# Hydrodynamic Shock Wave Effects on Protein Functionality

by

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# Abstract

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USDA Select bovine *Biceps femoris* (BF) samples were divided into four sections and randomly assigned to three hydrodynamic shock wave (HSW) treatments and a control. Different amounts of explosive (105 g, H1; 200 g, H2; 305 g, H3) were suspended in the center of the hemishell tank, 26.7 cm above the vacuum packaged beef placed on the bottom center of that water-filled tank and detonated, representing three HSW treatments. In addition, BF steaks (2.54-cm thick) from a different and limited common source (2 muscles) were packaged with each HSW designated BF section. These served as internal refernce steaks (IRS) for the six replications to determine if the HSW treatments physically altered the structural integrity of the meat. H1 and H3 decreased ($P<0.05$) Warner-Bratzler shear values of the IRS from 3.86 and 3.99 kg (controls) to 3.01 and 3.02 kg (HSW), respectively. H2 shear values, 3.86 (control) to 3.46 kg (HSW) were not different ($P> 0.05$). HSW and control BF sections were analyzed for protein solubility and then used to manufacture frankfurters formulated with 2.0% NaCl, 0.5 % sodium tripolyphosphate, 156 ppm sodium nitrite, 0.42 % sodium erythorbate, 2.0 % sucrose, and 25 % water. Frankfurters (cooked to 71 C) were evaluated for cooking yield, CIE L*a*b*, nitrosylhemochrome, Texture Profile Analysis (hardness, cohesiveness), and stress and strain (torsion testing). Compared to the control samples, the HSW did not affect ($P>0.05$) myofibrillar or sarcoplasmic protein solubility, cooking yield, or color. Textural properties and gel strength of the frankfurters were not affected ($P>0.05$) by the HSW. These results indicate that beef trim obtained from HSW processed meat can be used interchangeably with normal meat trim in the production of further processed meats since the functionality of meat protein is not affected significantly by the HSW process.

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**Table of Contents**

**Hydrodynamic Shock Wave Effects on Protein Functionality**

**ABSTRACT**

# LIST OF TABLES

# LIST OF FIGURES

# Chapter 1

## 1.1 Introduction

One of the major challenges of the beef industry is to market products without conventional aging and yet maintain acceptable tenderness. Over the years, scientists have been striving to improve tenderness by evaluating or using procedures such as hydrostatic pressure, electrical stimulation, proteolytic enzymes, skeletal alteration, and blade penetration. The hydrodynamic shock wave (HSW) treatment (Hydrodyne Process redesigned by John B. Long, U.S. patent #5,273,766 and #5,328,403) has been developed to instantaneously tenderize meat. This process utilizes an explosive to create a shock wave in a specially designed enclosed tank filled with water. The shock wave passes through the water and objects that are a mechanical impedance match to the water (Kolsky, 1980). Since meat is made up of approximately 75% water, the shock wave passes through muscle and ruptures selected cellular components. Sarcomeres are torn at the Z-line and A-band/I-band juncture, (Zuckerman and Solomon, 1998) in red meat. This rupturing of the cellular components appears to mainly affect the myofibrillar component. However, it is unlikely to affect connective tissue under testing conditions that have been used. (personal communication of Solomon 1997, reported by Raloff, 1998).

The HSW process has been utilized to effectively tenderize both cold-shortened and postrigor muscle (Solomon et al., 1997a and Solomon et al., 1997c). Solomon et al., (1997b) demonstrated that HSW processed beef 3 days postmortem was as effective in tenderizing beef as aging 17 to 35 days. Use of a plastic container with a steel plate on its

bottom to generate HSW was believed to play a major role in the tenderization of the meat.

Personal communication of Solomon as reported by Raloff, (1998) indicated that determining what parameters made earlier research with the use of garbage cans work so well, such as its geometry or shock wave propagation dynamics, could make this process repeatable. Determining these parameters and applying them to a commercial process may be a very effective tenderization method as well as a potential alternative for meat aging. If this process is commercialized, beef trim from muscle cuts treated with the HSW will likely be used in production of further processed products so that all of the muscle can be utilized. Thus, research is needed to determine the ability of meat proteins from HSW treated muscle to function in further processed products.

Although no research has been reported on how HSW affects protein functionality, those effects caused by hydrostatic pressure have been well documented. Hydroststic pressure is similar to the HSW treatment because pressure is utilized for tenderization (Kennick et al., 1980). Hydrostatics is the study of characteristics of liquids at rest or the force that a liquid imposes on a submerged object (Zobell and Kim, 1972). High hydrostatic pressure over an extended period of time (5 to 30 min) can increase tenderness as well as improve protein functionality characteristics and myofibrillar solubilization (McFarlane and McKenzie, 1976). Mandava et al. (1995) stated that hydrostatic pressure increased cooking yield and improved texture of low-salt and low-phosphate frankfurters. Hydrostatic pressure also improves the binding of comminuted meat and increases tensile strength (McFarlane et al., 1984). One major limitation of

hydrostatic pressure is that the process has not yet been designed so that it can function in a time efficient manner.

The first objective of this research was to determine the effects of the HSW process on cooking loss, cured color, cooked color, and texture of frankfurters manufactured from HSW processed beef. The second objective was to determine if this shock wave process affects protein solubility, individual proteins responsible for binding ability, and the cleavage of peptide bonds.

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# Chapter 2
## 2.1 Literature Review

### 2.1.1 Hydrodynamic Shock Wave Treatment

Hydrodynamics is the study of fluid motion and forces on solids in the fluids (Kolsky, 1963). In the Hydrodynamic shock wave (HSW) process, explosives are placed in water and detonated to create shock waves that are sent through the water and the meat samples placed in the water. The amount of explosive and distance of the explosive from the sample determines how effective the shock waves are in tenderizing the meat (Solomon, 1998). The shock wave travels through the water and any objects which are an acoustical match for the water (Kolsky, 1963). Meat contains approximately 75% water (Pearson, 1987), and therefore the shock wave will pass through the meat. As the shock waves travel through the meat, they rupture the cellular components. Sarcomeres are ruptured at the Z-line and A-band/I-band juncture (Zuckerman and Solomon, 1998), resulting in physical tenderization of red meat.

The Hydrodyne process redesigned by John B. Long (U.S. patent #5,273,766 and #5,328,403) uses hydrodynamic shock waves to tenderize beef (Solomon et al., 1997d). John B. Long first imagined this process over 30 years ago while floating in a pool. He observed that his body had approximately the same density as water. So, if a bomb was exploded in the water, the shock waves would go through his body, and they would also travel through his skeletal muscles (Raloff, 1998). This observation led him to start research on hydrodynamic shock waves (HSW) at a privately owned explosive site. At this site, he tenderized his first sample of meat with HSW. The process was then used to treat bone-in beef. It failed because bones bounce back shock waves inside the muscle tissue, turning it to mush (personal communication of Long reported by Donovan, 1998).

He shelved this technology for 20 years, and then teamed up with Stanford Klapper to form Hydrodyne Inc. headquartered in San Juan, Puerto Rico. Long determined that bone removal would allow the process to work, and since almost all meat is deboned before processing, this process could be of value to the industry (Donavan, 1998).

Godfrey (1970) originally patented the use of hydrodynamic shock waves to tenderize meat by the use of an explosive charge which generates and applies a shock front pressure propagated through a liquid medium at velocities exceeding the speed of sound. According to Long's U.S. Patents (#5,273,766 and #5,328,403), the principle was sound, but the tank and position of the meat in relation to the tank and explosive charge would present serious difficulties in the commercial tenderizing of meat. According to U.S. patents #5,273,766 and #5,328,403, the apparatus designed by John Long for commercial meat tenderization is a hemishell tank compared to that of Godfrey, which had a top to the tank that could not be used commercially.

Two different containers have been used in research pertaining to this process. The first container was a plastic container (garbage can) with a 208 L capacity (Solomon et al. 1997d) that was destroyed with each treatment (Raloff, 1998). This system has been used for research experiments to determine the capabilities of this process (U.S. patent #5,273,766). The second container was a steel hemishell tank (U.S. patent # 5,328,403) designed for the commercial process. Personal communication of Solomon reported by Raloff (1998) indicated that although this large commercial tank works, it does not work as well as the garbage cans. Not only did its treated meats come out less tender than those in the garbage can, but it was more variable in tenderization within a batch of treated meats. Recent studies by Solomon et al. (1999b,c) have verified this

personal communication reported by Raloff (1998). Solomon et al. (1999b) showed that the garbage can was more effective in tenderizing muscle than the steel hemishell container. According to Solomon et al. (1999c), the number of shock absorbers in the steel hemishell tank is one of the tank's parameters that affect its ability to tenderize meat. Solomon et al. (1999c) reported that the 1060 L tank with 4 shock absorbers was more effective in tenderizing meat than the same container with 8 or 16 shock absorbers as well as controls. Although, the 4 shock absorber tank was not as effective in tenderizing meat as the plastic garbage can.

Solomon et al. (1997d) were the first to report research on the use of hydrodynamic pressure to tenderize meat. In each of the following three experiments, the explosive used was a binary explosive composed of a liquid (nitromethane) and a solid (ammonium nitrate). All explosives were submerged in the water to a distance 30.5 cm away from the front surface of the meat. In the first set of experiments, 12 longissimus (LM) steaks (3.2 cm thickness) were removed from loins (both sides of five 2-yr-old Holstein cows, 24 h postmortem). Two steaks per loin were exposed to explosive levels of 50, 75, and 100 g of explosive in a single detonation. Two steaks per loin were treated as a control, two were treated twice by the shock wave using 50 g of explosive each time, and two frozen samples were treated with 75 g of explosive. These steaks were all treated in a plastic container (208 L, 51 cm diameter) with a steel plate placed on the bottom to reflect shock waves. All hydrodynamic shock wave treatments (HSW) reduced the shear value of the broiled steaks (71 C). However, the magnitude of improvement depended on the quantity of explosive used or the number of detonations performed on each meat sample. The control had a shear value of 7.8 kg while the 50, 75, and 100 g treatments

were 3.4, 4.0, and 2.6 kg. The treatment exposed to two shock waves had a shear value of 2.2 kg. The frozen steak had a shear value of 5.9 kg.

In the second set of experiments conducted by Solomon et al. (1997d), three fresh, whole USDA Select *Biceps femoris* (*BF*) were purchased from a grocery store. Eight steaks (3.2 cm thickness) were removed from each *BF*. Steaks were treated (single detonation, 50, 75, or 100 g of binary explosive) in 208 L, 51 cm diameter plastic containers with a steel plate placed on the bottom to reflect shock waves. As much as 19 to 30% improvement in broiled (71 C) BF shear force was observed compared to controls when the BF steaks were treated with the HSW. The control had a shear value of 4.3 kg while the 50, 75, and 100 g treatments were 3.3, 3.5, and 3.0 kg, respectively. These muscles were considered tender before treatment since they had shear force values less than 4.6 kg (Shackelford et al., 1991). Limited improvement would be expected on inherently tender meat, since the HSW affects the myofibrillar component of tenderness but does not affect connective tissue (Solomon, 1998).

In the third set of experiments conducted by Solomon (1997d), portions of boneless LM, BF, *Semimembranosus* (SM), and *Semitendinosus* (ST) from four 2 yr-old Holstein cows were hot-boned within 1.5 h postmortem, vacuum packaged, and stored for 1 day at 2 to 4 C. Muscle samples were frozen at –34 C and thawed before being treated with HSW. Each muscle was divided into two equal portions (20 cm length). One portion of the muscle was treated with HSW using 100 g of binary explosive. The 208 L, 51 cm diameter plastic containers were fitted with a steel plate placed on the bottom to reflect shock waves. Shear force of broiled (71 C) steaks were improved 66% (8.3 vs. 2.8 kg) in the LM, 59% (10.5 vs. 4.3 kg) in the SM, 53% (7.8 vs. 3.7 kg) for the

BF, and 56% (12.9 vs. 5.7 kg) in the ST comparing HSW with the control.  Locker and

Hagyard (1963) reported that hot boning the intact muscles and storing them for 1 day at

2 to 4 C before freezing resulted in high shear values indicating that "cold-shortening" of

the muscles occurred.  If the high shear values of the controls in Solomon et al. (1997d)

were due to cold-shortening, results indicate that the HSW process was effective at

significantly tenderizing "cold-shortened" meat, regardless of muscle origin.

O'Rourke et al. (1998) used the HSW process to determine to what extent it alters

pork tenderness and proteolysis during aging.  In the two studies conducted, the binary

explosive was placed at a distance from the front face of the meat such that the HSW

would apply approximately 70 MPa of force onto the front face of the samples.  The first

study involved 24 control and 24 HSW pork loins treated 1day postmortem.  The second

study consisted of paired loin pieces HSW treated 1day postmortem (12 controls and 12

HSW) and aged for 40 days.  The samples were treated with HSW in a plastic container

(208 L capacity, 51 cm diameter).  Shear values were determined on chops (1.27 cm

thick) cooked to an internal temperature of 72 C.  Myofibrils were isolated, and

electrophoresis was performed to determine proteolysis and to identify protein fragments

with different molecular weights.   In study 1, HSW treated pork was lower (33%) than

the controls (5.8 vs. 3.6 kg) in shear values.  HSW treated chops resulted in a 2.4 % lower

cooking loss than control chops.  In study 2, HSW treated pork was lower in shear force

(17 %) than control pork 1 day postmortem (4.3 vs. 3.5 kg) but not at 40 d postmortem

(3.8 vs. 3.6 kg).  HSW treatments immediately improved tenderness at one day

postmortem, but HSW treatments aged for 40 days were not different in tenderness than

control loins aged for 40 days.  Gel electrophoresis results illustrated that pork aged for

40 days undergoes sufficient proteolysis explaining its similarity in tenderness to HSW treated pork. Electrophoresis results also revealed that enhanced proteolysis does not appear to be the mechanism of tenderization in HSW-treated pork.

Solomon et al. (1997b) used 208 L capacity, 51-cm diameter plastic containers to determine the effect of HSW on tenderness of muscle in comparison to that of conventional aging. Four matched pairs of U.S. Choice boneless strip loins 72 h postmortem were equally divided into the following treatments: control- 3 days aged; HSW- 3 days aged with 100 g binary explosive, and aged for 17, 21, 28, and 35 days. Steaks were broiled to 71 C for shear force determinations. The explosive was placed 38 cm from the interface of the steel plate such that the HSW would apply approximately 100-130 MPa of force on to the front face of the samples. Aging strip loins from U.S Choice carcasses for 2 weeks to as much as 5 weeks reduced shear force between 24 to 37 % as compared to controls (Solomon et al., 1997b). The control samples required 4.8 kg compared to 3.0, 3.5, 3.5, and 3.2 kg for 17, 21, 28, and 35 days of aging, respectively. There were no differences among the four aging periods. Samples treated by the HSW process at 3 days post slaughter had a shear value of 3.2 kg compared to 4.8 for control samples at 3 days postmortem. This was a 33 % improvement in shear force which confirmed that the HSW was as effective in tenderizing meat as those found in the four aging periods evaluated.

