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To the Graduate Council:

I am submitting herewith a thesis written by George G. Wilson entitled "Water-binding capacity of myofibrillar proteins as influenced by sarcoplasmic protein extracts from normal and pale, soft, and exudative pork." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Riette L.J.M. van Laack, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by George G. Wilson III entitled "Water-binding Capacity of Myofibrillar Proteins as Influenced by Sarcoplasmic Protein Extracts from Normal and Pale, Soft, and Exudative Pork." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Riëtte L.J.M. van Laack

We have read the thesis
and recommend its acceptance:

Sharon L. Mattton

Clark J. Buckle

Accepted for the Council:

Lew Minkel

Associate Vice Chancellor and
Dean of the Graduate School

WATER-BINDING CAPACITY OF MYOFIBRILLAR PROTEINS AS INFLUENCED BY SARCOPLASMIC PROTEIN EXTRACTS FROM NORMAL AND PALE, SOFT, AND EXUDATIVE PORK

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

George G. Wilson III
December 1997

Abstract

Water retention is one determining factor in pork quality. The poor water-binding capacity (WBC) of Pale, Soft, and Exudative (PSE) pork has been associated with denaturation of sarcoplasmic and myofibrillar proteins. This study was conducted to determine the relevance of (denaturation of) sarcoplasmic proteins for WBC. PSE and normal myofibrils were combined with either sarcoplasmic extract from either PSE or normal samples. In addition, protein-free extracts from PSE and normal meat were combined with myofibrils from PSE and normal samples. The WBC of myofibrils combined with the sarcoplasmic extract from normal meat was significantly ($P<0.05$) higher than the WBC of myofibrils combined with sarcoplasmic extract from PSE meat. There was no difference between the WBC of myofibrils combined with the protein-free extracts from PSE and normal meat. It was concluded that the denatured sarcoplasmic proteins of PSE meat are not available to have a positive effect on the WBC of myofibrillar proteins. Results of this study provide an explanation for the low WBC of Red, Soft, and Exudative (RSE) pork.

TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
	Statement of the Problem	1
	Objectives of the Study	2
II	Literature Review	3
	Introduction	3
	Muscle Structure	4
	Pork Quality Defined	5
	Water-binding Capacity	8
	Proteins	10
	Ultimate pH and WBC	14
	Ionic Strength	15
	Conclusions	16
III	Materials and Methods	18
	Sample Collection	18
	Quality Evaluation Procedures	19
	Color Analysis	19
	Analysis of pH	20
	Analysis of Water-binding Capacity	20
	Protein Purification and Analysis	21
	Sarcoplasmic Protein Purification Process	21

Myofibrillar Protein Purification Process 22
SDS-PAGE Analysis 22
Moisture Analysis 24
Analysis of Myofibrillar Solubility . . . 25
Sarcoplasmic Extract Influence on WBC
of Myofibrils 26
Sample Pairing and Preparation 26
Myofibrillar and Sarcoplasmic Combination
Scheme 27
Determination of Myofibrillar Pellet WBC . 29
Myofibrillar WBC Influenced by
Protein-Free Sarcoplasmic Extract . . . 29
Sample Pairing and Preparation 29
Protein Removal from the Sarcoplasmic
Extract 30
SDS-PAGE Analysis of Protein-free Extract 30
Analysis of Sarcoplasmic
Protein-Free Extracts 31
Measurement of Conductivity in
Protein-free Extracts 32
Myofibrillar and Sarcoplasmic
Protein-free Extract Combination Scheme 32
Determination of Myofibrillar Pellet WBC . 34

Ionic Strength Influence on
Myofibrillar WBC 35
Statistical Analysis 36
IV Results and Discussion 37
Quality Measurements 37
Physical Characteristics of Samples . . . 37
Protein Measurements 37
SDS-PAGE Analysis of Myofibrillar Proteins 39
Moisture Analysis of Myofibrillar Pellets 42
Water-binding Capacity of Myofibrils 42
Influence of Sarcoplasmic Proteins 42
Influence of Ionic Strength 49
Conclusions 51
References 53
Vita 62

CHAPTER I

INTRODUCTION

1. STATEMENT OF THE PROBLEM

The quality defect known as Pale, Soft, and Exudative (PSE) has a tremendous economic impact, between $38 and 59 million per year, on the pork industry. The occurrence of PSE is associated with a combination of genetic and pre-slaughter environmental factors combined with the rate of muscle temperature and pH decline postmortem. Pre-slaughter stress and high muscle temperature have been associated with PSE. Rapid rates of muscle glycolysis at the time of death, brought on by stress, cause a rapid decrease in pH while the muscle remains at a high temperature. As a consequence, sarcoplasmic and myofibrillar proteins are denatured. Most of the evidence suggests that the myofibrillar proteins are responsible for the WBC of meat. PSE samples exhibit substantially lower myofibrillar protein solubility than samples classified as Red, Firm, and Non-exudative (RFN), also known as normal. This could explain the low water-binding capacity (WBC) of the PSE samples. However, samples classified as Red, Soft, and Exudative (RSE) have a high drip loss approaching that of PSE, whereas protein solubility of RSE samples is similar to that

of RFN samples. This does not support the theory that the myofibrillar proteins are solely responsible for WBC. Furthermore, there is a strong correlation between WBC and sarcoplasmic protein denaturation. Researchers have suggested that the denatured sarcoplasmic proteins are adsorbed onto the myofibrils, resulting in a reduction of WBC. Other researchers have suggested that the denaturation of the sarcoplasmic proteins interferes with the positive interaction with the myofibrillar protein WBC. The question is whether it is the proteins or another component of the sarcoplasmic extract that is interacting with the myofibrils to diminish the WBC. An understanding of the relationship between the sarcoplasmic fraction and myofibrils may help explain the diminished WBC of PSE, as well as of RSE meat.

2. OBJECTIVES OF THE STUDY

This study was designed to determine if the sarcoplasmic extract impacts the WBC of myofibrils. Furthermore, if the sarcoplasmic extract influences the WBC, then it would be determined if this influence was due to the proteins in the sarcoplasmic extract.

Chapter II

LITERATURE REVIEW

1. INTRODUCTION

The emphasis in the pork industry is to economically produce lean meat that is consistent in quality and safe. Pork quality expectations vary for each individual. However, quality can best be defined as a product that is appealing, while on display at the market, and provides a pleasing meal, both in taste and texture.

Pale, soft, and exudative (PSE) pork is a quality problem in the United States pork industry. The occurrence of PSE, occurring in approximately 16% of pork produced in the United States, has a tremendous economic impact on the pork industry; estimated annual losses are between $38 - 59 million (Kauffman, 1996; Meeker and Sonka, 1994). PSE negatively influences the acceptability of pork to consumers at the fresh meat counter, due to the poor color and the unsightly pool of exudate around the cuts. Moreover, when consumers purchase a fresh pork product that has the undesirable traits of PSE, they are likely to experience a dry product that is not tender. In addition, PSE is unacceptable to processors. PSE

meat is less functional than RFN for processed products such as canned hams and frankfurter type sausage products (Kauffman et al., 1992). PSE meat proteins have a limited gel strength, about 45% of that in normal meat, rendering PSE undesirable for processing (Camou and Sebranek, 1991).

The high incidence of PSE in the pork industry has stimulated research into the determinants of water-binding capacity (WBC). To enable the pork industry to achieve a consistent quality product, an understanding of the mechanisms that influence WBC, and ultimately quality differences, is essential.

2. MUSCLE STRUCTURE

The structure of muscle is ideally suited for the retention or holding of water. A muscle is enclosed in a thick sheath of connective tissue, the epimysium, and is divided into bundles of muscle fibers by a connective tissue network, called the perimysium. Each of these bundles of fibers consists of many individual muscle fibers (cells) which are wrapped with a thin layer of connective tissue, called the endomysium. These fibers contain myofibrils. The myofibrils consist of an arrangement of thick and thin filaments, with six thin filaments around each thick filament, and three thick

filaments around each thin filament. The thick filaments are bound together at the "M" line, while the thin filaments are bound together at the "Z" line. The region of a myofibril spanning from one "Z" line to another is called a sarcomere, and is the smallest functional contractile unit of muscle. The thick and thin filaments slide past each other during muscle contraction and relaxation, resulting in a decrease or increase in sarcomere length. The thick and thin filaments do not change in length, only the amount of overlap that exists. In meat, the filaments can no longer slide past each other due to the formation of irreversible cross bridges, created during rigor.

