APPLICATION AND EFFECT OF ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION ON BEEF HEART

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FORMAT OF THESIS

This thesis is presented in the Journal of Food Science style format, as outlined by the Oklahoma State University graduate college style manual. The use of this format allows for independent chapters to be suitably prepared for submission to scientific journals.

Chapter I

INTRODUCTION

In order to maximize profit in the beef industry, the greatest utilization of the carcass is necessary. Cattle tend to have a dressing percentage ranging from 60-64 %, which leaves a tremendous amount of edible and inedible byproducts for the industry to utilize.

Byproducts, as defined by the US meat industry, include everything but the dressed meat. The hide is the most valuable byproduct, making up 59% of the value of byproducts (Ockerman and Hansen 2000). Variety meats make up another portion of the byproduct industry. These edible byproducts can include the liver, tongue, heart, oxtail, kidney, and so on. In some countries blood is used as a food source, as well as blood meal, adhesives, and fertilizers.

Traditionally, the industry has been able to make meat and bone meal of the inedible byproducts for use in animal feeds. However, in 1997 a ban on the feeding of animal protein to ruminants (21 CFR 589.2000) was implemented in the United States to contain the spread of bovine spongiform encephalopathy (BSE). In addition, limits were placed on the use of brains and spinal cords due to concern about the transfer of BSE to humans.

The continual need for outlets for both edible and inedible byproducts led researchers to investigate new ways to utilize these products. Clemen (1927) outlined five requirements to effectively utilize byproducts. He stated that there must be: 1) a commercial means of transferring the byproduct to a usable end-product; 2) a market for

the end product; 3) an inexpensive byproduct of sizable amounts; 4) a method of storing the byproduct and end-product; 5) and usually a highly trained individual.

The traditional method of utilizing beef byproducts by rendering leaves the material heat-treated and stable with few functional properties. Ongoing research involves trying to make use of some of the byproducts before they are sent to rendering and heat-treated. An end-product that was not heat treated might retain some functional/gel forming characteristics that would add more value to the byproducts.

The Japanese have been improving the surimi process for hundreds of years. The advent of trawling in the early 1900's increased the development. By 1945 the framework for the kamaboko (fish cake-type products) industry had been set in the fish industry in Japan. However, it wasn't until the 1950's that several large fishing companies set up a large-scale batch processing system to commercially manufacture a fish sausage that had shelf stability. In the 1960's, after help from research institutions, the raw fish was converted to frozen surimi so the fish did not have to be delivered on a daily basis. The frozen surimi was washed of water-soluble components and then frozen with cryoprotectants to maintain functionality. Today the industry is still producing surimi and surimi based products with the crab cake analog being one of the most successful products.

In the 1990's there was increased interest in applying the surimi process to chicken and later to beef and sheep. Researchers started investigating the gelation process (Smyth and O'Neill 1997, Antonomanolaki and others 1999), changes in the composition (Kenney and others 1992, Ruiz and others 1993, Park and others 1996a), and the effects of added ingredients to these poultry and red meat surimi samples (Srinivasan

and Xiong 1996, Park and others 1996b, Wang and others 1997, Wang and Xiong 1998, Parkington and others 2000a, Parkington and others 2000b).

Another protein recovery system, using low ionic strength, was investigated to see if it would have practical application in the meat industry. In 1999 Hultin and Kelleher patented the acid solubilization/precipitation process. Kelleher and Hultin (2000) demonstrated that the process concentrated the myofibrillar proteins and significantly reduced the fat and other components in the chicken meat. Chang and others (2001) looked at gel formation of chicken muscle at low ionic strength and found that quality gels were obtained.

Mireles DeWitt and others (2002) determined the process parameters and procedures to utilize the acid solubilization isoelectric precipitation (SIP) on beef using beef heart as the model. Several studies, performed with the surimi process on beef, had also used beef heart as their model (Srinivasan and Xiong 1996, Wang and others 1997, Wang and Xiong 1998, Parkington and others 2000a, Parkington and others 2000b).

The objective of this study was to determine the composition and gel characteristics of protein recovered from beef heart utilizing the Acid-SIP process.

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CHAPTER II

REVIEW OF LITERATURE

Beef Byproducts

Beef carcasses traditionally have a dressing percentage of 60-64%. The rest is considered edible and inedible byproducts by the meat industry in the United States. The industry struggles to find uses for these products that will bring maximum value to each animal. The byproducts are usually classified into categories: hides, main ingredients, sausage material, pet food, animal feed, and fertilizer (Ockerman and Hansen 2000).

The hide is considered the most valuable byproduct from the animal with an estimated value of approximately $60 per hide, which is around 7.76% of the total value of the steer/heifer (USDA Market News 2003). According to Boyle (1995), the hide accounts for 59% of the animal's byproduct value. The majority of the hides in the US are converted to leather, but it has also been used as food, in cosmetics, and for medical prosthetics (Ockerman and Hansen 2000).

The edible byproducts (blood and organs) from cattle account for approximately 12% of the carcass weight. The most commonly used edible byproducts are the red viscera, or fancy meats, which refers to the liver, heart, kidney, tongue, and thymus. Acceptability for these and other byproducts from the beef carcass are dependent on the country and region. While the number of cattle slaughtered has remained fairly steady over the last 15 years (Nebraska Agricultural Statistics Service 2002), the use of byproducts for human consumption has decreased especially in developed countries. However, the US is a leading world exporter of offal products with almost $548 million

exported (United Nations Trade Data 2003) so continued use and development of processes that enhance byproduct utilization is important to US processors.

Organ meats are more perishable than the carcass because of the higher glycogen content and slight fat covering. Ideally, the organs should be removed within 30 minutes of bleeding the animal, but inspection usually occurs with the organs still attached so optimum time of removal cannot be obtained. Once removed, the organs should be chilled to limit microbial activity.

Beef Heart

The beef heart weighs around 1.4 kg and is sold as cap-on and cap-off. The cap-on has the bone-os cardis (cartilage) removed with the auricles remaining. The cap-off heart is denuded of the cartilages, auricles, aorta, pulmonary trunk, and some fat tissue. The basic composition of the raw beef heart cap-off is:

Table 1. Composition of Raw Beef Heart

MOISTURE	FAT	ASH	PROTEIN	CARBOHYDRATE[3]	CHOLESTEROL
75.56 %	3.78 %	1.03 %	17.05 %	2.58 %	140 mg

[1] Obtained from USDA National Nutrient Database for Standard Reference.
[2] Values based per 100 g edible portion.
[3] Carbohydrate determined by difference.

The heart is not considered tender and has a low bind value due to collagen (Ockerman and Hansen 2000, Pearson and Gillett 1996).

On the process floor after inspection, the hearts are usually washed for 10-15 minutes, hung, and chilled. Traditionally, heart is sold as a fresh or frozen product to be cooked long-term with moist heat. It is also used in stews or ground and mixed with other meat. Because of the high myoglobin content, heart meat adds color to finished products.

In June 2003 beef hearts brought $20/cwt on the domestic market and $21/cwt for the exports (USDA By-product Price Report 2003).

Rendering

Until the 1850's the meat industry usually considered the byproducts from slaughter facilities to be waste and would dispose of them. Individuals realized that some of the byproducts could be used as fertilizer supplying either nitrogen or phosphate. Their use of the offal products led to the start of rendering. Today wastes from slaughter and processing facilities, along with restaurant wastes and dead animals, are sent to rendering. There are four main types of rendering: digester wet rendering, dry batch rendering, continuous dry rendering, semi continuous process incorporating both wet and dry, and low temperature rendering (Ockerman and Hansen 2000).

In digester wet rendering a cooker is filled with the ground raw material. Steam is injected at 140 °C with 361 kPa pressure. This step lasts between 3-4 hours, and then to reduce emulsification, the pressure is slowly reduced. The treated material is removed with the fat drained out. The solid portion (cracklings) is pressed to remove excess liquid and dried. This process is not as popular as it once was as it has long cook times, is labor intensive, has low yields, and the viscera must be washed prior to processing. However, digester wet rendering does produce good quality tallow.

Dry batch rendering systems have steam-jacketed cookers with agitators to improve heat transfer. Raw material is ground, enclosed in the cookers, and cooked at lower temperatures than wet rendering in less time (1.5-2 hours). Fat is drained out, and the solids are pressed. After cooling the material is ground into meal. Dry batch rendering produces little loss of material as most of the steps are done in the same

container. The vent steam produced can be used to produce hot water to help reduce costs for the system's energy needs. However, this process has numerous drawbacks. The tallow is dark, the final lipid content in the meal is 10-16%, and it is very labor intensive. Additionally, it is hard to maintain the proper temperature of the cooker, the cookers are not efficient driers, and dry rendering cannot handle gelatinous material like slunks (unborn calves).

The continuous dry rendering is similar to dry batch except for how the raw material is added to the cooker. Raw material enters at one end of the cooker and exits as dry processed material at the other end. Due to cooker volume differences, heat transfer capabilities, and raw material, completion time varies. The dry processed material leaves the cooker, enters a percolator with a strainer, and then moves into the press. The remaining material is ground into meal. This process requires much less floor space than the other processes mentioned as well as having high yield and steam recovery from the cooker. Because of the high temperatures the tallow color is degraded, but since the process is not pressurized, it cannot hydrolyze hair and wool or sterilize. Continuous dry rendering has similar disadvantages to batch rendering except that it is not as labor intensive.

The continuous low temperature (mechanical dewatering) system takes minced raw material, which is placed in a low temperature cooker (coagulator, preheater, or melting section) where it is heated to 60-90 °C in 10-30 minutes. The tallow is released from the meat matrix, which allows a screw press to remove the tallow and water. The solids are sent to another cooker/drier to remove the remaining 40% moisture. The dried solids are then pressed and sent to the grinder. In this process the liquid tallow and water

are sent to an evaporator to remove the water and produce high quality tallow. This process uses approximately 40% less steam, can easily be automated, and produces meal with less than 8% fat. However, initial startup and maintenance is costly.

Low-temperature rendering phase separates the raw material. The tallow and wet solids are then processed separately at lower temperatures. Advantages to this process also eliminate the need to wash the raw material of paunch contents and dirt as the solids and tallow are separated prior to processing (Ockerman and Hansen 2000).

As of June 2003 price for inedible choice white grease averaged about $18.27/cwt with yellow grease costing about $14/cwt. Edible tallow was priced at $18.00/cwt. Beef meat and bone meal with 50% protein/ton ran $167.00/cwt with beef blood meal running around $358.00/cwt (USDA Tallow, Protein, and Hide Report 2003).

When the ruminant feed ban was implemented in the United States in 1997, the main outlet for the rendered meat and bone meal was lost. Today the majority of the meat and bone meal is sold to poultry producers. The majority of the yellow grease is still sold to feedlots in the Midwest United States. Further regulations restricted ruminant brains or spinal cords from entering the rendering line to help ensure that transmissible spongiform encephalopathy (TSE) prions did not inadvertently get added to feed stuffs. (Morgan 2003).

Surimi Process

For many generations the Japanese have investigated means to improve the process of making kamaboko (loaf or cake of ground or pureed, steamed fish) in order to create a more valuable product from less desirable fish. In the late 1800's and early 1900's larger fishing fleets began to develop which led to larger daily catches. This also

led to an increase in the less desirable fish, which is kamaboko's raw material, also being caught. Shelf life of the fish was very short so only locally caught fish could be used in the process. When kamaboko fish sausage began being made commercially in 1953, the need for a more shelf stable raw product was warranted. With the help of various research institutions, the kamaboko industry refined the process to obtain more stable raw material. Surimi, which means ground fish paste, takes flesh that has been mechanically separated from bone and skin (minced meat) and water-washes the minced meat to remove fat and water soluble components. The remaining flesh is pressed to remove excess water and the result is a product with concentrated myofibrillar proteins that has improved gel forming and water holding ability (WHA) over the original minced meat (Lanier and Lee 1992).

Today surimi made from Alaska polluck is preferred because of its abundance, accessibility, subtle flavor, and odor characteristics. However, there have been studies investigating the use of dark-fleshed fish such as Pacific whiting, bonito, and sardines to make surimi (Lanier and Lee 1992, Chung and others 1993, Morrissey and others 1993, Reppond and Babbitt 1997, Fernandez-Martin and others 1998). Typically, the surimi manufacture is performed aboard ships (within 12 hours of catching), as freshness of the fish is the most important requirement for the raw material. The fish to be used is sorted from the rest of the catch and washed. If the skin is not removed, the scales must be removed so they do not clog the deboning machines. Manual or automatic filleting is done to decapitate, eviscerate, and remove the backbone and roe if necessary. The fillets are then sent through the deboning process to separate and mince the flesh from bones, fins, and skin. Following the mincing step, the product is leached (remove minced meat

from water-soluble material, fat, and blood) in fresh water. Leaching is now a continuous system that contains a rotary screen to partially dewater the meat. The water to meat ratio may be as low as 6:1 on a ship but as high as 20:1 if processed later on land. This step promotes improved color, flavor, and gel strength of the surimi. The length of time the minced meat is in the leaching step and number of washings required is dependent of the type and freshness of the fish (Lanier and Lee 1992, Park and others 1997). At the refining step connective tissue, skin, and scales are removed from the mince. The refiner consists of a 1-3 mm screen and a screw-shaped rotor. Temperatures must be maintained at this step to maintain functionality of the surimi end product. To reduce the high moisture (90%) in the minced meat, a final dewatering step is performed to reduce moisture to around 82% (Lanier and Lee 1992). The final myofibrillar content is usually around 50% of the total proteins found in the end product (Park and others 1997).

The raw surimi, itself, has limited shelf life once frozen. The addition of cryoprotectants, such as sugar and sugar alcohols, extend shelf life by maintaining the functionality of the proteins (Lanier and Lee 1992, Park and others 1997). Generally, 4% sucrose and sorbitol, along with 0.2-0.3% polyphosphates, are used. When frozen with the cryoprotectants, the surimi is able to retain its gel forming ability for several months.

In the late 1980's surimi research started being performed on poultry, beef, and sheep. McKeith and others (1988) showed that surimi-like products made from beef, pork, and beef byproducts produced gels similar, if not better, than fish surimi. Fat reduction ranged from 60-99% depending on the meat source. Processing yield percentage revealed that while beef and pork had 45% yield, beef heart was not far behind with 38%. They concluded that beef heart would be the logical byproduct to use

for testing the surimi process. Park and others (1996a) further investigated the composition of beef and pork surimi. They found that potassium and magnesium were reduced, but the surimi process did not affect calcium, copper, zinc and iron. It was also shown that cholesterol remained unchanged in the water-washed products, which they attributed to the membranes remaining intact even with the extensive processing.

Srinivasan and Xiong (1996) looked at adding salt to the washing solution to help facilitate dewatering of beef heart surimi. They found the benefits of salt depended on the concentration of NaCl added and the pH of the solution. When at a lower pH or a high concentration the salt acted as a prooxidant. However, using 0.1M NaCl improved stability by inhibiting lipid oxidation.

Park and others (1996b) significantly enhanced the water holding capacity, gel forming strength, and cook hardness when salt was added to the surimi prior to cooking. They showed that the improvement was due to salt's unfolding effect on muscle proteins.

Smyth and O'Neill (1997) applied the surimi process to mechanically separated chicken. They found a significant reduction in fat, collagen, and calcium when the process was applied. However, after 14 days of frozen storage gel strength of the surimi product started to decrease. Wang and others (1997) demonstrated that cryoprotectants seem to increase lipid and protein oxidation in beef heart after 12 weeks of frozen storage when no antioxidants were added. Wang and Xiong (1998) and Parkington and others (2000) found that adding antioxidants with cryoprotectants helped control TBARS when stored in blast freezers for up to a year. Due to the washing conditions of the surimi process, beef heart is very susceptible to lipid oxidation. Therefore Parkington and others (2000) determined that using sodium phosphate buffer, buffer with salt, buffer with

propyl gallate, or buffer with sodium tripolyphosphate would still allow for slight oxidative changes on proteins while deterring lipid oxidation which they determined helped improve the gel strength over time.

Ruiz and others (1993) investigated using chuck muscles to produce surimi to make restructured steaks. Tenderness determined by an Instron Universal Testing Machine was not significantly different from other restructured steaks that had been made from closely trimmed meat. Desmond and Kenny (1998) also determined that beef heart surimi could be used in frankfurter formulations up to 15% without affecting the flavor or texture.

Major drawbacks to this procedure are the large amount of water used and the loss of organics into the water (Park and others 1997). In one study with sheep surimi, the meat was washed three times with a 1:5 ratio of meat to water (Antonomanolaki and others 1999).

Acid Solubilization Isoelectric Precipitation (SIP)

In 1999 Hultin and Kelleher patented a protein recovery process that took advantage of protein solubility differences to extract myofibrillar proteins from collagen, fat, and bone. Kelleher and Hultin (2000) demonstrated that by utilizing this process with light and dark chicken meat, they were able to significantly reduce the fat and produce a product with good gel strength. In their study the protein solubility of chicken breast muscle at pH 2.8 was almost 93%, but dark muscle fibers only had 72% solubility at pH 2.8. Using high centrifugation speeds (10000 x g), significant amounts of lipids and phospholipids were removed to help improve the stability of the product.