Zuckerman and Solomon (1998) incorporated 208 L containers with a metal plate on the bottom as discussed in previous studies for using the HSW process to tenderize meat. Boneless strip loins (USDA Select; 5 days postmortem) were cut into portions with the rib and loin end randomly assigned to either the HSW process (100g of binary

explosive, 30.5 cm from the front surface of the meat) or as controls.  Muscle samples

were removed from the treated and control samples (within 30 min) after HSW treatment

and fixed for transmission electron microscopy (TEM) evaluation.  The shear force

(broiled steaks, 71 C) of the HSW treatment was 37 % less than for the controls (5.4 vs.

3.4 kg).  The TEM observations illustrated that HSW treatment caused considerable

disruption of the myofibrillar lattice (lattice of filaments formed inside the myofibril).

These disruptions were seen in all HSW treated samples.  The most affected area was

near and within the I-band regions.  Portions of Z lines were observed attached to the A-

band on opposite sides of the fractures, resulting in a checkerboard pattern with no

discernable I-band.  The M-line in the control samples was less narrow and less distinct

than the HSW treated samples.  A-bands in fragmented sarcomeres had uneven edges as a

result of excessive stretching applied to the I-bands from the hydrodynamic pressure

caused by the HSW.  Observations of longitudinal gaps and splitting of myofibrils

occurred occasionally (Zuckerman and Solomon, 1998).  This structural change could be

due to the rapid high-pressure changes caused by the hydrodynamic shock wave where

no pressure equilibrium can be reached.  The authors went on to report that a slow

application of pressure, such as hydrostatic may allow for pressure equilibrium in which

molecules could adjust to their new environment by slow changes in the molecular

interaction level (Mordid, 1981).  Their results showed that the HSW caused increased

tenderness due to ultrastructural damage of the myofibril and substantiate those of

O'Rourke et al. (1998) which indicated that the HSW's mechanism of tenderization is not

proteolysis.

Using 208 L containers as previously described, Berry et al. (1997) tested the effects of the HSW on strip loins from U.S. Select grade beef. Previously frozen boneless beef strip loins (n=4 carcasses) were cut into portions and assigned to either the HSW process (100 g of binary explosive, 30.5 cm from the front surface of the meat) or as a control. Fresh boneless strip loins (n=7 carcasses) were also treated. The muscles were cut into sections and assigned to either the HSW process (80 g of binary explosive, 30.5 cm from the front surface of the meat) or as a control. The previously frozen loins treated with the HSW had a shear force of 2.0 kg compared to 2.8 kg for the control, a 29 % lower value. The trained panel tenderness rating was 7.0 for the HSW treated beef, which was a higher rating (more tender) than the control (6.1). The panel flavor and panel juiciness ratings were not affected by the HSW process. Panel flavor and panel juiciness values were 4.4 and 5.2 for the HSW treated samples and 4.4 and 5.4 for the control samples. The fresh strip loins treated with the HSW had a shear force of 3.3 kg compared to 4.3 kg for the controls, a 23% lower value. Panel tenderness rating was not affected by the HSW process (6.4, HSW; 5.8, control). The flavor and juiciness ratings were not affected by the HSW process. Flavor and juiciness values were 4.5 and 5.1 for the HSW treated samples and 4.4 and 5.3 for the control samples.

Using the 208 L plastic container, HSW treatments were used to investigate tenderness improvements in callipyge ovine *longissimus* muscle (LM) (Solomon et al. 1998). Ovine muscle (n=7 carcasses, 1 day aged) was selected from callipyge sheep because they have inherently tough muscle. These carcasses either received 21 V (rectangular wave), 60 Hz, 0.25 amps alternating current (electrical stimulation) immediately after slaughter or no stimulation. Stimulated carcasses were conditioned at

27 C until a pH<6.0 was obtained in the LM within 1 h.  The HSW process was also compared to $CaCl_2$ injection coupled with postmortem aging.  The HSW process involved using 100 g of binary explosive, 30.5 cm from the meat.  Callipyge LM samples treated with the HSW yielded shear values of 4.6 kg.  This resulted in a 41 % improvement from 1-day electrically stimulated callipyge carcasses (7.8 vs. 4.6 kg) and a 30 % improvement from 1-day non-stimulated callipyge carcasses (6.6 vs. 4.6 kg).  The HSW process had a shear value of 4.1 kg in non-callipyge lamb, a 38 % improvement in non-callipyge control lamb (6.6 kg).  Postmortem aging (22 d) of LM muscles in callipyge lambs had shear values of 4.5 kg, a similar value to the HSW treatment (4.6 kg).  In comparison, postmortem aging (22d) of LM in combination with $CaCl_2$ had a shear value of 4.3 kg.

There have been five studies published prior to 1999 on the use of the commercial steel tank (1060 L capacity, 4 shock absorbers) in the use of the HSW to tenderize meat.  Solomon (1997a) studied the effects of the HSW on cold-shortened beef.  Strip loins from five 2-yr-old Holstein cows were hot-boned (within 1 h postmortem), vacuum packaged, stored for 1 day at 2 C, and then frozen (-34 C).  HSW treatments were four different quantities of binary explosive (125, 160, 192, or 350 g) submerged in the tank to a distance 56 cm from the front face of the samples lying on the bottom of the tank.  The HSW treatments of 125, 160, 192, and 350 g had shear values of 5.2, 4.6, 4.3, and 3.5 kg compared to the control (8.3 kg).  The HSW in the large tank was successful in tenderizing the cold-shortened strip loins.

Solomon (1997c) excised strip loins from each carcass of six 2-yr-old Holstein cows at 24 h postmortem. The HSW treatment was with 350 g of binary explosive at 56

cm from the meat surface using the commercial, 1060 L capacity, steel tank with four shock absorbers.  The control treated strip loins had a shear value of 6.6 kg compared to 4.3 kg for the HSW, a 34 % decrease in shear value.

Solomon (1998) reported on use of the HSW to determine the effects of explosive level and explosive distance from the meat on the effectiveness of the HSW using the commercial tank (1060 L).  Strip loins and top rounds from 32 USDA Select carcasses 7 days postmortem were treated using 150 g, 56 cm from the front face of the sample; 350 g at, 56 cm from the front face; and 350 g, 46 cm from the front face.  For the strip loins, 350 g treatments at 46 cm and 56 cm decreased shear values when compared to the control steaks.  The 150 g treatment at 56 cm did not decrease shear values when compared to the control for the strip loins.  The control, 150 g at 56 cm treatment, 350 g at 56 cm treatment, and 350 g at 46 cm treatment had shear values of 4.6, 4.1, 3.8, and 3.4 kg, respectively.  These results infer that distance of the explosive to the sample was more important in reducing the shear value than the amount of explosive used in the process.  Shear values of top round muscles treated with the HSW were not different from the controls.  The controls, 150 g at 56 cm treatment, and 350 g at 56 cm treatment had shear values of 5.3, 5.3, and 4.7 kg, respectively.

O'Rourke et al. (1999) studied effects of hydrodynamic shock waves on muscle fiber orientation in relation to tenderness, aging and proteolysis in beef.  Paired semitendinosus muscles from 10 USDA Select beef carcasses were used.  HSW (350 g explosive, 46 cm from the sample) was implemented at 5 days postmortem.  The treated side was cut in half and assigned to either a vertical or horizontal positioning in respect to their muscle fibers orientation.  The 1060 L steel tank with four shock absorbers was

used.  Samples were removed from the controls at 5 and 15 days, horizontally oriented samples at 5 and 15 days, and vertically oriented samples at 5 days postmortem for shear force determinations and electrophoretic analysis.  Control 5-day samples (6.2 kg) were tougher than HSW treated samples (horizontal 5-day, 4.7 kg; 15-day, 4.6 kg; and vertical 5-day, 5.2 kg).  Control samples were tougher than 15-day control samples (4.4 kg). These results indicated that the HSW process effects on tenderness for 5-day horizontal samples were similar to the control at 15 days inferring that the HSW process on horizontal samples affects muscle tenderness similar to postmortem aging.  These results also reveal that the muscles with horizontal muscle fiber orientation in the HSW process were more tender than muscles with vertical fiber orientation.  These results were later substantiated by Solomon et al. (1999c).  Also, O'Rourke et al. (1999) discovered slightly higher proteolysis of HSW treated samples when compared to controls.

Meek  (1997) studied the effects of the HSW on decreasing broiler breast aging time.  Treated chicken breasts (five per bag) were treated with the following binary explosive to meat combinations: (200 g at 20 cm, 350 g at 22.5 cm, 275 g at 20 cm, and 350 g at 20 cm).  The commercial tank, 1060 L capacity was used.  The 350 g treatment at 20 cm from the sample face produced the greatest improvement in tenderness over the broiler breasts (6.0 vs. 4.3 kg) of all the treatments in the study when compared to the controls.  The traditionally aged, control breasts had a mean shear value of 3.1 kg.  It was concluded from this study that this process was effective in tenderizing early deboned broiler breasts, but it was not able to tenderize the broiler breasts as much as postmortem aging.

### 2.1.2 Hydrostatic Pressure

*Tenderness*

No research to date has been reported on the effects of hydrodynamic pressure on protein functionality. However, extensive research has been conducted on the effects of hydrostatic pressure on tenderness and protein functionality in beef as well as in other species. Hydrostatic pressure treatments are similar to hydrodynamic pressure in that they utilize high pressure to tenderize beef. Hydrostatic pressure differs from hydrodynamic shock waves in that it takes minutes to develop the high pressure while shock waves, in theory take a fraction of a millisecond.

Hydrostatics is the study of characteristics at rest or the force that a liquid imposes on a submerged object (Zobell and Kim, 1972). MacFarlane (1973) and Bouton et al. (1977) pioneered the use of high hydrostatic pressure to tenderize meat either in the prerigor or postrigor state. MacFarlane (1973) pressurized (100 MPa) different prerigor bovine muscles at 25 to 35 C for 2 to 4 min. He reported decreased shear force values from 17.1 and 12.6 kg to 2.2 and 2.5 kg, respectively in the *longissimus* (*LM*) and *Biceps femoris* (*BF*) muscles in comparison of the control and pressure treated samples. MacFarlane (1973) reported that the pressure applied, temperature of pressurization, and duration of pressurization could all affect the response of muscle to pressure. He also reported that pressure conditions used in his study lowered pH of the meat dramatically indicating that glycolysis was virtually complete shortly after the finish of pressurization. Similarly, Kennick et al. (1980) reported that 150 MPa pressure for 2 min at 35 C lowered pH to a typical 24 h pH value of below 5.8 in 1h. This lowering of pH favors proteolysis. Results demonstrated increased shortening of sarcomeres in raw muscle,

decreased cooking loss, increased sensory tenderness, and decreased sensory juiciness (MacFarlane, 1973). This unexpected difference of decreased cooking loss and decreased sensory juiciness could be due to decreased shortening of sarcomeres during cooking, even though pressure caused shortening of sarcomeres in the raw muscle.

Kennick et al. (1980) confirmed earlier research by MacFarlane (1973) that 150 MPa pressure for 2 min at 35 C decreased shear values of prerigor muscle samples that were treated within 1 h postmortem. Shear values were reduced in ovine LD (4.3 kg control vs. 2.6 kg pressure treated) and S*emimembranosus* (SM; 6.3 kg control vs. 2.3 kg pressure treated). Shear values also were reduced in bovine LD (8.4 kg control vs. 3.0 kg pressure treated), ST (6.2 kg control vs. 4.5 kg pressure treated), *Supraspinatus* (SS; 7.1 kg control vs. 4.4 kg pressure treated), and *Sternomandibularis* (STM; 15.0 kg control vs. 5.8 kg pressure treated). In contrast, MacFarlane et al. (1981) reported that pressure treatments of 150 MPa (3 h at 0 C) did not change shear values of postrigor (SM and LD) muscle or increased them, according to whether the muscle was in the stretched or contracted state. Examination of ultrastructure in this study revealed extensive change in the I-band and M-line region of pressure treated muscle. The authors stated that these results support a theory for contraction that increasing toughness is caused by increasing number of sarcomeres in which thick filaments have been compressed onto the Z-line, removing the I-band as a zone of weakness in the sarcomere. Similarly, Bouton et al. (1977) pressurized (100 MPa) prerigor and postrigor bovine BF and ST muscles for 4 min at 35 C. Pre-rigor pressurized muscle had a mean shear value of 4.7 kg and post-rigor, cold-shortened, pressurized beef had a mean shear value of 12.8 kg. Bouton et al. (1977) concluded that high pressure treatment may be able to overcome toughness of

myofibrillar origin without affecting the toughness caused by connective tissue so that tenderness of postrigor muscle that is already set up would not be affected. Bouton et al. (1980) studied the effect of prior aging treatments on shear properties of pressure-heat treated beef. The authors concluded that since aging of beef prior to the heat-pressure treatment did not provide an additional reduction in shear force, both aging and heat-pressure treatments affect the myofibrillar component of toughness.

Nishiwaki et al. (1996) isolated myofibrils from rabbit muscle. ATPase activity was observed between muscles excised immediately after death and pressurized up to 200 MPa for 5 min at 2 C. The BIMA (Biological Index of Myofibrillar Aging) value of the pressurized myofibrils reached the same level as myofibrils conditioned for seven days. Release of soluble materials from the myofibrils was accelerated by pressurization above 150 MPa. The SDS-PAGE analysis showed that the release of the constituents of thin filaments proceeded prior to the releasing of the constituents of thick filaments. This study demonstrated that high pressure caused accelerated aging in prerigor muscle to a level equivalent to muscle aged 7 days.

These studies confirm that hydrostatic pressure does decrease shear value of prerigor pressurized muscle but is not as effective in tenderizing postrigor pressurized muscle. Research demonstrates that prerigor pressure treatments accelerate the aging process of muscle. The ultrastructure, certain myofibrillar components, and mechanism of tenderization will be discussed to attempt to determine why hydrostatic pressure is effective in tenderizing prerigor muscle through rapid aging.

