This is to certify that the
dissertation entitled

Application of Whey Protein Polymers in Low Fat Beef Frankfurters

presented by

Jin-Shan Shie

has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Food Science

Major Professor's Signature

02 Sept 04

Date

APPLICATION OF PREHEATED WHEY PROTEIN POLYMERS IN LOW FAT BEEF FRANKFURTERS

By

Jin-Shan Shie

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

2004

ABSTRACT

APPLICATION OF PREHEATED WHEY PROTEIN POLYMERS IN LOW FAT BEEF FRANKFURTERS

By

Jin-Shan Shie

Functionality (water binding ability) of preheated whey protein isolate (WPI) in comminuted meat products was determined in this research project. The impact of adding preheated WPI on comminuted meat product attributes (textural properties and sensory attributes) was also investigated. The overall goal of this project was to investigate the applicability of preheated WPI in comminuted meat products. To achieve this goal, three separate studies were conducted.

Study I utilized response surface methodology in a β-lactoglobulin (LG) model system to determine the effect of heat denatured LG aggregate (HDLG) size on rheological properties and microstructure of LG gels. Response surface graphs predicted that LG gels with high gel hardness and low gel point could be produced by preheating LG solution at 90 °C, 40 min, pH 7.0. While larger HDLG aggregates produced LG gel with higher gel hardness, HDLG aggregates size did not affect microstructure of LG gels.

Study II focused on further investigating the effects of various preheating conditions on WPI in comminuted meat products and its effects on the functional properties of high added water, fat and standard meat emulsions. Three preheating conditions were chosen: 95 °C, 10 min, 95 °C, 40 min, and 85 °C, 80 min, and WPI was preheated at 8%, pH 7.0. Emulsion stability of high added fat, water emulsions prepared with the three preheated WPIs was higher than those prepared with unheated WPI

($p<0.05$). Increasing WPI amount from 3.5 to 7.0% also increased emulsion stability of high added fat and high added water emulsions ($p<0.05$). Among the three preheated WPIs, emulsions prepared with WPI preheated at 95 °C for 40 min had the best emulsion stability in both high added fat and high added water emulsions ($p<0.05$). While all beef meat emulsions were able to bind fat better, preheated WPI was a better water binder.

Study III verified the efficacy of preheated WPI in comminuted meat products. Whey protein isolate was preheated at 8% (w/w), 95 °C, 40 min, pH 7.0. Low fat (4%) beef frankfurters were manufactured with 35, 40 and 45% water, and preheated WPI was added at 3.5 and 7.0% level. At 40% added water level and 7.0% added WPI level, beef frankfurters prepared with preheated WPI had higher cooking yields with lower expressible moisture than those prepared with unheated WPI ($p<0.05$). Not only were cooking yields improved, but the textural properties of beef frankfurters prepared with preheated WPI had higher hardness, gumminess and chewiness values than those prepared with unheated WPI ($p<0.05$). During 56 days of refrigerated storage, beef frankfurters prepared with preheated WPI had less purge loss than those prepared with unheated WPI ($p<0.05$). Preheating of WPI was proven to be an effective method to improve functionality of WPI in comminuted meat products.

ACKNOWLEDGEMENTS

My graduate study in Michigan State University had been a tremendous learning experience. In the course of my study, I had been through three different major professors, and working with them was once in a life time learning opportunity. First, I would like to thank Dr. Denise Smith for accepting me to Michigan State University, without her opening the door for me; I could not have all the wonderful experiences. I also thank her for her advices and supports throughout the entire course of my study. After Dr. Smith joined University of Idaho, Dr. Gale Strasburg became my major professor. It is never easy to switch major professor, and I thank Dr. Strasburg for trying everything he can to help me and providing me a great working environment. Dr. Wesley Osburn served as my research advisor in the last two years of my study. I would like to express my deepest appreciation to him for guiding me to the right direction. It is him that I can get to where I am today, and I will always remember that.

Thanks go to my graduate committee, Dr Stanley Flegler, Dr. James Steffe, and Dr. Zeynep Ustunol, for their advices and guidance. I also thank Dr. Al Booren for his valuable suggestions and generosity in providing materials I needed for my research. MSU meat lab managers Mr. Tom Forton and Ms. Jennifer Dominguez, and Mr. Rodney Clark from MSU dairy plant, I thank them for helping me in manufacture of my frankfurters and preheated whey protein isolates.

To my friends and lab mates, Dr. Alicia Orta-Ramirez, Dr. Carolyn Ross, Dr. Manee Vittayanont, Dr. Sarah Smith Simpson, Dr. Virginia Vega-Warner, Dr. Winne Chiang, Dr. John Rogers, Ms. Jennifer Maurer, Ms. Fon Tuntivanich, Ms. Andrea Molengraft, I thank you all for your friendships and encouragements. Ms. Christine

Quinlan had helped me a great deal in my frankfurter sensory evaluation and manufacture; thanks go to her for her help and friendship. Dr. Osburn's undergraduate workers, Ms. Courtney Whitfield, Ms. Michelle Grabowski, Mr. Rory McClintock, Ms. Jayme Maderin, Mr. Dan Kiesling, Mr. Mark LaBar, Ms. Rachel Damore, you guys made my lab work easier and more enjoyable, and I thank you for all your help.

To my very special friend Dr. Hyo-Jung (Heather) Yoon, it's been my pleasure and privilege to know you. You have changed my life in a very positive way, and opened my eyes to look at the world from a different prospective. I have matured a lot through the daily interactions with you, and I thank you for all your energy and time.

Always last, but never least, to my family, I thank them for their understandings and never ending supports. Without them, it is impossible for me to finish my study. It is to them that this work is dedicated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Dairy protein products are high in protein content and low in fat. This unique characteristic has provided the dairy industry a great opportunity to meet the consumer demand for healthy and natural food products (Singh and others, 2000). Whey protein, one of the most used dairy proteins, is the by-product of cheese or rennet casein and acid casein manufacture. It is one of the most important non-meat ingredients in value-added meat products, and has been used primarily as a non-meat extender or binder in meat products to improve textural quality. Economically, addition of whey protein to meat products increases the yields, thereby decreasing the costs. The production of whey proteins is estimated in excess of one hundred million tons annually (de Wit, 1998). However, the high degree of variability in composition, functionality, and sensory properties of whey proteins has greatly limited their use by the food industry (Atughonu and others, 1998, Holt and others, 1999a, 1999b, Hung and Zayas, 1992, Langley and Green, 1989, Morr and Foegeding, 1990). In 2002, 450,000 tons of dry whey products were produced in the United States, but only 60% of the dry whey products were sold and leaving a 40% surplus (USDA, 2003).

Whey proteins have the ability to form gels when heated to temperatures above 70°C (Langely and Green, 1989). This characteristic of whey proteins can largely increase the textural quality of meat products. However, commercially available whey proteins do not form gels at cooking temperatures (60 to 70°C) used by meat processors, thus minimizing their influence on meat product texture. Upon the addition of salt or anions and under certain pHs, preheated whey protein polymers can form gels at ambient

temperature. This process is called cold-setting and whey protein gels formed under this condition are called cold-set gels (Barbut and Foegeding, 1993, Ju and Kilara, 1998a, 1998b, Mleko and Foegeding, 1999, Hongsprabhas and Barbut, 1997a, 1997b, Hongsprabhas and Barbut, 1998, Roff and Foegeding, 1996). In the formation of whey protein cold-set gels, whey proteins are initially heated (preheating step) above their denaturation temperatures (around 70°C); at this stage, whey proteins unfold and form various sizes of soluble aggregates. Upon the addition of salt or anions under certain pH ranges, the preheated whey protein aggregates can form gels (gelling step) well below the cooking temperatures used by the meat processors. Since salt is often added to processed meat products, the unique cold-set gelling ability of pre-heated whey protein polymers can be utilized to overcome the functional problem of whey proteins. Both preheating and cold-set gelling conditions are important to the rheological properties of whey protein cold-set gels, and different rheological properties of whey protein cold-set gels can be produced by manipulating the preheating and cold-set gelling conditions (Bryant and McClements, 2000a, 2000b, Chantrapornchai and McClements, 2002, Marangoni and others, 2000). When a food product with certain rheological properties is desired, food processors can take advantage of this unique characteristic of whey protein to fit their processing conditions.

The characteristics of whey protein aggregates under different heating conditions have been studied. It has been reported that the rheological properties of whey protein gels prepared with preheated whey proteins is affected by the size of whey protein aggregates (Hoffman and others, 1997, Ju and Kilara, 1998a, 1998b, MacLeod and

others, 1995, Mleko and Foegeding, 1999). However, the effect of size of whey protein aggregates on the textural attributes of meat products is still not fully understood.

β-Lactoglobulin (LG) is the main protein responsible for the gelling properties of whey proteins. Like whey proteins, when heated above its denaturation temperature, LG can form heat denatured LG (HDLG) soluble aggregates. The characteristics of LG have been studied extensively (Barbut and Foegeding, 1993, Bowland and Foegeding, 1995, Galani and Apenten, 1999, Harwalkar and Kalab, 1985, Hoffmann and Van Mil, 1997, Hoffmann and others, 1997, Hongsprabhas and Barbut, 1997a, 1997b, Hongsprabhas and Barbut, 1998, Iametti and others, 1996, Macleod and others, 1995, Manderson and others, 1998, McKenzie and Sawyer, 1967, McPhail and Holt, 1999, Roff and Foegeding, 1996, Verheul and others, 1998, Watanabe and Klostermeyer, 1976). However, there is a paucity of information regarding the relationship between the size of HDLG soluble aggregates and the textural properties of LG gels.

To maximize the use of preheated whey protein polymers in processed meat products, it is essential for meat processors to optimize the preheating condition for whey protein. Understanding the effect of preaggregation and the effect of aggregate size on rheological properties of whey protein gels would also be important for meat processors to choose the appropriate preheating condition. Our overall goal for this project is to optimize the preheating condition for whey proteins under conditions suitable for meat processing, and to verify the efficacy of the preheated whey protein polymers in emulsified meat products. To achieve this goal, three separate studies were conducted. In study I, response surface methodology was utilized in a LG model system to optimize the preheating conditions for whey protein. The effect of HDLG aggregate size on

rheological properties and microstructure of LG gels was also investigated. Study II further investigated the effects of various preheating conditions on whey protein isolate in emulsion stability of high added fat and water emulsions prepared with preheated whey protein isolate. In Study III, low fat frankfurters were manufactured to verify the efficacy of preheated whey protein isolate in comminuted meat products.

This dissertation is formatted as five chapters. Chapter 1 is the review of literature. Detailed materials and methods used in this project are described in Chapter 2. Chapter 3, 4 and 5 are formatted in manuscript style according to the Journal of Food Science. Chapter 5 is followed by recommended future research. Finally, appendices are provided with step by step procedures for all protocols used for each study.

CHAPTER 1

LITERATURE REVIEW

1.1. Whey proteins

Whey proteins, the by-product of cheese or rennet casein and acid casein manufacture, have been widely used in the food industry, such as in bakery products, dairy products, dressings, spreads, protein drinks, infant formula, surimi seafoods and processed meat products. Whey proteins are highly nutritious with a protein efficiency ratio of 3.0-3.2 (3.5 for hen eggs) and have a highly balanced amino acid profile. Research showed that some components found in acid liquid whey can serve as natural antioxidants (Colbert and Decker, 1991) and consuming whey proteins has been reported to contribute several health benefits in humans (Whey Protein Institute, 2004), such as:

1. Improved cardiovascular heath:

Studies in humans and animals have shown that hydrolyzed whey protein isolates may reduce blood pressure of borderline hypertension individuals (Sharpe and others, 1994). Whey proteins can also reduce cholesterol level in individuals with high blood cholesterol.

2. Reduced risk of certain type of cancers:

Whey proteins are rich in cysteine. Studies found that whey proteins can raise glutathione levels to provide a boost to the immune system (de Wit, 1998). For cancer patients, whey proteins can be added to a variety of foods and be used to reduce the risk of infection and improve the responsiveness of the immune system. Research has shown that consuming whey proteins can protect against certain types of intestinal tumors and reduce the risk of breast cancer in women (Bounous and others, 1991).

3. Control diabetes:

Whey protein products are low in carbohydrate and fat, so can be beneficial for diabetic patients (especially type 2 diabetes) who need to carefully monitor their food intakes and body weight.

4. Infant nutrition:

Breast-feeding is the best choice for infants. However, infant formulas containing whey proteins are the next best thing when breast-feeding is not possible. Whey proteins contain many of the same components found in human breast milk, and some whey protein based infant formulas have even been shown to reduce crying in colicky infants (Lucassen and others, 2000).

5. Healthy aging:

Maintaining lean body mass is another benefit of consuming whey proteins for seniors. As we age, we lose our lean body mass (losing muscles), so a decreased lean body mass in seniors is not uncommon. A recent study in Europe found that older men consuming whey protein showed greater protein synthesis than those who consumed casein (Fogelholm, 2003). This suggested that consuming whey proteins can help seniors to limit muscle loss over time.

6. Help wound healing:

An increased amount of protein is required when the body is healing after surgeries. Whey contains high quality proteins and is recommended by physicians following surgery or burn therapy. Anti-microbial compounds, such as lactoferrin are also found in whey protein, and this provides an extra benefit for the healing body (Kuwata, 1998).

Whey proteins were first introduced to the food market in 1977 and since then, the whey protein market has grown dramatically (Hood, 1985). Recently, the whey protein market has reached a plateau. The worldwide production of liquid whey is estimated around 118 million tons, and about 700,000 tons of solid whey proteins are available as valuable food ingredients (de Wit, 1998). However the high degree of variability in composition, functionality, and sensory properties of whey proteins has greatly limited their use by the food industry (Atughonu and others, 1998, Holt and others, 1999a, 1999b, Hung and Zayas, 1992, Langley and Green, 1989, Morr and Foegeding, 1990). In 2002, 450,000 tons of dry whey products were produced in the United States, but only 60% of the dry whey products were sold and leaving a 40% surplus (USDA, 2003).

Generally, there are two types of liquid whey (Mulvihill and Grufferty, 1997): sweet whey (minimum pH 5.6) and acid whey (maximum pH 5.1). Sweet whey is produced from coagulation of milk by rennet-type enzymes in the manufacture of cheeses and rennet casein. Acid whey is produced from coagulation of milk with acid in the manufacture of acid cheeses and acid casein. Milk composition, cheese variety, casein type and process conditions used in the manufacture of the cheese and casein greatly influence the composition of whey. Typically, about 50% of the total solids in the original milk are present in liquid whey. Whey proteins are composed of several small proteins, the composition and biological function of whey proteins in bovine milk are shown in Table 1.1 (de Wit, 1998).

1.2. Manufacture of whey protein

The technology to produce whey proteins has grown dramatically in the past two decades. Modern technologies have allowed the whey industry to easily separate the major components of cheese whey: water, lactose, ash, protein, and fat. The new technologies have also allowed the whey industry to tailor-make their whey proteins to fit the specific needs for the food industry.

Table 1.1. Composition and biological functions of whey proteins in bovine milk (Adapted from de Wit, 1998).

Whey protein	Weight contribution (g/L of milk)	Biological function (for calf)
β-lactoglobulin	3.2	Provitamin A (retinol) transfer
α-lactalbumin	1.2	Lactose synthesis
Bovine serum albumin	0.4	Fatty acid transfer
Immunoglobulins	0.8	Passive immunity
Lactoferrin	0.2	Bacteriostatic agents
Lactoperoxidase	0.03	Antibacterial agent
Enzymes (>50)	0.03	Health indicators
Protease-peptones	1-3	Antioxidant activity

Several steps involved in producing whey proteins include clarification, separation, pasteurization, crystallization, ultrafiltration-diafiltration, ion exchange, and drying (Mulvihill and Grufferty, 1997).

Clarification

To produce whey protein, first liquid whey needs to be recovered from the cheese or casein manufacturing operation. Clarification is the first step in manufacture of whey

proteins and is used to achieve low level of curd and prevent blocking of the heat exchanger. Clarification is done by a combination of settling, screening and simple centrifugation.

Separation

After the clarification step, fat is separated from the liquid whey. A self-discharging separator is used in this step to separate fat from the liquid whey. After the separation step, fat content in the liquid whey is normally around 0.02%.

Pasteurization

To maintain the quality and stability of liquid whey, the liquid whey is pasteurized immediately after the separation step. Temperature and time for the pasteurization are in the range of 72-75 °C and 15-20 sec, respectively. If the pasteurized liquid whey is not to be further processed right away, it is stored at 5 °C to minimize the growth of microorganisms (Young, 1985).

Reverse osmosis

After the pasteurization step, the liquid whey is evaporated under vacuum to increase the total solids to 40-60%. The vacuum evaporation is done at low temperatures (below 70 °C) to avoid denaturation of the proteins. Sometimes, reverse osmosis is applied before the vacuum evaporation to increase the efficiency of the evaporator. Reverse osmosis can increase the solid content of the liquid whey to about 20%.

Crystallization

To produce high quality, non-hygroscopic whey protein powders, liquid whey (40-60% total solids) is crystallized to remove lactose after reverse osmosis and vacuum evaporation. The concentrated liquid whey is cooled to 30 °C, and then crystallized in a

crystallization tank seeded with finely ground α-lactose monohydrate or well-crystallized whey powder. In the crystallization tank, the liquid whey is held for up the 4 hr, and then further cooled down to 10 °C at a very slow rate as low as 3 °C/hr. This process can produce up to 80% of the lactose as α-lactose monohydrate which is less hygroscopic than anhydrous β-lactose. After the crystallization, the crystallized lactose can be separated from the liquid whey by centrifugation.

Ultrafiltration-diafiltration

Ultrafiltration-diafiltration is used to increase the protein content of the liquid whey. Because microorganisms are also concentrated during ultrafiltration, the temperature during ultrafiltration is maintained above 50 °C to minimize the microbial growth. In diafiltration, water is added to the retentate, and lactose and minerals are washed out. As a result, the protein content of the retentate is increased (Young, 1985).

After ultrafiltration-diafiltration process, the retentate is pasteurized again (66-72 °C for 15 sec) to reduce the number of microbes.

Ion-exchange

The high ash content of liquid whey can have negative effects on the flavor of whey products. Demineralization of liquid whey results in products with low ash content. Minerals in liquid whey can be removed by ion-exchange process. Ninety percent of the minerals in liquid whey can be removed after the process. Combining with ultrafiltration-diafiltration, whey products containing 95% of protein can be produced (Huffman, 1996).

Drying

The final stage in producing whey products is drying. To produce non-caking, high solubility, and more functional whey powders, a multi-stage drying process is commonly practiced in the whey industry. Liquid whey is pre-crystallized before the first stage of drying; then the pre-crystallized whey is spray-dried to achieve 5-8% moisture content. Post-crystallization and the final drying step are done on a fluidized bed to produce whey powders with low bulk densities.

1.3. Types of whey proteins

Whey protein products are categorized on the basis of their composition; mostly they are grouped based on their protein contents (Huffman, 1996). A variety of whey protein products are listed in Table 1.2.

Table 1.2. Types of whey protein products.

Product name	Protein content (%)	Fat content (%)	Lactose content (%)	Ash content (%)
Whey powder	13	1	76	10
35% WPC[a]	34-35	4	53	8
50% WPC[a]	53	5	35	7
80% WPC[a]	80	4-7	7	4-7
Whey protein isolate	>90	1	1	3

[a]: WPC: Whey protein concentrate.

Because of the cost effects during whey processing, whey protein products are priced primarily based on their protein content. Products with higher protein content

such as whey protein isolate and 80% WPC are sold at higher prices than whey powder and 35 or 50% WPC.

The composition of whey products not only affects the price of whey products but also affects the functionality of the whey products. During spray drying lactose protects proteins from denaturation; hence whey protein products with low lactose contents often contain a higher proportion of denatured proteins. Residual fat from milk in whey products can be as high as 4-7% which can affect foaming properties. Lower fat content produces superior foaming properties. Mineral contents of whey products are also important for the functionality of whey products. Among all the minerals, calcium is the most important one. Both the concentration and ionic state of calcium affect the functionality of whey products. While a high concentration of free calcium can cause aggregation and gelation of the whey products during high intense heating, at a neutral pH, calcium phosphate can increase heat stability of the whey products. Because of the high variety and composition of whey products, no single whey product has all the functionalities needed by the food industry. Often whey products are combined to achieve the desired functionality (Jost, 1993).

1.4. Application of whey proteins in processed meat products

Natural foods are preferred by consumers and whey protein products are considered as natural foods. Stability, yield, texture, palatability and cost are the five major criteria for use non-meat proteins in meats. Other important factors include particle size, color and quality of the proteins (Hung and Zayas, 1992). The ability of whey proteins to form gels and provide a structural matrix for holding water, lipids, and

food ingredients is very useful in processed meat products. In the processed meat industry, whey protein is one of the most important non-meat ingredients and has been used primarily as a binder or extender in processed meat products to improve cooking yield and textural quality. Economically, addition of whey protein to meat products increases the yields, thereby decreasing the costs. Some functional benefits of whey protein products in processed meat products are listed in Table 1.3 (Keaton, 1999).

Table 1.3. Functionality of whey products in processed meats (From Keaton, 1999).

Function	Specific impact
Solubility	1.Smooth texture at most use levels 2.Creamy textures at high use rates 3.Reduced "gritty", "powdery" taste
Water binding	1.Binds and entraps water: improve cook yield 2.Provides body, texture 3.Improves sliceability
Viscosity	1.Thickening 2.Enhances body, texture
Gelation	1.Forms gel during heat processing 2.Animal/fat replacement possible
Emulsification	1.Forms stable, fat/oil emulsions 2.Prevents oiling-off and "fat caps" 3.Meat protein replacement
Foaming	1.Forms stable film 2.Provides structure
Browning	1.Enhances Maillard, non-enzymatic browning 2.Adds color, visual appeal
Flavor, aroma	1.Have little or no flavor of their own 2.Compatible with cooked meat flavors 3.Compatible with spice/seasoning blends
Nutrition	1.Superior amino acid profile 2.Can serve as source of calcium for enrichment

Currently in the United States, whey proteins are allowed to be used in processed meat products up to 3.5% in the finished product (USDA, 1999). The use of whey protein in low fat and non-fat meat products is more lenient and can be higher than 3.5% (U.S.D.A. policy memo 123, 1995). In non-standard meat products such as 'imitation" meat, there are no limits for the use of whey protein products (USDA, 1999). Also, no international standards limit the use of whey protein products in processed meat products. Whey proteins have been used in both restructured and comminuted types of meat products. In restructured meat products, whey proteins are used mainly to increase the binding ability. For comminuted meat products, whey proteins are used to increase emulsion stability and water holding capacity. The functional properties of whey proteins in processed meat products are described below.

1.4.1. Water binding ability and cooking loss

Any non-meat ingredients which can decrease cooking loss would be desired by meat processors. Early research demonstrated that high protein content of ultrafiltered whey proteins can increase juiciness and water binding ability in meat loaf (Lee and others, 1980). When added to knockwurst, whey protein concentrate proved to be a viable binder compared with soy protein isolate and calcium-reduced non-fat dry milk (Ensor and others, 1987).

Chen and Trout (1991a) reported that adding whey proteins (2.0%, whey protein concentrate) in restructured beef steaks decreased cooking loss. However, the use of whey proteins in restructured beef steaks can also increase non-meat flavors in the final products. Besides cooking loss, storage stability, sliceability, flavor and appearance are also important qualities in cooked ham. Thomsen and Pedersen reported (1993) that

when compared to carrageenan (0.4%), smoked hams prepared with Nutrilac HA-7570 whey protein concentrate (0.8% whey, 60% injection rate) had similar cooking loss, storage stability, and hardness value to those prepared with carrageenan. They suggested that when used in cooked hams, modified whey proteins can decrease cooking loss and increase storage stability of the products.

Whey proteins can also be used to improve emulsifying capacity and emulsion stability in comminuted meat products. Hung and Zayas (1992) reported that compared with all beef frankfurters (20% fat), beef frankfurters containing 3.5% whey protein concentrate had increased water holding capacity (from 0.375 to 0.507) and decreased cooking loss (from 11.75 % to 9.66%). The microstructure of beef patties prepared with whey protein concentrates (1 to 4%, w/w) was studied by El-Magoli and others (1995). They reported that myofibrils proteins started to shrink and coagulate around 60 °C; and as the cooking temperature increased, shrinkage and coagulation of the myofibrils proteins also increased. When whey proteins were added to the beef patties, shrinkage and coagulation of the myofibrils proteins were decreased by the presence of whey proteins. Whey proteins seemed to serve as a filler by occupying the intracellular and intercellular spaces. This protective action of whey proteins seemed to stabilize the protein matrix and retain water. When studied under an electron transmission microscope, frankfurters prepared with 3.5% whey protein concentrate had a fine protein-fat matrix with less coalescence of fat droplets (Atughonu and others, 1998). These results suggested that in emulsion-type meat products, whey proteins were able to incorporate into the protein-fat matrix and form a strong structure to hold water and fat. As a result, the cooking yield and water binding capacity increased in the products.

Preheating of whey proteins before adding to processed meat products is a new area that researchers have been working on. Preheated whey proteins decrease cooking loss and increase water holding capacity in poultry meat batters. Hongsprabhas and Barbut (1999) reported that at low salt levels (<1.5%), poultry meat batters prepared with whey protein isolate (10% (w/v) preheated at 80 °C for 30 min, pH 7.0) had a lower cooking loss than poultry meat batters prepared with unheated whey protein isolates. At 1.5% salt level, cooking loss was 1.23 and 17.47% for preheated and unheated whey protein, respectively. These results provide new research opportunities to explore the effects of preheating on the functional properties of whey proteins.

1.4.2. Textural properties

The increase in consumer awareness of the importance of a healthy diet has created a growing market for low fat meat products. Because of its high water and fat binding ability, whey protein products are good candidates for protein-based fat-replacers. El-Magoli and others (1996) investigated the use of whey protein concentrate (79.5% protein) as a fat-replacer in low fat ground beef patties. In their study, whey protein concentrate (0 to 4% addition) was added to ground beef patties formulated to contain 11 (patties with whey proteins) to 22% (control without whey concentrate) fat. At the 4% level, whey protein concentrate served as a fat-replacer without sacrificing product palatability and flavor. Hughes and others (1998) reported that adding whey proteins (3.0%) in low fat beef franks changed product textural properties. While the protein content was maintained constant (13%), fat content in beef franks was reduced from 12 to 5%. Reduced fat (5%) beef franks containing whey protein concentrate had similar sensory attributes compared to those without whey protein concentrate, and had

higher hardness, adhesiveness, gumminess and chewiness values than 12% fat beef franks. Pawar and others (2002) reported that additions of whey protein concentrates in goat patties also changed the textural properties of the patties. They stated that regardless of the cooking method (pan-frying, microwave oven cooking and hot-air oven cooking), as the amount of whey protein in the patties increased (0 to 30%) hardness and springiness values of the patties also increased.

1.4.3. Color

Because of its ability to extract myofibrilla proteins from meat, salt is essential in manufacturing processed meat products. However, salt has been reported to be associated with discoloration in fresh restructured meat products. Chen and Trout (1991b) investigated the possibility of using various binders to replace salt in restructured beef steaks. They reported that whey protein concentrate (2.0%) had the potential to be used in restructured meat products to partially replace salt (decreasing from 1.0% to 0.13 %) and phosphate (decreasing from 0.5% to 0.05%). Their results showed that surface discoloration of whey protein restructured beef steaks was similar to those without any salt and phosphate, and their color was stable during 12-weeks of frozen storage (-23°C).

Pink color in cooked, uncured turkeys is a problem for the poultry industry, since the pink color is associated with undercooking. Depending on the type and source of whey proteins, some whey proteins can decrease the pink color in cooked turkeys, while some can increase or do not change the pink color intensity. Slesinski and others (2000a, 2000b) reported that some whey proteins added with or without other milk proteins can decrease the pink color (CIE a^* value) in cooked ground turkey breasts. Three different whey protein concentrates were added (1.5 and 3.0%) to the turkey breasts. They

reported that after cooking to 80 °C, one whey protein concentrate (Alacen 882 from New Zealand Milk Products (NZMP)) was found to decrease the pink color (57% reduction), another (Alacen 841 from NZMP) did not change the pink color and another increased the pink color (Alacen 878 from NZMP) of cooked ground turkey breasts. At levels as low as 1.5%, whey proteins can effectively decrease the pink color, but the right type of whey protein must be used. Increasing lightness value (CIE L^* value) of the cooked turkey breasts can improve the overall appearance of the product. All the whey protein concentrates used in their studies increased the lightness value of the cooked turkey breasts. Similar results of reduced pink color in cooked ground turkey breasts prepared with whey proteins were reported by Sammel and Claus (2003). In their study three out of five commercial whey protein concentrates reduced the pink color of cooked ground turkey breast. To use whey protein to decrease the undesired pink color in cooked turkey breasts, meat processors need to carefully choose the source and type of whey proteins, and be aware that addition of some types of whey proteins may increase the pink color intensity.

1.4.4. Sensory characteristic

Hung and Zayas (1992) reported that at 3.5% addition, there was no difference in meaty aroma between beef franks prepared with or without whey proteins. Researchers have explored the possibility of using liquid whey in processed meat products. Marriott and others (1998) replaced 20 and 30% of the water in curing brines of restructured low-fat (4%) cured ham (240 ppm sodium nitrite) with unprocessed liquid whey. They indicated that replacing water with liquid whey did not change the sensory attributes of the restructured hams (juiciness, tenderness, flavor, and color). Yetim and others (2001)

substituted 25 to 100% of liquid whey for ice to manufacture beef franks. While franks manufactured with liquid whey (50, 75 and 100%) had higher emulsion stability, trained sensory panel can not detect any differences in sensory attributes (cohesiveness, pink color, taste and aroma, off-flavor) between control and liquid whey replaced franks.

Because of the mild flavor of whey proteins, whey proteins can be added to processed meat products to a high extent without changing the flavor of the final products. Hale and others (2002) compared sensory attributes of beef patties containing all meat and extruded whey proteins (a dry mix of 1 part cornstarch and 2 parts whey protein concentrate (80% protein content) with 1.69% calcium chloride dihydrate). They stated that their consumer sensory panels could not detect any differences in tenderness, juiciness, texture and flavor between all meat beef patties and patties containing 40% extruded whey proteins. The consumer sensory panels also showed that beef patties containing extruded whey proteins had an equal level of acceptability as all meat beef patties. The same research group (Taylor and Walsh, 2002) reported that the whey protein meatless patties had very high consumer acceptability and stated that wheat gluten and xanthan gum can be combined with whey proteins to develop more desirable meatless patties.

1.5. Whey protein cold-set gels

The ability of whey proteins to form gels at room temperature is called cold-setting. Cold-set gelation requires an initial preheating step to form denatured whey protein soluble aggregates, followed by incubation with additives (salts or acidulants). In the formation of cold-set gels, the initial heating step facilitates the formation of intra-

aggregate bonds. At this stage, whey proteins unfold and remain soluble due to the high electrostatic repulsion force. In the incubation step, the formation of inter-aggregate bonds is promoted and the electrostatic repulsion force is minimized by the additives. Heating exposes hydrophobic regions, sulfhydryl groups and disulfide bonds of whey proteins, and promotes formation of disulfide bonds and hydrophobic interactions. Salt and acid are hypothesized to promote association mainly through noncovalent bonds. Thus the two steps lead to a gel network formation as shown in Figure 1.1.

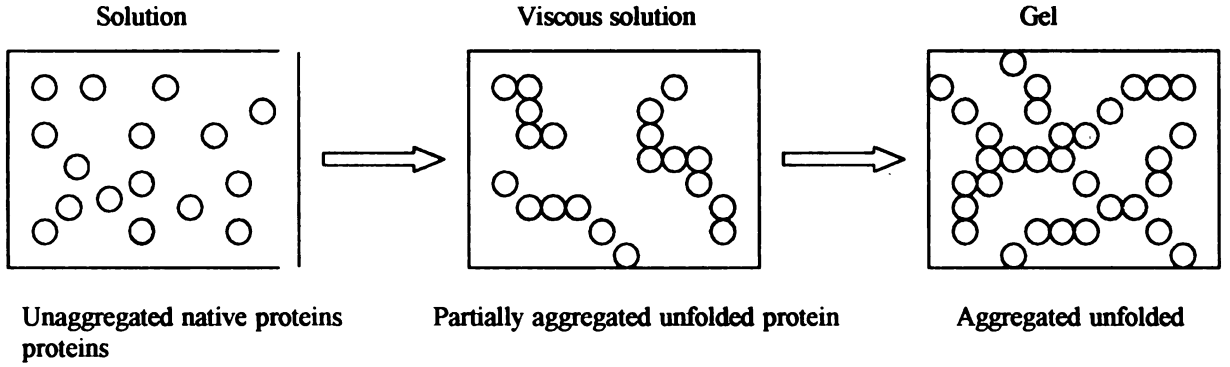


Figure 1.1. A schematic representation of whey protein cold-set gelation (From McClements and Keogh, 1995)

Both preheating and cold-set gelling conditions are important to rheological properties of whey protein cold-set gels. Research has shown that whey protein cold-set gels with different rheological properties can be produced by the adjustment of preheating and cold-set gelling conditions. The effects of preheating and cold-set gelling conditions on the rheological and functional properties of whey protein cold-set gels are discussed as below.

1.5.1. Pre-heating

1. Heating temperature and heating time:

To induce a whey protein cold-set gel, preheating temperature and time have pronounced effects on the rheological properties of the cold-set gels. Barbut and Foegeding (1993) studied the effects of preheating time (5 to 80 min) and temperature (70 to 90 °C) on the rheological properties of whey protein cold-set gels. They stated that preheating to temperatures higher than 70 °C was required to induce the cold-set gels. In the range of their studied temperature and time, preheating at higher temperature and longer time seemed to produce harder cold-set gels. Similar preheating effects on rheological properties of Ca^{2+} induced whey protein gels was reported by Hongsprabhas and Barbut (1996). They found that higher pre-heating temperatures resulted in higher water binding capacity and gel extensibility.

Heating temperature in the pre-heating step not only affects rheological properties of the cold-set gels, but also color of the cold-set gels. Hongsprabhas and Barbut (1996) reported that increasing preheating temperature (70 to 90 °C) in 10% (w/v, pH 7.0) whey protein solution increased translucency (L^* value decreased) of the whey protein gels. They suggested that when heated at higher temperatures, whey proteins were unfolded to a greater degree, and it promoted the formation of fine-stranded gels. Fine stranded gels reflect light better, hence the L^* value decreased.

2. Ionic strength and pH:

High electrostatic repulsion force between unfolded whey protein polymers is the main force to keep unfolded whey proteins soluble and from gelling. To maintain high electrostatic force, pH and ionic strength of the solution need to be carefully controlled. Bryant and McClements (2000a) investigated the influence of heating temperature (65 to 90°C), heating time (5 to 30 min), protein concentration (2 to 12 %) and pH (3 to 8) on

the rheology and appearance of heat-denatured whey protein solutions. Their results indicated that higher and longer heating temperature and time at higher pH resulted in higher apparent viscosity, and the rate of cold-gelation increased as the viscosity increased. Their study suggested that it's possible to produce whey protein gels with different textural properties by altering the preheating pH condition. Chantrapornchai and McClements (2002) reported that preheated whey protein solution remained in solution (heating 10% whey protein isolate at 80 °C for 15 min, pH 6.9) and no gel was formed when NaCl concentration was below 60 mM. As NaCl concentration increased above 60 mM, electrostatic repulsive force was not high enough to keep the unfolded proteins from interacting with each other. Consequently, heat-induced gels were formed. Hongsprabhas and Barbut (1996) stated that color of the cold-set gels was also affected by the concentration of ion in the preheating step. Regardless of the heating temperature, as the concentration of calcium increased, L^* value of the cold-set gels also increased.

3. Protein concentration:

Inter-molecular interaction is promoted when heating at a higher protein concentration, and a more ordered protein structure is formed. Both inter-molecular hydrophobic interactions and disulfide bond formation increase when protein concentration increases. Preheating whey protein solution (6 to 10% (w/v), preheated at 80 °C for 30 min, pH 7.0) at higher protein concentrations produced more rigid gels (Hongsprabhas and Barbut, 1997). The effect of protein concentration in the preheating step on the rheological properties of whey protein cold-set gels induced by $CaCl_2$ at 37 °C was investigated by Ju and Kilara (1998a). They indicated that the amount and size of soluble protein aggregates increased as the whey protein concentration increased (1-9%,

preheated at 80 °C for 30 min, pH 7). They also stated that the larger size of whey protein aggregates and higher concentration of whey protein in the preheating step led to increases in hardness of cold-set gels.

1.5.2. Cold-set gelling

1. Ionic strength and type of ions:

Addition of salt and ions decreases electrostatic repulsion force between unfolded whey protein molecules. Roff and Foegeding (1996) studied the effects of ion type on the gel strength of whey protein cold-set gels. Whey protein solutions (10% (w/v)) were preheated at 80°C for 30 min and then dialyzed (23±1 °C for 24 hr) against $CaCl_2$ and $BaCl_2$ at the concentration of 5 to 500 mmole/dm^3. Under the studied conditions, whey protein cold-set gels exhibited a general slow increase in shear stress with increasing ion concentration. A maximum gel stress was obtained at 75 mmol/ dm^3 and 20 mmol/ dm^3 for $CaCl_2$ and $BaCl_2$, respectively. At the same ion concentration, shear stress of $BaCl_2$ induced gel was always lower than $CaCl_2$ induced gel. A small decrease in water holding capacity was observed as the salt concentration increased. Hongsprabhas and Barbut (1998) suggested that the decreased water holding capacity may indicate that the gelation mechanism of cold-set gels is governed by the concentration of the ions. Similar results were reported using NaCl by Kitabatake and others (1996).

Depending on the cold-set gelation conditions, calcium can induce both aggregated and fine-stranded type of gels. Barbut (1995) reported that the gel structure of whey protein cold-set gels was determined by the concentration of calcium in the gels. Fine-stranded gels with high water holding capacity were formed when the calcium

concentration was lower than 180 mM/L. Thick aggregated gels with low water holding capacity were formed when the calcium concentration was higher than 180 mM/L.

Monovalent and divalent salt ions both can induce cold-set gels by decreasing electrostatic repulsion force. However, divalent salts (such as Ca^{2+}) are more effective than monovalent salt (such as Na^{+}). Bryant and McClements (2000b) reported that a lower amount of $CaCl_2$ was needed than NaCl to induce whey protein cold-set gels. They credited the cross-linking ability of Ca^{2+} for the increased ability in inducing cold-set gels. Marangoni and others (2000) also suggested that the cold-set gelation mechanisms of Ca^{2+} and Na^{+} induced gels were somehow different.

2. Temperature and time:

Several researchers have shown that incubation temperature and time affected rheological properties of whey protein cold-set gels. When reheated at higher temperatures, preheated whey protein isolate (preheated at 90 °C for 30 min, pH 7) solutions showed greater gelation rates and increased storage moduli (McClements and Keogh, 1995). Kitabatake and others (1996) reported the viscosity of preheated whey protein solutions (7.8% (w/v) heated at 80 °C for 30 min at pH 7.0) increased when the incubation time (20 to 120 min) and temperature (25 and 37 °C) increased. As temperature increased, the magnitude of hydrophobic interactions and movement of molecules increased. Under these conditions, unfolded whey protein molecules can interact with each other more freely. As a result, a stronger cold-set gel is formed (Bryant and McClements, 1998).

3. pH:

Besides addition of salts or ions, whey protein cold-set gels can also be induced through pH adjustment of the preheated whey solution. Ju and Kilara (1998b) indicated that pH of the preheated whey solution (preheated at 80 °C for 30 min, pH 7.0) needed to be lowered below 5.8 to induce whey protein cold-set gels. Mleko and Foegeding (1999) studied the pH effects on the viscosity of preheated whey protein solution (heating a 4% whey protein solution at pH 8.0). The apparent viscosity of the reheated whey protein solution was in the order of pH 6.0>6.5>7.0. Their result suggested that textural properties of preheated whey protein polymers can be modified by adjusting the pH in the reheating step.

4. Protein concentration:

As protein concentration increases, a stronger and harder gel is formed. Previous research has shown that higher concentration of whey protein produced harder whey protein cold-set gels. Hongsprabhas and Barbut (1997) stated that increased gel strength and water holding capacity was observed in whey protein gels with higher whey protein concentration (from 6 to 10%). Similar protein concentration effect on gel hardness of whey protein gels was also found by Ju and Kilara (1998b) and Marangoni et al (2000).

