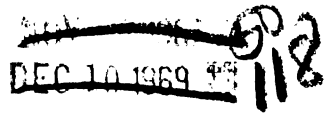
# EMULSIFYING AND OTHER PROPERTIES OF PORCINE MUSCLE TISSUE AS RELATED TO MICROBIAL CONTAMINATION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Ronald James Borton

1966



ABSTRACT

EMULSIFYING AND OTHER PROPERTIES OF PORCINE MUSCLE TISSUE AS RELATED TO MICROBIAL CONTAMINATION

by Ronald James Borton

For many years it has been known that bacteria caused spoilage of meat and meat products, but the affect of bacteria on the chemical properties of meats has not been studied very extensively. The purpose of this research was to study the influence of bacteria on some properties of muscle tissue through a 17 day storage period. In order to accomplish this, a method of obtaining a sample relatively free of bacteria was developed. Some contamination of the samples obtained by this method was noted but the contamination was slight when compared to the inoculated sample or to normal fresh meats.

Protein extractability was studied as protein is important in emulsification of fats in sausage products and nutritionally in fresh meats. Results indicated the protein extractability changed due to the factor of time but changed very little due to bacteria. The sarcoplasmic protein fraction tended to decrease during the period of rapid bacterial growth, but the bacteria had little or no effect on the myofibrillar, stroma, and nonprotein nitrogen (NPN) fractions.

The emulsifying capacity was also studied as emulsification of fat in a sausage product is important to sausage manufacturers. The emulsifying capacity appeared to decrease due to the bacteria growth in the sample.

The Extract Release Volume (ERV) was studied as it was reported to be an indicator of the amount of bacterial spoilage. Results of this study indicated that the ERV decreased inversely to the increase in bacteria numbers. However, the ERV appeared to be related to growth of bacteria rather than bacterial numbers as there was little difference between control and inoculated samples at 0 and 1 day of storage. Statistical analysis indicated there was a significant difference at $P < .01$ between the treatments as related to numbers of bacteria present at various storage periods.

# EMULSIFYING AND OTHER PROPERTIES OF PORCINE MUSCLE TISSUE AS RELATED TO MICROBIAL CONTAMINATION

By

RONALD JAMES BORTON

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1966

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Professor L. J. Bratzler for his guidance throughout the research and for his assistance in the preparation of this manuscript. He also wishes to thank Dr. Neil B. Webb (Director of Technology, Eckert Packing Company) for his advice, encouragement, and aide in accomplishing this research.

The author is grateful to his parents for their continual support and encouragement.

The author is indebted to the Eckert Packing Company, Defiance, Ohio, for the support furnished him by a special assistantship and providing space, equipment and materials for this project.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

# INTRODUCTION

Microorganisms have caused spoilage problems for the meat packer since the beginning of the meat packing industry. Ayres (1955) reviewed the work of many others concerning the spoilage problem. His review indicated that the spoilage organisms have been identified, the effects of microorganisms on physical properties such as odor, texture, flavor, water binding, sliminess, etc., have been established, and the origins of the spoilage microorganisms have been found.

Very little work has been expended to determine the effects of microorganisms on the chemical properties of muscle tissues. Davis (1965) proposed a method of aseptic slaughter and sample procurement involving surgical isolators which could provide control samples to compare with bacterially contaminated samples. He found he could hold control samples for at least 35 days at 2-5°C without any evidence of bacterial contamination. When he compared these aseptic samples with inoculated samples, his results indicated that bacteria may influence chemical properties as well as physical properties of muscles.

One chemical property which may be altered is protein extractability. Previous work indicated that one of the primary meat spoilage genera, Pseudomonas (Kirsh, et al., 1952; Ayres, 1955, 1956; Wolin et al., 1957; and Halleck et al., 1958) is a proteolytic organism (Camp and Van Der Zent, 1957; Van Der Zent, 1957; Peterson and Gunderson, 1960; and Witter, 1961) which would affect the extractability of the proteins.

A second chemical property which may be altered is the emulsifying capacity of the meat tissue. The emulsifying capacity is dependent on the amount and type of protein present (Swift et al., 1961; Swift and Sulzbacher, 1963; Hagerty et al., 1963; and Trautman, 1964). Thus, if the protein content is altered, the emulsifying capacity could be altered also.