### Myofibrillar Proteins and Connective Tissue

MacFarlane et al. (1986) reported that heat-pressure treatment (150 MPa at 60 C) broke down the myofibrillar protein, connectin, using a densitometer to quantify SDS gels. SM was excised within 0.5 h of slaughter, then immersed in water for 1 day to cold-shorten and enter rigor before pressure treatment was applied. Shear values reported in this study inferred that no decrease in tenderness was evident as a result of increased breakdown of connectin. This study rejects the theory that connectin is a major determinant of myofibrillar toughness. Pepstatin was injected in each sample to prevent the breakdown of connectin to compare to controls where connectin was broken down. Slight reduction in myosin was reported, but the authors stated that it could be due to the loss of myosin's ability to bind coomassie blue at 60 C. In contrast to this research, Kim et al. (1992) reported that the conversion of $\alpha$-connectin to $\beta$-connectin during conditioning has some influence on meat tenderization. In this study, rabbit skeletal muscle excised immediately after death was pressure treated (100 to 300 MPa) for 10 min at a temperature of 0 to 2 C. SDS-page and densitometer scans were used to determine changes in connectin. Though, this study revealed conversion of $\alpha$-connectin to $\beta$-connectin was induced by pressuriztion alone, it was not determined if the degradation of connectin caused by pressure was the same as the spontaneous breakdown of connectin causing tenderness during aging. MacFarlane et al. (1987) studied the effects of 150 MPa (1, 4 and 24 h) pressure on structural proteins at 30 C and 60 C from SM excised 1 h postmortem. At 24 h, densitometer scans of SDS gels reflected a presumed enzymatic breakdown of myosin.

Pressure treatment at 60 C rapidly destroyed the myofibrillar component of toughness, but reduced the component of connective tissue very slowly, if at all. Macfarlane et al. (1987) stated that because of this observation, this treatment method can be used to estimate the influence of both myofibrillar proteins and connective tissue on the toughness of meat.  Also, these authors stated that the 145,000 MW components appearing in densitometer scans of pressure treated samples (30 C) is not likely to be the mechanism involved in pressure-tenderization. This statement is made since samples treated at 60 C (1 h) essentially eliminated the myofibrillar influence on toughness but did not result in as great of an apparent yield of 145,000 MW components as achieved in 30 C pressure treatments.

Suzuki et al. (1993) suggested that pressure (100, 150, 100, and 300 MPa) for 5 min at 2 C had no significant effect on connective tissue. So, the pressure-induced tenderization of meat could be caused only by the improvement of actomyosin (myofibrillar) toughness attributed to the myofibrillar proteins.  Similarly, Dufour et al. (1996) reported that due to gelatin formation of collagen, it is unlikely that high pressure could be used to increase specific proteolysis of collagen.  Bouton et al. (1978) subjected beef ST samples to 150 MPa at 60 C for 30 min.  Shear, adhesion, isometric tension, and transition temperature were all determined.  These determinations showed that pressure stabilized connective tissue to the effects of heat.  These conclusions substantiate that the pressure-heat treatment is not likely to have a tenderizing effect, greater than that of heat alone on connective tissue.

Beilken et al. (1990) applied high pressure during heat treatment of selected beef muscles (SM and ST) and measured its effect on Warner-Bratzler shear force.  Both

contracted and stretched muscles were studied. Pressure treatment at 150 MPa during heat treatment prevented the development of the myofibrillar component of toughness. Pressure treatments at temperatures ranging from 40 C to 80 C had little or no effect on the connective tissue component of toughness other than to raise the temperature at which heat treatment alone produced a decrease in this component. Connective tissue contribution was reduced as cooking temperatures were raised into the region of 50 to 60 C, but was not evident in contracted muscle because of the large myofibrillar contribution. Research results indicated that no final conclusions have been made to what myofibrillar structural components in prerigor pressurized meat are responsible for tenderness. Results do suggest that myofibrillar toughness was overcome in the application of hydrostatic pressure, but not connective tissue toughness.

*Ultrastructure*

MacFarlane and Morton (1978) studied the effects of pressure treatment on the ultrastructure of pre- and postrigor ovine s*emimembranosus* muscles. The muscles were subjected to a pressure of 150 MPa for 15 min (3 C). Pressure treated postrigor samples were missing the M-band in the central region of the A-band inferring that proteins in the M-line were very susceptible to disaggregation under high pressure. Loss of integrity and aggregation of I-band filaments also occurred which presumably involved an F-G transformation of actin. Extensive structural disruption with contraction band formation was noted in prerigor pressure treated muscle. The authors suggested that a weakening of thin filaments and M-line bridges, when combined with a pressure-induced contraction, facilitates disruption of prerigor pressure-treated muscle.

Macfarlane et al. (1982) studied pressure induced length changes in muscles. Pressure (150 MPa) was applied for 20 min at varying temperatures to ovine SM. Prerigor muscles lengthened as pressure was applied but shortened when pressure was released. Postrigor muscles lengthened with application of pressure. These results infer that conditions which prevail in prerigor muscle are not as favorable for disaggregation of the myofilaments as those in postrigor muscle. These structural changes cause tenderness in prerigor muscle, but the mechanism that causes the sarcomere contraction and the myofibrillar structural changes is unknown.

*Mechanism of Tenderization*

Elgaism and Kennick (1982) studied the effect of 100 MPa pressure (2 min at 37 C) on meat microstructure. Disappearance of glycogen granules, appearance of swollen mitochondria, sarcoplasmic reticulum, and ruptured mitochondria were observed. The authors stated that these structural changes in mitochondria and sarcoplasmic reticulum should furnish the $Ca^{2+}$ which causes pressure-induced contractions in prerigor pressurized muscle. This infers that postrigor muscle would not contract upon pressurization since $Ca^{2+}$ is already depleted from the sarcoplasmic reticulum.

Horgan and Kupyers (1983) reported that pressure of 150 MPa (0 C and 35 C) for 2 min caused an increase in the activity of phosphorylase phosphatase and phosphorylase kinase causing rapid glycolysis. Horgan (1980) reported that the major effect of high pressure on sarcoplasmic reticulum was the loss of extra ATPase activity, essentially speeding up the aging process. Similarly, Horgan (1979) reported on ATPase activity of sarcoplasmic reticulum (SR) isolated from rabbit and bovine muscles subjected to prerigor pressure treatment. He isolated SR from prerigor muscle pressurized (150 MPa)

for 0 to 10 min at 35 C.  No calcium-activated ATPase activity was apparent in the isolated SR.  The author theorized that the destruction of the calcium pump ATPase was instrumental in causing the high rates of glycolysis in muscles after pressurization. Koohmarie et al. (1984) researched the possibility of the Calcium active factor (CAF) being the mechanism in which prerigor pressurized (PRP) muscle was tenderized. Evidence was presented on the weakening of the Z lines by PRP treatment which was the main action of the CAF.  However, other evidence suggesting the involvement of CAF, such as the 30,000 dalton component cannot be seen in PRP muscles.  The author stated from these conclusions that it is possible that CAF may not be the mechanism through which PRP enhances meat tenderness.

Kurth (1986) reported that high pressure appeared to protect Cathepsin B1 against heat inactivation.  Maximum activity was observed for 60 C and 150 MPa and was 12 times that observed at 60 C at atmospheric pressure.  These results suggest that an increase in the activity of Cathepsin B1 may account, at least in part, for the tenderization of meat by pressure-heat treatments.  This is interesting since the optimum pH for cathepsins is below 5.2 (Faustman, 1994).  Similarly, Homma et al. (1995) reported that high pressure (100, 200, and 300 MPa) for 5 min at 2 C on rabbit skeletal muscle increased the activity of calpains in muscle contributing to muscle tenderization. Homma et al. (1994) demonstrated that activity of cathepsin B, D, and L increased up to 400 MPa at 2 C for 5 min, then tended to decrease at 500 MPa.  The conclusions from this research were that this pressure induced an increase in the amount of protease activity in the muscle that was due to the release of the cathepsins from the lysosomes.  It can be concluded from the results of this section that the increased release of calcium and

increased activity of cathepsins caused rapid glycolysis involved in tenderizing prerigor muscle.  However, the exact mechanism remains unknown**.**

*Solubilization of Myofibrillar Proteins*

In the processing of further processed products, MacFarlane (1974) reported that pressure (100 MPa) increases solubilization of myofibrillar proteins in the presence of salt.  This increase in solubility improved binding strength of meat particles.  This was reflected through decreased cooking loss of the meat suspensions in the study.  In these studies, meat homogenates in saline solution were produced so that the aqueous phase of the homogenate was 0.5 M NaCl.  Pressure was applied to the homogenates and as pressure increased, myofibrillar protein solubility increased.  Similarly, MacFarlane and McKenzie (1976) used 150 MPa to solubilize myofibrillar proteins in saline solutions. Solubilization of proteins increased with time for up to 15 min.  Thereafter, improvements were negligible.  SDS-Page electrophoresis indicated that all proteins of the myofibril were solubilized except for myosin.  At this point, it is known that high pressure solubilizes myofibrillar proteins (MacFarlane 1973), but its effects on myosin need to be determined since this major protein is responsible for functionality of a comminuted meat product.

*Myosin*

Suzuki and MacFarlane (1984) studied the heat setting characteristics of myosin by pressure treatment.  These researchers prepared myosin in a 0.03 M NaCl solution that was pressure treated (150 MPa) for 10 min at 0 C.  Depolymerization of myosin occurred under pressure, such that myosin filaments accompanied by a conformational change of the monomer reaggregates in a different manner upon release of pressure than it was

before application of pressure. It was noted that this could occur because SH groups reactivity increased under high pressure. Nagashima et al. (1993) studied the effect of high hydrostatic pressure on the thermal gelation of squid mantle meat. The meat paste was compressed at 400 MPa for 20 min and had twice the breaking strength as the control treatment after heating at 90 C for 30 min. It was determined using SDS-PAGE that this pressure did not denature myosin heavy chains indicating that high pressure treatment before heating was effective in inducing thermal gelation of squid meat. Yamamoto et al. (1993) studied the effects of hydrostatic pressure-induced aggregation of myosin molecules in 0.5 M KCl at pH 6.0. The myosin solution did not form a gel by pressurization alone, though pressure-treated myosin formed a gel upon heating. Pressurization up to 210 MPa did not affect the gel strength or the microstructure of heat-induced gels. They also determined that increasing hydrostatic pressure induces conformational changes in the head portion of myosin molecules and following head to head interactions to form a daisy wheel shaped oligomer. Yamamoto et al. (1990) reported that pressures greater than 200 MPa caused gel formation of myosin filaments in 0.1 M KCL at pH 6. When pressure exceeded 200 MPa, the shaft of myosin filaments looked to be irregular and distorted suggesting that the formation of gel upon pressurization was closely related to the structural changes in the filaments. Structural changes in myosin did take place when pressure was applied that induces gel formation. Effects on myosin are such that improved protein functionality in further processed products could occur when high pressure is applied to meat systems with myosin present.

*Further Processed Meats*

Two types of studies have been conducted to test hydrostatic pressure effects on meat batter used for further processing of meats. The first type was the application of pressure to the meat batter before cooking. The second type was the application of pressure to the meat batter during cooking. Mandava et al. (1995) demonstrated that pressures greater than 200 MPa applied to the meat batter before cooking increased pH indicating denaturation of meat proteins. Pressurization in this study also increased cooking yield and improved texture using pressures of at least 100 MPa. These researchers concluded that high pressure could be used as a processing aid to improve the cooking yield and texture of low-salt, low fat, low phosphate, and high water-added meat products.

Similarly, MacFarlane et al. (1984) prepared patties made from comminuted meat and pressure treated them at 150 MPa at 0 to 3 C. Pressure treatment improved tensile strength of the patties. The magnitude of this increase was dependent on the intensity and duration of pressure treatment, salt concentration in the patty, and the pH value. This increase in tensile strength inferred that high pressure improved the binding of the meat patties. Some similarities were also noted between high-pressure effects and pyrophosphate effects on binding and reduction of cooking loss in comminuted meats, although pressure was more effective at a low pH. Elgaism et al. (1980) reported a decrease in cooking loss of intact beef treated with 150 MPa for 2 min. The improvement of these characteristics was inferred by research reporting that high pressure causes protein denaturation or aggregation which led to increased gel formation (Suzuki and MacFarlane, 1984; Okamoto et al., 1990). In contrast, Carballo et al. (1996)

and Colmenero et al. (1998) studied the effects of pressurization on meat batters while cooking and after cooking respectively. They both concluded from using pressures of 100 to 400 MPa that high pressure negatively affected the binding properties and Texture Profile Analysis parameters of the cooked products compared to their controls. These results differ from those of earlier research that showed improved textural characteristics in high-pressure treated raw batter.

### *Low-fat and High-fat Beef Patties*

Carballo et al. (1997) applied high hydrostatic pressure to low-fat beef patties to determine if that application would make their texture more like that of high-fat beef patties. The researchers stated that as fat content of beef patties was reduced while maintaining a constant level of protein content, the products tended to be harder and have less fat and water holding capacity. They reported that the effect of high pressures will not render the texture of low-fat patties any more like that of high-fat patties.

### *Emulsification Capacity*

Elgaism et al. (1982) studied the effect of prerigor pressurization on the emulsifying capacity of muscle protein. Application of 100 MPa for 2 min was applied to ovine *longissimus* muscle. Some differences in the emulsifying capacity between the control and pressure treated samples were noted, but the magnitude of the differences seems too small to be of practical importance.

### *Color and Myoglobin Effects*

Hydrostatic pressure effects on color were reported in Carlez et al. (1995) and Cheftel and Culioli (1997). Carlez et al. (1995) studied the effect of high pressure processing on color and myoglobin in minced beef. Meat was pressurized (200 to 500

MPa) for 10 min at 10 C.  It was determined that L* color values increased significantly

in the range of 200-350 MPa, while a* values decreased at 400-500 Mpa.  A whitening

effect occurred in the range of 200-350 MPa, due to globin denaturation and/or to heme

displacement or release, and oxidation of ferrous myoglobin to ferric metmyoglobin, at or

above 400 MPa.  Similarly, Cheftel and Culioli (1997) reported that pressures greater

than 250 MPa denatured myoglobin, decreased redness of raw beef, and affected cooked

color.  Due to myoglobin denaturation, cured color would be affected since the globin is

involved in the binding of nitric oxide to the heme ring.  These results are important

because they show that cooked and cured color could be affected due to denaturation of

myoglobin in both fresh and further processed products.