1.6. β-lactoglobulin

The composition of milk varies from species to species and also with the time since parturition. Milk proteins include caseins, whey protiens, milk fat globule membrane proteins and enzymes. Quantitatively, the main whey proteins represent about 20% of the total milk proteins and consist of α-lactalbumin (17%), β-lactoglobulin (LG)

(68%), immunoglobulin (7%), and bovine serum albumin (7%) (Imafidon and others, 1997). LG was first purified by Palmer (Palmer, 1934) and has become one of the most studied proteins. LG is biosynthesized within the secretory epithelial cells of the mammary gland under the control of prolactin. To date, no biological function for LG has been confirmed (Sawyer and others, 1998). The structure of the homodimer bovine LG shows a similarity with the plasma retinol binding protein and the odorant binding protein. This suggests that the role of LG may be connected with transport or accumulation of lipid-soluble biological components (Iametti and others, 1995). Native LG is a globular protein consisting of 162 amino acids in a single peptide chain with a molecular weight of 18 KDa. The molecule possesses two tryptophan residues (19, 61), two disulfide bonds ($S\text{-}S^{106\text{-}119}$ and $S\text{-}S^{66\text{-}160}$) and one free thiol group (121), which exhibits increased reactivity above 7. LG is negatively charged at pH > 5.2 and positively charged at pH < 5.2. LG molecule can occur in different associated forms, depending on both temperature and pH. Native LG occurs as a dimer form in solution between pH 5.2 (isoelectric point) and 7.5. Between pH 3.5 and 5.2, LG reversibly forms tetramers/octamers. When the pH is below 3.5 it can dissociate into monomers (Caessens and others, 1997).

1.7. Structure of β-lactoglobulin

Circular dichroism and infrared studies indicate that LG consists of an α-helix content of 10-15%, a β-structure content of 50% with turns accounting for 20%, and the remaining 15% representing amino acid residues in a random nonrepetitive arrangement without well-defined structure (Hosseini-nia and others, 1999). The structure of LG is an

antiparallel β-barrel made up of eight β-strands arranged in two groups of four. At the C-terminal end of the polypeptide chain there is a three-turn α-helix followed by a ninth β-strand (Sawyer and others, 1999). Six LG genetic variants (A, B, C, D, H and W) exist in the cow (*Bos taurus*). LG variants A and B are the most common in commercial preparations; they differ only in amino acid composition in two positions: 64Asp/Gly and 118Val/Ala for the A variant versus the B variant, respectively (Hambling and others, 1992). Jersey cattle in New Zealand and Australia have moderate levels of LG variant C (His^{59}), and its primary structure only differs from LG variant B (Gln^{59}) at residue 59.

1.8. Structural changes and aggregation of β-lactoglobulin

Many studies have been focused on the structural changes of LG during heating under a wide range of physicochemical conditions. Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry and circular dichroism (CD) spectroscopy are the most common techniques used to study the structural changes.

Upon heating, LG forms various sizes of denatured soluble aggregates. Verheul and others (1998) proposed a two successive step model for heat-induced denaturation and aggregation of LG.

Step 1: denaturation:

$$B \underset{k-1}{\overset{k1}{\rightleftarrows}} B^*$$

Step 2: aggregation:

$$B \text{ or } B^* + B_X^* \xrightarrow{k2} B_{X+1}^* \rightarrow \ldots\ldots \rightarrow B_m^*$$

Where B is the native state of LG, B^* is the partially unfolded state of LG, B_x^*, B_{x+1}^*, and B_m^* are LG aggregates consisting of x, (x+1), and m monomeric units, respectively. The unfolding and refolding are considered to be first-order reactions, which may involve a number of consecutive changes in the molecule. Upon unfolding of the molecule, the thiol group is exposed and becomes reactive, causing several irreversible aggregation reactions to take place; the aggregation reaction is a second-order reaction. The ability of LG to form aggregates through self-association is due to both specific recognition and stabilizing forces, such as hydrogen bonds, hydrophobic and electrostatic interactions (MacCleod and others, 1995). Although the conditions to form heat denatured LG (HDLG) soluble aggregates have been studied, the relationship between the size of soluble HDLG aggregates and the rheological properties of LG gels is still not clear yet (Vittayanont and others, 2002).

During heating, physicochemical factors affect the structural changes and aggregation of LG include heating temperature, pH, LG concentration, salt concentration and sugar content.

1.8.1. Heating temperature and heating time

Heating has been reported to change the structure of LG. When subjected to heat, LG undergoes intramolecular and intermolecular changes. Upon heating above 60 °C, LG molecule undergoes conformational changes and partially unfolds. Denaturation is then followed by irreversible aggregation such that the heat-induced process becomes irreversible. Watanabe and Klostermeyer (1976) studied the heat-induced changes in sulphydryl and disulfide levels of LG during heating. They reported that when 1% LG solution (in phosphate buffer at pH 7.0, ionic strength 0.1) was heated at 75 and 95 °C

from 5 to 40 min, the sulphydryl groups decreased with increasing time and temperature. The decrease in sulphydryl groups was reflected in the increase of disulfide groups. They also suggested that sulphydryl initiated disulfide bond exchange was responsible for the formation of high molecular weight LG aggregates during heating.

Cairoli and others (1994) investigated the reversible and irreversible structural changes of LG upon heating. Due to exposed hydrophobic regions, LG structural changes were no longer reversible when LG was heated above 60 °C (3.8 mg/ml in 50 mM phosphate buffer at pH 6.8). The exposure of LG hydrophobic regions was necessary for the formation of LG aggregates. A similar study was conducted by Iametti and others (1996); however, they indicated that the irreversible structural modification of LG (2.5 mg/ml, 50 mM phosphate buffer, pH 6.8) only occurred when the solution was heated above 70 °C. But the gel permeation and sodium disulfide polyacrylamide gel electrophoresis results confirmed the suggestion by Watanabe and Klostermeyer (1976) that disulfide exchange was the major force for the formation of LG aggregates.

Boye and others (1996) and Prabakarn and Damodaran (1997) studied the secondary structural changes of LG during heating. They both stated that heating changed the secondary structural of LG. Prabakarn and Damodaran (1997) further described that the critical change in the conformation of LG occurred at around 63-65 °C. At this temperature range, the buried sulphydryl group was exposed and the sulphydryl-disulfide interchange initiated leading to polymerization. At 63 °C 18-19% β-sheet was melted to aperiodic structure, and the melted β-sheet might be in the regions of amino acid residues 80-84, 89-97, 102-109, and 115-124.

The tightly packed structure of globular proteins is stabilized by intramolecular non-covalent and disulfide bonds. The hydrophobic clustering of certain non-polar residues is thought to enhance the thermal stability of globular proteins. Relkin (1998) examined the heat-induced conformational changes (4.5% LG heated at 20, 75 and 80 °C, pH 7.0, 0.1 M NaCl) and surface hydrophobicity of LG. They found that the surface hydrophobicity of heated LG solutions increased as the heating temperature increased. This result was in agreement with Cairoli and others (1994) who suggested that the surface-exposed hydrophobic-clusters formed during heating led to intermolecular hydrophobic forces were the major forces involved in the first step of the aggregation process.

Manderson and others (1999) utilized near–UV CD to study the effect of heat treatment on three genetic variants of LG (A, B and C). Under near–UV CD spectra, unheated LG A, B and C were all similar to one another (3.5 mg/ml in 26 mM sodium phosphate buffer, pH 6.7), but the heat stability of the three variants was in the order of C>A>B (at pH 6.7 and 7.4).

Increasing heating temperature and heating time generally increases LG aggregate size. Sharma and others (1996) purified LG (5%, w/v at pH 7.0) from whey protein concentrate and studied the relationship between heating temperature and LG aggregate size. They reported that as the heating temperature increased from 25 to 70 °C, the size of LG aggregates also increased. Five categories of LG aggregates were developed based on the aggregate size: aggregate 1 (1-9 nm), aggregate 2 (10-99 nm), aggregate 3 (100-599 nm), aggregate 4 (600-999 nm), and aggregate 5 (>1000 nm). At 35 °C, aggregate 1 (96.5% aggregate existed as aggregate 1) was the predominant aggregate type. At the

denaturation temperature of 70 °C, the amount of aggregate 1 decreased to 60% with a concomitant increase in aggregates 2 and 3. The authors explained that at 70 °C, LG undergoes irreversible and rapid denaturation causing exposure of its hydrophobic interior. This is seen in the sudden decrease at 70 °C of the small aggregate size (aggregate 1) with concomitant increases in aggregates of higher sizes, specifically aggregate 3. Similar results were also reported by Haque and Sharma (1997), Hoffmann and others (1996) and Aymard and others (1996b).

Heating time also has a strong influence on the size of LG aggregates. Le Bon and others (1999) reported that heating LG solution (18.6 g/L at pH 7.0 in 0.1 M ammonium acetate buffer) at 60 °C, LG aggregate size increased as the heating time increased.

Decreased LG aggregate size at higher heating temperatures was reported by Photchanachai and Kitabatake (2001). They indicated that increasing heating temperature to 113 °C (60 mg LG/mL, pH 6.4) increased LG aggregate size, but heating higher than 113 °C decreased bonding of the aggregates, therefore, LG aggregate size decreased.

1.8.2. pH

Early observations by McKenzie and Sawyer (1967) indicated that at different pHs, LG showed varying levels of conformation changes with different molecular size. Recent observation by Galani and Apenten (1999) and Busti and others (2002) confirmed the early finding by McKenzie and Sawyer.

The effect of pH (3.0 to 9.8) on the stability of sulphydryl and disulfide groups of LG (heating 1% LG solution at 75 and 95 °C for 20 min) was studied by Watanabe and

Klostermeyer (1976). The disulfide group level increased slightly up to pH 6.9 at both temperatures, but above 6.9 a remarkable decrease was observed, especially at 95 °C. They explained that cystine and cysteine residues of proteins in alkaline media are easily converted into degradation products such as H_2S and dehydroalanine, so the decreasing of sulphydryl and disulfide group levels at high pHs was expected.

At room temperature the secondary structure of native LG is composed predominantly of β-sheet which does not vary much over a wide pH range (2.75-10). Palazolo and others (2000) reported that LG seemed to be more flexible at high pH values, while a major rigidity was observed at low pHs. The secondary structure of LG started changing as the pH approached 10. Increasing pH from 3 to 9 at 25 °C (200 mg/ml) was reported to increase the formation of β-sheet structures. At pH 11, a decrease in α-helix and an increase in random coil could be detected (Matsuura and Manning, 1994). β-lactoglobulin was most susceptible to heat denaturation at higher pHs. At pH 9, the protein seemed to undergo partial denaturation even without heating (Boye and others, 1996). Manderson and others (1999) also reported that LG A (3.5 mg/ml) was more susceptible to unfolding when it was heated at a higher pH (8.1). Lefèvre and Subirade (1999) reported that the aggregation of LG was affected by pH and the intermolecular interactions between proteins and the degree of unfolding before aggregation were different at pH 7.4 and 4.4. Such molecular differences may lead to different gel microstructures.

Aymard and others (1996c) proposed that at neutral pH (7.0), the aggregation of LG occurred in two steps. In the first step, LG denatured and formed small particles through disulfide bond interchange, and the size of the small particles did not depend on

the initial protein concentration (1-3%), the temperature (70 and 76 °C) and the ionic strength (0.003 and 0.1 M NaCl). In the second step, the small globular particles interact with each other and form larger aggregates called "fractal structures".

Recent study by Surroca and others (2002) revealed the role of the free thiol group and disulfide bond interchange in the formation of LG aggregates at various pHs. In their study, they investigated the formation of LG aggregates (10 mg/ml at 68.5 °C) at two different pHs (4.9 and 6.7). They reported that thiol/disulfide bond exchange was the major force for LG aggregate formation at pH 6.7, but not at pH 4.7 (up to 6 hr heating).

Furthermore, after heating at pH 6.7 (up to 6 hr), only three different types of disulfide bonds were found: C121-C66, C121-C160 and C160-C160. This result suggested that initiating the formation of LG aggregates at pH 6.7, the free thiol group at C121 attacked the C66-C160 disulfide bond, as a result, three new disulfide bonds were formed. Under the studied condition C106-C119 disulfide bond was protected by the β-barrel of the LG structure, and it did not participate in the thiol/disulfide interchange. Hence C106-C109 disulfide bond was not responsible for the LG aggregate formation under the studied conditions. This findings were somewhat confirmed by Edwards and others (2002) who stated that after heating LG A solution (2.2 mM, pH 3.0) at 80 °C for 1 hr, C106-C119 disulfide bond still remained intact. Although C106-C119 disulfide bond did not participate in the thiol/disulfide bond exchange at lower heating temperatures (below 80 °C), higher heating temperatures could open up the protected C106-C119 disulfide bond. Croguennec and others (2003) reported that heating LG solution (7 g/L, pH 6.7) at 85 °C for 25 min, free sulfhydryl groups were formed from the breaking of C106-C119 disulfide bonds. Their results also indicated that at higher heating

temperatures, both disulfide bonds were involved in the thiol/disulfide bond exchange and responsible for the formation of LG aggregates.

Several studies have reported that pH has pronounced effects on the size and shape of LG aggregates upon heating. Harwalkar and Kalab (1985) reported that alkaline treatment (pH 11 for 10 min) of LG solution (1%) resulted in an alteration in the microstructure of LG aggregates. Heating (90 °C for 30 min) of alkaline treated LG solution at pH 2.5 and 6.5 resulted in less compact aggregates than those aggregates without alkaline treatment. They reported that LG aggregates formed at pH 4.5 were very compact, approximately 0.5 μm in diameter, and the globules subsequently fused and forming larger irregular shape aggregates. LG aggregates formed at pH 2.5 (0.2 μm) and 6.5 (0.2-0.3 μm) were denser and smaller than aggregates formed at pH 4.5, and the aggregates were linked by chain-like fibrous structures.

Aymard and others (1996a) and Kavanagh and others (2000) reported similar results. Aymard and others (1996 a) studied the structure of LG aggregates formed at pH 7.0 and 2.0 (heating at 70 and 90 °C for various times). At pH 2.0, the aggregates formed had a more elongated, rod-like structure containing very few branching points. However the aggregates formed at pH 7.0 had a globular shape. Kavanagh and others (2000) studied the shape of LG aggregates under different pHs (2, 2.5, 3, 7.0 and 9.0). They reported that after prolonged heating (3%, 6 hr at 80 °C), the aggregates formed under pH 2.0 and 2.5 had a long linear shape. Upon the addition of salt (0.05 M NaCl), much shorter linear aggregates were formed. As the solution pH moved away from 5.2 (isoelectric point of LG), more linear and extended aggregates were observed. Recently,

a British research group (Gosal and others, 2002) and a Japanese research group (Ikeda, 2003) reported similar results.

β-lactoglobulin heated at pHs considerably higher or lower than the isoelectric pH value of 5.2 and at low ionic strength (<0.1) remains in solution (denatured soluble aggregates). Xiong and others (1993) measured the turbidity to monitor the aggregation of LG solution (1.2 mg/ml) under different pHs (5.5, 5.75, 6.00, 6.25 and 6.5). They indicated that LG was most sensitive to aggregation when heated at pH 5.5 showing a transition temperature at 76 °C. As the pH increased, the transition temperature also increased. Maximum particle size of LG was observed at the isoelectric point at both 4 °C and room temperature (MacLeod and others, 1995). Intermolecular electrostatic repulsion is minimized when the pH of LG solution is close to its isoelectric point. Therefore, aggregation is enhanced through short-range bonding, such as hydrophobic and Van der Waals bonds (Xiong and others, 1993).

Haque and Sharma (1997) used an 8% (w/v) LG solution to study LG aggregate size at three different pHs (3.5, 7.0 and 9.0). Regardless of the pH, at room temperature (25 °C) native LG had an apparent diameter of 200 nm. The mean diameter of HDLG aggregates between 25 and 75 °C was in the order of pH 7.0>pH 3.5>pH9.0. The low association tendency at pH 9.0 was attributed to a reversible transition in molecular conformation that takes place around pH 7.5. Following this transition, thiol activity is increased. Such increased activity could make the molecule more rigid and unable to partake in spatial arrangement to accomplish optimum intermolecular contact, resulting in smaller LG aggregate size.

Several researchers have reported the role of thiol-disulfide exchange in the thermal aggregation of LG aggregates. Hoffmann and van Mil (1997, 1999) reported that in the pH range of 6.4-8.0, LG aggregates (10 g/L, heated at 65 °C) were formed mainly by intermolecular disulfide bonds, and even at pH 6.0 thiol/disulfide exchange reactions were involved, although to a lesser extent. Comparing LG aggregate size formed under different pHs, aggregates formed at pH 8.0 were smaller than those formed at pH 6.0. They suggested that in water at neutral and elevated pH values, the free thiol group played a crucial role in the heat-induced aggregation of LG by acting as an initiator of thiol/disulfide exchange reactions. The formation of intermolecular disulfide bonds prevented the reversibility of modifications in the tertiary structure of native LG. Schokker and others (1999) also indicated that at neutral pH, the heat-induced aggregation of LG (heating at 78.5 °C, 17 mg/mL, pH 7.0, ionic strength equivalent to 0.005 NaCl) occurred mainly through disulfide bonding and to a much lesser extent through non-covalent interactions. In the early stage of aggregation, LG dimers and oligomers were formed through thiol-disulfide exchange and thiol-thiol oxidation. Aggregate sizes from 10^5 to 2×10^6 Da were found at the later stage of aggregation. These large aggregates were probably formed by the incorporation of monomers and small aggregates into large aggregates through disulfide bonding.

Similar results were reported by Galani and Apenten (1999) who stated that thiol-disulfide interactions and hydrophobic associations were the two major bonding forces in LG aggregates formed at pH 6.7 (2 mg/mL, pH 6.7, 0.1 M Tris-HCl buffer, heated from 75 to 150 °C). Bauer and others (2000) characterized the intermediates formed by heating LG solutions (10 to 50 g/l in 10 mM sodium phosphate buffer, 67.5 °C) at pH 6.7

and 8.7. During heating, heat-modified LG dimers, trimers and tetramers were formed. At all stages of aggregation, LG dimers were the predominant oligomers. These oligomers reached a maximum in concentration when large aggregates (1000-4000 kg/mol) first appeared. After the oligomers reached the maximum in concentration, they started to decline with an increase in the amount of large aggregates, suggesting that the formation of the oligomers was essential to the aggregation process. Comparing different pHs (6.7 and 8.7), heat-modified oligomers were formed at a greater rate at the higher pH. The oligomers were formed mainly through disulfide bond interchange and the large aggregates were formed through noncovalent interaction.

1.8.3. Protein concentration

Intermolecular β-sheet formation is important in protein aggregation and LG concentration seems to have some influence on the structure of LG. Concentration-dependent increase in β-sheet structure was observed by Matsuura and Manning (1994). In the concentration range of 10-70 mg/ml (20 mM NaCl, pH 7.0 at 25 °C), LG solution prepared from higher LG stock solution had higher β-sheet content than those prepared from low LG stock solution.

Iametti and others (1995) used intrinsic tryptophan fluorescence to study the dependence of LG concentration on the heat-induced changes of LG tertiary structure. The irreversible modification of LG tertiary structure was not concentration dependent (in the temperature range of 70 to 85 °C, 50 mM phosphate buffer, pH 6.8), while the temperature required for the occurrence of protein swelling (the initial step in the formation of associated forms of the protein) increased as the protein concentration increased (3.8 to 16.0 mg/ml). The formation of intermolecular disulfide bond was

favored by increasing LG concentration. As the LG concentration increased LG aggregate size also increased, and the concentration dependence of LG aggregate size was more profound at higher heating temperatures. The dependence of LG aggregate size on LG concentration was also reported by Hoffmann and others (1996), Hoffmann and van Mil (1997), and Le Bon and others (1999).

LG concentration not only affects the size of LG aggregates but also the shape of LG aggregates. A study done by Kavanagh and others (2000) suggested that LG concentration strongly influenced the shape of LG aggregates. In their study, 2.7 and 3% of LG solutions were heated at 80 °C for 6 hr at pH 2.0. Aggregates formed from 2.7% LG solutions had a combination of short and long liner aggregates. However, only long linear aggregates were formed from 3% LG solution.

The rate of LG aggregation during heating was investigated by Nielsen and others (1996) and Schokker and others (2000). Both studies indicated that in the temperature range of 75 to 80 °C, higher protein concentrations (1.25-15% LG, 0.1 M NaCl, pH 2.5 and 7.0) resulted in increased rates and extents of aggregation.

1.8.4. Effect of ionic strength and type of ions

Electrostatic repulsion is presumably responsible for preventing or retarding heat-induced LG gel formation at low salt concentrations. Jeyarajah and Allen (1994) studied the calcium binding and salt-induced structural changes of native and preheated (heated to 80 °C for 15 min) LG. An ion-selective electrode was used to study the calcium binding in 1 mM LG solution and the structural changes were studied by fluorescence spectroscopy (0.1 mM LG solution in 3 mM Tris buffer, pH 7.4). Not only the addition of salts induced structural changes in LG and increased its apparent hydrophobicity,

reactive sulphydryl content of native and preheated LG also increased with increasing $CaCl_2$ concentration (1-15 mM). However, when NaCl (up to 25 mM) was added to the native LG, only very small change was observed in the fluorescence, and no further change was observed with the addition of 50 mM NaCl. They suggested that the larger change induced by the calcium salts, compared to sodium salts, was a result of LG local unfolding. And calcium must be participating in a specific interaction with LG. A possible interaction is the formation of intramolecular ion bridges.

During heating, high NaCl concentration can protect LG tertiary structure. A significant amount of LG tertiary structure was preserved when LG (70 mg/ml, pH 7.0) was heated (at 90 °C for 60 min) with 50 mM NaCl (Matsuura and Manning, 1994). The increased LG thermal stability was also observed by Boye and others (1996).

Both the concentration and type of salt affect the aggregation of LG. The effects of several types of ions on gel property and aggregate size of LG have been studied. Xiong and others (1993) found that at low concentrations of NaCl, addition of NaCl caused an upward shift in T_m (temperature at which protein-protein interaction is maximum) and an augmentation of the transition peak (an increase of 6.5 °C in 0.02 M NaCl) of LG (1.2 mg/ml at pH 6.0). But low concentrations of $CaCl_2$ decreased LG T_m (at 5 mM $CaCl_2$, T_m decreased from 83 to 75 °C). The increased T_m and enlarged peak size of LG in NaCl solution may have resulted from protein charge modifications, resulting in an alteration of protein-protein interactions. High NaCl concentrations are capable of modifying the conformation and decreasing the thermal stability of LG. However, increasing ionic strength may mask some exposed ionic groups and alter the electric double layer of LG resulted in facilitating chain interactions between LG

polypeptides. In contrast, $CaCl_2$ decreased LG aggregation temperature, particularly at low ionic strength. Ca^{2+} has the ability to form bridges between adjacent peptides, resulting in LG aggregation. Li and others (1994) also suggested that the LG aggregation occurred via different mechanisms in mono- and divalent ion solutions.

Hoffmann and others (1996) indicated that the aggregation of LG (50 g/L at pH 6.9) was very sensitive to small variations in ionic strength and ionic composition of the medium. The apparent diameter of LG aggregates and the rate of aggregation increased with the increasing salt concentration (at the concentration of 0 to 20 mM), and the effect of $CaCl_2$ was ten times stronger than that of NaCl. Increasing ionic strength not only reduced the intermolecular repulsion between molecules but also increased the denaturation temperature of the protein. The increased LG aggregation rate and aggregate size were probably due to the balance of the two effects. Similar results were also reported by McPhail and Holt (1999) and Schokker and others (2000).

Recently, the role of calcium in the aggregation of LG has been confirmed by a Dutch research team (Simons and others, 2002) who concluded that the effects of calcium in the aggregation of LG are the combination of (1) intermolecular cross-linking of adjacent molecules by forming protein-Ca^{2+}-protein units, (2) intramolecular electrostatic shielding of negative charges on the protein surface and (3) ion-induced conformation changes leading to altered hydrophobic interactions.

1.9. Formation of protein gels

The formation of heat-induced protein gels is composed of two major steps (Ferry, 1948). First, the denatured proteins unfold into random coils of peptide chains.

Upon protein denaturation, the unfolded peptide chains form three-dimensional networks with viscoelastic properties. Protein gels may be defined as three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner, resulting in the immobilization of large amount of water by a small proportion of protein (Mulvihill and Kinsella, 1987). Foegeding and Hamann (1992) proposed a general protein gelation model shown in Figure 1.2. In this model, upon heating native proteins denature and unfold followed by aggregation. When the amount of unfolded protein is high enough, the unfolded proteins interact with each other and form protein gels.

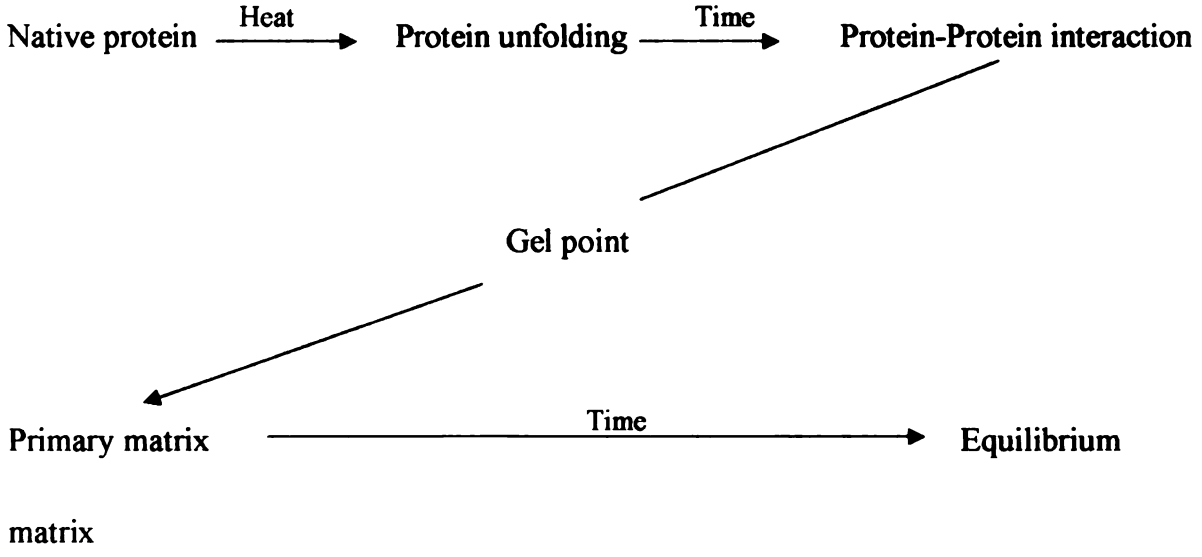


Figure 1.2. Heat-induced protein gelation model proposed by Foegeding and Hamann (1992).

Tombs (1974) presented two models for globular protein gels of denatured proteins: either random aggregation or a "string of beads" structure. β-lactoglobulin can form either type of gel depending on the gelling condition (Dio, 1993).

1.10. Factors affecting gelation of β-lactoglobulin gels

Factors affecting the gelation of LG, including pH, heating temperature, heating time, LG concentration, ionic strength and type of ion, will be discussed below.

1.10.1. β-lactoglobulin concentration

In order for proteins to form gels, certain concentrations of proteins are required depending on the type of protein and the environmental conditions. Vittayanont and others (2002) studied the gel property of heat-denatured LG aggregates (4%, w/v, preheated at 80 °C for 30 min in 0.05M phosphate buffer at pH 7.0) and native LG at different LG concentrations. They reported that upon heating of heat-denatured LG aggregates (0.6M NaCl and 0.05M phosphate buffer at pH 6.0 and heated to 71 °C), the storage modulus was 100, 860, and 3100 Pa for 1%, 2% and 3% of heat-denatured LG aggregates, respectively. The gel point of LG decreased as the LG concentration increased (1% at 63.0 °C and 3% at 42.0 °C) indicating that a stronger LG gel was formed at a higher LG concentration. Comparing heat-denatured LG aggregates and native LG, preheating decreased LG gel point and also increased LG gel rigidity.

1.10.2. pH

Two types of LG gels could be formed depending on the pH ranges. Near and above neutral pH, transparent, fine-stranded gels are formed. When the pH is close to the isoelectric point of LG, non-transparent particulate gels are formed. Both small and large deformation tests have been performed to study the rheological properties of LG gels. Stading and Hermansson (1990) used dynamic oscillatory test to study the rheological properties of LG gels formed at various pHs (2.5-9.0). β-lactoglobulin solutions (12%, w/v) were heated to 90 or 95 °C and held at the same temperatures for 60 min. Under

the conditions, aggregate gels with low gel point were formed at pH range of 4.0-6.0 while fine-stranded gels with high gel point were formed if the pH was higher or lower than that pH range. A two-step gelation was only observed at aggregate gels formed at pH 5.5. Phase angle of aggregate gels formed at pH 5.5 first decreased at 30 °C and the second decrease was observed at around 73 °C. This unique characteristic of aggregate gels may be used to differentiate between fine-stranded gels and aggregate gels. The aggregate gels were more strain sensitive and frequency dependent during gelation than fine-stranded gels, suggesting that aggregate gels were weaker than fine-stranded gels.

A large deformation test (tensile measurement) was also performed by this same research group (Stading and Hermansson, 1991) using the same heating conditions. At pH 4-6, opaque gels (aggregate gels) were formed, and gels formed below or above this pH range were transparent (fine-stranded gels). At low pHs (3.0-4.0) gels were brittle with low strain and stress at fracture and gels formed at high pHs (6.0-7.5) were rubber-like with high strain and stress at fracture. The results for these two studies were consistent with each other, showing that gels formed in the intermediate pH range (4.0-6.0) were weaker than gels formed in the high pH range (6.0-9.0).

Different microstructures of LG gels (heating 12% (w/v) LG at 90 °C for 60 min) formed at various pHs was examined by Langton and Hermansson (1992). White particulate gels were formed in the pH range of 4-6, and transparent fine-stranded gels were formed above or below the pH range. Images obtained from microscopy showed that the particulate gels had a very open structure and the particulate gels can be further divided into two categories: irregular network structures around pH 4.5 and regular network structures between pH 5 and 6. Between pH 5.5 to 6.0, the regular particulate

networks were composed of small particles. However, large particles were found in gels formed in the pH range of 5.0 to 5.5. The network of fine-stranded gels formed at pH 3.5 was regular and very dense with very small pores, short and thin strands and many strands were joined together in one junction. At pH 6.5-9.0, the network of fine-stranded gels was also regular, but composed of longer and more flexible fine strands.

Remarkable microstructural differences between LG gels formed in acid (3 and 5) and alkaline (7 and 8.6) pH were observed. Boye and others (1997) stated that under scanning electron microscopy (SEM) and transmission electron microscopy (TEM), compact spherical globules and their aggregates were the major components in gels formed at acid pHs (10% LG solution, heated at 90 °C for 30 min). The compact protein globules formed at acid pHs were loosely aggregated, and the void spaces in the gels were large. A very different type of gel structure was observed at gels formed at alkaline pHs. The proteins were more evenly distributed in the gel network and connected through narrow bridges. The gel network was more defined and organized with smaller void spaces. A similar cross-linking tendency of LG gels formed at pH 2 and 7 was reported by Takata and others (2000). At pH 2, LG molecules had a low tendency to cross-link and formed loosely tied networks. As pH increased to 7, cross-linking tendency of LG molecules also increased.

The gelation rate of LG gels increases as pH approaches 5.2. McSwiney and others (1994) stated that during heating of 10% LG solution (imidazole-HCl buffer containing 0.1 mol/dm^3 NaCl) at 75 °C, the increase in G' was faster at pH 7.0, than at pH 6.0, 8.0 and 9.0. No measurable changes in G' occurred until most of the protein

(80%) had formed covalently cross-lined aggregates, suggesting that soluble protein aggregates were formed prior to the formation of the gel networks.

Lefèvre and Subirade (2000) further investigated molecular differences in the formation of fine-stranded (formed at pH 2.8 and 7.8) and particulate (formed at pH 4.4 and 5.4) LG gels. They indicated that no matter the pH, the mechanism of the gelation for a given type of gel is basically the same. However, the gels of the same type formed under different pHs showed some spectral specificities and distinct molecular characteristics. In fine-stranded gels, LG molecules undergo extensive denaturation before aggregation, and the dissociation of dimers into monomers prior to aggregation is the characteristic of fine-stranded gels. Comparing with the particulate gels, LG remains in dimer form with little structural modification. Because the protein is more unfolded in the fine-stranded gels, intermolecular interactions and the hydrogen bonds between proteins are stronger in the fine-stranded gels. Two mechanisms for the formation of the two types of gels were illustrated. To form fine-stranded gels, LG dimers first dissociate to monomers followed by extensive denaturation of the protein. The dissociated and denatured monomers then interact with each other and finally form a three dimensional network. For the particulate gels, LG remains in dimer form with minor modification of the protein structure. The gelation process begins with the aggregation of dimers and also partial unfolding of the protein. The aggregation continues and finally the gel is formed.

1.10.3. Heating temperature and heating time

Heating rate affects the homogeneity of fine-stranded LG gels at pH 7.5 and 6.0 (Stading and Hermansson,1992). Heterogeneous LG gels were produced at the low

heating rate (0.017 °C/min, heated to 70 and 90 °C) and LG concentration. Gel structure formed at 12 °C/min heating rate had homogeneous evenly thick strands and both small and large pores were found in gel structure formed at 0.017 °C/min heating rate. Frequency dependence test indicated that gels formed at faster heating rate were less frequency-dependent than the gels formed at slower heating rate, suggesting gels formed at faster heating rate had more of the character of strong gels than gels formed at slower heating rate. As TEM images revealed, the inhomogeneity of LG gels was a result of microphase separation.

McSwiney and others (1994) studied the change of storage modulus (G') of bovine LG (10% in imidazole-HCl buffer containing 0.1 mol/dm^3 NaCl) under different heating temperatures. During heating at 70 °C (up to 60 min) no detectable change in G' was recorded. This result indicated that denaturation temperature of LG was higher than 70 °C. At 75 and 80 °C, G' increased as heating time increased.

1.10.4. Ionic strength and type of ions

Rheological and textural properties of LG gels are greatly affected by salt concentration and type of salt. Certain concentrations of salts are needed to produce self-supporting gels. Without adding salt, self-supporting gels were not formed even when 10% LG (pH 8.0) was heated at 90 °C for 30 min (Mulvihill and Kinsella,1988). However, in the presence of NaCl (25 mM) and $CaCl_2$ (5 to 100 mM), self-supporting gels were produced. LG gels with maximum compressive strength were formed with NaCl and $CaCl_2$ at concentrations of 200 and 10 mM, respectively. Further increasing NaCl and $CaCl_2$ concentration decreased the gel strength resulting in softer gels with lower water holding capacity.

Rheological properties of LG gels containing various concentrations of NaCl or $CaCl_2$ were examined by Foegeding and others (1992). LG gel produced with 20 mM $CaCl_2$ was more deformable and had higher gelation rate than LG gels produced with 100 mM NaCl (10% LG solution, heated at 80 °C for 30 min, pH 7.0). However after heating to 80 °C, a higher G' value was recorded on LG gel produced with NaCl.

Remondetto and others (2002) investigated the effects of iron concentration on the gelation of LG gels (8% LG preheated at 80 °C for 30 min at pH 7.0). They reported that the concentration of iron (10 to 40 mM) in the LG gels not only affected the water holding capacity, color and microstructure of the LG gels, but also the rheological property of the LG gels. LG gels with high water holding capacity may be produced at a low iron/protein ratio.

Recently, a new multistep calcium induced cold gelation of LG gels was developed by a Dutch research team (Veerman and others, 2003). LG solution (2%, w/w) was first preheated at pH 2 for 10 hr at 80 °C. The pH of the preheated LG solutions was adjusted to 7 or 8. Calcium was added in the final step of the cold gelation to induce the cold-set gels. This multistep calcium induced gelation process can produce LG gels at a much lower concentration. And their study suggested that this could be a new and more efficient method to modify the functional properties of whey proteins.

1.11. Summary of literature review

In today's food market, consumers demand more healthy and nutritious foods with reasonable prices. This trend in the food market has driven the food industry to develop food products which are high in protein and low in fat. Because of this

consumer demand, the processed meat industry has developed a variety of low fat meat products. One way to produce low fat meat products is by adding various non-meat binders and extenders (such as starches, soy proteins, whey proteins, and carrageenan). To increase the protein content in meat products, protein based binders and extenders are often used.

There are several protein based binders and extenders (such as soy proteins and milk proteins) available in today's food market. However, because of their limited functionalities, the processed meat industry is still searching for the perfect food ingredients to increase water binding capacity and textural properties in processed meat products.

Since the early 1980's, whey proteins have been used in a variety of processed meat products as a binder or extender to increase water binding capacity and textural properties. Currently in the U.S., up to 3.5% whey protein products are allowed to be used in meat products. However, because of the differences in functionalities, whey proteins are not used to their fullest potential. In 2002, only 60% of produced whey protein products were sold.

Preheated whey protein products have better water binding capacity and textural properties than unheated whey products. Preheated whey protein products can also be tailor-made (by adjusting preheating or gelling conditions) to fit the purpose for specific types of meat products. When producing different types of meat products, meat processors can take advantage of the high functional variability of preheated whey products to fit the needs for different types of meat products. So far, no comparisons of the functionalities of unheated and preheated whey proteins in processed meat products

have been studied. If preheated whey proteins proven to be more functional than unheated whey proteins, it will help the whey protein industry to develop more functional whey protein products for meat industry applications.

REFERENCES

Armstrong J, McKenzie HA, Sawyer WH. 1967. On the fractionation of β-lactoglobulin and α-lactalbumin. Biochem. Biophysics. Acta. 147: 60-72

Atughonu AG, Zayas JF, Herald TJ, Harbers LH. 1998. Thermo-rheology, quality characteristic, and microstructure of frankfurters prepared with selected plant and milk additives. J. Food Qual. 21: 223-228.

Aymard P, Durand D, Nicolai T. 1996a. A comparison of the structure of β-lactoglobulin aggregates formed at pH 7 and pH 2. Int. J. Polym. Anal. Charact. 2: 115-119.

Aymard P, Durand D, Nicolai T. 1996b. The effect of temperatures and ionic strength on the dimerisation of β-lactoglobulin. Int. J. Biol. Macromol. 19: 213-221.

Aymard P, Gimel, JC, Nicolai T, Durand D. 1996c. Experimental evidence for a two-step process in the aggregation of β-lactoglobulin at pH 7. J. Chim. Phsy. 93: 987-997.

Barbut S, Foegeding EA. 1993. Ca^{2+}-induced gelation of pre-heated whey protein isolate. J. Food Sci. 58: 867-871.

Barbut S, 1995. Effects of calcium level on the structure of preheated whey protein isolate gels. Can. Inst. Food Sci. Tech. 28:598-603.

Bauer R, Carrotta R, Rischel C, Øgendal L. 2000. Characterization and isolation of intermediates in β-lactoglobulin heat aggregation at high pH. Biophysical Journal. 79: 1030-1038.

Beuschel BC, Culbertson JD, Partridge JA, Smith DM. 1992a. Gelation and emulsification properties of partially insolubilized whey protein concentrates. J. Food Sci. 57 (3): 605-609, 634.

Beuschel BC, Partridge JA, Smith DM. 1992b. Insolubilized whey protein concentrate and/or chicken salt-soluble protein gel properties. J. Food Sci. 57 (4): 852-855.

Bounous G, Baptist G, Gold P. 1991. Whey proteins in cancer prevention. Cancer Letters. 57: 91.

Bowland EL, Foegeding EA. 1995. Effect of anions on thermally induced whey protein isolate gels. Food Hydrocolloids. 9: 47-56.

Boye JI, Ismail AA, Alli I. 1996. Effects of physicochemical factors on the secondary structure of β-lactoglobulin. J. Dairy Res. 63:97-109.

Boye JI, Ismail A, Harwalkar VR, Kalab M. 1997. Molecular and microstructural studies of thermal denaturation and gelation of β-lactoglobulin A and B. J Agric. Food Chem. 45: 1608-1618.

Bryant CM, McClements DJ. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. Tech. 9: 143-151.

Bryant CM, McClements DJ. 2000a. Optimizing preparation conditions for heat-induced whey protein solution to be used as cold-gelling ingredients. J. Food Sci. 65: 259-263.

Bryant CM, McClements DJ. 2000b. Influence of NaCl and $CaCl_2$ on cold-set gelation of heat-denatured whey protein. J. Food Sci. 65: 801-804.

Busti P, Scarpeci S, Gatti C, Delorenzi N. 2002. Use of fluorescence methods to monitor unfolding transitions in β-lactoglobulin. Food Res. Int. 35: 871-877.

Caessens PWJR, Gruppen SVH 1997. Method for the isolation of bovine β-lactoglobulin from a cheese whey protein fraction and physicochemical characterization of the purified product. Int. Dairy J. 7: 229-235.