Mireles DeWitt and others (2002) looked at applying the Acid-SIP process to beef to improve the composition and functionality of beef byproducts. Beef heart was used as a model as the heart is a smooth muscle with high levels of collagen and had responded well in the surimi studies. Procedures to isolate the myofibrillar proteins in beef were established, and verification of the concentration of the myofibrillar proteins was accomplished. To isolate the myofibrillar proteins, myosin solubility was shown to increase as a result of acidification under low ionic conditions. The pH of 1.5-3.5 appeared to be the optimum range of protein solubilization with maximum protein solubility volume being at 2.5 in regards to myosin and the 150 kDa bands. The pellet obtained from precipitation at the myofibrillar protein isoelectric point of 5.5 was shown to have around 36% myosin and 21% actin (major myofibrillar proteins). Composition results from the pellet demonstrated similar results to those seen with the chicken acid-SIP process. The collected pellet had significantly less fat, ash, collagen, and cholesterol than the untreated heart.

The purpose of this research was to investigate the compositional and functional properties of the proteins recovered from the Acid-SIP process.

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Wang B, Xiong YL. 1998. Functional stability of antioxidant-washed, cryoprotectant-treated beef heart surimi during frozen storage. J Food Sci 63:293-298.

Chapter III

CHARACTERISTICS AND COMPOSITION OF BEEF HEART WHEN TREATED BY ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION AND THE SURIMI PROCESS

J.M. JAMES AND C.A. MIRELES DEWITT

ABSTRACT

Composition and characteristics of beef heart (BH) when utilizing acid solubilization isoelectric precipitation (SIP) was studied and compared to surimi BH. Untreated BH, BH Acid-SIP, and BH surimi were adjusted to 78% moisture, with or without 2% NaCl. Proximate analysis, color, collagen, and cholesterol were evaluated. Differential scanning calorimetry (DSC) was used to characterize the samples' thermal behavior. Acid-SIP possessed less collagen and cholesterol than the control or surimi. Fat and ash were reduced by both treatments. Acid-SIP contained slightly more protein compared to the control and surimi. DSC results indicated Acid-SIP had different thermal properties than surimi and control. Based on results, red meat by-products treated with Acid-SIP improved composition and thermal characteristics.

INTRODUCTION

Maximizing utilization of the whole beef carcass has been a goal of the meat industry. Rendering was developed to produce a product from raw material that was not being utilized. The rendering process produces meat and bone meal and tallow. Due to the heat treatment involved in the rendering procedure, the end products have limited uses.

Several researchers have investigated applying the surimi process to beef and pork. They were able to concentrate the myofibrillar proteins with a water-washing and screening system to produce a product that had better gelling properties and stability than the original material (Park and others 1996, Parkington and others 2000).

Mireles DeWitt and others (2002) applied the acid solubilization isoelectric precipitation (SIP) method that Hultin and Kelleher (1999) patented to beef to verify the parameters and procedures for red meat. The objective of this study was to evaluate the composition and characteristics of beef heart treated by the Acid-SIP process and compare it to untreated and surimi processed beef heart.

MATERIALS AND METHODS

Preparation of Beef Heart

Fresh beef hearts (8-10) were collected from a local processing company, placed immediately in Ziploc bags on ice, and transported to a 5 °C walk-in cooler at Oklahoma State University. The hearts were held overnight prior to trimming off the cap, valves, and external fat. The hearts were cut into 5 cm slices and ground through a General MC-100 meat grinder (Red Goat Disposers, Murfreesboro, TN, USA) with a 5 mm extrusion die in refrigerated temperatures. The collected comminuted product was divided into 3 treatment groups: control (C), Acid-SIP (A), and surimi process (S).

Preparation of Surimi Beef Heart

The surimi samples were prepared according to Park and others (1996) with the following modifications. The beef heart was combined with cold deionized water (1:3 w/v), stirred for 15 min, and the deionized water was removed by straining through a 2

mm sieve. The meat was washed a total of 3 times in this manner. After the last water was removed, the meat was passed through a 2 mm ASTM sieve followed by a 1 mm ASTM sieve. Meat that passed through the 1 mm sieve was collected.

Preparation of Acid Solubilization Isoelectric Precipitation

The Acid-SIP samples were prepared according to Mireles DeWitt and others (2002). A 1:9 (w/v) mixture of beef heart to 2mM citrate buffer was blended in a Waring blender for 1 min. The pH was lowered to 2.5 with 2N HCl and centrifuged at 3300 x g at 4°C for 30 min (Beckman Model J-6M Swinging Bucket Rotor JS 4.2). Kelleher and Hultin (1999) used high-speed centrifugation to reduce the phospholipids in the recovered proteins. Preliminary work in our laboratory demonstrated lipid oxidation does not increase in the 2-3 day period under the conditions used to prepare the product for the Acid-SIP process. Since shelf-stability was not investigated in this study, lower speeds were used to facilitate recovery of larger amounts of protein. The supernatant was collected and raised to pH 5.5 with 2N NaOH to precipitate and recover the myofibrillar proteins. The pellet was collected after centrifugation at 3300 x g at 4°C for 30 min (Beckman Model J-6M Swinging Bucket Rotor JS 4.2).

Preparation of the Treatments

After the 3 treatments (control, Acid-SIP, and surimi) were prepared, each was centrifuged at 10000 x g for 15 min at 4 °C (Dupont Sorvall RC 5C Plus, Rotor 28 SLA-1500) to remove excess water from the sample. Cryoprotectants (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) were added according to Kelleher and Hultin (2000). The samples were adjusted to approximately pH 7 with 5% $NaHCO_3$ (Kelleher and Hultin 2000). Initial moisture was determined by oven drying (AOAC

1995). Samples were blast frozen overnight in vacuum-sealed Cryovac (Sealed Air Corp, Saddle Brooks, NJ) bags. The following morning the samples were tempered to 4 °C and equilibrated to 78% moisture. Each treatment was then split into 2 groups, no NaCl (O) and 2% NaCl (N), and mixed in a vacuum chopper (UMC 5 electronic, Stephan Machinery Corp., Columbus, OH, USA).

Analysis of Raw Treatments

Proximate analyses were performed (AOAC 1995) using procedure 960.39 for crude fat in meat with an indirect Soxhlet apparatus, procedure 992.15 for crude protein in meat with a Leco FP-428 (Leco Co., St. Joseph, MI, USA), and procedure 920.153 for ash of meat. Collagen levels were determined by an AOAC (1995) colorimetric method (990.26) for hydroxyproline in meat. Cholesterol was performed according to AOAC procedure 994.10 (1995) with modifications of injecting 5 μL sample into a HP 589 GC with Chemstation software (Hewlett-Packard, Palo-Alto, CA, USA) with a FID detector and a SPB-1 capillary column, 30 m x 0.25 mm x 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). Carrier gas was helium with a 50:1 split. Oven parameters were 190 °C for 2 min; ramp at 20 °C/min to 230 °C, hold for 3 min; ramp at 40 °C/min to 280 °C, hold for 11.8 min. Total run time was 20 min. Differential Scanning Calorimetry (DSC) values were obtained according to Fernandez-Martin and others (1997) with the following modifications. Around 15 mg of each sample, without added cryoprotectants or salt, was hermetically sealed and run on a calibrated Perkin-Elmer DSC-7 (Perkin Elmer LLC, Norwalk, CT). The method run consisted of a 1 min hold time at 22 °C, heated to 120 °C at 10 °C/min, held at 120 °C for 1 min, and cooled to 22 °C. An empty hermetically sealed pan was used as a reference for all samples. Results were analyzed

from 22-100 °C. Color values were obtained on the uncooked treatments with a Minolta Chroma Meter CR-300 (Ramsey, NJ) standardized with a white plate. Results were expressed as L* (lightness), a* (redness), and b* (yellowness). All analyses were repeated in at least duplicate for each of 3 replications.

Statistical Analysis

The data were analyzed for a completely randomized design using generalized least squares (PROC Mixed, SAS Institute, Cary, NC). The model included treatment and NaCl levels as main effects. The interaction between treatment and NaCl were included in the model. Mean separation was accomplished using Least Significant Difference.

RESULTS AND DISCUSSION

Composition & Characteristics

Proximate composition for the raw samples is shown in Table 2 on a wet basis and Table 3 on a dry basis. Reference values from the USDA Nutrient Database (Agricultural Research Service 2001) were slightly different from the starting material (control) as the heart cap was trimmed and not processed in this experiment. Moisture in our samples was adjusted to 78% to eliminate moisture differences in each treatment, and the heart was trimmed of exterior fat to facilitate recovery of larger amounts of protein with laboratory-scale equipment. Ash values from the control (1.04%) were similar to the USDA reference value (1.03%). Comparison of the treatments to the control showed that both treatments reduced fat and ash significantly. Park and others (1996) saw a similar reduction in fat with surimi-like beef. The raw crude protein with no salt from the

Acid-SIP process was significantly higher (66.20 ± 3.51%) than the surimi or control on a dry basis.

Collagen was reduced in the untreated beef heart by 80% when the Acid-SIP process was applied and by 40% with the surimi process. Mireles DeWitt and others (2002) also saw a decrease in collagen when the Acid-SIP process was applied. This reduction in collagen should allow the recovered proteins to have a higher binding value, which makes the meat more useful in processed formulations.

Cholesterol values on a wet basis from the control (85.92 ± 4.06 mg/100g) and surimi proteins (89.95 ± 0.64 mg/100g) were not significantly different. However, the Acid-SIP proteins (41.21 ± 5.99 mg/100g) had half as much cholesterol as the surimi and the control. Using higher centrifuge speeds in the Acid-SIP process than used in this study, Mireles DeWitt and others (2002) saw a similar trend with the untreated ground beef heart having higher cholesterol levels than the protein pellet that was extracted.

Past studies using DSC in red meat products and on myofibrillar proteins has shown myosin peaks around 65 °C, collagen and sarcoplasmic proteins around 65-70 °C, and actin peaks ranging from 67.8-81.5 °C (Feng and Xiong 2003, Fernandez-Martin and others 2000, Liu and others 2000, Fernandez-Martin and others 1997). In this study, the Acid-SIP proteins produced very repeatable results, while the control and surimi samples varied (Figure 1). A possible explanation is the Acid-SIP is more homogeneous with fewer non-myofibrillar components that might be interfering with the analysis. The control had peaks at 62.13 °C, 75.27 °C, and 77.08 °C that could represent the peaks seen in the other published reports. The surimi had a major peak starting at

Table 2. Composition of Trimmed, Ground Raw Beef Heart with Cryoprotectants and 0 or 2 % NaCl When Treated by Acid-SIP and the Surimi Process on a Wet Basis.

	CONTROL		ACID-SIP*		SURIMI	
	No NaCl	2% NaCl	No NaCl	2% NaCl	No NaCl	2% NaCl
Protein %	13.09 ± 0.35^{b}	12.98 ± 0.28^{b}	14.47 ± 0.70^{a}	14.51 ± 0.26^{a}	13.57 ± 1.06^{b}	13.53 ± 1.53^{b}
Fat %	0.54 ± 0.27^{a}	0.09 ± 0.09^{c}	$<0.01^{d\wedge}$	$<0.01^{d\wedge}$	0.19 ± 0.14^{b}	$<0.01^{d\wedge}$
Ash %	1.04 ± 0.09^{c}	2.79 ± 0.20^{a}	0.54 ± 0.03^{d}	2.69 ± 0.43^{a}	0.55 ± 0.05^{d}	2.22 ± 0.11^{b}
Collagen (mg/100g)	0.45 ± 0.08^{a}	0.40 ± 0.14^{a}	0.08 ± 0.09^{c}	0.07 ± 0.09^{c}	0.27 ± 0.12^{b}	0.26 ± 0.19^{b}
Cholesterol (mg/100g)	85.92 ± 4.06^{a}	80.77 ± 11.99^{a}	41.21 ± 5.99^{b}	40.00 ± 4.67^{b}	89.95 ± 0.64^{a}	84.29 ± 8.00^{a}

Data represent means ± standard deviation

[a,b,c,d]Means within same row without common subscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

^ Fat % too low for accurate reading.

Table 3. Composition of Trimmed, Ground Raw Beef Heart with Cryoprotectants and 0 or 2 % NaCl When Treated by Acid-SIP and the Surimi Process on a Dry Basis.

	CONTROL		ACID-SIP*		SURIMI	
	No NaCl	2% NaCl	No NaCl	2% NaCl	No NaCl	2% NaCl
Protein %	59.67 ± 2.11^{b}	55.62 ± 0.79^{c}	66.20 ± 3.51^{a}	61.71 ± 1.19^{b}	61.90 ± 4.29^{b}	56.81 ± 3.26^{c}
Fat %	2.48 ± 1.28^{a}	0.34 ± 0.49^{b}	$<0.01^{c\wedge}$	$<0.01^{c\wedge}$	0.85 ± 0.60^{b}	$<0.01^{c\wedge}$
Ash %	4.79 ± 0.42^{d}	12.00 ± 0.80^{a}	2.49 ± 0.11^{e}	10.93 ± 0.73^{b}	2.39 ± 0.14^{e}	9.29 ± 0.94^{c}
Collagen (mg/100g)	2.05 ± 0.35^{a}	1.70 ± 0.61^{b}	0.39 ± 0.41^{d}	0.32 ± 0.42^{d}	1.23 ± 0.62^{c}	1.23 ± 0.69^{c}
Cholesterol (mg/100g)	394.16 ± 13.48^{a}	346.55 ± 55.16^{b}	188.69 ± 28.95^{c}	170.19 ± 20.61^{c}	411.13 ± 20.28^{a}	357.36 ± 44.61^{b}

Data represent means ± standard deviation

^{a,b,c,d,e}Means,c within same row without common subscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

^ Fat % too low for accurate reading

69.22 °C. The surimi and control demonstrated similar enthalpy until around 60 °C. The Acid-SIP showed a steady increase in the endothermic heat flow until 91.43 °C. Fernandez-Martin and others (1997) reported that the process that caused the least amount of denaturation during heating would have the largest enthalpy. The Acid-SIP proteins are thought be unfolded during the solubilization step and do not reassociate back into their native form when brought back to the isoelectric point (Kristinsson 2002). Therefore, in the Acid-SIP proteins the DSC results did not indicate peaks where the major myofibrillar protein, myosin, was undergoing a change (unfolding) as was shown in the surimi and the control because the Acid-SIP proteins were already in that form.

Research has shown various beef cuts to have L* values of 29.0 to 44.6. In addition, the a* values in these studies ranged from 15.7 to 26.3 and the b* scores ranged from 9.5 to 21.5 (Brewer and Wu 1993; Boakye and Mittal 1996; Demos and Mandigo 1996; Wulf and Wise 1999). The control beef heart sample color values fell within these ranges with the exception of the b* value being slightly lower at 7.49 ± 2.91 (Table 4). Both the surimi process and the Acid-SIP process significantly lightened the meat when compared to the control. The control's a* value was approximately twice as high as the treated sample. This was anticipated as both treatment processes focus on concentrating the myofibrillar proteins thus removing a significant amount of the sarcoplasmic proteins (hemoglobin and myoglobin) that contribute to the red appearance in meat (Romans and others 2001). Acid-SIP and surimi had comparable b* values when no salt was added, but Acid-SIP with salt had b* values that were closer to the control with salt. Surimi a* and b* values were slightly higher than those reported for beef surimi by Park and others (1996).

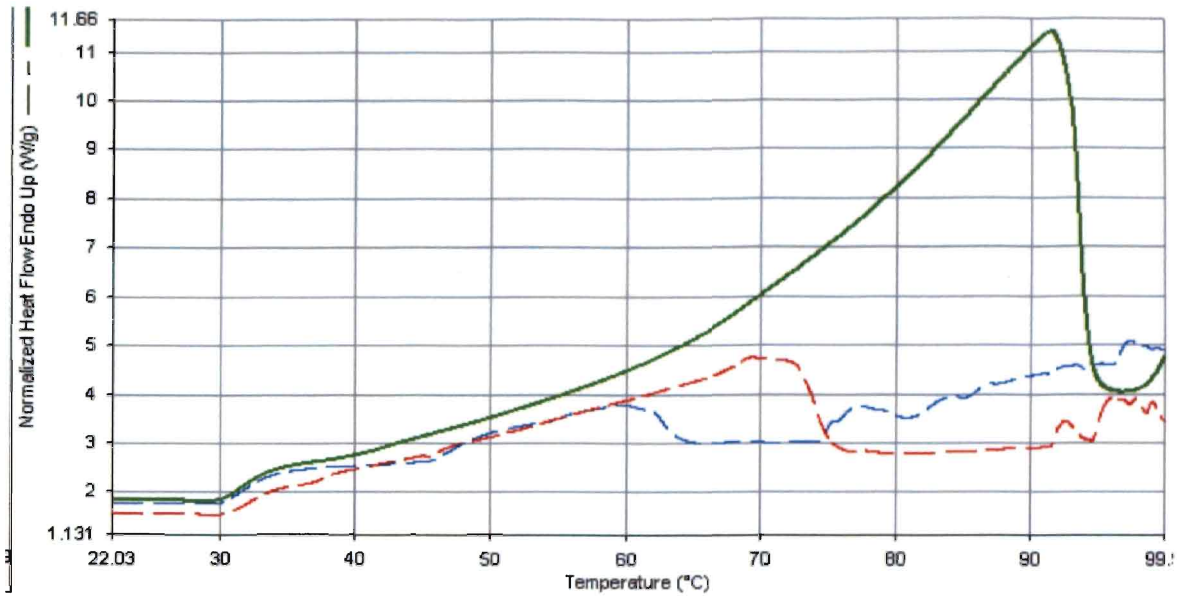

Figure 1. DSC thermal curves of Acid-SIP (green), Surimi (red) and Control (blue)

Table 4. Color Values of Trimmed, Ground Raw Beef Heart When Treated by Acid-SIP and the Surimi Process.