### 2.1.3 Protein Functionality in Meat

*Protein Properties*

Fukawaza et al. (1961) established that proteins are largely responsible for the

functionality characteristics of muscle foods.  According to Xiong and Kenney (1999),

protein functionality is any inherent or process-generated property of proteins that affects

physical and sensory characteristics of raw and finished products.  Functionality of meat

proteins has been shown to determine the properties of further processed products,

including comminuted products (Fukawaza et al., 1961a; Acton et al., 1983; Ashgar et

al., 1985; and Gordon and Barbut, 1992).  In comminuted meat products, the ability of

minced muscle to form a three-dimensional gel matrix, emulsify fat, and retain natural

and added water are some of the most important functional properties (Xiong and

Kenney, 1999).  Also, these authors stated that these properties influence product texture,

integrity, physical stability, cooking yield, appearance, and hence palatability and consumer acceptability. Similarly, Samejima et al. (1985) reported that water-holding and binding properties are the important factors that determine the quality of comminuted meat products. Schmidt (1987) defined binding strength as the force per unit cross-sectional area required to pull apart bound pieces of meat. It includes a measure of both the cohesive force exerted between the binding matrix and the meat pieces and the strength of the binding matrix itself. Ashgar et al. (1985) stated that the general consensus for the mechanism of gelation is that polypeptide chains cross-link to form five to six crystalline regions per molecule during gelation. Other molecules can move in between these links or strands, and they account for the flexibility of the gel.

Proteins within the muscle are generally classified into three groups: myofibrillar, sarcoplasmic, and stromal (Acton et al., 1983). Myofibrillar proteins constitute between 50 to 55 % of the total protein content, while the sarcoplasmic proteins account for approximately 30 to 34 % of the total protein. Gordon and Barbut (1992) conducted a study that indicated that the gel forming ability of the myofibrillar proteins was a major factor in stabilizing the fat in a comminuted product. Rust (1987) reported that myofibrillar proteins serve two functions in comminuted products: (1) to encapsulate or emulsify fat and (2) to bind water. Sufficient myofibrillar proteins are necessary in the comminuted product so that both of its functions are served. If all of the protein is used in emulsification, the water binding of the final product is low (Rust, 1987). Myosin in prerigor muscle and actomyosin in postrigor muscle are the principal myofibrillar proteins and are important in protein functionality (Acton et al., 1983). MacFarlane et al. (1977) expands this definition to myosin or actomyosin being the most important

myofibrillar protein responsible for water holding capacity and binding of meat pieces. Myoglobin, a sarcoplasmic protein, is functionally responsible for the color of fresh and cured meats (Acton et al., 1983).

### *Myosin and Actomyosin*

Fukawaza et al. (1961a and 1961b) studied the effects of myofibrillar proteins on binding in sausage. These researchers were the first to demonstrate that myofibrils without myosin had less binding strength than myofibrils with myosin. These were the first results that deemed myosin as the most important protein responsible for binding strength in further processed products. MacFarlane et al. (1977) compared myosin, actomyosin, and sarcoplasmic proteins as binding agents in meat products. It was determined that the binding strength of myosin was superior to that of actomyosin in salt solutions up to 1 M. The binding strength of sarcoplasmic proteins was too low to be measured. These results inferred that prerigor muscle has more protein functionality in the production of further processed products than postrigor muscle. Gordon and Barbut (1992) stated that myosin appears to act as an emulsifier even in its native state and formed a film of defined viscoelastic and mechanical properties at the oil-water interface. Yasui et al. (1980) studied the heat-induced gelation of myosin in the presence of actin. It was determined that a specific myosin to actin ratio was essential in developing a stronger gel than formed by myosin alone. The maximum strength was observed at a myosin:actin ratio (filamentous) of 2.7 which corresponds to the weight ratio of myosin to actin of 15.

**2.1.4 Factors Affecting Protein Functionality**

Gillett at al. (1977) studied the parameters affecting meat protein extraction effects on meat emulsion formation.  Increases in NaCl concentrations and mixing time were responsible for more salt soluble proteins.  These authors also concluded that salt soluble protein from fresh, uncooked frozen meat sources was highly correlated with emulsifying ability.  Also, freezing lowered the emulsifying ability of the meat.  The maximum protein extraction signifying the greatest emulsifying ability occurred at 7.2 C. Xiong and Blanchard (1993) studied the effects on viscoelastic properties of gels when polysaccharide gums (xanthan gum and alginates) are combined with salt soluble proteins (SSP) from chicken breasts.  Both xanthan gum and alginates hindered the gelation of SSP.  Using 0.6 M NaCl, pH around 6.0, a typical ionic environment for meat processing, sodium alginate and xanthan gum at 0.5 to 2.0 % decreased gel  strength (gel rupture force).  These gums had no effect on water binding.

*Salt*

Salt is the most common nonmeat adjunct added to further processed products.  It contributes flavor, preserves the product, and solubilizes myofibrillar proteins (Rust, 1987).  The ability of salt to solubilize the myofibrillar proteins is of vital importance to the successful manufacture of further processed products (Rust, 1987).  Salt solubilizes myofibrillar proteins by increasing the electrostatic repulsion between the filaments, and it alleviates some of the structural constraints of myofibrillar proteins.  Barbut and Findley (1989) tested the effects of using different salts in the stabilization of meat batters.  Results suggested that $Mg^{2+}$ ions destabilized the batter mainly by causing extensive precooking protein matrix aggregation and poor fat stabilization because of

insufficient protein film formation.  In this study, calcium ions destabilized batters by causing widespread protein aggregation during cooking, which led to extensive fat and water losses.  However, the use of NaCl and KCl to form stable meat batters was successful because they are monovalent cations.  KCl is not readily used in further processed products because it causes an astringent taste in the product (Claus et al., 1994).

*Muscle Fiber Type*

Xiong and Brekke (1991) determined that fast twitch (legs) and slow twitch (breast) myofibrils in chicken muscle are affected differently by rigor state, pH, and heating properties.  Muscle fiber type affected protein extractability and gelation properties of myofibrils.  Postrigor breast myofibrils demonstrated greater protein extractability and gel strength than prerigor breast myofibrils, but the reverse was found for leg myofibrils.  Optimum pH for gelation of chicken breast and leg myofibrils were 6.0 and 5.5, respectively.  In comparison, a pH value of 6.0 is considered optimal for gelation in pork and beef species (Yasui et al., 1980).  Heating at 1 C/min produced a stronger gel with chicken breast myofibrils than isothermal heating at 70 C (Xiong and Brekke, 1991).  The reverse was true in leg myofibrils.  It was concluded that muscle rigor state had more effect on protein extractability and gel strength for breast myofibrils than leg myofibrils.  Similarly, using rabbit skeletal muscle, Boyer et al. (1996) studied differences in heat-induced gelation of myofibrillar proteins and myosin from fast and slow-twitch rabbit muscles.  Proteins from slow-twitch muscle exhibited higher thermostability and lower gel strength than proteins from fast twitch muscle.  Slow twitch

myosin's gelling ability decreased in the absence of actin, but fast twitch myosin's gelling ability increased in its presence.

Samejima et al. (1992) studied effects of the postmortem aging period on the extractability of myofibrillar proteins from pork cardiac and rabbit skeletal muscles. Results indicated that pork cardiac myofibrils always exhibited lower solubility than those from rabbit skeletal muscles under identical conditions of pH, ionic strength, and temperature. Under these same conditions, cardiac myofibrils formed much weaker heat-induced gels than those produced by skeletal myofibrils. Myofibrils from 0 and 7 days postmortem muscles formed more rigid gels than those isolated from 3 days postmortem for both cardiac and skeletal muscles. Differences in cardiac and skeletal muscle gel formation is due to muscle fiber type. Cardiac muscle consists of red muscle fibers with poor functionality, and has only two forms of myosin to encapsulate fat (Samejima et al. 1992). Skeletal muscle has three variations of myosin (Samejima et al. 1992)

*pH*

Schmidt (1987) stated that protein extractability increases with pH elevation from 5.5 to 6.0, with 6.0 being the optimum (Yasui et al., 1980; Ishioroshi et al., 1979). Schmidt (1987) also stated that the addition of 2.5 % NaCl used to extract protein decreased pH by 0.1 to 0.2 units. Use of phosphates in a meat batter increases pH to enhance protein extractability and to offset the decrease in pH caused by NaCl (Schmidt, 1987). Samejima et al. (1985) conducted a study to predict binding in comminuted meat products by characterizing myofibrils (beef) properties with respect to gel formation and protein extractability. Ionic strength of the solution up to 0.6 M NaCl increased gel

strength and the addition of pyrophosphate in 0.3 M and 0.6 M NaCL ionic strength batters increased protein extractability and gel strength myosin composition.

*Collagen*

Kenney et al. (1992) reported on the effects of connective tissue and gelatin on the properties of low-salt, low-fat, restructured beef.  Raw and preheated connective tissue were useful for increasing tensile strength when added as 10 % of the formulation.  When used as 5 % of the formulation, only raw connective tissue was effective for increasing tensile strength.  Comparatively, Jones (1984) illustrated that the addition of collagen can improve yields and increase brittleness of a batter-type product, but a large amount of collagen can reduce stability, causing product defects such as fatting out and gel pockets.  In comparison, Samejima et al. (1969) reported that the addition of stromal proteins in the form of collagen reduced the gel strength of myofibrillar protein formulations.

*PSE/ Rigor State*

Pietrzak et al. (1997) studied the effects of a rapid rigor mortis process on protein functionality in turkey breasts.  These rapid rigor mortis processes produced turkey breasts similar to pale, soft, exudative (PSE) pork.  The PSE turkey breasts characterized by lower muscle ATP and higher lactate level compared with normal turkey breast.  Also, water holding capacity and cooking yields were lower in the PSE group as compared to the controls.  Less myosin could be solubilized from PSE than normal myofibril samples indicating that irreversible myosin insolubility due to low pH and high-temperature conditions causes the development of PSE turkey breast.  These conditions also caused poor protein functionality in the production of further processed products.

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# Chapter 3
# Hydrodynamic Shock Wave Effects on Protein Functionality

## 3.1 Abstract

The objective of this study was to determine the effects of Hydrodynamic shock waves on meat proteins responsible for protein functionality.  Frankfurters (cooked to 71 C) were evaluated for cooking yield, CIE L*a*b*, nitrosylhemochrome, Texture Profile Analysis (hardness, cohesiveness), and stress and strain (torsion testing).  Compared to the control samples, the Hydrodynamic shock waves (HSW) did not affect ($P>0.05$) myofibrillar or sarcoplasmic protein solubility, cooking yield, or color.  Textural properties and gel strength of the frankfurters were not affected ($P>0.05$) by the HSW. These results suggest that beef trim obtained from HSW processed meat can be used interchangeably with normal meat trim in the production of further processed meats since the functionality of meat protein is not affected significantly by the HSW process.

## 3.2 Introduction

The meat industry has been challenged to attain acceptable beef tenderness with decreased aging time.  Aging time increases storage, refrigeration, and labor costs (Meek, 1997).  The hydrodynamic shock wave (HSW) treatment (Hydrodyne Process redesigned by John B. Long, U.S. patent #5,273,766 and #5,328,403) has been developed to instantaneously tenderize meat.  The benefit of this process is that it can give top-quality tenderness to lower grade cuts when treated in a plastic garbage can (statement by Solomon and Berry; reported in Raloff, 1998).  This process utilizes an explosive to create a shock wave in either a plastic garbage can or a specially designed enclosed tank filled with water.  The shock wave passes through the water and objects that are a

mechanical impedance match to the water (Kolsky, 1980).  Since meat is made up of approximately 75% water, the shock wave also passes through muscle and ruptures selected cellular components.  Sarcomeres are ruptured at the Z-line and A-band/I-band juncture (Zuckerman and Solomon, 1998).

Research on this tenderization process has demonstrated an increase in tenderness and a decrease in required aging time (Zuckerman and Solomon, 1998; Solomon et al., 1997a).  However, no research has been reported concerning the effects of HSW on the functionality of meat protein destined for sausage manufacture, restructured meats, and ground beef.

Hydrostatic pressure effects on protein functionality have been well documented.  Results are similar to the HSW treatment in that pressure is utilized for tenderization (Kennick et al., 1980).  Hydrostatics is the study of characteristics of liquids at rest or the force that a liquid imposes on a submerged object (Zobell and Kim, 1972).  Mandava et al. (1995) found that hydrostatic pressure increased cooking yield and improved texture of low-salt and low-phosphate frankfurters.  High hydrostatic pressure over an extended period of time (5 to 30 min) increased tenderness as well as improved protein functionality characteristics and myofibrillar solubilization (MacFarlane and McKenzie, 1976).  Also, hydrostatic pressure improved the binding of comminuted meat and increases tensile strength (MacFarlane et al., 1984).  One major limitation of hydrostatic pressure is that the process has not yet been designed so that it can function in a time efficient manner.

The first objective of this research was to determine the effects of the HSW process on cooking loss, cured color, cooked color, and texture of frankfurters

manufactured from HSW processed beef. The second objective was to determine if this shock wave process affects protein solubility, individual proteins responsible for binding ability, and cleavage of peptide bonds.

## 3.3 Materials and Methods

### 3.3.1 Bovine Raw Materials

Bovine *Biceps femoris* (BF) muscles (N=6, from 3 animals) were purchased from a meat packing plant (Excel Corp.,Dodge City, KS). The muscle specifications were such that the muscle came from A maturity, USDA Select, Yield Grade 3 beef carcasses, aged 2 to 3 days, and a specified weight range of 4.5 to 6.0 kg for each muscle.

The proximal and distal end sections of the raw material were trimmed off (5.0 to 8.0 cm). The muscle was cut into four equal sections. Sections were stored (1 to 2 months) frozen (- 15 C) and thawed (4 C for 36 h) prior to treatment. Three sections were exposed to the hydrodynamic shock wave treatment with varying explosive levels at Hydrodyne Inc., in Buena Vista, Virginia. The other section served as a control. Location of sections was chosen in a random manner using a random numbers table so that all areas of the muscle were processed by each treatment. The sections were vacuum packaged in polyolefin resin bags (Product code B2650, Cryovac Division of Sealed Air Corp., Duncan, SC).

### 3.3.2 Hydrodynamic Shock Wave Process

The bagged samples were placed on the bottom, center of the hemishell tank (Model Beef hemishell-Unit 1, Hydrodyne Inc., San Juan, Puerto Rico) filled with water (10 C) . Explosive (nitromethane and ammonium nitrate) amounts of 105, 200, and 305 g (H1, H2, and H3, respectively) were placed in the center of the hemishell, 26.7 cm

above and from the center of the samples.  The tank was covered with a vented muffler, and the explosive was detonated.  Explosive amounts placed at this distance would theoretically (Pressure curves, developed by Hydrodyne, Inc.) create hydrodynamic shock waves with pressure fronts of 83, 104, and 124 MPa (12,000, 15,000, and 18,000 psi, respectively) that would pass through the water and beef.

