

EFFECT OF LOW-IONIC SODIUM CHLORIDE CON-
CENTRATIONS ON THE EXTRACTABLE SALT-
SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR PORCINE MUSCLE

By

ROGER GROOM JOHNSON

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1969

SEP 29 1969

EFFECT OF LOW-IONIC SODIUM CHLORIDE CON-
CENTRATIONS ON THE EXTRACTABLE SALT-
SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR PORCINE MUSCLE

Thesis Approved:

Robert L. Henrichson

Thesis Adviser

George W. Nuell

J. Brink

D. D. Durham

Dean of the Graduate College

724920

ACKNOWLEDGMENTS

The author expressed appreciation to Dr. R. L. Henrickson, Professor of Animal Science, under whose assistance and guidance this study was completed.

Appreciation is also extended to Drs. J. B. Mickle, G. W. Newell, J. V. Whiteman, and D. R. Rickansrud for assistance and suggestions during the preparation of this thesis.

The author acknowledges Mr. Earl D. Cagle, Mr. S. Gopal Reddy, and Mr. Curtis L. Kastner, and other people working in the Meat Science Laboratory for assistance and advice.

Special appreciation is extended to Janet L. Newman for weighing all samples and running the total protein determination.

Above all, the writer expresses deep gratitude to his wife, Deanne, and parents for their encouragement throughout this program of graduate study.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
Muscle Proteins	3
Myofibrillar Proteins	6
Myosin	7
Actin	8
Tropomyosin	9
Sarcoplasmic Proteins	10
Connective Tissue Proteins (Collagen, Elastin)	11
Animal Fat	12
Sodium Chloride in Meat	15
Water in Meat	16
Meat Emulsions	17
III. MATERIALS AND METHODS	22
Sampling Procedure	22
Part I	23
Part II	23
Salt-Soluble Protein Extraction	26
Salt-Soluble Protein Determination	26
Determination of pH	28
Total Protein Determination	28
Statistical Analysis	28
IV. RESULTS AND DISCUSSION	30
Part I	30
Part II	38
V. SUMMARY AND CONCLUSIONS	56
LITERATURE CITED	58
APPENDIX	62

LIST OF TABLES

Table	Page
I. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle.	31
II. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle	32
III. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle	34
IV. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle	34
V. Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels	39
VI. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels.	40
VII. Extractable Salt-Soluble Protein Content in Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels	42
VIII. Analysis of Variance for Extractable Salt-Soluble Protein Content in Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels	43
IX. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels.	44
X. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels.	45
XI. Extractable Salt-Soluble Protein Content in Pre-Rigor Low-pH Porcine Muscles at Three Sodium Chloride Levels.	47

LIST OF TABLES (Continued)

Table	Page
XII. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre-Rigor low-pH Porcine Muscle at Three Sodium Chloride Levels.	48
XIII. Extractable Salt-Soluble Protein Content in Post-Rigor Low-pH Porcine Muscles at Three Sodium Chloride Levels	49
XIV. Analysis of Variance for Extractable Salt-Soluble Protein Content in Post-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.	50
XV. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels	51
XVI. Analysis of Variance for Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscles At Three Sodium Chloride Levels	52
XVII. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle	63
XVIII. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels	64
XIX. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscles at Three Sodium Chloride Levels.	65
XX. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle	65
XXI. Total Protein Content in Normal and Low-pH Porcine Longissimus Dorsi Muscle	66

LIST OF FIGURES

Figure	Page
1. Flow Chart for Sampling Procedure.	24
2. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle.	32
3. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle	36
4. Extractable Salt-Soluble Protein Content in Normal-pH and Low- pH Pre-Rigor Porcine Muscle	36
5. Extractable Salt-Soluble Protein Content in Normal-pH and Low- pH Post-Rigor Porcine Muscle	37
6. Extractable Salt-Soluble Protein Content in Normal-pH Post- Rigor and Low-pH Pre-Rigor Porcine Muscle.	37
7. Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels	40
8. Extractable Salt-Soluble Protein Content in Post-Rigor Normal- pH Porcine Muscle at Three Sodium Chloride Levels	43
9. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels. . .	45
10. Extractable Salt-Soluble Protein Content in Pre-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels	48
11. Extractable Salt-Soluble Protein Content in Post-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels	50
12. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscles at Three Sodium Chloride Levels	52

LIST OF FIGURES (Continued)

Figure	Page
13. Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH and Pre-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.	54
14. Extractable Salt-Soluble Protein Content in Normal-pH and Low-pH Post-Rigor Porcine Muscles at Three Sodium Chloride Levels	54
15. Extractable Salt-Soluble Protein Content in Post-Rigor Normal-pH and Pre-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.	55

CHAPTER I

INTRODUCTION

Sausage is one of the oldest forms of processed food, and was common place some thousand years before Christ. The sausage industry in the United States has reached heights of achievement unknown by any other country of ancient or modern times. Today in the United States 2.2 billion pounds of meat annually goes into sausage manufacture, thus the per capita consumption is some 25 pounds per person. The increased demand for sausage products is due to the rapid development of new products, the desire for convenience food, and the advance in sausage technology.

Two of the chief problems in the sausage industry are, emulsion breakdown, sometimes called "greasing out", and the low emulsifying ability of the sausage meat. During processing fat renders from the meat and forms in little pockets in the sausage, or at other times the fat separation may be more complete. This is considered to be an emulsion breakdown. Also, there is a limited amount of fat which can be emulsified by the low amount of readily available salt-soluble protein content in the meat.

Due to the increasing cost of raw materials, there is a trend for greater economy in meat processing. This has led to the development of new processing methods. With the development of high temperature processing, there became a need for more knowledge about the advantage of using "hot" porcine muscle in sausage

emulsions. Pre-rigor bull meat has been used for years in sausage manufacture to increase the emulsifying and water binding ability of the products, also Trautman (1964) and Saffle and Galbreath (1964) have shown that pre-rigor meat had an advantage in meat emulsions. With the use of pre-rigor meat in sausage emulsions, there would likely be less chance for microbial contamination. Also the meat should possess greater emulsifying ability.

This study was designed to, (1) determine the advantage of pre-rigor porcine muscle over post-rigor porcine muscle in the yield of extractable salt-soluble protein, and (2) determine the level of sodium chloride which will provide the greatest amount of extractable salt-soluble protein in pre- and post-rigor porcine muscle. The longissimus dorsi muscle was used because of the ease and speed with which it could be excised. The extractable salt-soluble protein content was used as a measure of the emulsifying capacity.

CHAPTER II

REVIEW OF LITERATURE

The information reported herein will be confined, in general, to the chemistry and properties of; (1) muscle proteins, (2) myofibrillar proteins (actin, myosin, and tropomyosin), (3) sarcoplasmic proteins, (4) connective tissue proteins (collagen, elastin), (5) sodium chloride in meat, (6) water in meat, (7) animal fat, and (8) meat emulsions.

Muscle Proteins

Lean muscle has the following proximate percentage composition: protein, 20; fat, 9; moisture, 70; carbohydrates, less than 1; and ash, 1 percent. From the proximate analysis, one can note that protein is second only to water as the most abundant substance in animal tissues and is, without doubt, the most important constituent of the edible portion of meat (Giffee et al. 1960).

Proteins are complex substances of high molecular weight that contain, carbon, hydrogen, oxygen, nitrogen, and often, sulfur, as well as, phosphorous (McElroy, 1964). Hydrolysis of protein yields a mixture of closely related compounds, the alpha-amino acids. Among these are proline, which is actually an alpha-imino acid, and hydroxyproline, a derivative found in collagen. Their general structures are $RCH(NH_2)COOH$, where R stands for variety of side chains (Harrow and Mazur,

1966). The principle amino acids in fresh muscle are alpha-alanine, glycine, glutamic acid, and histidine (Tallon et al, 1954).

Except for the simplest amino acid, glycine, all are optically active and in each of them the carbon atom to which both the amino group and the carboxyl are attached, is the center of asymmetry.

Because an amino acid contains both a carboxyl and an amino group, it should be regarded as an "inner-salt" or a "zwitterion". What is actually present is an electric field between the two parts of the molecule. Amino acids are "dipole compounds", which are in the state of ionization but not of dissociation (Braverman, 1963).

Amino acids behave both as weak acids and weak bases, thus they are amphoteric substances. An amino acid such as glycine, therefore, can carry a positive and or a negative charge, depending upon the pH of the solution. At a certain pH, the molecule is electrically neutral, and this pH, at which the dipolar ion will not migrate either to the positive or negative pole in an electrical field, is called the "isoelectric point". The pH of the isoelectric point depends on the dissociation constants of the basic and acidic groups (McElroy, 1964).

In proteins the various amino acids are linked together by peptide bonds, (-CO-NH-). The carboxyl group of one amino acid is linked with the amino group of the second amino acid with elimination of H_2O , thus forming an amide of the second acid (Braverman, 1963). A compound containing two or more amino acids linked together by means of the peptide bond is called a polypeptide. The long polypeptide chain of a protein can fold in a number of ways to make unusual shapes and configurations, and in many cases additional bonds help stabilize the folded

structure.

There are several ways of classifying proteins, but solubility properties of the proteins are the most commonly used means of classification. Water, salt, alkaline and acid solutions, and ethanol are used for such separations. Classification based on solubility is as follows: (Harrow and Mazur, 1966).

Albumins. Water soluble proteins that may be precipitated from solution at high salt concentrations.

Globulins. Unlike albumins, these proteins are generally insoluble in salt free water, and soluble in dilute salt solutions, but insoluble in salt solutions at 30 to 50 percent saturation.

Glutelins. These are insoluble in neutral aqueous solutions but are soluble in dilute acid or alkali.

Gliadins. These are soluble in 70 to 80 percent ethanol and insoluble in water or absolute ethanol.

Histones. They are soluble in water and insoluble in dilute ammonia solutions.

Protamines. These proteins are soluble in water and are basic in character.

Proteins are made up of a number of amino acids linked together in definite sequence by peptide bonds. Some proteins may contain more than one peptide, and these are held together by specific cross links or disulfide bonds. The arrangement or sequence of the amino acids in the polypeptide is called the primary structure of the protein. In most proteins, the tightly coiled polypeptide chain produces a helical shape which we call the secondary structure of the protein molecule (McElroy, 1964). The vast majority of proteins do not behave as long fibers, but tend to be circular in shape. They consist of polypeptide chains folded or coiled in a reg-

ular pattern. This is the tertiary structure of a protein.

The most important property of proteins is that called "denaturation". Denaturation is a distinct change in the natural properties of the protein. Such changes may be caused by several factors, such as heat, strong acids and bases, certain solvents and solutes, ultraviolet rays, or heavy metals. All of these can bring about large changes in the molecule. Some proteins contain sulfhydryl groups hidden in the inner cores of the protein helix and when these agents break the bonds, the exposed protein chains unfold and become much more reactive and sensitive to side reactions, (Braverman, 1963). The protein may also lose its original properties due to denaturation. An example of this would be the loss of an enzymes activity.

Myofibrillar Proteins

Myofibrillar proteins are responsible for the filamentous organization of muscle and also directly participate in contraction. Their removal is accompanied by the disappearance of the myofilaments (Hanson and Huxley, 1953). The myofibrillar proteins are frequently denoted as the "structure proteins" or "insoluble proteins" of muscle. For their extraction, neutral salt solutions of high ionic strength are required, even though, after extraction, some of them are soluble at lower ionic strengths. The resistance to extraction is partly a result of associations and interactions between these proteins within the myofilaments. A high viscosity of the extract indicates the fibrous nature of the proteins. From myofibrils of striated and from most smooth muscles, three well identified components can be isolated; they are, myosin, actin, and tropomyosin (Szent-Gyorgyi, 1960). Actin, myosin, and tropomyosin comprise about 80 percent of the proteins of the myofibril of rabbit

skeletal muscle (Perry, 1956).

Myosin

Lawrie (1966) reported that myosin is the most abundant of the myofibrillar proteins. About 38 percent of the muscle proteins is myosin (Giffee, et al. 1960). Myosin is a fibrous protein that occurs in the "A" band of the muscle fibril. It contains bound Mg^{++} , but is also capable of binding Ca^{++} and K^+ (Harrow and Mazur, 1966). Giffee et al. (1966) reported that myosin shows a strong affinity for the divalent cations, calcium and magnesium, whereas the binding of sodium and potassium is somewhat less. Myosin is probably a dimer, with a molecular weight of the monomer being about 450,000. It is a long thin molecule, containing many dicarboxylic amino acids, and has an alpha-helical configuration (Harrow and Mazur, 1966). Each myofibril contains about 2,500 myosin filaments (Frandsen, 1966). Hanson and Huxley (1953) reported that each myosin filament is made up of 424 molecules of myosin. It is a highly asymmetric protein, with a ratio of length to diameter of about 100:1. Myosin has a relatively high charge, because it contains large amounts of glutamic and aspartic acids and a fair amount of dibasic amino acids. Since myosin has the amino acid composition that it does, it is a negatively charged protein at the physiological pH. The isoionic point of myosin is approximately pH 5.4 (Giffee, et al. 1960). Myosin has been found to be readily soluble at ionic strengths higher than 0.3. It has also been found that myosin is heat sensitive and aggregates readily at moderate temperatures. Myosin has a great tendency to surface denaturation. Engelhardt and Ljubimova (1939) reported that ATP-ase, the enzyme which liberates inorganic phosphate from ATP, could not be separated from

myosin, and seemed to be identical with it. It has since been shown that myosin does possess ATP-ase activity (Mommaerts, 1950). It has been observed that a rapid treatment of myosin with trypsin produced a water soluble product which showed no loss of enzyme or enzyme activity. While the general properties of the enzyme are unchanged, the ultra-centrifuge shows that an extremely rapid fission into two distinct components occur. The proteins have been called meromyosins. One of the components is a heavier meromyosin (H-meromyosin), while the other is a light meromyosin (L-meromyosin). The H-meromyosin contains all of the ATP-ase activity. The yields of the two proteins are, 43 percent for L- and 57 percent for H-meromyosin (Bailey, 1954).