Cairoli S, Iametti S, Bonomi F. 1994. Reversible and irreversible modifications of β-lactoglobulin upon exposure of heat. J. Protein Chem. 13(3): 347-354.

Chantrapornchai W, McClements DJ. 2002. Influence of NaCl on optical properties, large-strain rheology and water holding capacity of heat-induced whey protein isolate gels. Food Hydrocolloids. 16: 467-476.

Chen CM, Trout GR. 1991a. Sensory, instrumental texture profile and cooking properties of restructured beef steaks made with various binders. J. Food Sci. 56: 1457-1460.

Chen CM, Trout GR. 1991b. Color and its stability in restructured beef steaks during frozen storage: effects of various binders. J. Food Sci. 56: 1461-1464, 1475.

Colbert LB, Decker EA. 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Sci. 56: 1248-1250.

Croguennec T, Bouhallab S, Mollé D, O'Kennedy BT, Mehra, R. 2003. Stable monomeric intermediate with exposed Cys-119 is formed during heat denaturation of β-lactoglobulin. Biochem. Bio. Res. Commun. 301: 465-471.

De Wit JN. 1998. Nutritional and functional characteristics of whey proteins in food products. J. Dairy Sci. 81: 597-608.

Doi E. 1993. Gels and gelling of globular protein. Trends Food Sci. Tech. 4: 1-5.

Edwards PJB, Jameson GB, Palmano KP, Creamer LK. 2002. Heat-resistant structural features of bovine β-lactoglobulin A revealed b NMR H/D exchange observations. Int. Dairy J. 12: 331-344.

El-Magoli SB, Larola S, Hansen PMT. 1995. Ultrastructure of low-fat ground beef patties with added whey protein concentrate. Food Hydrocolloids. 9: 291-306.

El-Magoli SB, Larola S, Hansen PMT. 1996. Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. Meat Sci. 42: 179-193.

Ensor SA, Mandigo RW, Calkins CR, Quint LN. 1987. Comparative evaluation of whey protein concentrate, soy protein isolate and calcium-reduced nonfat dry milk as binders in an emulsion-type sausage. J. Food Sci. 1155-1158.

Ferry JD. 1948. Protein gels. Adv. Protein Chem. 4: 1-78.

Foegeding EA, Hamann DD. 1992. Physicochemical aspects of muscle tissue behavior. Ch. 8. *In physical chemistry of foods.* H.G. Schwartzgerg, and R.W. Hartel, (Eds), p 423-441. Marcel Dekker, Inc., NY.

Foegeding EA, Kuhn PR, Hardin CC. 1992. Specific divalent cation-induced changes during gelation of β-lactoglobulin. J. Agric. Food Chem. 40: 2092-2097.

Fogelholm D, 2003. Dairy products, meat and sports performance. Sports Medicine. 33: 615-631.

Galani D, Apenten RKO. 1999. Heat-induced denaturation and aggregation of β-lactoglobulin: kinetics of formation of hydrophobic and disulfide-linked aggregates. Int. J. Food Sci. Tech. 34: 467-476.

Gosal WS, Clark AH, Pudney PDA, Ross-Murphy SB. 2002. Novel amyloid fibrillar networks derived from globular protein: β-lactoglobulin. Langmuir. 18: 7174-7181.

Hale AB, Carpenter CE, Walsh MK. 2002. Instrumental and consumer evaluation of beef patties extended with extrusion-textured whey proteins. J. Food Sci. 67: 1267-1270.

Hambling SG, McAlpine AS, Sawyer L. 1992. β-lactoglobulin. In *advance dairy chemistry*: volume 1, proteins. Elsevier Science Publishers LTD. Barking, England.

Haque ZU, Sharma M. 1997. Thermal gelation of β-lactoglobulin AB purified from cheddar whey. 1. Effect of pH on association as observed by dynamic light scattering. J. Agric. Food Chem. 45: 2985-2963.

Harwalkar VR, Kalab M. 1985. Microstructure of isoelectric precipitates from β-Lactoglobulin solutions heated at various pH values. Milchwiss. 40: 665-668.

Hoffmann MAM, Roefs SPFM, Verheul M, Van Mil PJM, De Kruif KG. 1996. Aggregation of β-Lactoglobulin studied by *in situ* light scattering. J. Dairy Res. 63: 441-449.

Hoffmann MAM, van Mil JJM. 1997. Heat-induced aggregation of β-lactoglobulin: role of the free thiol group and disulfide bonds. J. Agric. Food Chem. 45: 2942-2948.

Hoffmann MAM, Sala G, Olieman C, de Kruif KG. 1997. Molecular mass distributions of heat-induced β-lactoglobulin aggregates. J. Agric. Food Chem. 45: 2949-2957.

Hoffmann MAM, van Mil JJM. 1999. Heat-induced aggregation of β-lactoglobulin as function of pH. J. Agric. Food Chem. 47: 1898-1905.

Holt C, Mcphail D, Nevison I, Nylander T, Otte J, Ipsen RH, Baur R, Ogendal L, Olieman K, de Druif KG, Leonil J, Molle D, Henry G, Maubois JL, Perez MD, Puyol P, Calvo M, Bur SM, Kontopidis G, McNae I, Sawyer L, Ragona L, Zetta L, Molinari H, Klarenbeek B, Jonkman M, Moulin J, Chatterton D. 1999a. Apparent chemical composition of nine commercial or semi-commercial whey protein concentrates, isolates and fractions. Int. J. Food Sci. Tech. 34: 534-556.

Holt C, Mcphail D, Nevison I, Nylander T, Otte J, Ipsen RH, Baur R, Ogendal L, Olieman K, de Druif KG, Leonil J, Molle D, Henry G, Maubois JL, Perez MD, Puyol P, Calvo M, Bur SM, Kontopidis G, McNae I, Sawyer L, Ragona L, Zetta L, Molinari H, Klarenbeek B, Jonkman M, Moulin J, Chatterton D. 1999b. Some physico-chemical properties of nine commercial or semi-commercial whey protein concentrates, isolates and fractions. Int. J. Food Sci. Tech. 34: 587-601.

Hongsprabhas P, and Barbut S. 1996. Ca^{2+} - induced gelation of whey protein isolate: effects of pre-heating. Food Res. Int. 29: 135-139.

Hongsprabhas P, Barbut S. 1997a. Structure-forming Processes in Ca^{2+}-induced whey protein isolate cold gelation. Int. Dairy J. 7: 827-834.

Hongsprabhas P, and Barbut S. 1997. Protein and salt effects on Ca^{2+} - induced cold gelation of whey protein isolate. J. Food Sci. 62: 382-385

Hongsprabhas P, Barbut S. 1998. Structure-forming process in Ca^{2+} - induced whey protein isolate cold gelation. Int. Dairy J. 7: 827-834.

Hongsprabhas P, Barbut S. 1999a. Effect of preheated whey protein level and salt on texture development of poultry meat batters. Food Res. Int. 32: 145-149.

Hongsprabhas P, Barbut S. 1999b. Use of cold-set whey protein gelation to improve poultry meat batters. Poultry Sci. 78: 1074-1078.

Hood RA. 1985. High-protein whey powder-marketing. J. Soc. Dairy Tech. 38 (4): 102-103.

Hsieh YL, Regenstein JM, Rao A. 1993. Gel point of whey and egg proteins using dynamic rheological data. J. Food Sci. 58(1): 116-119.

Huffman LM. 1996. Processing whey protein for use as a food ingredient. Food Tech. 60: 49-52.

Hughes E, Mullen AM, Troy DJ. 1998. Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. Meat Sci. 48: 169-180.

Hung SC, Zayas JF. 1992. Functionality of milk proteins and corn germ protein flour in comminuted meat product. J. Food Qual. 15: 139-152.

Iametti S, Cairoli S, De Gregori B, Bonomi F. 1995. Modification of high-order structures upon heating of β-lactoglobulin: Dependence on the protein concentration. J. Agric. Food Chem. 43:53-58.

Iametti S, De Grepori B, Vecchio G, Bonomi F. 1996. Modifications occur at different structural levels during the heat denaturation of β-lactoglobulin. Eur. J. Biochem. 237: 106-112.

Ikeda S. 2003. Atomic force microscopy and Raman scattering spectroscopy studies on heat-induced fibrous aggregates of β-lactoglobulin. Spectroscopy. 17: 195-202.

Imafidon GI, Farkye NY, Spanier AM. 1997. Isolation, purification, and alteration of some functional groups of major milk proteins: A review. Crit. Rev. Food Sci. Nutr. 37(7): 663-689.

Jeyarajah S, Allen JC. 1994. Calcium binding and salt-induced structural changes of native and preheated β-lactoglobulin. J. Agric. Food Chem. 42: 80-85.

Jost R. 1993. Functional characteristics of dairy proteins. Trends Food Sci. Tech. 4: 283-288.

Ju Z, Kilara A. 1998a. Effects of preheating on properties of aggregates and cold-set gels of whey protein isolate. J. Agric. Food Chem. 46: 3604-3608.

Ju Z, Kilara A. 1998b. Textural properties of cold-set gels induced from heat-denatured whey protein isolates. J. Food Sci. 63: 288-292.

Kavanagh GM, Clark AH. and Ross-Murphy, S.B. 2000. Heat-induced gelation of globular proteins: part 3. Molecular studies on low pH β-lactoglobulin gels. Int. J. Biol. Macromol. 28: 41-50.

Kuwata, H. 1998. Direct evidence of the generation in human stomach of anti-mocrobial peptide domain (lactoferricin) from ingested lactoferrin. Biochem. Biophys Acta. 1429: 129-141.

Keaton J. 1999. Whey protein and lactose products in processed meats. Applications monograph, U.S. Dairy Export Council.

Kitabatake N, Fujita Y, Kinekawa Y. 1996. Viscous sol and gel formation from process whey protein below 25 °C. J. Food Sci. 61: 500-503.

Langton M, Hermansson A. 1992. Fine-stranded and particulate gels of β-Lactoglobulin and whey protein at varying pH. Food Hydrocolloids. 5(6): 523-539.

Langley KR, Green ML. 1989. Compression and impact strength of gels, prepared from fractionated whey proteins, in relation to composition and microstructure. J. Dairy Res. 56: 275-284.

Le Bon C, Nicolai, T, Durand D. 1999. Growth and structure of aggregates of heat-denatured β-Lactoglobulin. Int. J. Food Sci. Tech. 34: 451-465.

Lee A, Cannon Y, Huffman DL. 1980. Whey protein concentrates in a processed meat loaf. J. Food Sci. 45: 1278-1279, 1304.

Lefèvre T, Subirade M. 1999. Structural and interaction properties of β-lactoglobulin as studied by FTIR spectroscopy. Int. J. of Food Sci. Tech. 34: 419-428.

Lefévre T, Subirade M. 2000. Molecular differences in the formation and structure of fine-stranded and particulate β-lactoglobulin gels. Biopolymers. 54: 578-586.

Li H, Hardin CC, Foegeding EA. 1994. NMR studies of thermal denaturation and cation-mediated aggregation of β-lactoglobulin. J. Agric. Food Chem. 42: 2411-2420.

Lucassen PLBJ, Assendelft WJJ, Gubbels JW, van Eijk JT, Douwes AC. 2000. Infantile colic: crying time reduction with a whey hydrolysates: a double-blind, randomized, placebo-controlled trial. Pediatrics. 106: 1349-1354.

MacLeod A, Fedio WM, Ozimek L. 1995. Aggregation of β-Lactoglobulin as a function of pH and temperature. Milchwiss. 50: 666-669.

Manderson GA, Hardman MJ, Creamer LK. 1998. Effect of heat treatment on the conformation and aggregation of β-Lactoglobulin A, B and C. J. Agric. Food Chem. 46: 5052-5061.

Manderson GA, Creamer LK, Hardman MJ. 1999. Effect of heat treatment on the circular dichroism spectra of bovine β-lactoglobulin A, B and C. J. Agric. Food Chem. 47: 4557-4567.

Marangoni AC, Barbut S, McGauley SE, Marcone M, Narine SS. 2000. On the structure of particulate gels: the case of salt-induced cold gelation of heat-denatured whey protein isolate. Food Hydrocolloids. 14: 61-74.

Marriot NG, Wang H, Claus JR, Graham PP. 1998. A research note: evaluation of restructured low-fat ham containing whey. J. Musc. Foods. 9: 201-207.

Matsuura JE, Manning MC. 1994. Heat-induced gel formation of β-lactoglobulin: a study on the secondary and tertiary structure as followed by circular dichroism spectroscopy. J. Agric. Food Chem. 42: 1650-1656.

McClements DJ, Keogh MK. 1995. Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. J. Sci. Food Agric. 69: 7-17.

McKenzie HA, Sawyer WH. 1967. Effect of pH on β-lactoglobulins. Nature. 214: 1101-1104.

McPhail D, Holt C. 1999. Effect of anions on the denaturation and aggregation of β-Lactoglobulin as measured by differential microcalorimetry. Int. J. Food Sci. Tech. 34: 477-481.

McSwiney M, Singh H, Campanella OH. 1994. Thermal aggregation and gelation of bovine β-lactoglobulin. Food Hydrocolloids. 8(5): 441-453.

Mleko S, Foegeding EA. 1999. Formation of whey protein polymers: effects of a two-step heating process on rheological properties. J. Text. Stud. 30: 137-149.

Morr CV, Foegeding EA. 1990. Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. Food Tech. 54: 100-112.

Mulvihill DM, Grufferty MB. 1997. Production of whey-protein-enriched products. *In Food Proteins and Lipids*. Damodaran, (Ed), p. 77-93. Plenum Press, New York.

Mulvihill DM, Kinsella JE. 1987. Gelation characteristics of whey proteins and β-lactoglobulin. Food Tech. 41(9): 102-111.

Muvihill DM Kinsella JE. 1988. Gelation of β-lactoglobulin: effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. J. Food Sci. 53(1): 231-236.

Nielsen BT, Singh H, Latham JM. 1996. Aggregation of bovine β-lactoglobulin A and B on heating at 75°C. Int. Dairy J. 6: 519-527.

Palazolo G, Rodriguez F, Farruggia B, Pico G, Delorenzi N. 2000. Heat treatment of β-lactoglobulin: structural changes studied by partitioning and fluorescence. J. Agric. Food Chem. 48: 3817-3822.

Palmer AH. 1934. The preparation of a crystalline globulin from the albumin fraction of cow's milk. J. Bio. Chem.104: 359-372

Pawar VD, Khan FA, Agarkar BS. 2002. Effect of fat/whey protein concentrate levels and cooking methods on textural characteristics of chevon patties. J. Food Sci. Technol. 39: 429-431.

Photchanachai S, Kitabatake N. 2001. Heating of β-lactoglobulin A solution in a closed system at high temperatures. J. Food Sci. 66(5): 647-652.

Prabakaran S, Damodaran S. 1997. Thermal unfolding of β-lactoglobulin: characterization of initial unfolding events responsible for heat-induced aggregation. J. Agric. Food Chem. 45: 4303-4308.

Qi X, Holt C, Mcnulty D, Clarke D, Brownlow S, Jones G. 1997. Effect of temperature on the secondary structure of β-lactoglobulin at pH 6.7, as determined by CD and IR spectroscopy: a test of the molten globule hypothesis. Biochem. J. 324: 341-346.

Relkin P. 1998. Reversibility of heat-induced conformational changes and surface exposed hydrophobic clusters of β-lactoglobulin: their role in heat-induced sol-gel state transition. Int. J. Biol. Macromol. 22: 59-66.

Rhee KS. 1978. Minimization of further lipid peroxidation in the distillation 2-thiobarbutiric test of fish and meat. J. Food Sci. 43: 1776-1778.

Remondetto CE, Paquin P. and Subirade, M. 2002. Cold gelation of β-lactoglobulin in the presence of iron. J. Food Sci. 67: 586-595.

Roff CF, Foegeding EA. 1996. Dicationic-induced gelation of pre-denatured whey protein isolate. Food Hydrocolloids. 10: 193-198.

Sammel LM, Claus JR. 2003. Whey protein concentrates effects on pink color development in a cooked ground turkey breast model system. Meat Sci. 65: 1293-1299.

Sawyer L, Brownlow S, Polikarpov I, Wu S. 1998. β-Lactoglobulin: Structural studies, biological clues. Int. Dairy J. 8: 65-72.

Sawyer L, Kontopidis G, Wu SY. 1999. β-lactoglobulin-a three dimensional perspective. Int. J. Food Sci. Tech. 34: 409-418.

Schokker EP, Singh H, Pinder DN, Norris GE, Creamer LK. 1999. Characterization of intermediates formed during heat-induced aggregation of β-lactoglobulin AB at neutral pH. Int. Dairy J. 9: 791-800.

Schokker EP, Singh H, Pinder DN, Creamer LK. 2000. Heat-induced aggregation of β-lactoglobulin AB at pH 2.5 as influenced by ionic strength and protein concentration. Int. Dairy J. 10: 233-240.

Sharma M, Haque ZU, Wilson WW. 1996. Association tendency of β-Lactoglobulin AB purified by gel permeation chromatography as determined by dynamic light scattering under quiescent conditions. Food Hydrocolloids. 10(3): 323-328.

Sharpe SJ, Gamble GD, Sharpe DN. 1994. Cholesterol-lowering and blood pressure effect of immune milk. Am J Clinical Nutrition. 59: 929-934.

Singh H, Ye A, Havea P. 2000. Milk protein interactions and functionality of dairy ingredients. Aus. J. Dairy Tech.. 55: 32-39.

Simons JFA, Kosters HA, Visschers RW, de Jongh HHJ. 2002. Role of calcium as trigger in thermal β-lactoglobulin aggregation. Arch. of Biochem. Biophys. 406: 143-152.

Slesinski AJ, Claus JR, Anderson-Cook CM, Eigel WE, Graham PP, Lenz GE, Noble RB. 2000a. Ability of various dairy proteins to reduce pink color development in cooked ground turkey breast. J. Food Sci. 65: 417-420.

Slesinski AJ, Claus JR, Anderson-Coo, CM, Eigel WE, Graham PP, Lenz, GE, Noble RB. 2000b. Response surface methodology for reduction of pinking in cooked turkey breast mince by various dairy protein combinations. J. Food Sci. 65: 421-427.

Stading M, Hermansson A. 1990. Viscoelastic behaviour of β-lactoglobulin gel structures. Food Hydrocolloids. 4(2): 121-135.

Stading M, Hermansson A. 1991. Large deformation properties of β-lactoglobulin gels structures. Food Hydrocolloids. 5(4): 339-352.

Stading M, Langton M, Hermansson A. 1992. Inhomogeneous fine-stranded β-lactoglobulin gels. Food Hydrocolloids. 6(5): 455-470.

Steventon AJ, Gladden LF, Fryer PJ. 1991. A percolation analysis of the concentration dependence of the gelation of whey protein concentrates. J. Text. Stud. 22: 201-218.

Surroca Y, Haverkamp J, Heck AJR. 2002. Towards the understanding of molecular mechanisms in the early stages of heat-induced aggregation of β-lactoglobulin AB. J. Chroma. 970: 275-285.

Takata S, Norisuye T, Tanaka N, Shibayama M. 2000. Heat-induced gelation of β-lactoglobulin. 1. Time-resolved dynamic light scattering. Macromolecules. 33: 5470-5475.

Taylor BJ, Walsh MK. 2002. Development and sensory analysis of a textured whey protein meatless patty. J. Food Sci. 67: 1555-1558.

Thomsen B, Pedersen M. 1993. Benefits of using whey protein concentrates in ham products. Eur. Food Drink Rev. 1993: 59-61.

Tomb MP. 1974. Gelation of globular proteins. Farad. Discus. Chem. 57: 158-202.

USDA. 1995. Policy Memo 123.

USDA. 1999. Code of Federal Regulation. Title 9.

USDA. 2003. National agricultural statistics service: Dairy products annual summary, USDA 2002.

Van Hoogstraten JJ. 1987. The marketing of whey products: A view from Europe "J si deux amours." In "Bulletin 212, Trends in Whey Utilization." p. 17. Int. Dairy Fed. Brussel, Belgium.

Veerman C, Baptist H, Sagis LMC, van der Linden E. 2003. A new multistep Ca^{2+} - induced cold gelation process for β-lactoglobulin. J. Agric. Food Chem. 51: 3880-3885.

Verheul M, Roefs SPFM, de Kruif KG. 1998. Kinetics of heat-induced aggregation of β-Lactoglobulin. J. Agric. Food Chem. 46: 896-903.

Vittayanont V, Steffe JF, Flegler SL, Smith DM. 2002. Gelling properties of heat-denatured β-lactoglobulin aggregates in a high-salt buffer. J. Agric. Food Chem. 50: 2987-2992.

Watanabe K, Klostermeyer H. 1976. Heat-induced changes in sulphydryl and disulphide levels of β-lactoglobulin A and the formation of polymers. J. Dairy Res. 43: 411-418.

Weber K, Osborn M. 1969. The reliability of molecular weight determinations by codicil sulfate polyacrylamide gel electrophoresis. J. Bio. Chem. 244: 4406-4412.

Whey Protein Institute. 2004. Benefits of whey proteins. Available from: www.wheyoflife.org. Accessed June 1, 2004.

Xiong YL, Dawson KA, Wan L. 1993. Thermal aggregation of β-lactoglobulin: Effect of pH, ionic environment, and thiol reagent. J. Dairy Sci. 76: 70-77.

Yetim H, Müller WD, Eber M. 2001. Using fluid whey in comminuted meat products: effects on technological, chemical and sensory properties of frankfurter-type sausages. Food Res. Int. 34: 97-101.

Young PR. 1985. High-protein whey powders: production. J. Soc. Dairy Technol. 38: 100-103.

CHAPTER 2

MATERIALS AND METHODS

This chapter provides detailed descriptions, procedures, and processes utilized during this research project. Study I focused on the heat denaturation of β-lactoglobulin aggregates. Study II determined the optimum preheating conditions for whey protein isolates. Study III verified the efficacy of preheated whey protein isolates in low fat beef frankfurters.

Study I: Heat denatured β-lactoglobulin aggregates: a study of the effect of preheating and aggregate size on microstructural and rheological properties of β-lactoglobulin gels

I. Experimental design and statistical analysis

A preliminary study was conducted to determine the ranges for heating time, heating temperature and the concentration of the LG solution. Based on the preliminary study, all the LG solutions were initially heated at a 7% (w/v) concentration using a water bath (PolyScience, model 9510, Niles, IL). Heated LG solutions were then diluted to 2% (using a 0.07 M sodium phosphate buffer containing 0.42 M NaCl, pH 6.5; final NaCl concentration: 0.3 M) and subjected to a second heating step as described later in small amplitude dynamic oscillatory test. During the second heating step, a small amplitude dynamic oscillatory test was performed to collect various rheological data (storage modulus, loss modulus, and gel point). The influence of the initial heating step (pH, heating time and heating temperature) on the rheology output during the second heating

step was determined using response surface methodology. A three factor, five level rotatable central composite design was applied to choose the conditions for the initial heating step. The central composite design contained fifteen combinations with one treatment combination (treatment 15) replicated six times. The three independent variables were assigned as time (X_1), pH (X_2) and temperature (X_3). Based on the preliminary study, the values for pH, heating time and heating temperature were chosen and shown in Table 2.1.

Response surface methodology was used to determine the effect of the three independent variables (pH, heating time and heating temperature) on storage modulus, loss modulus and gel point of LG gels. Second order polynomial regression models were generated using storage modulus, loss modulus and gel point as responses. The second order polynomial regression models were described as:

$$Y = B_0 + \sum_{i=1}^{k} B_I X_I + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i<j} B_{ij} X_i X_j$$

Where: B_0 was constant of the model

B_I (linear), B_{ii} (quadratic) ,B_{ij} (interaction) were parameter estimates of the model

X_i and X_j were the independent variables in the model

k was the number of factors

Y was the response of the model

Significance of total response surface regression equations was determined at a predetermined level ($P<0.05$), and R^2 value of the models was calculated. Response surface graphs were generated using all parameter estimates. Statistical analysis was done using SAS software system (SAS version 8.2, SAS Institute Inc., Cary, NC). Correlation between HDLG aggregate size and storage modulus and gel point of LG gels was analyzed using SAS Proc Corr procedure. Significance level was set at predetermined level of 5%.

Table 2.1. Initial preheating conditions for 7% (w/v) β-lactoglobulin solution.

	Variable levels		
Treatment	**Time (min)**	**PH**	**Temperature (°C)**
1	20.15	6.4	74.1
2	20.15	8.0	85.9
3	49.85	6.4	85.9
4	49.85	8.0	74.1
5	20.15	6.4	85.9
6	20.15	8.0	74.1
7	49.85	6.4	74.1
8	49.85	8.0	85.9
9	10.00	7.2	80.0
10	60.00	7.2	80.0
11	35.00	6.4	80.0
12	35.00	8.0	80.0
13	35.00	7.2	70.0
14	35.00	7.2	90.0
15	35.00	7.2	80.0

II. β-lactoglobulin (LG) purification

β-lactoglobulin was purified from fresh raw (unpasteurized) bovine milk according to the procedures by Armstrong and others (1967). Raw bovine milk was collected from Michigan State University Dairy farm. Ammonium sulfate (315.8 g/L) was added to the raw milk within 35-45 min period with constant stirring. After adding the ammonium sulfate, the solution was stirred for 2 h at room temperature (25 °C). The solution was then filtered through Whatman No.1 filters. The pH of the filtrate was adjusted to 3.5 using 1 M HCl and stirred for 1 hr at 25 °C. After the mixing, the solution was centrifuged at 13,200 $\times g$ for 40 min at 16 °C (Sorvall RC 5B Plus centrifuge, DuPont, Newtown, CT). The wet pellet was discarded and the pH of the supernatant adjusted to 6.0 with 1 M NH_4OH, and ammonium sulfate (243.2 g/L) was added to the solution. The solution was mixed for 2 hr at 25 °C and then centrifuged at 13,200 $\times g$ for 30 min. After centrifugation the wet pellet was collected and then centrifuged again at 34,800 $\times g$ for 20 min at 25 °C. After the final centrifugation, the pellet was dissolved in 0.12 M sodium acetate buffer (pH 5.2) and dialyzed against three changes (at least 100 times volume of the protein solution) of the same buffer for 24 hr. After the dialysis, the solution was centrifuged at 34,800 $\times g$ for 30 min. The pellet was discarded and the supernatant was dialyzed against three changes of deionized water for 24 hr. The purity of the purified LG solution was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified LG solution was then lyophilized using a Lyph-Lock Freeze Dry system (Model 77530, Labconco Co., Kansas City, MO). The lyophilized LG powder was stored at 4°C for future use.

III. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to check the purity of purified LG. A mini-Protean II Dual Slab Cell (Bio Rad Laboratories, Hercules, CA) was used to run the electrophoresis. A Tris (hydroxymethyl) aminomethane glycine electrode buffer (pH 8.3, and 0.1% SDS) was used to run the SDS-PAGE, and the acrylamide concentrations of stacking and resolving gels were 4% and 14%, respectively.

Preparation of LG sample

β-lactoglobulin solution was diluted to 4 mg/mL with sample buffer (0.0625M Tris pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.2% bromophenol blue). The diluted sample was then mixed using a vortex mixer at speed setting 3 for 30 second and then heated in boiling water for 5 min.

Running condition for SDS-PAGE

Each protein sample (10 μL) was loaded into a sample well and the gels were run at 90 mA current and 200 constant voltage for about 50 min until bromophenol blue tracing dye almost reached bottom of gels. Gels were stained for 20 min with Coomassie Brilliant Blue R250 solution (0.25% in 9:45:45 v/v/v of acetic acid : methanol : water) and de-stained overnight in acetic acid-methanol-water (6:4:7,v/v/v) solution. Standard molecular weight markers (Bio-Rad Laboratories, Hercules, CA) were used to determine unknown protein molecular weight by comparing their relative mobilities under the same electrophoretic conditions (Weber and Osborn, 1969). Purity of the purified LG was also compared with bovine milk LG purchased from Sigma Chemical Co. (St. Louis, MO, L0310 lot#114H7055).

IV. Determination of protein concentration

Purified LG powder (0.5 g) was dissolved in 5 ml deionized water. The pH of the LG solution was adjusted to the desired pH using either 0.1 M NaOH or 0.1 M NH_4OH. After the pH adjustment, the solution was stirred at 25 °C for 20 min and then filtered through a 0.2μm filter (Millipore, Bedford, MA). The concentration of the filtered LG solution was determined by spectrometric absorption (Varian Cary III double beam Spectrophotometer, Varian Ltd., Walnut Creek, CA) using an extinction coefficient ($E^{1\%}$) of 9.55 at 278 nm (Foegeding et al., 1992). After the protein concentration measurement, concentration of LG solution was adjusted to 7% using deionized water.

V. Small amplitude dynamic oscillatory testing for second heating step

Dynamic oscillatory tests were performed using a controlled stress rheometer (RS100, Haake, Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plate. After the initial heating step, the samples were diluted to 2% (w/v) (using a 0.07 M sodium phosphate buffer containing 0.42 M NaCl, pH 6.5) and loaded between the plate and base with a gap of 1.0 mm. The edge of the gap was covered by a few drops of corn oil (Mazola, Best Food, CPC international, Inc., Englewood Cilffs, NJ) to prevent evaporation. Temperature of the plate and base was controlled by a circulating water bath programmed as the temperature profile described below. The samples were held at 25 °C for 5 min and then heated from 25 to 71 °C at a rate of 1 °C/min. After reaching 71 °C, the samples were held for 30 min then cooled down to 25 °C within 10 min. Storage modulus (G') and loss modulus (G") were recorded continuously at a fixed frequency (0.464 Hz) using stresses (producing strains from 0.1-0.3%) within the range

of linear viscoelastic behavior based on stress sweeps performed at 71 °C and after cooling at 25 °C. Gel point temperature of LG was defined as the cross over point corresponding to temperature obtained by extrapolating the baseline storage modulus and the first 5 storage modulus data points that had values at least 5 times higher than the baseline storage modulus value. (Foegeding and others, 1992, Hsieh and others, 1993, Steventon and others, 1991).

VI. Scanning electron microscopy (SEM) of LG gels

After the initial heating step, the preheated LG solutions were diluted to 2% (w/v) (using a 0.07 M sodium phosphate buffer containing 0.42 M NaCl, pH 6.5). To prepare protein gels, 2.0 ml of diluted protein solution was transferred into a glass tube (12 mm × 75 mm) and sealed with Teflon™ tape. The sealed glass tubes were then heated in a programmed water bath (PolyScience, model 9510, Niles, IL) using the same temperature profile used in the small amplitude dynamic oscillatory test. Protein gels were cut into small pieces (1×2×2 mm). Small pieces of protein gels were prefixed for 3 hr in 2.0% glutaldehyde solution buffered with 0.1 M sodium phosphate pH 7.0. After the prefixation, the gels were postfixed at 4 °C overnight in 0.1% osmium tetraoxide solution. Fixed gels were then rinsed with 0.1 M sodium phosphate buffer (pH 7.0), and dehydrated in a graded series of ethanol (25, 50, 75 and 95%) for 20 min each followed by three 20 min changes in 100% ethanol. Dehydrated gels were dried using a carbon dioxide critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) and then coated with a 25-30 nm gold layer in an ion-sputter coater (Emscope Laboratories Ltd., Ashford, Kent, UK). Microstructures of prepared protein gels were examined with a

scanning microscope (JEOL, Model JSM-6400V, version 96-2, Tokyo, Japan) at a 15 mm working distance using an accelerating voltage of 12 KV at a magnification of 5000×. Gels were prepared in duplicate and three pieces from each gel were examined.

VII. Scanning electron microscopy of preheated LG aggregates (poly-L-lysine method)

Cover slips were coated with poly-L-lysine (Sigma, P 1399) for 5 min and then rinsed with distilled water. Preheated LG aggregates were fixed with an equal volume of 4% glutaldehyde solution (buffered with 0.1 M sodium phosphate, pH 7.0) at 4 °C for 1 hr. After the fixation, one drop of the suspension was placed on the coated cover slip and allowed to stand at 25 °C for 5 min. The cover slip was then washed with distilled water followed by dehydration, critical drying and coating as previously described except that a 15 nm layer of gold was coated. Preheated LG aggregates were examined at an 8 mm working distance using an accelerating voltage of 10 KV and 20,000× magnification. All samples were prepared in duplicate and three cover slips from each preparation were examined. Aggregate size was determined using analySIS program by measuring the inside area of circled aggregates (Soft Imaging System, GMBH, Muenster, Germany). All aggregates observed under the microscope were measured. The size distribution of the aggregates was calculated as percentage under each size category: Aggregate 1: $\leq$ 0.075 μm^2, aggregate 2: between 0.075 and 0.15 μm^2, aggregate 3: between 0.15 and 0.3 μm^2, aggregate 4: 0.3 and 0.5 μm^2, aggregates 5: $\geq$ 0.5 μm^2.

Study II: Selecting Preheating Conditions for Whey Protein Isolates

I. Experimental design and statistical analysis

To evaluate the use of preheated WPI for application in emulsified meat products, a four factor (WPI preheating condition), two level (WPI %) complete randomized design was used in this study. The four factors were assigned as: 1. WPI without preheating, 2. WPI preheated at 95 °C for 10 min, 3. WPI preheated at 95 °C for 40 min, and 4. WPI preheated at 85 °C, 80 min. Two WPI levels were set at 3.5 and 7.0%.

All experiments were replicated three times. To analyze the impact of WPI preheating condition and concentration on the emulsion stability of WPI and meat emulsions, emulsion stability of WPI and beef meat emulsions was measured. Differences in emulsion stability were statistically analyzed using two-way analysis of variance (ANOVA). Significant level of difference was set at a predetermined level (Tukey's adjusted p-value and $p<0.05$). Interactions between experiment factor and whey protein level were checked and significant difference was set at a predetermined 5% level. SAS LS means procedure was used to determine the means for proximate composition, solubility and pH of WPIs. Means were compared to determine significant difference using Tukey test with the mean square error at 5% level of probability (SAS version 8.2, SAS Institute Inc., Cary, NC).

II. Whey protein isolate (WPI) and ground beef

Two bags of 25 kg Alacen 895 whey protein isolate (WPI) was purchased from New Zealand Milk Products (Lot# 047U45283431314, Lemoyne, PA) and stored at 4 °C

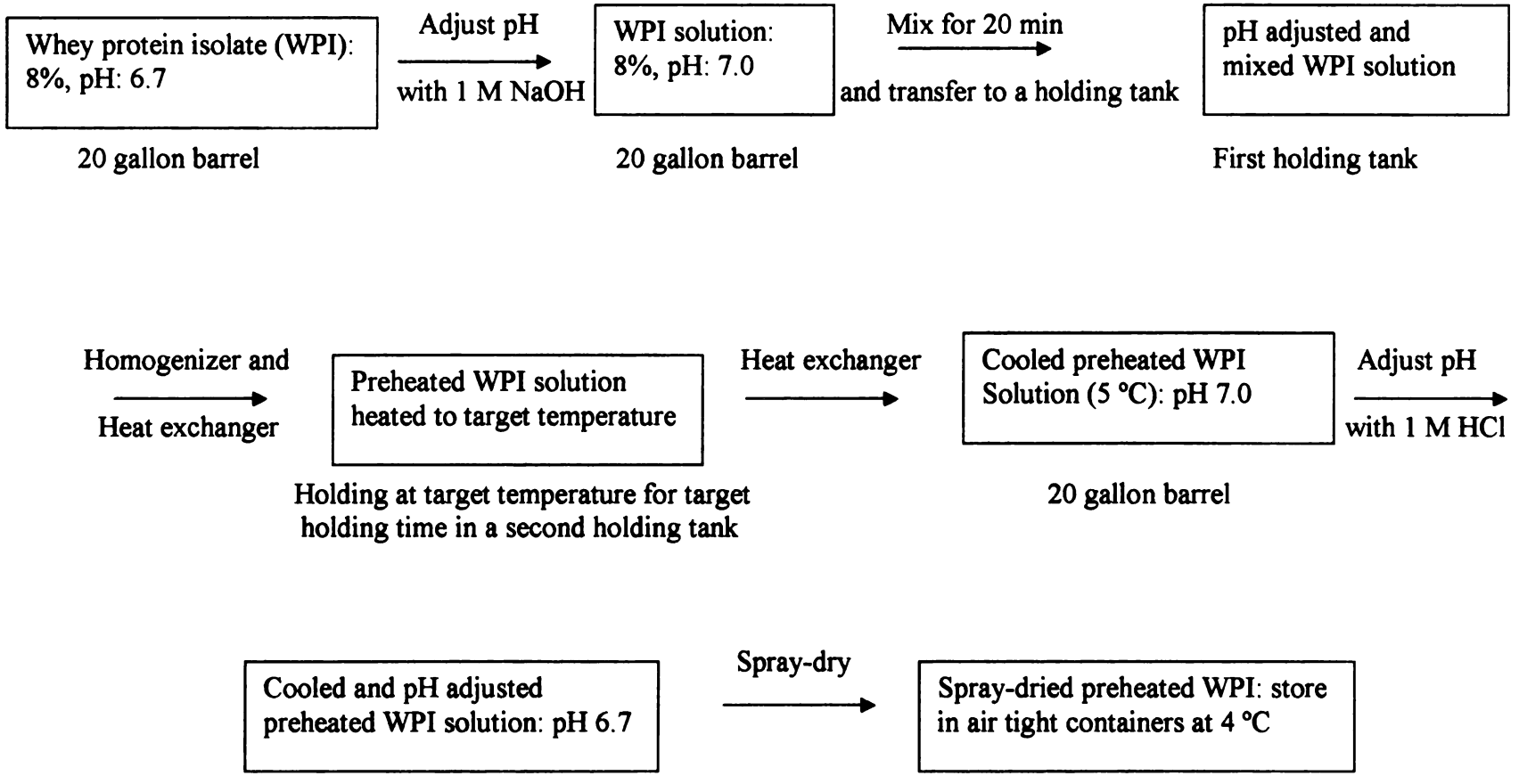
for two weeks before use. Alacen 895 whey protein isolates are undenatured, soluble whey proteins manufactured by ion exchange and ultrafiltration. Fresh 80/20 ground beef was purchased from a local meat company (Popoff Quality Food Service, Lansing, MI) and stored at 4 °C till use.

III. Manufacture of spray-dried preheated whey protein isolate

Spray-dried preheated WPI was manufactured as shown in Figure 2.1. Thirty liters of 8% (w/w) WPI solutions were prepared and mixed for 20 min using a hand agitator. Sodium hydroxide (1M) was used to adjust pH of the prepared WPI solutions to 7 before heating. The prepared WPI solutions were homogenized using a homogenizer (Superhomo homogenizer series 200, Cherry-Burrell corporation, Chicago, IL) and heated using a thin plate heat-exchanger (APV Crepaco Inc, Tonawanda, NY) to reach the target temperatures (85 or 95 °C). The heated WPI solutions were held in a 20 gallon holding tank for the target holding times (10, 40 or 80 min). During the holding times, steam was passed through the jacket of the holding tank to maintain the target temperatures (± 1.5 °C) and the WPI solutions were agitated constantly using a propeller stirrer. After the WPI solutions reached the target holding times, the preheated WPI solutions were cooled down to 5 °C by passing the preheated WPI solution through the heat-exchanger.

To spray-dry the preheated WPI solutions, pH of the solution was adjusted back to the original pH (6.7) using 1M HCl. The spray-drying process was carried out in the Michigan State University dairy plant using a Marriott Walker Corporation pilot plant spray dryer. The inlet and outlet temperatures of the spray-dryer were control at 135.0

Figure 2.1. Manufacture procedures for spray-dried preheated whey protein isolate



and 79.4 °C, respectively. After the spray-drying, the spray-dried preheated WPI were kept in air-tight jars and stored at 4 °C until use.

IV. Manufacturing of WPI and beef meat emulsions

High added fat WPI emulsions

Formulas for high added fat WPI emulsions are listed in Table 4.2. Applicable amounts of WPI and beef fat were mixed at 1200 rpm for 30 sec using a Stephan UMC 5 electronic vertical mixer (Stephan Machinery Corporation, Columbus, OH). Water and salt were then added and vacuum (35 mmHg) was applied. The mixture was mixed at 2100 rpm for 4.5 min. To increase homogeneity of the emulsions, the high added fat emulsions were chopped to a higher temperature around 30 ± 2 °C to melt the fat. Total mixing time was 5 min.

High added fat or high added water beef meat emulsions

Formulas for high added fat or high added water beef meat emulsions are listed in Table 4.3 and 4.4. Applicable amounts of beef, salt, and one half of water-ice were mixed at 1200 rpm for 30 sec using a Stephan mixer. The speed of the mixer was increased to 2100 rpm and the mixture was mixed for 1 more min to extract proteins. Phosphate, beef fat and the rest of the water-ice were added and vacuum (35 mmHg) was applied. After the vacuum was applied, the mixture was mixed for 3.5 more min. Total chopping time was 5 min and final emulsion temperature was around 11 ± 2 °C.