### 3.3.3 Internal Reference Steaks

Internal reference steaks (IRS), 2.54 cm thick were obtained from U.S. Select bovine *Biceps femoris* (BF), aged 2 to 3 days, with a specified weight range of 4.5 to 6.0 kg for each muscle.  IRS were packaged in the same vacuum bag (Product code B2650, Cryovac Division of Sealed Air Corp., Duncan, SC) along with each section of *BF* treated by the HSW used for the protein functionality determination.  The steaks were included to verify that the HSW process was functioning based on reported steak tenderization effects.  These steaks were obtained from two *BF* muscles coming from one animal different than those used to study protein functionality.  Starting from the proximal side of the muscle, nine consecutive pairs of IRS and control steaks were removed.  The HSW processed IRS and control steaks were cooked (AMSA, 1995), to an internal temperature of 71 C using a convection oven (Model Mark V, Blodgett Inc., Richmond, VA).  Six to eight cores (1.27 cm diameter) were removed parallel to the muscle fiber orientation from each steak after cooling to room temperature.  Shear force was determined using a Warner-Bratzler shear device mounted on an Instron universal testing machine (Model 1011, Instron Corp., Canton, MA) with a crosshead speed of 200 mm/min.  Mean values were calculated from six to eight cores removed from each steak.

### 3.3.4 pH and Fat Analysis

The pH of each *Biceps femoris* muscle was taken in triplicate to determine variation among muscles. Muscles were selected with a pH range of normal beef (5.3 to 5.8; Faustman, 1994) to minimize effects on protein functionality. The pH was determined by removing three 10-g samples from three similar anatomical locations on each of the *Biceps femoris* muscles and homogenizing in a stomacher (Model S10-400, Tekmar Company, Cincinatti, OH ) for 1 min in 100 mL of distilled deionized water. The pH was measured for the individual samples with a calibrated pH meter (Model AR25, Fisher Scientific, Pittsburgh, PA) and a pH electrode (Model 13-620-298, Fisher Scientific, Pittsburgh, PA).

Percentage fat (39.1.06, AOAC, 1995) was measured in triplicate for each *Biceps femoris* muscle using Foss-Let Fat Analyzer (Model 15310 Foss-Let, A/S N. Foss Electric, Denmark). Muscles were selected with fat percentages lower than 5 % to minimize the effect of fat content on protein functionality.

### 3.3.5 Frankfurter Processing

BF samples were ground (Model 4532, Hobart, Troy, OH) through a 1.25 cm plate. The ground beef (0.7 ±0.15 kg) was placed in a vacuum mixer (Model 4294, Stephan vertical-cutter/mixer, Stephan Machinery Corp., Columbus, OH) along with sodium chloride (2% meat weight basis, MWB), sodium tripolyphosphate (0.5% MWB), sodium nitrite (156 ppm), erythorbate (0.042 % MWB), sucrose (2.0 % finished weight basis, FWB), and water (25 % FWB). The pressure was set at 20 to 25 mm Hg and the raw materials (1.1 ±0.2 kg) were chopped for 3 min. Amount of products used as well as feasibility of the formulation was initially developed using Least Cost Formulator

software (Version IBMPC Basic 18.0, Least Cost Formulations Ltd., Inc., Virginia Beach, VA.).  Each replication of frankfurters was processed using this initial formulation.

The meat batter was filled into a cookie cutter (Marcato cookie cutter, Ampia Biscuits, Italy) and then stuffed into 32 mm fibrous casings (Rapid Peel Nojax, Viskase Co., Chicago, IL) and tied into 20 cm links.  Some frankfurters were inserted with thermocouples (Model 5100 datalogger, Electronic Controls Design, Inc. Milwaukee, OR) to monitor temperature during processing in the smokehouse.  The frankfurters were heat-processed to an internal temperature of 71 C.  The smokehouse schedule was: 15 min for dry bulb 60 C and wet bulb 49 C, 10 min for dry bulb 68 C and wet bulb 57 C, 10 min for dry bulb 74 C and wet bulb 63 C, 10 min for dry bulb 79 C and wet bulb 71 C, and finished at dry bulb 82 C and wet bulb 74 C until the internal temperature was achieved (about 4 min).  The frankfurters were immediately showered for 10 min, and then stored in covered plastic lugs at 4 C for 16 h prior to cooking loss determinations.

**3.3.6 Cooking Loss**

All of the frankfurters without thermocouples were used in determining cooking loss.  Cooking loss was reported as the percentage difference between the frankfurters prior to heat processing and the frankfurters chilled after processing.

**3.3.7 Torsion Testing**

A gelometer (Model 5xHBDVI+, Gel Consultants Inc., Raleigh, NC) was used to measure shear stress and shear strain of further processed products.  Frankfurters were stored (4 C) horizontally in plastic lugs for 2 days.  The frankfurters were then cut perpendicular to their length into 30 mm pieces using an adjustable plexiglass-cutting

device.  Styrene disks were glued with cyanoacrylate to each end of the 30 mm long

sausage sample  so that the grooves on each mounting disk were in line with each other.

The specimen milling machine (Model KCI-24A2, Bodine Elec. Co., Raleigh, NC) was

adjusted so that the sample was milled into a capstan-shaped object with an inside

diameter of 10 mm (Figure 1).

### 3.3.8 Texture Profile Analysis

Texture Profile Analysis (Bourne, 1978) was performed using an Instron

universal testing machine (Model 1011, Instron Corp., Canton, MA) to determine total

energy, hardness, springiness, cohesiveness, and chewiness.  Texture analyses were

performed on chilled (4 C) samples stored horizontally in plastic lugs for 1 day post-

processing.  Frankfurters were cut perpendicular to their length into 19 mm pieces and

were axially compressed to a height of 4.75 mm (75% compression) to determine total

energy and hardness.  Pieces were compressed twice to 50% to determine springiness

(b2, base width mm of second compression), cohesiveness (TE2/TE1 x 100, total energy

of first compression over the second), and chewiness (50% compression peak force x

springiness x cohesiveness/100).  The Instron was programmed for a load range of 50 kg

(100% of  50 kg compression load cell) with a crosshead speed of 100 mm/min and a

chart speed of 200 mm/min.

### 3.3.9 Instrumental Color Determinations

Cooked color of the frankfurters was evaluated by determining CIE L*a*b*

values with a chroma meter (Model CR-200, Minolta Camera Co., Ltd., Osaka, Japan)

and reflectance values in the visible range (400 to700 nm) using a spectrophotometer

(Model 2101PC Spectrophotometer, Shimadzu Inc., Kyoto, Japan).  Each device was

calibrated using a white plate (Model 20933026, Minolta Camera Co., Ltd., Osaka, Japan; CIE L* 97.91, a* -0.70, b* +2.44). The spectrophotometer was configured for a sampling interval of 1.0 nm, slit width of 2.0 nm, and fast scan speed. From the reflectance data, cured pigment (nitrosylhemochrome) was determined using the reflectance (R) ratio of %R at 650 nm divided by %R at 570 nm where a lower value indicated less pigment (Erdman and Watts, 1957).

### 3.3.10 Protein Solubility, Gel electrophoresis, and Collagen Determinations

Protein solubility was determined for both myofibrillar and sarcoplasmic proteins using a procedure adapted from Green et al. (1997). Differences were that the samples were ground, then frozen, and thawed for 30 min (4 C) before protein solubility was determined. Samples in Green (1997) were frozen intact, thawed out for 2 h (4 C), and ground before the procedure was performed. Determinations were performed in triplicate for each control and HSW treatment. Samples were removed from the raw muscle after treatment. Myofibrillar and sarcoplasmic protein extracts used from the protein solubility study were subjected to SDS-PAGE as described by Ashgar et al. (1986). Collagen concentration was quantified by determining hydroxyproline content (Bergman and Loxley, 1963) in the muscle using a procedure described by Hill (1966). Determinations were performed in triplicate for each control and HSW treatment. Samples were removed from the raw muscle after treatment.

### 3.3.11 Statistical Analysis

The experimental design was analyzed as a Randomized Complete Block Design with six replications of muscles and four treatments. Statistical analysis was performed using General Linear Model procedures (SAS, 1996) to determine significance (P<0.05)

of independent variable effects (HSW).  One sided t-tests were used to determine

differences in shear value (P<0.05) among internal reference steaks.

## 3.4 Results and Discussion

### 3.4.1 Muscle Variation

To control uniformity, samples from raw bovine *Biceps femoris (BF)* muscle were

specified to meet selection criteria for pH (5.3-5.7) and fat content (<5 %).  The

pH (5.4±0.04) and fat percentage (3.1±0.9) met those standards.  According to Faustman

(1994), 5.4 is a normal pH value for bovine *BF*.  The variation in pH of the beef was

controlled because pH has been reported to affect protein solubility (Hultin et al., 1997).

As pH decreases from 6.0 to 5.0, bovine myofibrillar protein solubility decreases (Yasui

et al., 1980), which can affect other functional properties of the meat proteins.  Fat

percentage was low for all muscles used resulting in small variation in fat among

muscles.  As fat percentage increases in a further processed product, protein percentage

decreases which inhibits its ability to encapsulate fat and bind water (Rust, 1987).

### 3.4.2 Internal Reference Steaks

In general, the hydrodynamic shock waves decreased shear values of bovine *BF*

steaks by 20 % (Figure 2).  These results confirm that the process was functioning similar

to published reports on beef tenderization effects.  Solomon et al. (1997b) reported a 19

to 30 % improvement in shear force for bovine *BF* muscles, and a 53 % improvement in

*BF* muscles that were cold shortened when cooked on an open-hearth Farberware broiler.

*BF* muscles are normally reported to have a lower reduction in shear values by HSW than

*Longissimus* muscles as well as other muscles with lower amounts of connective tissue.

All treatments in this study had shear values of less than 4.6 kg.  Shear values lower than

4.6 kg are considered acceptable in tenderness to consumers for *Longissimus dorsi* cooked on a Farberware broiler (Shackleford et al., 1991). A higher percentage reduction in shear values may have occurred if the control samples were inherently less tender. Solomon, quoted in Raloff (1998), reported that shear force values of 6, 8, 10, and 12 kg have the potential to be reduced to 3 to 4 kg shear force with HSW. His research differs from this study because he incorporated plastic containers with steel plates placed on the bottom. This research was performed in a steel hemishell-shaped tank.

H1 and H3 reduced ($P < 0.05$) shear values of *BF* steaks compared to control steaks. H2 did not reduce ($P > 0.05$) shear values. This difference compared to those of H1 and H3 could have been due to small sample sizes (n=6) and slight variations in connective tissue among steaks. *BF* muscles are known to be high in collagen and the collagen is not uniformly distributed (McCormick, 1994). Solomon quoted in (1998) stated that typically 100 grams of explosive, placed strategically was needed to tenderize meat in a plastic garbage can container. Nevertheless, greater reduction in shear force may not necessarily correlate with higher explosive amount since our results indicated H1 tenderized meat more effectively than H2. Solomon et al. (1997a) also reported that decreasing the distance of explosive to the meat reduced shear force more than increasing explosive amount. There were no differences ($P > 0.05$) in cooking loss among internal reference steaks and control steaks.

**3.4.3 Collagen Determinations**

Collagen content was not affected ($P > 0.05$) by the HSW process (Table 1). Connective tissue is composed of collagen which is highly cross-linked and not uniformly distributed in *BF* muscle (McCormick, 1994). High cross-linking may have

inhibited the shock wave treatment from disrupting the structure of collagen. If the collagen had not been uniformly distributed in the samples (MsCormick, 1994), protein functionality would have been affected since connective tissue amount affects protein solubility (Hultin et al., 1997). Lack of shock wave affect on collagen agrees with reports by Solomon in Raloff (1998), stating that the HSW does not affect connective tissue amount. Collagen amount was determined to assure that any differences in myofibrillar or sarcoplasmic protein functionality were due to the effect of the hydrodynamic shock wave treatment on these proteins and not differences in collagen content.

### 3.4.4 Protein Solubility

There were no differences (P>0.05) in myofibrillar and sarcoplasmic protein solubility among control and hydrodynamic shock wave treated bovine *Biceps femoris* muscle (Table 1). Myofibrillar proteins are responsible for the formation of further processed products including water holding capacity and textural properties (Fukawaza et al., 1961; Acton et al., 1983, Ashgar et al., 1985; and Gordon and Barbut; 1992). Solubility of sarcoplasmic proteins provides information about cooked and cured color because muscle pigment proteins, including myoglobin (Bandman 1987) are included in this class of proteins. Our results infer that no denaturation of myoglobin occurred. Cheftel and Culioli (1997) reported that pressures of 200 to 350 MPa for 2 to 5 min after the target pressure was reached were required to cause meat discoloration due to denaturation of myoglobin. These results substantiate data from our study. Our pressure level was less than 200 MPa and the detonation of the explosive occurred in much less time than 2 to 5 min (U.S. patent 5,273,766). Hydrodynamic shock waves have been shown to cause physical disruptions in the regions adjacent to the Z-lines and within the

A-bands actin/myosin interaction using wave pressure of approximately 60 to 70 Mpa (Zuckerman and Solomon, 1998) in a plastic container. These results combined with ours suggest that the degree of structural degradation of myofibrillar proteins caused by hydrodynamic shock waves was not sufficient to affect myofibrillar protein functionality.

MacFarlane et al. (1974) and MacFarlane and McKenzie (1976) reported that high pressure promotes the disaggregation or solubilization of myofibrillar proteins, which is important in the production of further processed products. They also reported that the solubilization of myofibrillar proteins was influenced by duration of pressurization and salt concentrations.

### 3.4.5 Gel Electrophoresis

Gel electrophoresis results support protein solubility data since no differences were determined among control and hydrodynamic shock wave treated bovine *Biceps femoris* muscles (Figure 3 and Figure 4). No proteolysis (breakdown of protein) of myosin or actin was visually apparent on the myofibrillar gels and no proteolysis of myoglobin was determined visually on the sarcoplasmic gels as a result of the hydrodynamic shock wave treatments compared to the control. In contrast, O'Rourke et al. (1998, 1999) reported slight proteolysis of porcine and bovine myofibrils using the hydrodynamic shock wave treatment. Differences between those results and our research could be due to different species and muscles studied. Pork loin muscle was used in their first study and bovine *semitendinosus* muscle was used in their second study. Also variations could be due to differences in the tanks used. Their first study was conducted in a plastic container (208 L capacity) with a steel plate placed on the bottom center of that container, and their second experiment was conducted in a 1060 L commercial tank.

### 3.4.6 Cooking Loss

There were no differences (P>0.05) in cooking loss among frankfurters processed from control and hydrodynamic shock wave treated Bovine *Biceps femoris* muscle (Table 2). Lack of differences in myofibrillar protein solubility, collagen concentrations, and gel electrophoresis substantiate these results. With higher myofibrillar protein solubility, less cooking loss is expected due to a higher water binding capacity. High collagen concentration can result in a higher cooking loss due to the low water holding capacity of native collagen. These results suggest that no loss in the industry due to yield of further processed products made from the trim of muscle treated with hydrodynamic shock waves would occur compared to untreated muscle.