Actin

Actin, along with myosin, forms the contractile component of muscle. Actin represents about 13 percent of the total muscle protein, or is present in muscle with respect to myosin in a ratio of 1:3. Its isoionic point is somewhat lower than that of myosin, occurring at pH 4.7 (Giffey, et al. 1960). Actin is unique among the fibrous muscle proteins, in that in the presence of salts, it aggregates, and in the absence of ions, it dissociates into monomers. While actin is soluble in water or low concentrations of neutral salt solutions, these solutions do not extract it readily from muscle. Agents which depolymerize actin, like potassium iodide, extract it readily (Szent-Gyorgyi, 1960). One property of actin that is very striking is that it can exist in two forms (Bailey, 1954). Extracted actin is in the globular form (G-actin). It can be converted into the fibrous form (F-actin) by the addition of 0.1 M KCL and traces of $MgCl_2$ (Szent-Gyorgyi, 1960). It is the F-actin which

combines with myosin to produce the contractile actomyosin of active or pre-rigor muscle and the inextensible actomyosin of muscle in rigor-mortis. G-actin consists of relatively small globular units having a molecular weight of about 70,000, and F-actin consists of the globular units aggregated end to end to form a double chain (Lawrie, 1966).

When solutions of actin and myosin are brought together, a complex actomyosin is formed. It is characterized by a viscosity higher than that of the sum of the component proteins, and by a high molecular weight. The solubility of myosin and actomyosin differs somewhat, and the difference can be used for separating the two proteins (Szent-Gyorgyi, 1960). The addition of ATP to solutions of actomyosin results in a decrease in viscosity. ATP only need be present in small amounts for the separation of actin and myosin to occur. The actomyosin complex also possesses ATP-ase activity (Harrow and Mazur, 1966).

Tropomyosin

Prolonged action by concentrated urea solution on myosin causes the formation of a large number of small molecules, of similar molecular weight, but with different amino acid composition, which are called tropomyosins. (Giffey, et al. 1960). Tropomyosin was discovered in 1946 by Bailey. Its amino acid composition is somewhat similar to myosin (Bailey, 1954) and like myosin, there are few free amino groups. It appears to be a cyclopeptide. Recently, it has been suggested that actin filaments are attached to the "Z" line by a mesh work of tropomyosin (Huxley, 1963). Tropomyosin appears to be a universally occurring component of the myofibril and has been prepared from a wide variety of muscles. It comprises about 5 percent of the myofibrillar proteins of rabbit muscle. No enzymatic activity has

been shown to be associated with it, and its contribution to the activity of the muscle cell is not known. Tropomyosin is remarkably resistant to acid, alkali, or heat treatment. It is not easily denatured at surfaces or by precipitation with non-polar solvents. Little tropomyosin is extracted from muscle with solvents of low-ionic strength, though after extraction, tropomyosin is readily soluble under the same conditions. The molecular weight of tropomyosin obtained from various animals depends on the ionic strength. Molecular weights of 60,000 to 150,000 have been obtained (Szent-Gyorgyi, 1960).

Sarcoplasmic Proteins

The sarcoplasmic proteins (myogen, globulin, myoglobin, and haemoglobin) are now known to represent a complex mixture of about 50 components, many of which are enzymes of the glycolytic cycle. Myogen and globulins make up 5.6 percent of muscle total protein, while myoglobin and haemoglobin make up 0.36 and 0.04 percent respectively (Lawrie, 1966). The sarcoplasmic proteins are extracted with the greatest ease and are frequently mentioned as the "soluble proteins" of muscle. These proteins occupy mostly the space between the myofibrils and can be brought into solution readily with water or with neutral salt solutions of low ionic strength. The solution thus obtained has a low viscosity. The sarcoplasmic proteins do not contribute significantly to the filamentous organization of muscle, and when they are removed, the characteristic morphological features of the different types of muscles remain apparently unaltered. The morphological characteristics can really be seen better in muscle preparations when the sarcoplasmic proteins have been removed. The sarcoplasmic proteins are not directly involved in the structural reor-

ganization which results in contraction. Their function is mainly in the metabolic activities of the cell. The percentage of sarcoplasmic proteins to the total proteins of muscle varies from species to species and depends on the embryonic development of the cell (Szent-Gyorgyi, 1960). Perry (1956) has reported that the sarcoplasmic proteins make up 20 to 30 percent of total muscle protein in striated rabbit muscle.

Connective Tissue Proteins

Collagen

Collagen may be defined by its amino acid chemistry. All collagen has a high glycine content which represents nearly one-third of the total amino acids. Also present are two amino acids which are unique to collagen, hydroxyproline and hydroxylysine (Piez, 1966). The hydroxyproline content of muscle is, therefore, frequently used as a measure of its connective tissue content. The protofibrils representing the ultimate structural units of collagen consist of a triple helix of amino acid chains, the most common sequence in these being glycine, proline or hydroxyproline, and other amino acids. When heated in water at 60 to 70°C, collagen fibers shorten to about one-third or one-quarter of their initial length. When the temperature is raised to about 80°C, collagen is converted into the water soluble molecule gelatin (Kramer and Little, 1955). Collagen can be solubilized with cold neutral salt solutions, and by dilute acetic acid or by citrate at pH 3.8 (Haurowitz, 1963). Collagen is insoluble in high-salt solutions (Lawrie, 1966). According to Grettie (1965), collagen appears to have an isoelectric point between pH 7.0 and 7.5.

Elastin

Elastin is the connective tissue that forms the yellow elastic fibers in muscle. Unlike collagen, it is not hydrolyzed on boiling with water and consequently shows little softening or dissolving upon cooking (Meyer, 1960). The name originated from its abundance of elastic fibers (Harrow and Mazur, 1966). Elastin is a rubber like protein which is normally present in animal connective tissues in small amounts, but this protein forms the major part of the tissue in structures such as the walls of arteries and in the elastic ligaments which support the heads of large ruminants (Partridge, 1966). Elastin fibers commonly exhibit branched structures. They are readily detected histologically because of their strongly acidophilic properties. Their resistance to the action of hot alkali and to pepsin permits their separation from collagen easily. Whereas pepsin has little elastolytic effect, trypsin has a great effect. Although elastin has a fibrous nature, it is very much different from collagen and the keratins. Approximately 90 percent of its amino acids have non-polar side chains, with about 50 percent of elastin being composed of glycine and alanine. The exceedingly low acidic and basic amino acid contents are apparently related to the elastic properties of the fibers. Available data suggests that there are no free N-terminal amino acids (Giffey, et al. 1960). Meyer (1960) reported that leucine, valine, phenylalanine, proline, and glycine make up 78 percent of the elastin molecule. It is similar to collagen in its small content of histidine, tyrosine, and tryptophan.

Animal Fat

Fats comprise 15 to 20 percent of the live weight of market hogs. These fats

provide a reservoir of energy for animals, since fat is the most concentrated form of energy available to animal life (Dugan and Slover, 1960). In animal tissues true fat is found primarily in the adipose tissues while the active tissues, those which use considerable oxygen and produce much carbon dioxide, contain relatively little fat in the lipid fraction and much more of the complex lipids and sterols (Meyer, 1960). Natural fats are composed principally of the glycerol esters of the straight chain carboxylic acids having an even number of carbon atoms. They, therefore, possess many of the characteristics of simple esters such as ethyl acetate. Since glycerol has three hydroxyl groups, it is possible to combine a single glycerol molecule with one, two, or three fatty acid molecules, thus forming mono-, di-, or tri-glycerides. Tri-glycerides predominate in meat fats, although small amounts of mono-, and di-glycerides may be present. If the three fatty acids are identical a simple tri-glyceride results. If they are different, the ester is called a mixed tri-glyceride (Dugan and Slover, 1960). Natural animal fats are composed principally of mixed tri-glycerides. In addition to the tri-glycerides, meat fats contain other substances in small amounts, seldom exceeding a few percent. Such components may be phospholipids in which phosphoric acid has replaced one of the fatty acids, steroids, protein fragments, free fatty acids, and water.

The fatty acids are divided into two groups, those that are saturated, and those that are unsaturated. They can easily be separated by the difference in the solubilities of their lead salts in 95 percent ethyl alcohol. Another distinction between the two groups is their reaction with iodine. The iodine number serves as an indication of the degree of unsaturation of a tri-glyceride. Saturated fatty acids are normal straight-chain acids with an even number of carbon atoms from C_2 to C_{26} .

Those with fewer than 12 carbon atoms are volatile. Of these the most widely distributed in nature are palmitic, lauric, and stearic acids.

The majority of oils from plant sources contain unsaturated fatty acids. This group also consists generally of straight-chain fatty acids with an even number of carbon atoms from C_{10} to C_{24} (Braveman, 1963).

The solubility of fats is important in many areas of processing. Fats, in general, are completely miscible with non-polar solvents such as petroleum ether, benzene, and some esters. They are virtually insoluble in polar solvents such as water, and they may be partially soluble in solvents of intermediate polarity such as alcohol and acetone.

The reaction of unsaturated components of fats with oxygen is one of the most important reactions in lipid chemistry. This reaction is responsible for the development of odors and flavors of rancidity and reversion. Oxidation takes place with oxygen from the air under the influence of heat, light, high energy radiation, and various pro-oxidant catalysts. Among the many reactions with oxygen, autoxidation is one of the most important. It was observed early that when meat fats became rancid, peroxides were developed to varying degrees. Study revealed that these were primarily hydroperoxides during the early stages of autoxidation. These hydroperoxides ($-OOH$) are found on the carbon atom adjacent to a double bonded carbon atom in a fatty acid molecule (Dugan and Slover, 1960). Oxidation in cooked meat is heme catalyzed (Younathan and Watts, 1959). It does not occur in invertebrate muscle which does not have heme pigments, and is stopped completely in skeletal muscle by converting the heme compounds to the cured meat pigments (Watts, 1962).

Unsaturated fatty acids are capable of existing in geometrically isomeric forms,

differing in their configuration about the double bonds. If both carbon chains are on the same side of the double bond, the form is termed "cis", if they are on opposite sides, it is called "trans". The acids occurring naturally in animal fats are, for the most part, the cis forms, although some trans acids have been detected in beef fats (Dugan and Slover, 1960).

Sodium Chloride in Meat

Salt helps in solubilizing proteins. Muscle proteins are separated on the basis of the salt concentration in which they are soluble (Giffey, et al. 1960).

DiMarco (1963) showed that the addition of sodium chloride to meat increased the water holding capacity of meat. He explained that the effect was due primarily to the chloride ion rather than the sodium ion. Disulfide bonds which linked the peptide chains may be split off by the binding of chloride, therefore, increasing water binding by both net charge effect and steric hindrance effect. When salt and water are added to meat, the proteins are dissolved and the meat becomes sticky. Upon heating, the dissolved proteins set up and bind the meat. This phenomenon is often referred to as "binding" of sausage or cured meat (Anonymous, 1965).

Zeigler (1962) pointed out that salt inhibited the growth of bacteria in meat products. In some products salt is more effective in conjunction with nitrite.

Brady et al. (1949) and Callow (1956) reported that salt is an effective dehydrating agent in the curing of meat, and as such, caused the meat to become dry and hard, thus decreasing the rate of microbial growth. Niven and Chesbro (1960) stated that a sufficient concentration of salt inhibited microbial growth as a result

of the increased osmotic pressure of the medium, which also was reflected in the lower water activity in food.

Salt adds flavor to meat products. Sodium chloride in combination with other curing agents has been found to have particular usefulness in lowering the thermal processing required to produce stable retorted meat foods (Niven and Chesbro, 1960).

Water in Meat

Lushbough and Schweigert (1960) reported that lean skeletal muscle has an average of 70 percent moisture. Garner (1966) reported that water comprised 60 to 95 percent of the total weight of a food and was by far the dominant constituent.

Except for a slight natural ionization which leads to the formation of minute amounts of hydrogen and hydroxyl ions, pure water consists of molecules made up of two atoms of hydrogen and one atom of oxygen. These molecules may be aggregated by weak forces in quasi-crystalline combinations whose size and form depend upon physical conditions in effect at the time (Matz, 1965). Water exists in meat and meat products in the free form and in the bound form.

DiMarco (1963) reported on the importance of water in meat curing. He pointed out that high water holding capacity resulted in many cases in a better quality product. He explained further that the water molecule behaved like a magnet because of the two positive charges on the hydrogen atoms and two negative charges on the oxygen atoms. Hence, the charges on a water molecule can attract or repel another water molecule. He showed that the reason why muscle tissue bound 70 to 75 percent of its weight in water was because of the attraction between

meat proteins and the molecular magnet, water. Hamm (1963) reported that water was immobilized within the meshes of the protein network in food. Matz (1965) stated that the texture of both dry and high moisture sausages were affected by the moisture content, although that in the high-moisture types with small particle size, the water content had a greater influence on the over-all texture than in dry products. Frazier (1958) explained the available moisture as it affected bacteria in foods. He indicated that water activity was in equilibrium with the relative humidity of the atmosphere about the food.

Meat Emulsions

Sorum (1963) has defined an emulsion as "any system in which one liquid is colloidally dispersed in another". Wilson (1960) reported that the physical structure and properties of a sausage mixture so resembled a true emulsion that the term is applied. A sausage emulsion has characteristics similar to an oil-in-water. Hansen (1960) studied meat emulsion formation. He stated, that a sausage batter is commonly referred to as an emulsion of fat globules in a continuous fluid protein phase. Carpenter and Saffle (1964) reported that the various constituents of a sausage emulsion - water, protein, and fat - have been identified as the continuous phase, emulsifying agent, and discontinuous phase, respectively. They also stated that the theory of sausage emulsions was: Lean meat is added to a chopper along with salt, other seasonings and water in the form of ice. Water and salt, aided by the action of the chopper serves to emulsify the mix, resulting in the fat particles being surrounded by protein and suspended in water.

