{h}	1.5	1.52^{de}	0.03	28.8^{fg}	0.8	1.43^{d}	0.03
EA	2	65.7^{dc}	2.6	1.45^{fg}	0.02	45.3^{d}	1.1	1.33^{e}	0.02
EA	3	61.0^{de}	1.8	1.29^{i}	0.03	47.3^{d}	2.3	1.17^{f}	0.03
MF									
EA	0	45.1^{gh}	1.3	1.44^{fg}	0.03	31.3^{f}	0.3	1.54^{bc}	0.02
EA	1	59.2^{ef}	3.6	1.46^{ef}	0.05	40.4^{e}	2.3	1.39^{d}	0.02
EA	2	77.4^{a}	4.6	1.39^{gh}	0.03	55.5^{b}	2.9	1.29^{e}	0.02
EA	3	72.2^{b}	3.6	1.21^{j}	0.05	59.7^{a}	4.4	1.07^{g}	0.02
Non-MF									
WPI	0	31.4^{i}	1.6	1.86^{a}	0.03	16.8^{i}	0.58	1.62^{a}	0.05
WPI	1	46.3^{gh}	2.6	1.60^{c}	0.05	28.8^{fg}	0.81	1.55^{bc}	0.03
WPI	2	55.4^{f}	2.7	1.42^{fgh}	0.03	39.0^{e}	1.65	1.39^{d}	0.03
WPI	3	49.4^{g}	3.8	1.27^{ij}	0.02	39.0^{e}	2.53	1.28^{e}	0.03
MF									
WPI	0	45.4^{gh}	1.8	1.74^{b}	0.05	26.1^{g}	0.31	1.57^{ab}	0.03
WPI	1	55.6^{f}	2.4	1.47^{ef}	0.04	37.8^{e}	2.35	1.49^{c}	0.02
WPI	2	69.7^{bc}	3.5	1.36^{h}	0.02	51.1^{c}	2.17	1.32^{e}	0.03
WPI	3	63.2^{de}	4.0	1.14^{k}	0.06	55.3^{b}	0.57	1.14^{f}	0.09

[a-i] Means in the same column with different superscripts are significantly different ($p < 0.05$). n=3

Table 4.4 Effects of microfluidization and NaCl levels on the storage modulus (G') of protein gels formed during temperature ramp at 3°C/min.

Protein	NaCl (g/100g)	Non-Microfluidized G' (Pa) Mean	SD	Microfluidized G' (Pa) Mean	SD
EA	0	6768^{n}	218	9830^{l}	402
EA	1	13955^{j}	458	15732^{i}	731
EA	2	17995^{gh}	602	19944^{f}	406
EA	3	17450^{h}	563	20528^{f}	472
SPI	0	18915^{f}	949	24137^{e}	571
SPI	1	24561^{e}	600	27113^{d}	138
SPI	2	27497^{cd}	579	30129^{b}	468
SPI	3	28311^{c}	1459	31269^{a}	299
WPI	0	3231^{p}	264	5234^{o}	234
WPI	1	4953^{o}	311	8340^{m}	395
WPI	2	12614^{k}	1210	14309^{j}	594
WPI	3	9464^{l}	199	13728^{j}	304

$^{a-p}$ Means in the same column with different superscripts are significantly different ($p < 0.05$). n=3

Table 4.5 Effects of microfluidization and NaCl levels on the storage modulus (G') of protein gels formed during temperature ramp at 1°C/min.

Protein	NaCl (g/100g)	Non-Microfluidized G' (Pa) Mean	SD	Microfluidized G' (Pa) Mean	SD
EA	0	7860^{p}	408	10753^{n}	276
EA	1	14584^{l}	305	16401^{j}	587
EA	2	18512^{i}	516	19812^{h}	133
EA	3	18986^{i}	484	20913^{g}	147
SPI	0	20385^{gh}	459	23349^{f}	444
SPI	1	26083^{e}	765	28539^{d}	358
SPI	2	28689^{d}	418	31067^{c}	504
SPI	3	32929^{b}	128	36401^{a}	515
WPI	0	4014^{r}	353	5724^{q}	215
WPI	1	6051^{q}	297	9317^{o}	342
WPI	2	13785^{m}	161	14719^{l}	137
WPI	3	13895^{m}	417	15397^{k}	440

$^{a\text{-}r}$ Means in both the column with different superscript are significantly different ($p < 0.05$). n=3

Table 4.6 Effects of microfluidization and Nacl levels on gel points[a] for protein dispersions heated at 3°C/min.

		Non-Microfluidized				Microfluidized			
		Gel Time (min)		Gel Temp (°C)		Gel Time (min)		Gel Temp (°C)	
Protein	NaCl (g/100g)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EA	0	15.6	0.01	66.4	0.01	14.6	0.01	63.3	0.03
EA	1	15.2	0.00	65.4	0.01	14.2	0.01	62.3	0.01
EA	2	14.9	0.01	64.4	0.00	13.5	0.00	60.2	0.01
EA	3	14.7	0.19	63.7	0.61	13.3	0.20	59.5	0.59
WPI	0	20.5	0.22	80.0	0.01	19.7	0.01	78.8	0.03
WPI	1	19.7	0.01	78.9	0.03	19.0	0.01	76.8	0.03
WPI	2	19.6	0.18	78.4	0.50	19.0	0.01	76.8	0.02
WPI	3	19.4	0.01	77.8	0.03	18.8	0.19	76.1	0.59

[a] Determined from small-deformation rheology at which G'=G". n=3

Table 4.7 Effects of microfluidization and Nacl levels on gel points[a] for protein dispersions heated at 1°C/min.