High added water WPI emulsions

Formulas for high added water WPI emulsions are listed in Table 4.5. Applicable amounts of WPI, water and salt were added to a Stephan mixer and mixed at 200 rpm for

30 sec. Vacuum (35 mmHg) was then applied and the mixture was mixed at 300 rpm for 4.5 min. To increase the solubility of WPI, WPI emulsions were chopped to a final temperature around 23 ± 3 °C, and total mixing time was 5 min.

V. Emulsion stability measurement

The prepared WPI or beef meat emulsions were stuffed into 50 ml polycarbonate tubes (Nalge Nunc International, Rochester, NY) using a hand stuffer. The prepared tubes were placed in a program controlled water batch (Model 9510, PolyScience, Niles, IL). A copper constantan thermocouple with 0.05 cm in diameter (Cole Parmer Instrument Company, Vernon Hills, IL) was inserted into the center of one of the tubes to monitor the temperature. The heating schedule was shown in Table 2.2. The tubes were cooked until the internal temperature reached 71.1 °C. After cooking, the tubes were chilled by placing them in an ice-water bath for 10 min. After chilling, the fluid released during cooking was collected by decanting fluid from the tubes through cheese cloth covered funnels for 5 min. Drained fluids were collected in 15 ml centrifugation tubes, and were centrifuged (Sorvall RC 5B plus, Sorvall Instruments, Newtown, CT) at 6,000 $\times$ *g* for 5 min. After centrifugation, the tubes were placed in a 4 °C cooler for 1 hr, and the volumes of released fat and water were recorded.

Table 2.2. Heating Schedule for Emulsion Stability Measurement

Time (min)	Temperature (°C)
20	60.0
20	71.1
15	76.6
90	82.2

VI. Solubility of whey protein isolate

WPI solubility was determined following method of Morr et al. (1985). WPI (500 mg) was weighed out in a 150 ml beaker. A small amount of 0.3 M NaCl solution was added to form a smooth paste. A total of 40 ml of 0.3 M NaCl solution was added, and the solution pH adjusted to 7.0. The solution was stirred with a stir bar at room 25 °C for 1 hr and transferred to a 50 ml volumetric flask. The volume of the solution was brought to 50 ml with 0.1 M NaCl. The solution was centrifuged at 20,000 $\times g$ for 30 min and the supernatant filtered through Whatman No. 1 filter into a beaker. The protein content of the filtered supernatant was determined by micro-Kjeldahl method (AOAC, 2000). Solubility of WPI was determined as follows:

Protein solubility = [supernatant protein concentration (mg/ml) × 50] /[sample weight (mg) × sample protein content (%) / 100] × 100

Micro-Kjeldahl protein content (AOAC method 930.29, 2000)

Ten ml of WPI solution was transferred to a Kjeldahl digestion tube. 1 Kjeltab digestion tab (Catalog# 13159B, Fisher Scientific, Pittsburgh, PA) and 5 ml of concentrated sulfuric acid were added to the digestion tube. The samples were heated using a digestion block (Tacator 2040 Digestor, Perstorp analytical company). The temperature of the digestion block was increased slowly until it reached 400°C and the samples were digested until the samples were clear. The digested samples were distilled using a Buchi 323 distillation unit (Brinkmann Instruments Inc., Westbury, NY)) and then titrated with HCl using a DMS Titrino titration unit (Brinkmann Instruments Inc.,

Westbury, NY). The amount of HCl used in the titration was recorded and the % protein content was calculated as:

$$\% \text{ protein content} = [(V_s - V_b) \times 1.4007 \times 6.38 \times N_{HCl}] / \text{weight of sample (g)}$$

V_s = the volume of HCl needed to titrate sample

V_b = the volume of HCl needed to titrate blank

N_{HCl} = normality of HCl

6.8: conversion factor for dairy protein

VII. Proximate composition and pH of WPI

Moisture content (AOAC method 927.05, 2000)

The moisture content of WPI samples was determined by the vacuum oven method. One gram WPI samples were weighed out into a round, flat bottomed aluminum dish. The dish was placed in a vacuum oven (GCA/Precision Scientific, model 19, Chicago, IL) at 100 °C and dried to constant weight (5 hr) under pressure ≤ 100 mmHg (4 inHg). Moisture content was calculated as:

% moisture = [(dish and sample weight before drying – dish and sample weight after drying) / sample weight] × 100

Fat content (AOAC method 932.06, 2000)

The fat content of WPI samples were determined by Mojonnier ether extraction method. One gram WPI samples were weighed out in a small beaker and 1 ml of H_2O was added to make a smooth paste. An additional 9 ml of H_2O was added and 1 ml of NH_4OH was added. The mixture was warmed on a steam bath. The warmed mixture was transferred to a Mojonnier flask and cooled at 25 °C for 10 min. To extract the fat, 10 ml of 95% alcohol and 25 ml of ether were added to the mixture. The mixture was shaken for 1 min and 25 ml of petroleum ether was added and the mixture shaken for an additional minute. The mixture was allowed to set at 25 °C for 5 min and the upper ether and pet ether layer was poured out in a drying dish. To complete the extraction, a second extraction was necessary. For the second extraction, the alcohol amount was changed to 4 ml, ether and petroleum ether amount was changed to 15 ml. The dish was dried at a 100 °C oven (Fisher Scientific, Model 725F) till constant weight. Fat content was calculated as following:

$$\% \text{ fat} = [(\text{dish weight after drying} - \text{empty dish weight}) / \text{sample weight}] \times 100$$

Ash content (AOAC method 930.30, 2000)

Crucibles were preheated at 550 °C for 2 hr to reach constant weight. One gram WPI samples were weighed out in a crucible. To prevent fire, samples were charred over a Bunsen burner until the smoke ceased. The crucible was then placed in a muffle furnace (Barnstead/Thermolyne type 1500 furnace, Dubuque, Iowa) at 550 °C until a constant weight was reached (about 16 hr). Ash content was calculated as:

% ash = [(ash and crucible weight – empty crucible weight) / sample weight] × 100

pH determination

An Accumet pH meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA) was used to measure pH of WPIs. Samples (10 ± 0.1g) were weighted out in a 400 ml beaker and 90 ml of deionized water was added. Samples were stirred at room temperature for 10 min, and pH of the WPI solution was then measured. All samples were measured in triplicate.

Study III: Verification of the efficacy of preheated whey protein isolates in low fat beef frankfurters

I. Experimental design and statistical analysis

To verify the efficacy of preheated whey protein isolate (WPI), a commercial beef frankfurter formula was used for this study. Beef frankfurters were formulated to contain 35, 40 or 45% water and 3.5 or 7.0% WPI as shown in Table 5.1. The experimental design used was a two-way analysis of variance with four treatment combinations (35% water with 3.5% preheated WPI, 40% water with 3.5% preheated WPI, 40% water with 7.0% preheated WPI and 45% water with 7.0% preheated WPI) and two control combinations (40% water with 3.5% unheated WPI and 40% water with 7.0% unheated WPI). The effect of preheating of WPI on quality attributes (cooking yield, emulsion stability, textural properties, purge loss, color, pH and lipid oxidation) of the frankfurters

were analyzed. Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure (SAS user's guide, version 8.2. Cary, NC: SAS Institute, Inc., 2002).

II. Ground beef, whey protein isolate and additives

Fresh 90/10 ground beef was purchased from a local meat company (Popoff Quality Food Service, Lansing, MI) and stored at 4 °C until use. Fresh ground beef came in 10 lb tubes, and 300 lbs (30 tubes) ground beef was ordered for the experiment. On each day, 10 tubes of the 30 tubes fresh ground beef were randomly selected and mixed at 4 °C for 30 sec using a mixer (Butcher Boy, Model 250F, Lasar Company Inc., St. Louis, MO). After mixing, a small amount of mixed fresh ground beef was collected for proximate composition. Optiform 4 sodium lactate was purchased from Purac America (Lincolnshire, IL). Whey protein isolate (WPI) (Alacen 895) was purchased from New Zealand Milk Products (Lot# 047U45283431314, Lenoyne, PA). FMC phosphate blend containing sodium tripolyphosphate, sodium hexametaphosphate and tetrasodium pyrophosphate was obtained from Astaris (St. Louis, MO) and frankfurter seasoning was obtained from A.C. LEGG Packing Co, Inc. (Birmingham, AL).

III. Manufacture of preheated whey protein isolates

Three batches of preheated WPI were manufactured as described in study II. Whey protein isolate (8%) was preheated at 95 °C for 40 min, pH 7.0. Preheated WPI was stored in air tight bags at 4 °C until use.

IV. Manufacture of beef frankfurters

Beef frankfurters were manufactured in the Michigan State University meat lab pilot plant (E. Lansing, MI). For three consecutive days, 6 batches of frankfurters (30 lb per batch, four treatments and two controls) were produced on each day. Except for beef meat, water/ice and Optiform 4 sodium lactate, all ingredients needed to manufacture frankfurters were weighed out in individual Whirl-Pack bags™ (Fishers Scientific USA, Pittsburg, PA) one day before the first day of manufacture. On each day, the order of frankfurter manufacture was randomized. To manufacture beef frankfurters, ground beef, half water/ice, and salt were mixed and chopped at 2000 rpm for 3 min using a bowl chopper (Seydelmann bowl chopper, model K64U-Va, Reiser Co, Inc., Canton, MA). After the first 3 min chopping, the rest of the water was then added. Other ingredients were added in the following order: phosphate blend, sugar, spice, whey protein isolates, Na erythorbate and curing salt. Sodium lactate was added last. After all the ingredients were added, the meat mixture was chopped at 4000 rpm for another 5 min to a temperature of 6-7 °C, emulsions were stuffed and linked into cellulose casing (30 mm in diameter, Brechteen Co., Chesterfield, MA) using a VEMAG stuffer (Model 500, Robert Reiser Co, Inc., Canton, MA). The linked frankfurters were put on smoke sticks. Two smoke sticks were used for each treatment and control. The position of the smoke sticks were randomized on the smoke truck and then cooked in a computer-controlled smokehouse (CGI Processing, Model A28-B0101, Automated Manufacturing, Cicero, IL) using the smokehouse schedule listed in Table 2.3.

After cooking, frankfurters were cold-showered for 20 min and chilled to 4.4 °C. The chilled frankfurters were peeled, vacuum packaged (5 frankfurters/bag) and placed in

Styrofoam boxes. Styrofoam boxes containing packaged frankfurters were stored at a 4 °C until further analyses.

Table 2.3. Smokehouse schedule

Stage	Time (min)	Internal (°C)	Smoke	Dry Bulb (°C)	Wet Bulb (°C)	Fan
1. Cooking	20	0	No	60	43.3	100
2. Cooking	20	0	Nature maple	71.1	51.6	75
3. Cooking	15	57.2	Nature maple	76.7	57.2	75
4. Cooking	90	71.1	No	82.2	76.6	100
5. Cold shower	20	54.4	No	1 min on	1 min off	100

V. Solubility and proximate composition for whey protein isolates

Solubility of preheated and unheated WPI was determined according to methods by Morr and others (1995) as described in study II. Protein, moisture, ash and fat contents of preheated and unheated WPI were determined according to AOAC methods 930.29A (micro-Kjeldahl method), 927.05 (vacuum oven drying method), 930.30 (dry ashing) and 932.06 (Mojonnier ether extraction method), respectively. All samples were measured in triplicate.

VI. Proximate composition for raw frankfurter emulsions and cooked frankfurters

After the chopping process and before stuffing, raw frankfurter emulsion was collected. Emulsions and frankfurters were packed in Whirl-Pack™ bags (Fishers Scientific USA, Pittsburg, PA) and frozen at a -10 °C freezer for at least 24 hr before

processing. Frozen samples were cut into small pieces and ground with dry ice into a fine powder using a Tekmar grinder (Tekmar Co, Cincinnati, OH), packed in opened whirl-pack bags at -10 °C freezer for at least 48 hr (to evaporate the dry ice), and sealed until further analysis.

Moisture, protein, fat and ash contents of samples were determined according to AOAC (2000) methods 950.46B (oven drying), 992.15 (combustion method, nitrogen measurement, Model FP-2000, LECO Co., St. Joseph, MO), 991.36 (Soxhlet ether extraction), and 920.153 (dry ashing), respectively, with small modifications. Appendix 7 describes the step by step procedures for methods used here. All samples were analyzed in triplicate.

VII. Back extrusion force of raw frankfurter emulsions

Raw frankfurter emulsions were collected after the chopping process. Back extrusion force of raw frankfurter emulsions was measured using a TA-HD*i* Texture Analyzer (Texture Technologies Co., Scarsdale, NY) attached to a 50 kg loading cell. Raw frankfurter emulsions (100 ± 0.1 g) were weighed out in an extrusion rig. The extrusion rig had an inner diameter and height of 52 mm and 100 mm, respectively. An extrusion plunger which was 40 mm in diameter was attached to a 50 kg loading cell. The extrusion plunger was advanced into the extrusion rig to a depth of 90 mm at a speed of 5 mm/sec. Frankfurter emulsions were analyzed at 4°C and extrusion force was recorded in Newtons. All samples were measured in triplicate.

VIII. Cooking yield

Cooking yield was measured according to Atughonu and others (1998). Stuffed and linked uncooked frankfurters were weighed before cooking. After cooking and chilling to 4.4 °C, frankfurters were removed from the smoke sticks and reweighed. Cooking yield was calculated as following:

Cooking yield % = (weight of cooked frankfurters / weight of uncooked frankfurters) × 100

VIIII. Emulsion stability

Emulsion stability of raw beef frankfurter emulsions was measured on the same day of manufacture. Raw frankfurter batter (33.3 ± 0.1 g) was stuffed into a 50 ml polycarbonate tube (Nalge Nunc International, Rochester, NY), and capped. A metal thermometer (Cole Parmer Instrument Company, Vernon Hills, IL) was inserted into the center of one of the stuffed tubes to monitor internal temperature of the batter. The tube inserted with thermometer was placed in the center of a program controlled water bath (Model 9510, PolyScience, Niles, IL) and all tubes were cooked until the temperature of the thermometer reached 71.1 °C. After cooking, fluid released during cooking was collected in 15 ml centrifugation tubes by decanting the fluid through cheese cloth covered funnels for 5 min. The tubes were centrifuged at 6, 000 $\times g$ for 5 min (Sorvall RC 5B plus, Sorvall Instruments, Newtown, CT) to separate water and fat layers. After centrifugation, centrifugation tubes were placed in a 65 °C water bath (Model 1268-52, Cole-Parmer instrument company, Chicago, IL) for 2 min. To solidify fat layers, tubes

were kept at 4 °C for 30 min and volumes of fat and water layers were recorded. Emulsion stability was calculated as:

Emulsion stability % = 100 – volume of total fluid (or fat, or water) released from 100 g of frankfurter emulsion

X. pH determination

An Accumet pH meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA) was used to measure pH of raw frankfurter emulsion and cooked frankfurter samples. Raw emulsions were mixed and cooked frankfurter samples were cut into small pieces. Sample (10 ± 0.1g) was collected in a 400 ml beaker and 90 ml of deionized water was added. Samples were homogenized for 1 min at setting 4 using a Polytron mixer (PT-35, Kinematica, AG, Switzerland). The pH of homogenized samples was measured at room temperature (22 °C). All samples were measured in triplicate.

XI. Thiobarbituric acid reactive substances (TBARS)

TBARS analysis was conducted to monitor oxidative rancidity. Four replicates were run for each sample according to methods by Rhee (1978). Frankfurters sample (10 g) was diced into small pieces and 50 ml of deionized water was added. Ten µl antioxidant solution (Tenox 5, BHA+BHT, Eastman chemical products, Inc, Kingsport, TN) was added before samples were homogenized for 1 min using Polytron mixer (PT-35, Kinematica, AG, Switzerland) at setting 4. Homogenized samples were transferred to 500 ml extraction flasks, four 4 mm glass beads (Fisher Scientific USA, Pittsburg, PA),

2.5 ml 4 M HCl, 1.0 ml sulfanilamide solution (0.5 g sulfanilamide in 100 ml water), 46.5 ml distilled water and 2 sprays of antifoam (Thomas Scientific, Swedesboro, NJ) were added. The prepared samples were distilled until 50 ml distillate was collected. Five ml distillate was mixed with 5 ml TBA reagent (0.72 g thiobarbituric acid with 250 ml water) and the mixed solution was boiled in boiling water bath (Model 9510, PolyScience, Niles, IL) for 30 min. The solution was transferred to a 96 well polypropylene plate (Fisher Scientific USA, Pittsburg, PA) and the absorbance of thiobarbituric acid reactive substances was read at 538 nm using a VERSA max microplate reader (Molecular Devices Corporation, Sunnyvale, CA). Absorbance was converted to mg malonaldehyde (MDA) /kg sample (TBARS value) using the following equation:

$$TBARS = A_{532nm} \times K \text{ (mg MDA/kg sample)}$$

Where K = (conc. in moles/5 ml of distillate $\times$ M.W.MDA $\times$ 10^7 $\times$ 100) / (Absorbance $\times$ wt. of sample $\times$ % recovery)

K is distillation constant and equal to 7.8 in this lab.

XII. Purge loss

On each sampling day, two packages of each treatment and control frankfurters were randomly selected and measured for purge loss. Each package of frankfurter was weighed before it was cut open and fluid was drained out. Excess fluid was blotted dry with paper towels and then the package was reweighed. Purge loss was calculated as:

Purge loss % = ((package weight before liquid draining – package weight after liquid draining) / package weight before draining) × 100

XIII. CIE L^*, a^*, and b^* value

A ColorTec PCM^{TM} Color Meter (Model 6482, ColorTec Associates, Clinton, NJ) with a 10° standard observer and an 8 mm reading orifice was used to measure L^* (lightness), a^* (redness), and b^* (yellowness) values of cooked frankfurters. Before measuring, the color meter was calibrated with standard white and black tiles. Cooked frankfurters were cut open to expose inner surfaces. L^*, a^*, and b^* values were measured by pressing tightly of the reading orifice of the color meter against the exposed inner surface of cooked frankfurters. On each sampling day, six frankfurters from each treatment were randomly selected and measured for color. And one reading for L^*, a^*, and b^* value was taken from each frankfurter.

XIV. Expressible moisture

Expressible moisture was measured according to Barbut (2002). Six frankfurters were used to measure expressible moisture, and 2 ± 0.1 g of samples were cut out from the center of each of the six frankfurters. The cut out frankfurter samples were placed in centrifuge tubes (9 mm in inner diameter and 60 mm in length). Bottom of the centrifuge tubes were covered with paper towels to prevent reabsorption of the fluid released during centrifugation. The prepared centrifuge tubes were centrifuged at 750 $\times g$ for 20 min at 4 °C. After centrifugation, frankfurter samples were removed from the tubes and weighed again. Expressible moisture was calculated as:

Expressible moisture % = ((sample weight before centrifugation – sample weight after centrifugation) / sample weight before centrifugation) × 100

XVI. Textural properties (two-cycle compression test)

Textural properties of cooked frankfurters were measured by two-cycle compression test using a TA-HD*i* Texture Analyzer (Texture Technologies Co., Scarsdale, NY). Ends of frankfurters were trimmed off, and frankfurters were cut into 20 mm long cores with skin on. Six frankfurters from each treatment were used and two cores from each frankfurter were analyzed for textural properties. The texture analyzer was calibrated before measurement and equipped with a 75 mm compression plate attached to a 50 kg loading cell. Samples were compressed to 35% of the original height at a speed of 5 mm/s, with 5 seconds interval between the first and second compression. All frankfurter samples were analyzed at 4 °C.

XVII. Consumer reheat yield

A cooked frankfurter was weighed and placed in 1000 ml of boiling water for 5 min. After reheating, the frankfurter was removed from the boiling water and cooled at 25 °C for 5 min, blotted dry and weighed again. Three frankfurters from each treatment were tested and consumer reheat yield was calculated as:

Consumer reheated yield = (weight of reheated frankfurter / weight of cooked frankfurter) × 100

XVIII. Sensory evaluation

Sensory attributes of cooked beef frankfurters were determined by a trained sensory panel. Five healthy panelists age between 28 and 65 (4 female and 1 male) were trained according to AMSA (1995) and Meilgaard and others (1991). All panelists had experiences in sensory evaluation and previously trained to evaluation various meat products. Before product evaluation, three training sections were held to familiarize the panelists with the attributes and evaluation procedures. An 8 point hedonic scale was used to measure 7 different sensory attributes: skin hardness, product hardness, springiness, juiciness, cohesiveness, off-flavor intensity and milk/whey coating. For skin hardness and product hardness, 1=extremely soft and 8=extremely hard. For springiness, 1=non-resilient and 8=extremely resilient. For juiciness, 1=extremely dry and 8=extremely juicy. For cohesiveness, 1=extremely crumbly and 8=extremely cohesive. For off-flavor intensity and milk/whey coating, 1=non-off-flavor and 8= abundant off-flavor, and 1=non milk/whey coating and 8= abundant whey/whey coating, respectively. Different brands and types of commercial frankfurters were used to train the sensory panel. Sensory panelists were instructed to test commercial frankfurters first. The scale of each sensory attribute on the commercial frankfurters was then decided after discussion among the panel. Koegel natural casing beef franks and Ball Park individually wrapped turkey franks had the highest and lowest skin hardness values, and Ball Park individually wrapped franks (made with beef, pork, and turkey) were assigned to have a 5 point on skin hardness, product hardness, and springiness.

Sensory evaluation was conducted in a climate controlled sensory evaluation room with partitioned booths (Department of Food Science, Michigan State University,

E. Lansing, MI). Red lights were used in the booths to mask the appearance bias during the evaluation, and the order of sample preparation was randomized within each section to minimize positional bias. Cooked frankfurters were heated for 8 min in closed packages in a stainless pan filled with 80 °C water heated by a double boiler (Broil King, Model BR7W, Winsted, CT). Before serving to the panel, heated cooked frankfurters were cut into 1 and 0.5 inch long sections and cooled in 4 oz Soufflé cups to 46 °C. Except for springiness, panelists were instructed to use 1 inch long frankfurter sections for all other sensory attributes. Panelists were trained to put frankfurter samples between incisors and use their incisors to measure skin hardness first. The same sample was then moved to the back of mouth and molars were used to measure cohesiveness. Cohesiveness, juiciness, product hardness values were decided after 15 chews. Expectorant cups, distilled water, apple juice, and unsalted soda crackers were provided to the panel to clean the palate between samples. Eighteen (four treatments, two controls, three replications) samples were evaluated in one day. The day was divided into three sections with six different samples in one replication were evaluated per section. For each section, the panelists were standardized with randomly selected one commercial frankfurter and one whey protein added frankfurter. The panel was also instructed to take a 15 min break between each section.

XIX. Sampling for quality attributes of beef frankfurters

Purge loss, pH, CIE L^*, a^*, b^* values, thiobarbituric acid reactive substances (TBARS) of cooked frankfurters were measured on day 1, day 7, day 14, day 28 and day 56 after the manufacture of frankfurters. Consumer reheat yield, expressible moisture

and textural properties of frankfurters were measured on day 56 after the manufacture. On each sampling day, two packages of frankfurters were randomly selected. Three frankfurters were then randomly selected from each package to measure pH, CIE color, TBARS value, expressible moisture, and textural properties. The four frankfurters left from the two packages were used to measure consumer reheat yield. All sensory evaluations were conducted on the same day which was 55 days after the first day of frankfurter manufacture. Two packages from each treatment and control were used for sensory evaluation.

CHAPTER 3

HEAT DENATURED β-LACTOGLOBULIN AGGREGATES: A STUDY OF THE EFFECT OF PREHEATING AND AGGREGATE SIZE ON MICROSTRUCTURAL AND RHEOLOGICAL PROPERTIES OF β-LACTOGLOBULIN GELS

ABSTRACT

A two-step heating process was applied to produce beta-lactoglobulin (LG) gels. The effects of the initial heating step on thermal aggregation, rheological and microstructural properties of LG aggregates and gels were studied. The influence of the initial heating step on rheology output of LG gels during the second step of heating was determined using response surface methodology. β-lactoglobulin gels with various storage moduli (G'), loss moduli (G") and gel point were produced through the manipulations of the initial heating conditions. Gel point of LG gels varied from 50.6 to 71.0 °C. After cooling to 25 °C, G' and G" of LG gels varied from 30.8 to 397.8 Pa and 1.8 to 38.1 Pa, respectively. Using the gel point, G' and G" of LG gels as the responses and pH, heating time and heating temperature as the independent variables, three response surface models were developed. The response surface models had a R^2 value of 0.97, 0.89 and 0.93, respectively. Response surface graphs predicted that LG gel with high G' and low gel point can be produced by preheating LG solution at 90 °C, 40 min, pH 7.0. After the first step of heating, various sizes and shapes of heat denatured LG aggregates were formed and larger sizes of aggregates produced stronger gels. However, there was no correlation found between the microstructure and initial heating condition of LG gels.

Chapter 3 is formatted in manuscript style according to the Journal of Food Science

Keywords: β-lactoglobulin aggregates, β-lactoglobulin gels, microstructure, preheating, rheological properties

Introduction

Beta-lactoglobulin (LG) is the main protein responsible for the gelling properties of whey proteins. Like whey proteins, when heated above its denaturation temperature, LG can form heat denatured LG soluble (HDLG) aggregates. The characteristics of LG have been studied extensively. Ionic strength, type of ion, LG concentration, pH, and heating temperature and heating time have all been reported to affect the polymerization, aggregation, and gelation of LG. Heating changes the structure of LG and exposes the buried SH group. The exposed SH group can initiate sulfhydryl-disulfide interchange leading to polymerization (Cairoli and others, 1994, Damodaran, 1997). The maximum particle size of HDLG aggregates is observed at the isoelectric point (5.2) and increasing salt concentration increases HDLG aggregation rate (MacLeod and others, 1995, Schokker and others, 2000). The dependence of HDLG aggregate size on LG concentration, heating temperature and time has been established. Increasing LG concentration, heating temperature, and time increases HDLG aggregate size (Hoffmann and others, 1996, La Bon and others, 1999, Sharma and others, 1996).

. Beta-lactoglobulin gels with various rheological properties could be produced by adjusting the heating conditions. Heating at high temperatures (>75 °C) and pHs (>7.0) produces LG gels with higher gel strain and stress (Stading and Hermansson, 1991, McSwiney and others, 1994). Although the characteristics of LG aggregates and gels have been studied extensively, the relationship between HDLG aggregate size and the rheological properties of LG gels is still not clear.

Recently, Ju and Kilara (1998a and 1998b) indicated that whey protein isolate gels can be induced by adding salts to preheated whey protein isolate solutions (preheated

at 80 °C for 30 min, pH 7.0). Vittayanont and others (2002) also reported that preheating LG solutions (4% LG heated at 80 °C, 30 min, pH 7.0) decreased LG gel point (58.5 °C, 2% (w/v) preheated LG at pH 6.5). When incorporated in poultry batters, preheated whey protein isolate proved to have better water holding capacity than non-preheated whey proteins in poultry meat batters (Hongsprabhas and Barbut, 1999a, 1999b). This characteristic of preheated whey protein can be beneficial to meat processors when a lower processing temperature is preferred.

To maximize the use of preheated whey protein polymers in processed meat products, it is essential for meat processors to optimize the preheating condition for whey protein. Since aggregate size could dominate the textural properties of whey protein gels, understanding the effect of aggregate size on rheological properties of whey protein gels would also be important for meat processors to choose the optimal preheating condition. Whey proteins are multi-component systems and the large number of proteins may cause ambiguity when interpreting results. Since LG is the major functional protein responsible for the gelling property of whey proteins, using LG as a model system would minimize confusion and develop our knowledge to optimize the preheating condition for whey proteins. To confirm the applicability of preheated whey proteins in processed meat products, this study was conducted at conditions (temperature: 71 °C, pH: 6.5, and salt concentration: 0.3 M NaCl) consistent with meat processing. The objectives for this study were to: 1). Optimize the preheating conditions of LG under conditions suitable for meat processing. 2). Study the effects of preheating on the rheological and microstructural properties of LG gels and 3). Investigate the relationship between HDLG aggregate size and the rheological properties of LG gels.

Materials and Methods

Experimental design and statistical analysis

A two-step heating process was used for this study. For the initial heating step, a preliminary study was conducted to determine the ranges for heating time, heating temperature and the concentration of the LG solution. All the LG solutions were initially heated at a 7% (w/v) concentration using a water bath (PolyScience, model 9510, Niles, IL). Heated LG solutions were then diluted to 2% and subjected to a second heating step. During the second heating step, a small amplitude dynamic oscillatory test was performed to collect various rheological data (storage modulus, loss modulus, and gel point).

The influence of the initial heating step (pH, heating time and heating temperature) on the rheology output during the second heating step was determined using response surface methodology. A three-factor, five-level rotatable central composite design was applied to choose the conditions for the initial heating step. The central composite design contained fifteen combinations with one treatment combination (treatment 15) replicated six times. The three independent variables were assigned as time (X_1), pH (X_2) and temperature (X_3). Based on the preliminary study, the values for pH, heating time and heating temperature were chosen and shown in Table 3.1.

Response surface methodology was used to determine the effect of the three independent variables (pH, heating time and heating temperature) on storage modulus (G'), loss modulus (G") and gel point of LG gels. Second order polynomial regression models were generated using storage modulus, loss modulus and gel point as responses. The second order polynomial regression models were described as:

$$Y = B_0 + \sum_{i=1}^{k} B_I X_I + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i<j} B_{ij} X_i X_j$$

Where: B_0 was constant of the model

B_I (linear), B_{ii} (quadratic),B_{ij} (interaction) were parameter estimates of the model

X_i and X_j were the independent variables in the model

k was the number of factors

Y was the response of the model

Significance of total response surface regression equations was determined at a predetermined level ($P<0.05$), and R^2 values of the models were calculated. Response surface graphs were generated using all parameter estimates. Statistical analysis was done using SAS software system (SAS version 8.2, SAS Institute Inc., Cary, NC). SAS Proc Corr procedure was used to correlate the relationship between HDLG aggregate size and G' and gel point of LG gel. Significant level was set at predetermined level of 5%.

β-lactoglobulin (LG) purification

Raw bovine milk was collected from Michigan State University Dairy Farm and LG was purified from the fresh raw (unpasteurized) bovine milk according to the procedures by Armstrong and others (1967). The purity of the purified LG solution was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified LG solution was then lyophilized using a Lyph-Lock Freeze Dry system (Model 77530, Labconco Co., Kansas City, MO). The lyophilized LG powder was stored at 4 °C for future use.

Table 3.1. Initial preheating conditions for 7% (w/v) β-lactoglobulin solution.

Treatment	Variable levels		
	Time (min)	PH	Temperature (°C)
1	20.15	6.4	74.1
2	20.15	8.0	85.9
3	49.85	6.4	85.9
4	49.85	8.0	74.1
5	20.15	6.4	85.9
6	20.15	8.0	74.1
7	49.85	6.4	74.1
8	49.85	8.0	85.9
9	10.00	7.2	80.0
10	60.00	7.2	80.0
11	35.00	6.4	80.0
12	35.00	8.0	80.0
13	35.00	7.2	70.0
14	35.00	7.2	90.0
15	35.00	7.2	80.0

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to check the purity of purified LG. A mini-Protean II Dual Slab Cell (Bio Rad Laboratories, Hercules, CA) was used to run the electrophoresis. A Tris (hydroxymethyl) aminomethane glycine electrode buffer (pH 8.3, and 0.1% SDS) was used to run the SDS-PAGE, and the acrylamide concentrations of stacking and resolving gels were 4% and 14%, respectively.

Preparation of LG sample

β-lactoglobulin solution was diluted to 4 mg/mL with sample buffer (0.0625M Tris pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.2% bromophenol blue), and then mixed using a vortex mixer and then heated in boiling water for 5 min.

Running condition for SDS-PAGE

Each protein sample (10 μL) was loaded into a sample well and the gels were run at 90 mA current and 200 constant voltage. Gels were stained for 20 min with Coomassie Brilliant Blue R250 solution (0.25% in 9:45:45 v/v/v of acetic acid : methanol : water) and de-stained overnight in acetic acid-methanol-water (6:4:7,v/v/v) solution. Standard molecular weight markers (Bio-Rad Laboratories, Hercules, CA) were used to determine unknown protein molecular weight by comparing their relative mobilities under the same electrophoretic conditions (Weber and Osborn, 1969). Purity of the purified LG was also compared with bovine milk LG purchased from Sigma Chemical Co. (St. Louis, MO, L0310 lot# 114H7055).

Determination of protein concentration

Concentration of LG solutions was determined by spectrometric absorption (Varian Cary III double beam Spectrophotometer, Varian Ltd., Walnut Creek, CA) using an extinction coefficient ($E^{1\%}$) of 9.55 at 278 nm (Foegeding and others, 1992).

Small amplitude dynamic oscillatory testing

A controlled stress rheometer (RS100, Haake, Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plate was used for dynamic oscillatory tests. Heated LG samples were diluted to 2% (w/v) and loaded between the plate and base with a gap of 1.0 mm. The edge of the gap was covered by a few drops of corn oil (Mazola, Best Food, CPC international, Inc., Englewood Cilffs, NJ) to prevent evaporation. The samples were held at 25 °C for 5 min, heated from 25 to 71 °C at a rate

of 1 °C/min, and held at 71 °C for 30 min. After 30 min holding, the samples were cooled to 25 °C within 10 min. Storage modulus (G') and loss modulus (G") were recorded continuously at a fixed frequency (0.464 Hz) using stresses (producing strains from 0.1-0.3%) within the range of linear viscoelastic behavior based on stress sweeps performed at 71 °C and after cooling at 25 °C. Gel point temperature of LG was defined as the cross over point corresponding to temperature obtained by extrapolating the baseline storage modulus and the first 5 storage modulus data points that had values at least 5 times higher than the baseline storage modulus value. (Foegeding and others, 1992, Hsieh and others, 1993, Steventon and others, 1991).

Scanning electron microscopy (SEM) of preheated LG gels

Preheated LG solutions were diluted to 2% (w/v) (using a 0.07 M sodium phosphate buffer containing 0.42 M NaCl, pH 6.5). To prepare protein gels, 2.0 ml of diluted protein solution was transferred into a glass tube (12 mm × 75 mm), sealed with Teflon™ tape and heated in a programmed water bath (PolyScience, model 9510, Niles, IL) using the same temperature profile used in the small amplitude dynamic oscillatory test. Protein gels were cut into small pieces (1×2×2 mm) and prefixed for 3 hr in 2.0% glutaldehyde solution (0.1 M sodium phosphate, pH 7.0). The gels were postfixed at 4 °C overnight in 0.1% osmium tetraoxide solution, rinsed and dehydrated in a graded series of ethanol followed by three 20 min changes in 100% ethanol. Dehydrated gels were dried using a carbon dioxide critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) and coated with a 25-30 nm gold layer in an ion-sputter coater (Emscope Laboratories Ltd., Ashford, Kent, UK). Microstructures of prepared protein gels were

examined with a scanning microscope (JEOL, Model JSM-6400V, version 96-2, Tokyo, Japan) at a 15 mm working distance using an accelerating voltage of 12 KV at a magnification of 5000×. Gels were prepared in duplicate and three pieces from each gel were examined.

Scanning electron microscopy of preheated LG aggregates (poly-L-lysine method)

Cover slips were coated with poly-L-lysine (Sigma, P 1399) for 5 min and rinsed with distilled water. Preheated LG aggregates were fixed with an equal volume of 4% glutaldehyde solution (buffered with 0.1 M sodium phosphate, pH 7.0) at 4 °C for 1 hr. One drop of the suspension was placed on the coated cover slip and allowed to stand at 25 °C for 5 min. The cover slip was then rinsed, dehydrated, and dried. A layer of gold (15 nm) was coated on the cover slip to avoid charging. Preheated LG aggregates were examined at an 8 mm working distance using an accelerating voltage of 10 KV and 20,000× magnification. All samples were prepared in duplicate and three cover slips from each preparation were examined. Aggregate size was determined using analySIS program by measuring the inside area of circled aggregates (Soft Imaging System, GMBH, Muenster, Germany). All aggregates observed under the microscope were measured. The size distribution of the aggregates was calculated as percentage under each size category: Aggregate 1: ≤ 0.075 μm^2, aggregate 2: between 0.075 and 0.15 μm^2, aggregate 3: between 0.15 and 0.3 μm^2, aggregate 4: 0.3 and 0.5 μm^2, aggregates 5: ≥ 0.5 μm^2.

Results and Discussion

Purification of LG

The electrophoretic pattern of LG extracted from bovine raw milk resolved by SDS-PAGE is shown in Figure 3.1. LG was the predominant protein (lane 4 and 5, molecular weight around 18 kDa) and little contamination was observed. Comparing Lane 6 (LG purchased from Sigma Chem. Co.), purity of purified LG was as good as LG purchased from Sigma Chem. Co .

Rheological properties of preheated LG solutions during second step of heating

Rheological properties of preheated LG solutions during the second heating step are shown in Table 3.2. This study intended to predict the rheological properties of preheated whey protein in processed meat products, so the second heating step was carried out at conditions suitable for meat processing (71 °C, 0.3 M NaCl, pH 6.5). Gel point of LG gels and storage moduli (G') and loss moduli (G") of LG after cooling to 25 °C were chosen to generate response surface models.. Storage moduli (G') and loss moduli (G") of diluted preheated LG solutions indicated that before the second heating step (G' and G" at 25 °C), there was no difference in storage moduli and loss moduli of diluted LG solutions. Throughout the second heating step, both G' and G" of all preheated LG solutions gradually increased, and G' was always higher than G". After cooling to 25 °C, LG gels with high G' also had low gel point.

After cooling to 25 °C, G' of LG gels varied from 30.8 to 397.8 Pa. Parameter estimates of second order polynomial response models are shown in Table 3.3. Response surface model generated using G' as response was significant (<0.05) and had a R^2 value

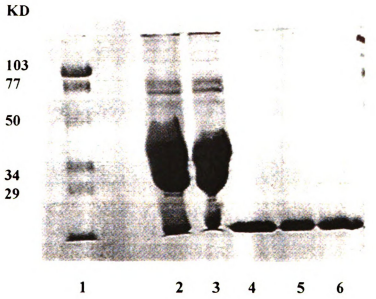


Figure 3.1. Electropherogram of bovine β-lactoglobulin (LG). Lane 1: molecular weight markers. Lane 2 and 3: bovine milk. Lane 4 and 5: LG purified from raw milk. Lane 6: LG purchased from Sigma Chemical Co..

Table 3.2. Least squares means for storage moduli, loss moduli and gel point of 2% preheated LG solution (0.3 M NaCl, 0.05 M phosphate buffer, pH 6.5) during second heating step.

Preheating condition			Storage modulus (Pa)				Loss modulus (Pa)				
Temperature (°C)	Time (min)	pH	At 25 °C	At 71 °C	At 71 °C for 30 min	At 25 °C after cooling	At 25 °C	At 71 °C	At 71 °C for 30 min	At 25 °C after cooling	Gel point (°C)
74.1	20.2	6.4	0.2	0.7	15.9	30.8	0.2	0.2	0.7	1.8	71.0
74.1	49.9	6.4	0.2	3.3	32.1	107.2	0.2	0.5	2.9	9.6	68.1
80.0	35.0	6.4	0.2	6.4	59.7	181.8	0.1	0.8	5.4	16.7	66.9
85.9	20.2	6.4	0.2	16.3	78.4	263.1	0.2	1.9	7.9	24.6	59.9
85.9	49.9	6.4	0.3	22.9	76.0	290.6	0.2	1.6	6.9	25.4	57.2
70.0	35.0	7.2	0.2	1.5	35.8	103.6	0.2	0.4	3.9	11.4	69.9
80.0	10.0	7.2	0.3	10.0	65.3	209.2	0.2	1.5	8.1	22.1	64.8
80.0	60.0	7.2	0.3	7.5	45.2	148.7	0.2	0.7	3.3	12.9	62.9
90.0	35.0	7.2	0.3	40.0	115.6	397.8	0.2	4.5	11.6	38.1	50.6
74.1	20.2	8.0	0.2	0.3	22.5	42.6	0.2	0.2	2.4	4.6	71.0
74.1	49.9	8.0	0.2	3.8	43.0	112.5	0.2	0.7	5.3	19.0	68.7
80.0	35.0	8.0	0.3	10.8	49.6	162.7	0.2	1.3	4.4	15.5	59.3
85.9	20.2	8.0	0.2	23.4	81.0	242.5	0.2	3.1	9.1	24.6	61.2
85.9	49.9	8.0	0.2	14.9	79.4	285.5	0.2	1.8	7.7	26.6	56.2
SEM[a]			0.0	3.0	7.3	27.8	0.0	0.3	0.8	2.6	1.9
80.0	35.0	7.2	0.3	13.3	70.14	251.1	0.2	1.3	6.3	23.6	59.5
SEM[b]			0.0	0.3	1.6	5.5	0.0	0.1	0.2	0.5	0.4

[a]: SEM: standard error of the mean for treatment combination.