3. PORK QUALITY DEFINED

Quality is a trait that may be defined differently by each consumer. The concept of quality is dependent upon an individual's background and can, and will, change over the years. To the consumer, acceptable pork is a product that looks, smells, and tastes good as well as is affordable. Acceptable meat quality is an ever-changing challenge for the industry.

Kauffman et al. (1990) pointed out that there are several factors that contribute to the definition of quality. They

suggest that quality exists in three levels. The first level is a "survival level", consisting of wholesomeness as it affects safety and image, and nutrition as highlighted by vitamins, proteins, and minerals contained in the product. A second level of quality, the "economy level," adds to the "survival level" of quality based on a minimum of shrinkage during processing and cooking. The final quality level, "the ideal level," adds to the previously mentioned quality characteristics in attractiveness such as color, convenience, and consistency and the best possible juiciness, tenderness, flavor, and texture.

Three primary factors are considered when determining pork quality: color, drip loss, and ultimate pH (pH_u). These three parameters have traditionally been used to classify pork quality into 3 groups: 1) normal, also known as Red, Firm, and Non-exudative (RFN), 2) Pale, Soft, and Exudative (PSE), and 3) DFD (Dark, Firm, and Dry). Recently, additional quality classifications have been discussed which include a class for Red, Soft, and Exudative (RSE) and a class for Pale, Firm, and Non-exudative (PFN).

For decades, it has been recognized that extremely rapid glycolytic rates, with low pH values (5.4 - 5.8) being achieved at high (body) temperatures, are associated with PSE conditions (Bendall and Wismer-Pedersen, 1962; Sayre and

Briskey, 1963). Briskey (1964) and Cassens et al. (1975) have reviewed the causes and the related changes that occur in PSE meat. The characteristics associated with DFD quality, a high pH_u, dark coloration, and high WBC, were discussed by Greaser (1986). The higher WBC is related to the high ultimate pH which is further from the myofibrillar isoelectric point causing increased repulsion and an enlargement in the microstructure for water to remain. The high pH_u is caused by the depletion of muscle glycogen before death, limiting the formation of post-mortem lactic acid. The mechanisms involved with the formation of PFN and RSE are not yet understood.

An objective definition for PSE has come about from subjective assessments of color, amount of drip loss, and a measurement of pH_u. Warner et al. (1997) used the following parameters to define samples: PSE, an $L^* > 50$, 48 h drip loss $> 5.0\%$, and an ultimate pH < 6.0; DFD, an L^* value < 42, drip $< 5\%$, and $pH_u \geq 6.0$; RFN, an L^* value = 42 - 50, drip $< 5\%$, and $pH_u < 6.0$; RSE, an L^* value = 52 - 58, drip $> 5.0\%$, and $pH_u < 6.0$.

4. WATER-BINDING CAPACITY

The ability of meat to hold water is important for several reasons. Water loss has an economic impact. The exudate is unattractive when it accumulates in the package, and soluble nutrients are lost with the exudate (Foegeding et al., 1996).

WBC has been defined as the ability of meat to retain its natural water in spite of the application of force (Hamm, 1960). Drip loss, i.e. the formation of exudate from meat under specific conditions without the application of force (besides gravity), from uncooked meat is a measure of WBC. Drip loss from a carcass is negligible. When broken into primal cuts, drip loss ranges from 0.1 - 1%; and when cut into steaks or fabricated, drip loss increases and may surpass 10% (Offer and Knight, 1988).

Lean meat consists of approximately 75% water, 20% protein and some fat. Much of the water in meat is classified as "free water" and is free to migrate throughout the muscle microstructure. Only about a tenth of the water in meat is bound to the proteins by hydrogen-bonds. The binding of this water to proteins is dominated by group-dipole interactions Wismer-Pedersen (1987). Water is located in three concentric locations around the protein: a primary shell dominated by

active groups on the protein, a secondary hydration shell corresponding to loosely bound water in a thin layer associated with the protein, and a surrounding region of unperturbed water. The remaining 90% of water must be held within the muscle in a different way (van Laack and Solomon, 1994). Myofibrils, having a three dimensional network of filaments, are well suited for holding this water. Offer and Knight (1988) conclude that a reasonable hypothesis for explaining changes in the WBC of meat is that they originate from volume changes in the myofibrils. Myofibril lattice spacing will only change by increasing or decreasing the space between myofibrils next to one another, creating a lateral expansion or shrinkage. The lateral spacing between the myofibrils, and hence the volume of the myofibrillar lattice, can be altered by changes in the ionic strength or by changes in pH. Changes in the volume based on the sarcomere length are unlikely, because the attached cross-bridges would prevent the sliding of the thick and thin filaments (Offer and Knight, 1988).

The limited amount of water bound to the proteins implies that it is unlikely that water loss from meat is related to changes in bound water. Since proteins account for about a fifth of the wet-weight of meat, and the weight of bound water is about half the weight of the protein, water bound (firmly

or loosely) to the protein in meat accounts at a maximum for 10% of the wet muscle weight (van Laack and Solomon, 1994). The amounts of water lost by drip, especially with PSE meat, would indicate that the drip originates from water held within myofibrillar structure (Offer and Knight, 1988). Offer and Trinick (1983) stated that variations in filament spacing due to changes in pH, sarcomere length, ionic strength, and osmotic pressure, range from 320 - 570Å. Variations in the filament spacing correspond to a three-fold change of volume in the myofibrillar structure. Thus, the amount of water retained/lost is dependent on the space available for water between the filaments.

5. PROTEINS

Offer and Trinick (1983) claim that it is reasonable that water is held in the muscle tissue by capillary forces, with most of the water being held in the interfilament spaces within the myofibrils. Hamm (1960) suggests that changes in WBC results from changes in the charges and structure of myofibrillar proteins. These changes in the charges and structure of the myofibrillar proteins are due to the influence of pH, ionic strength, and protein-protein interactions. Myofibrils are well suited for holding water

because of the three dimensional structure of the filaments. Myofibrils occupy approximately 70% of the volume of lean muscle. Therefore, most of the tissue water must be located within the filament structure.

It has been known for a long time that denaturation / solubility of proteins from PSE meat differs from that of normal pork (Sayre and Briskey, 1963; Boles et al., 1992). Changes in the conformation of the proteins' secondary, tertiary, and/or quaternary structures without the cleavage of the backbone structure (peptide bonds)constitute denaturation. Denaturation of a protein often results in a loss of functional properties, such as solubility. In meat, sarcoplasmic protein solubility and drip loss are closely correlated, while solubility of total and myofibrillar proteins and WBC are less closely related (Lopez-Bote et al., 1989; Warner, 1995).

Bendall and Wismer-Pedersen (1962) suggest that the watery condition of the pork is due to the adsorption of denatured sarcoplasmic proteins onto the surface of the myofibrils, thereby reducing the number of charged groups available for water-binding. Offer and Knight (1988) argue that this is unlikely. When globular proteins, such as sarcoplasmic proteins, denature and change their compact, folded, native conformation, they tend to entrap more water

instead of less. Using electron microscopic procedures Cassens et al. (1963a,b) found no evidence of denatured sarcoplasmic proteins on the myofibrils.