Hansen (1960) reported that, since the fat globule membrane is formed only

from salt-soluble extracts, it appears to be composed, at least in part, of the salt-soluble proteins myosin and actomyosin. Wilson (1960) stated that the salt-soluble protein, myosin, acts as the primary emulsifying agent in sausage emulsions. Price (1964) also suggested that the contractile proteins in red muscle were the proteins involved in emulsion formation. Trautman (1964) studied the rate of fat separation to determine the relative emulsifying capacity of ham muscle proteins. In this study he found that the salt-soluble proteins were the most effective fat emulsifiers. The salt-soluble residues and water-insoluble proteins were found to possess very little emulsifying power, and were not influenced by the post-mortem time. Voegeli (1966) suggested that the proteins must be soluble to be an effective fat emulsifiers. Helmer and Saffle (1963) indicated that the salt-soluble proteins were the primary emulsifying agents in sausage emulsions. Swift et al. (1961) studied the capacity of various meat for emulsifying fat. They showed, microscopically, that membranes formed by salt-soluble proteins consisted of thicker layers than those formed by water-soluble proteins. Hegarty et al. (1963) found that actin, myosin, and actomyosin had greater emulsifying capacities than did the sarcoplasmic proteins, but at the pH of normal fresh meat (5.6-5.8), the sarcoplasmic fractions produced the most stable emulsions. Swift et al. (1961) reported that the water-soluble proteins did have a low emulsifying capacity, but that the action of sodium chloride appeared to enhance the tendency of these proteins to stabilize emulsions. Swift and Sulzbacher (1963) studied some factors affecting meat proteins as emulsion stabilizers. They found that sodium chloride increased the emulsifying capacity of the water-soluble proteins by unfolding their structure, thus extending their ability to enclose fat globules in membranes. The emulsifying capacity of the salt-

soluble proteins was not increased by the addition of sodium chloride. Carpenter and Saffle (1964) reported on the emulsifying capacity of various meat trimmings based on a unit of soluble protein. Barton et al. (1968) studied the emulsifying capacity of several sausage meat trimmings. They reported that chopped beef cheek meat, with 3 percent sodium chloride, added approximately 20 hours prior to emulsion preparation, had a greater emulsifying efficiency, based on a unit of protein, than did beef cheek meat with no sodium chloride added, but did not emulsify a greater amount of fat when based on amount of oil emulsified per gram of sample. Thus, the addition of sodium increased the emulsifying efficiency of the proteins without a decrease in emulsion stability.

Trautman (1964) found that pre-rigor protein extracts from porcine muscle had improved emulsifying power as compared to post-rigor extracts. As an explanation for this, it was found that the pre-rigor extracts contained a higher percent of the protein as salt-soluble protein. Within the procedure used for comparison of these proteins, he concluded that the pre-rigor proteins are several times as effective in fat emulsification as the post-rigor proteins. This study suggested the desirability of using pre-rigor meat in certain formulation where rendering was a problem.

Saffle and Galbreath (1964) made quantitative determinations of salt-soluble proteins in various types of meat. They found that the pH of the meat had a significant effect on the amount of salt-soluble protein which could be extracted. Any rise in the pH from the isoelectric point of the meat proteins resulted in an increased amount of protein which could be extracted. The amount of salt-soluble protein in this study was 50 percent greater in pre-rigor beef than in beef 48 hours post-mortem. If frozen, it was found that the amount of salt-soluble protein decreased

approximately 9 percent as compared with the 48-hour post-rigor meat. It was suggested that a possible cause for the decreased amount of soluble protein might be that some denaturation occurred during freezing which caused a decrease in the solubility of the protein. They also noted that portions of the beef carcass having high contents of collagen and elastin show a lower quantity of salt-soluble protein as compared with portions of the beef carcass having the least amount of collagen and elastin. It was also noted that skeletal and cardiac muscle contained considerably more soluble protein than smooth muscle meat. This is in agreement with the findings of Borton et al. (1968).

Meyer et al. (1964) studied the effects of lecithin, oleic acid and eight commercial food emulsifiers in frankfurter emulsions to determine their value in preventing fat separation. The results of this study showed that an increase in emulsifier concentration resulted in a less stable processed product.

Pearson et al. (1965) studied the emulsifying capacity and stability of soy-sodium proteinate, potassium caseinate, and non-fat dry milk. They found that the soy-sodium proteinate and potassium caseinate were most effective as emulsifiers at a high pH (10.5) and tended to have the greatest emulsifying capacities at low ionic strengths. At lower concentrations, non-fat dry milk had the greatest emulsifying capacity of any of the protein additives in the approximate pH range of meat (5.4-5.6).

Helmer and Saffle (1963) studied the effect of chopping temperature on the stability of sausage emulsions. They found that emulsions were stable at chopping temperatures of 60°F, but that there was emulsion breakdown to some extent in all emulsions chopped to 70°F, and complete breakdown occurred in every case for

emulsions chopped to 80°F and 90°F.

Saffle and Galbreath (1964) and Wierbicki et al. (1956) have reported similar quantitative methods of determining the salt-soluble protein content of chilled meat.

CHAPTER III

MATERIALS AND METHODS

Eleven market weight hogs (5 Hampshire barrows, 4 Hampshire gilts, 2 Yorkshire gilts) all with normal (pre-rigor) pH muscle ($\text{pH} > 6.2$ at 30 minutes post-mortem) were used. Also four market weight hogs (1 Hampshire barrow and 3 Yorkshire barrows) all with low (pre-rigor) pH muscle ($\text{pH} < 5.8$ at 30 minutes post-mortem) were included, making a total of 15 animals. The same analysis was run on both populations. The 15 animals ranged in weight from 86.2 to 113.4 kg and were obtained from the Oklahoma Agricultural Experiment Station herd. The animals were from similar managerial background and ranged in age from 22 to 50 weeks. The animals were delivered to the Meat Laboratory approximately one hour before slaughter. Prior to stunning, the animals were washed with warm water (35°C). Each animal was stunned, using a Cervin Model MM electrical tool, shackled by one leg, raised from the floor, and bled in the conventional manner. The animals were skinned and eviscerated as rapidly as possible. The carcasses were split, washed thoroughly, and the leaf fat was removed. Pre-rigor muscle, in this study, is defined as that muscle which has either been analyzed or treated within two hours post-mortem. Post-rigor muscle is that chilled muscle which has either been analyzed or treated approximately 26 hours post-mortem.

Sampling Procedure

The sides were placed on a cutting table, and a section of the longissimus dorsi muscle from the 8th to the 13th vertebrae was removed. Right and left sides were alternated from one animal to the next. The longissimus dorsi muscle was excised from the five rib section approximately 15 minutes post-mortem. All fat and connective tissue was removed from the outside of the muscle. Each muscle was then ground once through a 3 mm plate and mixed thoroughly in a plastic beaker.

This study was conducted in two parts. Part I provided a comparison of the extractable salt-soluble protein content in pre- and post-rigor muscle. Part II is a comparison of the extractable salt-soluble protein content in pre- and post-rigor muscle treated with three sodium chloride levels. The sampling procedure for these two parts are as follows:

Part I

Approximately 30 minutes post-mortem two - 10 g pre-rigor muscle samples were weighed into 50 ml beakers using a Mettler balance. After weighing, the beakers were covered with saran wrap to prevent moisture loss from the samples. Each sample, was then analyzed (45 minutes post-mortem) for its extractable salt-soluble protein content. Twenty-four hours later, after the ground meat had gone into rigor-mortis, two post-rigor samples were weighed, handled in the same manner, and analyzed (24 hours post-mortem) for extractable salt-soluble protein content (Figure 1).

Part II

While the two pre-rigor samples were being analyzed for extractable salt-

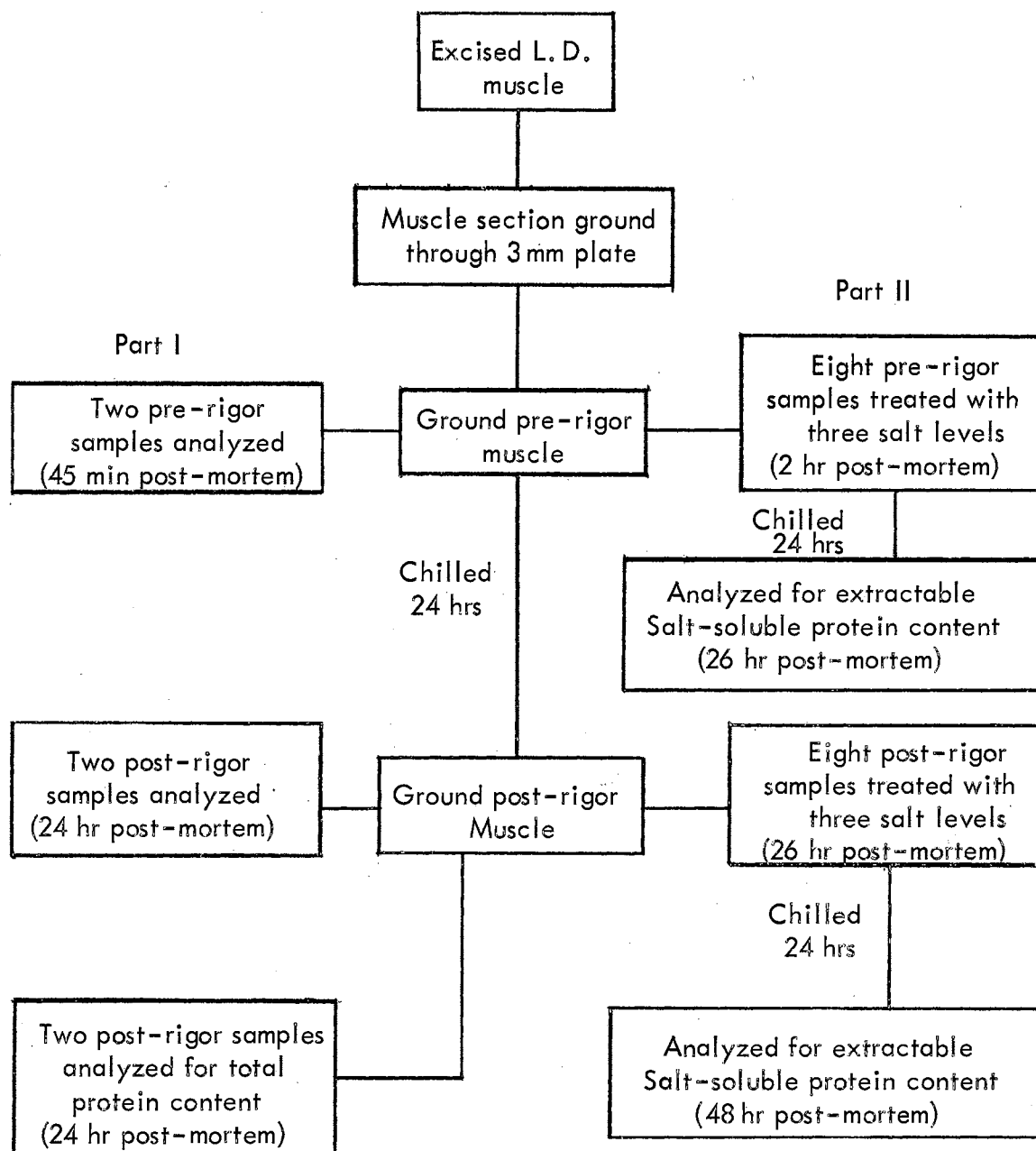


Figure 1. Flow Chart for Sampling Procedure.

soluble protein (Part I), a technician weighed eight - 10 g pre-rigor samples into 50ml beakers, each carefully covered after weighing with saran wrap to prevent moisture loss. When all eight samples were weighed (2 hours post-mortem), the samples were randomly assigned in duplicate to three sodium chloride treatments. Two of the samples received 2ml of distilled water, two received 2ml of a 5 percent sodium chloride solution, two received 2ml of a 10 percent sodium chloride solution, and the remaining two received 2ml of a 15 percent sodium chloride solution. Two ml of the 5 percent sodium chloride solution provided one percent sodium chloride by weight of the meat. The 10 and 15 percent solutions gave 2 and 3 percent sodium chloride in the meat by weight, respectively. The 2ml of distilled water was used as a control. The sodium chloride solutions were stirred into the ground pre-rigor meat for approximately 30 seconds for each sample. After mixing, the eight beakers were again covered with saran wrap to prevent moisture loss, and were placed into a 2°C cooler for a period of 24 hours. Twenty-four hours after the treatment had been applied (26 hours post-mortem), the eight samples were analyzed for extractable salt-soluble protein content.

At the same time that the pre-rigor treated samples were being analyzed, a technician weighed out eight - 10 g post-rigor samples. They were treated in the same manner as the eight pre-rigor samples, placed in the 2°C cooler, and analyzed for extractable salt-soluble protein content 24 hours after being treated (48 hours post-mortem) (Figure 1). The sodium chloride used in the different saline solution was pure granulated brine salt, and the solutions were kept at room temperature.

Salt-soluble Protein Extraction

The procedure used for extraction of the salt-soluble protein was similar to that of Saffle and Galbreath (1964). Extraction of the salt-soluble protein was accomplished using a 3 percent saline solution, in an extraction solution to muscle ratio of 8:1. The sodium chloride used in the extraction solution was of reagent grade.

Ten ml of the extraction solution was pipetted into each beaker contained the samples to aid in the removal of the muscle from the beaker. The sample plus the 10 ml of extraction solution was then placed in a 300 ml omni-mixer can. Seventy ml of extraction solution were used to wash out any tissue left in the 50 ml beaker. Washings were carefully poured into the omni-mixer can containing the respective sample. The 10 gm sample plus the 80 ml of extraction solution was then blended in a Sorvall omni-mixer for a period of four minutes with the omni-mixer can in an ice-water bath to prevent protein denaturation. The homogenate was poured into a 200 ml autoclaveable polypropylene centrifuge bottle. Any homogenate left in the omni-mixer can was then washed into the centrifuge can with distilled water. The homogenate was centrifuged for 10 minutes at 16,300 X G in a Serval Model RC2-B refrigerated ultra-centrifuge. The supernatant was decanted into another centrifuge bottle and centrifuged again for 10 minutes at 16,300 X G. The supernatant was filtered through a cheese cloth filter into a 200 ml volumetric flask. The fluid was made to volume with distilled water and the extractable salt-soluble protein and distilled water were mixed thoroughly. All extraction procedures were carried out at 2°C and all chemicals were chilled to 2°C. The samples were extracted at random.

Salt-soluble Protein Determination

The procedure used to determine extractable salt-soluble protein content was by the Biuret test (Layne et al. 1957). The Biuret reagent was made by dissolving 1.5 gm of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 6 gm of $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ in 500 ml of water. To this mixture 300 ml of 10 percent NaOH (carbonate free) was added. This solution was diluted to 1 liter with water, and stored in a paraffin-lined bottle. The protein standard was made by dissolving 1 g of bovine serum albumin in distilled water and made to 100 ml volume. This gave a protein standard with the concentration of 10 per ml.

The procedure for the Biuret test was as follows: To 1 ml of a solution containing 1 to 10mg of protein per ml add 4ml of Biuret reagent, mix by swirling, and allow to stand for 30 minutes at room temperature. Determine optical density at 550mu. One ml of the protein extract was used to determine protein content.