A third property, the Extract Release Volume, which could be considered a chemical property, could also change with bacterial growth (Jay, 1964; and Jay and Kontou, 1964).

This study was initiated to determine:

1. If a more economical yet efficient means of obtaining a sample relatively free from microbial contamination could be found.
2. The effect of microorganisms on protein extractability.
3. The effect of microorganisms on emulsifying capacity.
4. The effect of microorganisms on the Extract Release Volume (ERV).

# LITERATURE REVIEW

## Microorganisms

### Spoilage

Ayres'(1955) review of meat microbial studies indicated that muscle tissues from or on the carcasses of bovine, porcine, and ovine species provide most of the nutrients required by microorganisms. Also, he indicated the sources of microbial contamination are such that microbial growth is a serious problem to the meat packer. In his review, he listed the normal defenses of an animal against microbial infection as: 1. Skin and mucous membranes; 2. Hair and cilica; 3. Gastric juice; 4. Digestion; and 5. Localization of an infection if it begins. After death of the animal, these defensive mechanisms are lost and microbial contamination can take place. The same author indicated animals carry heavy loads of microorganisms on the hide, hair, skin, and hooves. Two studies he reviewed indicated the skin carried an average of 3.91 million aerobes, 100 million anaerobes, and 100 yeasts and molds per sq. cm. of skin surface. Jensen and Hess (1941) and Ayres (1956) reported similar results. Another source of contamination indicated by Ayres (1955) was the rumen and intestines of the bovine and ovine species and the intestines of the porcine species. If workers were careless when eviscerating the animals, the carcass became contaminated.

Contamination of the carcass by the workmen and the equipment used in processing the carcass was also found by Ayres (1955, 1956). Every handling of the product increased the microbial load on or in the carcass.

Jensen and Hess (1941) reported the sticking knife carried bacteria into the blood stream. The organisms were either contaminants of the knife or were on the skin and hair surrounding the sticking area and forced into the animal with the sticking knife. This contamination by the sticking knife was also reported by Ayres (1955, 1956). Jensen and Hess (1941) also reported that the scalding tank provided another means of contamination of hogs. They reported the deep tissues became more easily contaminated if the heart was still beating when the hog was dropped into the scalding vat as the microbially loaded water would be circulated in the animal's system. Ayres (1955, 1956) also found the scalding tank a source of contamination. Other sources of contamination listed by Ayres (1955, 1956) include the air in the packing plants, the water used in processing, and the sawdust used on the cooler floors.

Kirsh et al. (1952) reported that ground beef purchased from retail stores had been contaminated so that the total aerobic counts ranged from 1-95 million per gram of tissue. When these bacteria were classified, it was found that the non-pigmented Pseudomonas-Aerobacter group dominated the flora with Lactobacillus and cocci organisms also present. The findings were similar to those of Ayres (1955, 1956), Wolin et al. (1957), and Halleck et al. (1958). Wolin et al. (1957) found that the initial contamination was primarily Gram-positive rods but after incubation at 20°C under moist conditions this changed to non-pigmented, Gram-negative, aerobic rods having polar flagella which are characteristic of Pseudomonas. This trend was also noted by Halleck et al. (1958).

Lepovetsky et al. (1953) studied the bacteria found in the deep tissues. They studied 23 beef animals and found bacteria in 15 of 23 lymph nodes, 3 of 23 bone marrow samples, and 2 of 23 muscle samples. The lymph nodes act as a filtering system in an animal and thus trap bacteria found in an animal's system. Twelve different genera of bacteria were isolated from the lymph nodes including Aerobacter and Pseudomonas. The three marrow samples with bacteria contamination were contaminated by bacteria of the genera Aerobacter, Corynebacterium, and Pseudomonas. The two contaminated muscle samples had isolates of the genera Pseudomonas and Streptococcus. This study indicated that deep spoilage of a tissue probably will start at the lymph nodes as they are the primary source of bacteria in the deep tissues.