### 3.4.7 Cooked Color

Cooked color of the frankfurters was not affected (P>0.05) by treating bovine *BF* with the HSW (Table 2). CIE a* values indicating redness of products is related to myoglobin in the beef. Denaturation of myoglobin prior to production of further processed products would affect cooked color since this is a sarcoplasmic protein responsible for meat pigmentation (Bandman, 1987). No difference observed in cooked color supports the sarcoplasmic protein solubility results. Cheftel and Culioli (1997) reported that pressures greater than 250 MPa caused loss of red color in fresh meat due to myoglobin denaturation.

### 3.4.8 Cured Color

There were no differences (P>0.05) in cured color pigment(nitrosylhemochrome) of frankfurters among control and hydrodynamic shock wave treated *Biceps femoris* muscle (Table 2). Denaturation of myoglobin can cause differences in cured color since

this protein is involved in the binding of nitric oxide to the heme ring responsible for cured color formation (Fox, 1987). No proteolysis of sarcoplasmic proteins in gel electrophoresis and no differences in sarcoplasmic protein solubility further substantiate the cured color results.

### 3.4.9 Texture Analysis

The hydrodynamic shock wave process did not affect (P>0.05) product texture of frankfurters (Table 3). In comparison, Mandava et al. (1994) demonstrated that high hydrostatic pressure applied to sausage batters improved the textural characteristics in low-salt, low phosphate, and high water added frankfurters that were exposed to 150 to 200 MPa for 5 min at 10 C. The reported key factors for texture improvements were pressure level, temperature, and time (Mandava et al., 1994).

Though no differences (P>0.05) were detected in gelometer torsion testing, potential trends suggest that the control sample tended to be slightly more brittle and tougher than hydrodynamic shock wave treated samples (Figure 5). In a comparison of the shear values of internal reference steaks (IRS) with torsion testing values, shear stress of the frankfurters was less as shear values of IRS decreased. Interestingly, shear stress of H2 tended to be higher than H1 and H3 which was similar to the WBS results of the IRS.

## 3.5 Conclusions

Meat protein characteristics were not affected by the hydrodynamic shock wave treatment. Trim removed from hydrodynamic shock wave tenderized beef (Hydrodyne process) can be used interchangeably with normal beef trim in the production of further processed products.

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Table 1- Collagen and protein solubility means[a] from a finely chopped meat made from hydrodynamic shock wave treated and control bovine *Biceps femoris* muscles.

| Treatment[b] | Collagen[c] | | | Protein solubility[d] | | |
|---|---|---|---|---|---|---|
| | SOLUBLE (mg/g) | INSOLUBLE (mg/g) | Percent Soluble | MYO (mg/g) | SARC (mg/g) | TOTAL (mg/g) |
| Control | 0.97 | 8.68 | 10.1 | 182.3 | 53.7 | 235.9 |
| H1 | 0.92 | 7.84 | 10.5 | 183.2 | 52.7 | 235.8 |
| H2 | 0.95 | 8.22 | 10.4 | 180.6 | 52.4 | 233.0 |
| H3 | 1.02 | 8.58 | 10.6 | 179.2 | 53.1 | 232.3 |
| S.E. | 0.04 | 0.38 | 0.14 | 2.59 | 0.73 | 2.76 |

[a] Means within a column are not different (P>0.05)

[b] Explosive amounts of 105, 200, and 305 grams (Treatments H1, H2, H3, respectively) were placed in the center of the hemishell, 26.7 cm above the meat. These explosive amounts placed at this distance would theoretically (Pressure tables, Hydrodyne Inc.) create shock waves with pressure fronts of 83, 104, and 124 MPa (12,000, 15,000, and 18,000 psi) that would pass through the water and beef

[c] Collagen: SOLUBLE: amount of soluble collagen per gram of meat
    INSOLUBLE: amount of insoluble collagen per gram of meat
    Percent Soluble: (soluble collagen/total collagen) X 100

[d] Protein Solubility: Protein solubility was determined using a procedure adapted from Green (1997).
    MYO = soluble myofibrillar proteins
    SARC = soluble sarcoplasmic proteins
    TOTAL = soluble myofibrillar and sarcoplasmic proteins

Table 2-Means[a] for quality attributes of frankfurters made from hydrodynamic shock wave treated and control bovine *Biceps femoris* muscles.

| Treatment[b] | Cooking Loss[c] (%) | Cooked Color CIE values[d] | | | Nitrosylhemochrome[e] |
| --- | --- | --- | --- | --- | --- |
| | | L* | a* | b* | |
| Control | 5.1 | 58.9 | 20.1 | 8.4 | 3.0 |
| H1 | 5.3 | 57.7 | 20.6 | 8.3 | 3.1 |
| H2 | 4.9 | 58.4 | 20.2 | 8.3 | 3.0 |
| H3 | 4.7 | 57.3 | 20.0 | 8.2 | 2.9 |
| S.E. | 0.41 | 0.14 | 0.12 | 0.05 | 0.015 |

[a] Means within a column are not different ($P > 0.05$)
[b] Explosive amounts of 105, 200, and 305 grams (Treatments H1, H2, H3, respectively) were placed in the center of the hemishell, 26.7 cm above the meat. These explosive amounts placed at this distance would theoretically (Pressure tables, Hydrodyne Inc.) create shock waves with pressure fronts of 83, 104, and 124 MPa (12,000, 15,000, and 18,000 psi) that would pass through the water and beef.
[c] The percentage difference based on weight of the frankfurters prior to heat processing and chilled after heat processing.
[d] Lightness, redness, and yellowness of cooked frankfurter was measured using CIE L*, a*, b*, respectively.
[e] Nitrosylhemochrome was determined using the reflectance ratio of %650nm/%570 nm where a lower value indicated less pigment (AMSA, 1991).

Table 3- Texture Analysis value means[a] for frankfurters made from hydrodynamic shock wave treated and control bovine *Biceps femoris* muscles.

| | Texture Profile Analysis[c] | | | | | | Gelometer[d] | |
| | 50 % compression | | | | 75% compression | | | |
| Treatment[b] | Spring (mm) | Cohes | Chewi (kg*mm) | Peak force (kg) | Total Energy (kg*mm) | Hard- ness (kg) | Shear stress (kPa) | Shear strain |
|---|---|---|---|---|---|---|---|---|
| Control | 8.67 | 56.4 | 85.6 | 17.1 | 196.1 | 38.4 | 50.1 | 1.42 |
| H1 | 8.68 | 58.4 | 84.4 | 16.1 | 181.6 | 35.1 | 46.4 | 1.40 |
| H2 | 8.71 | 52.9 | 85.8 | 18.0 | 183.2 | 36.0 | 48.0 | 1.36 |
| H3 | 8.73 | 55.3 | 83.1 | 17.4 | 159.6 | 31.0 | 45.8 | 1.38 |
| S.E. | 0.025 | 0.56 | 1.67 | 0.30 | 3.00 | 0.63 | 3.96 | 0.072 |

[a] Means within a column are not different (P>0.05)

[b] Explosive amounts of 105, 200, and 305 grams (Treatments H1, H2, H3, respectively) were placed in the center of the hemishell, 26.7 cm above the meat.  These explosive amounts placed at this distance would theoretically (Pressure tables, Hydrodyne Inc.) create shock waves with pressure fronts of 83, 104, and 124 MPa (12,000, 15,000, and 18,000 psi) that would pass through the water and beef.

[c] Texture Profile Analysis:

    Spring = Springiness, base width (mm) of second compression

    Cohes = Cohesiveness = (total energy of second compression divided by total energy of first compression) x 100

    Chewi = Chewiness = (hardness (at 50% compression, peak force) x springiness x cohesiveness) divided by 100

    Peak Force = The peak force during the the first compression cycle (50 % compression)

    Total Energy= The total energy of the positive force area during the first compression

    Hardness = The peak force during the first compression cycle (75 % compression)

[d] Gelometer: Shear stress and shear strain were determined using a torsion tester manufactured by Gel Consultants, Inc. Raleigh, North Carolina

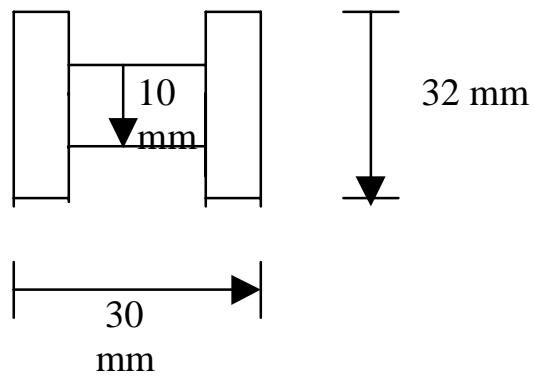Fig. 1- Dimensions and shape of sample tested in the gelometer.
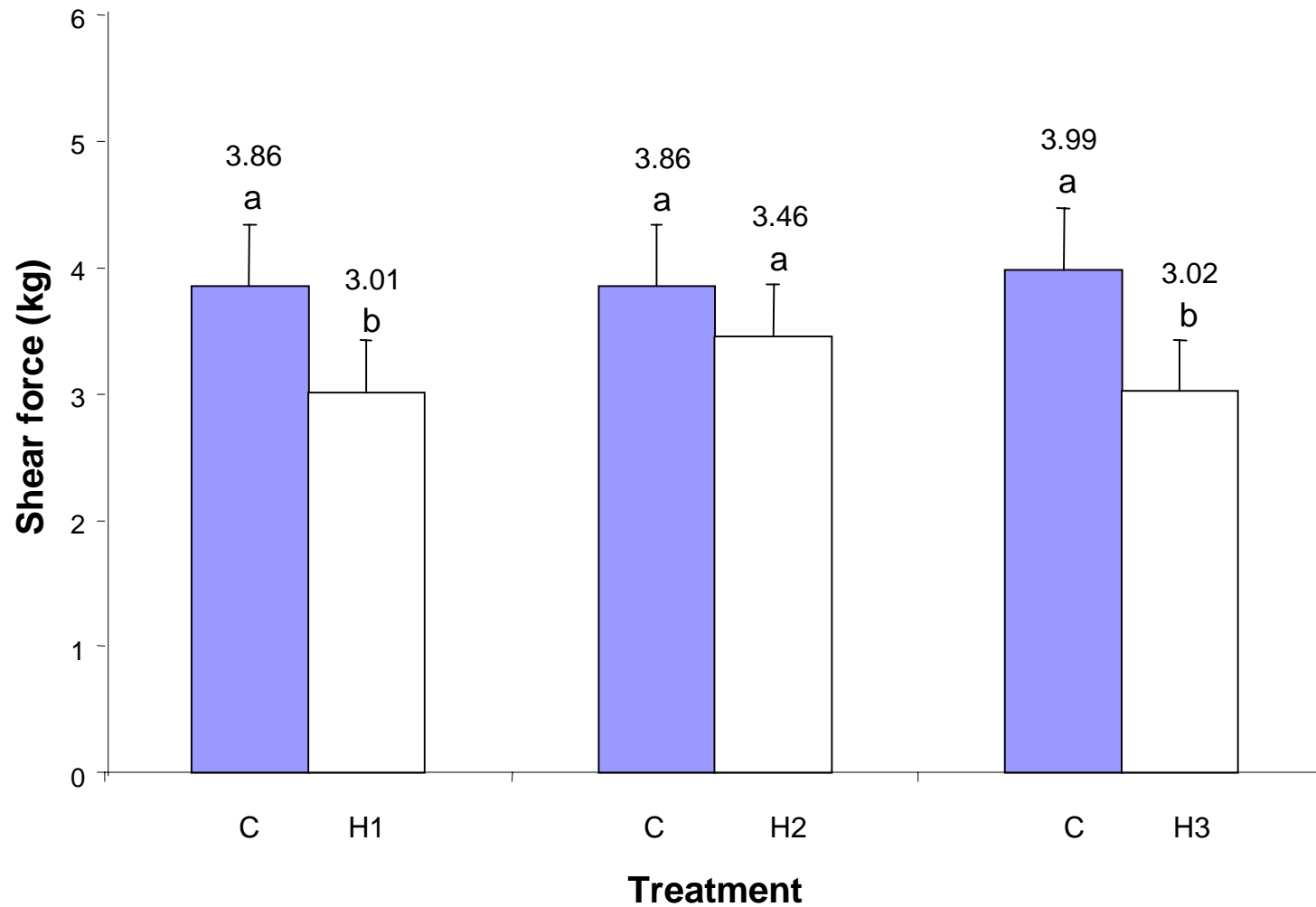
Figure 2 - Effects of hydrodynamic shock waves on the shear force of 2.54 cm thick steaks treated by detonating 105 grams (H1), 200 grams (H2), and 305 grams (H3) of explosive versus controls. Bar means within a pair with unlike letters are different (P<0.05). Standard error lines project above bars.