TREATMENT[^]	L*[x]	a*[y]	b*[z]
C *O*	31.80 ± 3.50[c]	19.05 ± 2.56[a]	7.49 ± 2.91[b]
A *O*	38.05 ± 1.19[a]	6.28 ± 0.34[c]	11.43 ± 1.34[a]
S *O*	36.38 ± 2.95[a]	9.86 ± 2.05[b]	10.29 ± 2.55[a]
C *N*	29.14 ± 1.47[d]	20.08 ± 4.54[a]	8.23 ± 2.31[b]
A *N*	36.18 ± 0.81[a]	4.87 ± 0.68[c]	8.56 ± 1.48[b]
S *N*	33.92 ± 0.58[b]	10.98 ± 1.30[b]	11.37 ± 1.07[a]

[^] (C) Control; (A) Acid-SIP; (S) Surimi; (*O*) No NaCl; (*N*) 2% NaCl.
[x] L*: 0 = Black, 100 = White.
[y] a*: Negative values = Green, Positive Values = Red.
[z] b*: Negative Values = Blue, Positive Values = Yellow.
Data represent means ± standard deviation.
[a,b,c,d]Means within same column without common superscript are different ($p<0.05$).

CONCLUSION

Based on results, the application of either one of the protein recovery systems, the surimi process or Acid-SIP, significantly reduced fat, ash, and collagen. Acid-SIP also reduced cholesterol. By further processing low value beef products or byproducts a less fat, lower cholesterol sample may be obtained. The Acid-SIP compositional data applied with knowledge from textural studies will give an indication of the ability to use the extracted proteins in formulations for low fat, low cholesterol food or pet food.

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Chapter IV

GEL ATTRIBUTES OF BEEF HEART WHEN TREATED BY ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION AND THE SURIMI PROCESS

J.M. JAMES AND C.A. MIRELES DEWITT

ABSTRACT

Beef heart left untreated, prepared using acid solubilization-isoelectric precipitation (SIP), or prepared by the surimi process was equilibrated for moisture and two salt levels. Samples were stuffed, cooked, and chilled. Cook yield, water holding ability (WHA), Texture Profile Analysis (TPA), composition, and color were evaluated. Acid-SIP and surimi samples improved gel attributes when compared to the control. Acid-SIP without NaCl and surimi with NaCl showed very similar texture characteristics for all gel attributes. The addition of salt to Acid-SIP further enhanced the gel. Based on the results, Acid-SIP has the ability to maintain, if not improve, gel strength properties in addition to reducing fat and ash.

INTRODUCTION

Meat recovery systems are being investigated to find alternatives for animal by-products sent to rendering and low valued products. Hultin and Kelleher (1997) introduced a nonthermal process to solubilize proteins and separate out the fat and ash while leaving the proteins in a functional form (Kelleher and Hultin 2000). When applied to fish muscle, proteins from this nonthermal acid solubilization process were shown to have improved gel functionality (Kristinsson 2002).

Previous research in our laboratory determined that this acid solubilization-isoelectric precipitation (Acid-SIP) process could be applied to beef byproducts such as beef heart. Parameters and procedures for obtaining concentrated proteins from this method were established for beef using beef heart as the model. Through SDS-PAGE it was shown that the pellet obtained consisted mainly of myosin and actin (the major myofibrillar proteins) (Mireles DeWitt and others 2002).

Myofibrillar proteins have long been known to be important in the formation of gels in meats. With the addition of salt to natural meat proteins, the myofibrillar proteins are extracted to form a surface-protein matrix that determines the strength of the bond formed by heating (Pearson and Gillett 1996). Salt is also known to destabilize the native protein structure prior to adding heat (Park and Lanier 1990). However, the Acid-SIP process affects the native state of the protein by electrostatic repulsion when the pH is lowered to solubilize the myofibrillar proteins (Stenesh 1993). Therefore it is thought that gel strength of unsalted Acid-SIP proteins should be greater than the unsalted, non-processed proteins from the same source.

The objective of this study was to determine the gel composition and attributes of beef proteins obtained by the Acid-SIP process and compare them to gels from another meat recovery system, the surimi process. Changes in the gel attributes due to the addition of salt were also investigated.

METHODS AND MATERIALS

Preparation of Beef Heart

Fresh beef hearts (8-10) were collected from a local packing company less than 30 miles away, placed immediately in Ziploc bags on ice, and transported to a 5 °C walk-in cooler at Oklahoma State University. The hearts were held overnight prior to trimming off the cap, valves, and external fat. The hearts were cut into 5 cm slices and ground through a General MC-100 meat grinder (Red Goat Disposers, Murfreesboro, TN, USA) with a 5 mm extrusion die. Preparation of the hearts was conducted in a 5 °C walk in cooler. The collected comminuted product was divided into 3 treatment groups: control (C), Acid-SIP (A), and surimi process (S).

Preparation of Surimi Beef Heart

The surimi samples were prepared in a 5 °C walk-in cooler according to Park and others (1996) with the following modifications. The beef heart was combined with cold deionized water (1:3 w/v), stirred for 15 min, and the water was removed by straining through a 2 mm sieve. The meat was washed a total of 3 times in this manner. After the last water was removed, the meat was passed through a 2 mm ASTM sieve followed by a 1 mm ASTM sieve. The surimi-like sample was collected from the 1 mm sieve.

Preparation of Acid Solubilization Isoelectric Precipitation

The Acid-SIP samples were prepared in a 5 °C walk-in cooler according to Mireles DeWitt and others (2002) with the following modifications. A 1:9 (w/v) mixture of beef heart to 2mM citrate buffer was blended in a Waring blender for 1 min. The pH was lowered to 2.5 with 2N HCl and centrifuged at 3300 x g at 4°C for 30 min (Beckman

Model J-6M Swinging Bucket Rotor JS 4.2, Palo Alto, CA). High-speed centrifugation (~10000 x g) has been used in this process (Kelleher and Hultin 1999) to help stabilize recovered proteins through reduction of phospholipids. Preliminary work in our laboratory has demonstrated the Acid-SIP process itself does not increase lipid oxidation in the 2-3 day period used to prepare the product in these studies. Since the focus of this study was the effect of the process on texture, not shelf-life stability, lower speeds were used to facilitate recovery of larger amounts of protein. The supernatant was collected and raised to pH 5.5 with 2N NaOH to precipitate and recover the myofibrillar proteins. The pellet was collected after centrifugation at 3300 x g at 4°C for 30 min (Beckman Model J-6M Swinging Bucket Rotor JS 4.2).

Preparation of the Treatments

After the 3 treatments (control, Acid-SIP, and surimi) were prepared each was centrifuged at 10000 x g for 15 min at 4 °C (Dupont Sorvall RC 5C Plus, Rotor 28 SLA-1500, Newtown, CT) to remove water from the sample. Cryoprotectants (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) were added according to Kelleher and Hultin (2000). The samples were adjusted to approximately pH 7 with 5% $NaHCO_3$ (Kelleher and Hultin 2000). Initial moisture was determined by oven drying (AOAC 1995). The samples were blast frozen overnight in vacuum-sealed Cryovac (Sealed Air Corp, Saddle Brooks, NJ) bags. The following morning the samples were tempered to 4 °C and equilibrated to 78% moisture. Each treatment was then split into 2 groups, No NaCl (O) and 2% NaCl (N), and mixed in a vacuum chopper (UMC 5 electronic; Stephan Machinery Corp. Columbus, OH, USA).

Preparation of the Gels

Each of the 6 groups was stuffed (American Harvest Jerky Works Kit, with 12.7 mm horn attachment, The Metal Ware Corp, Two Rivers, WI) into two-21 mm cellulose casings (Viskase, E-Z Peel® Nojax, Willowbrook, IL) around 16 cm long. The gels were cooked in a 90 °C water bath for 30 min, and then chilled (4 °C) overnight.

Analysis of Cooked Gels

Proximate composition was determined (AOAC 1995) for crude fat in meat (960.39), crude protein in meat (992.15) with a Leco FP-428 (Leco Co., St. Joseph, MI, USA), and ash of meat (920.153). Collagen levels were determined by an AOAC (1995) colorimetric method (990.26) for hydroxyproline in meat and converted to collagen. Color values were obtained with a Minolta Chroma Meter CR-300 (Ramsey, NJ) on 3 slices per link. The results were expressed as L* (lightness), a* (redness), and b* (yellowness).

Texture characteristics were evaluated. Cook yield and water holding ability (WHA) were determined according to Daum-Thunberg and others (1992). Cook yield was calculated by the following equation: cook yield % = (wt cooked gel / wt raw) * 100. For WHA a gel slice, 1.5 ± 0.15g, was placed on 3 dried, preweighed filter papers and centrifuged at 30600 x g (Dupont Sorvall RC 5C Plus, Rotor 28 SLA-1500, Newtown, CT) for 15 min at 4 °C. The filter papers were reweighed and calculated as follows: WHA = (total g H_2O – g H_2O lost during centrifugation) / (g protein). Texture was further evaluated with the TA-XT2i Texture Analyzer (Texture Technologies, Inc., Scarsdale, NY, USA/Stable Micro Systems, Godalming, Surrey, UK) with three randomly sliced 2 cm long segments per link tempered to room temperature. A texture

profile analysis (TPA) was conducted. The program allowed the probe (2.5 cm acrylic cylinder probe) to have a double compression into the sample with a 10 s delay between the 2 descents. The probe descended into the geometric center of the slice to a distance of 12 mm at a rate of 2 mm per s to measure the tertiary texture attributes. Values were calculated as (www.texturetechnologies.com):

Hardness = Peak Force of the 1st Compression

Cohesiveness = Area 2nd Compression / Area of 1st Compression

Gumminess = Hardness x Cohesiveness

Springiness = Length of 2nd Compression / Length of 1st Compression

Chewiness = Gumminess x Springiness

Resilience = Area Withdrawal of 1st Compression / Area of 1st Compression

All tests were evaluated in at least duplicate for each of the 3 replications.

Statistical Analysis

The data were analyzed for a completely randomized design using generalized least squares (PROC Mixed, SAS Institute, Cary, NC). The model included treatment and NaCl levels as main effects. The interaction between treatment and NaCl were included in the model. Mean separation was accomplished using Least Significant Difference.

RESULTS AND DISCUSSION

Composition

Proximate composition for the gels is shown in Table 5 on a wet basis and Table 6 on a dry basis. Fat was significantly reduced when treated by either the Acid-SIP or the

surimi process. The Acid-SIP process reduced the fat around 75% and 85% compared to the surimi and the control respectfully. Ash was also significantly reduced by both treatments. Total crude protein levels all fell within 70-77% on a dry basis. Collagen results showed the Acid-SIP process significantly reduced the undesirable collagen in the sample. The surimi process also reduced collagen (0.47 ± 0.11 mg / 100 g) but showed higher amounts than the Acid-SIP (0.13 ± 0.02 mg / 100 g) on a dry basis. Collagen is known to cause a problem called short meat where there is an imbalance of myosin to collagen. In processed meat products, fat particles are covered with myosin or collagen. When heat processed, the collagen converts to gelatin and exposes the fat particle allowing a fat cap or jelly pocket to form (Pearson and Gillett 1996). The concentrated myofibrillar proteins from the Acid-SIP process should help prevent the imbalance of myosin to collagen.

Color

L* value results (Table 7) demonstrated processing by either method lightens the sample. The addition of salt to Acid-SIP further lightened the cooked proteins. The a* value as expected showed the control was significantly redder than the treatments. With the sarcoplasmic proteins (hemoglobin and myoglobin) being reduced in the Acid-SIP and surimi, one expects to obtain lower red values than the control (Romans and others 2001). The b* values were minimally affected by treatment. The addition of salt slightly influenced the color value of each treatment except for the surimi L* and a* values.

Cook Yield

In Figure 2, the Acid-SIP with and without NaCl had significantly higher (98.17 ± 0.81% and 99.57 ± 3.71% respectively) cook yield than the surimi with and without NaCl

Table 5. Composition of Trimmed, Ground Cooked Beef Heart When Treated by Acid-SIP and the Surimi Process on a Wet Basis.

	CONTROL		ACID-SIP*		SURIMI	
	No NaCl	2% NaCl	No NaCl	2% NaCl	No NaCl	2% NaCl
Protein %	18.66 ± 2.08^{a}	15.14 ± 0.77^{b}	14.89 ± 0.88^{b}	14.84 ± 1.65^{b}	15.16 ± 3.47^{b}	15.04 ± 1.95^{b}
Fat %	1.59 ± 0.15^{a}	0.98 ± 0.17^{b}	0.15 ± 0.07^{d}	0.12 ± 0.06^{d}	$0.80 \pm 0.23^{b,c}$	0.58 ± 0.35^{c}
Ash %	0.76 ± 0.09^{c}	1.15 ± 0.37^{a}	0.39 ± 0.03^{d}	$0.87 \pm 0.14^{b,c}$	0.48 ± 0.04^{d}	0.96 ± 0.21^{b}
Collagen (mg/100 g)	0.16 ± 0.02^{a}	0.18 ± 0.02^{a}	0.03 ± 0.00^{c}	0.03 ± 0.02^{c}	0.10 ± 0.01^{b}	0.11 ± 0.02^{b}

Data represent means ± standard deviation

Means within same row without common superscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Table 6. Composition of Trimmed, Ground Cooked Beef Heart When Treated by Acid-SIP and the Surimi Process on a Dry Basis.

	CONTROL		ACID-SIP*		SURIMI	
	No NaCl	2% NaCl	No NaCl	2% NaCl	No NaCl	2% NaCl
Protein %	76.47 ± 4.37^{a}	70.85 ± 3.96^{b}	76.86 ± 2.87^{a}	75.80 ± 7.52^{a}	71.66 ± 2.94^{b}	71.19 ± 6.60^{b}
Fat %	6.07 ± 0.49^{a}	4.27 ± 0.94^{b}	0.68 ± 0.34^{d}	0.56 ± 0.31^{d}	$3.76 \pm 0.60^{b,c}$	2.64 ± 1.32^{c}
Ash %	3.13 ± 0.31^{c}	5.34 ± 1.55^{a}	2.01 ± 0.18^{d}	$4.45 \pm 0.67^{b,c}$	2.37 ± 0.43^{d}	4.60 ± 1.07^{b}
Collagen (mg/100 g)	0.59 ± 0.10^{b}	0.75 ± 0.08^{a}	0.13 ± 0.02^{d}	0.12 ± 0.02^{d}	0.47 ± 0.11^{c}	0.47 ± 0.11^{c}

Data represent means ± standard deviation

Means within same row without common superscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Table 7. Color Values of Trimmed, Ground Cooked Beef Heart When Treated by Acid-SIP and the Surimi Process.

TREATMENT^	L*	a*	b*
C *O*	47.60 ± 2.54^{c}	11.08 ± 0.73^{b}	$14.14 \pm 0.67^{a,b}$
A *O*	48.75 ± 1.08^{b}	7.13 ± 0.29^{d}	14.33 ± 0.71^{a}
S *O*	52.01 ± 0.99^{a}	$7.17 \pm 0.60^{c,d}$	13.94 ± 0.54^{b}
C *N*	45.96 ± 0.68^{d}	12.35 ± 0.46^{a}	12.63 ± 0.37^{d}
A *N*	52.18 ± 0.54^{a}	5.29 ± 0.83^{e}	12.93 ± 0.74^{d}
S *N*	51.71 ± 2.13^{a}	7.26 ± 0.38^{c}	13.36 ± 0.33^{c}

^ (C) Control; (A) Acid-SIP; (S) Surimi.
(*O*) No NaCl; (*N*) 2% NaCl.
Data represent means ± standard deviation
Means within same column without common superscript are different ($p<0.05$)

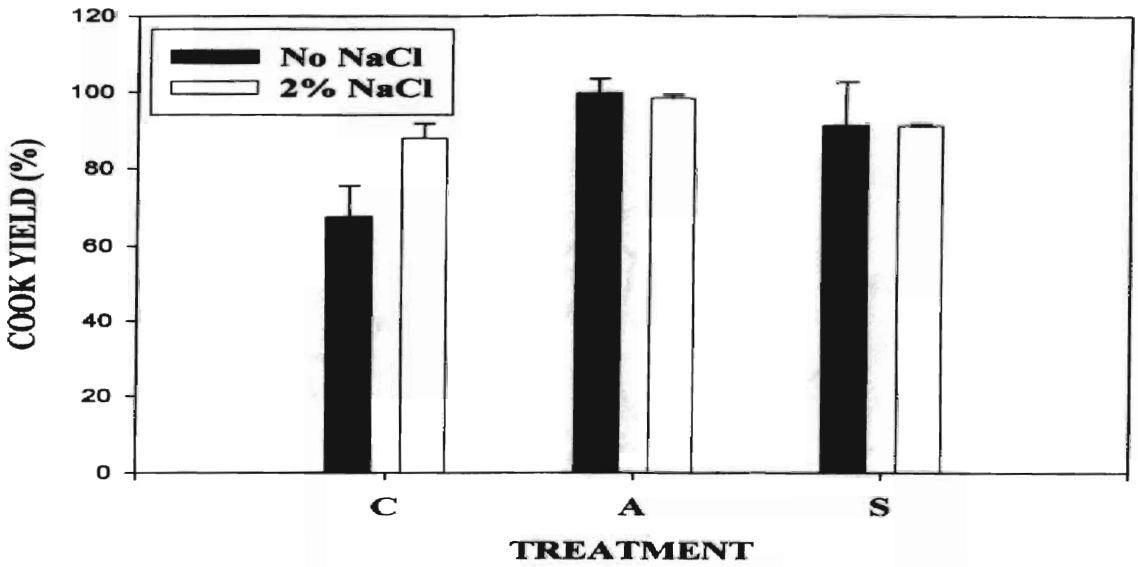

Figure 2. Cook Yield Percentage of Cooked Beef Heart Gels. Cook Yield % = (Wt Cooked Gel / Wt Raw) * 100 (Daum-Thunburg and others 1992). (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

(91.08 ± 0.58% and 91.28 ± 11.34%) and the control with and without NaCl (87.85 ± 3.96% and 67.52 ± 8.16%). The control without salt was significantly lower than all the other treatments supporting the theory that processing improves the attribute.