Another theory about the involvement of sarcoplasmic proteins and WBC was proposed by Tornberg et al. (1993). They suggested that the denatured sarcoplasmic proteins aggregate and cannot pass through the cell membrane (as long as it is intact). Proteins being polyelectrolytes, give rise to an osmotic effect. As the proteins begin to aggregate, the osmotic effect decreases. As the osmotic effect declines, the water in the cell moves out of the cell. Once outside the cell, this water will not flow back, due to the lack of space created by the muscle contraction at the onset of rigor. In beef muscle, the influence of sarcoplasmic proteins on the osmotic pressure is minimal; the sarcoplasmic proteins contribute about 2.4 mOs to the total, 480 - 540 mOs (post-rigor), osmotic pressure of beef (Winger and Pope, 1980-1981). It seems unlikely that a 0.5% portion of the overall osmotic pressure can be accountable for a drip loss of greater than 6%.

The impact of the sarcoplasmic extract on the water binding of myofibrils was studied by Monin and Laborde (1985); a positive effect on WBC was observed. This positive interaction between sarcoplasmic compounds and myofibrillar

proteins on WBC has also been suggested by Bendall and Wismer-Pedersen (1962). Monin and Laborde did not determine if the positive influence of the sarcoplasmic extract could be attributed to the proteins or to the ions of the extract. The proteins of the sarcoplasmic extract may be involved in a protein-protein interaction, or the ions present in the extract could contribute to the WBC by influencing the filament repulsion/spacing. A change in the filament spacing of the myofibrils caused by the sarcoplasmic extract would support the hypothesis suggested by Offer and Knight (1988). Their hypothesis states that the gains and losses of water in meat originates from volume changes in the myofibrillar lattice. In rigor muscle the myofibrils are unlikely to change in sarcomere length due to the attachment of cross-bridges. These cross-bridges will prevent the sliding of the thick and thin filaments to lengthen or shorten the sarcomere. In contrast, side-to-side thick filament spacing can change substantially. Changes in ionic strength and or pH can influence the center-to-center spacing of the filaments. If the result is lateral expansion of the filament lattice, more water is firmly held. Conversely, lateral shrinkage of the filament spacing expels water from the myofibril lattice and, since the expelled water is less-firmly held, it can be easily lost from the meat.

The occurrence of RSE has raised questions about the mechanisms behind the WBC of myofibrils. The WBC of RSE is similar to that of PSE, although protein solubility and myosin denaturation characteristics of RSE samples are similar to RFN. The only protein denaturation measurement that differentiates between RSE and RFN samples was the diminished solubility of phosphorylase and creatine kinase in RSE meat (Warner, 1995).

6. ULTIMATE pH AND WBC

Prior to slaughter, the pH of an animal is about 7.0. Following slaughter, the pH declines to around 5.5 as the carcass passes through rigor. Ultimate pH (pH_u) is important to the WBC of meat. The pH_u is dependent, in part, on the glycogen content at the time of slaughter. A high pH_u, as in DFD meat, is due to depletion of muscle glycogen before death, limiting the post-mortem formation of lactic acid (Greaser, 1986). DFD pork has a high pH_u (>6.0) resulting in a high WBC. This higher WBC is related to the high pH_u, which is further from the myofibrillar isoelectric point, causing increased repulsion and increased filament spacing. At pH values of 5.0-5.1, near the isoelectric point of the myofibrillar proteins, the WBC is at a minimum. At the isoelectric point, the net

charge of the proteins is zero, causing a minimum of repulsion. When the pH is higher than the isoelectric point, the WBC increases due to increased repulsion of the myofibrillar filaments, resulting in increased spacing (Offer and Knight, 1988). Irving et al. (1990) reported that filament spacing increased linearly with pH increase from 40 to 46 nm in the pH range of 5.2 and 6.4. Above pH 6.4, there was no further increase in the filament spacing.

Warriss and Brown (1987) suggest that differences in pH_u could only explain about 15% of the variation in drip loss, while van Laack et al.(1994) found that pH_u explained 24% of the variation in WBC. Warner (1995) reported a correlation between pH_u and percent drip of -0.77. The decrease in the WBC with a decrease in pH is reversible. This is attributed to changes in the interfilament spacing caused by a change in the repulsive forces between myofibrillar proteins, by adjusting pH.

7. IONIC STRENGTH

Ionic strength has been shown to have an influence on filament spacing by changing the repulsive forces (April et al., 1972; Yu et al., 1984). When the pH of meat is greater than 5, the net charge on thick and thin filaments is

negative, resulting in a repulsion between filaments. At very low salt concentrations between 0.0 - 0.1 M, raising the salt concentration decreases the filament spacing, as if the salt were covering the charges on the filaments (Offer and Trinick, 1983). An increase in the concentration of salt results in the salts competing with the proteins for water. When the water is removed from the proteins, they tend to precipitate and fall out of the solution, a process known as "salting out".

Hamm (1986) suggests that divalent cations such as Ca^{2+} and Mg^{2+} lower WBC of meat. These cations were thought to reduce the electrostatic repulsion between the negatively charged groups by creating a screening effect. The screening effect would cause the structure to tighten, resulting in a shrinkage of the interfilament space and a loss of WBC. Divalent cations occur in different fractions of muscle tissue. Offer and Trinick (1983) found that $MgCl_2$ had no major influence on the concentration at which NaCl caused swelling of myofibrils.

8. CONCLUSIONS

Achieving consistent pork quality is important to the pork industry. Pork quality is defined by the consumer by the appearance of the product in the retail market and the meat

texture when consumed. Pork that has the quality traits of PSE is undesirable to both consumers and processors. With the relatively high incidences of PSE in the industry, attempts have been made to improve the genetics and handling conditions of the animals to limit the incidences of PSE. Also, there is continuing research to determine and understand the mechanisms involved in the increased drip loss associated with PSE meat. The correlation between muscle protein denaturation and poor WBC is not fully understood. The association may be attributed to several factors. However, which factors are most important, and if they can be controlled, has yet to be determined. The high degree of drip associated with very limited protein denaturation in RSE meat has yet to be explained.

The intent of this literature review is to provide a basis of what is known about the WBC of pork and indicate what has yet to be determined. The intent of this thesis is to address the question of what portion of the sarcoplasmic extract, proteins or ions, has an influence on myofibrillar WBC.

Chapter III

MATERIALS AND METHODS

1. SAMPLE COLLECTION

Approximately 24 h after slaughtering, pork longissimus samples were selected from the boning line at a commercial slaughter plant. Based on subjective visual and filter paper drip evaluations, six PSE and six normal samples were selected. Filter paper (S & S filter paper, 4.5 cm circles, Schleicher and Schuell, Keene, NH), used to give a subjective indication of exudation, was placed on a freshly cut surface for approximately 10 sec. Samples with a visually pale appearance in which the filter paper adsorbed a large amount of moisture were considered to be PSE. After a longissimus muscle was selected, a portion, 20 cm in length, from the sirloin-end of the loin was removed. These portions were placed in individual bags, stored in ice chests on ice, and transported to the University of Tennessee Meat Laboratory.

2. QUALITY EVALUATION PROCEDURES

To confirm that the samples collected were correctly classified as PSE or normal, each sample was analyzed for color, pH, WBC, protein solubility, and by SDS-PAGE. Initially, measurements of the sample pH, color, and filter-paper-drip were reviewed to establish the classification. The other analysis were used for further confirmation, and conducted at a later time. Samples with pH values ≤ 5.6, $L^* \geq 55.0$, and filter paper absorbance ≥ 90 mg were classified as PSE. Samples with parameters of $L^* < 55.0$, and filter paper absorbance < 90.0 mg were considered normal. Any sample with a $pH_u > 6.0$ was excluded.

Color Analysis

Color CIE L*, a*, and b* values were measured using a Minolta Spectrophotometer (CM-508; Minolta Co, Ramsey, NJ). The Minolta was set for a light source of D_{65}, and an observer angle of 2°. An approximately 2 cm thick slice was taken from each loin sample and used for color measurements. This slice was set out in a refrigerated room, and exposed to the air for an hour prior to the color measurements, to allow the sample to bloom and the color to stabilize. Measurements were made at three locations on the sample surface to obtain a

representative average. An L* value greater than 55.0 was considered indicative of PSE.