The concentration was calculated from the formula:

$$\text{Protein concentration (mg/ml)} = (K) (\text{O.D.}) (\text{Dilution factor});$$

$$\text{where } K = \frac{1}{\text{average O.D./mg protein}}$$

$$\text{Protein concentration (mg/gm of sample)} =$$

$$\frac{\text{Protein conc. (mg/ml)} \times 200}{\text{sample weight}}$$

Extractable salt-soluble protein content was also calculated as percent extractable salt-soluble protein:

$$\text{Percent extractable salt-soluble protein} =$$

$$\frac{\text{Salt-soluble protein content (mg/gm sample)}}{\text{Total protein content (mg/gm sample)}}$$

Determination of pH

The pH values of the ground muscle were determined with the use of a Corning Model 10 pH meter. Pre-rigor pH was determined approximately 30 minutes post-mortem, and post-rigor pH was determined after 24 hours. The electrode was inserted directly into the ground muscle at three different places and an average of three reading was recorded as the pH of the muscle.

Total Protein Determination

Duplicate total protein determinations were conducted on each muscle. The muscle used in the determination came from the post-rigor ground muscle (Figure 1). Total protein analysis was determined by the Macro-Kjeldahl nitrogen determination method (A.O.A.C., 1965).

Statistical Analysis

Part I

A randomized complete block design was used. Comparisons between pre- and post-rigor muscle were determined by using an Analysis of Variance and F test, with animals representing blocks (Steel and Torrie, 1960). The error term in the analysis used for testing was not a true estimate of experimental error per se, but an estimate of the animal by treatment interaction.

Part II

A randomized complete block design with a factorial arrangement of treatments

was used. An Analysis of Variance and F test aided in determining the effect of animal and treatments, and their interactions. The error term in the analysis used for testing was not a true estimate of experimental error per se, but an estimate of the animal by treatment combination interaction (Steel and Torrie, 1960).

Comparisons between normal-pH and low-pH animals were made by the use of the T-test (Steel and Torrie, 1960).

CHAPTER IV

RESULTS AND DISCUSSION

The mean and standard error were calculated for the extractable salt-soluble protein content of the pre- and post-rigor lean tissue and for each sodium chloride treatment. The discussion and data for Part I were separated into the comparisons between extractable salt-soluble protein content of pre- and post-rigor normal-pH and low-pH meat, and into the comparisons between normal-pH and low-pH pre- and post-rigor muscles. The discussion and data for Part II were separated into the effect of various sodium chloride treatments on both pre- and post-rigor normal-pH and low-pH meat, and into the comparisons of the various sodium chloride treatments between normal-pH and low-pH muscles. Comparisons were tested using either the Analysis of Variance or the T-test.

Extractable protein content, expressed as percent salt-soluble protein of total protein, is shown in the Appendix (p. 62) for Parts I and II. The results are expressed in mg of salt-soluble protein per gram of sample. Extractable salt-soluble protein content presented in the results and discussion is expressed in mg/g of sample to eliminate the extra source of error which occurred in the determination of the total protein.

A highly significant difference ($P < .01$) was found in the extractable salt-soluble protein content between the pre- and post-rigor normal-pH muscles (Tables I and II). This was to be expected, and is in agreement with the findings of Saffle and Galbreath (1964), who reported that the extractable salt-soluble protein con-

TABLE I

EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR NORMAL-pH PORCINE MUSCLE

Animal No.	Pre-Rigor		Post-Rigor	
	Protein ^c	pH	Protein	pH
1	128.71	6.70	78.89	5.40
	126.87		77.58	
2	124.83	6.45	55.92	5.25
	125.53		57.03	
3	108.88	6.55	58.30	5.38
	109.88		58.31	
4	102.59	6.55	65.66	5.45
	104.30		64.51	
5	113.67	6.50	68.14	5.45
	112.06		68.74	
6	111.89	6.20	71.72	5.52
	108.77		70.01	
7	119.67	6.45	67.63	5.38
	117.04		66.46	
8	122.99	6.20	79.55	5.65
	126.82		78.89	
9	106.38	6.42	52.80	5.26
	104.91		51.34	
10	95.73	6.41	62.32	5.35
	97.93		62.88	
11	122.48	6.45	84.63	5.32
	125.87		83.32	
Mean	114.45 ^a	6.44	67.48 ^b	5.40
S.E.	2.87	-	2.87	-

^{ab}Highly significant difference ($P < .01$) between muscles.

^cExpressed in mg of protein per g of sample.

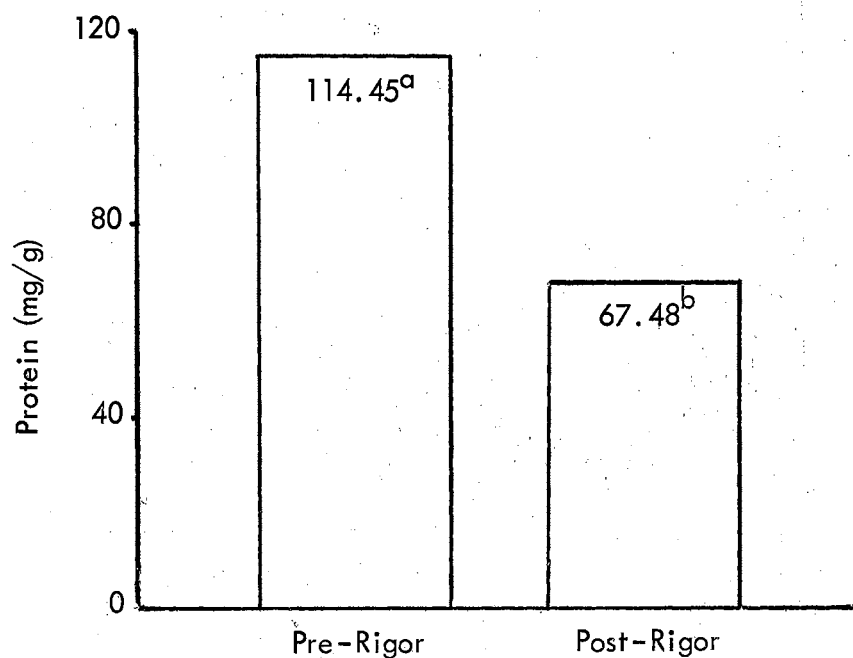
TABLE II

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE- AND POST-RIGOR NORMAL-pH PORCINE MUSCLE

Source	d. f.	S. S.	M. S.	F
Total	43	28,450.82	-	-
An.-Treat. Comb.	21	28,413.95	1,353.05	14.9492**
Animal	10	3,248.82	324.88	3.5890**
Treatment	1	24,260.00	24,260.00	268.0367**
Animal X Treatment	10	905.13	90.51	-
Within-An. X Treat. -Comb.	22	36.87	1.68	-

*P<.05

**P<.01



^{ab}Highly significant difference (P<.01) between pre- and post-rigor muscles.

Figure 2. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle.

tent in pre-rigor beef was 50 percent greater than post-rigor beef. The pH of the pre-rigor muscle was much farther away from the isoelectric point of the muscle proteins than the pH of the post-rigor muscle, thus allowing for greater protein extraction. In this study, the extractable salt-soluble protein content in pre-rigor muscle was found to be 69.61 percent greater than that in the post-rigor muscle. Significant animal differences ($P < .05$) were noted (Table II). Differences among animals in extractable salt-soluble protein content were probably due to inherent differences in muscle pH, intra-muscular fat, hemoglobin, and myoglobin content. The extractable salt-soluble protein content in pre- and post-rigor normal-pH muscles is shown graphically in Figure 2.

The difference in extractable salt-soluble protein content between pre- and post-rigor low-pH muscles was found to be significant ($P < .05$) (Tables III and IV). This was to be expected since the pH of the pre-rigor muscle was consistently higher than the pH of the post-rigor muscle (Table III). It should be noted that the pre-rigor low-pH muscle only contained 7.30 percent more extractable salt-soluble protein than the post-rigor low-pH muscle, whereas the pre-rigor normal-pH muscle contained 69.61 percent greater extractable salt-soluble protein than the post-rigor muscle. This was anticipated since the difference between the pH of the pre- and post-rigor low-pH muscles was much less than the difference between the pH of the pre- and post-rigor normal-pH muscles (Tables I and III). A highly significant difference ($P < .01$) was noted for animals (Table IV). The inherent variations between muscle pH, intra-muscular fat, hemoglobin and myoglobin content may account for the wide differences. The difference in extractable salt-soluble protein content of pre- and post-rigor low-pH muscles is shown as a bar graph in

TABLE III
EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN
PRE- AND POST-RIGOR LOW-pH
PORCINE MUSCLE

Animal No.	Pre-Rigor		Post-Rigor	
	Protein ^c	pH	Protein	pH
1	63.21	5.65	53.46	5.31
	62.11		56.11	
2	82.42	5.85	75.47	5.46
	81.12		77.45	
3	69.69	5.55	66.64	5.45
	69.10		66.09	
4	74.01	5.80	70.36	5.34
	74.62		71.53	
Mean	72.04 ^a	5.71	67.14 ^b	5.39
S.E.	1.11	-	1.11	-

^{ab}Significant difference ($P < .05$) between muscles.

^cExpressed in mg of protein per g of sample.

TABLE IV
ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN PRE- AND POST-RIGOR LOW-pH PORCINE MUSCLE

Source	d.f.	S.S.	M.S.	F
Total	15	1,002.85	-	-
An. - Treat. Comb.	7	994.73	142.10	28.71**
Animal	3	883.98	293.66	59.33**
Treatment	1	95.89	95.89	19.37*
Animal X Treatment	3	14.86	4.95	-
Within-An. X Treat. - Comb.	8	8.12	1.02	-

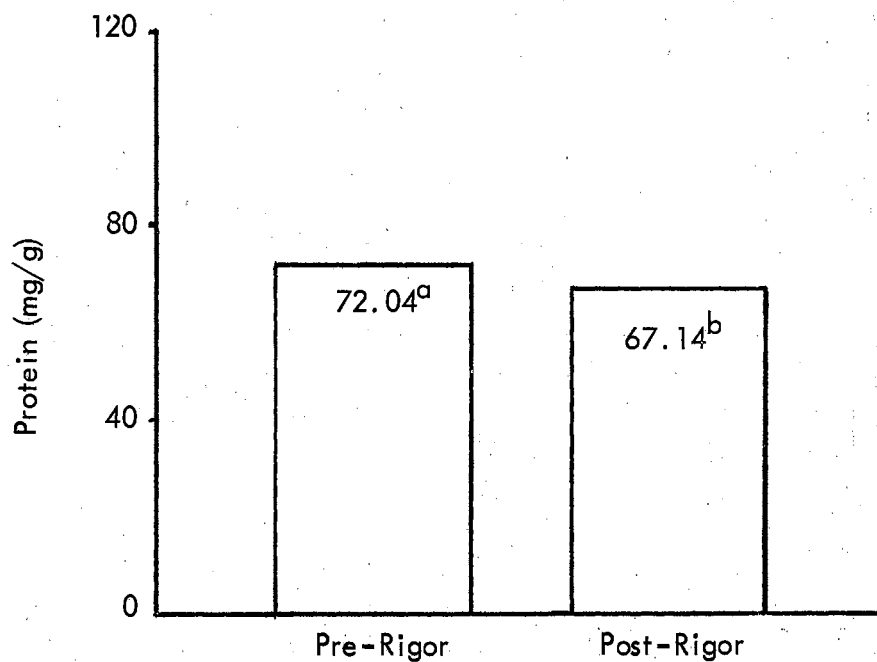
* $P < .05$
** $P < .01$

Figure 3.

A graphic comparison between extractable salt-soluble protein content of pre-rigor normal-pH and pre-rigor low-pH muscles is given in Figure 4. A significant difference ($P < .05$) was found to exist between the pre-rigor normal-pH and low-pH muscles in extractable salt-soluble protein content. The pre-rigor normal-pH muscle contained 58.87 percent greater extractable salt-soluble protein content than did the pre-rigor low-pH muscle. This can be explained by the much higher pH of the pre-rigor normal-pH muscle (Tables I and III). It can be seen that the pre-rigor low-pH muscle reacted very similarly to that of the post-rigor normal-pH muscle, due to its low initial pH (Figures 2 and 4).

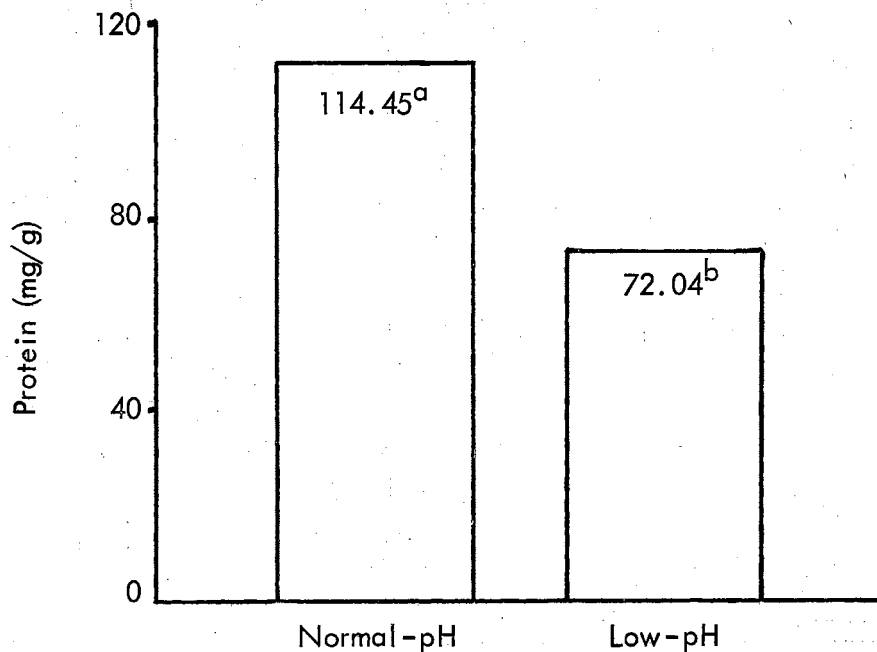
A comparison between extractable salt-soluble protein content of post-rigor normal-pH and post-rigor low-pH muscles is given in Figure 5. The post-rigor normal-pH muscle had a slightly higher extractable salt-soluble protein content than the post-rigor low-pH muscle, however, the difference between the treatment means was not significant.