Water Holding Ability (WHA)

Water holding ability is shown in Figure 3. Treatment by Acid-SIP or the surimi process improves WHA. The addition of salt significantly improved the WHA except in the Acid-SIP (2.18 ± 0.49g/g protein without NaCl; 2.40 ± 0.44g/g protein with NaCl). Generally, when a meat product has the fat reduced water becomes difficult to bind (Pearson and Gillett 1996), but these results show that when the fat was reduced by Acid-SIP process the gel was still able to maintain its WHA. The Acid-SIP with and without NaCl and the surimi with salt were comparable and significantly higher than the other treatments. The control without NaCl had poor WHA (1.17 ± 0.22g/g protein).

Texture Characteristics

Springiness values (Figure 4) for the control without salt were significantly less (0.73 ± .07 mm) than the other treatments especially the Acid-SIP without salt and surimi with salt (both 0.91 ± 0.02 mm). Trends between treatments for hardness, gumminess, cohesiveness, chewiness, and resilience (Figures 5-9) were similar. The control without NaCl and the surimi without NaCl had significantly lower values in these attributes than the Acid-SIP with and without salt and the surimi with salt. For every attribute tested with the Texture Analyzer the control and surimi saw a very significant statistical increase in the attribute when NaCl was added. Traditionally, one of salt's main functions in the formulation of processed meats is protein extraction to allow proteins to bind in a product (Romans and others 2001). However, the addition of salt to the Acid-

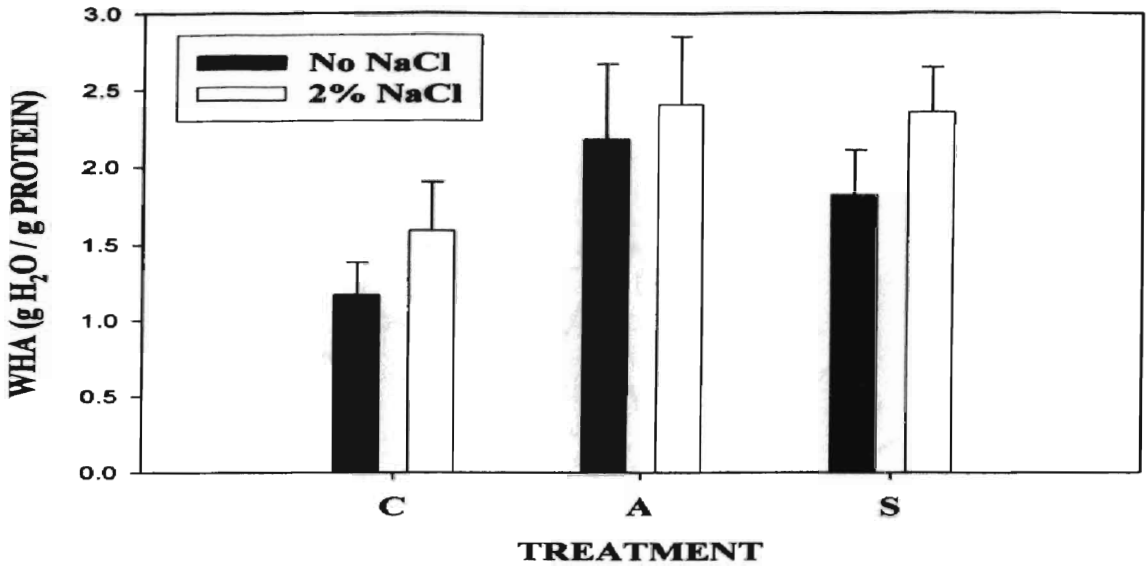

Figure 3. Water Holding Ability (WHA) of Cooked Beef Heart Gels. WHA = (Total g H2O – g H2O Lost During Centrifugation) / (g Protein) (Daum-Thunberg and others 1992). (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

SIP gels had only a slight effect on the texture attributes. The Acid-SIP values of chewiness and cohesiveness were similar to samples from beef semitendinosus muscle (Palka 2003). Hardness values for all of the samples appear to be less than is found for bologna (30% fat) and low fat bologna made with konjac or soy protein isolate (SPI). The other attributes were also slightly less compared to those seen in the SPI and konjac bologna samples (Chin and others 1999, Chin and others 2000). The difference seen could be attributed to the addition of other ingredients such as carrageenan and starch into the formulated bologna.

The tests performed demonstrated the Acid-SIP proteins have not lost their gel binding ability due to treatment. Therefore, with their unique gel properties and composition, Acid-SIP recovered proteins could be utilized in formulations of processed meats.

CONCLUSION

Further processing a low binding product such as beef heart by the surimi process and Acid-SIP improved the gel attributes. Acid-SIP, both with and without NaCl, had comparable or better characteristics than surimi with NaCl and control with NaCl. Salt played only a minor role in the Acid-SIP gels so further research could be done to investigate using Acid-SIP proteins in low sodium formulations to maintain proper texture. A practical application may be to apply the Acid-SIP process to enhance the quality and functionality of mechanically separated meats utilized in the formulations of processed meats.