Analysis of pH

The ultimate pH (pH_u) was measured using an invasive method using an Orion Model 250A hand-held pH meter (Orion, Boston, MA). The pH probe was inserted into the sample approximately 3 cm deep and held until the pH reading stabilized. A pH_u of < 5.6 was one of the parameters required for a PSE sample.

Analysis of Water-binding Capacity

The WBC of each sample was determined using two different methods. One 4 cm thick section, from each sample, was used for both methods. The filter paper method developed by Kauffman et al. (1986), a rapid method based on weight gained by the filter paper, was the first method conducted. A freshly cut sample was set out for 10 min to allow the surface moisture from the sample to equilibrate. After the equilibration time, a preweighed 4.5 cm circle filter paper was placed on the surface of the sample for 10 sec and then reweighed. Samples with a weight gain of > 90 mg were classified as PSE. The second method is a variation of a method outlined by Honikel (1987). Following completion of the

filter paper method, a core was cut from each sample using a 4 cm in diameter coring device. The individual cores were weighed and suspended, from fish hooks, and stored at 0-4°C in plastic boxes for 48 h. After 48 h, the samples were removed from the fish hooks and reweighed to determine drip loss. The remaining section of each loin was ground and used for further analysis.

3. PROTEIN PURIFICATION AND ANALYSIS

Sarcoplasmic Protein Purification Process

A 60 g portion of the ground sample was homogenized in 5 volumes (300 mL) of 0.03 M phosphate buffer, pH 7.4, using a Waring blender (Waring Products Division, New Hartford, CT) for 30 sec on the high speed setting. The homogenate was stored on ice for one hour, and then centrifuged (Sorvall RC2-B, Sorvall Inc, Newton, CT) for 15 min at 2000xg with a temperature setting of 4°C. Following the centrifugation, the supernatant was filtered through #1 Whatman paper. The filtered supernatant, containing sarcoplasmic proteins and ions, was analyzed for protein content (Gornall et al., 1949). As a standard for the biuret procedure bovine serum albumin (BSA; Sigma Chemical Co, St. Louis, MO) was used.

Myofibrillar Protein Purification Process

A second portion of the ground sample was used to obtain purified myofibrils, following a procedure described by Warner (1995). A 50 g ground portion of the sample was combined with 500 mL rigor buffer (75 mM KCl, 5 mM K-phosphate, 2 mM EGTA, 2 mM $MgCl_2$, pH 7.2) and homogenized for 30 sec in a Waring blender. After homogenation, the solution was centrifuged for 15 min at 1000xg. Following the centrifugation, the supernatant was discarded; the pellet was resuspended in rigor buffer (using a Polytron PT 10/35, Brinkmann Instruments, Inc, Westbury, KY set on medium speed for 15 sec) and centrifuged again. This process was repeated a total of four times. After the final centrifugation, the pellet was resuspended in 250 mL rigor buffer. A portion of the final suspension was used for protein content analysis with the biuret procedure. BSA was used as the standard. Also, a portion of each myofibril extraction was stored in glycerated rigor buffer (50% rigor buffer, 50% glycerol) in the freezer until analysis with SDS-PAGE.

SDS-PAGE Analysis

SDS-PAGE was used to verify that samples had been classified correctly. PSE myofibrillar preparations analyzed with SDS-PAGE will have an identifiable band of phosphorylase

unlike RFN samples which will not have phosphorylase present. A portion of the final myofibril suspension was prepared for SDS PAGE analysis. The samples were centrifuged for 15 min at 1000xg. Following centrifugation the supernatant was discarded, and the remaining pellet was resuspended in a 0.0625 M Tris buffer, pH 6.8. Subsequently, the protein concentration of the suspension was determined using the biuret procedure. The procedure requires that the standard be made with the same concentration of Tris:water as will be present in the samples (Tris tends to interfere with the biuret procedure). After determining the concentration of each suspension, the concentration was adjusted to 4 mg/mL by diluting with 0.0625 M Tris. Equal volumes, 200 µL of each, protein suspension and 2x sample buffer (0.0625 M Tris, pH 6.8, 20% glycerol, 10% mercaptoethanol, 4% SDS, 0.02% bromophenol blue) were mixed and vortexed (Baxter Scientific Products S/P S8223-1) on medium speed for 15 sec. The samples suspensions were heated for 10 min at 95°C. Subsequently, the samples were vortexed again, then frozen, in 2 mL microfuge tubes, at -20°C until analysis.

The procedures followed for the electrophoretic analysis were essentially those outlined by Fritz et al. (1989). A 12% gel, pH 8.8, was used with a 3% stacking gel, pH 6.8. The gels were loaded with 20 µL of each sample containing 4 µg of

protein. The gels were run using a Hoefer S600 vertical slab unit (Hoefer, San Francisco, CA) with a current of 40mA for 4 h. Sigmamarkers M 4038 Wide Range (Sigma, San Francisco, CA) were used as a standard for molecular weight comparison. At the end of the run, when the dye reached the bottom of the gel, the gels were removed from the glass slabs by disassembling, and the gels were placed in a staining solution (0.025% Coomassie Brilliant Blue R250, 50% methanol, 9.2% acetic acid). The gels were stained for 12 h, at which time the staining solution was poured off and a destaining solution was added (10% methanol, 7.5% acetic acid). The destaining solution was changed every 2 to 4 h, until the background was clear. Photographs were taken of the destained gels using a 35 mm camera and a light box to preserve the image of the gel. After the photographs were taken, the gels were stored in zip lock freezer bags with 10 mL destain solution.

Moisture Analysis

From each myofibrillar suspension, a portion, calculated to yield 400 mg myofibrillar protein, was centrifuged for 15 min at 1000xg. After the centrifugation process, the supernatant was discarded. The remaining pellet was used for moisture analysis. For the analysis, drying pans were stored in a drying oven prior to use, to assure that they were free

from moisture. The pans were weighed, and the weights recorded. Then, a 1 g portion from each pellet was added to the pan, and the combined weight recorded. For each sample, this was done in triplicate. A 1 g portion of pellet should contain approximately 100 mg of myofibrils. Following the weighing of the pans and sample, the pans were placed in the vacuum drying oven set at 60°C with a vacuum of 20 - 25 psi. Samples were dried for a 24 h period. When the samples were removed from the oven, they were placed in a desiccator to cool and prevent any moisture pickup. When the samples and pans had cooled to room temperature, they were reweighed. The moisture content of the myofibrillar pellet was calculated and reported ({[wt of wet pellet - wt of dry pellet] / wt of wet pellet} x 100).

Analysis of Myofibrillar Solubility

Solubility of the myofibrils was determined using a portion of the myofibril extract that had been stored in the freezer in glycerated rigor buffer. The samples were removed from the freezer and 5 mL was pipetted into 50 mL centrifuge tubes along with 15 mL rigor buffer. The mixture was vortexed on the high speed setting for 30 sec. Following the mixing, the solution was centrifuged for 15 min at 1000xg. After centrifugation, the supernatant was discarded and the pellet

was resuspended in 20 mL rigor buffer, mixed and centrifuged two more times, to wash out the glycerol. After the final washing, the myofibrillar pellet was resuspended with 5 mL rigor Buffer. The concentration of protein of the final suspension was determined using the biuret procedure. For the determination of soluble myofibrillar protein, a portion of the final suspension, sufficient to yield 100 mg protein, was pipetted into 50 mL centrifuge tubes and centrifuged at 1000xg for 15 min. After the centrifugation the supernatant was poured off and the pellet was mixed with 25 mL of a solution containing 50% 1.1 M KI and 50% 0.1 M K-phosphate, pH 7.4. This suspension was incubated at 4°C for 12 h. Subsequently, the solution was filtered through #1 Whatman paper. The protein content of the filtrate was determined using the biuret procedure, with the protein content reported as mg soluble protein per 100 mg of myofibrils.