Pseudomonas and Aerobacter bacteria seem to be the primary source of meat spoilage (Ayres, 1960). Kirsh et al. (1952) noted a sour odor in beef after 8-12 days of refrigerated storage. The aerobic count when the odor was noted was 500,000,000 per gram which was close to the critical slime value of $6 \times 10^7$ reported by Ayres (1960) and substantiated by Jay and Kontou (1964). Halleck et al. (1958) reported a foul odor when Pseudomonas-Aerobacter bacteria were incubated on veal infusion agar. As Pseudomonas and Aerobacter seemed to be the primary meat spoilage organisms, a brief review of their characteristics would be helpful.

Witter (1961), in a review of psychrophilic bacteria, found Pseudomonas and Aerobacter primarily soil contaminants, i.e., they are normally soil organisms. He reported psychrophiles do not survive pastuerization,

are inhibited more extensively by hypochlorites than quarternary ammonia compounds, can grow in salt concentrations of 4 percent, are sensitive to antibiotics, and are sensitive to pH reduction. Pseudomonas bacteria are proteolytic bacteria as reported by many workers. Peterson and Gunderson (1960) found Pseudomonas fluorescens to have extracellular and endocellular proteolytic enzymes. They found at lower temperatures of incubation that the extracellular enzymes reacted more slowly as less enzyme was produced due to a slower rate of growth. The enzymes were very active during the logarithmic phase of growth. Van Der Zant (1957) reported extracellular enzymes were most active when the viable cell count was highest. Also he reported a pH of 6.5 to 8.0 resulted in the greatest activity. When the enzymes were heated to 60°C for 3 minutes they were inactivated. The greatest enzymatic activity was noted at temperatures of 30-50°C. Peterson and Gunderson (1960) found the endocellular enzymes to be 3 times more active at 10°C than at 35°C. Camp and Van Der Zant (1957) reported optimum endocellular enzyme activity at a pH of 7.0 to 8.0, but disagreed with Peterson and Gunderson's results as they had optimum activity at 35°C and a decrease below 25°C. Camp and Van Der Zant (1957) reported endocellular enzymes were not influenced by heating for 8 minutes at 60°C at a pH of 7.0. Hurley et al. (1963) reported the presence of ferrous ion increased the proteolytic activity of Pseudomonas fluorescens. Ayres (1960) found Pseudomonas grew well up to a temperature of 25°C. He also reported at temperatures below 10°C pseudomonads were the only bacteria growing on meat samples.

Extract Release Volume (ERV)

Jay and Kontou (1964) evaluated a method for the rapid determination of the microbial quality of fresh meat. They found a high correlation between the extract-release volume (ERV) and the number of bacteria per gram. Jay (1964b) found the ERV decreased in a straight line relationship as the bacteria numbers increased. He found a mean ERV value of 24.6 ml corresponded with the onset of spoilage detectable by organoleptic means. The bacterial numbers at this point averaged $3 \times 10^8$ bacteria per gram. Jay and Kontou (1964) found the mean ERV value of sample rejection by a trained panel was 30.4 ml which corresponded to $7 \times 10^7$ bacteria per gram. Price et al. (1965) found the onset of spoilage occurred when the ERV values were between 30 and 40 ml and below 30 ml spoilage was definite and bacterial numbers were very high.

Jay (1964a) reported that an ERV was obtained at temperatures varying from 7-37°C. He also found the maximum ERV was obtained at pH values between 5.0 and 5.8 while no ERV was obtained below pH 4.9 or above pH 11.0. Price et al. (1965) found the environmental and slurry temperatures varied the ERV. They reported the temperature was controlled most efficiently by running the procedure at cooler temperatures (2-6°C). Fat content below 20% did not affect ERV values in beef according to Jay (1964a). However, high amounts of fat tended to increase the ERV value. Price et al. (1965) found the fat content and storage time of beef had an interaction which influenced the ERV value. They also stated the fat content of pork had no significant effect on ERV values.