		Non-Microfluidized				Microfluidized			
		Gel Time (min)		Gel Temp (°C)		Gel Time (min)		Gel Temp (°C)	
Protein	NaCl (g/100g)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EA	0	42.5	0.15	62.4	0.15	40.4	0.14	60.3	0.12
EA	1	40.9	0.09	60.7	0.09	38.9	0.12	58.8	0.11
EA	2	40.3	0.12	60.2	0.09	38.4	0.09	58.3	0.10
EA	3	40.0	0.11	59.9	0.11	37.8	0.11	57.8	0.12
WPI	0	56.7	0.09	76.6	0.10	54.6	0.07	74.5	0.08
WPI	1	55.0	0.09	74.9	0.09	52.3	0.15	72.2	0.17
WPI	2	54.3	0.09	74.2	0.10	51.9	0.09	71.8	0.10
WPI	3	53.4	0.11	73.3	0.11	51.3	0.50	71.2	0.49

[a] Determined from small-deformation rheology at which G'=G". n=3

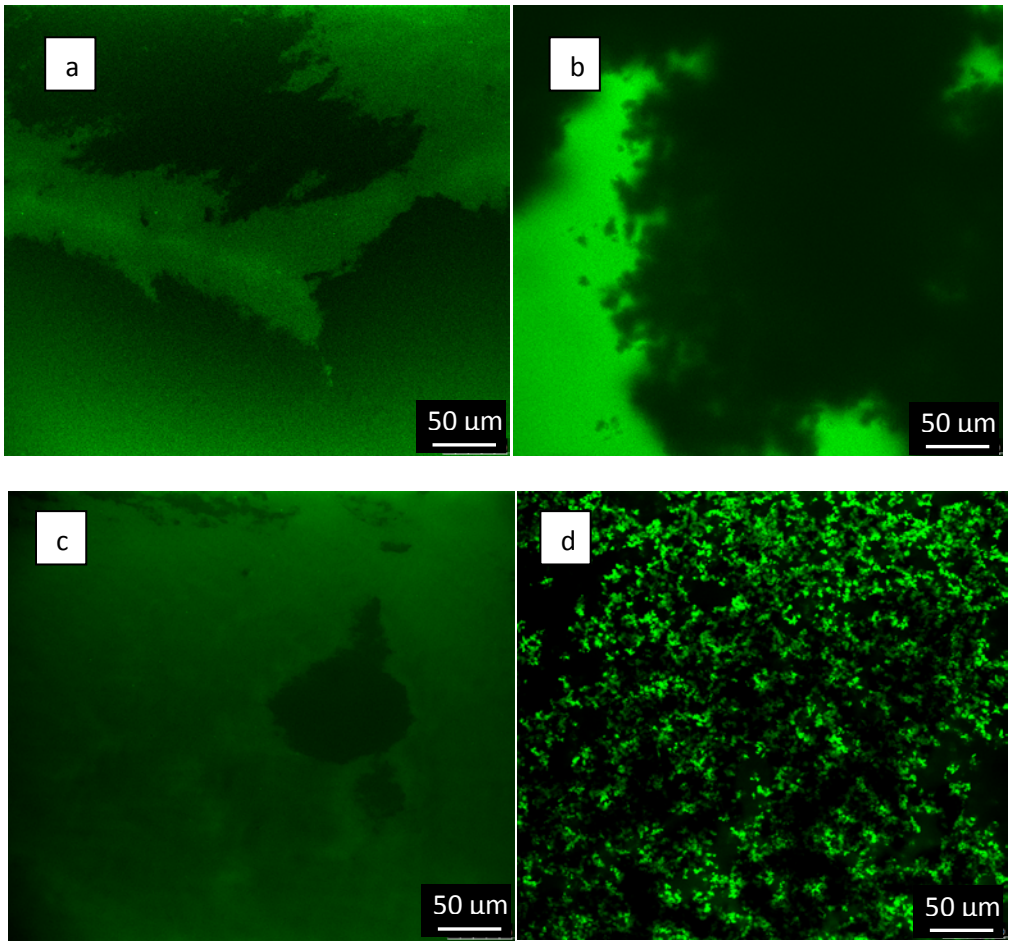

Fig 4.1 Confocal laser scanning microscopic (CLSM) images of Whey Protein Isolate gel formed due to protein aggregation (a) non-microfluidized, 0% NaCl, (b) non-microfluidized, 3% NaCl, (c) microfluidized, 0% NaCl, (d) microfluidized, 3% NaCl.

CHAPTER 5

EFFECT OF MIOCROFLUIDIZATION AND PROTEIN LEVELS ON THE QUALITY OF PORK SAUSAGE.

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Abstract

Microfluidized (MF) dispersions of soy protein isolate (SPI), whey protein isolate (WPI) and egg albumin (EA) were added as binders at 0% (control) 1%, 2% and 3% by weight to 80% lean pork to formulate pork sausages. The quality was assessed for sausages made with different levels of MF and non-MF proteins. Cook loss, expressible moisture, Warner Bratzler shear force, texture profile analysis, color analysis and torsion tests were performed. Cook loss and expressible moisture decreased significantly ($p < 0.05$) as the level of protein increased. At the same level of protein, MF resulted in less cook loss and expressible moisture. The addition of proteins caused greater cutting force, hardness and cohesiveness while reducing; however, at the same level of protein MF did not result in any differences in these parameters. Torsion tests showed an increase in shear stress and fracture modulus at failure with increases in added protein. Color L^* and b^* values increased with added protein level, whereas a^* value decreased with protein level. Sausages with MF-SPI were preferred over sausages with non-MF SPI and control.

Key words: Microfluidization, proteins, pork sausage.