4. SARCOPLASMIC EXTRACT INFLUENCE ON WBC OF MYOFIBRILS

Sample Pairing and Preparation

To determine the influence of sarcoplasmic proteins on the WBC of the myofibrils, the following procedures were developed. Random pairs of samples, each pair consisting of one PSE sample and one RFN sample, were utilized. For example

PSE sample #1 was paired with RFN sample #1, PSE #2 with RFN #2, and so on. For each sample, a portion of the myofibril suspension, equal to 300 mg myofibrillar protein, was pipetted into each of nine 50 mL centrifuge tubes. Subsequently, the tubes were centrifuged for 15 min at 1000xg at 0-4°C. Following centrifugation, the supernatant was discarded. The tubes, containing the pellets, were inverted for 30 min to allow any free liquid to drain. After 30 min, each tube and pellet was weighed and placed on ice.

Myofibrillar and Sarcoplasmic Combination Scheme

Once the tubes with the pellets had been weighed, the sarcoplasmic extracts were combined with the myofibrillar pellets. The myofibrillar pellets from a PSE sample were combined with either 17.7 mL of its own sarcoplasmic protein extract, or with 17.7 mL sarcoplasmic protein extract from the paired normal sample, or with 17.7 mL of 0.03 M phosphate buffer, pH 7.4. Volumes of sarcoplasmic extract and phosphate buffer added to the myofibrillar pellets were 5.9 mL for each 100 mg of myofibrils, based on the dilution factor from the sarcoplasmic extraction. The sarcoplasmic extracts were made with 5 volumes of buffer per 1 g of sample. In order to maintain the original ratio of sarcoplasmic proteins to myofibrillar proteins (1:1) the ratio of sarcoplasmic extract

to myofibrillar pellet should be 5.9 mL sarcoplasmic extract to 100 mg myofibrillar pellet. Each combination was made in triplicate. For example, each PSE sample had a set of 9 tubes with myofibrillar pellets.

- 3 combined with its own PSE sarcoplasmic extract
- 3 combined with sarcoplasmic extract from the paired normal sample
- 3 combined with the phosphate buffer as a control.

A similar scheme was followed for the paired normal samples. Combination schemes for the other five pairs of PSE and normal samples were the same as described above. After the combination of the pellets and the appropriate extracts, each tube was vortexed 15 sec at the highest speed setting and placed back on ice. Once the samples had all been vortexed, the pH of each tube was adjusted (using 1 M HCl or 1 M KOH) to the pH_u of the meat from which the particular myofibrillar pellet originated. It is known that pH has an influence on the WBC of myofibrils. Before adjustment, when combined (myofibrils and the sarcoplasmic extract), the pH was in the range of 6.2, due to the influence of the extraction buffers and the buffering capacity of the myofibrils. The pH adjustments were made to eliminate any impact that pH might have on WBC. After the pH had been adjusted, the centrifuge tubes were capped and stored on ice at 0-4°C for 24 h.

Determination of Myofibrillar Pellet WBC

At the completion of the 24 h incubation, the water binding of the myofibrils was determined. The samples were centrifuged for 15 min at 1000xg and the supernatant discarded. Once the supernatant had been discarded, each tube was inverted for 30 min to drain any free fluid that still remained in the tube. Subsequently, the tubes were weighed. Weight, gained or lost by the pellet during the incubation, was recorded as a percentage of the weight gained or lost/mg myofibril, and considered the WBC of the pellet.

5. MYOFIBRILLAR WBC INFLUENCED BY PROTEIN-FREE SARCOPLASMIC EXTRACT

Sample Pairing and Preparation

The same pairs and combination scheme, as were used in the combination of myofibrils and sarcoplasmic extract experiments, were utilized for this part of the study. For each sample a portion of the final myofibril suspension was pipetted into a centrifuge tube to obtain 300 mg of myofibrils, based on protein concentration, in each of 6 tubes. Subsequently, they were centrifuged for 15 min at 1000xg at a temperature between 0-4°C. Following centrifugation, the supernatant was discarded. The tubes,

containing the pellets, were inverted for 30 min to allow any free liquid to drain. After 30 min, each tube and pellet were weighed and placed on ice.

Protein Removal from the Sarcoplasmic Extract

Proteins from the sarcoplasmic extract were removed for this experiment. Protein removal was accomplished by heating 150 mL extracts (in Erlenmeyer flasks) in an 80°C water bath for 30 min. This resulted in denaturation of the protein. Subsequently, the samples were filtered through #1 Whatman paper, the filtrates were then centrifuged for 15 min at 2000xg. Upon completion of centrifugation, the supernatant was refiltered through #1 Whatman paper, and stored on ice. This method was tested, and can effectively remove more than 97% of the proteins from the solution, without changing the conductivity. The solutions were tested for protein content, using the biuret procedure, and had protein levels less than 2.5 mg/g of meat, i.e. less than 2.5% of the original sample. The protein-free extracts were also subjected to SDS-PAGE for analysis.

SDS-PAGE Analysis of Protein-free Extract

For verification that proteins were removed from the sarcoplasmic extract, the protein-free extract was subjected

to SDS-PAGE analysis. After the protein extraction process had been completed, a 2 mL portion of each sample was placed in a centricon-3 concentrator (AMICON Company, Beverly, MA). The samples were centrifuged at 10,000xg until the volume was concentrated to 200 μL. The concentrated samples were combined with 2x sample buffer, and heated for 10 min at 95°F. The samples were then frozen at -20°C until they could be analyzed by SDS PAGE. For analysis the samples were removed from the freezer, thawed, again heated to 95°C for 10 min, and then cooled prior to loading onto the gel. The electrophoretic procedures used were similar to those outlined by Fritz et al. (1989). The samples were run in 15% acrylamide, pH 8.8, resolving gels with 3% acrylamide, pH 6.8, stacking gels. The gels were run with a constant 40 mA current for 4 h. Samples and a standard, Sigmamarkers M 4038 Wide Range run for molecular weight comparison, were loaded onto the gel in 20 µL portions with a concentration of 4 µg protein.

Analysis of Sarcoplasmic Protein-free Extracts

In an effort to determine if the extracts were free from sarcoplasmic proteins, the samples were analyzed using both SDS-PAGE and the biuret procedure. The biuret procedure indicated that there was an average concentration of peptides equivalent to 0.43 mg/mL of protein. There was no significant

difference (P>0.05) between PSE and RFN protein-free extracts, with protein contents of 0.42 and 0.46 mg/mL, respectively. After concentrating the extracts 10x, they were subjected to SDS-PAGE and produced gels that showed no sign of proteins in the MW range of 6,500-205,000 (Figure 1). This would indicate that the peptide bonds detected using the biuret procedure are part of very small proteins or fragments.

Measurement of Conductivity in Protein-free Extracts

The conductivity of the solutions was determined as a measure of the ionic strength of the extracts. A 30 mL portion from each protein-free sarcoplasmic extract was placed in 50 mL centrifuge tubes and the conductivity measured in milli Siemens per centimeter (mS/cm) using an ORION Model 122 conductance meter (Orion, Boston, MA).