The non-significant difference in extractable salt-soluble protein content between pre-rigor low-pH and post-rigor normal-pH muscles is shown graphically in Figure 6. No significant difference was expected since the pH of the pre-rigor low-pH muscle very closely approached the pH of the post-rigor normal-pH muscle (Tables I and III). During the course of the experiment it was also observed that the texture and water holding capacity of the pre-rigor low-pH muscle resembled very closely the texture and water holding capacity of the post-rigor normal-pH muscle and post-rigor low-pH muscle. This was considered to be due to its low initial pH.



^{ab}Significant difference ($P < .05$) between pre- and post-rigor muscles.

Figure 3. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH Porcine Muscle.



^{ab}Significant difference ($P < .05$) between normal-pH and low-pH muscles.

Figure 4. Extractable Salt-Soluble Protein Content in Normal-pH and Low-pH Pre-Rigor Porcine Muscle.

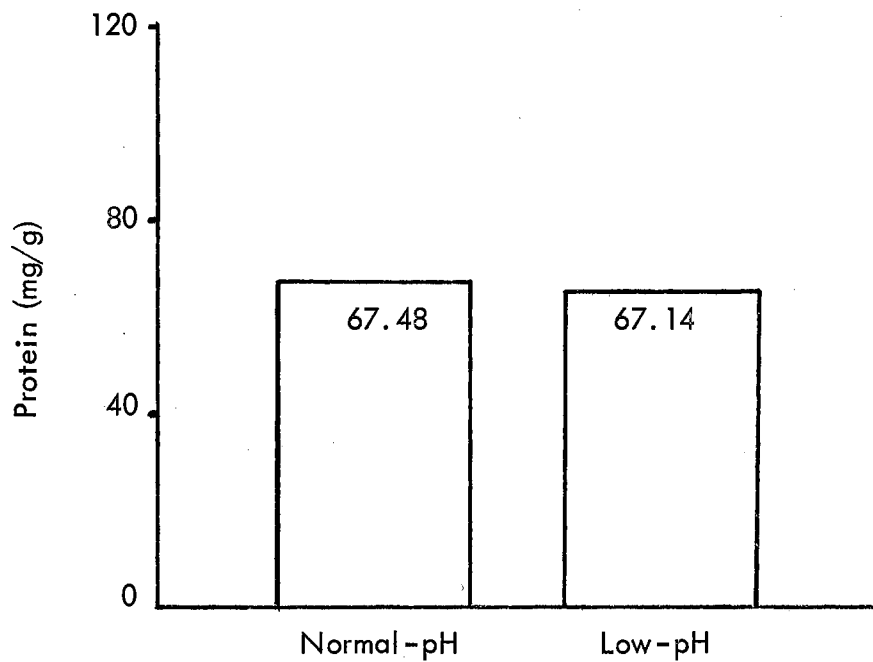


Figure 5. Extractable salt-Soluble Protein Content in Normal-pH and Low-pH Post-Rigor Porcine Muscle

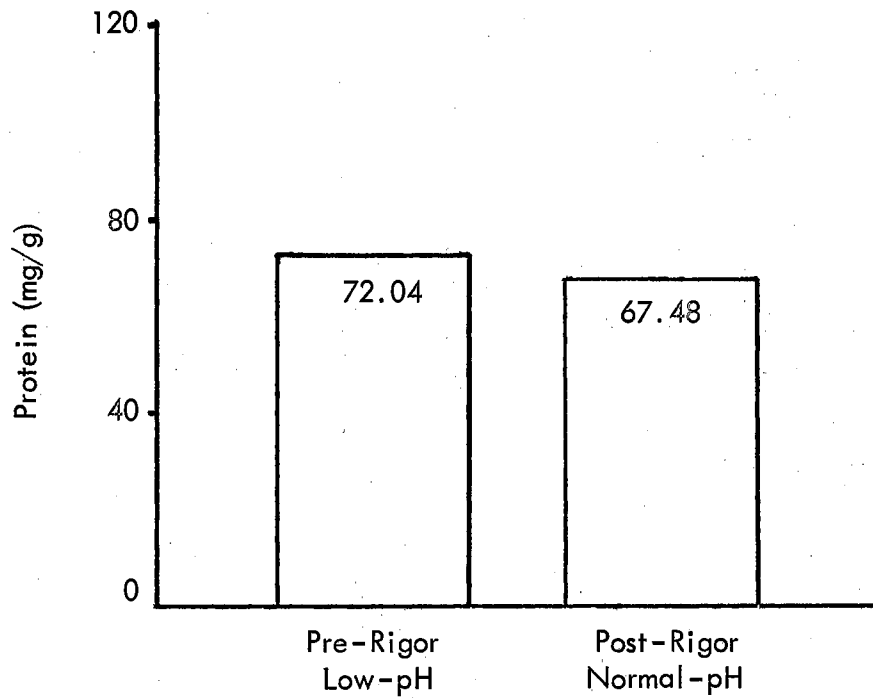


Figure 6. Extractable Salt-Soluble Protein Content in Normal-pH Post-Rigor and Low-pH Pre-Rigor Porcine Muscle

Part II

The extractable salt-soluble protein content of pre-rigor normal-pH muscle at three sodium chloride levels is given in Table V. The corresponding Analysis of Variance is presented in Table VI, and a plot of the means at each sodium chloride level is given in Figure 7.

Differences in the extractable salt-soluble protein content among the three sodium chloride levels were found to be highly significant ($P < .01$) (Tables V and VI). Orthogonal comparisons were made among the means of the salt-soluble protein content at the three sodium chloride levels. A test of linearity proved highly significant ($P < .01$), showing that as the added amount of sodium chloride increased, so did the amount of extractable salt-soluble protein (Figure 7). The test for the quadratic effect was found to be non-significant. The 1 percent sodium chloride treatment increased the amount of extractable salt-soluble protein 3.35 percent over the zero level, while the 2 and 3 percent sodium chloride treatments increased the extractable salt-soluble protein content 11.41 and 11.67 percent, respectively. The sharp increase in extractable salt-soluble protein at the 2 and 3 percent treatments can be explained by the fact that 2 percent sodium chloride tended to block the onset of rigor mortis (Hamm, 1958). The formation of the actomyosin complex does not have a chance to occur, and the salt-soluble proteins are in a more soluble state, allowing for greater extraction.

The 3 percent concentration may have caused protein denaturation to some degree resulting in a decrease in protein solubility. Further work is necessary to show if this is really what has occurred. Highly significant differences ($P < .01$) were noted in extractable salt-soluble protein content among animals (Table VI).

TABLE V
 EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN
 PRE-RIGOR NORMAL-pH PORCINE MUSCLE
 AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Sodium Chloride (%)			
	0	1	2	3
	mg/g of sample			
1	92.80 94.95	94.12 93.52	100.66 100.63	90.01 89.43
2	59.75 59.71	62.48 62.48	68.79 67.04	74.07 73.45
3	61.09 60.47	63.91 63.86	72.03 71.43	72.01 72.03
4	70.86 69.12	73.20 69.08	75.65 73.82	79.35 77.48
5	67.01 65.31	71.08 71.07	81.51 81.58	82.20 83.45
6	72.36 72.96	75.34 75.98	80.92 82.25	77.87 79.09
7	67.62 67.63	73.44 72.86	80.17 80.80	79.54 80.13
8	78.33 80.20	81.47 81.44	83.37 82.71	84.04 85.31
9	55.38 52.85	55.90 55.38	58.52 59.59	60.68 60.67
10	64.00 63.44	66.20 64.56	70.18 69.05	70.19 70.16
11	82.05 82.61	82.12 82.62	87.75 87.77	89.13 89.79
Mean ^b	70.02	72.37	78.01	78.19
S.E.	1.18	1.18	1.18	1.18

^aSodium chloride content of sample by weight.

^bHighly significant differences ($P < .01$) among sodium chloride levels.

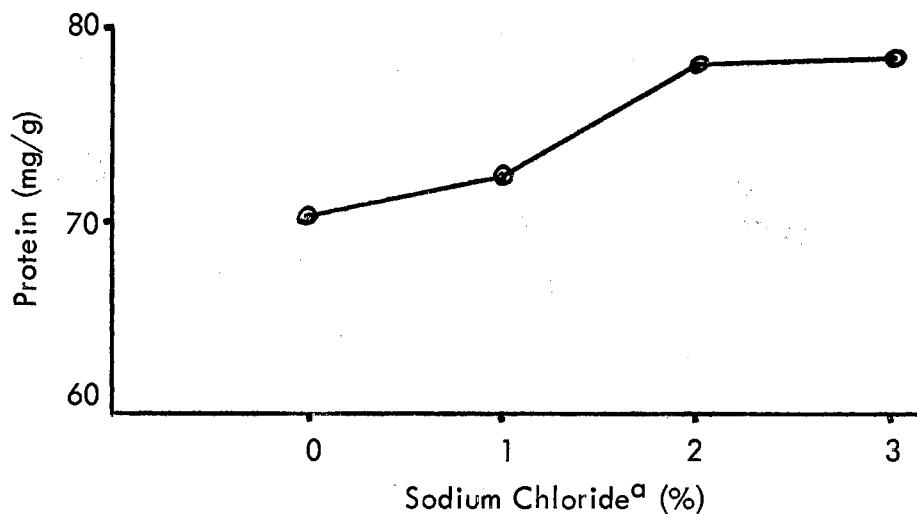
TABLE VI

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN PRE-RIGOR NORMAL-pH PROCINE
MUSCLE AT THREE SODIUM CHLORIDE LEVELS

Source	d.f.	S.S	M.S.	F
Total	87	10,090.01	-	-
An. - Treat. Comb.	43	10,057.74	233.90	15.40**
Animal	10	8,493.07	840.31	55.32**
Treatment	3	1,108.92	369.64	24.33**
Linear	1	998.57	998.57	65.74**
Quadratic	1	25.91	25.91	1.63 ^{ns}
Cubic	1	84.44	84.44	5.56*
Treat. Comb. X An.	30	455.75	15.19	-
Within-An. X Treat. Comb.	44	32.27	0.73	-

*P < .05

**P < .01

^{ns}Non-significant (P < .05)

^aHighly significant differences (P < .01) among sodium chloride levels.

Figure 7. Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH Muscle at Three Sodium Chloride Levels.

Here again animal differences are probably due to inherent differences in muscle pH, intramuscular fat, hemoglobin content, and myoglobin content.

Differences among the sodium chloride levels in extractable salt-soluble protein content of post-rigor normal-pH muscle were found to be highly significant ($P < .01$) (Tables VII and VIII). The test for linearity was also highly significant ($P < .01$), showing that as the amount of sodium chloride increased, the amount of salt-soluble protein extracted decreased (Figure 8). The 1, 2, and 3 percent sodium chloride treatments contained 1.17, 2.81, and 7.05 percent less extractable salt-soluble protein than did the zero percent level, respectively. The post-rigor muscle proteins are tied up in the actomyosin complex and the pH of the post-rigor muscle is at the isoelectric point of the muscle proteins, thus the proteins are in a less soluble state when the sodium chloride is added. It appeared that the more sodium chloride that was added caused a decrease in the solubility of the post-rigor muscle proteins even further, and possibly the 3 percent level even caused some protein denaturation. These results agree with the findings of Borton *et al.* (1968), who reported that 3 percent sodium chloride added to beef cheek meat 20 hours before emulsion preparation decreased the emulsifying capacity of the meat when the emulsifying capacity was expressed as, amount of oil emulsified per gram of meat. This indicated that less salt-soluble protein was available in the sodium chloride treated meat than in the non-treated meat. Animal differences were noted to be highly significant ($P < .01$) for extractable salt-soluble protein content (Table VIII).

Extractable salt-soluble protein content in pre- and post-rigor normal-pH muscle at three levels of sodium chloride is given in Table IX. The corresponding Analysis of Variance is presented in Table X. Figure 9 shows a plot of the means

TABLE VII
 EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN
 POST-RIGOR NORMAL-pH PROCINE MUSCLE
 AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Sodium Chloride (%)			
	0	1	2	3
	mg/g of sample			
1	86.59	87.08	89.05	86.46
	86.46	87.07	89.15	86.46
2	62.48	63.03	58.04	59.12
	61.93	63.01	59.14	55.90
3	58.40	58.45	54.73	51.12
	58.96	58.40	55.77	51.60
4	63.86	63.91	63.27	58.37
	62.76	63.83	63.30	57.25
5	67.39	65.74	65.74	63.60
	67.99	66.31	65.19	63.05
6	70.99	66.33	67.44	60.71
	68.08	67.45	65.22	58.58
7	65.70	63.58	58.62	60.25
	65.72	62.98	59.16	59.74
8	83.05	83.73	84.34	81.80
	82.37	83.05	83.70	83.08
9	48.95	45.58	44.12	41.31
	49.43	46.01	44.61	40.85
10	58.77	56.68	59.27	56.13
	59.30	55.62	58.29	55.64
11	84.92	85.49	83.61	83.63
	83.63	86.86	84.91	84.28
Mean ^b	68.07	67.28	66.21	63.59
S.E.	0.78	0.78	0.78	0.78

^aSodium chloride content of sample by weight.

^bHighly significant differences ($P < .01$) among sodium chloride levels.

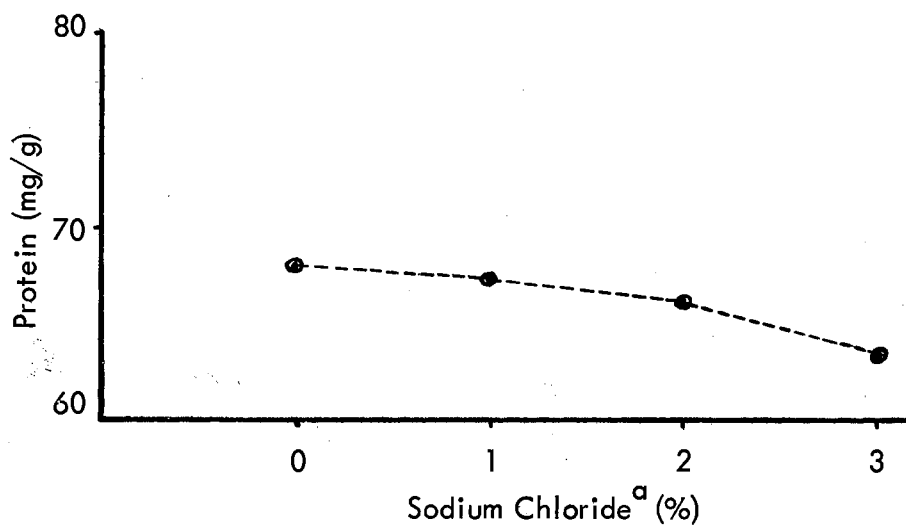
TABLE VIII

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN POST-RIGOR NORMAL-pH PORCINE
MUSCLE AT THREE SODIUM CHLORIDE LEVELS

Source	d.f.	S.S.	M.S.	F
Total	87	14,626.10	-	-
An. -Treat. Comb.	43	14,601.95	339.58	51.69**
Animal	10	14,152.25	1,415.23	215.41**
Treatment	3	252.46	84.15	12.81**
Linear	1	232.22	232.22	35.35**
Quadratic	1	18.45	18.45	2.81 ^{ns}
Cubic	1	1.80	1.80	0.27 ^{ns}
Treat. Comb. X An.	30	197.24	6.57	-
Within-An. X Treat. Comb.	40	24.15	0.55	-

**P < .01

^{ns} Non-Significant (P < .05)



^a Highly significant differences (P < .01) among sodium chloride levels.