Aseptic Muscle Sampling

Weiser et al. (1954) used antibiotics to reduce the bacterial numbers to prevent deep spoilage of beef tissue. They infused the beef rounds with antibiotics by a method similar to arterial pumping of hams and thus reduced the bacterial population to practically zero. The bacteria in the lymph nodes were reduced from 100 millions recorded in the controls to ten thousands in the infused rounds. Also a method of infusing the antibiotic in the animal via the jugular vein during the slaughter operation was very successful. Aureomyocin was the antibiotic used and it degenerated during storage and was not present in the tissue when it reached the consumer.

Ayres (1956) and Zender et al. (1958) found normal muscle tissue in the living animal was sterile or relatively free of living microorganisms. Earlier workers (Jansen and Hess, 1941; and Lepovetsky et al., 1953) found bacteria in muscle samples. The low numbers recovered, however, and the methods used for slaughter indicated a greater possibility of contamination than the microorganisms being present in the living muscle tissue.

The germ-free work being conducted is based on the fact that the embryo of the various animals are free of microorganisms. Landy et al. (1961) claimed any laboratory animal used had bacteria, fungi, viruses, etc. in its system. These people were especially concerned with the digestive tract. Meyer et al. (1964) found pigs could be taken from the sow under sterile conditions and reared using sterile isolators, equipment, food, and water so there was no evidence of bacteria, fungi, viruses,

etc. in any of the pigs' physiological systems. Ockerman et al. (1964) slaughtered and eviscerated germ-free mice in a sterilized isolator with sterilized equipment. The mouse carcasses were stored in sealed sterilized tubes and no bacterial contamination was found. However, the carcasses did deteriorate due to proteolysis with those carcasses stored at higher temperature deteriorating at a more rapid rate.

Zender et al. (1958) followed a very precise and aseptic means of excising muscle samples from rabbits and lambs. Using this method, they obtained samples which were practically void of contamination. Davis (1965) combined the methods of Zender et al. (1958) and Ockerman et al. (1964). He slaughtered beef aseptically using sterilized sticking knives, one to cut the shaved, bacteriocidally washed skin, and the second to sever the carotid arteries. Other slaughter and eviscerating procedures were normal except a large patch of the hide was left intact over the loin area. This area was scrubbed with warm water and a bacteriocidal soap, rinsed, scrubbed again, and finally rinsed with 90% ethanol before storing the unsplit carcass in a cooler. After the carcass had cooled sufficiently, the whole rough loin, after another alcohol rinse, was placed on a cart and a sterile isolator was attached to it and secured. Then by working through the gloves attached to the isolator a slit was made in the isolator and through the hide so the hide could be rolled from the exposed muscle. The muscle was excised, ground, and placed in sterile containers with all operations taking place in the isolators. The samples were then stored up to 35 days at a temperature of 2-5°C with no evidence of bacterial contamination.

## Proteins

Skeletal muscle consists of fibers enclosed in a sheath of perimysium permeated with fat deposits and connective tissues. The contractile element is the fiber portion of the muscle. The fibers are multinucleated and are composed mainly of the myofibrillar proteins, myosin, actin, actomyosin, and tropomyosin. The perimysium sheath, fatty deposits, connective tissues, nerve tissues, and vascular tissues are composed of proteins which are known as the stroma proteins. The intercellular material or sarcoplasma is a liquid material containing proteins which are known as the sarcoplasmic proteins (Helander, 1957; and Whitaker, 1959).

### Sarcoplasmic Proteins

Helander (1957) identified sarcoplasmic proteins as those muscle proteins which are soluble in water or low concentrations of salt and are characterized as globular, low viscosity, and low molecular weight proteins. He stated the primary sarcoplasmic proteins were myogen, myoalbumin, globulin X, and myoglobin. Whitaker (1959) stated the sarcoplasmic proteins were primarily enzymes. Scopes and Lawrie (1963) found 35 bands on starch gel electrophoresis from beef sarcoplasmic fractions. During the process of rigor mortis some of the bands were lost. Fujimaki and Deatherage (1964) fractionated sarcoplasmic protein of beef skeletal muscle on an ion-exchange cellulose chromatography column and found at least 14 different fractions. They also noted a loss in the number of peaks and the level of the peaks during storage and freeze-drying. Aberle

and Merkel (1966) found 15 definable bands in starch gel electrophoretic patterns from the sarcoplasmic fraction of bovine muscle. Their results indicated that the bands increased in intensity and new zones appeared during aging.