Myofibrillar and Sarcoplasmic Protein-free Extract Combination Scheme

Once the tubes with the pellets had been weighed, the sarcoplasmic protein-free extracts were combined with the myofibrillar pellets. Each PSE sample was paired with a normal sample. For example PSE sample #1 was paired with RFN sample #1, PSE #2 with RFN #2, and so on. The myofibrillar pellets from a PSE sample were combined with either 17.7 mL of their

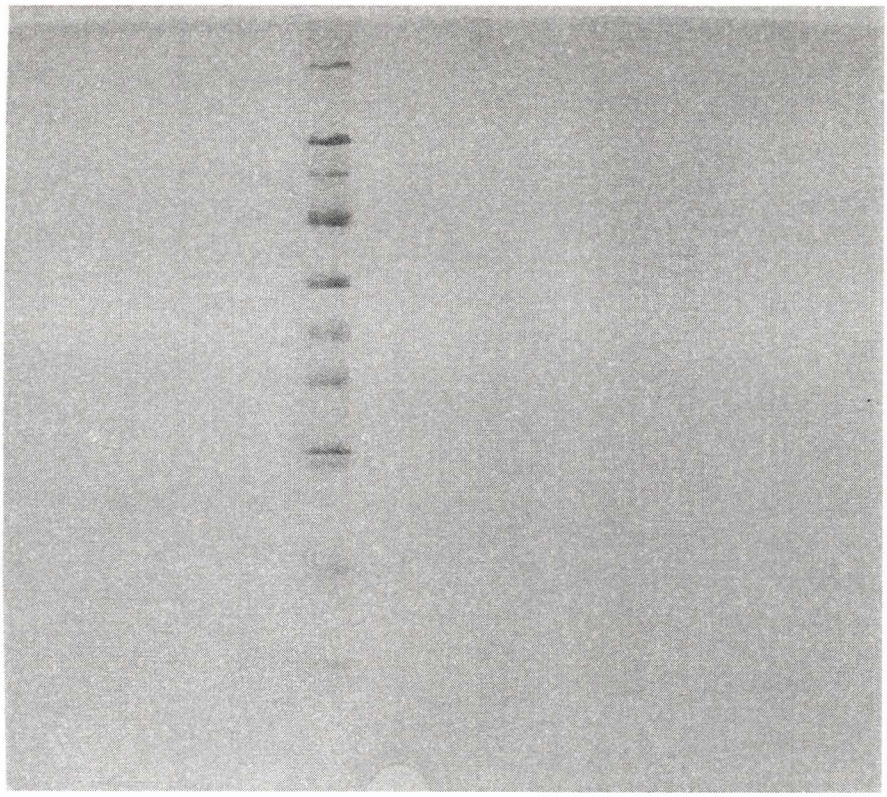

Figure 1: SDS-PAGE 15% gel, patterns showing the results of sarcoplasmic extracts after protein removal. SIGMAMARKER wide range standard is in lane 5. PSE samples were run in lanes 4, 6, 7, 11, 12, 13, and RFN samples in lanes 1, 2, 3, 8, 9, and 10. Molecular weights of standard are indicated (kilodaltons).

own sarcoplasmic protein-free extract, or with 17.7 mL sarcoplasmic protein free extract from the normal sample. The combination of phosphate buffer with the myofibrils was not repeated. Each combination was made in triplicate. For example, each PSE sample had a set of 6 tubes with myofibrillar pellets.

- 3 combined with its own PSE sarcoplasmic extract
- 3 combined with sarcoplasmic extract from the paired normal (RFN) sample

(The same scheme was followed for the paired normal sample.) Combination schemes for the other 5 pairs of PSE and normal samples were the same as described previously. After the combination of the pellets and the appropriate extracts, each tube was vortexed for 15 sec at the highest speed setting, and placed back on ice. Once the samples had all been vortexed, the pH of each tube was adjusted (using 1 M HCl or 1 M KOH) to the pH_u of the meat from which the particular myofibrillar pellet originated. After the pH has been adjusted appropriately the samples were stored on ice for 24 h.

Determination of Myofibrillar Pellet WBC

At the completion of the 24 h incubation period, the water uptake of the myofibrils, as influenced by the protein-free extract, was determined. Samples were centrifuged for 15

min at 1000xg and the supernatant was discarded. Subsequently, the tubes were inverted for 30 min to drain any free fluid that may have remained. The tubes and pellets were reweighed. Weight gained or lost by the pellet during the incubation was recorded as a percentage of the weight gained/mg myofibrils, and considered the WBC of the pellet.

Ionic Strength Influence on Myofibrillar WBC

To determine what influence changes in ionic strength would have on myofibrillar protein WBC, the following procedures were developed. In each of 16 tubes per sample, 300 mg of myofibrils was pipetted into a centrifuge tube. The suspension was centrifuged for 15 min at 1000xg. At the completion of the centrifugation, the supernatant was discarded and the pellets were resuspended in 17.7 mL of one of a set of phosphate buffers. The buffers had been adjusted, using calcium chloride, so the conductivity ranged from 5.3 to 8.3 mS in increments of 0.5 mS. In addition, one buffer was adjusted to 15 mS. Sample sets, in duplicate, were incubated overnight. After the incubation the samples were centrifuged for 15 min at 1000xg. Following the centrifugation, the supernatant was discarded, and the WBC of the myofibrils determined and reported as a percentage of weight gained/lost per mg myofibrillar pellet.

6. STATISTICAL ANALYSIS

To evaluate the differences between the 2 classes of pork SAS Procedure Mixed (SAS Institute, Inc. 1989) was used to analyze the data. The experimental design consisted of a randomized block design with replication. Samples were blocked based on sample classification, PSE or RFN. Ionic strength and WBC was evaluated between classes of pork. The influence of sarcoplasmic extract, sarcoplasmic proteins and ionic strength on WBC of myofibrils was evaluated to determine if there was a significant impact on WBC by either.

CHAPTER IV

RESULTS AND DISCUSSION

1. QUALITY MEASUREMENTS

Physical Characteristics of Samples

Samples were physically measured for surface lightness (L*), pH_u, drip loss, and fluid exudate. These measurements were used to classify each selected sample as either PSE or normal (RFN). The least squares means values for pH_u, lightness, drip loss, and surface exudate were significantly ($p<0.05$) different between the two classes (Table 1). The PSE samples had a surface L* value approximately 11.1 units higher, a pH_u of 0.4 units lower, a 48 h drip loss almost 4 times higher, and 80.0 mg more surface exudate than RFN samples. These results are in agreement with published reports on differences between the two quality classes(Lopez-and Bote et al., 1989; Kauffman et al., 1993; van Laack et al., 1994; Warner, 1995; Warner et al., 1997).

Protein Measurements

The protein content and solubility of samples were determined measured to confirm the classification of each

Table 1: Quality Measurements on Loin Samples for Quality Classification[1].

Quality Class[2]	N	pH_u	Lightness (L*)	Drip Loss (%)	Surface Exudate (mg of fluid)
PSE	6	5.3 ± 0.1^a	62.6 ± 0.9^a	8.9 ± 0.5^a	131 ± 15^a
RFN	6	5.7 ± 0.1^b	49.5 ± 0.5^b	2.4 ± 1.0^b	51 ± 10^b

[1] Least squares means ± standard errors.

[2] Quality classes used are PSE = Pale, soft, and exudative and RFN = Red, firm, and non-exudative.

[ab] Least squares means within a column with different superscripts are different ($p<0.05$).

sample. Sarcoplasmic protein solubility was 22 mg lower(35% difference) in the PSE samples than in the RFN samples (Table 2). Myofibrillar solubility was determined using the purified myofibrillar extract that had been stored in a glycerated buffer in the freezer. Myofibrils of PSE samples had a significantly lower ($P<0.05$) solubility than myofibrils from RFN samples (Table 2). Several researchers have reported differences in sarcoplasmic and myofibrillar protein solubility of the two quality classes (Sayre and Briskey, 1963; Lopez-Bote et al., 1989; Boles et al., 1992; Warner, 1995).

SDS-PAGE Analysis of Myofibrillar Proteins

SDS-PAGE was used as another confirmational procedure in the classification of samples. SDS-PAGE gels of the myofibrillar proteins showed distinct protein bands of phosphorylase (M.W. 97,000) in the PSE samples but not in the RFN samples (Figure 2). Presence of the sarcoplasmic protein phosphorylase in the myofibrillar fraction of PSE samples has been reported before (Boles et al., 1992; Warner, 1995; Warner et al., 1997). The major sarcoplasmic proteins that become denatured in PSE meat are phosphorylase (Fischer et al., 1979) and creatine phosphokinase (Scopes, 1964). Phosphorylase and creatine phosphokinase both tend to denature under conditions

Table 2: Sarcoplasmic and Myofibrillar Solubility of PSE and RFN Samples.[1]

Quality Class[2]	N	Sarcoplasmic Solubility[3]	Myofibrillar Solubility[4]
PSE	6	41±3.1[a]	26±4.7[a]
RFN	6	64±5.3[b]	50±2.7[b]

[1] Least squares means ± standard errors.
[2] PSE = pale, soft, and exudative and RFN = red, firm, and non-exudative.
[3] Sarcoplasmic protein solubility measurements are presented as mg of protein per g of sample.
[4] Myofibrillar protein solubility measurements are expressed as mg per 100 mg myofibrillar protein.
[ab] Within columns, means with different superscripts are significantly different ($P<0.05$).