Figure 8. Extractable Salt-Soluble Protein Content in Post-Rigor Normal-pH Muscle at Three Sodium Chloride Levels.

TABLE IX
EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR NORMAL-pH PORCINE MUSCLE
AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Pre-Rigor ^b				Post-Rigor ^c			
	Sodium Chloride ^d (%)							
	0	1	2	3	0	1	2	3
	mg/g of sample							
1	92.80	94.12	100.66	90.01	86.50	87.08	89.05	86.46
	94.95	93.52	100.63	89.43	86.46	87.07	89.15	86.46
2	59.75	62.48	68.79	74.07	62.48	63.03	58.04	59.12
	59.71	62.48	67.04	73.45	61.93	63.01	59.14	55.90
3	61.09	63.91	72.03	72.01	58.40	58.45	54.73	51.12
	60.47	63.86	71.43	72.03	58.96	58.40	55.77	51.60
4	70.86	73.20	75.65	79.35	63.86	63.91	63.27	58.37
	69.12	69.08	73.82	77.48	62.76	63.83	63.30	57.25
5	67.01	71.08	81.51	82.20	67.39	65.74	65.74	63.60
	65.31	71.07	81.58	83.45	67.99	66.31	65.19	63.05
6	72.36	75.34	80.92	77.87	70.99	66.33	67.44	60.71
	72.96	75.98	82.25	79.09	68.08	67.45	65.22	58.58
7	67.62	73.44	80.17	79.54	65.70	63.58	58.62	60.25
	67.63	72.86	80.80	80.13	65.72	62.98	59.16	59.74
8	78.33	81.47	83.37	84.04	83.05	83.73	84.34	81.80
	80.20	81.44	82.71	85.31	82.37	83.05	83.70	83.08
9	55.38	55.90	58.52	60.68	48.95	45.58	44.12	41.31
	52.85	55.38	59.59	60.67	49.43	46.01	44.61	40.85
10	64.00	66.20	70.18	70.19	58.77	56.68	59.27	56.13
	63.44	64.56	69.05	70.16	59.30	55.62	58.29	55.64
11	82.05	82.12	87.75	89.13	84.92	85.49	83.61	83.63
	82.61	82.62	87.77	89.79	83.63	86.86	84.91	84.28
Mean	70.02	72.37	78.01	78.19	68.07	67.28	66.21	63.59
S.E.	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42

^aSodium chloride content of sample by weight

^{bc}Highly significant difference ($P < .01$) between pre- and post-rigor muscles.

^dSignificant differences ($P < .05$) among sodium chloride levels.

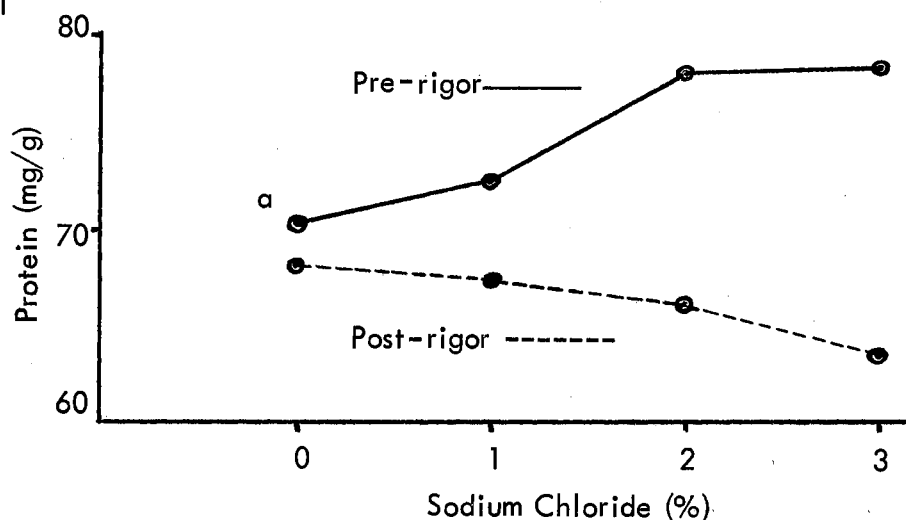
TABLE X

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN PRE- AND POST-RIGOR NORMAL-pH PORCINE
MUSCLE AT THREE SODIUM CHLORIDE LEVELS

Source	d. f.	S. S.	M. S.	F
Total	175	27,790.18	-	-
An. - Treat. Comb.	87	27,733.15	318.77	14.37**
Animal	10	21,744.97	2,174.50	97.99**
Treat. Comb.	7	4,434.85	633.55	28.55**
Pre- and Post-Rigor Muscles (A)	1	3,073.47	3,073.47	138.51**
Sodium Chloride Level (B)	3	233.34	77.78	3.51*
A X B	3	1,128.04	376.01	16.95**
Treat. Comb. X An.	70	1,553.33	22.19	-
Within-An. X Treat. Comb.	88	57.03	0.65	-

*P < .05

**P < .01



^a Means at the zero level are not significantly different.

Figure 9. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Normal-pH Porcine Muscle at Three Sodium Chloride Levels.

at each sodium chloride level for extractable salt-soluble protein content for both pre- and post-rigor normal-pH muscles.

Differences in extractable salt-soluble protein content between the pre- and post-rigor normal-pH muscles was found to be highly significant ($P < .01$) (Tables IX and X). No significant difference was found between pre- and post-rigor normal-pH muscles in extractable salt-soluble protein content at the zero percent sodium chloride level, although a significant difference ($P < .05$) was found between the pre- and post-rigor muscles at the 1, 2, and 3 percent sodium chloride levels (Figure 9). No difference was expected at the zero percent level since neither pre- nor post-rigor samples had sodium chloride added. Also, it can be noted from Table X and Figure 9 that the interactions between rigor-mortis condition and the various sodium chloride treatments was found to be highly significant ($P < .01$), thus showing that pre- and post-rigor normal-pH muscle responded differently to the addition of sodium chloride in the amount of salt-soluble protein that was extracted (Figure 9).

Significant differences ($P < .05$) were found among the sodium chloride treatments in extractable salt-soluble protein content in pre-rigor low-pH muscle (Tables XI and XII). The test for linearity was found to be highly significant ($P < .01$), indicating that as the added amount of sodium chloride increased, the amount of salt-soluble protein which could be extracted decreased (Figure 10). The 1, 2, and 3 percent sodium chloride treatments contained 3.14, 6.07, and 9.00 percent less extractable salt-soluble protein than did the zero percent level, respectively. One explanation for these results is that the pH of the pre-rigor low-pH muscle was very low, nearing the isoelectric point of the muscle proteins, thus the pro-

TABLE XI
EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-RIGOR LOW-pH
PORCINE MUSCLES AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Sodium Chloride (%)			
	0	1	2	3
	mg/g of sample			
1	57.16	47.80	47.84	46.80
	56.64	49.80	46.77	47.77
2	76.79	74.98	75.63	71.43
	75.65	74.42	74.39	72.02
3	62.69	62.66	60.99	59.86
	62.64	61.51	58.23	58.76
4	70.37	73.91	70.95	67.50
	72.17	72.78	68.70	65.84
Mean ^b	66.76	64.73	62.94	61.25
S.E.	1.29	1.29	1.29	1.29

^aSodium chloride content of sample by weight.

^bSignificant differences ($P < .05$) among sodium chloride levels.

teins, were in a less soluble state, approximately that of the proteins of the post-rigor normal-pH muscle. As the amount of added sodium chloride was increased, the solubility of the proteins was decreased to an even greater extent. The test for the quadratic effect was found to be non-significant (Table XII). Highly significant animal differences ($P < .01$) were again noted in extractable salt-soluble protein content (Table XII).

The same results were also found for the post-rigor low-pH muscle at the three sodium chloride levels. These results are presented in Tables XIII and XIV, and in Figure 11.

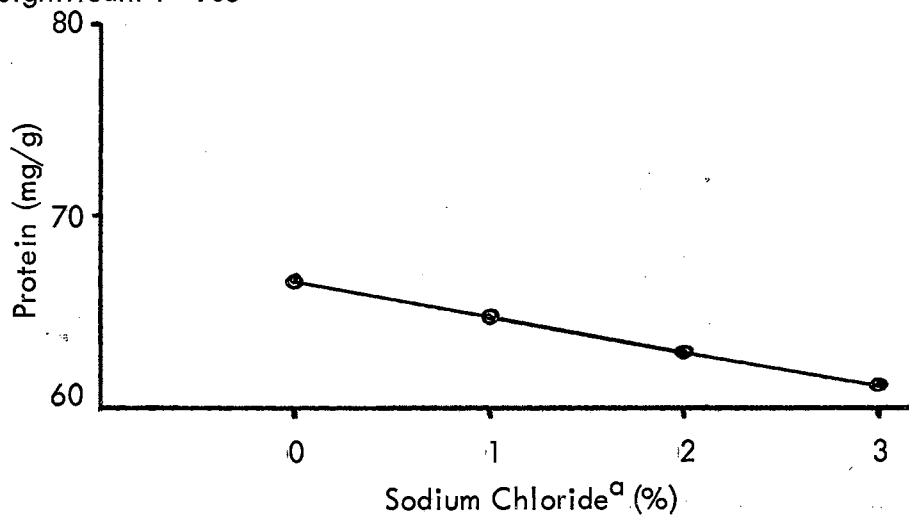
TABLE XII

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN PRE-RIGOR LOW-pH PROCINE MUSCLE
AT THREE SODIUM CHLORIDE LEVELS

Source	d.f.	S.S.	M.S.	F
Total	31	3,040.44	-	-
An. -Treat. Comb.	15	3,024.28	201.62	22.96**
Animal	3	2,810.44	936.81	106.69**
Treatment	3	134.84	44.95	5.12*
Linear	1	134.60	134.60	15.33**
Quadratic	1	0.23	0.23	0.26 ^{ns}
Cubic	1	0.01	0.01	0.00 ^{ns}
Treat. Comb. X An.	9	79.00	8.78	-
Within-An. X Treat. Comb.	16	16.16	1.01	-

*P < .05

**P < .01

^{ns} Non-significant P < .05

^a Significant differences (P < .05) among sodium chloride levels.

Figure 10. Extractable Salt-Soluble Protein Content in Pre-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.

TABLE XIII

EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN POST-RIGOR LOW-pH PORCINE MUSCLES AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Sodium Chloride (%)			
	0	1	2	3
	mg/g of sample			
1	53.83	50.34	50.31	49.81
	51.80	49.85	49.83	49.30
2	74.91	72.53	71.28	68.95
	74.94	73.14	70.17	67.76
3	68.04	69.25	65.05	62.08
	66.82	71.79	66.83	60.08
4	69.66	66.29	62.48	61.44
	69.08	66.27	62.49	61.95
Mean ^b	66.14	64.92	62.31	60.17
S.E.	1.25	1.25	1.25	1.25

^aSodium chloride content of sample by weight.

^bHighly significant differences ($P < .01$) among sodium chloride levels.

Extractable salt-soluble protein content in pre- and post-rigor low-pH muscle at three sodium chloride levels is given in Table XV. The corresponding Analysis of Variance is presented in Table XVI, and a plot of the extractable salt-soluble protein content means is shown in Figure 12.

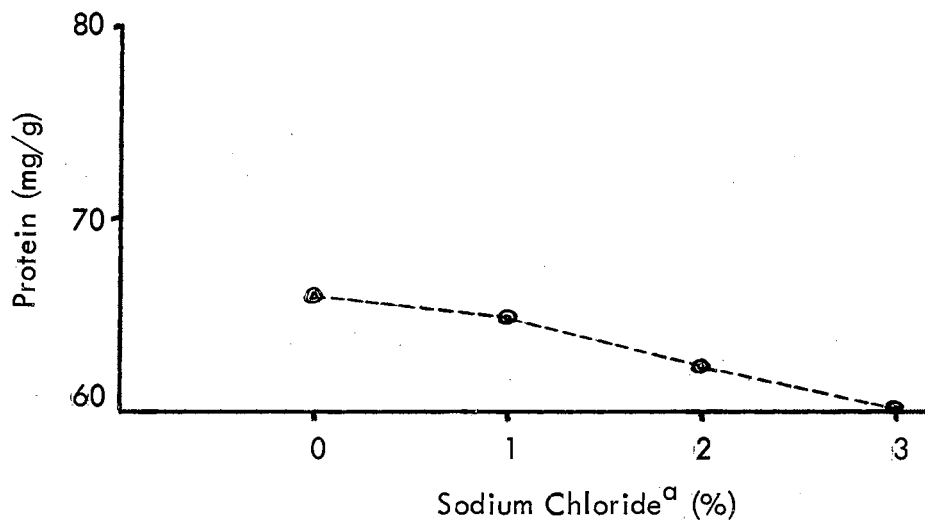
It can be noted from Table XV and Figure 12 that the extractable salt-soluble protein content of the pre-rigor low-pH muscle was slightly higher than the extractable salt-soluble protein content of the post-rigor low-pH muscle at all sodium chloride levels except at the 1 percent level, where the means were approxi-

TABLE XIV

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN POST-RIGOR LOW-pH PORCINE MUSCLE
AT THREE SODIUM CHLORIDE LEVELS

Source	d.f.	S.S.	M.S.	F
Total	31	2,180.16	-	-
An. -Treat. Comb.	15	2,167.59	144.51	23.05**
Animal	3	1,939.79	646.60	103.13**
Treatment	3	171.33	57.11	9.11**
Linear	1	168.22	168.22	26.83**
Quadratic	1	1.69	1.69	0.27 ^{ns}
Cubic	1	1.42	1.42	0.23 ^{ns}
Treat. Comb. X An.	9	56.47	6.27	-
Within-An. X Treat. Comb.	16	12.57	0.79	-

**P < .01

^{ns} Non-significant P < .05

^a Highly significant differences (P < .01) among sodium chloride levels.