[b]: SEM: standard error of the mean for replicated treatment combination

Table 3.3 Parameter estimates and analysis of variance of the second order polynomial regression for three factors and three responses.

Response	Storage modulus after cooling to 25 °C (pa)		Loss modulus after cooling to 25 °C (pa)		Gel point (°C)	
Source	Parameter estimate	Prob > |T|	Parameter estimate	Prob > |T|	Parameter estimate	Prob > |T|
Constant	247.51	0.015	23.23	0.039	59.46	0.005
Time	14.32	0.222	1.18	0.1654	-3.10	0.165
Temperature	158.75	0.72	13.75	0.8283	-10.61	0.343
pH	-2.77	0.025	1.21	0.009	-0.77	0.007
Time × time	-63.05	0.019	-5.14	0.100	4.47	0.023
Temperature × time	-26.99	0.395	-6.91	0.099	2.31	0.323
Temperature × temperature	8.45	0.717	2.07	0.481	0.88	0.609
pH × time	1.90	0.918	1.64	0.482	-0.14	0.916
pH × temperature	-9.067	0.626	-2.33	0.328	0.14	0.913
pH × pH	-60.158	0.001	-5.52	0.095	3.88	0.003
Model R^2 value	0.97		0.89		0.93	
Model p value	<0.001		0.008		<0.001	

of 0.97. Response surface graph of G' (Figure 3.2a) shows that when LG solution was preheated at 90 °C, increasing preheating time to around 40 min and pH to around 7.0 gradually increased G' of LG gels. As preheating time and pH continued to increase, G' of LG gels tended to decrease. Figure 3.2b shows the effect of heating temperature and pH on G' of LG gels. As shown in the graph, in the studied temperature range (70 to 90 °C), G' increased as preheating temperature increased. Highest G' was observed when LG was preheated at pH 7.0.

Response surface graphs of G" of LG gels are shown in Figure 3.3. After cooling to 25 °C, G" of LG gels varied from 1.8 to 38.1 Pa. Figure 3.3a shows that preheating at 90 °C, varying preheating time and pH did not have profound effect on G" of LG gels. However, preheating LG solution for 40 min, increasing preheating temperature increased G" of LG gels. Similar to G' of LG gels, highest G" was observed when LG was preheated at pH 7.0 (Figure 3.3b).

Gel point of LG gels varied from 50.6 to 71.0 °C. Response surface model of LG gel point was significant (<0.05) and had a R^2 value of 0.93. Response surface graph of LG gel point (Figure 3.4a) shows that increasing preheating time to around 40 min and pH to around 7.0 decreased gel point of LG gels, which then decreased at longer preheating time and higher preheating pH. Increasing preheating temperature decreased gel point of LG gels (Figure 3.4b).

Barbut and Foegeding (1993) investigated the effects of preheating time and temperature on the rheological properties of whey protein cold-set gels. In the range of the studied temperature (70 to 90 °C) and time (5 to 80 min), preheating whey protein solution (8% (w/v) whey protein isolate, pH 7.0) at higher temperature and longer time

Figure 3.2. Response surface graphs of significant ($p<0.05$) total regression equation for storage modulus (after cooling to 25 °C) of LG gels.

Figure 3.2a. Effect of heating time and pH at 90 °C.

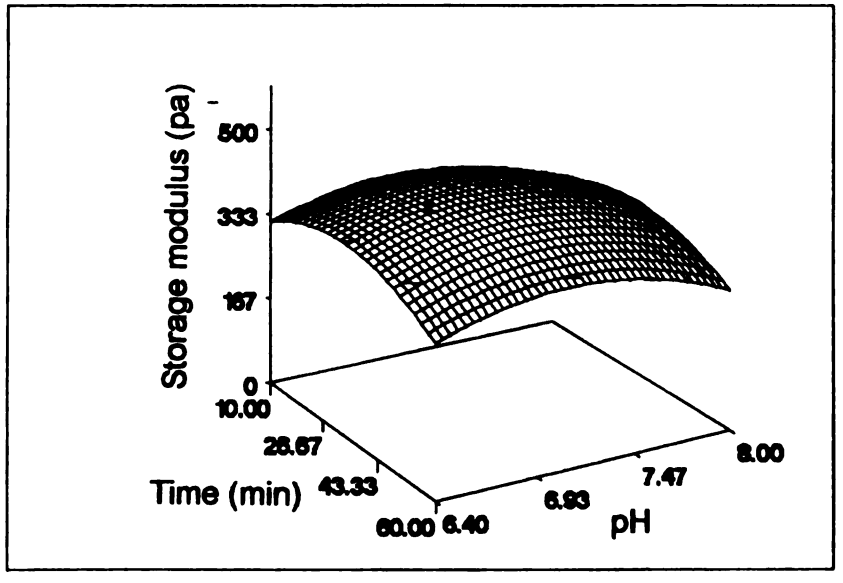


Figure 3.2b. Effect of heating temperature and pH at 40 min.

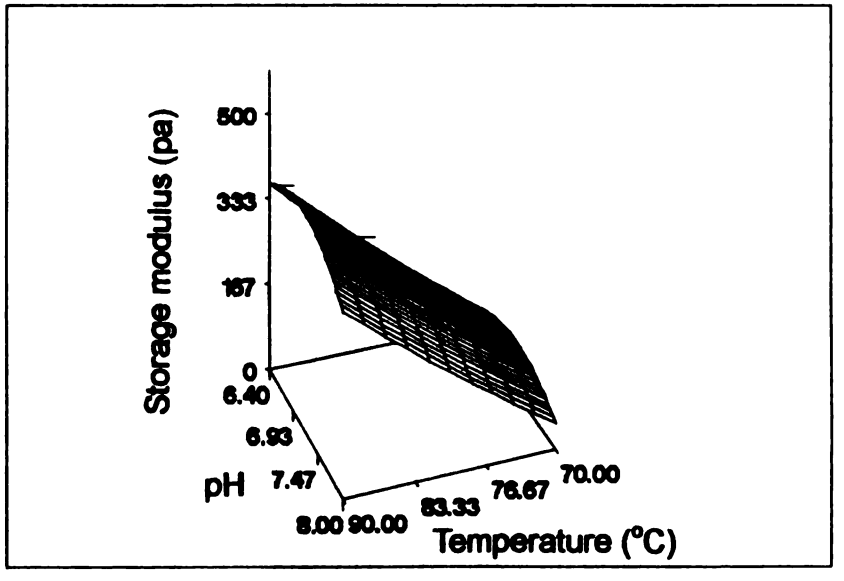


Figure 3.3. Response surface graphs of significant ($p<0.05$) total regression equation for loss modulus (after cooling to 25 °C) of LG gels.

Figure 3.3a. Effect of heating time and pH at 90 °C.

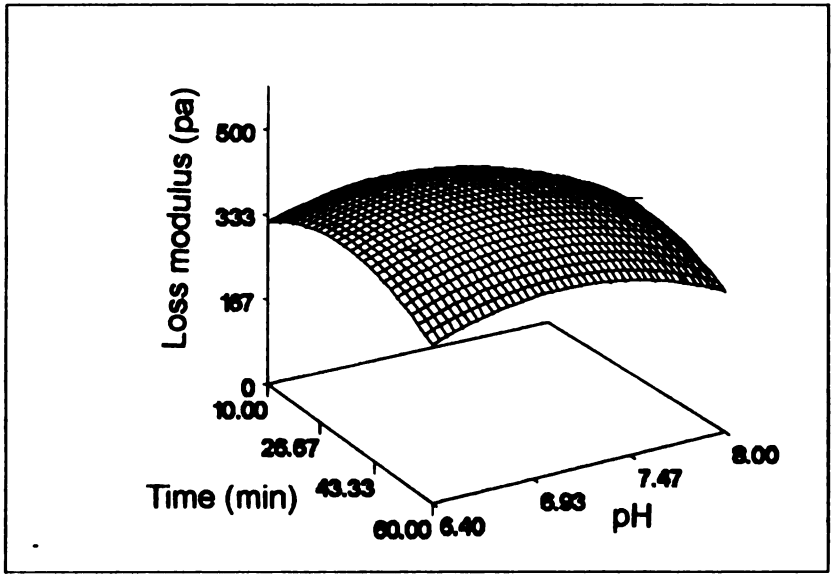


Figure 3.3b. Effect of heating temperature and pH at 40 min

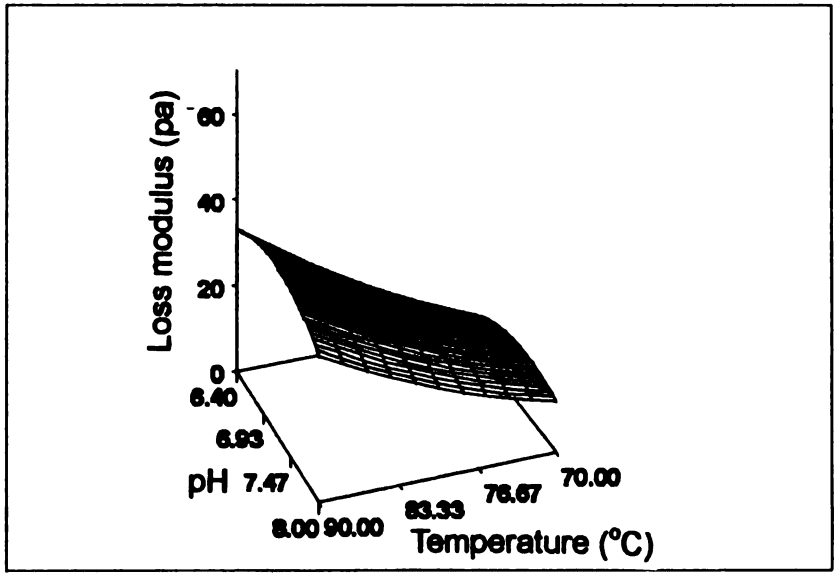


Figure 3.4. Response surface graphs of significant ($p<0.05$) total regression equation for gel point of LG gels.

Figure 3.4a. Effect of heating time and pH at 90 °C.

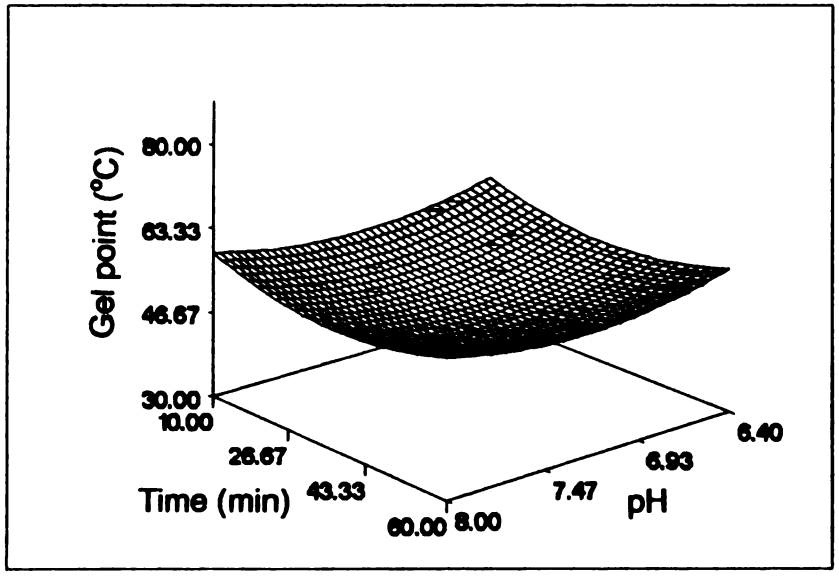
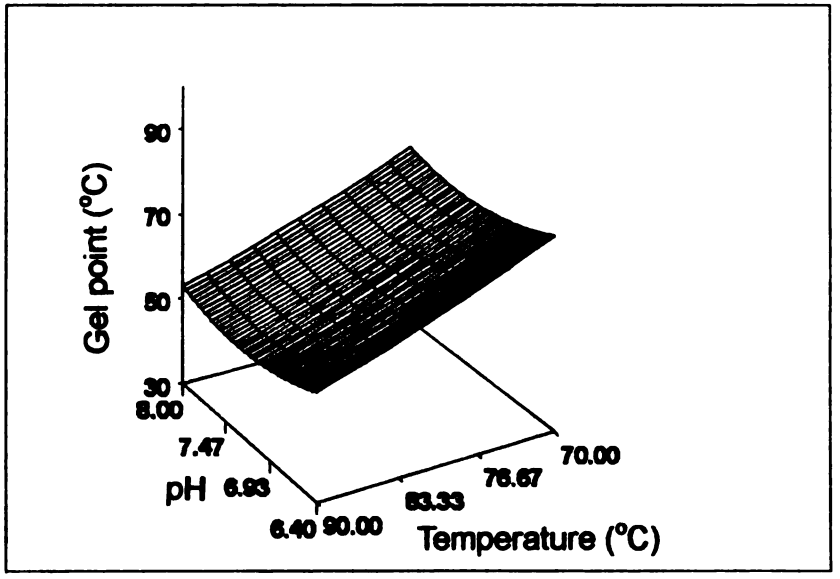


Figure 3.4b. Effect of heating temperature and pH at 40 min.



produced harder whey protein isolate gels. Hongsprabhas and Barbut (1996) also stated the same preheating effects on the textural properties of Ca^{2+} induced whey protein gels. Bryant and McClements (2000) examined the influence of holding temperature (65 to 90 °C), holding time (5 to 30 min), protein concentration (2 to 12 wt%) and pH (3 to 8) on the rheology and appearance of heat-denatured whey protein isolate (WPI) solutions. They reported that WPI gels with various gelation times (gelled within 5 min to 12 hr after addition of 0.2 M NaCl) can be produced by varying the preheating conditions. All these studies indicated that preheating conditions of whey protein (or LG) can change rheological properties of whey protein gels (or LG gels). When a food product with certain rheological properties is desired, food processors can use this unique characteristic of whey protein to fit their processing conditions. The results of this study further confirmed that under the conditions suitable for meat processing (71 °C, 0.3 M NaCl, pH 6.5), LG gels with various rheological properties can be produced through the manipulation of the preheating step.

Effect of preheating temperature, time, and pH on aggregation and gelation of LG

Figure 3.5 shows microstructure of LG at the three different pHs. As shown in Figure 3.5, even before heating, size and shape of the unheated LG changed as the pH changed. Caessens and others (1997) reported that depending on pH, LG can occur in different associated forms. At pH above 7.5 or below 3.5, LG occurred as monomers and as the pH moved to its isoelectric point (5.2), LG reversibly formed tetramers/octamers. Size of LG monomers and dimers are smaller than 0.0002 μm^2 (Haque and Sharma, 1997, Sharma and others, 1996). Our results indicated that at pH 8.0 and 7.2, mostly LG

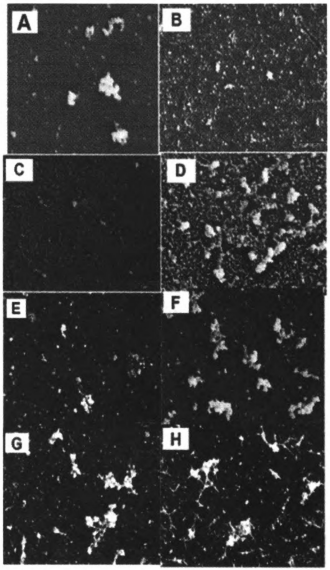


Figure 3.5. SEM images of β-lactoglobulin aggregates produced under various heating conditions. A: Control at pH 6.4. B: Control at pH 7.2. C: Control at pH 8.0. D: Preheated at 70 °C, 35 min, pH 7.2. E: Preheated at 80 °C, 35 min, pH 6.4. F: Preheated at 80 °C, 35 min, pH 7.2. G: Preheated at 80 °C, 35 min, pH 8.0. H: Preheated at 90 °C, 35 min, pH 7.2. Marker bar = 1 μm.

monomers and dimers were observed. As pH decreased to 6.4, association tendency of LG increased, and some large rounded shape LG aggregates were observed

Size distribution of heat denatured LG (HDLG) aggregates is presented in Table 3.4. Heat denatured LG aggregates were categorized into 5 categories. Aggregates smaller than 0.075 μm^2 were categorized as aggregate 1. Size of aggregate 2 was between 0.075 and 0.15 μm^2. Size of Aggregate 3 and 4 was between 0.15 and 0.3 μm^2 and 0.3 and 0.5 μm^2, respectively. Aggregates larger than 0.5 μm^2 were categorized as aggregate 5. Size distribution of HDLG aggregates was calculated as a percentage under each aggregate category.

As shown in Table 3.4, before heating at pH 8.0 and 7.2, all of the LG aggregates were $\leq 0.15\ \mu m^2$. As pH decreased to 6.4, some larger sizes of LG aggregates started to form. At pH 6.4, 16.7% of the LG aggregates were larger than $0.15\mu m^2$. Table 3.4 also indicates that no matter the preheating condition, preheating clearly changed size distribution of HDLG aggregates for all three different pHs. When heat was applied, the amount of small size HDLG aggregates decreased and the amount of large size HDLG aggregates increased.

Because no LG aggregate larger than 0.15 μm^2 was formed before heating at pH 8.0 and 7.4, HDLG aggregates were further divided into two groups. As shown in Table 3.5, size of aggregate A was smaller than 0.15 μm^2 and size of aggregate B was larger than 0.15 μm^2. The percentage of these two groups of HDLG aggregates was used to elucidate the relationship between HDLG aggregate size and rheological properties of LG gels. The effects of heating pH, temperature and time on HDLG aggregate size and

Table 3.4. Size distribution of LG aggregates produced under different preheating conditions

Preheating condition			Aggregate size distribution (%)[f]				
Temperature (°C)	Time (min)	pH	Aggregate 1[a]	Aggregate 2[b]	Aggregate 3[c]	Aggregate 4[d]	Aggregate 5[e]
Control at pH 6.4			75.8	7.5	3.5	5.5	7.5
85.9	49.9	6.4	60.3	15.5	11.6	9.1	3.8
85.9	20.2	6.4	51.3	19.0	22.3	8.0	0.0
80.0	35.0	6.4	76.2	8.5	9.0	3.0	3.0
74.1	49.9	6.4	73.5	13.5	6.0	6.0	1.0
74.1	20.2	6.4	74.0	12.8	8.8	2.6	2.6
Control at pH 7.2			93.3	6.8	0.0	0.0	0.0
90.0	35.0	7.2	41.2	31.6	18.2	4.0	5.1
80.0	60.0	7.2	61.4	20.5	10.0	6.0	2.5
80.0	35.0	7.2	29.2	24.6	31.6	6.3	9.3
80.0	10.0	7.2	50.0	16.4	22.9	5.0	6.0
70.0	35.0	7.2	32.5	32.5	29.8	6.0	0.0
Control at pH 8.0			90.0	10.0	0.0	0.0	0.0
85.9	49.9	8.0	42.3	19.3	19.5	15.8	3.5
85.9	20.2	8.0	44.0	33.5	14.5	7.5	0.0
80.0	35.0	8.0	59.3	15.3	12.8	5.2	7.7
74.1	49.9	8.0	70.6	16.0	11.1	0.0	2.4
74.1	20.2	8.0	84.5	7.0	5.2	4.8	0.0

[a]: Aggregate 1: aggregate size <0.075 μm^2

[b]: Aggregate 2: aggregate size between 0.075-0.15 μm^2

[c]: Aggregate 3: aggregate size between 0.15-0.3 μm^2

[d]: Aggregate 4: aggregate size between 0.3-0.5 μm^2

[e]: Aggregate 5: aggregate size >0.5μm^2

[f]: Aggregate size distribution was presented as percentage of each aggregate

Table 3.5. Size distribution of HDLG aggregates and rheological properties of LG gels produced under different preheating conditions.

Preheating condition			Aggregate size distribution[d]		Rheological property	
Temperature (°C)	Time (min)	pH	Aggregate A[a] (%)	Aggregate B[b] (%)	Storage modulus[c] (Pa)	Gel point (°C)
Control at pH 6.4			83.3	16.7	-	-
85.9	49.9	6.4	75.8	24.2	290.6	57.2
85.9	20.2	6.4	70.3	29.7	263.1	59.9
80.0	35.0	6.4	84.7	15.3	181.8	66.9
74.1	49.9	6.4	87.0	13.0	107.2	68.1
74.1	20.2	6.4	86.8	13.2	30.8	71.0
Control at pH 7.2			100.0	0.0	-	-
90.0	35.0	7.2	70.8	29.2	397.8	50.6
80.0	60.0	7.2	81.9	18.1	148.7	62.9
80.0	35.0	7.2	54.9	45.1	251.1	59.5
80.0	10.0	7.2	66.4	33.6	209.2	64.8
70.0	35.0	7.2	65.0	35.0	103.6	69.9
Control at pH 8.0			100.0	0.0	-	-
85.9	49.9	8.0	61.5	38.5	285.5	56.2
85.9	20.2	8.0	77.5	22.5	242.5	61.2
80.0	35.0	8.0	74.5	25.5	162.7	59.3
74.1	49.9	8.0	86.6	13.4	112.5	68.7
74.1	20.2	8.0	95.2	4.8	42.6	71.0

[a]: Aggregate A: aggregate size <0.15 μm^2

[b]: Aggregate B: aggregate size >0.15 μm^2

[c]: Storage modulus: storage modulus of LG gels after cooling to 25 °C

[d]: Aggregate size distribution was presented as percentage of each aggregate

shape were first analyzed, and the percentage of aggregate B was then statistically correlated with gel point and storage modulus (after cooling to 25 °C) of LG gels.

Effect of preheating pH on HDLG aggregate size and shape

Upon heating, the unfolding of LG molecules exposes LG thiol groups causing several aggregation reactions to take place. When unheated, decreasing pH increased the amount of large LG aggregates. However, under the heating conditions used in this study, adjusting pH did not seem to have a clear effect on the HDLG aggregate size. For example, at preheating conditions of 85.9 °C, 49.9 min, increasing pH produced more aggregate B. However, at preheating conditions of 85.9 °C, 20.2 min, increasing pH produced more aggregate A. At 85.9 °C, 49.9 min, increasing pH from 6.4 to 8.0 increased aggregate B amount from 24.2 to 38.5%. At 85.9 °C, 20.2 min, increasing pH from 6.4 to 8.0 decreased aggregate B amount from 29.7 to 22.5%.

Under the studied conditions, the change in pH may not be severe enough to affect the size distribution of HDLG aggregates. Haque and Sharma (1997) reported similar results. They reported that when 8% (w/v) LG was heated between 70 and 90 °C (5 min) at pH 3.5, 7.0 and 9.0, no clear correlation was found between pH and HDLG aggregate size. However, when LG was pre-incubated at high pH, different result was found. Harwalkar and Kalab (1985) reported that HDLG aggregates (pre-incubated at pH 11for 10 min at 22 °C, and then heated at 90 °C for 30 min) formed at pH 2.5 and 6.5 were smaller than those formed at pH 4.5.

SEM images of HDLG aggregates (Figure 3.5) show that changing pH in the preheating step changed HDLG aggregate shape, particularly when LG was preheated at high temperatures. When preheating pH was increased, HDLG aggregate shape changed

from a rounded shape to long and elongated shape. A clear transition in HDLG aggregate shape can be observed when LG was heated at 80 °C for 35 min. At pH 6.4, HDLG aggregates had a round shape. As pH increased, HDLG aggregate shape became irregular, and more thin and elongated HDLG aggregates were produced at pH 8.0. Similar results were reported by other researchers. Kavanagh and others (2000) stated that when pH moved away from isoelectric point of LG (5.2), HDLG aggregates had a rod like shape. Ikeda (2003) indicated that HDLG aggregates (produced by heating at 80 °C for 90 min) formed at pH 2 were thin and long, but HDLG aggregates formed at pH 7 had a round and globular shape. Electrostatic repulsion force increases when pH moves away from isoelectric point of LG. The increase in electrostatic repulsion force made HDLG molecules more rigid and unable to partake in spatial arrangement to accomplish optimum intermolecular contact, consequently thin and rod like HDLG aggregates were formed when pH moved away from 5.2.

Effect of preheating temperature on HDLG aggregate size and shape

Preheating temperature had profound effects on HDLG aggregate size. As shown in Table 3.5, increasing preheating temperature produced more aggregate B. At pH 8.0, preheating LG solution at 74.1 °C for 20.2 and 49.9 min only produced 4.8 and 13.4% of aggregate B, respectively. However, when preheating temperature increased to 85.9 °C, 22.5 and 38.5% of aggregate B was produced. The same phenomenon was also observed at pH 6.4. Similar results were also reported by Haque and Sharma (1997), Hoffmann and others (1996) and Aymard and others (1996). Sharma and others (1996) suggested that the increased HDLG aggregate size at high temperatures resulted from a balance of hydrophobic interactions of unfolded LG molecules, sulphydryl-disulfide exchange and

thermodynamic stability. Since association of HDLG aggregates would favor thermodynamic stability in the system, large HDLG aggregates were produced when temperature increased.

Increasing heating temperature also changed HDLG aggregate shape. As the preheating temperature increased from 70 to 80 and 90 °C (at pH 7.2, 35 min), HDLG aggregates changed from a more rounded shape to irregular, thin and linear shape. SEM image shows (Figure 3.5) that partial gel structure (filament-like gel network connections between HDLG aggregates) was observed when LG solution was preheated at 90 °C. Interestingly, LG gel produced with this shape of aggregate had lowest gel point and highest G' after cooling (25 °C).

Effect of preheating time on HDLG aggregate size and shape

Our results indicated that increasing preheating time changed HDLG aggregate size only when LG solutions were preheated at pH 8.0 and 7.2, and prolonging heating time would decrease HDLG aggregate size. At pH 8.0, prolonging heating time produced more large HDLG aggregates (Table 3.5). Preheating at 74.1 and 85.9 °C for 20.2 min, produced 4.8 and 22.5% aggregate B, respectively. Under the same temperatures and increasing preheating time to 49.9 min, 13.4 and 38.5% of aggregate B was produced. When heated at 80 °C, pH 7.2, as heating time increased from 10 to 35 min, the amount of aggregate B increased from 33.6 to 45.1%. However, the amount of aggregate B decreased from 45.1 to 18.1% when heating time further increased from 35 to 60 min, indicating that prolonging heating may break down large HDLG aggregates. Similar observation was found by Le Bon and others (1999), Photchanachai and Kitabatake (2001), and Schokker and others (2000).

Under the studied conditions, SEM images show (Figure 3.5) that increasing heating time did not change HDLG aggregate size. Regardless of heating time, HDLG aggregates produced with same heating temperature and pH had similar shape.

Correlation between HDLG aggregate size and rheological properties of LG gel

Table 3.5 shows LG gels produced with larger HDLG aggregates had lower gel point and higher G' values after cooling to (25 °C). McClements (1995) suggested that disulfide bond formation was most important in determining the final gel rigidity in whey protein isolate gels produced with preheated whey protein isolate. More sulphydryl groups were available when LG was heated at alkaline conditions (Manderson and others, 1999). As heating temperature and time increase, more sulphydryl-disulfide bond exchange and formation are observed (Croguennec and others, 2003, Prabakarn and Damodaran, 1997, Surroca and others, 2002). All these results suggested that more sulphydryl groups were available for building gel networks when LG was preheated for a longer heating time at higher temperatures and pHs. The increased G' and decreased gel point of LG gels were probably due to the increased disulfide bond formation during the initial heating step.

The correlations between HDLG aggregate size and gel point and G' of LG gels were statistically analyzed. The effects of HDLG aggregate size on G' and gel point of LG gels were both significant ($p<0.05$). HDLG aggregate size had a positive correlation with G' of LG gels (correlation coefficient = 0.62) indicating that increasing HDLG size produced LG gels with higher G' value after cooling (25 °C). A negative correlation between HDLG aggregate size and LG gels point was found (correlation coefficient = −0.58) confirming that increasing HDLG aggregate size produced LG gels with lower gel

point. Similar effect of increased aggregate size on increased gel hardness of whey protein isolate cold-set gels (gels induced at 37° C by adding salt in preheated whey protein isolate solution) was reported by Ju and Kilara (1998a).

Effect of preheating temperature, time and pH on microstructure of LG gels

Protein gel structure can be categorized into two types: fine-stranded gels and particulate aggregate gels. Depending on the gelation conditions, salt can induce both aggregated and fine-stranded type of LG gels and type of LG gels formed can be influenced by type and concentration of salt. At high salt concentration, aggregated gel can be formed composed of short and thick strands. On the other hand, when salt concentration is low, fine-stranded gels are formed (Barbut 1995, Marangoni and others, 2000, Roff and Foegeding, 1996). Under the gelling condition used in this study (pH 6.5, 0.3 M NaCl, 0.05 M phosphate buffer), HDLG aggregates formed white opaque gels. The opaque gel color was due to light scattering by HDLG aggregate strands indicated globular or aggregated gels (Hongsprabhas and Barbut 1998).

Microstructures of LG gels produced with HDLG aggregates preheated at different preheating conditions are shown in Figure 3.6. Although size and shape of preheated whey protein aggregates have been reported to affect gel structure of whey protein cold-set gel (Hongsprabhas and Barbut, 1998b), HDLG aggregate size did not have a large affect on the microstructure of our globular LG gels. And changing heating conditions in the preheating step did not change the microstructure of LG gels.

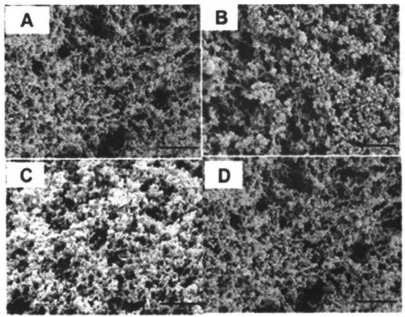


Figure 3.6. SEM images of β-lactoglobulin (LG) gels formed with heat denatured β-lactoglobulin aggregates produced under different preheating conditions. A: Preheated at 90 °C, 35 min, pH 7.2. B: Preheated at 80 °C, 35 min, pH 7.2. C: Preheated at 74.1 °C, 49.9 min, pH 8.0. D: Preheated at 80 °C, 35 min, pH 6.4. LG was preheated at 7% and diluted to 2% with 0.42 M NaCl, 0.05 M phosphate buffer, pH 6.5. Marker bar = 5 μm.

Conclusions

LG gels with various rheological properties were produced through the manipulations of the preheating conditions. Increasing preheating temperature increased LG gel hardness and decreased LG gel point. After the first step of heating, various sizes and shapes of HDLG aggregates were formed and large HDLG aggregates tended to produce more elastic LG gels. However, there was no correlation found between LG microstructure and preheating condition of LG.

To our knowledge, this is the first study focused on the relationship between HDLG aggregate size and rheological properties of LG gels under conditions suitable for meat processing and all experiments in this study were designed to mimic meat processing conditions. The results of this study suggested that preheating treatment can increase the potential use of whey proteins in processed meat products when lower meat processing temperatures are favored. Under the studied conditions, response surface graphs predicted that preheating LG solution at 90 °C, 40 min, pH 7.0 would produce LG gels with high G' and low gel point. This provides some background information to choose preheating conditions for whey protein in application of processed meat products. However, more research is needed to verify the applicability of the preheating conditions in whey proteins.

References

Aymard P, Durand D, Nicolai T. 1996. The effect of temperatures and ionic strength on the dimerisation of β-lactoglobulin. Int. J. Biol. Macromol. 19: 213-221.

Bryant CM, McClements DJ. 2000. Optimizing preparation conditions for heat-induced whey protein solution to be used as cold-gelling ingredients. J. Food Sci. 65: 259-263.

Barbut S, Foegeding EA. 1993. Ca^{2+}-induced gelation of pre-heated whey protein isolate. J. Food Sci. 58: 867-871.

Barbut S. 1995. Effects of calcium level on the structure of preheated whey protein isolate gels. Can. Inst. Food Sci. Tech. 28:598-603.

Busti P, Scarpeci S, Gatti C, Delorenzi N. 2002. Use of fluorescence methods to monitor unfolding transitions in β-lactoglobulin. Food Res. Int.. 35: 871-877.

Caessens PWJR, Gruppen SVH. 1997. Method for the isolation of bovine β-lactoglobulin from a cheese whey protein fraction and physicochemical characterization of the purified product. Int. Dairy J. 7: 229-235.

Cairoli S, Iametti S, Bonomi F. 1994. Reversible and irreversible modifications of β-lactoglobulin upon exposure of heat. J. Protein Chem. 13(3): 347-354.

Croguennec T, Bouhallab S, Mollé D, O'Kennedy BT, Mehra R. 2003. Stable monomeric intermediate with exposed Cys-119 is formed during heat denaturation of β-lactoglobulin. Biochem Biophys. Res. Commun. 301: 465-471.

Doi E. 1993. Gels and gelling of globular protein. Trends Food Sci. Tech. 4: 1-5.

Foegeding EA, Kuhn PR, Hardin CC. 1992. Specific divalent cation-induced changes during gelation of β-lactoglobulin. J. Agri. Food Chem. 40: 2092-2097.

Galani D, Apenten RKO. 1999. Heat-induced denaturation and aggregation of β-lactoglobulin: kinetics of formation of hydrohobic and disulfide-linked aggregates. Int. J. Food Sci. Tech. 34: 467-476.

Haque ZU, Sharma M. 1997. Thermal gelation of β-lactoglobulin AB purified from cheddar whey. 1. Effect of pH on association as observed by dynamic light scattering. J. Agric. Food Chem. 45: 2985-2963.

Harwalkar VR, Kalab M. 1985. Microstructure of isoelectric precipitates from β-Lactoglobulin solutions heated at various pH values. Milchwiss. 40: 665-668.

Hoffmann MAM, Roefs SPFM, Verheul M, Van Mil PJJM, De Kruif KG. 1996. Aggregation of β-Lactoglobulin studied by *in situ* light scattering. J. Dairy Res. 63: 441-449.

Hoffmann MAM, van Mil JJM. 1997. Heat-induced aggregation of β-lactoglobulin: role of the free thiol group and disulfide bonds. J. Agric. Food Chem. 45: 2942-2948.

Hoffmann MAM, van Mil JJM. 1999. Heat-induced aggregation of β-lactoglobulin as function of pH. J. Agric. Food Chem. 47: 1898-1905.

Hongsprabhas P, Barbut S. 1996. Ca^{2+} - induced gelation of whey protein isolate: effects of pre-heating. Food Res. Int. 29: 135-139.

Hongsprabhas P, Barbut S. 1998a. Ca^{2+} - induced gelation of whey protein isolate: effect of two-stage gelation. Food Res. Int. 30: 523-527.

Hongsprabhas P, Barbut S. 1998b. Structure-forming process in Ca^{2+} - induced whey protein isolate cold gelation. Int. Dairy J. 7: 827-834.

Hongsprabhas P, Barbut S. 1999a. Effect of preheated whey protein level and salt on texture development of poultry meat batters. Food Res. Int. 32: 145-149.

Hongsprabhas P, Barbut S. 1999b. Use of cold-set whey protein gelation to improve poultry meat batters. Poultry Sci. 78: 1074-1078.

Hsieh YL, Regenstein JM, Rao A. 1993. Gel point of whey and egg proteins using dynamic rheological data. J. Food Sci. 58(1): 116-119.

Ikeda S. 2003. Atomic force microscopy and Raman scattering spectroscopy studies on heat-induced fibrous aggregates of β-lactoglobulin. Spectroscopy. 17: 195-202.

Ju Z, Kilara A. 1998a. Effects of preheating on properties of aggregates and cold-set gels of whey protein isolate. J. Agric. Food Chem. 46: 3604-3608.

Ju Z, Kilara A. 1998b. Textural properties of cold-set gels induced from heat-denatured whey protein isolates. J. Food Sci. 63: 288-292.

Kavanagh GM, Clark AH, Ross-Murphy SB. 2000. Heat-induced gelation of globular proteins: part 3. Molecular studies on low pH β-lactoglobulin gels. Int. J. Biol. Macromol. 28: 41-50.

Langton M, Hermansson A. 1992. Fine-stranded and particlate gels of β-Lactoglobulin and whey protein at varying pH. Food Hydrocolloids. 5(6): 523-539.

Le Bon C, Nicolai T, Durand D. 1999. Growth and structure of aggregates of heat-denatured β-Lactoglobulin. Int. J. Food Sci. Tech. 34: 451-465.

Manderson GA, Creamer LK, Hardman MJ. 1999. Effect of heat treatment on the circular dichroism spectra of bovine β-lactoglobulin A, B and C. J. Agric. Food Chem. 47: 4557-4567.

Marangoni AC, Barbut S, McGauley SE, Marcone M, Narine SS. 2000. On the structure of particulate gels: the case of salt-induced cold gelation of heat-denatured whey protein isolate. Food Hydrocolloids. 14: 61-74.

MacLeod A, Fedio WM, Ozimek L. 1995. Aggregation of β-Lactoglobulin as a function of pH and temperature. Milchwiss. 50: 666-669.

McClements DJ, Keogh MK. 1995. Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. J. Sci. Food Agric. 69: 7-17.

McKenzie HA, Sawyer WH. 1967. Effect of pH on β-Lactoglobulins. Nature. 214: 1101-1104.

McSwiney M, Singh H, Campanella OH. 1994. Thermal aggregation and gelation of bovine β-lactoglobulin. Food Hydrocolloids. 8(5): 441-453.

Mleko S, Foegeding EA. 1999. Formation of whey protein polymers: effects of a two-step heating process on rheological properties. J. Text. Stud. 30: 137-149.

Photchanachai S, Kitabatake N. 2001. Heating of β-lactoglobulin A solution in a closed system at high temperatures. J. Food Sc. 66(5): 647-652.

Prabakaran S, Damodaran S. 1997. Thermal unfolding of β-lactoglobulin: characterization of initial unfolding events responsible for heat-induced aggregation. J. Agric. Food Chem. 45: 4303-4308.

Roff CF, Foegeding EA. 1996. Dicationic-induced gelation of pre-denatured whey protein isolate. Food Hydrocolloids. 10: 193-198.

Schokker EP, Singh H, Pinder DN, Norris GE, Creamer LK. 1999. Characterization of intermediates formed during heat-induced aggregation of β-lactoglobulin AB at neutral pH. Int. Dairy J. 9: 791-800.

Schokker EP, Singh H, Pinder DN, Creamer LK. 2000. Heat-induced aggregation of β-lactoglobulin AB at pH 2.5 as influenced by ionic strength and protein concentration. Int. Dairy J. 10: 233-240.

Sharma M, Haque ZU, Wilson WW. 1996. Association tendency of β-Lactoglobulin AB purified by gel permeation chromatography as determined by dynamic light scattering under quiescent conditions. Food Hydrocolloids. 10(3): 323-328.

Stading M, Hermansson A. 1990. Viscoelastic behaviour of β-lactoglobulin gel structures. Food Hydrocolloids. 4(2): 121-135.

Stading M, Hermansson A. 1991. Large deformation properties of β-lactoglobulin gels structures. Food Hydrocolloids. 5(4): 339-352.

Steventon AJ, Gladden LF, Fryer PJ. 1991. A percolation analysis of the concentration dependence of the gelation of whey protein concentrates. J. Text. Stud. 22: 201-218.

Surroca Y, Haverkamp J, Heck AJR. 2002. Towards the understanding of molecular mechanisms in the early stages of heat-induced aggregation of β-lactoglobulin AB. J. Chroma. 970: 275-285.

Vittayanont V, Steffe JF, Flegler SL, Smith DM. 2002. Gelling properties of heat-denatured β-lactoglobulin aggregates in a high-salt buffer. J. Agric. Food Chem. 50: 2987-2992.

Weber K, Osborn M. 1969. The reliability of molecular weight determinations by codicil sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406-4412.

Xiong YL, Dawson KA, Wan L. 1993. Thermal aggregation of β-lactoglobulin: Effect of pH, ionic environment, and thiol reagent. J. Dairy Sci. 76: 70-77.