Hill (1962) found sarcoplasmic protein accounted for 15-20% of the total nitrogen in bovine muscle, 20-25% of the total nitrogen in porcine muscle, and approximately 25% of the total nitrogen in ovine muscle. Hegarty (1963) found similar results for bovine muscle as did Davis (1965). Topel (1965) found similar results for porcine muscle. The amount of sarcoplasmic protein extracted depended on many environmental and physiological conditions including temperature, pH, age of carcass, degree of rigor mortis, etc.

Seven different articles have reported that with aging sarcoplasmic proteins decrease in all types of muscle. Scharpf and Marion (1964) reported this decrease in turkey muscle. Khan and Van Den Berg (1964a) found a decrease in the amount of sarcoplasmic proteins in chicken muscle due to aging. Porcine muscle also showed this decrease according to Sayre and Briskey (1963) and McLoughlin (1963). Fujimaki (1962), Goll et al. (1964) and Davis (1965) also found beef muscle sarcoplasmic protein decreased in amount during aging. Hegarty (1963) found an increase in the amount of sarcoplasmic protein from 0 to 24 hours post mortem and little change in the amount from 24 hours to 7 days post mortem in beef muscle. Aberle and Merkel (1966) reported a decrease in the sarcoplasmic fraction from 0 hour throughout a 336 hour storage post

mortem for the semi-tendinosus muscle of beef. However, they reported no change in the amount of sarcoplasmic protein of the beef longissimus dorsi muscle. Scopes and Lawrie (1963) and Scopes (1964) reported evidence of a sarcoplasmic precipitation on the myofibrillar proteins such that some of the sarcoplasmic proteins were extracted with the myofibrillar fraction. Scharpf and Marion (1964) reported some sarcoplasmic proteins were carried from the muscle sample with the exuded moisture. Most workers incorporated the exuded moisture into the muscle samples before testing.

The pH of the sample influenced the amount of sarcoplasmic protein extracted (Scopes, 1964). At pH values below 5.0, less than 10% of the protein was extractable. pH values of 5.6-5.7 gave the highest total extractability (sarcoplasmic and myofibrillar fractions) with little change at higher pH values. Helander (1957) found a pH of 7.4 was best for extracting sarcoplasmic proteins. Scopes and Lawrie (1963) reported that the pH fall associated with the onset of rigor mortis could account for part of the sarcoplasmic precipitation noted in the aging of the muscle. Scopes (1964) reported storage temperatures near 37°C caused some denaturation of proteins and thus a loss in extractability. Khan and Van Den Berg (1964) reported there was no variation due to the influence of temperatures ranging from 0° to 5°C.

Topel (1965) reported pork muscles which were extremely soft and watery had a lower portion of extractable sarcoplasmic proteins than did the normal pork muscles. Khan and Van Den Berg (1964a) and Scharpf and

Marion (1964) reported higher sarcoplasmic fractions in the lighter muscles than in the darker muscles of chicken and turkey. This relationship held true for the "red meats" as reported by Hill (1962). The darker beef had a lower sarcoplasmic content than the lighter pork and lamb. Davis (1965) found that bacterially inoculated beef muscle had a higher sarcoplasmic content than the aseptic sample throughout the 35 day storage period. At the end of the 35 day storage period, the mean difference was 4 mg of protein per gram of fresh sample.

Myofibrillar Proteins

Helander (1957) identified the myofibrillar proteins as those muscle proteins not soluble in water or low salt concentrations but soluble in high salt concentrations. He characterized these proteins as fibrous, highly viscous, high molecular weight muscle proteins. They are the contractile proteins; myosin, actin, and their aggregation, actomyosin, plus tropomyosin and X-protein (Whitaker, 1959). Hill (1962) reported the myofibrillar protein fraction comprised approximately 55% of the total nitrogen of beef muscle, 53% of the total nitrogen of lamb muscle, and 56% of the total nitrogen of pork muscle. Davis (1965) found similar results for beef while Hegarty (1963) reported myofibrillar proteins accounted for about 66% of the total nitrogen in beef. Topel (1965) reported results similar to Hill's for pork.