Introduction

Soy, whey, egg albumen and a host of other non-meat proteins may be added to sausage as binders and extenders, which results in improved texture and increased water holding capacity in lean meat sausage. However, there is a limit to how much non-meat protein can be added to the batter, determined both by consumer acceptance and by regulations. According to FSIS directive 7620-3, additives in sausage are limited to 3.5% of the finished product weight. Soy protein isolate (SPI) and sodium caseinate are limited to 2% of the finished product weight due to their relatively high protein percentage. In addition, at higher levels these added proteins can result in unacceptable flavor. Chin and others (2000) reported that addition of 2% SPI in low-fat bologna did not impart detectable off-flavors. Sensory flavor scores dropped drastically as the level of textured soy protein was increased in wiener-type products (Sofos and Allen 1977; Sofos and others 1977). Some improvement in flavor was observed by changing seasoning mixes in the batter (Noda and others 1977) or by treating the soy with moist heat before incorporation (Das and others 2006). Off-flavor can also be reduced by removing oil-body associated proteins and polar lipids from soy protein isolate using ultracentrifugation (Samoto and others 1998). In general, limiting added protein to less than 2% will circumvent flavor problems and the product will be within regulatory compliance. Typically, sausages have a pH between 5.0 and 6.5 and in this pH range globular proteins such as soy, egg albumin and whey do not completely unfold when heated at less than 100 °C (Doi 1993). However, in the meat industries sausages are processed at temperatures well below 100 °C.

Added proteins can interact with myosin proteins to a greater extent in comminuted meats if the protein structure can be unfolded by other means such as lowering pH, enzymatic hydrolysis or surface modifications. Modified proteins often have greater solubility and higher EAI (Emulsifying Activity Index) values (Pearce and Kinsella 1978). Surface modifications of the protein induces unfolding which facilitates the formation of intramolecular and intermolecular crosslinks (Yuan and others 2011). High-shear processing is a promising method to modify protein structure and may allow for creation of several interaction sites on the protein molecule (Hu and others 2011, Dissanayake and Vasiljevic 2009). High-shear microfluidization (MF) is one such method in which fluid suspensions are pumped at high-pressure (150-250 MPa) through an interaction chamber, causing the fluid to split into two streams at 180° before rejoining. Prior MF of proteins may provide a means in which they can be incorporated at lower levels in meat products while providing equivalent improvement in physical properties. Some studies have shown the effectiveness of MF on altering protein functionality in other foods. Van Hekken and others (2007) processed full-fat and low-fat milk for preparation of cheese using MF at three different pressures. They observed that the hardness of cheese made from MF milk decreased as the pressure was increased. Softer and juicier product can be expected with the incorporation of proteins sheared at high-pressure in a microfluidizer (Apichartsrangkoon 2003; Apichartsrangkoon and others 1999). In addition, rapid high-shear processing can reduce browning and off-flavors.

In the present study, reduced-fat pork sausages were formulated using 80% lean pork meat with added MF and non-MF proteins at various levels of incorporation. The

objective of the study was to compare the effectiveness of MF and non-MF soy protein isolate (SPI), egg albumin (EA) and whey protein isolate (WPI) incorporated in sausage batter in improving the quality of the finished product. This involved the assessment of moisture binding properties, color, basic rheological properties, texture attributes and sensory quality of the cooked sausage.

Material and methods

Microfluidization of protein dispersions

Protein susspensions were prepared by hydrating dried powders (15g/100g) in deionized water to prepare dispersions of SPI (Pro-Fam 932, ADM, Decatur IL), WPI (Protient, St. Paul, MN,) and EA (P-39, Henningsen Foods Inc. Omaha, NE). After mixing, a venturi aspirator was used the to remove air bubbles from the protein suspensions. Prepared protein dispersions were processed using a Microfluidizer Prcessor (Model M-140K, Microfluidics International Corporation, Newton, MA) with a throttling valve attachment. All protein dispersions were microfluidized at 30,000 PSI (207 MPa) with a single pass.

Preparation of sausage

Fresh pork (80% lean) was obtained from the UGA Meat Science Technology Center (Athens, GA) and stored at 4°C. The meat was weighed and chopped using a Kramer and Grebe bowl chopper (Biedenkopf, Germany) and other dry ingredients were added and mixed in with a Koch A-80 rotary mixer (UltraSource, Kansas City, MO). A smoked sausage seasoning mix was used (Blend 2525-W, A. C. Legg Inc.) that contained salt, red pepper, sage, sugar, dextrose and black pepper. Once mixed, the batter was

passed through a model 4046 5-HP meat grinder (Hobart, Troy, OH)equipeed with a 3/16-in grinder plate. A Vemag Robot 500 (Machinebau, Germany) was used to fill the batter in to 30-32 mm hand-pulled hog casings (International Casings Group, Inc, Chicago, IL). All processing was done in a cold room at 4°C. The three different MF protein dispersions were added at three levels (1%, 2% and 3% w/w) to create 9 different sausage formulations. A control sample was also prepared without any added protein. The encased sausages were smoke-cooked using an Model 1000 smoke oven (Alkar Inc. Lodi, WI). The cook schedule was: 40 min at 43°C to dry the casings; 2 h at 54°C/20%RH for initial smoking, followed by 2 h at 60°C/30%RH; cooking to an internal temperature of 75°C (165°F) at 88%RH, with a 1 min hold time to ensure product safety; 10 min cold shower at 10°C. Cooked sausages were refrigerated at 4°C overnight. Physical tests and analysis were performed the following day after bringing the sausages to room ~21°C.