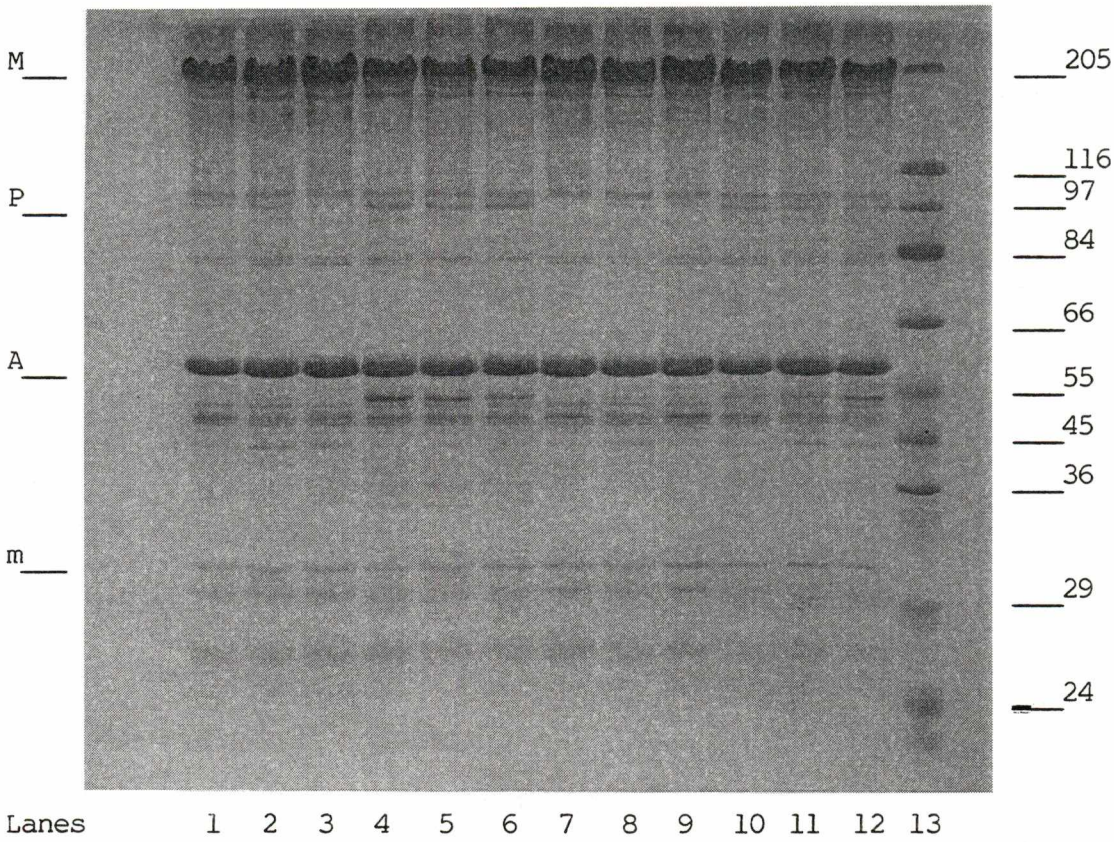

Figure 2: SDS-PAGE 12% gel, patterns showing the differences between PSE and RFN myofibrillar extracts. PSE samples are in lanes 4, 5, 6, 10, 11, 12, and RFN samples are in lanes 1, 2, 3, 7, 8, and 9. The SIGMAMARKER wide range standard is in lane 13 with the molecular weights indicated (kilodaltons). Protein bands identified are M = myosin, p = phosphorylase, A = actin, m = myosin light chain 1.

conducive to PSE meat, and upon denaturation become insoluble in low salt concentration buffers, such as those used in extracting sarcoplasmic proteins. The phosphorylase is very evident in the columns of the SDS-PAGE gel containing the PSE myofibrillar extracts. The creatine phosphokinase is much less evident due to incomplete resolution from the adjacent band of actin.

Moisture Analysis of Myofibrillar Pellets

Following the extraction of the myofibrils from the ground meat samples, a moisture analysis was performed on the myofibrillar pellets. PSE samples had a significantly ($P<0.05$) lower moisture content than RFN samples, 89.3±0.7% and 92.5±0.3%, respectively. This is consistent with the decreased water-binding capacity found in PSE meat.

2. WATER-BINDING CAPACITY OF MYOFIBRILS

Influence of Sarcoplasmic Proteins

The WBC of myofibrils from RFN samples when combined with the sarcoplasmic protein extract from the PSE sample, was significantly lower ($P<0.05$) than the WBC of the RFN myofibrils combined with the sarcoplasmic fraction from the same sample (Table 3). The WBC of the myofibrils from the PSE

Table 3: Water-Binding Capacity[1] of Myofibrils Influenced by Sarcoplasmic Extracts[2]

Sarcoplasmic Extract Source	N	Myofibril Source[3] PSE	RFN
PSE[4]	6	1.3±0.4[ac]	1.9±0.4[b]
RFN	6	2.6±0.4[b]	2.8±0.4[a]
PSE Protein-Free[5]	6	0.7±0.4[c]	1.8±0.4[b]
RFN Protein-Free	6	0.9±0.4[c]	1.2±0.4[b]

[1] Water-binding capacity measured as mg water gained per mg myofibrils.

[2] Least squares means ± standard errors.

[3] PSE = Pale, Soft, Exudative and RFN = Red, Firm, Non-exudative.

[4] PSE and RFN extracts consisted of the sarcoplasmic fraction that was extracted utilizing 0.03 M phosphate buffer in a 5:1 ratio with each sample.

[5] Protein-free extracts were sarcoplasmic extracts with the proteins removed through denaturation.

[abc] Within columns, means with different superscripts are significantly different ($P<0.05$).

samples combined with their own sarcoplasmic extract, was significantly lower ($P<0.05$) than the WBC of PSE myofibrils combined with the sarcoplasmic extract from a RFN sample (Table 3).

The influence of the sarcoplasmic proteins on WBC became evident when the sarcoplasmic protein-free extracts were combined with the myofibrils. WBC of myofibrils from the RFN samples combined with RFN sarcoplasmic protein-free extract was not significantly different ($P>0.05$) from WBC of RFN myofibrils combined with the sarcoplasmic protein-free extracts from the PSE samples (Table 3). There was a significant difference ($P<0.05$) between the RFN WBC of myofibrils combined with the protein-containing sarcoplasmic extract from RFN samples and the WBC of RFN myofibrils combined with sarcoplasmic protein-free extracts from either RFN or PSE samples.

The results were similar when the PSE myofibrils were combined with the sarcoplasmic protein-free extracts. There was no significant difference ($P>0.05$) between the PSE myofibrillar water-binding capacity combined with the sarcoplasmic protein-free extract from their own samples compared to the WBC of myofibrils combined with the sarcoplasmic free extracts from the paired RFN samples (Table 3). PSE myofibrillar water-binding capacity when combined with

sarcoplasmic proteins from the PSE samples was not significantly different (P>0.05) from WBC when combined with the protein-free extract (Table 3).

Both RFN and PSE myofibrils incubated with the protein containing RFN extracts had a higher (approximately 50%) WBC than when incubated with the PSE sarcoplasmic extract. This would indicate that the sarcoplasmic proteins that are denatured in the PSE sarcoplasmic extract are not available to have a positive influence on the WBC of myofibrils. The RFN and PSE myofibrils incubated with the protein-free extracts had a lower (approximately 50%) WBC than the myofibrils incubated with the extract from PSE samples. This would suggest that not only do the sarcoplasmic proteins that denature in PSE meat have an influence on the WBC of myofibrils, the other proteins in the sarcoplasmic fraction also have a positive influence on myofibrillar WBC. A comparison of the WBC of myofibrils incubated with protein-containing extracts and protein-free extracts suggest that the proteins in the sarcoplasmic fraction interact with the myofibrils and play an important role in WBC.