The amount of myofibrillar protein extractable from a muscle sample depends on environmental and physiological conditions similar to the sarcoplasmic proteins. It should be pointed out that much of the work

done concerning proteins of the muscle reports on the extractable proteins which would include the sarcoplasmic and myofibrillar protein fractions. This work will be discussed in this section as 70-80% of the extractable proteins are myofibrillar proteins.

The length of time a sample was stored had very little effect on the myofibrillar fraction according to Goll et al. (1964). Kronman and Winterbottom (1960) reported that 10-30% of the soluble nitrogen was lost during a 7 day aging period for beef. Moorjani (1962) found a loss of soluble protein when storing fish in crushed ice. The loss in solubility was thought to be due to a protein-protein interaction. Khan and Van Den Berg (1964a) found a slight decrease in the solubility of the myosin fraction but no change in the actomyosin fraction. Davis (1965) reported the myofibrillar fraction increased during the first 10 days of storage and then decreased slowly until the 35th day of storage when the myofibrillar content was the same as 48 hours post mortem or 0 days storage.

The state of rigor mortis influences the extractability of the myofibrillar fraction. Saffle and Galbreath (1964) found pre-rigor beef had 50% more salt-soluble proteins than beef at 48 hours post mortem. Actomyosin is formed at the onset of rigor mortis according to Wierbicki et al. (1956) and Whitaker (1959). This is supported by Connell (1962) who found a reduction in the extractability of myosin during the formation of the actin-myosin bond in rigor mortis. Baliga et al. (1962) found a decrease in the salt-soluble fraction during the first 5 days post mortem

which was coupled with the onset of rigor. This first decrease was recovered as the resolution of rigor took place. Then at 16 days a decrease in the salt-soluble fraction was noted again which was not recoverable. This reaction was due to denaturation of the salt-soluble proteins. Khanand Van Den Berg (1964b) found similar results in poultry except the process was quicker due to a faster onset of rigor. They found a minimum amount of extractability 4-8 hours post mortem which corresponded with the onset of rigor mortis. After rigor mortis was resolved in approximately two days the extractability reached a maximum and held fairly constant for ten days of aging.

Helander (1957) reported satisfactory myofibrillar extraction at pH values ranging from 6.5 to 9.0. A low ultimate pH of meat resulted in lower nitrogen content in the myofibrillar extract according to McLoughlin (1963) and Sayre and Briskey (1963). Both papers also reported that higher muscle pH caused less loss of extractability due to changes in rigor mortis and storage time. Kromman and Winterbottom (1960) found that during freezing of a bovine muscle sample the myofibrillar fraction decreased due to denaturation of the protein. Khan et al. (1963) also indicated that the proteins denatured during frozen storage of chicken muscle caused a loss in myofibrillar solubility. At temperatures of 0-5°C, Khan and Van Den Berg (1963a) found little difference in the extractability of myofibrillar proteins. In beef samples, a high temperature (above 35°C) resulted in a severe loss of the myofibrillar fractions (Sayre and Briskey, 1963). Topel (1965) found that pork tissue which

was extremely soft and watery had a significantly lower quantity of myofibrillar protein. Davis (1965) reported that aseptic tissue had a slightly lower myofibrillar protein content for the first 10-15 days of storage than inoculated tissue. However, by the 35th day of storage the myofibrillar content of the aseptic tissue was higher than that of the inoculated tissue.

Stroma Protein

The stroma proteins are the connective tissues, nerve tissues, and vascular systems of the muscle tissue (Helander, 1957). He identified these proteins as being insoluble in either water or high salt concentrations. Whitaker (1959) identified the stroma proteins as collagen, elastin, reticulum, and ground substance. Hill (1962) reported that the stroma fraction contained 12-18% of the total nitrogen of bovine muscle, 8-12% of the total nitrogen of ovine muscle, and 7-10% of the total nitrogen of porcine muscle. Hegarty (1963) found the stroma content of bovine muscle to be slightly lower at 8% of the total nitrogen. Davis (1965) reported results similar to Hill's for bovine muscle. Topel (1965) reported similar results for porcine muscle.