Cooking loss

Cooking loss was calculated from the weights before (w_i) and after cooking (w_f), and expressed as a percentage:

$$Cook\ Loss = \frac{w_i - w_f}{w_i} \times 100 \quad (1)$$

Expressible moisture

Samples for expressible moisture were prepared by cutting sausages in to 15 mm thick slices then cutting transversely with a cylindrical corer (20 mm diameter). The TA-XT2i texture analyzer (Stable Microsystems, Surrey UK) equipped with a 3-inch clylindrical plate was used to compress the sample between two Whatman No-1 filter

papers. Samples were compressed at 2 mm/s to 50% deformation, then held for 30 s in this position. The difference in weight before (w_o) and after compression (w_c) was used to calculate expressible moisture:

$$Expressible\ Moisture = \frac{w_o - w_c}{w_o} \times 100 \quad (2)$$

Texture analysis

Warner Bratzler shear tests

For tests of shear strength, cooked sausages were cored to obtain 1-inch long and 16 mm diameter samples. A Warner Bratzler blade with trianglular insert (TA-7) was mounted on the TA-XT2i texture analyzer and lowered through the samples at 4 mm/s. Analyses of maximum shear force were taken as a measure of tenderness.

Texture profile analysis

Texture attributes attained under simulated chewing were measured using texture profile analysis (TPA). Cooked sausage samples 16 mm in diameter and 15 mm long were prepared by slicing and coring the sausages. The TA-XT2i, fitted with a 3-inch (7.62 cm) diameter aluminum cylinder plate (TA-30) was used to compress the sample twice to 50% deformation at 2 mm/s. The test speed and post-test speed was kept same so that the areas under the curve would be comparable for both compression and withdrawal cycles. The maximum force during the first compression cycle was reported as hardness, the ratio of areas of the second and first compression cycles was reported as cohesiveness, and the negative area in the first withdrawal cycle was reported as adhesiveness.

Color analysis

Color analyses was done on samples cut to ~1 cm slices. Samples were placed on a flat surface and analyzed from above with a Minolta Chroma-Meter series CR-200 (Minolta, Japan). Color values in the CIELAB L*, a* and b* system were recorded.

Torsion test

The relative strength of the meat gels was tested using a torsion gelometer, which supplies a uniform twisting force until the sample fractures. Samples 20 mm in diameter and 25 mm high were prepared by cutting and coring the sausages. Each sample was mounted on two notched styrene discs with the help of cyanoacrylate adhesive and made into hour glass shapes using a gel grinder (Gel Consultants, Raleigh,N.C., U.S.A). The Torsion Gelometer, built on a Brookfield viscometer, was used to twist the sample at 2.5 rpm and the generated shear stress and shear strain were measured up to the point of material fracture. Failure shear stress was measured to determine the strength of gels, whereas failure shear strain was measured for the deformability of gels. The ratio of shear stress and shear stain at failure was recorded as the failure modulus G. The slope ratio was calculated as failure modulus G divided by the modulus at 30% failure stain.

Sensory analysis

A ranking test was used to assess consumer preference for the differently treated samples. Panelists (n=50) were recruited from staff and students at the University of Georgia. Samples were cooked and kept at 40°C before presentation to panelists. Non-

parametric analysis was performed using the Friedman-type statistic from the rank data in a randomized block:

$$T = ([12/bt(t+1)]\sum_{j=0}^{t} X_j^2) - 3b(t+1) \quad (3)$$

where b is the number of panelists, t is the number of samples and X_j is the rank sum corresponding to the sample. A critical value for multiple comparisons, LSD_{rank} was calculated to find the significant difference (Meilgaard and others 2007).

Statistical analysis

All data, except from the sensory tests, were analyzed using SAS statistical software (SAS 9.1, 2008) using analyses of variance (ANOVA). Fisher's protected LSD test was performed to determine if any significant differences existed amongst the treatment groups. Statistical significance was expressed at $p < 0.05$ level.

Results and Discussions

Cook loss and expressible moisture

For all three proteins, cook loss and expressible moisture decreased with increasing protein level (Table 5.1). The cook loss for control was 24.1%. For non-MF proteins, cook loss ranged from 20.0 to 17.9% for SPI, 23.3 to 21.6% for EA, and 23.6 to 22.5% for WPI. SPI was the most effective at reducing the amount of liquids lost during cooking. Reducing cook loss is a major advantage as less product weight is loss, and the cooked meat will be more tender and juicy. Expressible moisture is another measure of water holding properties as it shows how the cooked meat will retain liquid even when

subject to compressive stresses. Again, addition of the non-meat proteins reduced expressible moisture. While control had 5.21% expressible moisture, SPI samples had 4.44 to 3.54%, EA samples 4.62 to 3.84%, and WPI samples 4.44 to 3.66%.

At the same level of added protein, MF proteins resulted in products with lower cook loss and expressible moisture. Thus, prior MF gave juicier products with higher yield. While non-MF SPI gave 20.0-17.9% cook loss, MF SPI resulted in 17.8-14.0%. Similarly, non-MF EA resulted in 23.3-21.6%, while MF versions resulted in 21.9-19.7% cook loss. For non-MF WPI values ranged from 23.6-21.6%, while for MF WPI they ranged from 21.9-19.9%. Sausages with 3% SPI had the least cook loss of all samples (14.0%). Differences in expressible moisture for sausages with non-MF and MF proteins were not large. In most cases, sausages with MF proteins had less expressible moisture, Again, this suggests that those products with MF proteins have better moisture holding properties, and it will maintain it better in the meat where it can later be released and perceived as juiciness by consumers.