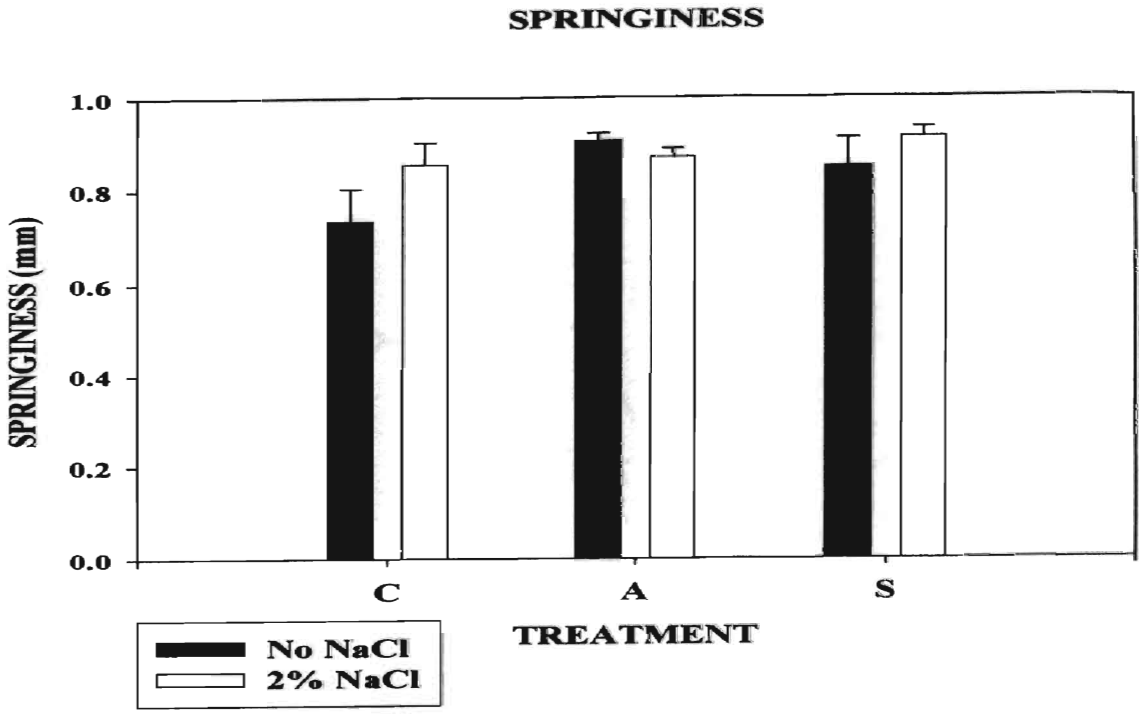

Figure 4. Springiness of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

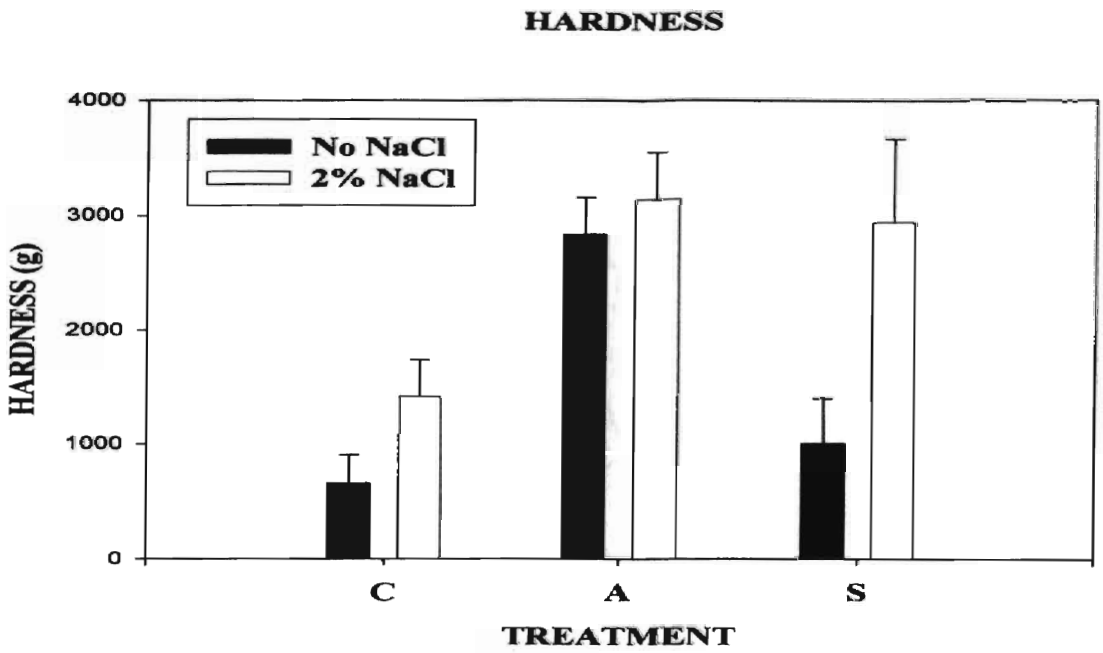

Figure 5. Hardness of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

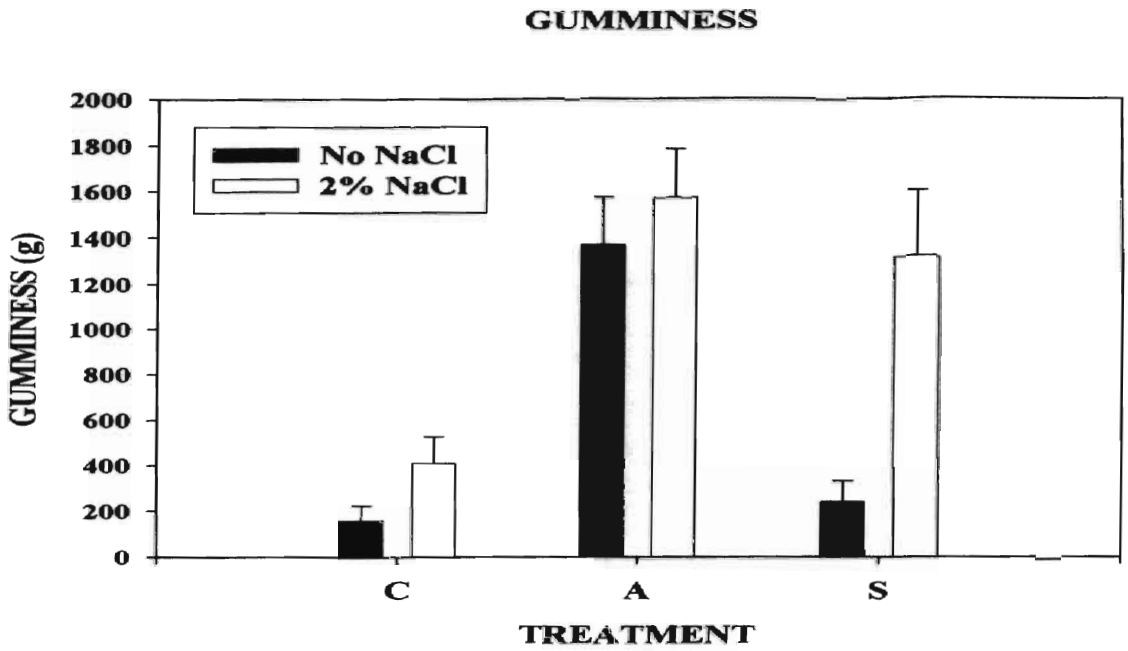

Figure 6. Gumminess of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

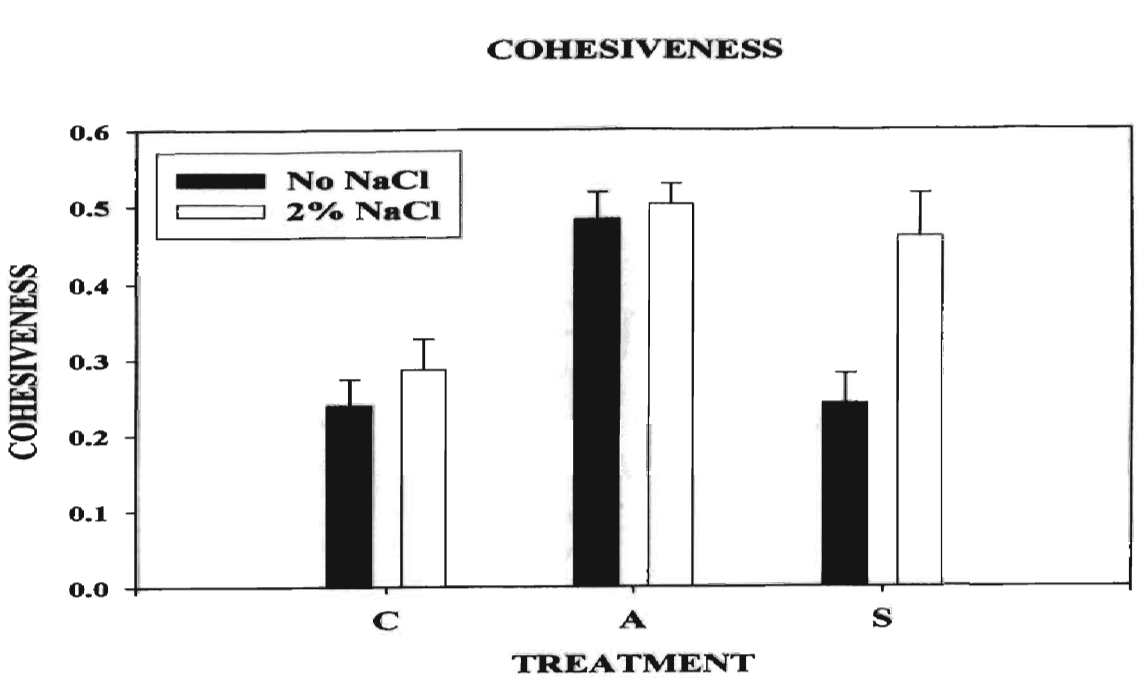

Figure 7. Cohesiveness of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

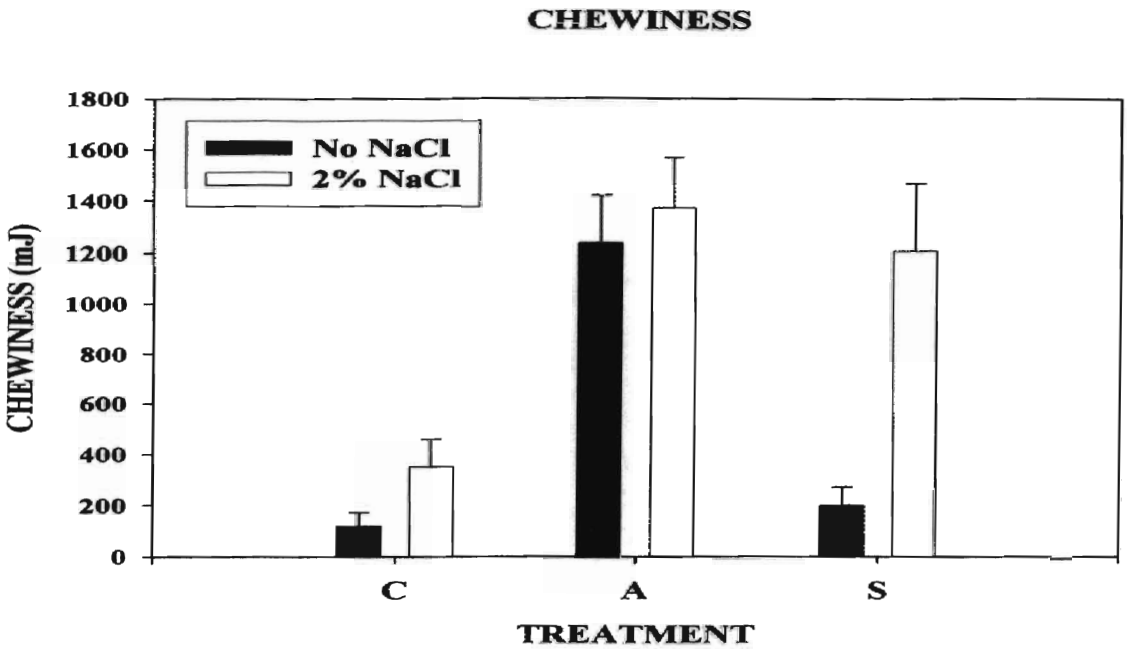

Figure 8. Chewiness of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

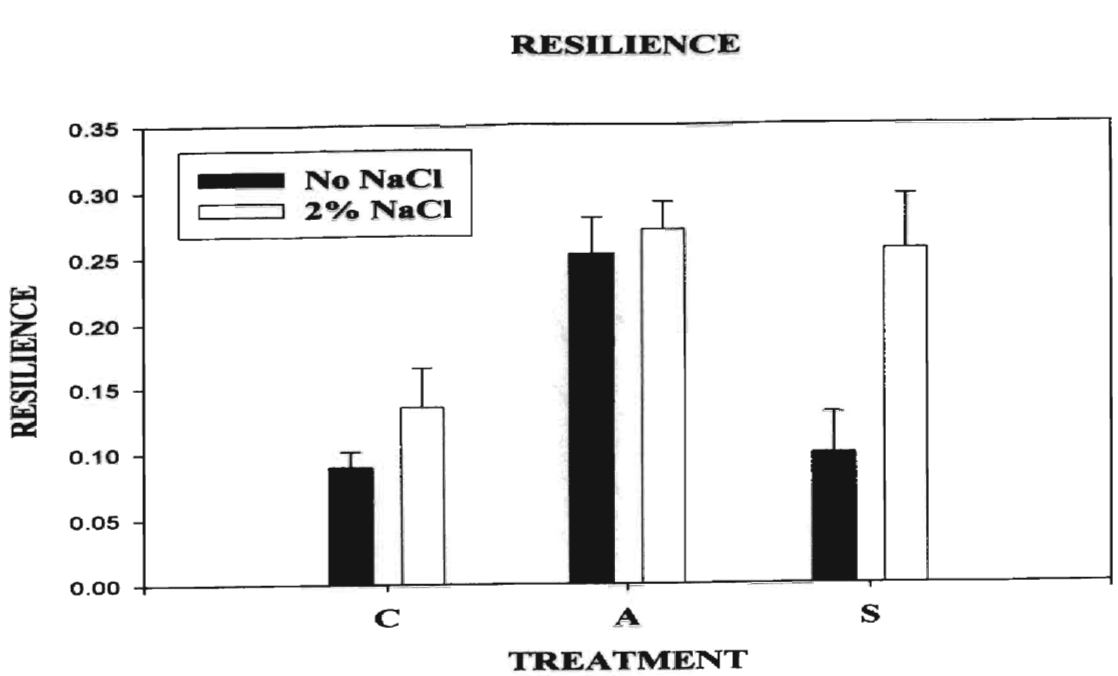

Figure 9. Resilience of Cooked Beef Heart Gels. (C) Control; (A) Acid-SIP; (S) Surimi. Data Represent Means ± Standard Deviation.

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CHAPTER V

SUMMARY OF RESULTS AND CONCLUSIONS

The composition of the raw beef heart treated samples (Acid-SIP and surimi) had much less fat, ash, collagen, and cholesterol than the control. Thermal characteristics of the raw samples from the differential scanning calorimeter (DSC) showed that the Acid-SIP proteins had undergone the least amount of denaturation during the test. Color of the raw samples was affected as the proteins that give meat its red color (myoglobin and hemoglobin) were removed when concentrating the myofibrillar proteins in each of the treatments.

The cooked gels showed that the water binding ability and functionality of the treated samples were not lost due to processing. Cook yield, WHA, and texture attributes as determined by a texture analyzer were improved with processing. The improved composition (less fat, ash, and collagen) created by both treatment processes played a role in improving the texture properties of the low binding beef heart. The Acid-SIP gels with and without salt and the surimi gels with salt performed similarly. The addition of NaCl significantly helped the texture attributes of both the control and the surimi, but had minimal effect on the Acid-SIP properties.

The DSC results from the raw Acid-SIP sample in conjunction with the functional properties demonstrated in the textural study further support the thought that the process leaves the myofibrillar proteins in an unfolded state. This allows the proteins to bind to other components to form a gel matrix.

Perhaps Acid-SIP proteins could be used much in the same manner that has been suggested for beef surimi. However, the Acid-SIP concentrate did not require salt to

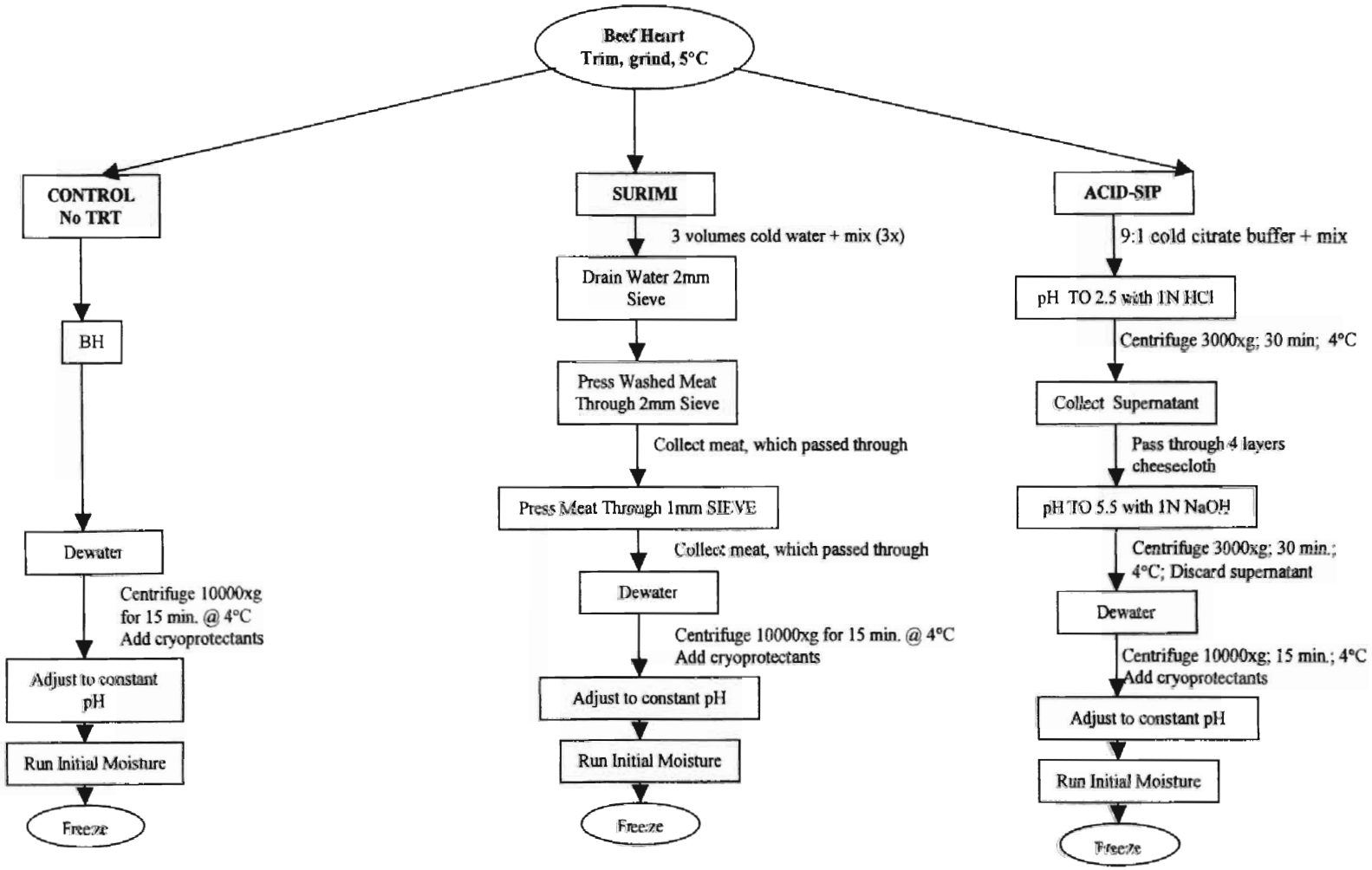
yield adequate composition or texture properties. Therefore, Acid-SIP concentrates could be added to formulations as a filler (much like soy is used) to improve nutritional content, but reduce the need for salt to unfold the myofibrillar proteins to create the gel matrix. Salt could then be added as a flavor ingredient at lower levels. This would produce a processed product that potentially could be lower in fat and sodium with texture attributes traditionally enjoyed.

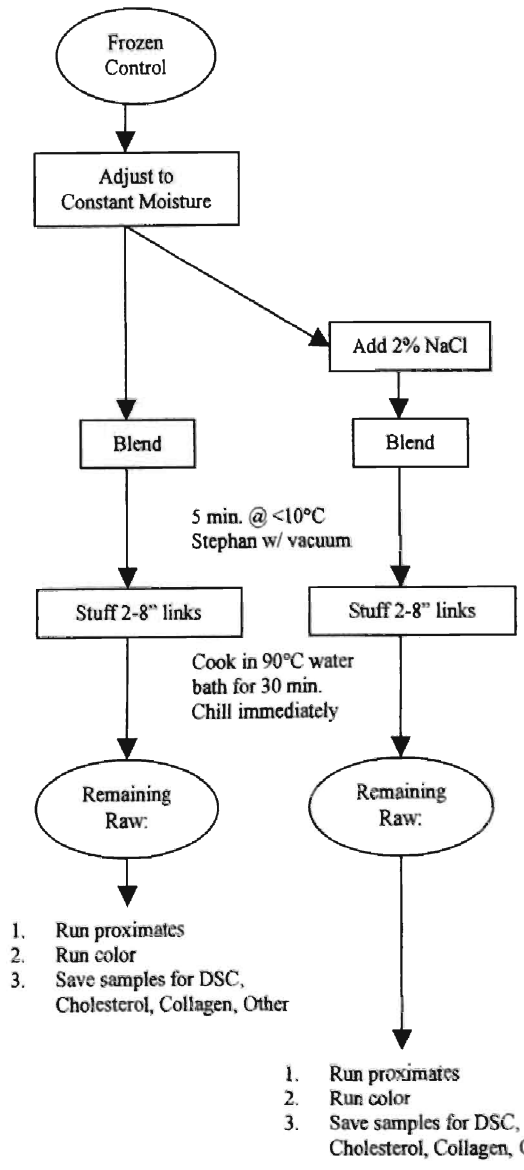
CHAPTER VI

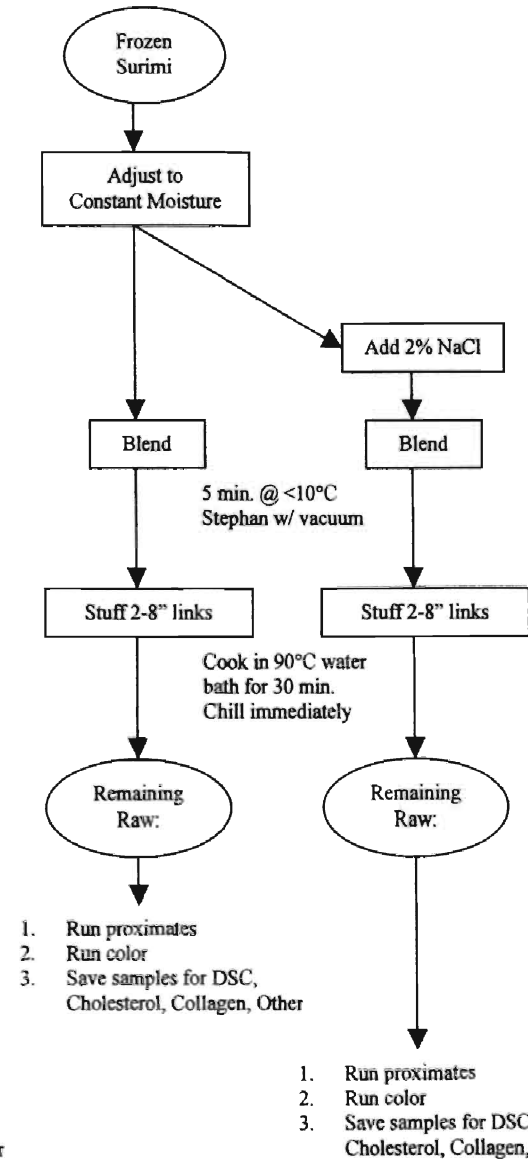
APPENDIX

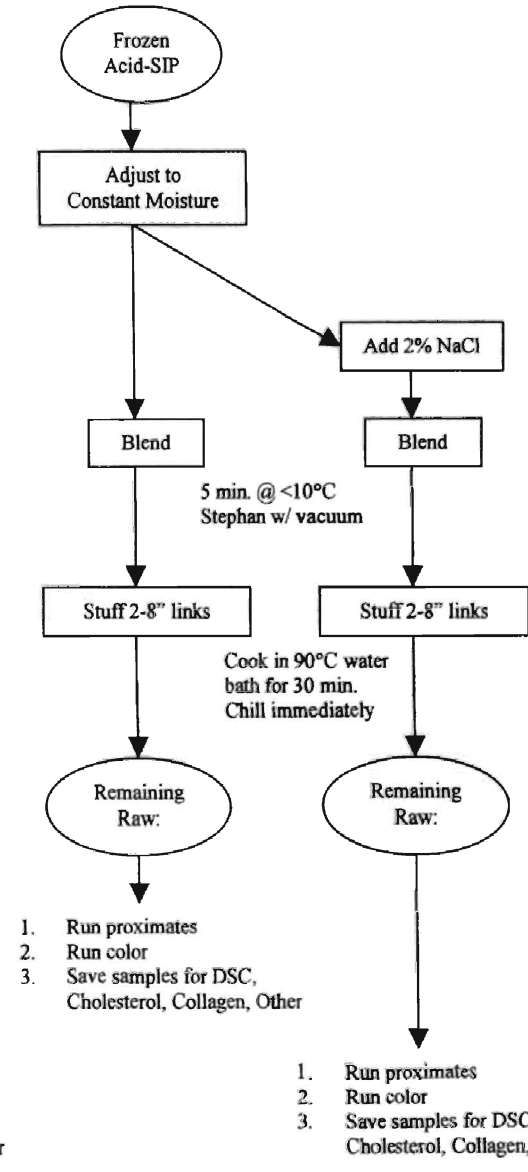
APPENDIX A

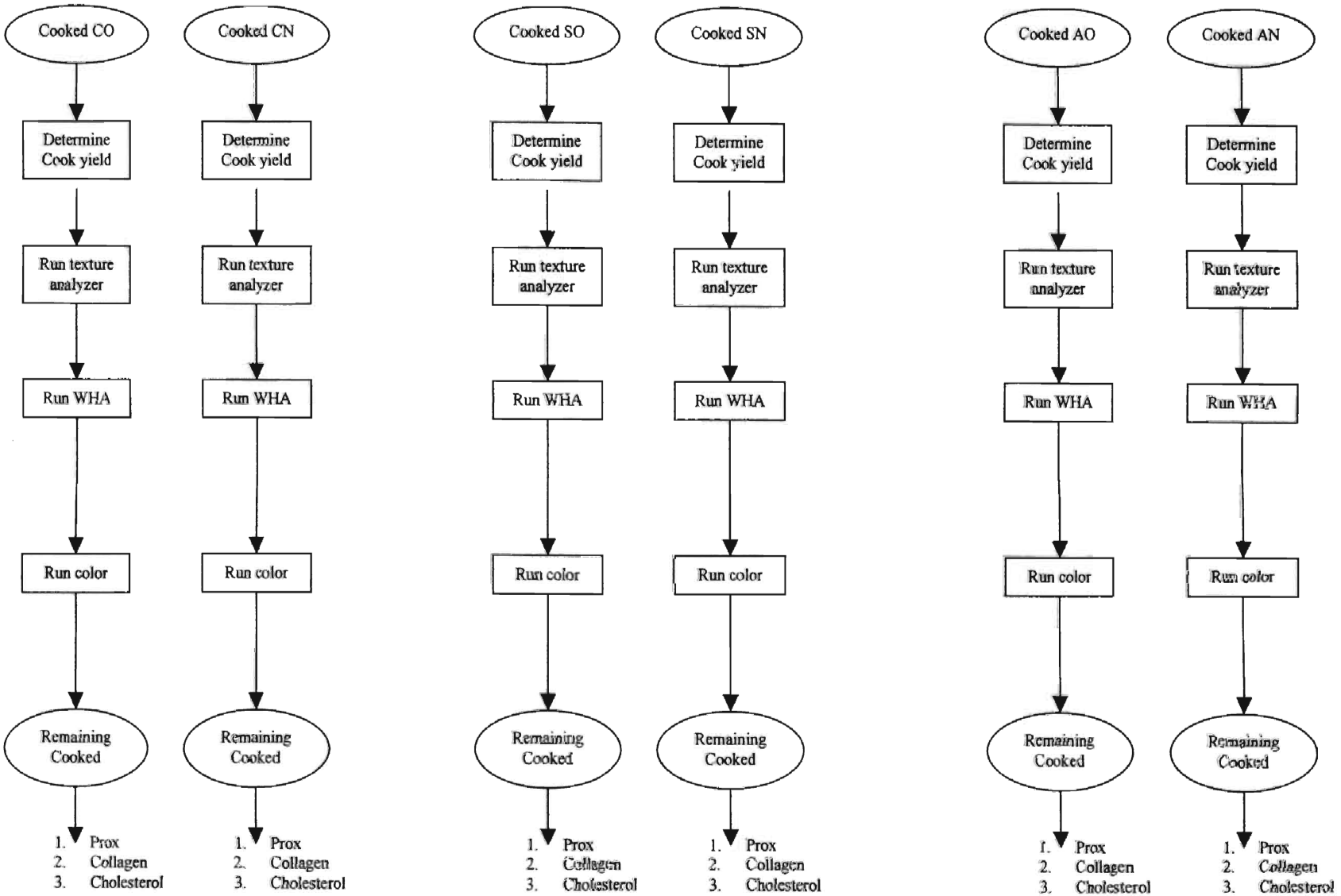
SCHEMATIC OF EXPERIMENTAL DESIGN




Frozen Control
Adjust to Constant Moisture
Add 2% NaCl
Blend
Blend
5 min. @ <10°C
Stephan w/ vacuum
Stuff 2-8" links
Stuff 2-8" links
Cook in 90°C water bath for 30 min.
Chill immediately
Remaining Raw:
Remaining Raw:
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other