As a control the myofibrils, PSE and RFN were combined with the sarcoplasmic extraction buffer, 0.03 M phosphate, in much the same way as Monin and Laborde (1985) did. When the buffer was combined with the PSE myofibrils the myofibrillar

WBC was not significantly different (P>0.05) than the WBC of the myofibrils combined with the sarcoplasmic extracts from either the PSE or RFN samples. However, PSE myofibrils combined with the buffer was significantly different (P<0.05) from the WBC of the PSE myofibrils combined with the protein-free extracts from the PSE and RFN meat. WBC of RFN myofibrils were combined with the buffer and was not significantly different (P>0.05) from the WBC of the RFN myofibrils combined with the RFN sarcoplasmic protein-containing extract. The WBC of the RFN myofibrils combined with the buffer was significantly higher (P<0.05) from the WBC of the RFN myofibrils combined with the sarcoplasmic protein-containing PSE extract. The WBC of the RFN myofibrils combined with the protein-free extracts from the PSE and RFN meat samples were significantly lower (P<0.05) from the WBC of the RFN myofibrils combined with the 0.03 M phosphate buffer. These results were expected considering the low salt concentration 0.03 M of the buffer.

Monin and Laborde (1985) reported that the water-binding of myofibrils was higher when incubated with extracts containing sarcoplasmic proteins. They compared the influence of the sarcoplasmic extracts with that of a 0.05 M phosphate buffer. They used a 0.03 M phosphate buffer for the sarcoplasmic extract and did not take into account what, if

any, influence the difference in salt concentrations (0.05 vs. 0.03 M) might have on the water-binding capacity. Offer and Trinick (1983) reported that at very low salt concentrations, between 0.0-0.1 M, raising the salt concentration decreases the filament spacing and, thus, the water-binding capacity. Bendall and Wismer-Pedersen (1962) also reported an increased water-binding of myofibrils in the presence of sarcoplasmic protein-containing extract. Neither Monin and Laborde (1985) nor Bendall and Wismer-Pedersen (1962) determined if the influence on the water-binding of myofibrils could be attributed solely to the sarcoplasmic proteins or to other compounds associated with the extract.

Over the years it has been suggested that denaturation of sarcoplasmic proteins contributes to the decreased water-binding capacity of PSE meat. It has been suggested that the denatured proteins interact with the myofibrillar proteins and thus interfere with the water-binding capacity. Also, it has been suggested that the denatured sarcoplasmic proteins are adsorbed onto the surface of the myofibrils, interfering with the myofibrillar solubility and reducing the number of charged groups available for proton and water-binding (Bendall and Wismer-Pedersen, 1962; Boles et al., 1992). Monin and Laborde (1985) suggested, that the denatured proteins would not have a negative effect on the WBC of myofibrils, but would

act by suppressing the positive effect of proteins in the ability to bind water. The research presented here would indicate that the sarcoplasmic proteins do in fact have a positive influence on the water-binding capacity of myofibrils, which coincides with the theory presented by Monin and Laborde (1985). It is evident that when the myofibrils from RFN samples were combined with the sarcoplasmic extracts from PSE samples or with the protein-free extracts from either quality class, the WBC was lower than when combined with the non-denatured sarcoplasmic protein extract from the RFN sample. The positive influence of the sarcoplasmic proteins on the WBC of myofibrils can be seen in the increased WBC of the PSE myofibrils combined with the RFN sarcoplasmic extract instead of the PSE sarcoplasmic extract. Also, the PSE myofibrils incubated with the RFN sarcoplasmic extract had higher WBC than PSE myofibrils with PSE extract. This would suggest that the precipitated sarcoplasmic proteins (phosphorylase and creatine kinase) are not available to positively interaction with the myofibrils, but, in the non-denatured form are available to interact with the myofibrils to improve the WBC of the myofibrils.

Influence of Ionic Strength

To determine if the influence of the sarcoplasmic extract on the water-binding capacity of myofibrils could in fact be attributed to the sarcoplasmic proteins, ionic strength, measured as conductivity, was assessed in both protein-free extracts. There was no significant difference ($P>0.05$) between the conductivity of the PSE and RFN extracts. The PSE extracts had conductivity measurements that averaged 6.37 ± 0.10 mS while the RFN extracts had an average conductivity of 6.18 ± 0.10 mS .

Although, there was no difference determined in conductivity between the extracts, RFN and PSE, the effect of small variations in ionic strength on the WBC of myofibrils was tested. Myofibrils were incubated with a series of buffers of incremental ionic strength levels. WBC of the myofibrils (Table 4) was determined after the incubation in the various buffers. There was no significant difference ($P>0.05$) between the WBC of the myofibrils at any of the ionic strength levels. This supports the conclusion that the sarcoplasmic proteins influence the WBC of the myofibrils.

Table 4: Influence of Ionic Strength on WBC[1] of Myofibrils from PSE and RFN Samples[2]

Conductivity Levels (mS)	Sample Type	
	PSE	RFN
5.3	0.8±0.4[a3]	0.0±0.2
5.5	1.0±0.5	0.8±0.2
6.3	0.8±0.4	0.7±0.1
6.8	0.8±0.3	0.3±0.3
7.3	0.3±0.2	0.2±0.01
7.8	0.5±0.1	0.4±0.2
8.3	0.3±0.2	0.1±0.5
15.0	0.0±0.1	0.3±0.02

[1] Water-binding capacity measured as mg water gained per mg of myofibrils.
[2] PSE = pale, soft, and exudative and RFN = red, firm, and non-exudative.
[3] Means ± standard errors.
[a] Within columns, means were not found to be significantly different (P>0.05).

3. CONCLUSIONS

Methods were developed to examine the influence of various components of the sarcoplasmic fraction on the WBC of myofibrils. It was determined that sarcoplasmic proteins which are sensitive to denaturation, have a positive effect on the WBC of myofibrils. It was also shown that the proteins that are not sensitive to denaturation in the sarcoplasmic fraction have a positive influence on myofibrillar WBC. The influence of the ionic strength on the WBC of myofibrils was determined to be insignificant in the range tested (5.3 - 15 mS).

Understanding the influence of the sarcoplasmic fraction on the WBC of myofibrils can be used to help explain the diminished WBC of RSE quality pork. It has been shown that denaturation of phosphorylase and creatine kinase is associated with RSE quality. RSE meat has a water loss similar to PSE meat. It is proposed that the high drip loss seen in PSE meat is the result of the low pH_u, sarcoplasmic and myosin denaturation. The drip loss found in the RSE meat may be linked to denatured sarcoplasmic proteins such as phosphorylase and creatine kinase.

In summary, knowledge was gained about the influence of the ionic strength and the sarcoplasmic fraction on the WBC of myofibrils. This knowledge gain has contributed to our basic understanding of the WBC of poor quality pork. More research

is needed to fully explain the effect proteins in the sarcoplasmic fraction have on myofibrillar WBC. Also, research should be conducted to determine under what conditions the sarcoplasmic proteins begin to denature. The ability to control the denaturation by methods such as rapid chilling could limit the amount of poor quality pork in the industry.

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Vita

George G. Wilson was born in Plattsburg, New York on December 3, 1958. After completing high school, he spent fifteen years working in various restaurants and hotels. While working in Seattle, Washington he completed an Associate of Applied Science degree in Data Processing, 1983. Following several years of food service management, he decided to pursue a career change and entered the University of Tennessee in 1992. In December, 1995 he received the Bachelor of Science degree in Food Science and Technology with a minor in Business. He entered the Master's program at the University of Tennessee in Food Science and Technology in January, 1996 and worked as an Research Associate in Meat Science. He completed the Master's Program in December, 1997.

On August 4, 1997, George accepted a position with ConAgra Frozen Foods in Omaha, Nebraska as a Food Technologist. Currently, he is working in the product development department, creating products for the food service industry.