Though cook loss was lower in EA compared to WPI formulations the expressible moisture was lower in WPI suggesting WPI can retain moisture better in the network compared to EA post cooking. Higher expressible moisture in EA formulation compared to SPI and WPI can be due to greater degree of structural degradation in EA compared to WPI and SPI over time (Privalou 1989). Several work suggested that most binder's results in improvement in yield due to reduction in cook loss (Youssef and Barbut 2011; Yang and others 2007; Bejosano and Corke 1998) and expressible moisture (Pietrasik 1999; Pietrasik and Janz 2009; Pietrasik and Janz 2010; Pietrasik and others 2007; Pietrasik and Li-Chan 2002) whereas others suggested a no effect of additive in the

sausage (Sofos and others 1977; Ensor and others 1987). The latter case was observed in regular sausages with high level of fat whereas the former observed in reduced or low fat formulations. In the low or reduced fat formulation where formation of good emulsion is an issue additive such as proteins fill the gap as they have tendencies to form network among themselves and with muscle proteins thereby help in emulsion stability. Microfluidization of these proteins resulted in further improvements in the yield due to modification of protein at secondary and tertiary level which also expose the hydrobhobic group and increase the surface hydrophobicity (Iordache and Jelen 2003; Sanchez and others 1997; Sanchez and others 1999). Increase in surface hydrophobicity allows for more surfaces for interaction with other protein molecule and muscle protein and result in formation of protein network which can potentially bind more water and show further enhancement over non microfluidized proteins (Liu and Tang 2011; Shen and Tang 2012; Tang and Liu 2013).

Cutting force and Texture analysis

Values for the Warner Bratzler shear force are shown in Table 5.2, along with textural attributes measured by texture profile analysis. Control sausage had a maximum shear force of 403.9 g. Sausages with added SPI had somewhat larger shear force than control (466.6-652.9 g), and these increased with the level of protein added. For EA proteins the range was 358.9-483.4 g, and for WPI 322.3-451.4. For EA and WPI, samples with 1 or 2% protein had lower shear values than control, while those with 3% had larger values than control. Interestingly, there were no differences in shear forces for sausages with non-MF protein versus their MF protein counterparts. Shear force is one measure of tenderness in muscle foods. In particular, it measures the force to cut through

a sample. This is most closely related to the force needed to cut through a meat sample with a knife, or to shear through it with the incisors in the mouth.

For TPA, samples were compressed two times at 50% deformation. The sample hardness increased with added protein. For control sausage the hardness was 1537 g, for non-MF SPI 1943-2413 g, for EA 1728-2014 g, and for WPI 1679-1894 g. Thus, in all cases added protein increased the measured hardness, but as noted above 1-2% of EA or WPI decreased the cutting force. Thus, one possibility is that the added protein increases hardness by adding aggregates or a more extensive gel that cannot be easily moved by normal forces, while at the same time may weaken the intermolecular forces that resist shear forces that tend to pull it apart.

Cohesiveness also increased with added protein. For the control sausage, the cohesiveness was 0.24, and ranged from 0.31-0.42 for non-MF SPI, 0.28-0.30 for EA and 0.26-0.29 for WPI. Thus, sausage with non-MF SPI was clearly more cohesive than control, while those with EA or WPI were slightly more cohesive. While both shear force and cohesiveness are measures of how easily a sample comes apart, the first is accomplished through transverse shear forces, the latter through normal compressive forces. As with hardness, cohesiveness was not significantly different between sausages with non-MF proteins versus their MF protein counterparts. The control sausage did have some level of adhesiveness (66.6 gs). This might be traced to some of the extracted proteins that create a slightly sticky surface. In general, the addition of non-MF protein reduced adhesiveness, and samples with more protein were less adhesive. Thus, for non-MF SPI values ranged from 40.3-22.9 gs, for EA 54.8-35.6 gs, and for WPI 63.7-37.1 gs.

This may be somewhat related to the ability of these proteins to help retain more moisture within the product, thus limiting the amount that comes to the surface and contributes to stickiness. As with the other TPA attributes, the adhesiveness of sausage made with non-MF proteins was not different than for those with the same type and level of MF protein. The water binding and texture data suggests that MF can assist in the production of a juicy sausage with higher yield without appreciably changing the texture profile.(Ciron and others 2010) found similar results when they worked on microfluidization of milk to produce low fat yogurt. On the other hand (Cavender and Kerr 2013) working with full fat ice-cream with microfluidized locust bean gum and xanthan gum found that microfluidization increase hardness, adhesiveness and cohesiveness. (Ciron and others 2011; Ciron and others 2012) found significant difference in the perceived texture of the reduced fat yogurt due to microfluidization in a descriptive sensory test. No difference in the texture can be attributed to increase in the volume corresponding to the increase in the network due to microfluidization rather than formation of a tight pack network.

Torsion test

Torsion tests were also conducted on the samples as these are often felt to give a more accurate representation of the gel strength. Results for the shear stress and strain at failure are shown in Table 5.3. For control, the shear stress and strain at failure were 23.22 kPa and 1.08, respectively. The addition of protein increased the stress at failure, and stress increased with the level of protein. For sausage with non-MF SPI, stress values ranged from 36.85-49.69 kPa, while for EA samples they ranged from 28.08-38.84 kPA, and for WPI from 26.46-32.22 kPa. Thus, samples with SPI resist cutting, are harder under compression and resist twisting forces to a greater extent than control or those with

EA or WPI. In general, there were no measurable differences in the strain (that is, the relative change in dimension) at failure due to the added proteins.