Figure 11. Extractable Salt-Soluble Protein Content in Post-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.

TABLE XV

EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR LOW-pH PORCINE MUSCLE AT
THREE SODIUM CHLORIDE LEVELS^a

		Pre-Rigor				Post-Rigor			
Animal	Sodium Chloride ^b (%)								
No.	0	1	2	3	0	1	2	3	
1	57.16	47.80	47.84	46.80	53.83	50.34	50.31	49.81	
	56.64	49.80	46.77	47.77	51.80	49.85	49.83	49.30	
2	76.79	74.98	75.63	71.43	74.91	72.53	71.28	68.95	
	75.65	74.42	74.39	72.02	74.94	73.14	70.17	67.76	
3	62.69	62.66	60.99	59.86	68.04	69.25	65.05	62.08	
	62.64	61.51	58.23	58.76	66.82	71.70	66.83	60.80	
4	70.37	73.91	70.95	67.50	69.66	66.29	62.48	61.44	
	72.17	72.78	68.70	65.84	69.08	66.27	62.49	61.95	
Mean	66.76	64.73	62.94	61.25	66.14	64.92	62.31	60.17	
S.E.	1.29	1.29	1.29	1.29	1.29	1.29	1.29	1.29	

^aSodium chloride of sample by weight.

^bHighly significant differences ($P < .01$) among sodium chloride levels.

mately the same. The differences among the means of extractable salt-soluble protein content of the pre- and post-rigor low-pH muscle at all sodium chloride levels was found to be non-significant. It can also be noted from Table XVI and figure 12 that no significant interaction was found between rigor-mortis condition and sodium chloride levels, thus indicating that pre- and post-rigor low-pH muscle responded the same to the various sodium chloride treatments.

Significant differences ($P < .05$) were found to exist between the means of ex-

TABLE XVI

ANALYSIS OF VARIANCE FOR EXTRACTABLE SALT-SOLUBLE PROTEIN
CONTENT IN PRE- AND POST-RIGOR LOW-PH PORCINE
MUSCLE AT THREE SODIUM CHLORIDE LEVELS

Source	d. f.	S.S.	M.S.	F
Total	63	5,225.13	-	-
An. -Treat. Comb.	31	5,196.49	167.63	9.09**
Animal	3	4,498.20	1,499.40	81.27**
Treat. Comb.	7	310.80	44.40	2.41 ^{ns}
Pre- and Post-Rigor Muscles (A)	1	4.62	4.62	0.25 ^{ns}
Sodium Chloride Level (B)	3	302.84	100.95	5.47**
A X B	3	3.34	1.11	0.06 ^{ns}
Treat. Comb. X An.	21	387.49	18.45	-
Within-An. X Treat. Comb.	32	28.64	0.90	-

**P<.01

^{ns}Non-significant P<.05

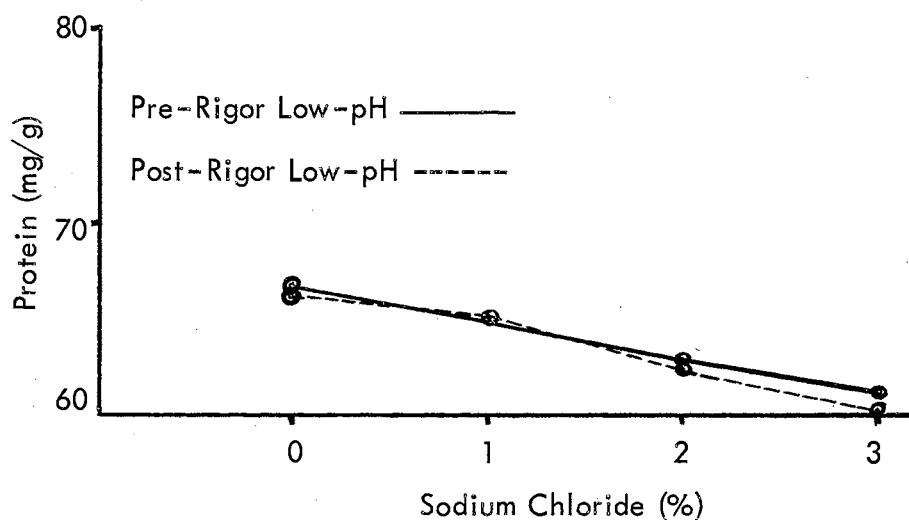
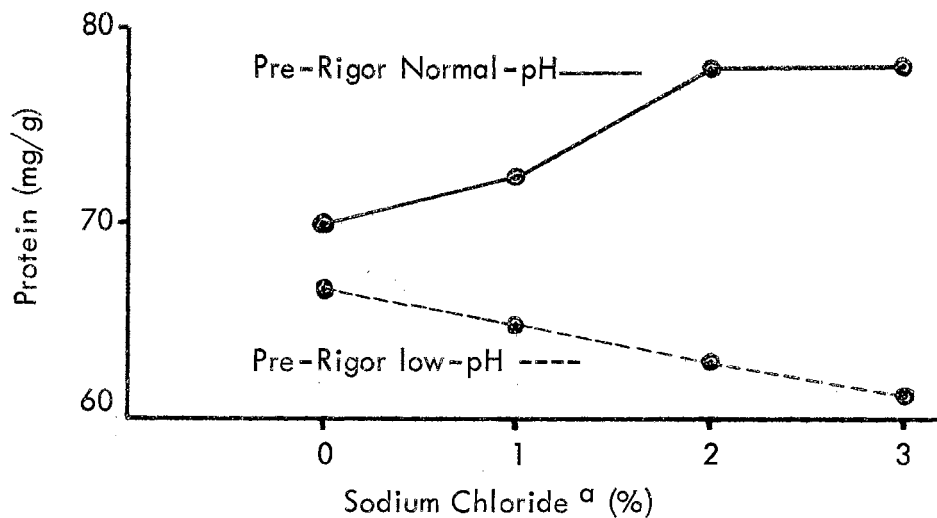


Figure 12. Extractable Salt-Soluble Protein Content in Pre- and Post-Rigor Low-pH porcine Muscles at Three Sodium Chloride Levels.

tractable salt-soluble protein content of pre-rigor normal-pH and pre-rigor low-pH muscles at the 0, 1, 2, and 3 percent sodium chloride levels (Figure 13). This indicated that the pre-rigor low-pH muscle responded approximately the same as the post-rigor normal-pH muscle to the various sodium chloride treatments (Figures 9 and 13).

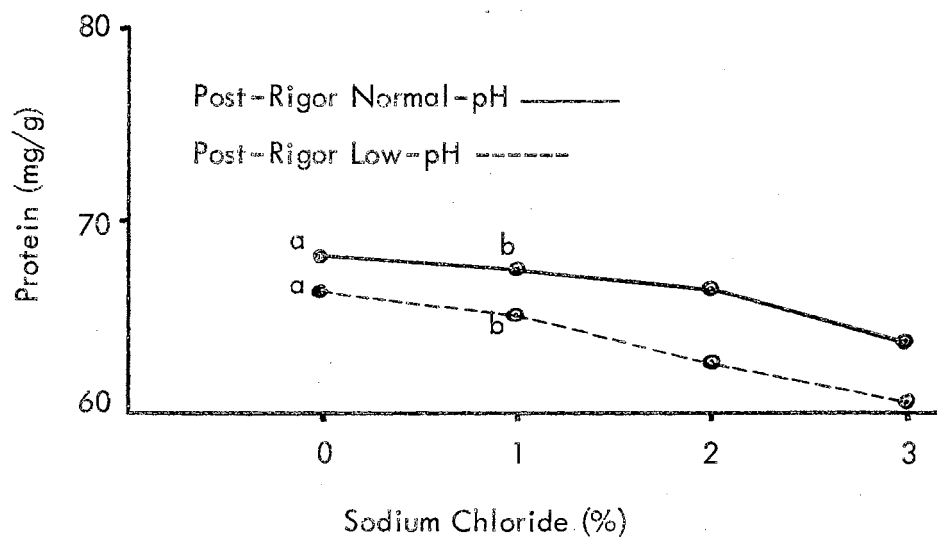
Extractable salt-soluble protein content at the 2 and 3 percent sodium chloride levels between the post-rigor low-pH and post-rigor normal-pH was found to be significantly different ($P < .05$). No significant differences were found at the 0 and 1 percent sodium chloride levels (Figure 14).

Comparisons were made at each sodium chloride level between the pre-rigor low-pH and post-rigor normal-pH muscle (Figure 15). The post-rigor normal-pH muscle was slightly higher at all sodium chloride levels, but differences between extractable salt-soluble protein content means were non-significant at all levels except the 2 percent sodium chloride level, where the difference was found to be significant ($P < .05$).



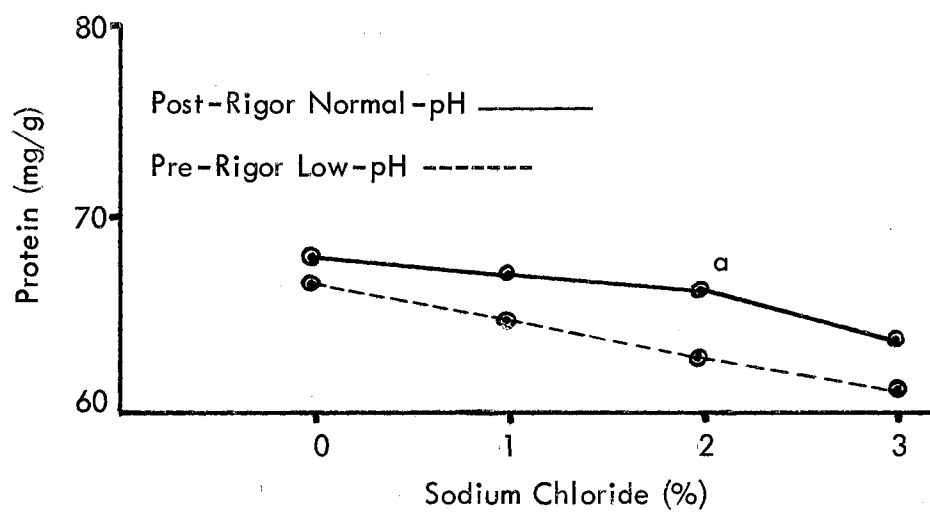
^aSignificant differences ($P < .05$) at all sodium chloride levels between pre-rigor normal-pH and pre-rigor low-pH muscles.

Figure 13. Extractable Salt-Soluble Protein Content in Pre-Rigor Normal-pH and Low-pH Porcine Muscles at Three Sodium Chloride Levels.



^{ab}Means with same superscript are not significantly different.

Figure 14. Extractable Salt-Soluble Protein Content in Normal-pH and Low-pH Post-Rigor Porcine Muscle at Three Sodium Chloride Levels.



^aMeans are significantly different ($P < .05$) at the 2 percent level.

Figure 15. Extractable Salt-Soluble Protein Content in Post-Rigor Normal-pH and Pre-Rigor Low-pH Porcine Muscle at Three Sodium Chloride Levels.

CHAPTER V

SUMMARY AND CONCLUSIONS

Eleven market weight hogs with normal pre-rigor pH muscles ($\text{pH} > 6.2$, 30 minutes post-mortem) were used. Four other market weight hogs all with low pre-rigor pH ($\text{pH} < 5.8$, 30 minutes post-mortem) were included, making a total of 15 animals. The same analysis was run on both populations. The 15 animals ranged in weight from 86.2 kg to 113.4 kg and were obtained from the Oklahoma Agricultural Experiment Station herd. All animals were slaughtered at the Meat Laboratory, skinned, and eviscerated as rapidly as possible. A section of the longissimus dorsi muscle from the 8th to 13th vertebrae was removed, freed of external fat, connective tissue, and ground. Pre-rigor muscle, in this study, is defined as that muscle which has either been analyzed or treated within two hours post-mortem. Post-rigor muscle is that chilled muscle which has either been analyzed or treated approximately 26 hours post-mortem.

This study was conducted in two parts. In Part I, duplicate 10 g pre-rigor and post-rigor samples for each treatment were analyzed for extractable salt-soluble protein content. In Part II, duplicate 10 g pre-rigor samples were treated with 0, 1, 2, and 3 percent sodium chloride, chilled for 24 hours, and analyzed for extractable salt-soluble protein content 24 hours later (26 hours post-mortem). Also duplicate 10 g post-rigor samples were treated with the three levels of sodium chlor-

ide, chilled another 24 hours, and analyzed for extractable salt-soluble protein content (48 hours post-mortem). Samples were extracted at 2°C for salt-soluble protein at random using the Biuret test.

A highly significant difference ($P < .01$) was noted in the extractable salt-soluble protein content between the pre- and post-rigor normal-pH muscles. The extractable salt-soluble protein content between the pre- and post-rigor low-pH muscles was significantly different ($P < .05$), although not as great as in the normal-pH muscle.

Differences in the extractable salt-soluble protein content in pre-rigor normal-pH muscle varied significantly ($P < .01$) among the sodium chloride levels. As the sodium chloride increased the extractable salt-soluble protein content increased. Highly significant differences ($P < .01$) were found in the extractable salt-soluble protein content among the three sodium chloride levels of post-rigor normal-pH muscle. In the post-rigor muscle as the level of sodium chloride increased, the amount of salt-soluble protein which could be extracted decreased. A highly significant interaction ($P < .01$) was found to exist between rigor mortis condition and sodium chloride level.

Pre- and post-rigor low-pH muscles were found to differ significantly ($P < .01$) among the sodium chloride levels in extractable salt-soluble protein content. In both the pre- and post-rigor muscles, as the amount of sodium chloride increased, the extractable salt-soluble protein content decreased. Differences among animals in extractable salt-soluble protein content were found to be highly significant ($P < .01$) in both phases of the work. The repeatability of the procedure was found to be 0.97.