CHAPTER 4

SELECTING PREHEATING CONDITIONS FOR WHEY PROTIEN ISOLATE IN APPLICATION OF HIGHLY COMMINUTED MEAT PRODUCTS

ABSTRACT

Emulsion stability of high added fat and high added water emulsions prepared with preheated whey protein isolate (WPI) and beef meat was investigated. Three preheating conditions for WPI: 1). Preheating at 95 °C, 40 min, 2). Preheating at 95 °C, 10 min, 3). Preheating at 85 °C, 80 min, were selected. WPI was preheated at 8% (w/w) and pH 7.0. WPI without preheating treatment was used as control. Using a standard meat emulsion formula, WPI was added at 3.5 and 7% level. Emulsion stability of high added fat and high added water emulsions prepared with WPI were significantly affected by WPI preheating condition and WPI level ($p<0.05$). Preheating WPI increased emulsion stability of both high added fat and high added water emulsions prepared with WPI ($p<0.05\%$). Increasing WPI level from 3.5 to 7.0% also increased emulsion stability of high added fat and high added water emulsions prepared with WPI ($p<0.05$). Among the three preheated WPIs, emulsion prepared with WPI preheated at 95 °C for 40 min had the best emulsion stability and emulsion prepared with WPI preheated at 85 °C for 80 min had the worst emulsion stability. While beef meat emulsions were more effective at binding fat, preheated WPI emulsions were more effective at binding water. The results of this study demonstrated the potential of preheated WPI in emulsified meat products, especially when a high added water emulsion formula is used.

Chapter 4 is formatted in manuscript style according to the Journal of Food Science

Keywords: preheating, whey protein isolate, emulsion stability, frankfurters

Introduction

The ability of whey proteins to form gels has increased their use in meat products as binders or extenders to increase yield and decrease cost. However, the high gelation temperature (above 70 °C) of whey proteins has been an obstacle for meat processors since most whey proteins do not form gels under meat processing temperatures (60 to 70 °C). As a result, whey proteins are not used to their fullest potential.

To decrease the gelation temperature of whey proteins, researchers found that preheating whey proteins can effectively decrease their gelation temperature (Barbut and Foegeding, 1993, Bryant and McClements, 2000a). Not only the gelation temperature of whey proteins is decreased after preheating treatment, other functional properties of whey proteins are also modified (Bryant and McClements, 1998, Hongsprabhas and Barbut, 1996, 1997, Ju and Kilara, 1998a, 1998b, Kitabatake and others, 1996). Recently, a two-step heating process (a pre-heating step, and a gelling step) of whey proteins was employed to improve the quality attributes of poultry meat batters. When incorporated in poultry meat batters, the meat batters prepared with preheated whey protein isolate (10% whey protein isolate (w/v) preheated at 80 °C for 30 min, pH 7.0) had higher cooking yield and water holding capacity than those prepared with unheated whey protein isolate (Hongsprabhas and Barbut, 1999a, 1999b). Since increased cooking yield and water holding capacity are desired by the meat processors, this finding has opened the door for meat and whey processors to find a better way to utilize whey proteins.

To maximize the use of preheated whey protein polymers, researchers suggested that whey proteins can be tailor-made for specific meat products (Beuschel and others, 1992a, 1992b). Shie and others (2002) utilized response surface methodology to study

the impact of preheating on rheological properties of β-lactoglobulin (LG) (major protein responsible for gelling properties in whey) gels prepared with the preheated LG. They reported that LG gels with various rheological properties can be produced through the manipulation of the preheating conditions. They also stated that under conditions suitable for meat processing, LG gels with low gel point and high gel hardness could be produced by preheating LG at pH 7.0 and 40 min. Hendrix and others (2002) investigated textural properties of salt soluble beef protein (SSP) gels prepared with preheated whey protein isolate (WPI, preheated at 8% (w/v)). The mixed gels prepared with WPI preheated at 95 °C tended to have low expressible moisture. When SSP was incorporated with WPI preheated at 95 °C for 10 min and 85 °C, 80 min, the mixed gels tended to have high gel hardness and high gel stress, respectively. Both studies were carried out under conditions suitable for manufacturing of emulsified meat products (heating to 68 and 71 °C, 0.3 to 0.4 M NaCl, pH 6.5).

The objective of this study was to: 1). Evaluate the use of preheated WPI in high added fat and high added water meat and WPI emulsions. 2). Test the applicability of preheated WPI in emulsified meat products. To achieve the objectives, a standard meat emulsion formula was used. The two major protein components in the formula: meat protein and whey protein; were separated to evaluate the fat binding and water binding abilities of the individual protein. Base on the previous findings on LG gels and SSP and preheated WPI mixed gels, three preheating conditions for whey protein isolate: 1). 95 °C, 40 min, pH 7.0. 2). 95 °C, 40 min, pH 7.0. 3). 85 °C, 80 min, pH 7.0; were selected to be further tested for their applicability in emulsified meat products.

Materials and Methods

Manufacturing and spray-drying of preheated whey protein isolate

Alacen 895 whey protein isolate (WPI) was purchased from New Zealand Milk Products (Lot# 047U45283431314, Lemoyne, PA) and stored at 4 °C until use. To manufacture large quantities of preheated WPIs, preheated WPIs were manufactured using a heat exchanger and industrial scale spray-dryer. Preheated WPI was manufactured at Michigan State University Dairy plant as described in Chapter 2. 8% WPI (w/w) solution was preheated according to the preheating conditions. The preheated WPI was spray-dried and stored in air-tight containers at 4 °C until use.

Manufacturing of WPI and beef meat emulsions

Table 4.1 shows the basic beef frankfurter formulation used to formulate WPI and beef meat emulsions. Four different formulas were created from the basic formula to produce high added fat and high added water WPI and beef meat emulsions.

Table 4.1. Beef frankfurter formulation

	Control		3.5% Whey isolate		7.0% Whey isolate	
Ingredient	%	Weight (g)	%	Weight (g)	%	Weight (g)
Beef (80/20)	40.02	400.20	36.52	365.20	33.02	330.20
Water	35.00	350.00	35.00	350.00	35.00	350.00
Fat	20.00	200.00	20.00	200.00	20.00	200.00
Salt	1.80	18.00	1.80	18.00	1.80	18.00
Corn Syrup Solids	2.10	21.00	2.10	21.00	2.10	21.00
Whey protein isolate	0.00	0.00	3.50	35.0	7.00	70.00
Spice (no salt added)	0.60	6.00	0.60	6.00	0.60	6.00
FMC phosphate blend	0.30	3.00	0.30	3.00	0.30	3.00
Na Erythorbate	0.03	0.30	0.03	0.30	0.03	0.30
Cure Salt	0.15	1.50	0.14	1.40	0.13	1.30
Total	100.00	1000.00	100.00	1000.00	100.00	1000.00

High added fat WPI emulsion

Table 4.2 shows the formulas for high added fat WPI emulsions. These formulas were created to evaluate the fat holding ability of WPI when WPI was the only protein source. In manufacturing beef frankfurter emulsions, usually one half of the total amount of water in the formula is used to assist in protein extraction, so only one half of the total amount of water was used to make the emulsions. In this high fat WPI emulsion, half of the water was replaced by fat (175 g).

Applicable amounts of WPI and beef fat were mixed at 1200 rpm for 30 sec using a Stephan UMC 5 electronic vertical mixer (Stephan Machinery Corporation, Columbus, OH). Water and salt were then added and the mixture was mixed under vacuum (35 mm Hg) at 2100 rpm for 4.5 min. Final emulsion temperature was around 30 ± 2 °C.

Table 4.2. Formulation for high added fat whey protein isolate emulsions[1]

Ingredient	3.5% whey protein isolate		7.0% whey protein isolate	
	Weight (g)	%	Weight (g)	%
WPI	35	8.49	70	15.7
Beef fat	200	48.5	200	44.7
Water	175	42.5	175	39
Salt	2	0.5	2	0.5
Total	412	100	447	100

[1] Formulas determined from original beef frankfurter formula. Beef, phosphate, corn syrup solids, spices, erythorbate, cure salt, one-half water volume (175 g) and 87.5% salt (16 g) removed. Total fat (200g), one-half water (175 g) and 12.5% of total salt (2 g) of original beef frankfurter formulation combined with WPI to manufacture fat emulsions.

High added fat or high added water beef meat emulsion

Table 4.3 and 4.4 show the formulas for high added fat and high added water beef meat emulsions. In these formulas, beef meat was the only protein source (400 g). Four percent of salt has been found to be the optimum amount to extract meat protein. For this

reason, 4% of salt (16 g, based on beef meat weight) was used in these formulas to extract meat protein and 3 g of phosphate from the original formula was also added.

To produce beef meat emulsions, applicable amounts of beef, salt, and one half of water-ice were first mixed for 1.5 min. Phosphate, beef fat and the rest of the water-ice were added and the mixture was mixed for 3.5 more min under vacuum (35 mm Hg). Final emulsion temperature was around 11 ± 2 °C.

Table 4.3. Formulation for high added fat beef meat emulsion[1]

Ingredient	Weight (g)	%
Beef meat	400	50.3
Beef fat	200	25.2
Water	175	22.1
Salt	16	2
Phosphate	3	0.4
Total	794	100

[1] Formula determined from original beef frankfurter formula. Corn syrup solid, spices, erythorbate, cure salt, one-half water volume (175 g) and 12.5% salt (2 g) removed. Total fat (200 g), one-half water (175 g), phosphate (3 g), and 87.5% salt (16 g – 4% concentration when added to beef protein) of original beef frankfurter formulation combined with beef (400 g) to manufacture fat emulsion.

Table 4.4. Formulation for high added water beef meat emulsion[1]

Ingredient	Weight (g)	%
Beef meat	400	52.0
Water	350	45.5
Salt	16	2.1
Phosphate	3	0.4
Total	769	100

[1] Formula determined from original beef frankfurter formula. Fat, corn syrup solids, spices, erythorbate, cure salt, and 12.5% salt (2 g) removed. Total water (350 g), phosphate (3 g), and 87.5% salt (16 g – 4% concentration when added to beef protein) of original beef frankfurter formulation combined with beef (400 g) to manufacture high added water emulsion.

High added water WPI emulsion

A high added water emulsion was formulated as shown in Table 4.5. All the water from the basic emulsion formula was used with no fat added.

Applicable amounts of WPI, water and salt were added to a Stephan mixer and mixed for 5 min under vacuum. Final emulsion temperature was around 23 ± 3 °C.

Table 4.5. Formulation for high added water whey protein isolate emulsions[1]

Ingredient	3.5% whey protein isolate		7.0% whey protein isolate	
	Weight (g)	%	Weight (g)	%
WPI	35	9.1	70	16.6
Water	350	90.4	350	82.9
Salt	2	0.5	2	0.5
Total	387	100	422	100

1.Formulas determined from original beef frankfurter formula. Beef, fat, phosphate, corn syrup solids, spices, erythorbate, cure salt and 87.5% salt (16 g) removed. Total water (350 g) and 12.5% of total salt (2 g) of original beef frankfurter formulation combined with WPI to manufacture high added water emulsions.

Emulsion stability measurement

Prepared WPI or beef meat emulsions (33.3 g) were stuffed into 50 ml polycarbonate tubes using a hand stuffer (Nalge Nunc International, Rochester, NY). The prepared tubes were cooked in a program-controlled water bath (Model 9510, PolyScience, Niles, IL) until the internal temperature reached 71.1 °C. After cooking, the tubes were chilled to 0 °C, and the fluid released during cooking was collected in 15 ml centrifugation tubes. The centrifugation tubes were centrifuged (Sorvall RC 5B plus, Sorvall Instruments, Newtown, CT) at 6,000 $\times g$ for 5 min. The volumes of the total liquid released, fat released and water released were then recorded.

Solubility and proximate composition of whey protein isolate

Solubility of WPI was determined following method by Morr and others (1985) as described in Chapter 2. Protein, moisture, ash and fat content of preheated and unheated WPI were determined according to AOAC methods 930.29A (micro-Kjeldahl method),

927.05 (vacuum oven drying method), 930.30 (dry ashing) and 932.06 (Mojonnier ether extraction method), respectively. All samples were measured in triplicate.

pH determination

An Accumet pH meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA) was used to measure pH of WPI. Samples (10 ± 0.1g) were weighed out in a 400 ml beaker with 90 ml deionized water, stirred at room temperature for 10 min. Whey protein isolate solution pH was then measured. All samples were measured in triplicate.

Experimental design and statistics analysis

All experiments were replicated three times. For each replication, three samples were analyzed. To evaluate the use of preheated WPI for application in emulsified meat products, a four factor (1. WPI without preheating, 2. WPI preheated at 95 °C for 10 min, 3. WPI preheated at 95 °C for 40 min, and 4. WPI preheated at 85 °C, 80 min), two level (3.5 and 7.0% WPI) complete randomized design was used in this study. Emulsion stability of WPI and beef meat emulsions was measured to analyze the impact of WPI preheating condition and concentration on the emulsion stability of WPI and meat emulsions. Interactions between experiment factor and WPI level were checked and significant level of difference was set at a predetermined level (Tukey's adjusted p-value and $p<0.05$) (SAS version 8.2, SAS Institute Inc., Cary, NC).

SAS LS means procedure was used to determine the means for pH, solubility and proximate compositions of WPI. Means were compared to determine significant

difference using Tukey test with the mean square error at 5% level of probability (SAS version 8.2, SAS Institute Inc., Cary, NC).

Results and Discussion

Proximate composition and pH of unheated and preheated WPIs are shown in Table 4.6. Alacen 895 WPI had very low fat and ash contents (0.58 and 2.45%, respectively). The fat content of WPIs decreased slightly after preheating. There was no difference ($p<0.05$) in ash or protein contents of all WPIs. Unheated WPI had a pH around 6.7 and all three preheated WPIs were preheated at pH 7.0. To minimize the pH difference between unheated and preheated WPIs, after the preheating step, pH of the preheated WPIs was adjusted back to 6.7. Although pH of preheated WPI solutions was adjusted back to 6.7 before spray-drying, WPIs preheated at 95 °C, 10 min and 95 °C, 40 min had higher ($p<0.05$) pH than unheated and WPI preheated at 85 °C, for 80 min. While moisture content of WPI decreased, protein content of WPI increased after preheating. Differences found in moisture and protein content between unheated and heated WPIs may due to combination of the spray-drying and storage condition. Unheated WPI may absorb some moisture during the storage and this may contribute to the differences in proximate composition between unheated and heated WPIs.

Beuschel and others (1992a) reported that after preheating of liquid whey, solubility of the whey protein concentrate decreased. Similar results were observed in this study. The solubility of spray-dried preheated WPIs decreased and WPI preheated at 85 °C for 80 min had the lowest solubility (23.1%) among all preheated WPIs. Heating exposed hydrophobic regions of whey protein and degree of denaturation in preheated WPIs depended on the heating time and heating temperature (Bryant and McClements, 1998). The decreased solubility in preheated WPIs was due to the combination of the exposed hydrophobic sites and denaturation.

Table. 4.6. Least square means for proximate composition, solubility and pH of WPI prepared with different preheating condition[1].

Preheating Condition	Without preheating	95°C,10min[d]	95°C,40min[d]	85°C,80min[d]	SEM[e]
Moisture %	9.37[a]	5.52[b]	5.50[b]	5.17[c]	0.04
Fat %	0.58[a]	0.39[b]	0.27[c]	0.24[c]	0.03
Ash %	2.45[a]	2.82[a]	2.43[a]	2.55[a]	0.14
Protein %	88.41[a]	91.89[a]	91.36[a]	91.07[a]	2.43
Solubility %	92.1[a]	31.5[b]	26.8[c]	23.1[c]	1.57
pH	6.73[c]	6.78[b]	6.82[a]	6.71[c]	0.01

[a-c]: Presence of different letters within same row indicates values which differed significantly within each

[d]: WPI was preheated at 8% (w/w), pH: 7.0

[e]: Standard error of the mean (SEM)

Emulsion stability of high fat emulsion

Figure 4.1 shows the emulsion stability of high added fat WPI and beef meat emulsions. Emulsion stability of high added fat emulsions was significantly ($p<0.05$) affected by both WPI level and WPI preheating conditions. Figure 4.1a shows total fluid released from WPI and beef meat emulsions after cooking. Beef meat emulsion had a better emulsion stability than WPI emulsions. Only 9.7 ml of total fluid was released after cooking. Among all WPIs, emulsions prepared with unheated WPI had the worst emulsion stability and emulsions prepared with WPI preheated at 95 °C, 40 min had the best emulsion stability.

As WPI amount increased, emulsion stability of WPI emulsions increased. At 3.5% WPI level, after cooking, 14.1 ml of total fluid was released from WPI emulsion prepared with WPI preheated at 95 °C, 40 min. When whey protein level increased to 7.0%, only 1.5 ml of total fluid was released after cooking.

Figure 4.1b shows the amount of fat released after cooking. The amount of fat released followed the same trend as the amount of total fluid released, and interestingly, there was no fat released from the beef meat emulsion after cooking.

The amount of water released after cooking from beef meat and WPI emulsions is shown in Table 4.7. The amount of water released from WPI emulsion prepared with WPI without preheating treatment was significantly higher ($p<0.05$) than those prepared with preheated WPIs. The most water released (9.7 ml) was found in the beef meat emulsion. Comparing the amount of total fluids, water and fat released, our results indicated that WPI emulsions were able to hold water better and beef meat emulsions were able to hold fat better.

Figure 4.1. Two way interactions ($p<0.05$) for emulsion stability of high added fat emulsions prepared with unheated, or preheated whey protein isolate (WPI), or beef meat.

Figure 4.1a. Total liquid released.

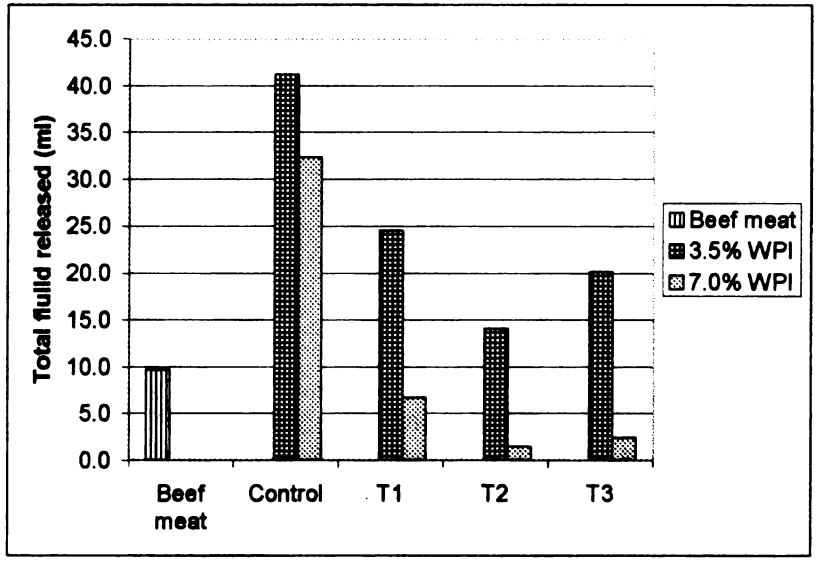
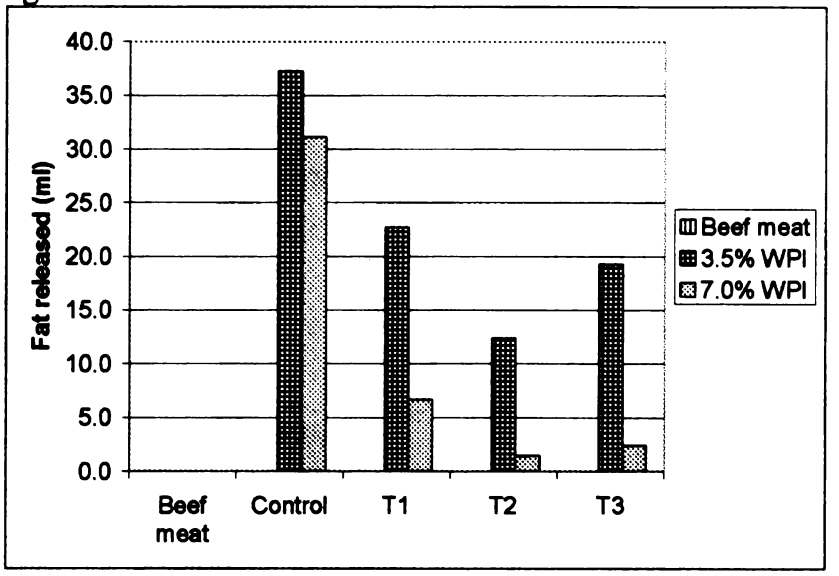


Figure 4.1b. Fat released.



Control: whey protein isolate without preheating treatment. T1: whey protein preheated at 95 °C, 10 min. T2: whey protein isolate preheated at 95 °C, 40 min. T3: whey protein isolate preheated at 85 °C, 80 min. Total liquid, fat and water released is based on 100 g of sample weight.

Table 4.7. Least square means for emulsion stability of high added water or high added fat emulsions prepared with unheated, or preheated WPI, or beef meat.

Emulsion type (fluid released)	Control[c]	Treatment 1 95 °C, 10 min	Treatment 2 95 °C, 40 min	Treatment 3 85 °C, 80 min	SEM[d]	Beef meat	Whey protein level 3.5%	Whey protein level 7.0%	SEM[d]
High added fat (water released in ml[e])	4.91[a]	0.9[b]	0.9[b]	0.4[b]	0.57	9.7[a]	2.1[b]	0.25[b]	1.02
High added water (fat released in ml[e])	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0	1.9[a]	0[b]	0[b]	0.43

[a-b]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[c]: Control: whey protein isolate without preheating treatment

[d]: Standard error of the mean (SEM)

[e] : Total liquid, fat and water released is based on 100 g of sample weight.

Regenstein and Imm (1998) suggested that dispersed whey proteins could help immobilize water and fat in the emulsion system. The increased emulsion stability of high fat preheated WPI emulsions was probably caused by the exposed hydrophobic regions of preheated WPI. The exposed hydrophobic sites of preheated WPI interacted with fat molecules and stabilized them in the emulsions. This resulted in the higher emulsion stability of high fat preheated WPI emulsions.

Emulsion stability of high water emulsion

Both WPI level and WPI preheating condition affected emulsion stability of high added water emulsions (<0.05). Figure 4.2a shows total fluid released after cooking from high added water WPI and beef meat emulsions. Among all WPIs, WPI emulsions prepared with unheated WPI had the worst emulsion stability. At 3.5% WPI level, 100ml of fluid was released from emulsion prepared with unheated WPI. WPI emulsion prepared with WPI preheated at 95 °C, 40 min had the best emulsion stability, as only 6.7 ml of total fluid was released after cooking. As WPI level increased to 7.0%, no fluid was released from emulsions prepared with preheated WPIs, and 0.6 ml of total fluid was released from WPI emulsion prepared with unheated WPI. For beef meat emulsions, 23.5 ml of total liquid was released after cooking.

Figure 4.2b shows the amount of water released from WPI and beef meat emulsions. As no fat was added in high added water WPI emulsions, amount of water released was same as total fluid released in high added water WPI emulsions. For high added water beef meat emulsion, 21.6 ml of water was released after cooking. Amount

Figure 4.2. Two way interactions ($p<0.05$) for emulsion stability of high added water emulsions prepared with unheated, or preheated whey protein isolate (WPI), or beef meat.

Figure 4.2a. Total liquid released.

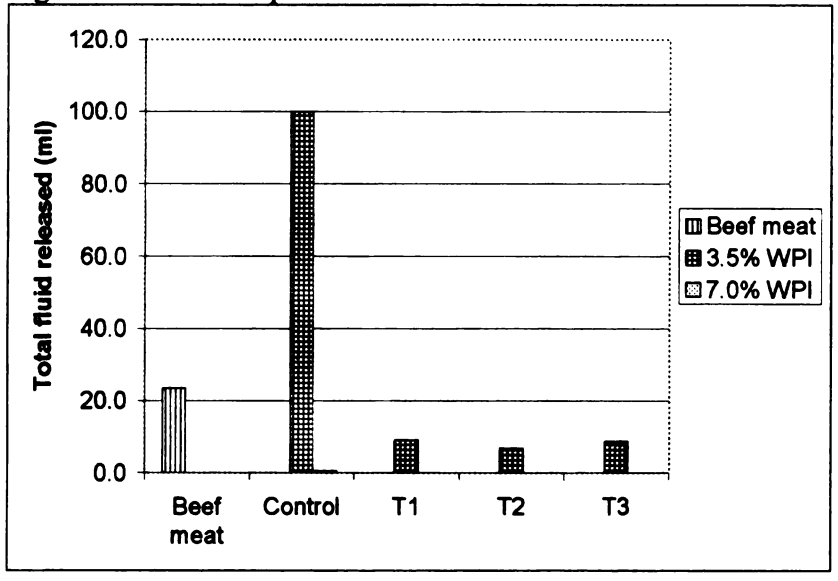
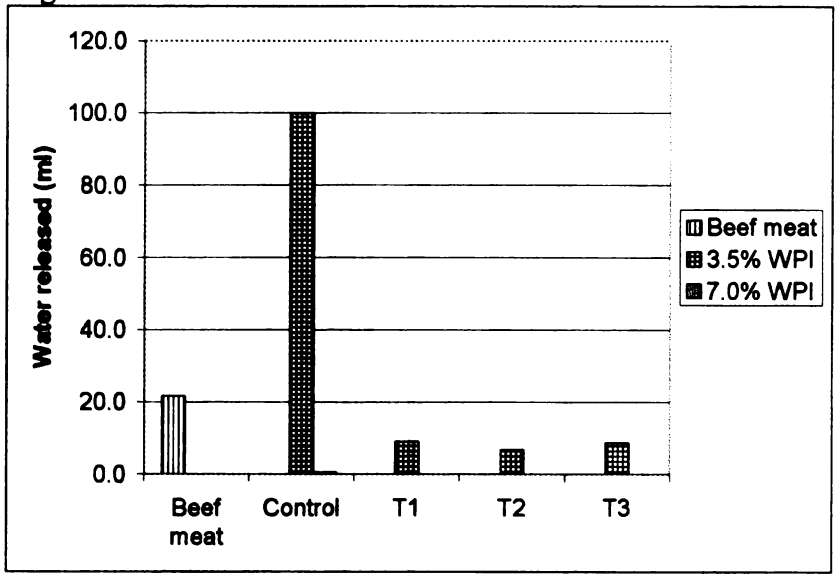


Figure 4.2b. Water released.



Control: whey protein isolate without preheating treatment. T1: whey protein preheated at 95 °C, 10 min. T2: whey protein isolate preheated at 95 °C, 40 min. T3: whey protein isolate preheated at 85 °C, 80 min. Total liquid, fat and water released is based on 100 g of sample weight.

of fat released from high added water WPI and beef meat emulsion is shown in Table 4.7 and 1.9 ml of fat was released from beef meat emulsion after cooking.

Under the studied conditions, at 3.5% WPI level, unheated WPI was not able to gel, as a result, all fluid was released after cooking. Preheating WPI decreased gelation temperature of WPI and increased water holding capacity of WPI.

. Gelation temperature of whey proteins is affected by concentration of whey proteins. As the concentration of whey protein increased, gelation temperature of whey protein decrease (Errington and Foegeding, 1998, Tang and others, 1993, Tang and others, 1995a, 1995b). Although unheated WPI was not able to gel at 3.5% WPI level, at 7.0% WPI level, all WPIs formed gels after cooking. A small amount of water (0.6 ml) was released from emulsion prepared with unheated WPI, and no water was released from emulsions prepared with preheated WPIs. These results suggested that although all WPIs were able to gel at 7.0% WPI level, protein matrix formed with preheated WPI was stronger than those formed with unheated WPI.

Conclusions

Preheating of WPI increased emulsion stability of high added fat and high added water emulsions prepared with WPIs. Among all three preheated WPIs, WPI emulsions preheated with WPI preheated at 95 °C for 40 min (pH 7.0) had the best emulsion stability in both high added fat and high added water emulsions ($P<0.05$). Increasing WPI amount also increased emulsion stability of high added fat and high added water emulsions prepared with WPIs. While beef emulsions were able to hold fat better, preheated WPI emulsions were able to hold water better. The high water holding ability

of preheated WPIs can be utilize to improve cooking yield and water holding capacity in emulsified meat products; especially in high added water emulsified meat products, such as frankfurters.

This study was designed to test the applicability and functionality of preheated whey protein polymers in emulsified meat products. Although preheated whey protein polymers showed superior water holding capacity than unheated whey protein, only partial emulsion formula was used and the experiments were carried out under carefully controlled lab conditions. The lab conditions intended to mimic the commercial meat processing conditions (processing procedures, cooking procedure and temperature). However, under commercial meat processing conditions and the present of other non-protein ingredients, the interactions between meat proteins and whey proteins are more complicated. The improved functionality of preheated whey protein polymers may be compromised due to the processing conditions used to produce a product. More researches are needed to verify the efficacy of preheated whey protein polymers in emulsified meat products under commercial meat processing conditions.

References

Barbut S. 1995. Effects of calcium level on the structure of preheated whey protein isolate gels. Can. Inst. Food Sci. Tech. 28:598-603.

Barbut S, Foegeding EA. 1993. Ca^{2+}-induced gelation of pre-heated whey protein isolate. J. Food Sci. 58: 867-871.

Beuschel BC, Culbertson JD, Partridge JA, Smith DM. 1992 a. Gelation and emulsification properties of partially insolubilized whey protein concentrates. J. Food Sci. 57 (3): 605-609, 634.

Beuschel BC, Partridge JA, Smith DM. 1992 b. Insolubilized whey protein concentrate and/or chicken salt-soluble protein gel properties. J. Food Sci. 57 (4): 852-855.

Bryant CM, McClements DJ. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. Tech. 9: 143-151.

Bryant CM, McClements DJ. 2000a. Optimizing preparation conditions for heat-induced whey protein solution to be used as cold-gelling ingredients. J. Food Sci. 65: 259-263.

Chantrapornchai W, McClements DJ. 2002. Influence of NaCl on optical properties, large-strain rheology and water holding capacity of heat-induced whey protein isolate gels. Food Hydrocolloids. 16: 467-476.

Damodaran S, Anand K. 1997. Sulfhydryl-disulfide interchange-induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. J. Agric. Food Chem. 45: 3813-3820.

Errington AD, Foegeding EA. 1998. Factors determining fracture stress and strain of fine-stranded whey protein gels. J. Agric. Food Chem. 46: 2963-2967.

Hendrix KM., Williams CJ, Manson AR, Smith DM. 2002. Properties of combination gels prepared from beef salt-soluble protein and preheated whey protein isolate. IFT Annual Meeting Book of Abstracts; 2002 June 16-18; Anaheim, CA. Chicago, IL.: Institute of Food Technologists. Abstract nr 76D-11.

Hongsprabhas P, Barbut S. 1996. Ca^{2+} - induced gelation of whey protein isolate: effects of pre-heating. Food Res. Int. 29: 135-139.

Hongsprabhas P, Barbut S. 1997. Protein and salt effects on Ca^{2+} - induced cold gelation of whey protein isolate. J. Food Sci. 62: 382-385

Hongsprabhas P, Barbut S. 1999a. Effect of preheated whey protein level and salt on texture development of poultry meat batters. Food Res. Int. 32: 145-149.

Hongsprabhas P, Barbut S. 1999b. Use of cold-set whey protein gelation to improve poultry meat batters. Poultry Sci. 78: 1074-1078.

Ju Z, Kilara A. 1998a. Effects of preheating on properties of aggregates and cold-set gels of whey protein isolate. J. Agric. Food Chem. 46: 3604-3608.

Ju Z, Kilara A. 1998b. Textural properties of cold-set gels induced from heat-denatured whey protein isolates. J. Food Sci. 63: 288-292.

Kitabatake N, Fujita Y, Kinekawa Y. 1996. Viscous sol and gel formation from process whey protein below 25 °C. J. Food Sci. 61: 500-503.

Langley KR, Green ML. 1989.Compression and impact strength of gels, prepared from fractionated whey proteins, in relation to composition and microstructure. J. Dairy Res. 56: 275-284.

Mleko S, Foegeding EA. 1999. Formation of whey protein polymers: effects of a two-step heating process on rheological properties. J. Text. Stud. 30: 137-149.

Imm JY, Regenstein JM. 1998. Emulsification of commercial dairy proteins with exhaustively washed muscle. J. Food Sci. 63: 1012-1017.

Shie JS, Strasburg GM, Smith DM. 2002. Heat denatured β-lactoglobulin aggregates: a study of the effects of preheating and aggregates size on the microstructure and rheological properties of β-lactoglobulin gels. J.R. Brunner Protein Symposium, May 15-17, E. Lansing, MI.

Tang Q, McCarthy O, Munro P. 1993. Oscillatory rheological study of the gelation mechanism of whey protein concentrate solutions: effects of physicochemical variables on gel formation. J. Dairy Res. 60: 543-555.

Tang Q, McCarthy O, Munro P. 1995a. Oscillatory rheological study of the effects of pH and salts on gel development in heated whey protein concentrate solutions. J. Dairy Res. 62: 469-477.

Tang Q, McCarthy O, Munro P. 1995b. Effects of pH on whey protein concentrate gel properties: comparisons between small deformation (dynamic) and large deformation (failure) testing. J. Text. Stud. 26: 255-272.

CHAPTER 5

EFFICACY OF PREHEATED WHEY PROTEIN POLYMERS IN LOW FAT BEEF FRANKFURTERS

ABSTRACT

Efficacy of spray-dried preheated whey protein isolate (WPI, preheated at 8% (w/v), 95 °C for 40 min, pH 7.0) in emulsified meat products was evaluated using a commercial beef frankfurter formula. In the formula water was added at 35, 40 and 45% level, and WPI was added at 3.5 and 7.0% level. At 40% added water level and 7.0% added WPI level, beef frankfurters prepared with preheated WPI had higher cooking yield, lower expressible moisture than those prepared with unheated WPI ($p<0.05$). Textural properties measured by two-cycle compression test indicated that beef frankfurters prepared with preheated WPI had higher hardness, gumminess and chewiness values than those prepared with unheated WPI ($p<0.05$). Sensory attributes of beef frankfurters conducted by a trained sensory panel also indicated that preheating WPI had significant effects ($p<0.05$) on product hardness and springiness of beef frankfurters. TBARS, pH and L^*, a^* and b^* values of beef frankfurters remained stable during 56 days of storage at 4 °C. During the storage, product purge loss gradually increased, and frankfurters prepared with preheated WPI had less purge loss then those prepared with unheated WPI ($p<0.05$). The results of this study showed that preheating WPI can alter WPI functionalities in emulsion type of meat products.

Chapter 5 is formatted in manuscript style according to the Journal of Food Science

Keywords: preheated whey protein polymers, cooking yield, textural properties, sensory attributes

Introduction

A variety of non-meat ingredients have been used to improve flavor, texture, appearance, and nutritional value of comminuted meat products such as frankfurters. Whey protein is one of the most important non-meat protein ingredients in value added meat products. Several health benefits have been reported by consuming of whey protein products (Whey Protein Institute, 2004). Historically, whey proteins have been used primarily as binders and extenders in processed meat products. Economically, addition of whey protein to meat products increases the yields, thereby decreasing the costs.

Whey protein concentrates (WPC) and whey protein isolates (WPI) are the two common types of whey products. These two whey products have been used in a variety of processed meat products. Early research has shown that addition of whey proteins increased juiciness and water binding in meat loaf and cooking yield in restructured beef steaks (Chen and Trout, 1991a, Lee and others 1980). Studies have shown that sensory and texture attributes of meat products can also be altered by the addition of whey proteins (Chen and Trout, 1991a, Hughes and others, 1998, Pawar and others, 2002, Slesinski and others, 2000a, 2000b).

Although whey proteins have been used in the meat industry since early 1980s, commercial available whey proteins do not form gels at meat processing temperatures (60 to 70 °C) and the high degree of variability in composition, functionality, and sensory properties of whey proteins has been a challenge to food industry (Atughonu and others 1998, Holt and others, 1999a, 1999b, Hung and Zayas, 1992, Langley and Green, 1989, Morr and Foegeding, 1990). In 2002, only 60% of the dry whey products produced in the United States was sold (USDA, 2003).

Upon addition of salt, preheated whey proteins have the ability to form gels at temperatures well below the meat processing temperatures. Since salt is often added to processed meat products, the unique cold-set gelling ability of pre-heated whey protein polymers can be utilized to overcome the functional problem of whey proteins. Both preheating and gelling conditions are important to the rheological properties of whey protein gels, and different rheological properties of whey protein gels can be produced by adjusting the preheating and gelling conditions (Bryant and McClements, 2000a, 2000b, Chantrapornchai and McClements, 2002, Marangoni and others, 2000). Shie (2004) reported that under meat processing conditions, emulsion stability of high added fat and water emulsions prepared with preheated WPI (8% (w/w) WPI preheated at 95 °C, 40 min, pH 7.0) was superior than those prepared with unheated. Emulsion stability of the emulsions also increased as preheated WPI amount increased (3.5 to 7.0%). The flexibility and improved functionality of preheated whey protein polymers give meat and whey industries a new prospect to utilize whey products. However, no research has conducted to verify the efficacy of preheated whey protein polymers in commercial meat products.

The objective of this study was to verify the efficacy of preheated whey protein polymers in comminuted type of meat products. To achieve this objective, a commercial beef frankfurter formula was used for this study, and WPI was preheated at 95 °C for 40 min (pH 7.0). The specific objectives of this study were to: 1). Evaluate quality, sensory attributes and textural properties of low fat beef frankfurters prepared with preheated and unheated WPI 2). Evaluate stability of low fat beef frankfurters prepared with preheated and unheated WPI during 56 days refrigerated storage (4 °C).

Materials and Methods

Ground beef, whey protein isolate and additives

Fresh 90% lean/10% fat (90/10) ground beef was purchased from a local meat company and stored at 4 °C until use. Sodium lactate was purchased from Purac America (Lincolnshire, IL). Alacen 895 whey protein isolates were purchased from New Zealand Milk Products (Lot# 047U45283431314, Lenoyne, PA). FMC phosphate blend was obtained from Astaris (St. Louis, MO) and frank seasoning was obtained from LEGG Packing Co, Inc. (Birmingham, AL).

Manufacture of preheated whey protein isolates

To prepare preheated WPI, 8% WPI (w/w) was dissolved with reverse osmosis water. The pH of the solution was adjusted to 7.0 with 1 M NaOH, and stirred at 1500 rpm for 15 min using a Rotostat mixer (Model 80XP63SS, Admix Inc., Londonderry, NH). Both preheating and spray-drying procedures took place in Michigan State University Dairy plant (E. Lansing, MI).

Preheating of whey protein isolates

The pH adjusted WPI solution was heated to 95 °C using a thin plate heat-exchanger (APV Crepaco Inc., Tonawanda, NY), and maintained at the same temperature (± 1.5 °C) in a double jacketed holding tank for 40 min. During the 40 min holding time, the WPI solution was agitated constantly using a propeller stirrer. After the 40 min holding time, the preheated WPI solution was cooled to 5° C.

Spray-drying of preheated whey protein isolates

Before spray-drying, pH of the preheated WPI solution was adjusted to 6.7 with 1 M HCl. Preheated WPI solution was then spray-dried using a Marriott Walker Corporation pilot plant spray dryer. The spray-dried preheated WPI were packaged in air-tight plastic bags and stored at 4 °C until use.

Manufacture of beef frankfurters

Table 5.1 presents the formulas used to manufacture beef frankfurters. Beef frankfurters were manufactured in Michigan State University meat lab pilot plant (E. Lansing, MI). Applicable amounts of beef meat, half water/ice, and salt were mixed and chopped at 2000 rpm for 3 min using a silent bowl chopper (Seydelmann bowl chopper, model K64U-Va, Reiser Co, Inc., Canton, MA). The rest of the water was then added. Other ingredients were added in the following order: phosphate blend, sugar, spice, WPI, Na erythorbate, curing salt and sodium lactate. The meat mixture was chopped at 4000 rpm for another 5 min to a temperature of 6-7 °C. The meat emulsions were stuffed and linked into cellulose casing (30 mm in diameter, Brechteen Co., Chesterfield, MA) using a VEMAG stuffer (Model 500, Robert Reiser Co, Inc., Canton, MA), and cooked in a smokehouse (CGI Processing, Model A28-B0101, Automated Manufacturing, Cicero, IL) using the smokehouse schedule listed in Table 2.4.

Cooked frankfurters were chilled to 4.4 °C, peeled, vacuum packaged (5 frankfurters/bag), and stored in Styrofoam boxes. Styrofoam boxers containing packaged frankfurters were stored at a 4 °C cold room until further analyses.

Table 5.1. Beef frankfurters formulations with different amounts of water and whey protein isolate.