Figures 3 and 4 (gfigures 495 kb)

**Figure 5: Texture Map for Torsion tester adopted from Lanier (1986) and Hamann (1987). Samples are frankfurters made from bovine *Biceps femoris* muscle treated with the HSW process at 100 grams (C), 105 grams (H1), 200 grams (H2), and 305 grams (H3) of binary explosive.**

**APPENDIX**

# Determination of residual, soluble, and total collagen
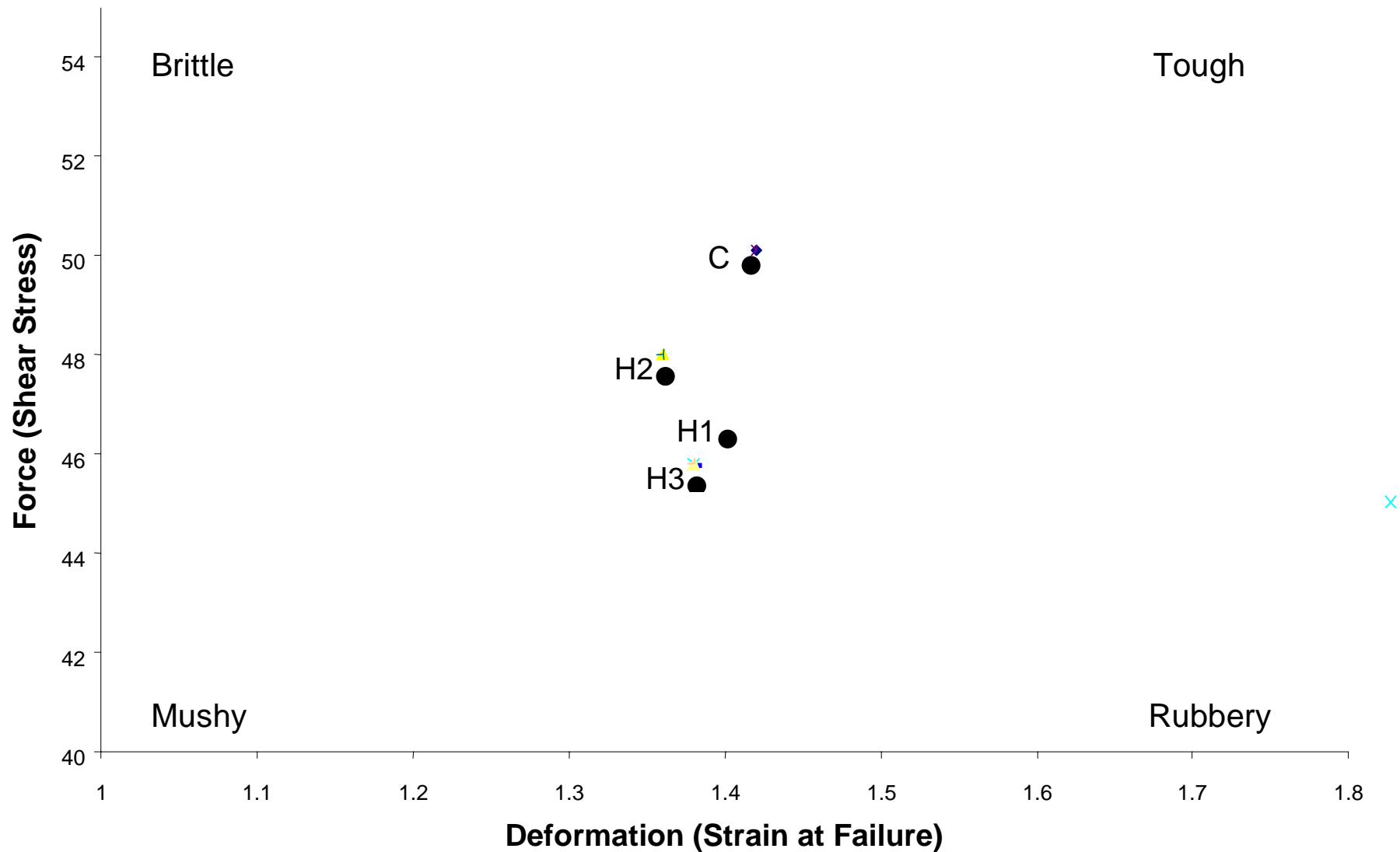
Modified by M.W. Schilling 1999

The following procedure is designed to carry out collagen analysis on four samples done in triplicate for beef out of the *Biceps femoris* muscle. The procedure is divided into three segments and needs either 2 or 3 days to perform.

### DAY ONE

1. Collect the following equipment and chemicals.
   a) 12 centrifuge tubes (50 mL), tubes that will fit into the refrigerated centrifuge (Model RC-5B, Sorvall, Newton, CT) into lab using rotor FAS 21C
   b) 12 glass rods
   c) 24 screw top test tubes (50 mL) with 24 teflon coated screw tops
   d) 12 N HCL (fully concentrated HCL)
   e) ¼ Strength Ringer's solution (400 mL)

**Stock Ringer's Solution:** 7.0 g NaCl, 0.026 g $CACl_2$, 0.35 g KCL brought to 1000 mL with dd $H_2O$

**¼ Strength Ringer's Solution:** Prepare ¼ Strength Ringer's Solution by mixing one part of Ringer's Stock Solution with 3 parts dd $H_2O$

2. Heat the water bath to 77 C.

3. Set the centrifuge temperature at 2 C.

4. Weigh out 0.9-1.1 g freeze-dried, ground samples (use mortar and pestle to grind) into 50 mL centrifuge tube. Sample weight will be based on a sample that weighed 4 g before freeze drying. If a sample was 75% moisture, weigh out 1.0 g of freeze dried sample since it would have been 4.0 g before freeze drying.

5. Pipette out 12 mL of ¼ strength Ringer's solution into each centrifuge tube. Suspend a glass rod in each tube and stir each sample to mix. Leave the stirring rods in the tubes.

6. Place the tubes in the waterbath at 77 C. Heat for 70 min, stirring at 10, 20, 30, 40, 50, 60, and 70 min intervals.

7. Remove from waterbath and allow to cool for 30 min at room temperature. Remove the stirring rods, **rinsing each tube with the same amount of Ringer's solution to make it easy to balance out the centrifuge**.

8. Balance the tubes with Ringer's solution and place them in the pre-cooled centrifuge (rotor FAS-21C). Centrifuge for 10 min at 2 C and 6000G (about 7200 rpm for FAS21C rotor).

9. Remove tubes from centrifuge and decant (pour) the supernatant liquid in 50 mL screw top test tubes with teflon coated screw tops.

10. Add 4 mL of ¼ strength Ringer's solution to the residue and stir. Add a second 4 mL aliquot rinsing each of the glass rods, balance and centrifuge again for 10 min.

11. Decant the supernatants into the screw top tubes as in step 9. Save the residuals.

12. Add 8 mL of dd $H_2O$ to the residue, stir with the glass rod and transfer to 50 mL screw top test tubes with teflon coated caps. Rinse the centrifuge with 10 mL 12N HCL and pour into respective test tubes. Rinse and add with HCL under the hood with it on. Do not smell the HCL to avoid illness.

13. Add 16 mL of 12N HCL to each supernatant tube.

14. Screw caps on the 12 residual tubes and 12 supernatant tubes. Screw the caps on tightly and then loosen them a half turn. Autoclave overnight at 138 KPa pressure and 121 C. Place a sign on the autoclave with your name and number to be called if the autoclave is not running properly and so that no one will turn the autoclave off.

# DAY 2

15. Allow slow exhaust of pressure in the autoclave to prevent sample loss due to overflowing. Allow this until the temperature is below 60 C in the autoclave. Remove the tubes and cool them to room temperature in the hood.

16. While the test tubes are cooling collect the following equipment and chemicals.
    a) Alkaline decolorizing carbon
    b) 12 vacuum Erlenmeyer flasks (250 mL). Do supernatant or residual first, clean flasks after second day procedure by rinsing good with hot water and then rinsing well with distilled water. Let the flasks air dry. Then do the procedure for the supernatant or residual depending on which one you have already completed.
    c) 2 vacuum filters (5.5 cm)
    d) rubber tubing and vacuum
    e) 50 mL burette
    f) 12 funnels
    g) methyl red indicator solution
    h) 12 graduated cylinders (250 mL). Clean between supernatant and residual filtering in accordance with directions in b).
    i) 5 N NaOH
    j) 24 screw cap test tubes (20 mL)

k) 48 filter papers (whatman # 1 size 5.5 cm diameter and 12.5 cm diameter-24 each)
l) pH meter calibrated at pHs of 4 and 7

**Preparation of Methyl Red Indicator: Dissolve 0.02 g methyl red granules in 100 g of 95 % methanol (85 mL methanol and 5 mL deionized water).**

17. Add 0.5 g decolorizing carbon to each supernatant and swirl to mix.

18. Add 1.0 g decolorizing carbon to each residual and swirl to mix.

19. Filter samples through 5.5 cm diameter filter paper in a vacuum filter. Rinse distilled water through the filter paper twice. Also rinse distilled water through the vacuum filter and clean with a kimwipe between uses.

20. Add seven drops of methyl red indicator, mix, and titrate with 5 N NaOH to a yellow end point. At this point a gel-like precipitate appears. Measure pH and adjust pH to between 6.0 and 7.0 using 4 N HCL.

21. Filter the contents of the Erlenmeyer into a 250 mL graduated cylinder using Whatman filter paper # 1, 12.5 cm diameter.

22. Dilute the supernatants to 150 mL and filtrates from residual samples to 1000 mL. Put parafilm over container and invert to mix. The residuals were diluted to 500 mL but a few samples fell outside of the standard curve. Dilution to 1000 mL is recommended.

23. Save about 20 mL of filtrate from each cylinder into 20 mL screw cap test tubes. Properly label the samples and screw the caps on. Samples are now ready for hydroxyproline analysis and can be stored in the refrigerator for up to four days.

### THIRD DAY

24. Collect the following equipment/chemicals/reagents
    a) Stock oxidant solutions A and B
    b) Stock Erlich's Reagent
    c) Isopropanol
    d) Stock Hydroxyproline solution
    e) Pipettes (1 mL and 5 mL)
    f) Fisher Vortex Genie 2
    g) Screw cap test tubes (10 or 20 ml)
    h) Stir plates and stir bars
    i) 2.5 L bottle to pour waste in. Do not pour Erlich's reagent, oxidant solution, and samples treated with those chemicals down the drain. Pour them in a designated collagen procedure waste bottle.

**Preparation of Hydroxyproline Solution:**
Dissolve 0.1 g of solid hydroxyproline in 1000 mL of 0.001N HCL and store in the refrigerator.  Discard after one month.

**Preparation of standard hydroxyproline solutions:**
Prepare 7 working standards: 0,2,4,6,8,10, and12 µg/ml. For example, 2 mL stock hyddroxyproline solution brought to 100 mL volume makes 2 µg/mL standard solution. These standards should be refrigerated and are good for 1 month.

**Preparation of stock oxidant solution:**

**Solution A.** 3.5 grams Cloramine T dissolved in 50 mL of distilled water.  **Store in refrigerator and Discard after one month.**

**Solution B.** Acetate/Citrate Buffer: 34.4 g sodium acetate anhydrous, 37.5 g trisodium citrate dihydrate, 5.5 g citric acid monohydrate and 385 mL isopropanol.  Dilute to 1000 mL with deionized water.  Check the pH! If it is not close to 6, adjust with acetic acid or 5 N NaOH.  **Store at room temperature and discard after one month.**

**Oxidant Solution for today's Analysis:** Mix 1 volume of solution A with 4 volumes of solution B. Make Fresh daily before use.  Possession of 24 samples and 7 standards, and the need for 1 ml for each sample, necessitates 31 mL for analysis.  Recommendations include 40 mL for easy pipetting as well as  any mistakes.  40 mL is made by mixing 8 mL of solution A with 32 mL of solution B.

**Preparation of Stock Erlich's Reagent:**
**Solution A:** Dissolve 10 g of p-dimethylaminobenzaldehyde (DABA) in 12 mL of 70 % perchloric acid.  Use mask while weighing DABA and mix the reagent in the perchloric acid hood (room 124).  **Store in refrigerator for up to one month but discard if it turns green.**

**Erlich's Reagent for today's analysis:** Mix 3 volumes of solution A with 13  volumes of isopropanol.  Presence of 24 samples and 7 standards each require 2 mL for a total of 62 mL.  Prepare 80 mL in case of mistakes and for ease in pipetting.  Prepare 80 mL by mixing 15 mL of solution A and 65 mL of isopropanol.

25. Take the samples and standards out of the refrigerator and bring them to room temperature.  Let them sit out for around 30 to 45 min. Turn on the waterbath at 60 C.

26. Add 1 mL of samples, blank (DD water), or standard to each tube.

27. Add 2 mL of isopropanol to each sample and vortex

28. Add 1 mL of oxidant solution, vortex and allow it to stand for 4 min.

29. Add 2 mL of Erlich's reagent and vortex well.  Pipette Erlich's reagent very slowly to prevent acidic solution from retracting into the pipette.

30.  Screw caps to limit evaporation and heat the tubes in the water bath at 60 C for 25 min.

31. Turn the spectrophotometer on to let it warm up.

32. Cool the tubes in running tap water for 4.5 min.  Remove the caps and vortex to mix well.

33. Measure the absorbance at 558 nm against 0 µg/mL blank.  Measure all absorbances within 30 min after heating is done. Check the blank 3 or 4 times during the analysis to make sure the spec is working properly.

34.  Discard all waste materials in a waste container.  Pour unused collagen samples down the drain but do not do so with those treated with isopropanol, Erlich's reagent, and oxidant solution.

## CALCULATIONS

1. From absorbances for standards, prepare a regression of µg/mL on the x-axis and absorbance on the Y-axis.  Use absorbance to obtain µg/mL for each sample from the regression equation provided by a spreadsheet program.

2. Multiply µg/mL by total volume to which the sample was diluted (150 mL for supernatants and 1000 mL for residual).  Divide this value by the grams of sample (4.00) to get µg of hydroxyproline / g sample.

3. Convert hydroxyproline to collagen by multiplying the supernatants by 7.52 and the residuals by 7.25 to µg collagen / g of sample.

4. Divide by 1000 to convert µg collagen/ g to mg collagen / g.  (For *Biceps femoris* muscle, I have seen values reported from 6 to 9 mg collagen/ g  for residuals and 0.7 to 1.0 mg collagen/ g for supernatant).

5. Report as soluble collagen (supernatants), insoluble collagen (residual), total collagen (soluble+insoluble) and % soluble collagen (soluble/total*100).

# Gelometer Procedure

1) Process sausage project in fibrous casings.

2) Cut the sausage longitudinally into **3.0 cm** pieces using adjustable plexiglass cutting device

3) Using superglue, glue styrene disks to each end of the 3.0 cm sausage sample so that the grooves on each disk are in line with each other.

4) Adjust specimen milling machine so that it mills the sample to an inside diameter of **1.0 cm**.

5) Place samples one at a time in the milling machine and mill the cylindrical piece of sausage into a capstan-shaped sample with an inside diameter of 1 cm.
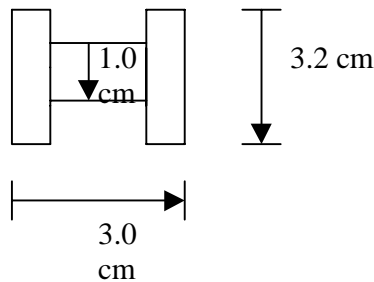


6) Turn on the gelometer and 486 computer. Open up Windows! Go to File Manager and double click on **torsion.exe.**

7) Zero the gelometer on the computer. Using the up and down arrows on the gelometer, set the gelometer for **2.5 rpm. Press** the enter button when the gelometer says remove spindle because the gelometer doesn't use a spindle.

8) Place the sample in the gelometer so that the bottom styrene disk fits in the pins on the gelometer. Before adjusting, verify that the top circle of the gelometer does not touch the sample. Using the switch on the back of the gelometer, rotate the top circle so that the pins that fit in the grooves on the styrene disk are slightly behind the grooves on the styrene disk. This will permit a negative value for force on the gelometer. A negative value is necessary or the gelometer will not test the sample. Adjust the sample upwards into the grooves by turning the adjuster on the bottom side of the gelometer so that the pins on the top circle are fitting into the grooves on the styrene disk. If the force value is negative, preferably $-0.3 \pm 0.2$, click on test sample on the computer screen. Verify that the data is being saved on the disk in the **A drive.** Test as many samples as desired, but always change the file name with a separate sample I.D.

9) The most critical is the shear stress and the shear strain. Shear stress correlates with sensory hardness, and shear strain correlates with sensory cohesiveness.