Frozen Surimi
Adjust to Constant Moisture
Add 2% NaCl
Blend
Blend
5 min. @ <10°C
Stephan w/ vacuum
Stuff 2-8" links
Stuff 2-8" links
Cook in 90°C water bath for 30 min.
Chill immediately
Remaining Raw:
Remaining Raw:
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other


Frozen Acid-SIP
Adjust to Constant Moisture
Add 2% NaCl
Blend
Blend
5 min. @ <10°C
Stephan w/ vacuum
Stuff 2-8" links
Stuff 2-8" links
Cook in 90°C water bath for 30 min.
Chill immediately
Remaining Raw:
Remaining Raw:
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other
1. Run proximates
2. Run color
3. Save samples for DSC, Cholesterol, Collagen, Other


Cooked CO
Cooked CN
Cooked SO
Cooked SN
Cooked AO
Cooked AN
Determine Cook yield
Run texture analyzer
Run WHA
Run color
Remaining Cooked
1. Prox
2. Collagen
3. Cholesterol

APPENDIX B
BEEF HEART ACID-SIP PROCESS VALUES

SAMPLE[a]	WT[b]	INITIAL pH[c]	TARGET pH[d]	pH[e]	AMT HCl ADDED[f]	SUPERNATANT pH[g]	TARGET pH[h]	pH[i]	AMT NaOH ADDED[j]	FINAL WT[k]
1a	400	4.91	**2.5**	2.49	49.5	2.82	**5.5**	5.51	39.6	355.9
1b	400	5.50	**2.5**	2.51	50.8	2.61	**5.5**	5.50	44.9	364.5
1c	400	5.07	**2.5**	2.50	48.6	2.81	**5.5**	5.51	40.6	362.1
1d	400	5.12	**2.5**	2.51	50.1	2.82	**5.5**	5.51	39.7	346.0
2a	400	5.20	**2.5**	2.50	56.3	2.74	**5.5**	5.50	38.3	367.3
2b	400	4.99	**2.5**	2.50	55.1	2.97	**5.5**	5.51	39.6	383.2
2c	400	4.95	**2.5**	2.51	57.2	2.85	**5.5**	5.52	41.3	354.8
2d	400	4.95	**2.5**	2.51	57.3	2.78	**5.5**	5.52	39.5	360.7
3a	400	4.95	**2.5**	2.51	39.9	2.74	**5.5**	5.51	49.5	350.5
3b	400	5.05	**2.5**	2.49	32.3	2.65	**5.5**	5.52	50.3	373.7
3c	400	5.11	**2.5**	2.50	35.7	2.52	**5.5**	5.50	51.3	379.6
3d	400	4.99	**2.5**	2.51	36.8	2.51	**5.5**	5.51	49.6	345.7
3e	400	4.89	**2.5**	2.50	37.3	2.68	**5.5**	5.51	51.3	361.6
AVG	400	5.05		2.50	46.68	2.73		5.51	44.27	361.97
ST DEV	0	0.16		0.01	9.07	0.13		0.01	5.30	11.82

[a] Number is trial run. Letter is replication for that day.
[b] Weight (g) of ground heart.
[c] pH of ground heart blended with 3600mL of 4 °C 2mM citrate buffer.
[d] Determined by Mireles DeWitt and others (2002) as the optimum pH for solubilizing myofibrillar proteins for beef heart.
[e] pH after 2N HCl was added.
[f] Amount of 2N HCl added to blended heart and citrate buffer to drop the pH to around 2.5.
[g] pH of supernatant obtained after centrifugation and discarding the pellet.
[h] Isoelectric point of myofibrillar proteins (Pearson and Gillett 1996).
[i] pH of supernatant after 2N NaOH was added.
[j] Amount of 2N NaOH added to supernatant to raise pH to around 5.5.
[k] Final weight of protein pellet obtained after centrifugation prior to dewatering the sample.

APPENDIX C

CRYOPROTECTANTS ADDED

SAMPLE[a]	WT[b]	pH[c]	SUCROSE (g)	SORBITOL (g)	NaTPP[d] (g)	pH[e]	AMT $NaHCO_3$[f]	pH[g]	FINAL WT[h]
1 C	1000.0	6.33	40.00	40.00	3.00	6.56			1050
1 A	644.0	4.96	25.76	25.76	1.93	5.96	55.0	6.91	701.6
1 S	1207.5	6.56	48.30	48.30	3.62	6.90			1276.0
2 C	1000.0	6.01	40.00	40.00	3.00	6.47			1067.3
2 A	627.0	4.87	25.08	25.08	1.88	5.93	35.0	6.90	692.8
2 S	710.8	6.22	28.43	28.43	2.13	6.50			754.9
3 C	1000.0	6.41	40.00	40.00	3.00	6.50			1049.5
3 A	807.7	4.86	32.31	32.31	2.42	5.99	47.5	7.00	870.0
3 S	1167.7	6.84	46.71	46.71	3.50	6.93			1200.0

[a] Number is trial run. C=Control, A=Acid-SIP, S=Surimi.
[b] Weight (g) of sample after dewatering.
[c] pH of sample.
[d] NaTPP=Sodium Tripolyphosphate.
[e] pH after cryoprotectants added
[f] Amount of 5% $NaHCO_3$ added to Acid-SIP sample.
[g] pH of Acid-SIP proteins after NaHCO3 was added.
[h] Weight of sample frozen.

APPENDIX D

INITIAL MOISTURE

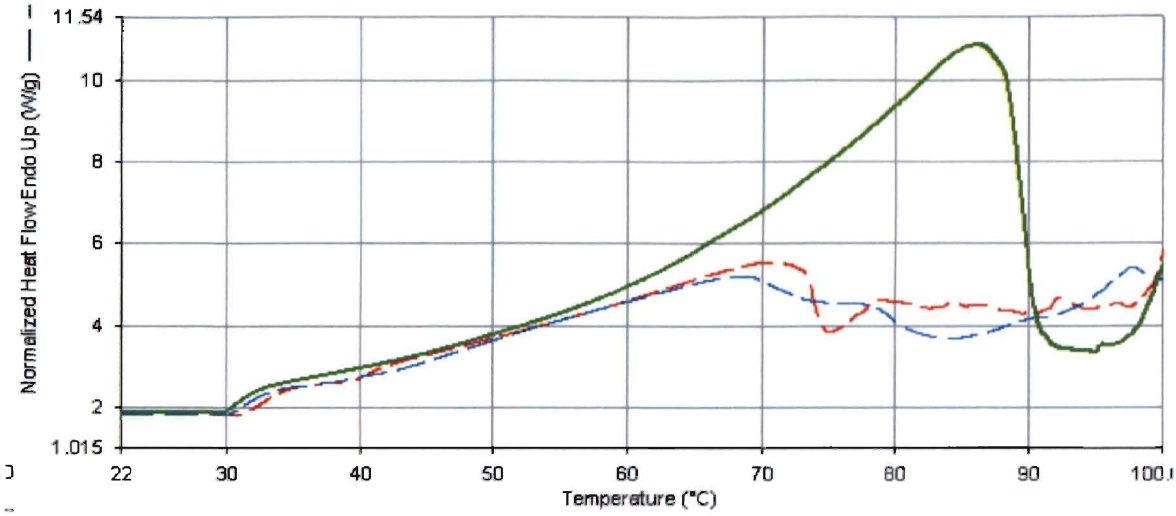
SAMPLE[a]	INITIAL % MOISTURE[b]
1C	74.01
1A	75.12
1S	77.53
2C	73.00
2A	75.46
2S	73.70
3C	74.14
3A	74.93
3S	77.43

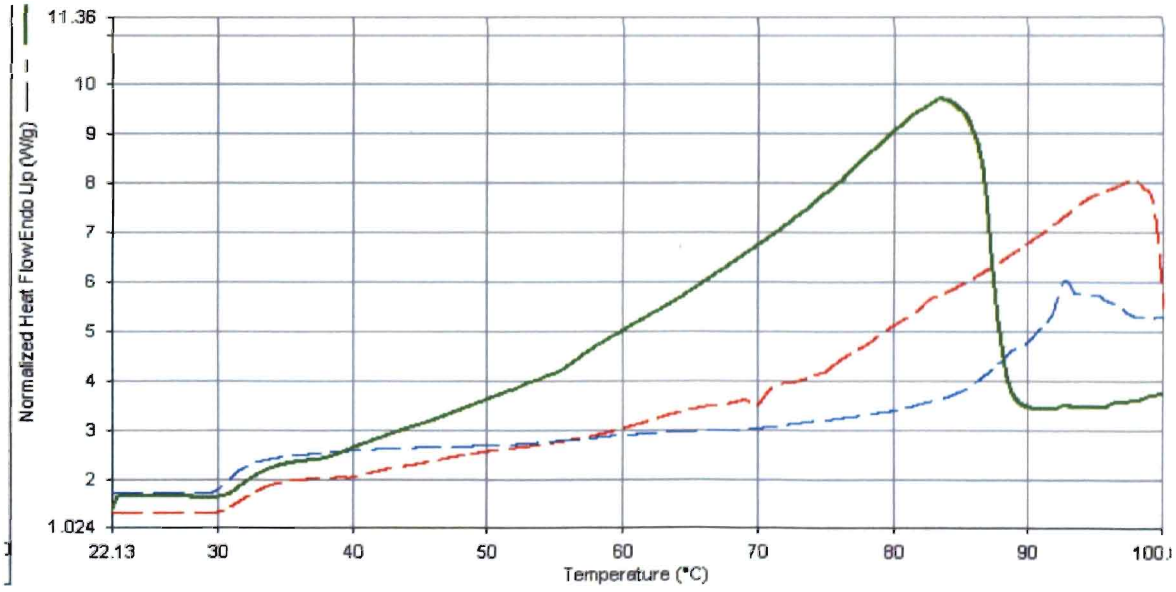
[a] Number is trial run. C=Control, A=Acid-SIP, S=Surimi.
[b] % moisture of samples with cryoprotectants prior to equilibration

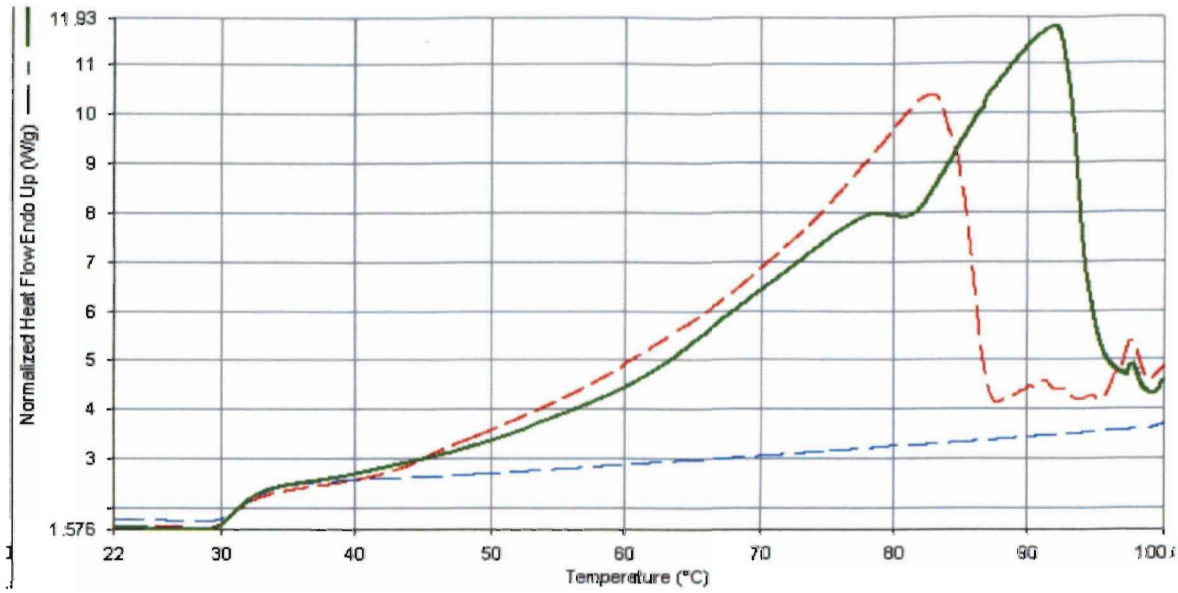
APPENDIX E

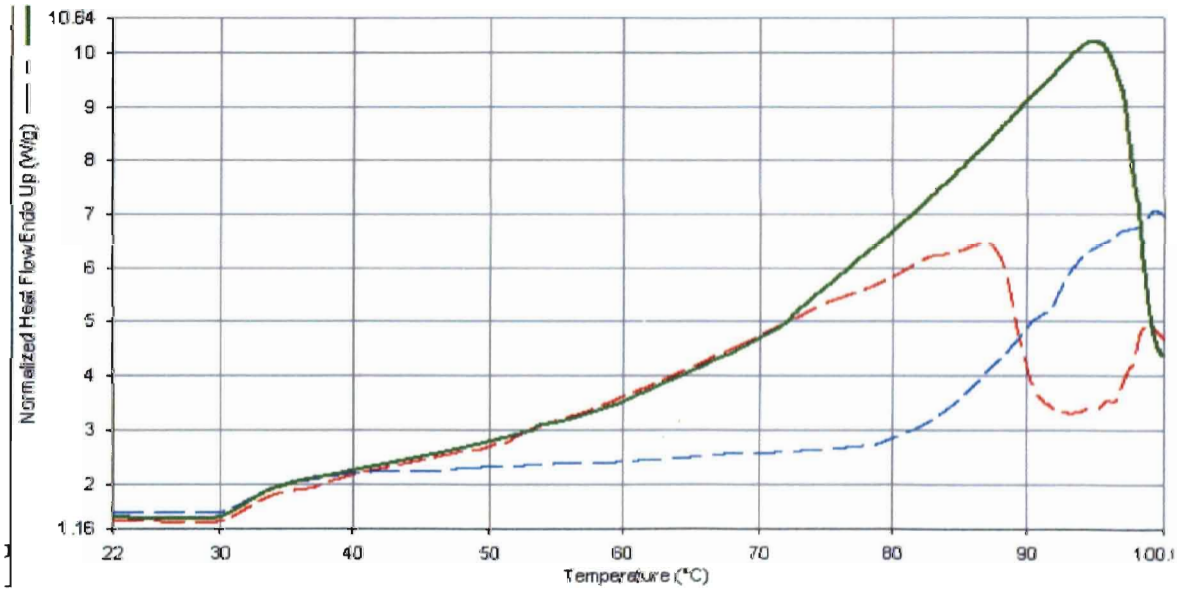
DIFFERENTIAL SCANNING CALORIMETRY (DSC) CURVES
See also Figure 1

No cryoprotectants or salt have been added to the sample.
ACID-SIP = GREEN CURVES
SURIMI = RED CURVES
CONTROL = BLUE CURVES






Normalized Heat Flow Endo Up (W/g)
11.93
11
10
9
8
7
6
5
4
3
1.576
22
30
40
50
60
70
80
90
100
Temperature (°C)