Ingredient (%)	Treatment				Control	
	1	2	3	4	1	2
	35% water 3.5% WPI[a]	40% water 3.5 WPI[a]	40% water 7.0% WPI[a]	45% water 7.0% WPI[a]	40% water 3.5% WPI[a]	40% water 7.0% WPI[a]
Beef (90/10)	55.00	50.00	46.51	41.52	50.00	46.51
Water and ice	33.70	38.70	38.70	43.70	38.70	38.70
Salt	1.80	1.80	1.80	1.80	1.80	1.80
Sodium lactate[c]	3.30	3.30	3.30	3.30	3.30	3.30
Sugar	1.50	1.50	1.50	1.50	1.50	1.50
Control WPI[a]	0.00	0.00	0.00	0.00	3.50	7.00
Preheated WPI[b]	3.50	3.50	7.00	7.00	0.00	0.00
Spice	0.55	0.55	0.55	0.55	0.55	0.55
FMC phosphate	0.50	0.50	0.50	0.50	0.50	0.50
Na Erythorbate	0.03	0.03	0.03	0.03	0.03	0.03
Cure salt	0.14	0.13	0.12	0.10	0.13	0.12
Total	100.00	100.00	100.00	100.00	100.00	100.00

[a]: Whey protein isolate (WPI)

[b]: Preheated whey protein isolates were preheated at 95 °C for 40 min at pH 7.0

[c]: Sodium lactate: Optiform 4 sodium lactate

Solubility and proximate composition for whey protein isolates

Solubility of WPI was determined according to methods by Morr and others (1995). Protein, moisture, ash and fat content of preheated and unheated WPI were determined according to AOAC methods 930.29A (micro-Kjeldahl method), 927.05 (vacuum oven drying method), 930.30 (dry ashing) and 932.06 (Mojonnier ether extraction method), respectively. All samples were measured in triplicate.

Proximate composition for raw frankfurter emulsions and cooked frankfurters

Raw frankfurter emulsions and frankfurters were packed in Whirl-Pack™ bags (Fisher Scientific USA, Pittsburg, PA) and frozen at a -10 °C freezer for at least 24 hr before processing. Frozen samples were cut into small pieces and ground with dry ice into a fine powder using a Tekmar grinder (Tekmar Co, Cincinnati, OH), packed in opened whirl-pack bags at -10 °C freezer for at least 48 hr (to evaporate dry ice) until further analysis.

Moisture, protein, fat and ash content of samples were determined according to AOAC (2000) methods 950.46B (oven drying), 992.15 (combustion method, nitrogen measurement, Model FP-2000, LECO Co., St. Joseph, MO), 991.36 (Soxhlet ether extraction), and 920.153 (dry ashing), respectively, with small modifications. Samples were analyzed in triplicate.

Back extrusion force of frankfurter emulsions

Back extrusion force of frankfurter emulsions was measured using a TA-HD*i* Texture Analyzer (Texture Technologies Co., Scarsdale, NY). Frankfurter emulsions

(100 ± 0.1 g) were weighed out in an extrusion rig which had an inner diameter of 52 mm and 100 mm in height. An extrusion plunger (40 mm in diameter) was attached to a 50 kg loading cell and advanced into the extrusion rig to a depth of 90 mm at a speed of 5 mm/sec. Extrusion force was recoded in Newtons.

Cooking yield

Cooking yield was measured and calculated according to Atughonu and others (1998).

Cooking yield % = (weight of cooked and chilled frankfurters / weight of uncooked frankfurters) × 100

Emulsion stability

Frankfurter emulsions (33.3 ± 0.1 g) were stuffed into a 50 ml polycarbonate tube (Nalge Nunc International, Rochester, NY) and cooked to an internal temperature of 71.1 °C using a program controlled water bath (Model 9510, PolyScience, Niles, IL). After cooking, fluid released during cooking was collected in 15 ml centrifugation tubes and centrifuged at 6, 000 $\times g$ for 5 min (Sorvall RC 5B plus, Sorvall Instruments, Newtown, CT). Volumes of fat and water were recorded. Emulsion stability was calculated as:

Emulsion stability % = 100 – volume of total liquid drained out from 100 g of frankfurter batter

pH determination

An Accumet pH meter (AB 15, Fisher Scientific, Co., Pittsburgh, PA) was used to measure pH of raw frankfurter emulsion and cooked frankfurter samples. Samples (10 ± 0.1g) were weighed in a 400 ml beaker with 90 ml of deionized water. Samples were homogenized for 1 min at setting 4 using a Polytron mixer (PT-35, Kinematica, AG, Switzerland). The pH of homogenized samples was then measured.

Thiobarbituric acid reactive substances (TBARS)

TBARS analysis was conducted to monitor oxidative rancidity. Four replicates were run for each sample according to methods by Rhee (1978).

Purge loss

Two packages of frankfurters from each treatment were randomly selected and measured for purge loss. Each package of frankfurters was weighed before and after fluid was drained out. Purge loss was calculated as:

Purge loss % = ((package weight before liquid draining – package weight after liquid draining) / package weight before draining) × 100

CIE L^*, a^*, and b^* value

A ColorTec PCM^{TM} Color Meter (Model 6482, ColorTec Associates, Clinton, NJ) with a 10° standard observer and an 8 mm reading orifice was used to measure L^* (lightness), a^* (redness), and b^* (yellowness) values of cooked frankfurters. The color

meter was calibrated first and color of cooked frankfurters then measured. Cooked frankfurters were cut open to expose inner surfaces. L^*, a^*, and b^* values were measured by pressing tightly of the reading orifice of the color meter against the exposed inner surface of cooked frankfurters.

Expressible moisture

Expressible moisture was measured according to method by Barbut (2002) and calculated as:

Expressible moisture % = ((sample weight before centrifugation – sample weight after centrifugation) / sample weight before centrifugation) × 100

Textural properties (two-cycle compression test)

Textural properties of cooked frankfurters were measured by two-cycle compression test using a TA-HD*i* Texture Analyzer (Texture Technologies Co., Scarsdale, NY). The texture analyzer was equipped with a 75 mm compression plate attached to a 50 kg loading cell. Frankfurters were cut into 20 mm long cores and compressed to 35% of the original height at a speed of 5 mm/s. There was a 5 seconds interval between the first and second compression. All samples were analyzed at 4 °C.

Consumer reheat yield

A cooked frankfurter was weighed before and after reheating in 1000 ml of boiling water for 5 min. Consumer reheat yield was calculated as:

Consumer reheated yield = (weight of reheated frankfurter / weight of cooked frankfurter) x 100

Sensory evaluation

The sensory panel (n=5, age between 28 and 65, 4 female and 1 male) was trained according to AMSA (1995) and Meilgaard and others (1991). All panelists were experienced in sensory evaluation and previously trained to evaluation various meat products. Before product evaluation, three training sections were held to familiarize the panelists with the attributes and evaluation procedures. An 8 point hedonic scale was used by the trained sensory panel to evaluate 7 different sensory attributes: skin hardness, product hardness, springiness, juiciness, cohesiveness, off-flavor intensity and milk/whey coating, where 1=extremely soft and 8=extremely hard, 1=non-resilient and 8=extremely resilient, 1=extremely dry and 8=extremely juicy, 1=extremely crumbly and 8=extremely cohesive, 1=non-off-flavor or non milk/whey coating and 8= abundant off-flavor or abundant whey/whey coating.

Sensory evaluation was conducted in a climate controlled sensory evaluation room with partitioned booths (Department of Food Science, Michigan State University, E. Lansing, MI). Red lights were used in the booths to mask the appearance bias during the evaluation, and the order of sample preparation was randomized within each section to minimize positional bias. Cooked frankfurters were heated for 8 min in closed packages in a stainless pan filled with 80 °C water heated by a double boiler (Broil King, Model BR7W, Winsted, CT). Prepared frankfurters were cut into 1 and 0.5 inch long sections and cooled in 4 oz Soufflé cups to 46 °C before serving to the sensory panel.

Except for springiness, panelists were instructed to use 1 inch long frankfurter sections for all other sensory attributes. Expectorant cups, distilled water, apple juice, and unsalted soda crackers were provided to the panel to clean the palate between samples. Eighteen (four treatments, two controls, three replications) samples were evaluated in one day. The day was divided into three sections with six different samples in one replication were evaluated per section. For each section, the panelists were standardized with randomly selected one commercial frankfurter and one whey protein added frankfurter. The panel was also instructed to take a 15 min break between each section.

Sampling for quality attributes of beef frankfurters

Purge loss, pH, CIE L^*, a^*, b^* values, and TBARS of cooked frankfurters were measured on day 1, day 7, day 14, day 28 and day 56 after the manufacture of frankfurters. Consumer reheat yield, expressible moisture and textural properties of frankfurters were measured on day 56 after the manufacture. On each sampling day, two packages of frankfurters were randomly selected. Three frankfurters were then randomly selected from each package to measure pH, CIE color, TBARS value, expressible moisture, and textural properties. The four frankfurters left from the two packages were used to measure consumer reheat yield. All sensory evaluations were conducted on the same day which was 55 days after the first day of frankfurter manufacture. Two packages from each treatment and control were used for sensory evaluation.

Experimental design and statistics analysis

A commercial beef frankfurter formula was used for this study to verify the efficacy of preheated WPI. Beef frankfurters were formulated to contain 35, 40 or 45% water and 3.5 or 7.0% WPI as shown. The experimental design used was a two-way analysis of variance with four treatment combinations (35% water with 3.5% preheated WPI, 40% water with 3.5% preheated WPI, 40% water with 7.0% preheated WPI and 45% water with 7.0% preheated WPI) and two control combinations (40% water with 3.5% unheated WPI and 40% water with 7.0% unheated WPI). Frankfurters prepared with unheated WPI were used as controls to monitor functionality changes in WPI after preheating. The effect of preheating of WPI on quality attributes (cooking yield, emulsion stability, textural properties, purge loss, color, pH and lipid oxidation) of the frankfurters were analyzed. Difference among attribute means was determined with a predetermined level of significance ($P<0.05$) using Tukey's Least Significant Difference procedure (SAS user's guide, version 8.2. Cary, NC: SAS Institute, Inc., 2002).

Results and Discussion

Proximate composition and solubility of whey protein isolate

Whey protein isolate used in this study was high in protein and low in fat and minerals. Table 5.2 shows that before preheating treatment Alacen 895 WPI had a protein content of 88%, and a moisture content of 9.4%. After preheating treatment, protein content of WPI slightly increased to 89.74% and moisture content slightly decreased to 7.44%.

After preheating, solubility of WPI decreased from 92.05 to 21.81%. Beuschel and others (1992a) also reported that after preheating of liquid whey protein concentrate, solubility of whey protein concentrate decreased. Preheating partially denatured WPI, and depending on the preheating conditions, solubility of the preheated WPI may vary.

Proximate composition of raw frankfurter emulsions and cooked frankfurters

Table 5.3 shows proximate composition and pH of raw frankfurter emulsions and cooked frankfurters prepared with different amounts of water and WPI. Water content, WPI content and preheating of WPI did not affect pH of raw frankfurter emulsions. Unheated WPI had a pH around 6.7. Although preheated WPI was produced by preheating at pH 7.0, after preheating, pH of the preheated WPI was adjusted back to its original pH of 6.7. This minimized the pH difference between different treatments and controls. After cooking, pH of all treatments and controls increased slightly, and control 2 frankfurters prepared with 40% water and 7.0% unheated WPI had significantly ($p<0.05$) higher pH than other treatments and control. Before cooking, moisture content of raw emulsions ranged from 74.82 (control 2) to 77.32% (treatment 2), and only small

Table 5.2. Least square means for proximate composition and solubility of whey protein isolate and 90/10 fresh ground beef.

	Unheated whey protein isolate	Preheated whey protein isolate[a]	90/10 fresh ground beef
Moisture (%)	9.40	7.44	73.13
Protein (%)	87.97	89.74	18.63
Fat (%)	0.58	0.46	7.38
Ash (%)	2.44	2.29	0.94
Solubility (%)	92.05	21.81	-

[a]: preheated whey protein isolate was preheated at 95 °C for 40 min, pH 7.0.
[b]: Standard error of the mean

Table 5.3. Least square means for proximate composition and pH of raw frankfurter emulsions and cooked frankfurters prepared with different amounts of water and whey protein isolate.

Composition (%)		Treatment[e] 1 35% water 3.5% WPI[h]	Treatment[e] 2 40% water 3.5 WPI[h]	Treatment[e] 3 40% water 7.0% WPI[h]	Treatment[e] 4 45% water 7.0% WPI[h]	Control[f] 1 40% water 3.5% WPI[h]	Control[f] 2 40% water 7.0% WPI[h]	SEM[g]
Moisture	Raw emulsion	76.10^{b}	77.32^{a}	75.20^{cd}	75.92^{bc}	76.79^{ac}	74.82^{d}	0.21
	Cooked frank	69.70^{b}	69.44^{b}	69.56^{b}	69.92^{b}	71.09^{a}	69.26^{b}	0.23
Protein	Raw emulsion	15.10^{c}	14.18^{d}	16.49^{a}	15.77^{b}	14.51^{d}	16.84^{a}	0.11
	Cooked frank	18.01^{c}	19.20^{ab}	19.98^{a}	18.90^{b}	17.47^{c}	19.74^{a}	0.19
Ash	Raw emulsion	3.95^{a}	3.83^{c}	3.95^{a}	3.91^{ab}	3.85^{bc}	3.91^{ab}	0.02
	Cooked frank	4.51^{a}	4.28^{b}	4.41^{ab}	4.40^{ab}	4.27^{b}	4.33^{ab}	0.05
Fat	Raw emulsion	3.22^{a}	3.08^{ab}	2.72^{c}	2.83^{bc}	2.98^{abc}	2.69^{c}	0.08
	Cooked frank	4.45^{a}	4.00^{abc}	3.92^{bc}	3.62^{bc}	4.04^{ab}	3.54^{c}	0.11
pH	Raw emulsion	6.21^{c}	6.21^{bc}	6.23^{ab}	6.23^{ab}	6.20^{c}	6.23^{a}	0.01
	Cooked frank	6.25^{b}	6.26^{b}	6.28^{b}	6.28^{b}	6.27^{b}	6.30^{a}	0.01

[a-d]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[e]: Whey protein isolate used in treatment was preheated at 95 °C for 40 min at pH 7.0.

[f]: Whey protein isolate without any preheating treatment was used in control

[g]: Standard error of the mean (SEM)

[h]: Whey protein isolate (WPI)

differences were found in moisture content among different treatments. In this study we formulated different amounts of water and WPI in different treatments, and the decreased water amount was replaced by beef meat. Beef meat proximate composition showed that the ground beef contained 73.13% moisture. Because of the high moisture content of the beef meat, there was only small difference in the moisture content in different treatments.

Based on the formula, treatment 3 and control 2 should have the highest protein contents, and our results indicated that before cooking the protein content in treatment 3 (16.49%) and control 2 (16.84%) were significantly higher ($p<0.05$) than other treatments. Although, preheating slightly increased protein contain of WPI, there was no significant difference ($p<0.05$) in protein content between treatments and controls containing same amount of WPI.

Beef meat was the only fat source used in the study. Before cooking, treatment 1 had the highest fat content (3.22%) than any other treatments. This result was expected since treatment 1 had the highest beef content in the formula.

Due to cooking loss, other than moisture content, all other compositions (protein, ash, fat) increased after cooking. Moisture content of all frankfurters decreased to around 70%, and treatment 3 and control 2 had the highest protein contents (19.98 and 19.97%, respectively). Although ash content also increased, there was only very small difference in ash content among different treatments and controls.

Quality attributes of raw frankfurter emulsions

Quality attributes of raw frankfurter emulsions were presented in Table 5.4. Back extrusion force (maximum force required to accomplish extrusion) is measured as an

Table 5.4. Least square means for quality attributes of raw frankfurter emulsions and cooked frankfurter yield prepared with different amounts of water and whey protein isolate.

	Treatment[e]				Control[f]		
	1	2	3	4	1	2	
Attribute	35% water 3.5% WPI[h]	40% water 3.5 WPI[h]	40% water 7.0% WPI[h]	45% water 7.0% WPI[h]	40% water 3.5% WPI[h]	40% water 7.0% WPI[h]	SEM[g]
Back extrusion force (N)	14.67[b]	11.45[c]	16.76[a]	11.92[c]	7.67[d]	6.72[d]	0.24
Cooking yield (%)	87.95[b]	84.75[ab]	84.47[ab]	82.21[abc]	78.57[bc]	77.57[c]	1.32
Emulsion stability (%)	99.63[a]	99.33[a]	99.67[a]	99.33[a]	99.27[a]	97.87[b]	0.16

[a-d]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[e]: Whey protein isolate used in treatment was preheated at 95 °C for 40 min at pH 7.0.

[f]: Whey protein isolate without any preheating treatment was used in control

[g]: Standard error of the mean (SEM)

[h]: Whey protein isolate (WPI)

index of textural quality of raw frankfurter emulsions and is influenced by the viscoelasticity of the emulsions (Bourne, 2002). Back extrusion force of raw frankfurter emulsions indicated that even before cooking raw frankfurter emulsions prepared with different amounts of water and WPI had different rheological properties. Emulsion prepared with 40% water and 7.0% preheated WPI had the highest back extrusion force (16.76 N), and control prepared with 40% water and 7.0% unheated WPI had lowest back extrusion force (6.72 N) ($p<0.05$). As the water content in the formula increased or as the WPI content in the formula decreased, back extrusion force also decreased. In the treatment at 3.5% WPI level, back extrusion force decreased as water content increased. Back extrusion force decreased from 14.67 to 11.45 N, when the water content increased from 35 to 40%. The same phenomenon was also observed in the treatment at 7.0% WPI level. However, in controls as WPI amount increased, the back extrusion force decreased.

Paoletti and others (1995) suggested that back extrusion force can be used as an indication to measure protein content in emulsified WPC. Our results confirmed that protein contain of raw frankfurter emulsions prepared with preheated WPI can be differentiated by back extrusion force. Back extrusion force is also an indication for the strength of protein matrix, the higher the force is the stronger the protein matrix. Back extrusion force results suggested that preheated WPI incorporated with meat protein better than unheated WPI, as a result, the stronger protein matrixes were formed when preheated WPI was used. Because WPI used in controls were not preheated, as the meat protein in the formula decreased, the back extrusion force also decreased.

Cooking yield is affected by both the nature of the meat product and relative humidity/temperature combinations used during processing (Smith and Brekke, 1985). Regardless of the water and WPI content in the formula, all 4 treatments prepared with preheated WPI had higher cooking yield than the controls prepared with unheated WPI. At the same water and whey protein content, treatments prepared with preheated WPI had higher cooking yield than control prepared with unheated WPI (about 6% higher). Treatment 1 (35% water and 3.5% preheated WPI) had the highest cooking yield (87.95%), and as the water content increased, cooking yield decreased. However, there was no significant difference ($p<0.05$) in cooking yield between treatment 2 (84.75%) and 3 (84.47%), although WPI content was increased from 3.5 to 7.0% in treatment 2 to treatment 3.

This result suggested that at 40% water level cooking yield of whey protein added frankfurters reached a plateau at 3.5% WPI level and further increase of whey protein in the formula would not increased the cooking yield. Preheating of WPI exposed the hydrophobic regions of WPI and increased the surface reactivity of WPI. This allowed preheated WPI to re-orient more easily than unheated WPI (Lucca and Tepper, 1994). Hongsprabhas and Barbut (1999a, 1999b) reported that at low salt level (<1.5%) poultry batter prepared with preheated WPI had higher cooking yield than those prepared with unheated WPI. They suggested that preheated WPI had more exposed negative charges than unheated WPI, and the more negative charged WPI may help in forming a stronger protein matrix in holding water and fat. Although control 2 had 3.5% more unheated WPI than control 1, cooking yield in control 1 did not differ from control 2 ($p<0.05$). Gelation temperature of whey protein is around 75 °C, but final cooking temperature of

beef frankfurters was only around 71.1 °C. Unlike preheated WPI, unheated WPI did not complete the gelation process. Hence increasing unheated WPI content did not increase the cooking yield.

Emulsion stability followed the same trend as cooking yield. However, it did not correlate well with the cooking yield results. All frankfurter emulsions had emulsion stability above 99% except control 2 with 40% water and 7.0% unheated WPI which had the least emulsion stability (97.87%). Unlike cooking yield, emulsion stability was measured in a closed system using 50 ml polyethylene tubes cooked in a water bath. When fluid was released during cooking, the closed polyethylene tubes did not allow released fluid to drain away, and this may create a change for reabsorption. Depending on the speed of the fan in the smoke house, cooking in a smoke house would allow higher evaporation of moisture during cooking. These reasons combined may explain the low correlation between emulsion stability and cooking yield. Similar results were reported in frankfurters prepared with fluid whey by Yetim and others (2001). Substituting ice/water with unprocessed liquid whey increased emulsion stability of frankfurter emulsions. Increasing the amount of liquid whey also increased frankfurter emulsion stability. But only small difference in emulsion stability between frankfurter emulsions substituted with 0 and 100% liquid whey was observed (86.50 and 90.00%, respectively).

Quality attributes of cooked frankfurters

Water held within a protein matrix can be divided into three major categories: bound, immobilized, and free water. Free water can be relatively easily squeezed out and is measured by expressible moisture (Barbut 2002). A better emulsified protein system

would have a better ability to hold liquid (water and fat) when an outside force such as centrifugation force is applied. Hence expressible moisture can be used to measure the stability of the protein matrix. Quality attributes of cooked frankfurters were listed in Table 5.5. Hongsprabhas and Barbut (1999b) reported that water holding capacity of poultry batters increased when preheated WPI (preheated at 80 °C for 30 min, pH 7.0) was added, and water holding capacity of poultry batters prepared with preheated WPI was higher than those prepared with unheated WPI. Similar findings were observed here. Expressible moisture results indicated that when frankfurters manufactured with preheated WPI retained water better than those manufactured with unheated WPI, and as the added water amount increased expressible moisture also increased. Expressible moisture results also confirmed that unheated WPI did not incorporate well with meat protein. At the same water content, when the meat protein amount decreased, the amount of decreased meat protein was replaced by WPI. Because the unheated WPI was not able to stabilize the protein matrix, as unheated WPI amount increased, expressible moisture % also increased.

Consumer reheat yield is an important quality attribute for consumers. A desired product would have high consumer reheat yield and also good product appearance after consumer reheat. To test the desirability and practicability of the WPI added frankfurters, consumer reheat yield was measured according to a recommended cooking method by a commercial frankfurter company. All frankfurters had high consumer reheat yield (>99%). The appearance of frankfurters did not change (no discoloration or structure breakdown) after reheating, indicating that preheating of WPI did not change consumer reheat yield and after reheating frankfurters prepared with preheated WPI had

Table 5.5. Least square means for quality attributes of cooked beef frankfurters prepared with different amounts of water and whey protein isolate.

	Treatment[e]				Control[f]		
	1	2	3	4	1	2	
Attribute	35% water 3.5% WPI[i]	40% water 3.5 WPI[i]	40% water 7.0% WPI[i]	45% water 7.0% WPI[i]	40% water 3.5% WPI[i]	40% water 7.0% WPI[i]	SEM[g]
Expressible moisture (%)	13.96^{d}	17.34^{bc}	15.42^{cd}	18.31^{b}	17.97^{b}	20.74^{a}	0.51
Consumer reheat yield (%)	99.62^{a}	99.01^{b}	99.51^{ab}	99.59^{ab}	99.28^{ab}	99.31^{ab}	0.14
Hardness (kg)[h]	17.17^{a}	14.44^{b}	18.26^{a}	12.72^{bc}	10.91^{c}	8.41^{d}	0.47
Cohesiveness[h]	0.25^{a}	0.25^{a}	0.24^{ab}	0.24^{b}	0.24^{ab}	0.23^{c}	0.01
Gumminess (kg)[h]	4.42^{a}	3.63^{b}	4.44^{a}	3.05^{bc}	2.66^{c}	1.90^{d}	0.14
Chewiness (kg)[h]	4.50^{a}	3.71^{a}	4.35^{a}	2.74^{b}	2.55^{b}	1.64^{c}	0.20

[a-d]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[e]: Whey protein isolate used in treatment were preheated at 95 °C for 40 min at pH 7.0.

[f]: Whey protein isolate without any preheating treatment was used in control

[g]: Standard error of the mean (SEM)

[h]: Hardness, cohesiveness, gumminess and chewiness values were measured by two-cycle compression test

[i]: Whey protein isolate (WPI)

the same desirability in consumer reheat yield as frankfurters prepared with unheated WPI.

Structure of meat products can be affected by addition of non-meat proteins. Non-meat proteins interact directly with meat protein occupying interstitial spaces in the protein matrix (Lanier, 1991). Textural properties of beef franks measured by two-cycle compression test were listed in Table 5.5. A texture profile analysis curve can be constructed by plotting force during compression against time. Hardness is defined as the peak force during the first compression. Frankfurter hardness values increased as the preheated WPI amount increased. However, when unheated WPI amount increased, hardness value decreased. Although there was no significant difference ($p<0.05$) in hardness value between treatment 1 and treatment 3, treatment3 preheated WPI had the highest hardness value (18.26 kg) among the 4 different treatments. As the water amount increased, hardness value decreased. At 7.0% preheated whey protein level, there was a sudden drop in frankfurter hardness when water content of frank formula increased from 40 to 45%. Comparing same amount of added water and WPI, preheating of WPI significantly increased frankfurter hardness value ($p<0.05$). Frankfurters prepared with 7.0% WPI and 40% water, when preheated WPI was used instead of unheated WPI, frankfurter hardness value increased more than double from 8.41 to 18.26 kg. Atughonu and others (1998) reported that all-meat frankfurters had higher compression force than frankfurters prepared with variety of milk proteins. They explained that all-meat frankfurters had more disulfide bond linkages, therefore a firmer product was produced. If the difference in gelation temperature between non-meat protein and meat protein is

big enough, coagulation of the non-meat protein might occur. As a result, the hardness of the product is decreased.

The ratio of the area under second compression to the area under first compression is defined as cohesiveness value. Only small difference was observed in cohesiveness value among different treatments and controls. Gumminess is the product of hardness and cohesiveness, and chewiness is the product of gumminess and springiness. Because only small differences were found in cohesiveness value among different treatments and controls, not surprisingly, gumminess and chewiness values followed the trend of hardness value.

Several other studies also reported that addition of whey protein in meat products changed textural properties of the products. WPC was proven to be a viable binder for knockwurst alternative by providing similar textural attributes in comparison to soy protein and calcium-reduced nonfat dry milk (Ensor and others, 1987). Although a slight decrease in hardness value was observed in restructured ham prepared with liquid whey (Marriott et al., 1998), Hughes et al. (1998) reported that hardness, gumminess and chewiness values of frankfurters increased with the addition of WPC (3%).

Storage stability of whey protein beef frankfurters

Stability of WPI added frankfurters was evaluated by TBARS, pH, color and purge loss. Purge loss and L^*, a^* and b^* values during 56 days refrigerated storage are shown in Table 5.6. For frankfurters prepared with preheated WPI, L^* value significantly ($p<0.05$) increased as the water and WPI amount in the formula increased. However, for frankfurters prepared with unheated WPI, L^* value decreased when WPI amount

Table 5.6. Least square means for L^*, a^*, b^* values and purge loss for cooked beef frankfurters prepared with different amounts of water and whey protein isolate (WPI).

Measurement	Days					SEM[h]	Treatment				Control		SEM[h]
							1	2	3	4	1	2	
	1	7	14	28	56		35% water 3.5% WPI	40% water 3.5% WPI	40% water 7.0% WPI	45% water 7.0% WPI	40% water 3.5% WPI	40% water 7.0% WPI	
L^*	50.07[a]	49.96[a]	49.97[a]	49.95[a]	49.48[b]	0.08	49.48[b]	50.02[c]	50.81[b]	51.39[a]	48.97[e]	48.63[f]	0.09
a^*	4.21[a]	4.02[b]	3.94[bc]	3.97[bc]	3.90[c]	0.03	4.11[ab]	3.99[bc]	3.89[c]	3.69[d]	4.19[a]	4.17[a]	0.03
b^*	12.20[c]	12.95[b]	12.96[b]	13.16[ab]	13.33[a]	0.06	12.73[c]	12.95[c]	13.49[b]	13.88[a]	12.14[d]	12.33[d]	0.07
Purge loss (%)	-	2.18[b]	2.36[b]	2.51[ab]	3.05[a]	0.16	1.57[c]	3.24[ab]	1.70[c]	2.11[c]	2.78[b]	3.77[a]	0.19

[a-d]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[h]: Standard error of the mean (SEM)

increased. Frankfurter a^* value slightly decreased as water and preheated WPI amount increased, and no difference was found in a^* value in frankfurters prepared with unheated WPI. Cooking yield result showed that preheated WPI had better water holding ability than unheated WPI. Because of the extra water held in the protein matrix, light was easier to pass through the protein matrix and the redness color of meat was diluted by extra water. This may result in higher L^* value and lower a^* value in frankfurters prepared with preheated WPI. No significant difference was found in cooking yield in frankfurters prepared with unheated WPI, so the extra WPI in the protein matrix decreased the lightness value. Other studies also reported that when whey protein was added in frankfurters, L^* value increased and a^* value decreased (Atughonu and others, 1998, Hughes and others 1998). Although the exact mechanism of how whey protein decreases redness color in meat products is still not clear, Sammel and Claus (2003) suggested that whey protein may interact with pink-color-generating ligands, inhibiting their ability to produce pink color. No significant difference ($p<0.05$) was found in yellowness value (b^*) in frankfurters prepared with unheated WPI, and increasing preheated WPI amount increased yellowness value of frankfurters.

Santiago and others (2004) reported that frankfurter formulations with higher meat protein content had lower purge loss. Purge loss result indicated that preheated WPI had a better purge control than unheated WPI, and increasing preheated WPI amount significantly ($p<0.05$) decreased purge loss. At 40% water and 7.0% WPI, preheated WPI decreased purge loss more than 50% ($p<0.05$). Purge loss was 1.70 and 3.77 for frankfurters prepared with preheated and unheated WPI, respectively. During 56 days of refrigerated storage, while purge loss and b^* value slowly increased, L^* and a^*

values gradually decreased. And there was a significant difference ($p<0.05$) in L^*, a^* and b^* values between day 1 and day 56. The decreasing of L^* and a^* values during the storage may be due to the combination effects of water loss and oxidation of hemoglobin. Fox and others (1967) suggested that during storage free oxygen may transport slowly into the package. Available reductant in frankfurters would be depleted through oxidation, and eventually resulted in color loss.

Two-way interaction for pH and TBARS with storage day during 56 days storage was significant ($p<0.05$) and shown in Figure 5.1. Regardless of the preheating, increasing WPI amount tended to increase the pH. However, Yetim and others (2001) reported that pH of frankfurters prepared with liquid whey did not differ from frankfurters prepared with ice. Frankfurter pH tended to increase as the storage day increased. WPI had a pH of 6.7, and pH of beef meat was around 5.5. Frankfurter pH increased as meat was replaced by WPI. Although pH of frankfurters tended to increased during storage, only small increase (0.1) was observed in all frankfurters through out the whole 56 days storage period.

Lipid oxidation (TBARS) values maintained low during the 56 days storage. On day 7 TBARS value increased for all frankfurters and then decreased on day 14. Lipid oxidation value for treatment 1 with highest fat content increased most (from 0.14 to 0.25 mg malonaldehyde/Kg frank). After day 14 TBARS value tended to increase slowly with storage day. At the end of storage (day 56), treatment 1 had the highest TBARS value (0.28 mg MAD/Kg sample). It was unclear why TBARS values increased on day 14 and then decreased. On the same sampling day, all TBRAS readings for all frankfurter samples were taken with one measurement on one 96 well plate. The increased TBARS

Table 5.7. Least square means for sensory attributes of cooked beef frankfurters prepared with different amounts of water and whey protein isolate.

	Treatment[f]				Control[g]		
	1	2	3	4	1	2	
Attribute[e]	35% water 3.5% WPI[i]	40% water 3.5 WPI[i]	40% water 7.0% WPI[i]	45% water 7.0% WPI[i]	40% water 3.5% WPI[i]	40% water 7.0% WPI[i]	SEM[h]
Skin hardness	6.67 [a]	6.07 [ab]	6.27 [ab]	6.20 [ab]	5.80 [ab]	5.40 [b]	0.24
Product hardness	6.40 [a]	6.13 [a]	5.93 [ab]	5.33 [bc]	5.13 [cd]	4.60 [d]	0.17
Springiness	6.20 [a]	5.93 [ab]	5.67 [abc]	5.20 [bc]	5.07 [c]	4.93 [c]	0.21
Juiciness	4.33 [ab]	4.93 [a]	3.73 [b]	4.40 [ab]	4.73 [a]	5.13 [a]	0.21
Cohesiveness	5.07 [ab]	4.60 [b]	4.80 [ab]	5.00 [ab]	4.93 [ab]	5.80 [a]	0.29
Off-flavor intensity	1.00 [a]	1.00 [a]	1.00 [a]	1.00 [a]	1.00 [a]	1.00 [a]	0.00
Milk/whey coating	1.20 [b]	1.27 [ab]	1.47 [ab]	1.93 [a]	1.27 [ab]	1.13 [b]	0.16

[a-d]: Means having different superscripts within same rows are significantly different ($p<0.05$)

[e]: Sensory attributes were evaluated using 8 point hedonic scale:

1=extremely soft/ Non-Resilient/dry/ Crumbly/Non- off-flavor/No-milk/whey coating

8=extremely hard/Resilient/ Juicy/Cohesive/ Abundant off-flavor/Abundant milk/whey coating

[f]: Whey protein used in treatment was preheated at 95 °C for 40 min at pH 7.0.

[g]: Whey protein without any preheating treatments was used in control

[h]: Standard error of the mean (SEM)

[i]: Whey protein isolate (WPI)

values for all frankfurter samples on day 14 may be a systemic error. Comparing other research result (Sommers and others, 2003), TBARS value was low. Whey proteins are able to scavenge peroxyl radicals and chelate ions (Tong and others, 2000), the relatively low and stable TBARS values through out the storage may due to antioxidant activity of WPI. Pena-Ramos and Xiong (2003) stated that 2% WPI in cooked pork patties significantly decreased TBARS values during 8 days storage at 4 °C. Although no all-meat frankfurters were used as control in this study, the low TBARS value was desirable. Sensory results correlated well with TBARS value since there was no sensory off-flavor in any of the frankfurters produced.

Sensory attributes

Frankfurter sensory attributes evaluated by a trained sensory panel were listed in Table 5.7. Skin hardness, product hardness correlated well with hardness value measured by two-cycle compression test ($p<0.05$), except that treatment 1 had the highest skin hardness, and product hardness value. In all frankfurters prepared with preheated WPI, as the water amount increased and WPI amount decreased skin hardness, product hardness and springiness values decreased. However, skin hardness, product hardness and springiness of frankfurters prepared with unheated WPI decreased as the unheated WPI content increased. Comparing frankfurters prepared with preheated and unheated WPI, at the same WPI and water level, skin hardness, product hardness, and springiness of frankfurters prepared with preheated WPI were higher than those prepared with unheated WPI. Hughes and others (1998) and Marriott and others (1998) reported similar sensory results in unheated WPC. Ensor and others (1987) used a consumer sensory

Figure 5.1. Two way interactions ($p<0.05$) for pH and TBARS of cooked beef frankfurters prepared with different amounts of water and whey protein isolate during 56 days refrigerated storage.

Figure 5.1a: pH

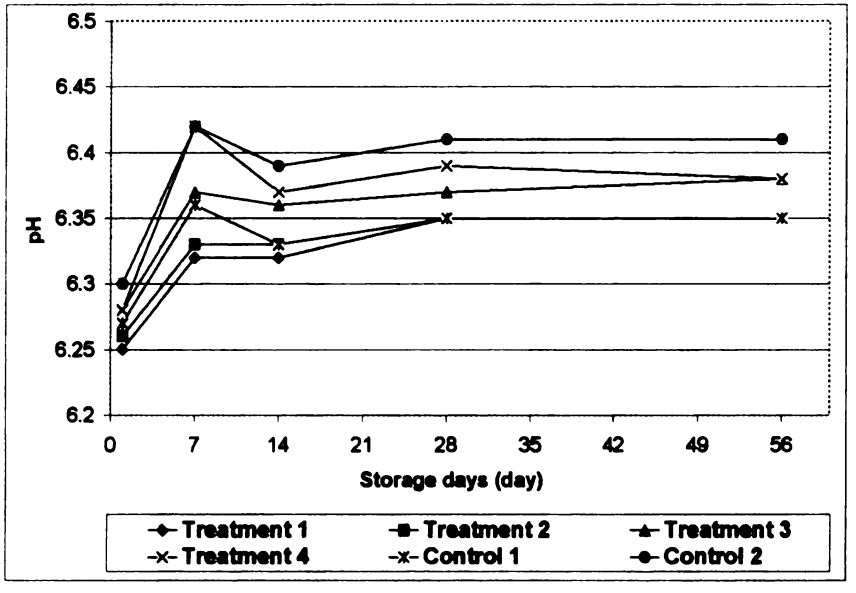
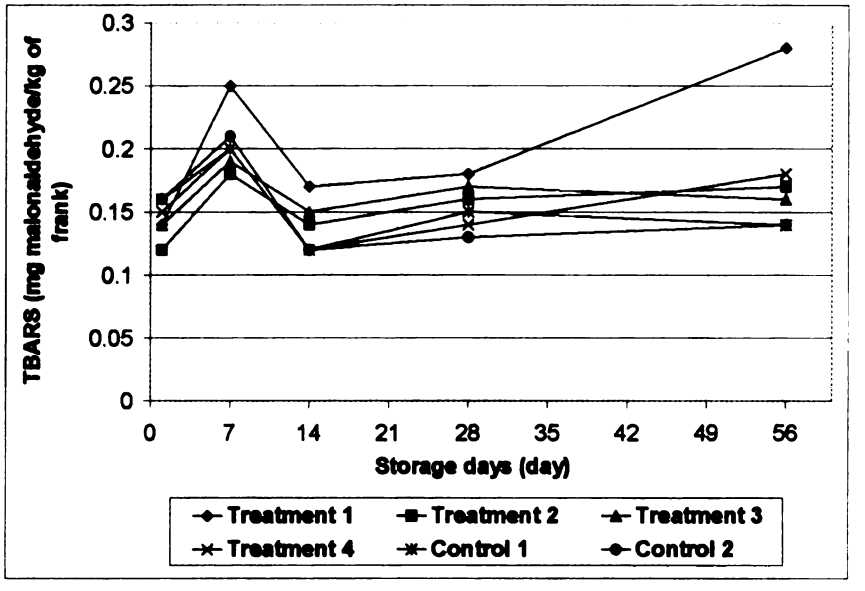


Figure 5.1b: TBARS



Treatment 1: 35% water, 3.5% preheated whey protein isolate. Treatment 2: 40% water, 3.5% preheated whey protein isolate. Treatment 3: 40% water, 7.0% preheated whey protein isolate. Treatment 4: 45% water, 7.0% preheated whey protein isolate. Control 1: 40% water, 3.5% unheated whey protein isolate. Control 2: 40% water, 7.0% unheated whey protein isolate.

panel to study the desirability of knockwurst prepared with WPC. Consumer panel preferred product prepared with WPC rather than all-meat product.

Although there was no significant difference ($p<0.05$), the juiciness of frankfurters increased as the water content in the formula increased. Control 2 prepared with 40% water and 7.0% unheated WPI had the highest juiciness value. Control 2 also had the highest cohesiveness value. Before serving to the sensory panelists, frankfurters were reheated in package in a pan of 80 °C water for 8 min. This reheating step may help to complete the gelation process of unheated WPI. As a result, a stronger protein matrix was formed. Hence the cohesiveness value of frankfurters increased. Cooking yield result showed that preheated WPI bound water better than unheated WPI. At 40% water and 7.0% WPI, juiciness of frankfurters prepared with unheated WPI was significant ($p<0.05$) higher than those prepared with preheated WPI. This was probably because preheated WPI was able to absorb excess water in the protein matrix, resulting in a drier mouth-feel (Comer and others, 1986). Similar dry mount-feel was also observed in 3.5% WPC frankfurters (Hung and Zayas, 1992). While juiciness of frankfurters was reported to be decreased by adding whey protein, juiciness of other whey protein added meat products was not affected by the presence of whey protein (Yetim and others, 2001).

Depending on the whey protein amount, the mild flavor of whey protein has been reported to have no or little effect on the flavor profile of meat products (El-Magoli and others, 1996, Hale and others, 2002, Hughes and others, 1998, Hung and Zayas, 1992, Marriott and others, 1998). Sensory off-flavor result agreed with those findings and none off-flavor was detected in all frankfurters. Even though frankfurters prepared with 45% water and 7.0% preheated WPI had significant ($p<0.05$) higher milk/whey coating than

other frankfurters, the score was low, only up to 1.93 on a 8 point scale, and all other frankfurters only had very mild milk/whey coating (ranged from 1.13 to 1.47).