# Protein Solubility Protocol

## Meat Preparation

Thaw the ground, chopped sample for 30 min, or until it can be cut without loss of fluid.

## Sarcoplasmic Assay

1. In triplicate, place 1 g samples into centrifuge tubes.

2. Add 10 mL of 0.025 M potassium phosphate buffer solution to the samples in the centrifuge tubes (buffer solutions must be kept in an ice bath).

3. Homogenize using Virtishear, speed 40, for three: 4-second burst (performed in an ice bath).

4. Place on a shaker, 150 rpm, for 3 h at 4 C.

5. Centrifuge at 2600 x g for 30 min at 4 C

6. Use the supernatant for protein determination.

7. Use the Biuret protein determination procedures.

## Myofibrillar Assay

1. In triplicate, place 1 g samples into centrifuge tubes.

2. Add 10 mL of 1.1 M KI/ 0.1 M potassium phosphate buffer solution to the samples in the centrifuge tubes (buffer solutions must be kept in an ice bath).

3. Homogenize using Virtishear, speed 40, for three: 4-second burst (performed in an ice bath).

4. Place on a shaker, 150 rpm, for 3 hat 4 C.

5. Centrifuge at 2600 x g for 30 min at 4 C.

6. Use the supernatant for protein determination.

7. Use the Biuret protein determination procedures.

*Procedure for making biuret reagent*

- 1.5 g of $CuSO_4$
- 6.0 g of NaKTartrate
- add 400 mL of 10 % NaOH to 500 mL of $CO_2$ free distilled water
  (prepare DI water under vacuum) - this will make 1 L of Biuret reagent

*Procedure for making buffer solutions*

0.025 M Potassium phosphate buffer

1. Add 4.355 g of Potassium phosphate to Deionized water to make up 1 L of solution.

2. Agitate on stirrer until all of the solid dissolves in solution.

3. Adjust pH to 7.2 $\pm$ 0.05 and increase pH by adding 5N NaOH until desired pH is reached. Decrease pH by adding 5N HCL until desired pH is reached.

1.1M KI/ 0.1 M potassium phosphate buffer

1. Add 17.42 g of potassium phosphate and 182.6 g of Potassium iodide to deionized water to make up 1 L of solution.

2. Agitate on a stirrer until all of the solid dissolves in solution

4. Adjust pH to 7.2 $\pm$ 0.05 and increase pH by adding 5N NaOH until desired pH is reached. Decrease pH by adding 5N HCL until desired pH is reached.

### *Biuret Protein Determination Procedures*

*General Requirements*

1. Perform standard curve

*Sarcoplasmic*

1. Take 1 mL of the sarcoplasmic supernatant and add to 4 ml of biuret.

2. Vortex.

3. Let stand undisturbed for 30 min.

4. Read using spectrophotometer at 550 nm.

5. Save supernatant to be used in SDS-page gel electrophoresis.

*Myofibrillar*

1. Perform a 1:10 dilution, dilution performed by taking a 0.5 mL sample of the supernatant and adding 4.5 mL of the 1.1 MKI/0.1 M potassium phosphate buffer, mixing well.

2. Take a 1 mL aliquot of the diluted sample and add to 4 ml biuret.

3. Vortex.

4. Let stand undisturbed for 30 min.

5. Read using spectrophotometer at 550 nm.

6. Save supernatant to be used in SDS-page gel electrophoresis.

## *Standard Curve Procedure*

1. Use 100 g/L BSA as stock solution.

2. Use .05 M NaCl as buffer solution.

3. Make concentration of stock solution range from 0 to 10 mg/mL.

4. Use the following chart for the standard curve:

| Conc (mg/mL) | Stock BSA (mL) | Buffer Sol. (mL) | Biuret (mL) | Final Volume (mL) |
|---|---|---|---|---|
| 0 | 0.0 | 1.0 | 4.0 | 5.0 |
| 1 | 0.1 | 0.9 | 4.0 | 5.0 |
| 2 | 0.2 | 0.8 | 4.0 | 5.0 |
| 4 | 0.4 | 0.6 | 4.0 | 5.0 |
| 6 | 0.6 | 0.4 | 4.0 | 5.0 |
| 8 | 0.8 | 0.2 | 4.0 | 5.0 |
| 10 | 1.0 | 0 | 4.0 | 5.0 |

5. Vortex tubes.

6. Let stand for 30 min.

7. Read using spectrophotometer at wavelength 550 nm.

8. Perform standard curve in duplicate.

9. After receiving the readings of the standard curve. Use Excel to find the average absorbance for each concentration. Perform a regression analysis on the data. If $R^2$ is not 0.98 or higher, redo the standard curve. Use this data to determine the concentration of sarcoplasmic and myofibrillar protein samples.

# Gel Electrophoresis Protocol

1. Make stock solutions according to pages 15-17 in **Hoefer Mighty Small II User Manual** for SE 250 and SE 260 Mini-Vertical Gel Electrophoresis Units. **Filter the solutions through a Gelman 0.45 $\mu$m filter attached to a 50 mL syringe.**

2. Prepare protein samples by mixing 1 mL of myofibrillar and sarcoplasmic samples from protein solubility with 1 mL of 2X sample treatment buffer, heating each sample for 90 s in water boiling in a beaker stored in a hood with air flowing through it. Store the samples in an ultra low freezer at -80 $C^o$ for up to 6 months.

3. Prepare the dual gel caster according to pages 2-3 in **Hoefer Mighty Small SE 245 Dual Gel Caster User Manual.**

4. Prepare the gel

   a. Using a 250 mL Erlenmeyer flask, prepare 10 % acrylamide separating gel according to the Laemmli gel recipe **Hoefer Mighty Small II User Manual page 18. Deareate the solution before adding the initiator and the catalyst, and add initiator and catalyst just prior to pouring the gel (Temed and ammonium persulfate are the catalyst and initiator).** Pipette gel (using 1000 $\mu$L pipette) between the gel plates to a height of three cm from the top. *Let it solidify for at least one hour.* Put a thin layer of n-butanol on top of the separating gel to protect from oxidation. Wash out n-butanol with

distilled water after solidification.

b. Using a 25 or 50 mL Erlenmeyer flask, prepare 4 % stacking gel according to **Hoefer Mighty Small II User Manual page 18. Make sure you deareate the solution before adding the initiator and the catalyst, and add initiator and catalyst just prior to pouring the gel (Temed and ammonium persulfate are the catalyst and initiator).** Pipette gel (using 1000 µL pipette ) between the plates to the top of the white plate. Place comb with eight 0.75 um teeth between the two plates. It is very important that no bubbles are underneath the teeth of the comb. *Allow the stacking gel to solidify for at least 1 h.*

5.  Transfer the gel sandwich from the dual caster to the buffer chamber

a. Remove the comb from the gel sandwich.

b. Carefully place the gel sandwich into the buffer chamber. Clamp the sample in place and fill the buffer chamber with tank buffer both behind and in front of the gels.

c. Use a cooling system to run cold water (4 C) in between the gels through the middle of the buffer chamber to keep the gels cooled..

6.  Load Samples

a. Determine amount of sample based on how much protein extracted in the protein solubility study using 3.6 µL as the mean value.

b. Use a 10 µL syringe to load samples into each well. Be careful not to puncture the bottom of the well with your syringe needle. Rinse the syringe with tank

buffer between sample loadings.  Keep a record of order of that samples were

placed in wells.  Verify the presence of a reference standard to read your gels against.

c. Verify that the lower and upper buffer chambers are completely filled with tank

buffer.  Place the safety lid on the unit and plug the power supply into the buffer

chamber.  Run the gel at 40 mA, constant current for one hour or until tracking
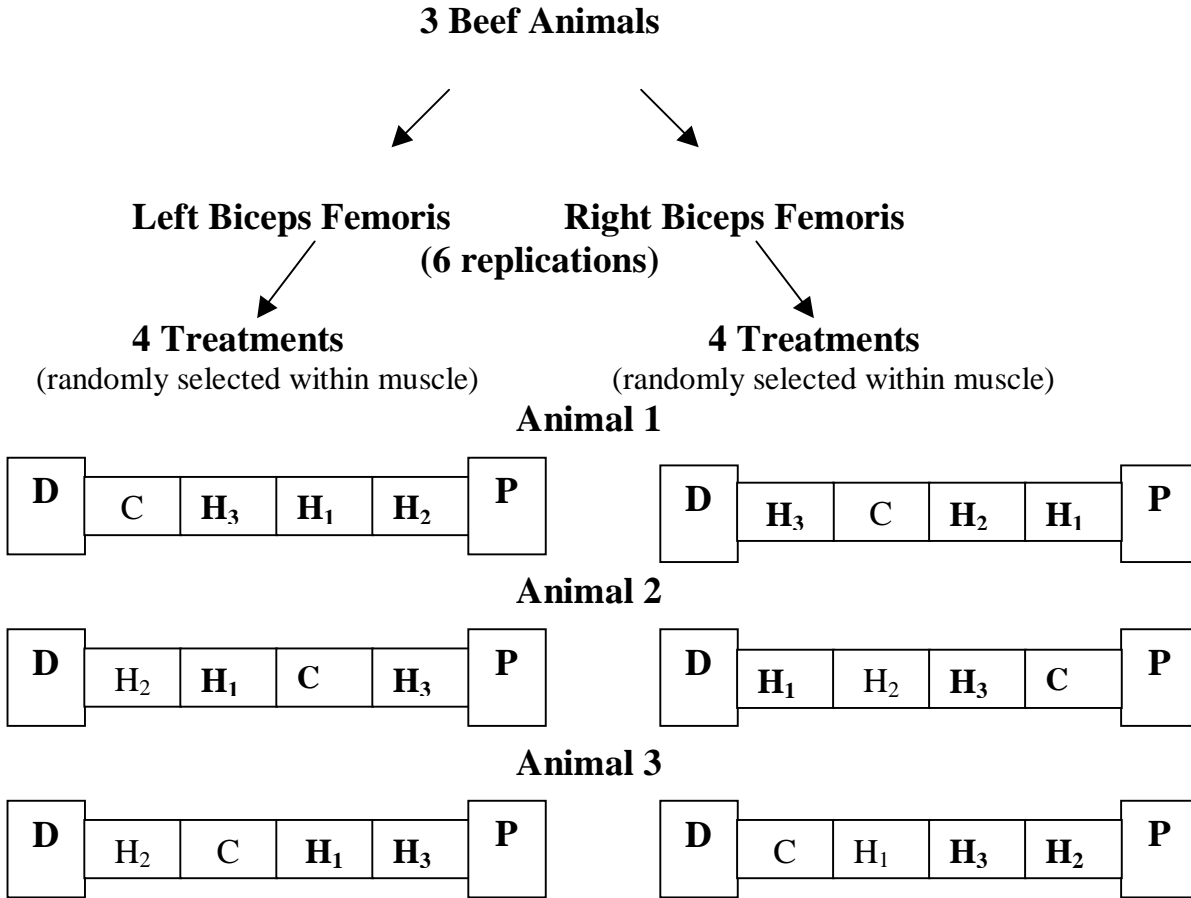
dye runs to the bottom of the gel.


7. Staining the gel:Remove the gel from the gel plates very carefully!  Use distilled water

if necessary.  Place the gel in Coomassie stain solution overnight.


8. Destain the gel

a. Remove sample from Stain solution and place in Destaining Solution 1

(40% methanol, 7 % acetic acid, 1 litre) for 20 min and place on a staining

plate and stir the staining solution with a magnetic stirrer.

b. Remove sample from Destaining Solution 1 and place in Destaining Solution 2

(7 % acetic aci, 5% methanol, 1 liter) for 2 h under the same conditions as Destaining

Solution 1.


9. Documentation of the gel: Place the gel in 1 % glycerol in a white pan.  Take the gel

outside in the sun and take a picture of it.  Special lighting can be used in the lab to take a

picture if weather outside does not permit.

**3 Beef Animals**

**Left Biceps Femoris**   **Right Biceps Femoris**
**(6 replications)**

**4 Treatments**        **4 Treatments**
(randomly selected within muscle)   (randomly selected within muscle)

**Animal 1**

| D | C | $H_3$ | $H_1$ | $H_2$ | P |
|---|---|---|---|---|---|

| D | $H_3$ | C | $H_2$ | $H_1$ | P |
|---|---|---|---|---|---|

**Animal 2**

| D | $H_2$ | $H_1$ | C | $H_3$ | P |
|---|---|---|---|---|---|

| D | $H_1$ | $H_2$ | $H_3$ | C | P |
|---|---|---|---|---|---|

**Animal 3**

| D | $H_2$ | C | $H_1$ | $H_3$ | P |
|---|---|---|---|---|---|

| D | C | $H_1$ | $H_3$ | $H_2$ | P |
|---|---|---|---|---|---|

**P = Proximal end of muscle**

**D = Distal end of muscle**

**C=Control, no hydrodynamic shock wave treatment**

**$H_1$=Hydrodynamic shock wave treatment, with 105 grams explosive 26.7-cm from the sample**

**$H_2$=Hydrodynamic shock wave treatment, with 200 grams explosive 26.7-cm from the sample**

**$H_3$=Hydrodynamic shock wave treatment, with 305 grams explosive 26.7-cm from the sample**

**Fig- 1. Explanation of the experimental design of dependent variables for study on the effect of hydrodynamic shock waves on protein functionality in further processed meat products.**

Table 1- Mean[a] percentage fat and pH values for each raw bovine *Biceps femoris* used as a measure of variation among replications

| Replication | pH[b] | fat[c] (%) |
| --- | --- | --- |
| 1 | 5.32 | 3.8 |
| 2 | 5.37 | 3.6 |
| 3 | 5.32 | 1.5 |
| 4 | 5.34 | 3.9 |
| 5 | 5.39 | 3.1 |
| 6 | 5.41 | 2.9 |

[a] Mean: average of three measurements taken from three similar locations on each bovine *Biceps femoris* muscle.
[b] pH: measurement taken using ten grams of muscle stomached in 100 mL water.
[c] fat%: determined using Foss-Let procedure.

# Vita

Mark Wesley Schilling was born on October 28th, 1975 in Oak Ridge, Tennessee, the son of Robert M. and Carolyn S. Schilling. He graduated from Oak Ridge high school in June, 1993. He later married Jennifer Knowles on May 29th, 1999.

He studied Food Science and Technology at Virginia Polytechnic Institute and State University where he received a Bachelor of Science in May, 1997 and where he continued in graduate studies in August, 1997. He received his Master of Science in Food Science and Technology in the fall of 1999.

He is a member of the American Meat Science Association and the Institute of Food Technologists. In August of 1999, he plans to begin work on a Ph.D. degree where he will conduct research on muscle foods with a heavy application of statistics.