With two exceptions, sausage with MF proteins did not have higher shear force at failure than those with non-MF proteins. Samples with either 3% SPI or 3% EA had somewhat higher forces: 52.92 versus 49.69 kPa for SPI and 41.14 versus 38.84 kPa for EA. MF did not significantly alter the measured shear strain values at failure. (Ashie 1999) also reported no difference in the shear strain due to high pressure treatment. Highest shear stain can be observed at protein concentration closer to critical gel concentration (Foegeding and others 1995). Since all the formulations were containing proteins well above critical gel concentration therefore no difference in the shear stain was evident. Changes in the shear stress and shear strain was independent of each other and similar to the results obtained by (Bottcher and Foegeding 1994). Slope ratio for all the treatments was significantly higher than control (1.25). Slope ratio increased very slightly due to microfluidization and not significant at any level of protein. All the sausage formulation exhibited strain hardening as the slope ratio was greater than 1 in every case (Gwartney and others 2004).

Color analysis

Color values for the sausages prepared with added non-MF and MF proteins are shown in Table 4. The mean L* for control was 42.33, while L* values were higher for samples with added protein, and increased with protein level. L* ranged from 43.39-45.03 for samples with non-MF SPI, 43.24-44.19 for those with EA, and 43.54-44.89 for those with WPI. Thus, although the differences were very small, samples with added

protein were somewhat lighter than those without. Samples with added protein had slightly lower a* values and slightly higher b* values. In practical terms, this means that samples with added protein were slightly lighter, a little more yellow than red, and with less color saturation. However, the differences were so small that they would not likely be noticed by consumers. Microfluidizatoin did not cause any appreciable change in a* and b* value but decreased L* value for SPI at all three levels. In general lightness of meat protein increase with high pressure above 200 MPa but at very high hydrostatic pressure above 300MPa color get stabilized and color difference which is a function of L*, a* and b* values for control and treatment decreases substantially (Shigehisa and others 1991; Carlez and others 1995; Jung and others 2003). Addition of protein in the meat batter result in decrease in L* value because the color of added proteins like SPI, WPI and EA are lighter (Pietrasik and Li-Chan 2002; Ahmad and others 2010). Reduction of redness and increase in yellowness can also be attributed to addition of these pale to cream color proteins. All proteins were processed at 207 MPa pressure of microfluidization and no visible change in color was observed.

Sensory

Preference test was performed for sausages with 2% non-microfluidized SPI and microfluidized SPI against control. Sausages with microfluidized SPI was preferred over non-microfluidized SPI and control (data not shown). Greater acceptability in terms of texture and appearance was reported by (Sikes and others 2009) for high pressure treated sausage with lower salt compared to non pressure treated. Sausage with microfluidized SPI was slightly softer compared to non-microfluidized SPI formulation. (Mor-Mur and

Yuste 2003) reported preference of pressure treated sausage over non pressure treated and in some cases difference was not discernible.

Conclusions

High shear microfluidization can improve functional properties of SPI, WPI and egg albumin thereby allowing them to interact with meat protein to provide improved emulsification and greater gel strength. This results in increased water holding capacity and improved texture of the pork sausage. Microfluidization as a technique can be used to change the surface structure of protein, which can result in cook loss reduction and higher yield at a lower level of incorporation in the sausage. Incorporation of microfluidized protein resulted in lower cook loss and lower expressible moisture without any significant change in texture profile and color of the sausage. Acccpetability for sausage with microfluidized SPI was higher than sausages with non- microfluidized SPI and control.

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Table 5.1. Effect of microfluidization and protein level on the Cook loss (%) and expressible moisture (%) of the pork sausage.

Treatments		Cook Loss (%)		Expressible Moisture (%)	
		Mean	SD	Mean	SD
C	Control	24.14^{a}	0.55	5.21^{a}	0.24
NMF	SPI 1%	20.01^{fg}	0.60	4.44^{cd}	0.20
NMF	SPI 2%	19.22^{g}	0.43	4.13^{gh}	0.04
NMF	SPI 3%	17.93^{h}	1.10	3.54^{l}	0.10
NMF	EA 1%	23.32^{abc}	0.22	4.62^{b}	0.06
NMF	EA 2%	22.56^{cd}	0.25	4.26^{efg}	0.06
NMF	EA 3%	21.57^{e}	0.06	3.84^{ij}	0.10
NMF	WPI 1%	23.65^{ab}	0.30	4.44^{cd}	0.15
NMF	WPI 2%	22.96^{bc}	0.32	4.12^{gh}	0.03
NMF	WPI 3%	22.53^{cd}	0.18	3.66^{kl}	0.06
MF	SPI 1%	17.81^{h}	0.23	4.31^{def}	0.09
MF	SPI 2%	14.62^{i}	0.19	4.09^{gh}	0.07
MF	SPI 3%	14.00^{i}	0.45	3.30^{m}	0.06
MF	EA 1%	21.94^{de}	0.28	4.51^{bc}	0.14
MF	EA 2%	21.21^{e}	0.48	4.19^{fg}	0.04
MF	EA 3%	19.71^{fg}	0.19	3.76^{jk}	0.07
MF	WPI 1%	21.94^{de}	0.63	4.39^{cde}	0.04
MF	WPI 2%	20.26^{f}	0.29	4.17^{fg}	0.09
MF	WPI 3%	19.86^{fg}	0.15^{fg}	3.98^{hi}	0.03

$^{a\text{-}m}$ Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

Table 5.2. Effect of microfluidization and protein level on the Warner Bratzler cutting force, Hardness, adhesiveness and cohesiveness of the pork sausage.