Very few reports are available on the effect of environmental and physiological changes on the stroma fractions. Davis (1965) found the stroma fraction decreased in bacterially inoculated beef muscle during a 35 day storage period while the stroma fraction of the aseptic sample remained constant or increased slightly. Most other workers studied

exclusively the effect of various conditions on the myofibrillar, sarcoplasmic, and non-protein nitrogen fractions.

Non-protein Nitrogen (NPN)

Some of the nitrogen present in a muscle sample is not incorporated as a part of the protein material. This nitrogen is present as amino acids, ammonia, peptides, etc. All of these compounds are water soluble so they are fractionated with the sarcoplasmic proteins. To determine the amount of NPN, the sarcoplasmic fraction is treated with a 20% trichloracetic acid solution which percipitates the proteins. The nitrogen content determined on the remaining extract is the NPN.

Hill (1962) found that NPN accounted for 11-13% of the total nitrogen in beef, pork, and lamb. Hegarty (1963) found beef to have 9-10% of the total nitrogen in the NPN fraction. Davis (1965) found NPN values in these ranges for beef. Topel (1965) reported results similar to those of Hill for pork. NPN is influenced primarily by proteolytic activity. There are two sources of proteolytic activity in a muscle sample; proteolytic enzymes found in the muscle and proteolytic activity of enzymes from outside sources such as bacteria. Khan et al. (1963), Van Den Berg et al. (1963), and Khan and Van Den Berg (1963a,b) reported the NPN content of chicken muscle increased during a storage time of 5 weeks with bacterial growth kept to a minimum with chemical treatment. Scharpf and Marion (1964) obtained the same results with turkey muscle. Sharp (1963) found during storage of rabbit and beef muscle there was an increase in the amount of NPN. Aberle and Merkel (1966) also found this increase in

beef. Davis (1965) found aseptic beef samples had an increase in NPN during a 35 day storage period but bacterially inoculated beef had a greater increase in NPN content.

Emulsifying Capacity

An emulsion is a dispersion of one liquid in another, the liquids being immiscible (Jirgensons and Straumanis, 1962). An emulsion can be prepared by shaking the liquids together causing a dispersion of one liquid into the other. However, this emulsion would be very unstable, so emulsifying agents would be used to increase stability. These agents lower interfacial tension and aid the formation of stable droplets which are then surrounded by the continuous phase of the emulsion.

A meat emulsion may not be a true emulsion in that some solid material is present in the muscle tissue. Hansen (1960) found that a meat emulsion was essentially a fat or oil dispersed in water with the protein of the muscle tissue acting as the emulsifying agent. Studies by Pearson et al. (1965) indicated that protein from extenders such as nonfat dry milk, soy sodium proteinate, and potassium caseinate, though they emulsify fat provided very little emulsifying capacity to a meat emulsion containing them. Meyer et al. (1964) found the emulsifying agents used in chemical work, such as the diglycerides and some of their derivatives, do not aid in the formation of a meat emulsion. Actually these workers found such emulsifiers incorporated in a meat emulsion hindered the formation of a stable emulsion. They thought there were two reasons for the lack of stability. First, the emulsifiers caused the fat droplets

to become so small the protein present in the emulsion could not cover the droplets. Second, they found evidence of a reaction between the protein and the emulsifiers causing denaturation of the protein and thus the emulsion broke down.

Since Hansen (1960) has found that proteins are the primary emulsifying agents in meat emulsions, others have studied this aspect of sausage emulsions. Swift et al. (1961), while devising a method for determining emulsifying capacity, found the salt soluble proteins were efficient emulsifying proteins. Fukæ;awa (1961) found the salt-soluble myosin A was essential in the emulsification of fat in a sausage. Trautman (1964) reported pre-rigor meat had higher emulsifying capacities than post rigor meat. He studied this thoroughly and found pre-rigor meat had a higher amount of salt-soluble protein which accounted for the greater emulsifying capacity. He found the water-soluble proteins formed weak emulsions which were readily separated into oil and water phases. These results were not in agreement with those of Hegarty et al. (1963). They found the sarcoplasmic proteins formed a very stable emulsion at the pH of normal fresh meat. They also reported salt-soluble proteins produced very stable emulsions.