LITERATURE CITED

- Anonymous. 1965. Modern Meat Curing Practice. B. Heller and Company. pp. 7.
- A.O.A.C. 1965. Official Methods of Analysis. 10th ed. Assoc. Official Agric. Chemists. Washington, D. C. pp. 346.
- Bailey, K. 1954. "Structure Proteins." The Proteins II. B., Neurath, H. and Bailey, K. Academic Press Inc., New York, New York. pp. 951.
- Borton, R. J., N. B. Webb, and L. J. Bratzler. 1968. "Emulsifying Capacities and Emulsion Stability of Dilute Meat Slurries from Various Meat Trimmings." Food Technol. 22:506.
- Brady, D. E., F. H. Smith, L. N. Tucker and T. N. Blumer. 1949. "Characteristics of Country-Style Hams as Related to Sugar Content of Curing Mixture." Food Res. 14:303.
- Braverman, J. B. S. 1963. Introduction to the Biochemistry of Foods. Elsevier Publishing Company. New York, New York. pp. 119.
- Callow, E. H. 1956. "The Action of Salts and Other Substances Used in the Curing of Bacon and Ham." British J. Nutr. 1:269.
- Carpenter, J. A., and R. L. Saffle. 1964. "A Simple Method of Estimating the Emulsifying Capacity of Various Sausage Meats." J. Food Sci. 29:774.
- DiMarco, R. G. 1963. "Role of Additives in Curing." Meat. 29:32.
- Dugan, L. R., and H. T. Slover. 1960. "Fats." The Science of Meat and Meat Products. W. H. Freeman and Company, San Francisco, California. pp. 309.
- Engelhardt, V. A., and M. N. Ljubimova. 1939. "Myosine and Adenosinetriphosphatase." Nature. 144:668.
- Franson, R. D. 1966. Anatomy and Physiology of Farm Animals. Lea and Febiger, Philadelphia, Pennsylvania. pp. 175.
- Frazier, R. J. 1958. Food Microbiology. McGraw-Hill Book Company, New York, New York. pp. 341.

LITERATURE CITED (Continued)

- Garner, R. G. 1966. "Water Activity in Relation to Foods." Presented to Assoc. Southern Agri. Workers. Jackson, Mississippi.
- Giffee, J. W., M. C. Urbain, J. B. Fox, W. A. Landmann, A. J. Siedler and R. A. Sliwinski. 1960. "Proteins." The Science of Meat and Meat Products. W. H. Freeman and Company, San Francisco, California. pp. 56.
- Grettie, D. P. 1965. "The Isoelectric Points of Collagen and Gelatin." J. Am. Leather Chemists Assoc. 60(10):572.
- Hamm, R., and R. Grau. 1958. "Z. Lebensmitt. Untersuch." 108:280. (Quoted by Lawrie, 1966).
- Hamm, R. 1963. "The Water Imbibing Power of Foods." Recent Adv. Food Sci. Biochem. and Biophysics. 3:218.
- Hansen, L. J. 1960. "Emulsion Formulation of Finely Comminuted Sausage." Food Technol. 14:565.
- Hanson, J., and H. E. Huxley. 1953. "Structural Basis of the Cross-Striations in Muscle." Nature. 172:530.
- Harrow B., and A. Mazur. 1966. Textbook of Biochemistry. W. B. Saunders Company, Philadelphia, Pennsylvania. pp. 15, 493.
- Haurowitz, F. 1963. The Chemistry and Function of Proteins. 2nd ed. Academic Press Inc., New York, New York. pp. 212.
- Hegarty, G. R., L. J. Bratzler, and A. M. Pearson. 1963. "Studies on the Emulsifying Properties of Some Intracellular Beef Muscle Proteins." J. Food Sci. 28:663.
- Helmer, R. L., and R. L. Saffle. 1963. "Effect of Chopping Temperature on the Stability of Sausage Emulsions." Food Technol. 17:1194.
- Huxley, H. E. 1963. "Electron Microscope Studies on the Structure of Natural and Synthetic Protein Filaments from Striated Muscle." J. Mol. Biol. 7:281.
- Kramer, H., and K. Little. 1955. Nature and Structure of Collagen. Butterworths, London. pp. 33.
- Lawrie, R. A. 1966. Meat Science. Pergaman Press, New York, New York. pp. 70.

LITERATURE CITED (Continued)

- Layne, E., S. P. Colowick, and N. O. Kaplan. 1957. "Methods in Enzymology." 111:447.
- Lushbough, C. H., and B. S. Schweigert. 1960. "The Nutritive Content and the Nutritional Value of Meat and Meat Products." The Science of Meat and Meat Products. Freeman and Company, San Francisco, California. pp. 185.
- Matz, S. A. 1965. Water in Foods. The AVI Publishing Company, Westport, Connecticut. pp. 1.
- McElroy, W. D. 1964. Cell Physiology and Biochemistry. Prentice-Hall Inc., Englewood Cliffs, New Jersey. pp. 12.
- Meyer, J. A., W. L. Brown, N. E. Geltner, and J. R. Guinn. 1964. "Effect of Emulsifiers on the Stability of Sausage Emulsions." *Food Technol.* 18:138.
- Meyer, L. H. 1960. Food Chemistry. Reinhold Publishing Corporation, New York, New York. pp. 178.
- Mommaerts, W. F. 1950. Muscular Contraction. Interscience Publishers Inc., New York, New York. pp. 77.
- Niven, C. F. Jr., and W. R. Chesbro. 1960. "Meat Preservation". The Science of Meat and Meat Products. W. H. Freeman and Company, San Francisco, California. pp. 307.
- Partridge, S. M. 1966. "Elastin." Physiology and Biochemistry of Muscle as Food. Briskey. The University of Wisconsin Press, Madison, Wisconsin. pp. 327.
- Pearson, A. M., M. E. Spooner, G. R. Hegarty, and L. J. Bratzler. 1965. "The Emulsifying Capacity and Stability of Soy Sodium Proteinate, Potassium Caseinate and Non-Fat Dry Milk." *Food Technol.* 19:103.
- Perry, S. V. 1956. "Relation Between Chemical and Contractile Function and Structure of the Skeletal Muscle Cell." *Physio. Rev.* 36:1.
- Piez, K. A. 1966. "Collagen." Physiology and Biochemistry of Muscle as Food. Briskey. The University of Wisconsin Press, Madison, Wisconsin. pp. 317.
- Price, J. F. 1964. "Meat Emulsion." *Proceedings: Meat Processing Conference*. 1964.
- Saffle, R. L., and J. W. Galbreath. 1964. "Quantitative Determination of Salt-

LITERATURE CITED (Continued)

- Soluble Protein in Various Types of Meat." *Food Technol.* 18:1943.
- Sorum, C. H. 1963. Fundamentals of General Chemistry. 2nd ed. Prentice-Hall Inc., Englewood Cliffs, New Jersey. pp. 430.
- Steel, R. G., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company Inc., New York, New York.
- Swift, C. L., C. Lockett, and H. J. Fryer. 1961. "Comminuted Meat Emulsions: The Capacity of Meats for Emulsifying Fats." *Food Technol.* 15:468.
- Swift, C. L., and W. L. Sulzbacher. 1963. "Comminuted Meat Emulsions: Factors Affecting Meat Protein as Emulsion Stabilizers." *Food Technol.* 17:224.
- Szent-Gyorgyi, A. G. 1960. "Protein of the Myofibril." Structure and Function of Muscle II, Bourne. Academic Press, New York, New York.
- Tallon, H. H., S. Moore, and W. H. Stein. 1954. *J. Biol. Chem.* 211:927. (Quoted by Lawrie, 1966).
- Trautman, J. C. 1964. "Fat Emulsifying Properties of Pre-Rigor and Post-Rigor Protein." *Food Technol.* 18:1065.
- Voegeli, M. M. 1966. "Meat Emulsions." *Proceedings: Meat Processing Conference*. 1966.
- Watts, B. M. 1962. "Meat Products." Lipids and Their Oxidation. The AVI Publishing Company, Westport, Connecticut. pp. 205.
- Wierbicki, E., L. E. Kunkle, V. R. Cahill, and F. E. Deatherage. 1956. "Post-Mortem Changes in Meat and Their Possible Relation to Tenderness Together With Some Comparisons of Meat From Heifers, Bulls, Steers, Diethylstilbestrol Treated Bulls and Steers." *Food Technol.* 10:80.
- Wilson, G. D. 1960. "Sausage Products." The Science of Meat and Meat Products. W. H. Freeman and Company, San Francisco, California. pp. 373.
- Younathan, M. T., and B. M. Watts. 1959. "Relationship of Meat Pigments to Lipid Oxidation." *Food Res.* 24:728.
- Zeigler, P. T. 1962. The Meat We Eat. The Interstate Printers and Publishers Inc., Danville, Illinois.

APPENDIX

TABLE XVII

EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR NORMAL-pH PROCINE MUSCLE^a

Animal No.	Pre-Rigor Protein (%)	Post-Rigor Protein (%)
1	49.57	30.38
	48.86	29.88
2	58.04	26.00
	58.37	26.52
3	55.57	29.75
	56.08	29.76
4	53.20	34.05
	54.09	33.45
5	54.61	32.74
	53.84	33.02
6	57.58	36.91
	55.97	36.03
7	59.66	33.72
	58.35	33.13
8	54.16	35.03
	55.84	34.74
9	53.73	26.67
	52.98	25.93
10	48.51	31.58
	49.62	31.86
11	52.82	36.50
	54.29	35.93
Mean	54.35	31.98

^a Percent salt-soluble protein of total protein.

TABLE XVIII
 EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
 AND POST-RIGOR NORMAL-pH PROCINE MUSCLE
 AT THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Pre-Rigor				Post-Rigor			
	Sodium Chloride (%)							
	0	1	2	3	0	1	2	3
	Protein (%)							
1	35.74	36.25	38.77	34.66	33.32	33.54	34.30	33.30
	36.57	36.02	38.76	34.44	33.30	33.54	34.34	33.30
2	27.78	29.05	31.98	34.44	29.05	29.30	26.99	27.49
	27.76	29.05	31.17	34.15	28.79	29.30	27.50	25.99
3	31.18	32.62	36.76	36.75	29.80	29.83	27.93	26.09
	30.86	32.59	36.46	36.76	30.09	29.80	28.46	26.33
4	36.74	37.96	39.23	41.15	33.12	33.14	32.81	30.27
	35.85	35.82	38.28	40.18	32.54	33.10	32.82	29.69
5	32.19	34.15	39.16	39.49	32.37	31.58	31.58	30.55
	31.38	34.14	39.19	40.09	32.66	31.85	31.32	30.29
6	37.24	38.77	41.64	40.07	36.53	34.13	34.70	31.24
	37.54	39.10	43.32	40.70	35.03	34.71	33.56	30.14
7	33.71	36.61	39.97	39.65	32.75	31.70	29.22	30.04
	33.72	36.32	40.28	39.95	32.76	31.40	29.49	29.78
8	34.49	35.87	36.71	37.01	36.57	36.87	37.14	36.02
	35.31	35.86	36.42	37.56	36.27	36.57	36.86	36.58
9	27.97	28.23	29.55	30.65	24.72	23.02	22.28	20.86
	26.69	27.97	30.10	30.64	24.97	23.23	22.53	20.63
10	32.43	33.54	35.56	35.57	29.78	28.72	30.03	28.44
	32.14	32.71	34.99	35.55	30.05	28.18	29.53	28.19
11	35.39	35.42	37.84	38.44	36.62	36.87	36.06	36.07
	35.63	35.63	37.85	38.72	36.07	37.47	36.62	36.35
Mean	33.11	34.26	36.95	37.12	32.14	31.72	31.19	29.89

^aSodium chloride of sample by weight.

TABLE XIX
EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR LOW-pH PORCINE MUSCLE AT
THREE SODIUM CHLORIDE LEVELS^a

Animal No.	Pre-Rigor				Post-Rigor			
	Sodium Chloride (%)							
	0	1	2	3	0	1	2	3
	Protein (%)				Protein (%)			
1	28.07	23.47	23.49	22.98	26.43	24.72	24.70	24.46
	27.81	24.45	22.97	23.46	25.44	24.48	24.47	24.21
2	34.97	34.14	34.43	32.52	34.11	33.02	32.45	31.39
	34.44	33.88	33.87	32.79	32.12	33.30	31.95	30.85
3	29.07	29.06	28.28	27.76	31.55	32.11	30.16	28.79
	29.05	28.52	27.00	27.25	30.98	33.25	30.99	27.86
4	29.84	31.34	30.09	28.63	29.54	28.11	26.50	26.06
	30.60	30.87	29.14	27.92	29.30	28.11	26.50	26.27
Mean	30.48	29.47	28.66	27.91	30.18	29.64	28.47	27.49

^aSodium chloride of sample by weight.

TABLE XX
EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE-
AND POST-RIGOR LOW-pH PORCINE MUSCLE^a

Animal No.	Pre-Rigor Protein (%)	Post-Rigor Protein (%)
1	31.04	26.25
	30.50	27.55
2	37.53	34.36
	36.93	35.26
3	32.32	30.91
	32.04	30.65
4	31.39	29.84
	31.64	30.86
Mean	32.92	30.71

^aPercent salt-soluble protein of total protein.

TABLE XXI

TOTAL PROTEIN CONTENT IN NORMAL AND LOW-pH
PORCINE LONGISSIMUS DORSI MUSCLE^a

Animal No.	Normal-pH Protein (%)	Low-pH Protein (%)
1	25.97	20.37
2	21.51	21.96
3	19.59	21.56
4	19.28	23.58
5	20.82	-
6	19.43	-
7	20.06	-
8	22.71	-
9	19.80	-
10	19.74	-
11	23.19	-
Mean	21.10	21.87

^aPercent total protein of sample.

VITA

Roger Groom Johnson

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF LOW-IONIC SODIUM CHLORIDE CONCENTRATIONS ON
THE EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT IN PRE- AND
POST-RIGOR PORCINE MUSCLE

Major Field: Food Science

Biographical:

Personal Data: Born in Muskogee, Oklahoma, May 20, 1945, the son of
Albert J. and Clara E. Johnson. Married Deanne L. Hutchens.

Education: Graduated from Muskogee Central High School in 1963. Attended
Northeastern State College, Talequah, Oklahoma 16 months. Attended
Oklahoma State University, Stillwater, Oklahoma and was awarded the
Bachelor of Science degree May, 1967 with a major in Animal Science.

Experience: Research Assistant in Food Science, Oklahoma State University,
1967-68.

Professional Organizations: Member of the Institute of Food Technologists,
Phi Sigma Epsilon, The Northeastern State Chapter; and Brothers of the
Flame.