Normalized Heat Flow Endo Up (W/g)
10.64
10
9
8
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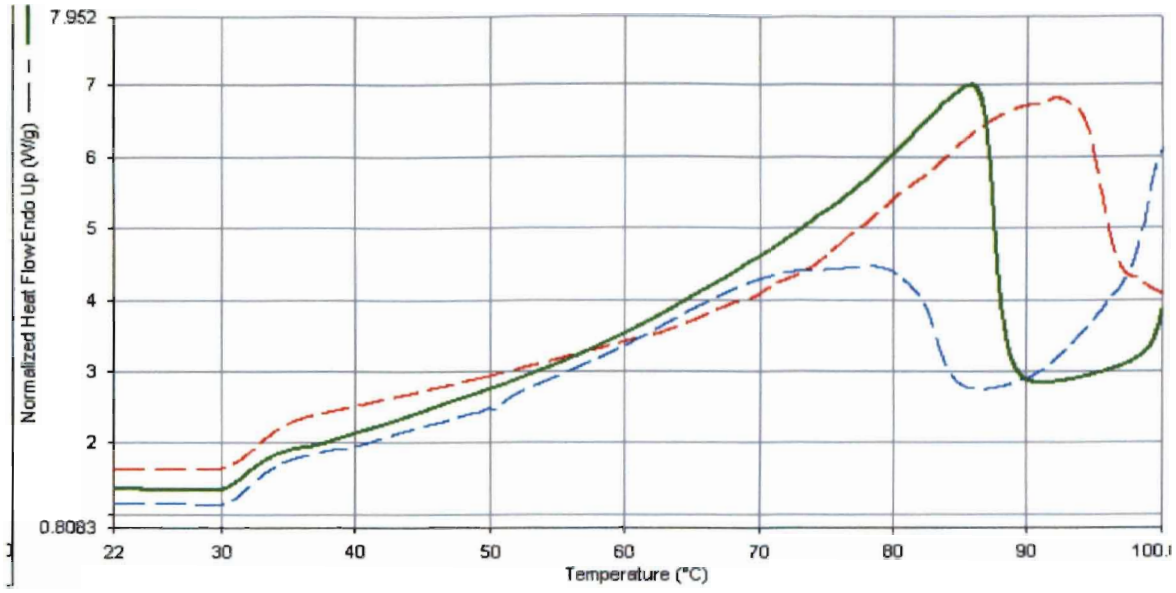
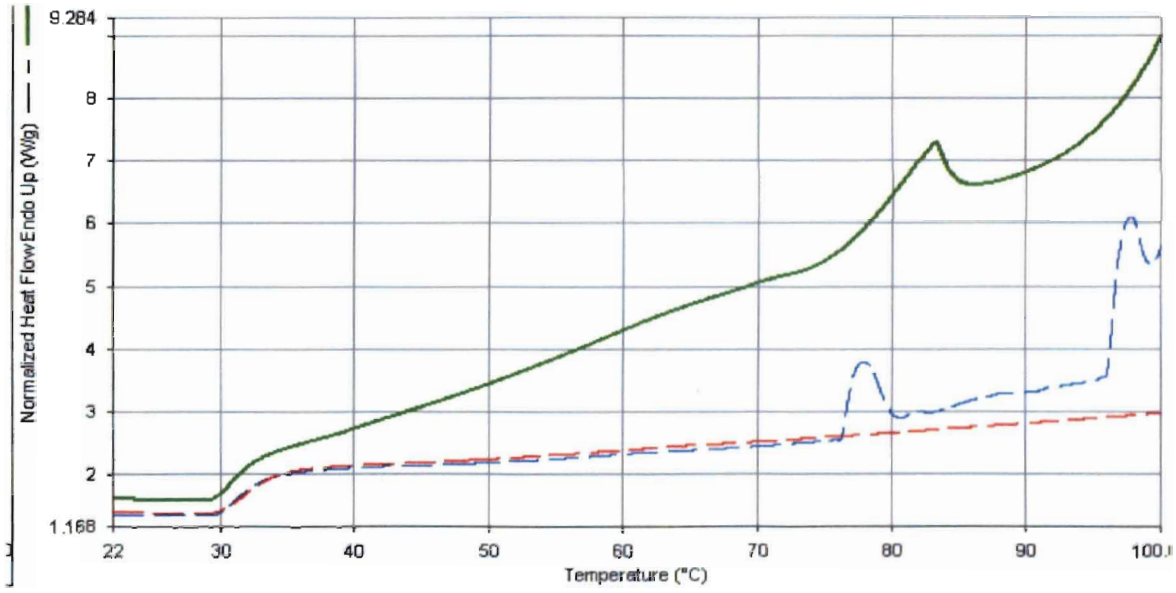
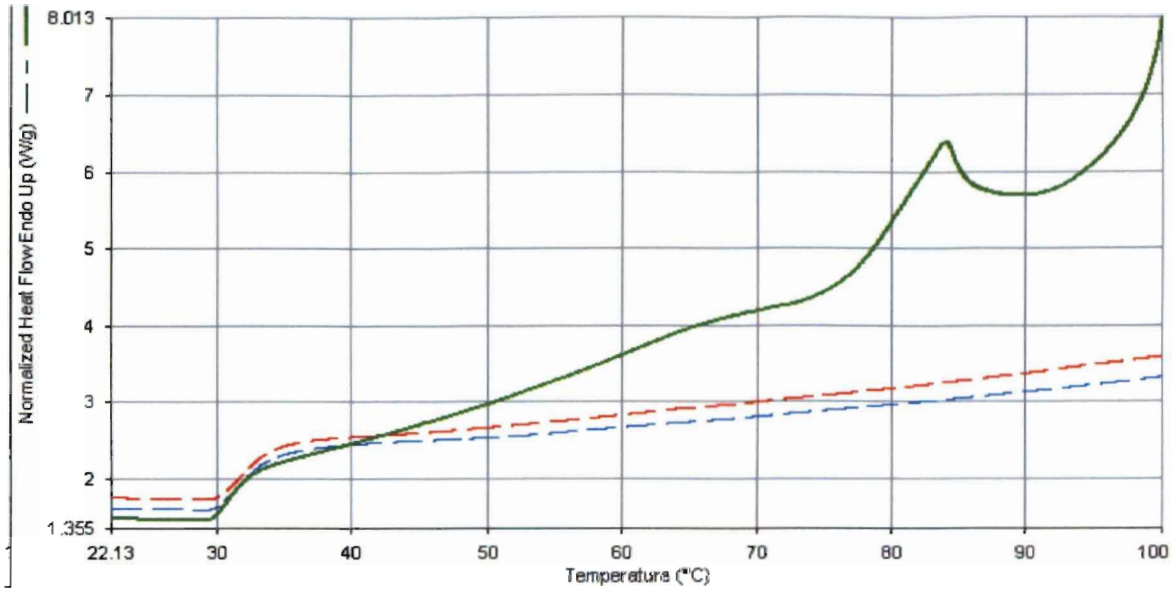
3
2
1.16
22
30
40
50
60
70
80
90
100
Temperature (°C)


7.952
7
6
5
4
3
2
0.8083
Normalized Heat Flow Endo Up (W/g)
22
30
40
50
60
70
80
90
100.
Temperature (°C)


9.284
8
7
6
5
4
3
2
1.168
Normalized Heat Flow Endo Up (W/g)
22
30
40
50
60
70
80
90
100.
Temperature (°C)


8.013
7
6
5
4
3
2
1.355
Normalized Heat Flow Endo Up (W/g)
22.13
30
40
50
60
70
80
90
100
Temperature (°C)

APPENDIX F

BEEF HEART TEXTURE PROPERTIES
COOK YIELD & WATER HOLDING ABILITY

TREATMENT^	COOK YIELD %*	WHA (g H_2O/ g PROTEIN)*
CONTROL *O*	67.52 ± 8.16^{a}	1.17 ± 0.22^{a}
CONTROL *N*	87.85 ± 3.96^{b}	1.59 ± 0.31^{b}
ACID-SIP *O*	99.57 ± 3.71^{c}	2.18 ± 0.49^{c}
ACID-SIP *N*	98.17 ± 0.81^{c}	2.40 ± 0.44^{c}
SURIMI *O*	91.28 ± 11.34^{b}	1.82 ± 0.29^{b}
SURIMI *N*	91.08 ± 0.58^{b}	2.35 ± 0.29^{c}

^ (*O*) No NaCl; (*N*) 2% NaCl.

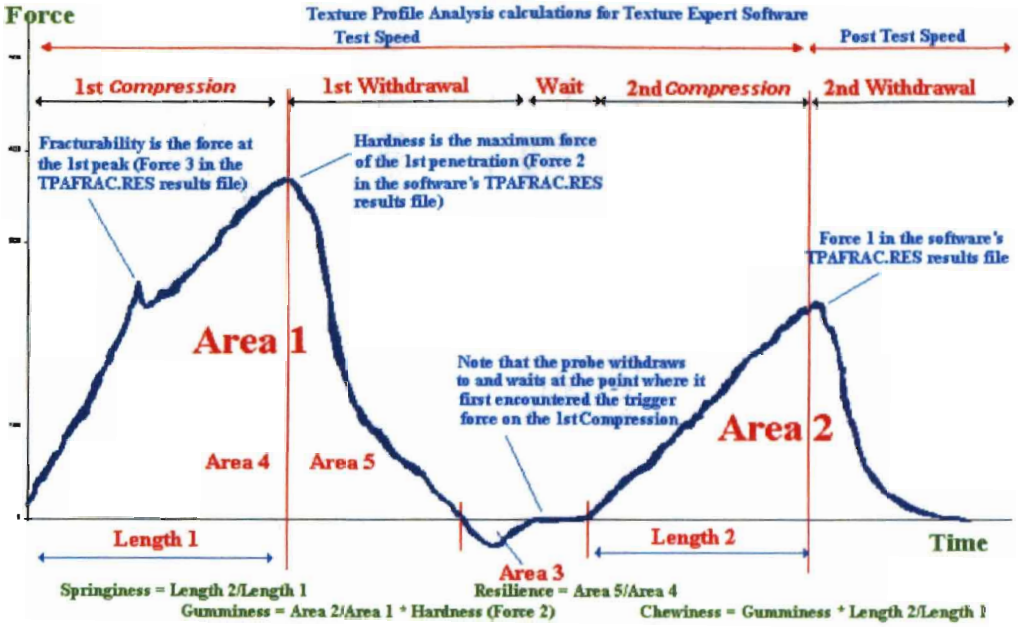
Data represent mean ± standard deviation.

[a,b,c] Means within same column without common subscript are different ($p<0.05$).

* Determined according to Daum-Thunberg and others (1992).

Appendix G

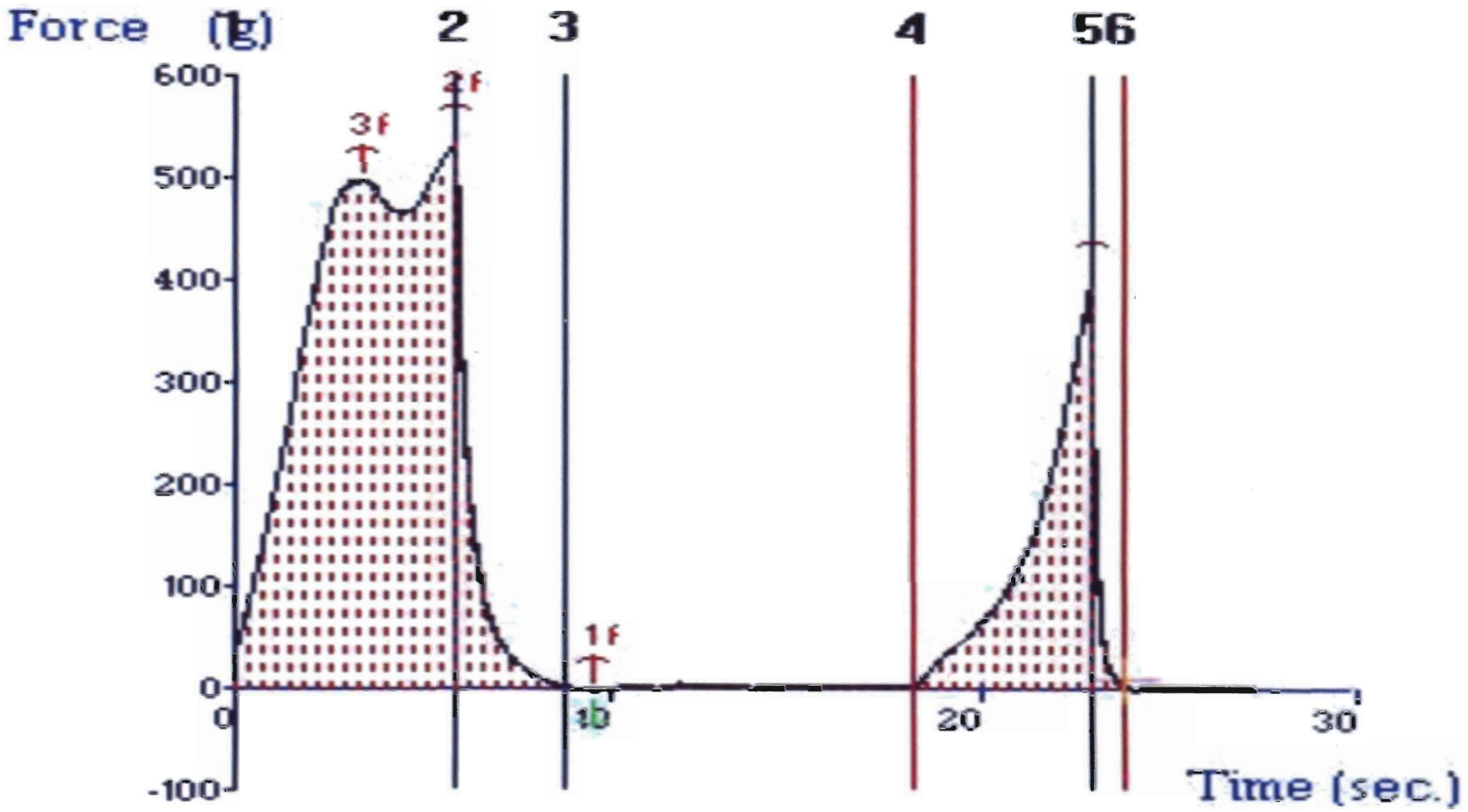
BEEF HEART TEXTURE PROPERTIES
Texture Profile Analysis Calculations
www.texturetechnologies.com



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

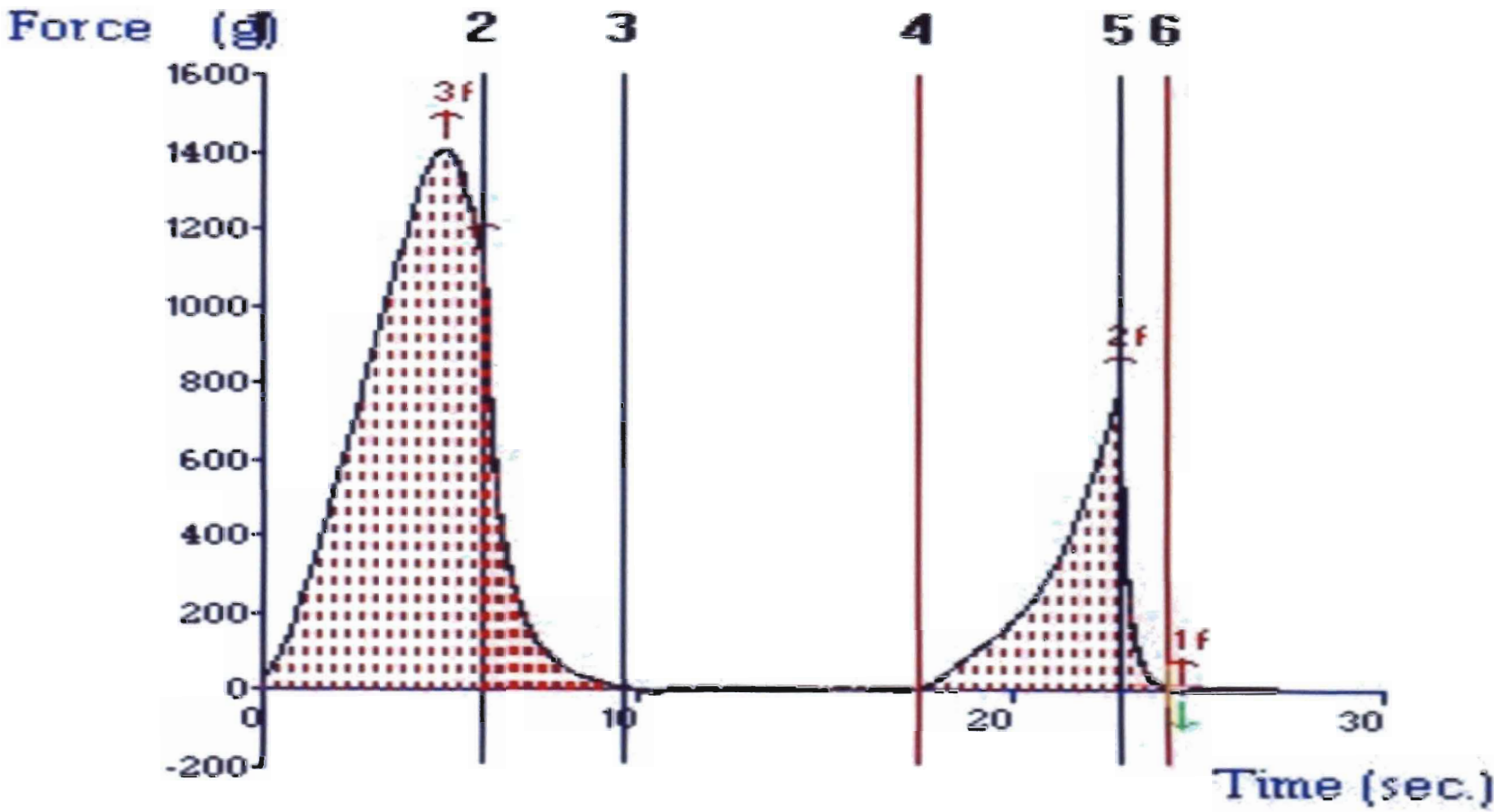
Representative TPA of Control Beef Heart without NaCl