Treatments		Cutting Force (g)		Hardness (g)		Adhesiveness (gs)		Cohesiveness	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	Control	402.87^{fg}	12.14	1536.47^{l}	66.61	69.09^{a}	11.69	0.24^{j}	0.02
NMF	SPI 1%	466.62^{cd}	9.29	1942.67^{de}	68.53	40.29^{gh}	2.41	0.31^{cde}	0.02
NMF	SPI 2%	584.35^{b}	5.28	2092.53^{b}	87.11	27.43^{i}	1.00	0.37^{b}	0.02
NMF	SPI 3%	652.92^{a}	13.31	2413.47^{a}	58.21	22.88^{i}	1.34	0.42^{a}	0.02
NMF	EA 1%	358.88^{ij}	13.52	1727.87^{ijk}	27.96	54.78^{cd}	4.70	0.28^{ghi}	0.01
NMF	EA 2%	426.85^{e}	7.05	1816.50^{gh}	18.57	47.29^{ef}	1.63	0.28^{ghi}	0.01
NMF	EA 3%	483.36^{c}	13.55	2014.47^{cd}	31.69	35.56^{h}	2.69	0.30^{efg}	0.01
NMF	WPI 1%	322.28^{k}	10.47	1678.90^{k}	29.04	63.72^{ab}	4.82	0.26^{ij}	0.01
NMF	WPI 2%	377.85^{hi}	8.46	1779.53^{ghi}	17.86	54.04^{d}	3.16	0.27^{hi}	0.01
NMF	WPI 3%	451.40^{d}	14.71	1894.10^{ef}	49.74	37.14^{h}	1.43	0.29^{fgh}	0.01
MF	SPI 1%	453.71^{d}	15.06	1899.90^{ef}	50.77	37.11^{h}	1.26	0.33^{c}	0.02
MF	SPI 2%	568.61^{b}	12.62	2052.00^{bc}	35.14	25.71^{i}	1.12	0.39^{b}	0.01
MF	SPI 3%	638.77^{a}	25.67	2421.60^{a}	33.04	22.92^{i}	0.49	0.44^{a}	0.02
MF	EA 1%	346.25^{j}	10.91	1747.27^{hijk}	14.75	51.21^{def}	2.97	0.27^{hi}	0.01
MF	EA 2%	417.50^{ef}	18.38	1840.83^{fg}	46.66	46.17^{fg}	1.68	0.28^{ghi}	0.01
MF	EA 3%	466.01^{cd}	8.62	2032.80^{bc}	53.10	35.77^{h}	2.01	0.32^{cd}	0.03
MF	WPI 1%	324.83^{k}	5.43	1703.10^{jk}	19.43	60.45^{bc}	3.16	0.27^{i}	0.01
MF	WPI 2%	384.69^{gh}	9.40	1755.33^{hij}	16.38	52.32^{de}	3.93	0.28^{ghi}	0.01
MF	WPI 3%	461.01^{d}	9.38	1908.53^{ef}	13.95	35.34^{h}	1.46	0.31^{def}	0.02

[a-l] Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

Table 5.3. Effect of microfluidization and protein level on the shear stress, shear strain, fracture modulus (G) and slope ratio at failure of the pork sausage.

Treatments		Shear Stress at Failure (kPa) Mean	SD	Shear Strain at Failure Mean	SD	G at Failure (kPa) Mean	SD	Slope Ratio at Failure (0.30) Mean	SD
C	Control	23.11^{k}	2.31	1.08^{abcd}	0.05	21.47^{k}	2.08	1.25^{k}	0.03
NMF	SPI 1%	36.85^{e}	1.47	1.05^{abcd}	0.04	35.14^{ef}	2.34	1.46^{defg}	0.02
NMF	SPI 2%	43.56^{c}	1.03	1.05^{abcd}	0.02	41.36^{c}	1.51	1.50^{cd}	0.04
NMF	SPI 3%	49.69^{b}	1.61	1.10^{abc}	0.05	45.34^{b}	1.45	1.57^{ab}	0.02
NMF	EA 1%	28.08^{hij}	1.00	1.05^{abcd}	0.03	26.86^{ij}	1.65	1.38^{hij}	0.03
NMF	EA 2%	33.94^{fg}	1.05	1.07^{abcd}	0.05	31.64^{g}	0.71	1.45^{defg}	0.05
NMF	EA 3%	38.84^{e}	0.50	1.06^{abcd}	0.01	36.53^{e}	0.38	1.55^{ab}	0.07
NMF	WPI 1%	26.46^{j}	0.64	1.08^{abcd}	0.04	24.60^{j}	1.05	1.38^{ij}	0.02
NMF	WPI 2%	29.19^{hi}	0.47	1.07^{abcd}	0.07	27.26^{ij}	1.42	1.43^{efgh}	0.02
NMF	WPI 3%	32.22^{g}	0.50	1.05^{abcd}	0.05	30.62^{gh}	1.14	1.43^{efg}	0.03
MF	SPI 1%	38.69^{e}	1.23	1.04^{bcd}	0.02	37.10^{de}	1.69	1.50^{cd}	0.02
MF	SPI 2%	45.56^{c}	0.76	1.11^{ab}	0.05	41.24^{c}	2.44	1.52^{bc}	0.02
MF	SPI 3%	52.92^{a}	1.69	1.08^{abcd}	0.04	49.07^{a}	3.08	1.58^{a}	0.02
MF	EA 1%	29.27^{hi}	0.90	1.11^{a}	0.05	26.42^{ij}	1.69	1.41^{ghij}	0.04
MF	EA 2%	34.49^{f}	2.22	1.07^{abcd}	0.04	32.22^{g}	0.87	1.46^{def}	0.04
MF	EA 3%	41.14^{d}	1.54	1.03^{cd}	0.02	39.83^{cd}	1.96	1.57^{a}	0.03
MF	WPI 1%	27.37^{ij}	0.93	1.10^{ab}	0.04	24.83^{j}	1.24	1.37^{j}	0.02
MF	WPI 2%	29.96^{h}	0.28	1.07^{abcd}	0.04	28.02^{hi}	1.03	1.42^{fghi}	0.03
MF	WPI 3%	33.44^{fg}	1.13	1.03^{d}	0.02	32.59^{fg}	1.58	1.47^{cde}	0.02

[a-i] Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

Table 5.4. Effect of microfluidization and protein level on the color of the pork sausage.