Conclusions

Preheating WPI was proven to be an effective method to improve functionality of WPI in beef frankfurters. Cooking yield of beef frankfurters increased when preheated WPI was used instead of unheated WPI. At same WPI level, as water content increased cooking yield decreased. Textural properties of frankfurters were altered when preheated WPI was added. Hardness value of frankfurters prepared with preheated WPI was higher than those prepared with unheated WPI. Thiobarbituric acid reactive substance value, pH and L^*, a^* and b^* values of frankfurters prepared with both unheated and preheated WPI remained stable during 56 days storage. While some sensory attributes were different among frankfurters prepared with unheated and preheated WPI, no off-flavor and only mild milk/whey flavor was detected in beef frankfurters.

This study was designed specifically to verify the efficacy of preheated WPI in comminuted meat products. The results of this study confirmed that quality attributes and textural properties of comminuted meat products can be improved by using preheated WPI instead of unheated WPI. Since functionality of preheated WPI can be manipulated by changing both preheating and gelling conditions, more research should be conducted to explore the applicability of preheated WPI in other type of meat and food products.

References

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental measurements of fresh meat. American Meat Science Association and National Livestock and Meat Board. Chicago, IL.

AOAC. 2000. Meat and meat products. Hess, J. Ed. *In Official Methods of Analysis of AOAC International.* 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Atughonu AG, Zayas JF, Herald TJ, Harbers LH. 1998. Thermo-rheology, quality characteristic, and microstructure of frankfurters prepared with selected plant and milk additives. J. Food Qual. 21: 223-228.

Barbut S. 2002. Measuring sensory and functional properties. *In Poultry products processing.* Boco Raton, Fl.: CRC press Inc.. p.467-514

Beuschel BC, Culbertson JD, Partridge JA, Smith DM. 1992a. Gelation and emulsification properties of partially insolubilized whey protein concentrates. J. Food Sci. 57 (3): 605-609, 634.

Bryant CM, McClements DJ. 2000a. Optimizing preparation conditions for heat-induced whey protein solution to be used as cold-gelling ingredients. J. Food Sci. 65: 259-263.

Bryant CM, McClements DJ. 2000b. Influence of NaCl and $CaCl_2$ on cold-set gelation of heat-denatured whey protein. J. Food Sci. 65: 801-804.

Bourne M. 2002. Principles of objective texture measurement. In *food texture and viscosity.* 2nd ed. New York, NY: Academic press. p.107-188.

Chantrapornchai W, McClements DJ. 2002. Influence of NaCl on optical properties, large-strain rheology and water holding capacity of heat-induced whey protein isolate gels. Food Hydrocolloids. 16: 467-476.

Chen CM, Trout GR. 1991a. Sensory, instrumental texture profile and cooking properties of restructured beef steaks made with various binders. J. Food Sci. 56: 1457-1460.

Comer FW, Chew N, Lovelock L. 1986. Comminuted meat products: functional and microstructural effects of fillers and meat ingredients. Can. Inst. Food Sci. Technol. J. 19: 68-74.

Ensor SA, Mandigo RW, Calkins CR, Quint LN. 1987. Comparative evaluation of whey protein concentrate, soy protein isolate and calcium-reduced nonfat dry milk as binders in an emulsion-type sausage. J. Food Sci. 52: 1155-1158.

El-Magoli SB, Laroia S, Hansen PMT. 1996. Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. Meat Sci. 42: 179-193.

Fox JB, Townsend WE, Ackerman SA, Swift CE. 1967. Cured color development during frankfurter processing. Food Tech. 21: 386-3392.

Hale AB, Carpenter CE, Walsh MK. 2002. Instrumental and consumer evaluation of beef patties extended with extrusion-textured whey proteins. J. Food Sci. 67: 1267-1270.

Holt C, Mcphail D, Nevison I, Nylander T, Otte J, Ipsen RH, Baur R, Ogendal L, Olieman K, de Druif KG, Leonil J, Molle D, Henry G, Maubois JL, Perez MD, Puyol P, Calvo M, Bur SM, Kontopidis G, McNae I, Sawyer L, Ragona L, Zetta L, Molinari H, Klarenbeek B, Jonkman M, Moulin J, Chatterton D. 1999a. Apparent chemical composition of nine commercial or semi-commercial whey protein concentrates, isolates and fractions. Int. J. Food Sci. Tech. 34: 534-556.

Holt C, Mcphail D, Nevison I, Nylander T, Otte J, Ipsen RH, Baur R, Ogendal L, Olieman K, de Druif KG, Leonil J, Molle D, Henry G, Maubois JL, Perez MD, Puyol P, Calvo M, Bur SM, Kontopidis G, McNae I, Sawyer L, Ragona L, Zetta L, Molinari H, Klarenbeek B, Jonkman M, Moulin J, Chatterton D. 1999b. Some physico-chemical properties of nine commercial or semi-commercial whey protein concentrates, isolates and fractions. Int. J. Food Sci. Tech. 34: 587-601.

Hongsprabhas P, Barbut S. 1999a. Effect of preheated whey protein level and salt on texture development of poultry meat batters. Food Res. Int. 32: 145-149.

Hongsprabhas P, Barbut S. 1999b. Use of cold-set whey protein gelation to improve poultry meat batters. Poultry Sci. 78: 1074-1078.

Hughes E, Mullen AM, Troy DJ. 1998. Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. Meat Sci. 48: 169-180.

Hung SC, Zayas JF. 1992. Functionality of milk proteins and corn germ protein flour in comminuted meat product. J. Food Qual. 15: 139-152.

Langley KR, Green ML. 1989. Compression and impact strength of gels, prepared from fractionated whey proteins, in relation to composition and microstructure. J. Dairy Res. 56: 275-284.

Lanier TC. 1991. Interactions of muscle and non-muscle protein affecting heat-set gel rheology. In *Interactions of food proteins*. Washington, DC: ACS Series. p. 454.

Lee A, Cannon Y, Huffman DL. 1980. Whey protein concentrates in a processed meat loaf. J. Food Sci. 45: 1278-1279, 1304.

Lucca PA, Tepper BJ. 1994. Fat replacers and the functionality of fat in foods. Trends Food Sci. Tech. 5: 12-19.

Marangoni AC, Barbut S, McGauley SE, Marcone M, Narine SS. 2000. On the structure of particulate gels: the case of salt-induced cold gelation of heat-denatured whey protein isolate. Food Hydrocolloids. 14: 61-74.

Marriott NG, Wang H, Claus JR, Graham, PP. 1998. A research note: evaluation of restructured low-fat ham containing whey. J. Musc. Foods. 9: 201-207.

Meilgaard M, Civille GV, Carr BT. 1991. Sensory Evaluation Techniques. Boca Raton, Fl.: CRC press Inc.

Morr CV, German B, Kinsella JE, Regenstein JM, Van Bure JP, Kilara A, Lewis BA, Mangino ME. 1985. A collaborative study to develop a standardized food protein solubility procedure. J. Food Sci. 50: 1717-1718.

Morr CV, Foegeding EA. 1990. Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. Food Tech. 54: 100-112.

Paoletti F, Nardo N, Saleh A, Quaglia GB. 1995. Back extrusion test on emulsions stabilized with whey protein concentrates. Food Res. Int. 28: 616-619.

Pawar VD, Khan FA, Agarkar BS. 2002. Effect of fat/whey protein concentrate levels and cooking methods on textural characteristics of chevon patties. J. Food Sci. Tech. 39: 429-431.

Pena-Ramos EA, Xiong YL. 2003. Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci. 64: 259-263.

Rhee KS. 1978. Minimization of further lipid peroxidation in the distillation 2-thiobarbutiric test of fish and meat. J. Food Sci. 43: 1776-1778.

Sammel LM, Claus JR. 2003. Whey protein concentrates effects on pink color development in a cooked ground turkey breast model system. Meat Sci. 65: 1293-1299.

SAS Institute, Inc. 2002. SAS user's guide, version 8.2. Cary, NC: SAS Institute.

Santiago LG, Aringoli EE, Sabbag NG, Bnaldo AG, Gonzalez RJ. 2004. Kinetics of physico-chemical and microbiological changes during storage of commercial frankfurters. Food Sci. Tech. Int. 10: 109-118.

Shie JS. 2004. Application of preheated whey protein polymers in low fat beef frankfurters. Ph.D. dissertation. Michigan State University. E. Lansing, MI.

Slesinski AJ, Claus JR, Anderson-Cook CM, Eigel WE, Graham PP, Lenz GE, Noble RB. 2000a. Ability of various dairy proteins to reduce pink color development in cooked ground turkey breast. J. Food Sci. 65: 417-420.

Slesinski AJ, Claus JR, Anderson-Cook CM, Eigel WE, Graham PP, Lenz GE, Noble RB. 2000b. Response surface methodology for reduction of pinking in cooked turkey breast mince by various dairy protein combinations. J. Food Sci. 65: 421-427.

Smith DM, Brekke CJ. 1985. Characteristics of low-salt frankfurters produced with enzyme-modified mechanically deboned fowl. J. Food Sci. 50: 308-312, 320.

Sommers CH, Fan X, Handel AP, Sokorai KB. 2003. Effect of citric acid on the radiation resistance of *Listeria monocytogenes* and frankfurter quality factors. Meat Sci. 63: 407-415.

Tong LM, Sasaki S, McClements DJ, Decker EA. 2000. Mechanisms of the antioxidant of a high molecular weight fraction of whey. J. Agric. Food Chem. 48: 1473-1478.

USDA. 2003. National agricultural statistics service: Dairy products annual summary, USDA 2002.

Whey Protein Institute. 2004. Benefits of whey proteins. Available from: www.wheyoflife.org. Accessed June 1, 2004.

Yetim H, Müller WD, Eber M. 2001. Using fluid whey in comminuted meat products: effects on technological, chemical and sensory properties of frankfurter-type sausages. Food Res. Int. 34: 97-101.

RECOMMENDATIONS FOR FUTURE RESEARCH

The results of this study demonstrated that preheated whey protein isolate (WPI) can be tailor-made to fit processing conditions suitable for emulsified meat products. The tailor-made WPI not only had improved functionality (water holding and fat holding capacities), when applied in beef frankfurter, it also increased cooking yield and altered textural properties of beef frankfurters. Preheating of WPI was proven to be an effective method to improve functionality of WPI in beef frankfurters.

In this study WPI was used as the sole non-meat protein extender in the emulsion formula. In commercial emulsified meat products, more than one non-meat binders or extenders are often used. Other non-meat protein extenders such as soy proteins, caseins, and dry milk proteins can be used in the combinations with WPI and the synergistic effects of different combinations of these non-meat protein extenders could be studied. It is possible that when used in the combinations with other non-meat protein extenders, the effects of these extenders on textural and quality attributes of meat products may increase. Other than non-meat protein binders, polysaccharide stabilizers such as alginates, pectins and carrageenans can also act synergistically with non-meat protein extenders to stabilize meat emulsions. The feasibility of developing different combinations of these non-meat protein extenders and polysaccharide stabilizers in application in meat products could also be explored.

Since preheated WPI functionality can be changed by both preheating and gelling conditions, this great flexibility of preheated WPI would allow meat processors to use preheated WPI to a great extent. Milk protein extenders can be applied in three ways: 1)

As a powder at the beginning of the emulsifying process; 2) As a water solution; 3) As a pre-emulsion prepared from milk protein, water and fat. In this study, WPI was added as a powder at the beginning of the comminuting process. Since gelling conditions of preheated WPI can also be optimized for certain food products, the method of application of preheated WPI in meat products could also affect quality attributes of meat products. Preblending of milk protein with technologically difficult-to-stabilize fats, such as chicken fat and beef suet and tallow can increase emulsion stability of emulsified meat products. Since preheating WPI increased fat holding capacity of WPI, preblending preheated WPI with these technologically difficult-to-stabilize fats may increase emulsion stability of emulsified meat products to a further extent. Research in this area should be conducted to confirm the preblending effect of preheated WPI and fat on emulsion stability of emulsified meat products.

In this study, all experiments were designed to fit the processing conditions suitable for emulsified meat products. When used in restructured type meat products, whey protein is served as a binder. The interactions between preheated whey proteins and meat proteins in restructured meat products are similar but also may be different to those in emulsion type meat products. To maximize the potential use of preheated whey protein polymers in restructured meat products, the preheating conditions for whey protein can also be tailor-made for restructured meat products. It would be interesting for researchers to optimize the preheating conditions for whey protein under the conditions specific for restructured meat products.

Finally, the use of preheated whey proteins can also be explored in other food products. The decreased textural qualities in surimi seafood have been reported to due to

the endogenous proteases found in surimi. Whey proteins have showed the ability to inhibit the activity of these endogenous proteases and improve the textural qualities of surimi seafood. In the present study of whey protein, not only textural qualities of whey proteins are improved, cooking yields are also increased. Like processed meat products, the production of surimi seafood involves a heat-set gelation step. The new improved functionality of preheated whey proteins would also be beneficial to surimi processors, especially a cold-set incubation step has been reported to increase the textural properties in both surimi seafood and gels prepared with preheated whey protein.

APPENDICES

APPENDIX 1: Small Amplitude Dynamic Oscillatory Testing

Rheometer: Haake RS100 rheometer, Karlsruhe, Germany

Analysis Procedures:

1.Turn on nitrogen gas, the nitrogen gas pressure should be set at 37 psi. Let the machine stabilize for 5 min.

2. Turn on water bath by switch the on switch.

3. Turn on automatic fan and cooling by switch on button on the side of the water bath.

4. After the machine has been warmed up for 5 min, attach measurement plate and base to the rheometer.

5. Turn on computer.

6. Choose oscillatory mode.

7. Under "measurement" set up gap size, plate size and type, program.

8. After all parameters are set, load sample between the plate and base.

9. Under "operation" move plate to the measurement position.

10. Start measurement by "start measurement"

11. If a temperature profile is used, "PG" button on the water should be pressed.

12. When finish analysis, press "save", then type in file name you want to save to.

13. Go to "evaluation", then "file", then "load", load the file you just saved.

14. Under "print", choose "file" then "A" to save your file in the disc.

15. When finish, lift the plate by choose "lift/control", then "operation", then "lift"

16. Remove the plate and base.

17. Turn off computer, then water bath, then the nitrogen gas.

APPENDIX 2: Scanning Electron Microscopy

Operating instructions for JSM 6400V provided by the Center for Electron Optics

---

Scanning electron microscope: JEOL scanning electron microscope, Model JSM-6400V, version 96-2, Tokyo, Japan

Caution:

1. Never change the accelerating voltage with the filament saturated. Change the accelerating voltage only when the filament is desaturated to the preheat value.

2. Never turn the filament knob faster than the directions describe.

3. At the 8 mm working distance nothing should extend more than 2 mm above the top of the sample hold.

Analysis procedures:

Start-up

1. Turn up the brightness on CRTs 1 and 2

2. Check the vacuum (10^{-7} range).

3. Check the heat/preheat light (on). This is located on the box to the right of the SEM.

Sample insertion

1. Working distance 39, X-25, Y-35, Tilt-0, Rotation-0.

2. Place samples in the sample holder, adjust height, attach holder to the sample insertion rod.

3. Pull the rod back into the spring clip. Place rod on the port, press the red button, and wait till the light goes out. Do not wait. After the light goes out, the port is no longer pumped. If you wait, the vacuum will decrease to dangerously low levels.

4. Turn the flat on the knob to the front and pull it to the right.

5. Push the rod in, place sample holder on the rail, unscrew the rod, and pull it out.

6. Push the knob to the left then turn the flat up.

7. Push the red button, wait till the vacuum is gone, and remove the rod.

Sample removal

1. First desaturate filament and turn off the accelerating voltage

2. Working distance 39, X-25, Y-35, Tilt-0, Rotation 0.

3. Pull rod back into spring clip. Place the rod on the port, press the red button, and wait till the light goes out.

4. Turn the flat on the knob to the front and pull it to the right.

5. Push the rod in, screw the rod into the holder, then pull the rod all the way out.

6. Push the knob to the left then turn the flat up.

7. Push the red button, wait till the vacuum is gone, and remove the rod.

Shut-down

1. Desaturate filament, and sample should be removed.

2. Turn down the brightness of CRTs 1 and 2.

3. Turn off the hanging lamp.

APPENDIX 3: Programmed Water Bath Procedure

Water bath: PolyScience Circulating water bath, Model 9510, Niles, IL

Procedures:

1. To begin entering program, press FCN then #5. Select what program number you want to name the program (1 or 2).

2. Upon number selection you will see the word transferring. Select #1= display, edit and write a program. This will then allow you to enter temperatures and time points. Press enter after each item.

3. Up to 10 temperature/time steps can and must be entered. Repeat last step until you reach step 10.

4. After passing all 10 steps enter number of cycles you wish the water bath to go through (1-999 times).

5. At end of program select soak or power off when done. Selecting soak allows for water bath to hold at final constant temperature entered indefinitely.

6. Select #1 or 2 to store your new program.

7. Water bath is now programmed and ready to conduct process.

APPENDIX 4: Proximate Composition for Whey Protein Isolate

Protein content (Micro-Kjeldahl, AOAC method 930.29,2000)

1. Weight 0.1 g sample in digestion tube and add 1 Kjeldahl tab and 5 ml concentrate sulfuric acid.

2. Digest sample slowly till digest is clear.

3. Distill digested sample using Buchi 323 distillation unit.

4. Titrate distillate using DMS Titrino titration unit.

Procedures for Buchi 323 distillation unit

1. Make sure the caps of distilled water and NaOH tanks are air tight and the drain pipes are inserted and secured properly in the sink.

2. Open the distilled water cap and switch on the unit (at the back of the instrument).

3. Wait till the "wait" flash signal goes off. Set the amount of dispense water to 20 ml, and NaOH to 25 ml, and distill time to 5 min.

4. Fill a Kjeldahl digestion tube with 1/3 distilled water and the boric acid dispensing glass cup with 70 ml water.

5. Press "preheat" button to warm up and boil water.

6. Repeat step 5 twice.

7. Dispense 60 ml of 4% boric acid into the cup and place it in the unit.

8. Insert one tube to the unit.

9. Press "start" button. At the end of distillation, the ammonia in the tube will be trapped into the boric acid.

Procedures for DMS Titrino titration unit

1. Make sure HCl amount is enough for the entire assay.

2. Remove the pH cove and wash the pH probe with distilled water and place the pH probe in the boric acid cup trapped with ammonia from sample.

3. Press "start" and wait till it finishes titrating.

4. Number shows on the screen is the amount of HCl in ml used in titration.

% protein content is calculated as:

$$\% \text{ protein content} = [(V_s - V_b) \times 1.4007 \times 6.38 \times N_{HCl}] / \text{weight of sample (g)}$$

V_s = the volume of HCl needed to titrate sample

V_b = the volume of HCl needed to titrate blank

N_{HCl} = normality of HCl

6.8: conversion factor for dairy protein

Moisture content (AOAC method 927.05, 2000)

1. Weight 1 g sample into a round, flat bottom aluminum dish.

2. Place the dish in a vacuum oven (< 100 mmHg) at 100 °C for 5 hr or till sample reaches constant weight. Moisture content is calculated as:

$$\% \text{ moisture} = [(\text{dish and sample weight before drying} - \text{dish and sample weight after drying}) / \text{sample weight}] \times 100$$

Fat content (AOAC method 932.06, 2000)

1. Weight out 1 g sample in a small beaker and add 1 ml of deionized water to make a smooth paste.

2. Add 9 more ml of deionized water and 1 ml of NH_4OH.

3. Warm the mixture on a steam bath, and then transfer the warmed up mixture to a Mojonnier flask and cool.

4. All 10 ml 95% alcohol and 25 ml of ether then shake the mixture for 1 min.

5. Add 25 ml of pet ether and shake the mixture for 1 min.

6. Set the mixture at 25 °C for 5 min and transfer the upper ether and pet ether layer to a round bottom aluminum drying dish.

7. For second extraction, repeated step 4 to 6 and decrease alcohol amount to 4 ml, and ether and pet ether amount to 15 ml.

8. Set the dish under ventilation hood for 30 min and then dry the dish at a 100 °C oven till constant weight. Fat content is calculated as following:

$$\% \text{ fat} = [(\text{dish weight after drying} - \text{empty dish weight}) / \text{sample weight}] \times 100$$

Ash content (AOAC method 930.30, 2000)

1. Weight 1 g sample in a crucible and char the sample till smoke ceases.

2. Put the crucible in a muffle furnace at 550 C for 16 hr. Ash content is calculated as:

$$\% \text{ ash} = [(\text{ash and crucible weight} - \text{empty crucible weight}) / \text{sample weight}] \times 100$$

APPENDIX 5: Emulsion Stability

1. Stuff 33.3 g sample into a 50 ml polycarbonate tube.
2. Insert a thermometer into the tube.
3. Put prepared tubes in a program controlled water bath.
4. Cook tubes till the temperature reaches the target temperature and time.
5. Place cooked tubes in a water/ice bath for 10 min.
6. Decant fluid from tubes through cheese cloth coved funnels.
7. Collect fluid in 15 ml centrifuge tubes and centrifuge tubes at 6,000 × g for 5 min.
8. Record total volume of the fluid, and also volume of fat and water.

APPENDIX 6: Solubility of Whey Protein Isolate

1. Weight 0.5 g sample in a 150 ml beaker.

2. Add a small amount of 0.3 M NaCl to make a smooth paste.

3. Add 40 ml more of 0.3 NaCl and adjust pH of the solution to 7.0.

4. Transfer the solution to a 50 ml volumetric flask and bring the volume of the solution to 50 ml with 0.3 M NaCl.

5. Stir the solution at 25 °C for 1 hr and centrifuge the solution at 20, 000 × g for 30 min.

6. Filter the supernatant through Whatman No.1 filter.

7. Determine the protein content of the filtered supernatant by micro-Kjeldahl method. Solubility of WPI was determined as following:

Protein solubility = [supernatant protein concentration (mg/ml) × 50] /[sample weight (mg) × sample protein content (%) / 100] × 100

APPENDIX 7: Proximate Composition for Raw Frankfurter Batters and Cooked Frankfurters

---

Moisture content (oven drying method, AOAC method 950.46B, 2000)

1.Place a medium weigh boat on scale and zero. This is to keep the scale clean. Add paper labeled with sample ID and paperclip. Record the weight then tare the scale.

2.Add 2 grams (± .03g) of thoroughly mixed sample to the paper. Once desired weight is reached record weight and fold over top. Place flat on tray. Do all samples in triplicate. Do not stack samples on tray. This will hinder the drying process.

3. Once tray is full, place in drying oven set at 100°C for 20 - 24 hours.

4. After drying, place samples using latex gloves or tongs in desiccators to cool completely before weighing. Once cool, weigh samples and record. This is your final weight for moisture and your initial weight for fat analysis. Use the following formula to determine the percent moisture in your samples:

$$\text{Moisture (\%)} = \frac{\text{wet sample wt.} - \text{dry sample wt.}}{\text{wet sample wt.}} \times 100$$

Fat content (Soxhlet ether extraction, AOAC method 991.36, 2000)

1. Take samples from moisture analysis and place in extraction tubes. Make sure that all the samples are below the level where the ether drains off (curved glass on outside of tube).

2. Add petroleum ether to clean boiling flasks until about ¾ full. Add 2 to 3 glass beads as a boiling aid.

3. Connect the extraction flask to the boiling flask and Soxhlet apparatus. Place parafilm on the joint. Mount both to the condensing units on top of extraction flasks using parafilm around joint.

4. Turn on condensing water so it runs at a steady stream.

5. Set Rheostats on high and run for 24 hours.

6. Place ether soaked samples onto a tray in a hood for 2 hours to allow ether to dissipate.

7. Place samples in drying oven for 5 to 10 min to remove any possible moisture then place in desiccators for 1/2 hour to cool.

8. Weigh and record the weight of the samples. Calculate fat on wet basis with the following equation:

$$\text{Fat (\%)} = \frac{\text{dry sample wt.} - \text{extracted sample wt.}}{\text{wet sample wt.}} \times 100$$

Ash content (dry ashing, AOAC method 920.153, 2000)

1. Weight 1 g sample in a crucible and char the sample till smoke ceases.

2. Put the crucible in a muffle furnace at 550 C for 16 hr. Ash content is calculated as:

$$\text{\% ash} = \frac{[(\text{ash and crucible weight} - \text{empty crucible weight})}{\text{sample weight}} \times 100$$

Protein content (combustion method, AOAC method 992.15, 2000)

1. Weigh out approximately 1 gram of powdered meat into the tared crucible. Write the weight and sample ID on the side of the crucible with pencil.

2. After weighing out samples, dry for 18 to 20 hours in the drying oven at 100°C. This removes moisture that can cause internal malfunctions with the Leco Protein Analyzer. Do not reweigh samples. Enter wet weight into computer.

Procedures for the LECO FP 2000 Nitrogen Analyzer

1. Open valves completely on oxygen, helium and compressed air tanks. Make sure tanks have adequate levels of gas (gauge should read >100psi) and that the pressure out of the tanks are set at 40 psi.

2. Press escape on upper left hand corner of touch screen until "front panel" comes up and then press it. On right hand side of screen a section labeled "analysis gas" can be found. Push the "on" button to turn gasses on to the machine. Check to see that your furnace temperature is 1050°F (located on left part of screen).

3. Wait about 5 minutes for all gasses to equilibrate then start your leak tests. Press escape from the front panel located in upper left corner. A screen with several icons will appear. Press "maintenance". This will bring up helium leak test, combustion leak test and ballast leak test icons. Press the helium leak test. If it passes move onto the combustion leak test. Run a ballast test only if there is a leak in the combustion system. Once finished, start running blanks.

4. Run several air blanks through to purge the system. To do this escape from the "maintenance" section and push the "analyze" icon. On the bottom of the screen you will see several commands. Push "select ID code". Move the highlighted line using the arrows to "blanks". Then push exit on bottom. Then push manual weight. This will bring up a touch screen with 0.2000000 on it. Push the enter button at least 10 times to bring up 10 rows of 0.20000. Then push analyze. The machine will run through these ten samples. Numbers should come down to about <0.2000% protein. Wait until several blanks have approximately the same protein content. Then run EDTA samples.

5. Weigh approx. 1.0 g EDTA samples out in the ceramic boats and write the weight on the side in pencil (at least four decimal places).

6. Select "manual weight" and put your weight into the machine pressing enter after each entry. Once weights are entered, press analyze. Follow the directions on the touch screen. Push your first sample into the chamber about one half inch so the door doesn't catch the boat. Push okay on the screen when it asks you to place your sample in the chamber. The next message will tell you to wait because the system is purging. Then the machine will then tell you to push the boat into the chamber. The machine will combust and analyze the sample in approximately 4-5 minutes.

7. Once blanks are at an acceptable number, run 5-6 EDTA samples (approximately 1.0g) to verify machine is operating properly. EDTA is 59.9% protein. The samples should come out to be 59.9% +/- 0.2%.

8. If there is more variation in the percentages than 0.2%, DRIFT the samples to equilibrate the percentages. To drift, press escape until you reach the front panel. Press Calibrate, then press Drift Correction. A new screen will pop up; press Carbon and then OK. Select the closest samples from the list of results by using the up/down arrows and "Include Result" button at the bottom of the screen. Pressing the "Include Result" will highlight the result and use it to recalibrate the machine. Once weights are all selected, choose "Process Results" at the bottom of the screen. Another screen will pop up asking if you would like to save the new calibration. Choose "Yes."

9. After the Drift Correction escape to the front panel and choose Analyze. Continue running EDTA samples to ensure that the machine is working properly. Once it is functioning properly, you may run your test samples. Analyze samples as described in step 7.

APPENDIX 8: Two-Cycle Compression Settings

Texture Analyzer :	TA-HD*i* Texture Analyzer Texture Technologies Corporation, Scarsdale, NY
Software:	Texture Expert Version: 1.22
TA-HD*i* Settings:	
Test Mode:	TPA
Option:	Return to Start
Pre-Test Speed:	4.00 mm/s
Test Speed:	5.0 mm/s
Post-Test Speed:	5.0 mm/s
Pre-Travel Distance:	40 mm
% Compression:	Sample compressed to 35% of original height
Trigger Type:	Return
Data Acquisition Rate:	200 pps
Attachment/Accessory:	TA-30; 75mm aluminum plate, 10mm tall 50 kg load cell TA-90; Heavy duty platform

APPENDIX 9: TA-HD*i* Texture Analyzer Calibration and Analysis Procedures

---

Calibration Procedure:

Machine Calibration

1. Turn the texture analyzer (TA) on. The power button is located on the bottom right side toward the front.

2. Log on to texture analyzer program on computer (Texture Expert Analyzer) found on computer desktop.

3. Turn TA key to the "run" position.

4. Remove any attachments or platforms that are present on the TA.

5. Attach calibration weight hanger attachment and weight hanger.

6. Turn TA key to machine configuration.

7. Press "ENT (enter)" until you reach the screen that determines the load cell weight (Cell).

8. Press " +/-" to acquire appropriate load cell weight. For example: 50 kg load cell will be indicated by "50" on screen.

9. Turn TA key back to "run" position and then back to machine configuration. This saves settings in TA.

10. Press the "calibrate" key, then "enter".

11. When TA screen reads the appropriate weight put the actual weight on the TA weight hanger. For example: 50 kg load cell will utilize a 10 kg weight, 5 kg load cell utilizes a 2 kg weight.

12. Press "calibrate" and when screen reads "done", switch TA key back the to "run" position.

13. Remove the actual weight from hanger but do not remove the weight hanger. Do not put the weight away because you will be using it in the computer calibration.

Computer Calibration

1. Go to heading that reads "TA".

2. Click on "Calibrate Force".

3. Press "ok". The computer will then ask you to place the actual weight on the hanger.

4. Once the weight is placed onto the hanger press "ok".

5. The computer will then say "calibration successful". If this is not indicated, or if the calibration unsuccessful, re-calibrate the machine.

6. Remove the weight and the hanger from the TA.

7. Attach the platform to the TA and the appropriate attachment. For Example: For the Kramer shear test attach the 5-blade attachment to TA and from Gel hardness attach TA-10 attachment.

8. When using the WBS attachment an extra set up step is required, if you are not using the WB attachment you may skip this step. Using the up and down arrows on the TA control board lower the WB blade into the slit on the platform until you can feel the blade poke through the platform with the tips of your fingers. Run quick tests and move the platform to make the force in kg as close to 0.000 as possible. This reduces the friction during the analyses.

9. The TA is now ready to analyze samples.

Analysis Procedure and Setting up the Computer Files:

1. Create a personal file for data collection – go to computer desktop.

2. Click "My Computer"

3. Go to Drive "C:\"

4. Click on "My Documents"

5. Open the folder in which you wish to save you results. To create a new folder, Right click and scroll to "new" chose the "folder" option.

6. Name your folder. (Example: Set 1) If you choose a file name that is too long the computer will not read it. (Example: Shear Set 1)

7. In the Texture Expert Program go to your selected project (the minimized window in the bottom left hand corner of the screen, this is the project window.

8. Under settings, push dotted button (ellipses) and make sure your correct folder is selected. Do the same for macro and results. If your desired settings are not entered you must go to a folder with previous tests in it. Copy the macro file, setting files, as well as a result file to your folder. This is done so the computer knows which format to follow.

9. Push "Restart" on the Texture Expert Analyzer Program. You may receive an error message because you have not created any results yet.

10. Under TA go to "Settings" and include the appropriate settings for the test if different than the settings listed there.

11. Click on the "TA" heading on the computer screen. Select "run test". Check to where the results are being sent. The path in black on the middle of the screen indicates this. (Example: C:\mydocu\wbs\johnson\set1 will put the results on the C:\ drive under My Documents in Johnson's folder under set 1) If the path is incorrect it can be changed by pressing on the ellipses dots in the large white box. Continually clicking on the top of this screen will take you back to the C:/ drive. Click on the folder into which you wish your results to be placed.

12. Enter the ID of the sample you will analyze under "File ID". Set the "file number" to one so it can count the samples. A low number is necessary because too many characters in the file name will cause an error message to appear. Set the file number to one each time you open a new spreadsheet for results.

13. Place the sample on the machine to be analyzed.

14. Enter the ID of the sample. Dates can be entered under "Batch ID".

15. Press "ok" on the Run Test screen to begin analyzing.

16. You will be prompted to save the results from the previous test. Click "ok".

17. Repeat steps 13-16 as necessary.

18. The analyzer may begin to run slowly after several analyses. If this occurs you should start a new spreadsheet. To start a new spreadsheet click on the results window to make it active. Select "Save" from the File menu. Then hit the "X" button on the top right corner of the screen. Do not select "exit", as this will close the Texture Analyzer Program. You do not need to open a new spreadsheet. One will start as soon as you run the next test. To get back to the analyzing screen click on the graph page to make that window active.

19. If at anytime the "run test" option is not available go back to the Project screen (the first screen) and click "restart". This should cause the "run test" option to be available again.

APPENDIX 10: Cooking Yield

1. Weigh linked uncooked frankfurters in a meat lug before smoke house cooking

2. After cooking, chill cooked frankfurters to 4.4 °C.

3. Weigh cooked chilled frankfurters.

4. Percent cook yield is determined by the following calculation:

$$\% \text{ cook yield} = \frac{\text{Weight of cooked frankfurter}}{\text{Weight of uncooked frankfurter}} \times 100$$

APPENDIX 11: pH Determination for Raw and Cooked Frankfurters

1. Homogenize 10 gram diced sample with 90 ml of deionized water in a 500 ml plastic beaker with Polytron mixer (PT-35, Kinematica, AG, Switzerland) set on speed setting 4 for 1 min. Rinse and blot dry Polytron bit between each sample.

2. Measure pH using an Accumet Scientific pH meter calibrated using buffers 4.0 and 7.0.

3. Rinse pH meter probe with distilled, deionized water between sample readings.

APPENDIX 12: Purge Loss

1. Remove specified day vacuum package from cooler and weigh for total package weight.

2. Drain out released fluid from package.

2. Remove frankfurters, blot dry frankfurters with paper towel, and reweigh.

3. Dry package with paper towel and reweigh the whole package

4. Percent purge loss is determined using the following calculation:

$$\% \text{ purge loss} = \frac{\text{Total pkg. wt.} - (\text{Dry pkg. wt} + \text{Dry meat wt.})}{\text{Total pkg. wt.}} \times 100$$

APPENDIX 13: Thiobarbituric Acid Reactive Substances (TBARS)

Rhee, KS. 1978. Minimization of further lipid peroxidation in the destillation 2-thiobarbutiric acid test of fish and meat. J Food Sci 43:1776-1778.

Tarladigis, GG, Wats, BM, Younthan, MT, Dugan, L Jr. 1960. J Am Oil Chem 37:44-48.

Zipser, MW, Watts, BM. 1962. Lipid oxidation (TBA) methods. Food Technol 16(7):102.

Reagents:

1. TBA Reagent

Prepare the amount of TBA Reagent needed for your samples according to the table below:

Thiobarbituric Acid	Total Vol. Water and Acid
1.4416 g	500 ml
0.7208 g	250 ml
0.5766 g	200 ml
0.2883 g	100 ml
0.1442 g	50 ml

Dissolve the Thiobarbituric Acid (Eastman Organic Chemicals) in the distilled water, about 2/3 the total volume. Place flask in sonic cleaner (several minutes) and shake occasionally until TBA is dissolved. Allow reagent to come to room temperature then bring to volume. Store in cooler, may be kept for 2 days.

2. HCl Solution:

Make volume as needed; 1:2, HCl : H_2O (v/v).

3. Antifoam (Thomas®, Swedeboro, NJ):

The use of antifoam may not be necessary depending on the product. Fish and egg require antifoam while poultry does not.

4. Sulfanilamide Reagent (Cured Meat Only):

Dissolve 0.5 g Sulfanilamide and 20ml Conc. HCl in 100 ml volumetric flask. Bring to volume with distilled water. NOTE: Store in dark bottle, will discolor with age.

Procedures:

1. Add 10 g of diced sample to 100 ml plastic bottle containing 50 ml distilled water plus 10 µl antioxidant solution (Tenox 5 – food grade BHA+BHT).

2. Homogenize sample plus solution using Polytron mixer (PT-35, Kinematica, AG, Switzerland) on speed setting 4 for 1 minute (Homogenized samples can be held in cooler if needed).

3. Into 500 ml extraction flasks, add glass beads (Fisher Scientific, Pittsburgh, PA), homogenized meat sample, 2.5 ml HCl solution, and if necessary antifoam and/or sulfanilamide solution. (1 ml if > 100 ppm nitrite; 2 ml if > 200 ppm nitrite.)

Note: total volume is 50 ml + 2.5 ml + 47.5 ml = 100 ml

Or 50 ml + 2.5 ml + 1.0 ml + 46.5 ml = 100 ml

Or 50 ml + 2.5 ml + 2.0 ml + 45.5 ml = 100 ml

4. Turn on condenser water and place graduated cylinders under spouts.

5. Connect extraction flasks to distilling tubes.

6. Turn heat control knobs to HI.

7. Distill and collect 50 ml of the distillate.

8. Transfer distillate to 50 ml centrifuge tubes, cap and hold in refrigerator for TBA reaction. (can be held for 18 hours).

9. Invert each test tube containing the 50 ml distillate and pipette 5 ml into each of 2 tubes labeled "A" and "B". Prepare 2 blanks by pipetting 5 ml distilled water into both tubes labeled "A" and "B".

10. Add 5 ml of TBA Reagent into each tube containing 5 ml of sample and into both blanks. Thoroughly mix each tube using Vortex mixer (American Scientific Products, McGaw Park, IL).

11. Turn water bath on 100° C.

12. Place tubes in test tube rack and immerse into boiling water bath (model 9510 PolyScience, Sorvall Co., Niles, IL) for 30 minutes.

13. Turn on plate reader.

14. When the tubes are done heating in the water bath cool them in ice for at least 10 minutes.

15. Mix each test tube with sample for 10 seconds using Vortex mixer (American Scientific Products, McGaw Park, IL).

16. Pipette 200ul into well on plate (done in duplicate).

17. Place plate in plate reader and set up plate reader. Click on plate reader on computer, click on experiment 1. Go to "set up" and set the appropriate wavelength and chose end point analysis. Go to "template" and set up blank and unknowns according to plate. (Wavelength to 538 nm for cured meat and 530 for fresh meat) Read samples within 1 hour.

18. Convert % T to optical density and multiply by the constant 7.8 (7.6 for poultry) to convert to mg malonaldehyde/1000 g of sample, i.e. TBA Number.

Absorbance was converted to mg malonaldehyde (MDA) /kg sample (TBARS value) using the following equation:

$$TBARS = A_{532nm} \times K \text{ (mg MDA/kg sample)}$$

Where K = (conc. in moles/5 ml of distillate × M.W.MDA × 10^7 × 100) / (Absorbance × wt. of sample × % recovery)

K is distillation constant and equal to 7.8 in this lab.

APPENDIX 14: Color Measurement (CIE L^*, a^*, and b^* value)

Color meter: ColorTec PCM, ColorTec Associates, Inc, Clinton, NJ

Analysis Procedures:

Calibration:

1. Turn on color meter.
2. Press mode to Pick
3. Up to Setup
4. Down to Full Calibrate
5. Mode to Measure Black tile,
6. Press black tile against measuring orifice
7. Hit Go key when ready
8. Next you will be prompted to "Measure White Ready Hit mode"
9. Place white tile against measure orifice
10. Press Go key when Ready.
11. Now you are done with calibration

Sample measurement:

1. Press sample against measuring orifice
2. Hit Go key when ready
3. Use Kimwipes to clean measuring orifice between measurements.

APPENDIX 15: Trained Sensory Panel Ballot

Low Fat Beef Frankfurter Descriptive Attribute Ballot

Name_____ Rep_____ Date___

Sample	Skin Hardness	Product Hardness	Springiness	Juiciness	Cohesiveness	Off-Flavor Intensity	Milk/Whey Coating or Residue
Warm Up							
Warm Up							

Skin Hardness/ Product Hardness	**Springiness**	**Juiciness**	**Cohesiveness**	**Off Flavor Intensity/ Milk/Whey Coating/Residue**
8 Extremely Hard	8 Extremely Resilient	8 Extremely Juicy	8 Extremely Cohesive	8 Abundant
7 Very Hard	7 Very Resilient	7 Very Juicy	7 Very Cohesive	7 Moderately Abundant
6 Moderately Hard	6 Moderately Resilient	6 Moderately Juicy	6 Moderately Cohesive	6 Slightly Abundant
5 Slightly Hard	5 Slightly Resilient	5 Slightly Juicy	5 Slightly Cohesive	5 Moderate
4 Slightly Soft	4 Slightly Non-Resilient	4 Slightly Dry	4 Slightly Crumbly	4 Slight
3 Moderately Soft	3 Moderately Non-Resilient	3 Moderately Dry	3 Moderately Crumbly	3 Traces
2 Very Soft	2 Very Non-Resilient	2 Very Dry	2 Very Crumbly	2 Practically None
1 Extremely Soft	1 Extremely Non-Resilient	1 Extremely Dry	1 Extremely Crumbly	1 None