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

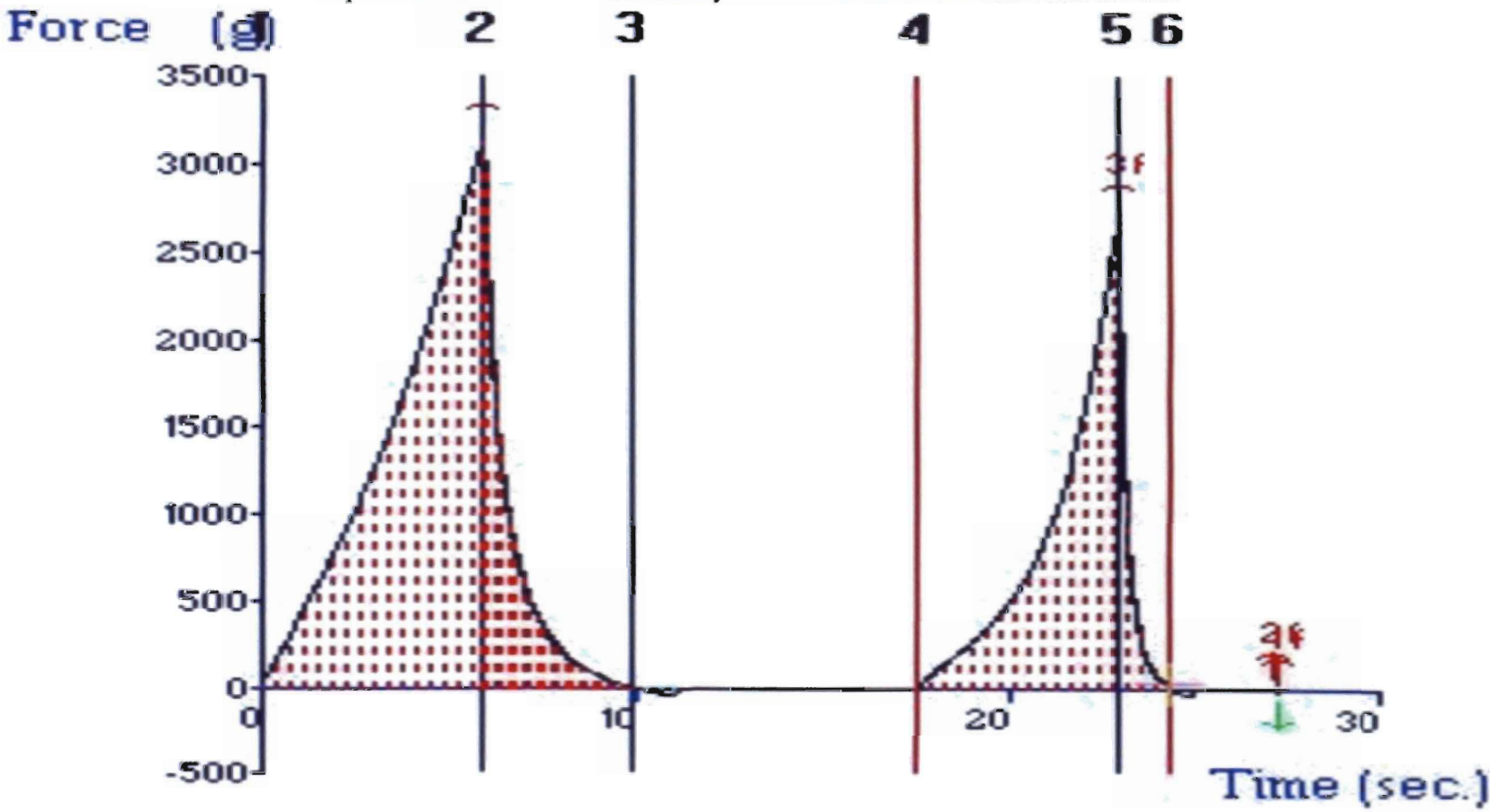
Representative Texture Profile Analysis of Control Beef Heart with NaCl



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

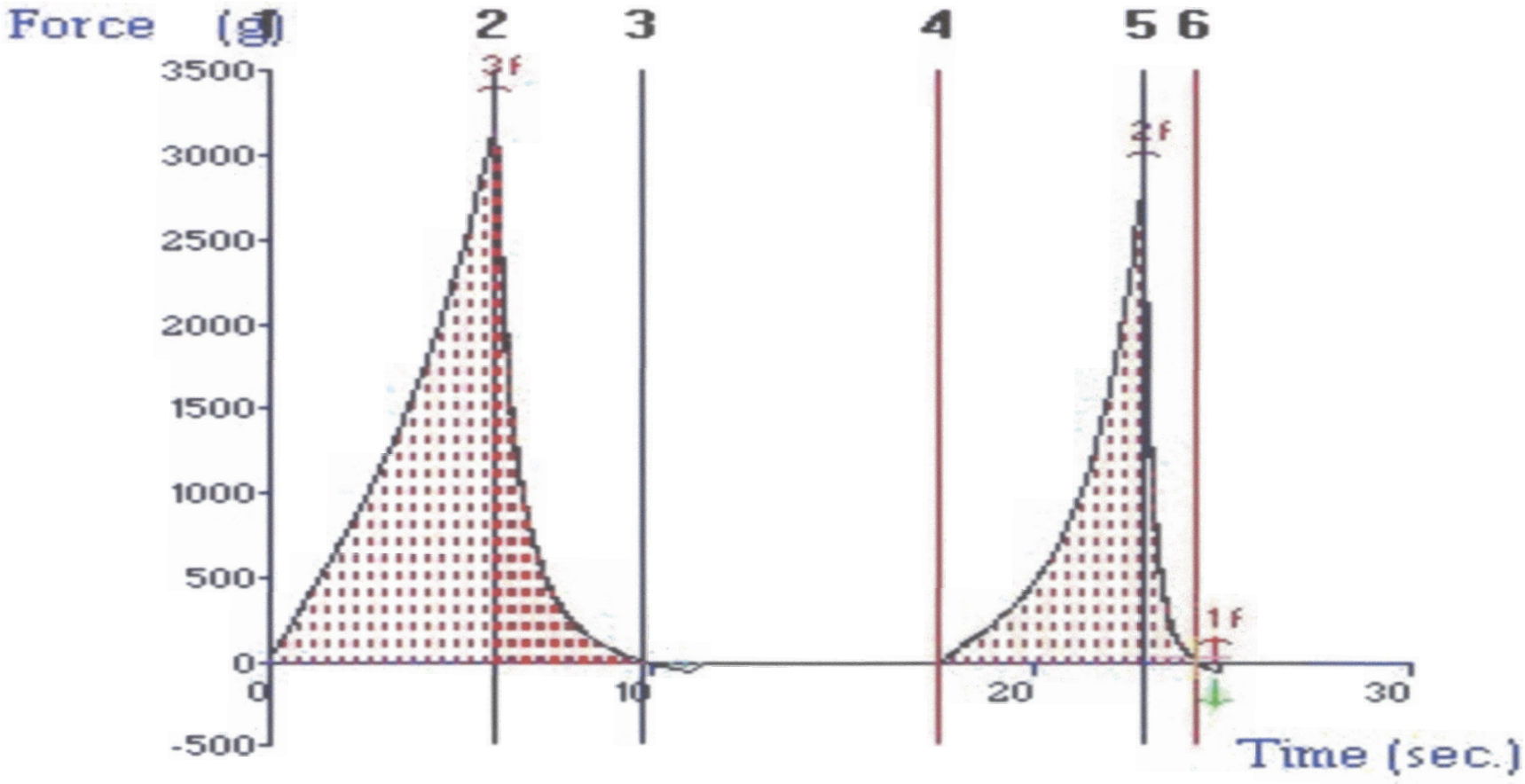
Representative Texture Profile Analysis of Acid-SIP Beef Heart without NaCl



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

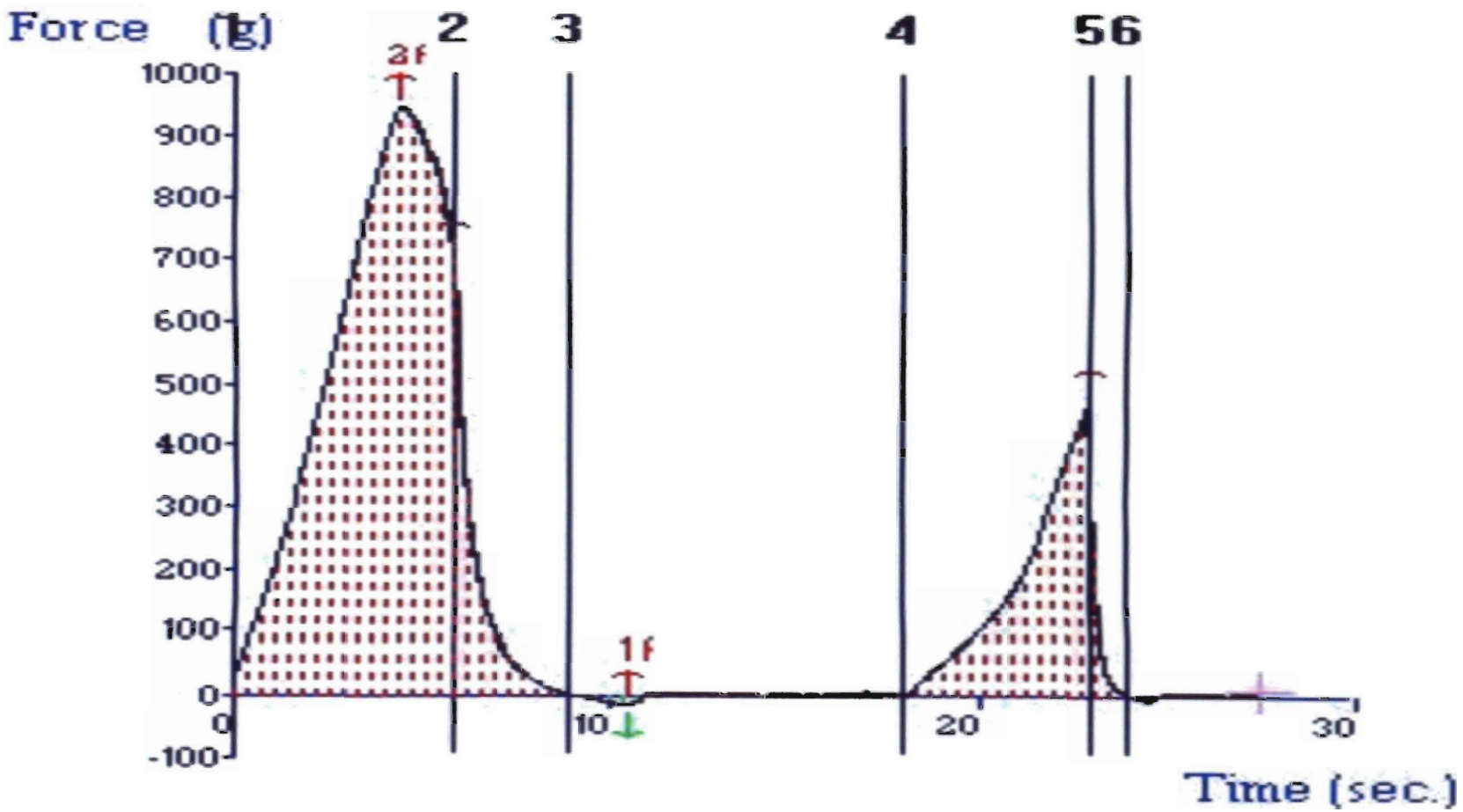
Representative Texture Profile Analysis of Acid-SIP Beef Heart with NaCl



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

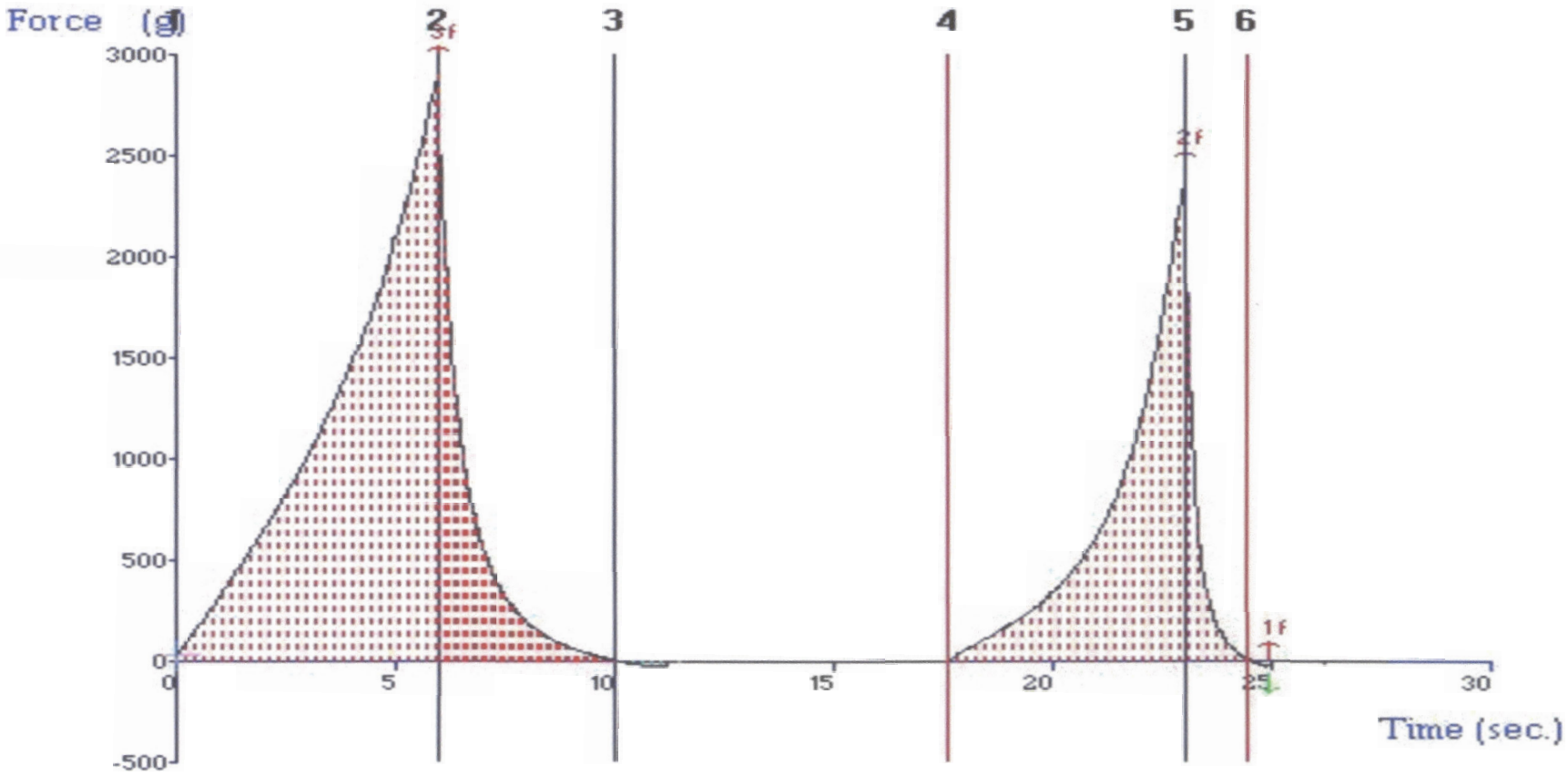
Representative Texture Profile Analysis of Surimi Beef Heart without NaCl



APPENDIX H

TEXTURE PROFILE ANALYSIS (TPA)

Representative Texture Profile Analysis of Surimi Beef Heart with NaCl



APPENDIX I

BEEF HEART TEXTURE PROPERTIES
TEXTURE PROFILE ANAYLSIS (TPA)

TREATMENT^	HARDNESS (g)	SPRINGINESS (mm)	COHESIVENESS	GUMMINESS (g)	CHEWINESS (mJ)	RESILIENCE
C *O*	652.87 ± 249.79[a]	0.73 ± 0.70[a]	0.24 ± 0.04[a]	157.87 ± 64.25[a]	118.40 ± 52.49[a]	0.09 ± 0.01[a]
C *N*	1407.07 ± 325.43[c]	0.86 ± 0.05[b]	0.29 ± 0.04[b]	406.55 ± 119.41[b]	349.54 ± 108.87[b]	0.13 ± 0.03[b]
A *O*	2834.14 ± 324.07[d]	0.91 ± 0.02[c]	0.48 ± 0.04[d]	1369.08 ± 203.71[c]	1239.88 ± 179.36[c]	0.25 ± 0.03[c]
A *N*	3132.90 ± 417.77[e]	0.87 ± 0.02[b]	0.50 ± 0.03[d]	1569.10 ± 214.10[d]	1369.70 ± 199.28[d]	0.27 ± 0.02[d]
S *O*	998.60 ± 392.37[b]	0.85 ± 0.06[b]	0.24 ± 0.04[a]	237.30 ± 92.41[a]	199.11 ± 70.86[a]	0.10 ± 0.03[a]
S *N*	2937.59 ± 729.84[d,e]	0.91 ± 0.02[c]	0.46 ± 0.06[c]	1324.51 ± 278.21[c]	1207.98 ± 258.46[c]	0.25 ± 0.04[c]

^ (C) = Control; (A) = Acid-SIP; (S) = Surimi
(*O*) = No NaCl; (*N*) = 2% NaCl

Data represent mean ± standard deviation.

[a,b,c,d,e] Means within same column without common superscript are different ($p<0.05$).

(2)

VITA

Jennie Marie James

Candidate for the Degree

Master of Science

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Personal Data: Born in Clovis, New Mexico, November 6, 1978, the daughter of Louis and Elaine James

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Professional Organizations: American Meat Science Association, Institute of Food Technologists