Treatments		L		a		B	
		Mean	SD	Mean	SD	Mean	SD
C	control	42.33^{k}	0.09	26.37^{a}	0.08	14.32^{h}	0.13
NMF	SPI 1%	43.39^{hi}	0.04	24.52^{b}	0.16	15.77^{ed}	0.14
NMF	SPI 2%	44.14^{de}	0.06	23.67^{d}	0.08	16.13^{c}	0.14
NMF	SPI 3%	45.03^{a}	0.19	22.67^{g}	0.07	16.70^{a}	0.14
NMF	EA 1%	43.24^{j}	0.04	24.54^{b}	0.27	15.58^{fg}	0.12
NMF	EA 2%	43.52^{gh}	0.06	23.79^{cd}	0.02	15.84^{d}	0.07
NMF	EA 3%	44.19^{cd}	0.04	23.31^{f}	0.16	16.31^{b}	0.11
NMF	WPI 1%	43.54^{g}	0.05	24.67^{b}	0.14	15.55^{g}	0.08
NMF	WPI 2%	44.26^{cd}	0.13	23.76^{d}	0.09	16.29^{bc}	0.17
NMF	WPI 3%	44.89^{ab}	0.03	23.38^{ef}	0.05	16.71^{a}	0.02
MF	SPI 1%	43.37^{ij}	0.06	24.55^{b}	0.08	15.61^{efg}	0.07
MF	SPI 2%	43.79^{f}	0.09	23.69^{d}	0.05	16.16^{bc}	0.05
MF	SPI 3%	44.78^{b}	0.12	22.63^{g}	0.10	16.59^{a}	0.04
MF	EA 1%	43.39^{hi}	0.04	24.68^{b}	0.03	15.57^{fg}	0.06
MF	EA 2%	43.54^{g}	0.05	23.83^{cd}	0.05	15.88^{d}	0.04
MF	EA 3%	44.04^{e}	0.11	23.43^{ef}	0.06	16.18^{bc}	0.11
MF	WPI 1%	43.58^{g}	0.07	24.62^{b}	0.09	15.74^{def}	0.09
MF	WPI 2%	44.32^{c}	0.13	23.95^{c}	0.06	16.20^{bc}	0.20
MF	WPI 3%	44.86^{b}	0.06	23.49^{e}	0.08	16.59^{a}	0.09

$^{a-k}$ Means in the same column with different superscript are significantly different ($p < 0.05$). n=3

CHAPTER 6

CONCLUSIONS

Main focus of this dissertation was to evaluate the role of three very commonly available globular protein viz. soy protein isolate, whey protein isolate and egg albumin as binders in one very commonly consumed comminuted meat product, pork sausage. Prepared pork sausages with and without these proteins at different levels were analyzed for water holding capacity using cook yield and expressible moisture as indicator. Sausages were also evaluated for texture using texture profile analysis and Warner Bratzler shear test. Torsion test was done to get true stress and strain at failure. Effects of protein addition on color of the sausages were also analyzed. Further, effect of high pressure microfluidization was studied to observe the change in protein functionality as evident through the change in their rheological and gelling behavior at different level of NaCl concentration. Confocal laser scanning microscope was used to observe the difference between microfluidized and non microfluidized protein gels with and without NaCl. Finally the effect of addition of microfluidized protein in the sausage batter was analyzed post cooking for cooking quality by observing cook yield, expressible moisture, cutting force, TPA and torsion test. Sensory test was performed on selected formulations.

Results of this study indicated that SPI, WPI and egg albumin can be successfully added to the reduced fat pork sausage batter to improve the cook yield and texture without appreciably changing the color and sensory attributes of the sausages. Higher level of incorporation lead to off flavor development and lighter color products. Sensory

data suggested 2% SPI incorporation to get highest overall acceptability among the treatments together with significantly lower cook loss compared to control.

Increase in NaCl concentration resulted in increase in particle size due to increase in protein aggregation whereas microfluidization resulted in decrease in particle size and uniform distribution of protein aggregates. Gel hardness and storage modulus of all the proteins at all level of NaCl was increased whereas strain hardening decreased due to microfluidization. Therefore microfluidization can potentially be used for changing the functional properties of proteins. Sausage incorporated with microfluidized protein resulted in significantly lower cook loss and expressible moisture when compared with sausage with non-microfluidized protein.

Soy protein isolates, egg albumin and whey protein isolates were successful in reducing the cook loss and increasing the yield and their increased incorporation showed significant increase in the desirable effect to a point after which any further addition lead to undesirable flavor development and decrease in consumer acceptability. At 2% level soy protein isolate formulation was acceptable due to almost bland flavor of SPI, whereas egg albumin and whey protein isolate was not acceptable at those levels due to off flavor development.

Microfluidization proved to be a very useful tool in modifying SPI, WPI and egg albumin and resulted in significantly increased yield compare to non microfluidized formulations. Since all three protein worked really well as binder to a varying degree in the pork sausage and microfluidization resulted in providing added flexibility in the formulation a least cost formulation and coat analysis will be pertinent next step of study. Also studies targeting the masking of flavor or off flavor reduction by utilizing various

techniques such as microfluidization, can be useful. Ultramicroscopic study utilizing TEM, SEM will give ample insight in to the protein matrix in the gel.