Swift et al. (1961) found the emulsifying capacity of meat increased with an increasing concentration of salt. Swift and Sulzbacher (1963) carried their work a little further and reported that the water-soluble proteins had increases in emulsifying capacity with increasing concentrations of salt. Vold and Groot (1962, 1964) studied this phenomena in laboratory emulsions and reported that the salt is adsorbed at the interface forming a more stable emulsion. Thus salt is important in the formation of meat emulsions.

Chopping temperature is another important factor in the formation of meat emulsions. Hansen (1960) used a slow chop and found at temperatures below 11°C the protein matrix was not formed, and above 23°C the matrix broke down by denaturation. Chopping temperatures around 15-19°C proved to form more stable emulsions with the desired size of fat droplet. Helmer and Saffle (1963) found similar results using a high speed chopper. Hansen (1960) and Helmer and Saffle (1963) reported that chopping for extended periods of time caused a decrease in the size of the fat globule and thus a weakening of the emulsion. This weakening was due to the overextension of the protein.

Davis (1965) found the emulsifying capacity of beef inoculated with microorganisms had a slight increase during a 35 day storage period at 2-5°C. This inoculated muscle also had a higher emulsion capacity than the aseptic tissue after the 5th day of storage. The aseptic tissue had a constant emulsifying capacity throughout the storage period.

## EXPERIMENTAL METHODS

### Slaughter

The 180-230 lb. pigs used in this study were produced by the Michigan State University Farms and brought into the Meat Laboratory the evening before or on the morning of slaughter. The animals were stunned with an electric stunner and then hoisted by one rear leg. The sticking area of the neck was scrubbed thoroughly with a warm solution of pHisoHex bacteriocidal soap. An alcohol-flame sterilized knife was used to stick the hog which was then allowed to bleed to death by heart action. The hog was placed in the scalding tank to loosen the hair and then transferred to the dehairer where the hair was removed. The evisceration, dropping the head, and further cleaning of the carcass were accomplished in a normal manner. The carcass was not split, however. Before placing the carcass in the cooler, it was thoroughly rinsed with alcohol. The slaughter procedure was similar to that used by Davis (1965) except shaving the neck was not accomplished due to the difficulties encountered.

### Excising the Muscle Sample

The carcass was chilled in the cooler for 20 hrs after which it was removed and again rinsed with alcohol. The carcass was placed on a kraft paper covered table where the shoulders and hams were removed at normal locations from the unsplit middle. The middle was then placed on the table so the midline of the external fat cover was easily accessible. Using a sterilized knife, a cut was made down the midline of the loin

backfat cover, then two cuts were made perpendicular to the midline cut and about 5-8 cm in from each end. The backfat was then stripped and rolled back to expose the longissimus dorsi muscle which was then excised in 3 cm pieces and placed in sterile containers. The second muscle was excised following the same procedure. The person handling the knife wore sterile disposable plastic gloves during the sample procurement. The gloves were changed and the sterilized knife replaced by another between excising the first and second muscles.

The tissue excised from each side of the loin was kept separate but was treated similarly during the excising operations. The excised samples were transferred to a 4-6°C cooler where they were ground through a sterilized pre-chilled grinder (4-6°C) into sterilized Mason quart jars. A two millimeter plate was used for grinding the sample. The jars containing the ground sample were placed in an insulated plastic bag with dry ice and then transported to the Eckert Packing Company Laboratory, Defiance, Ohio, for further study.

The excising procedure was similar to that used by Zender et al. (1958) Davis (1965) used a comparable method but the excising and grinding took place in sterilized isolators.

Inoculation of the Sample

One loin sample was removed from the Mason jar and placed on a sterile tray. Using an alcohol-flame sterilized atomizer, 50 ml of sterile APT broth were sprayed over the sample and mixed into the sample with a sterilized fork. This sample was then divided into six sterilized

sample bottles, each containing approximately 200 grams of sample. Five of the bottles were placed in a refrigerator case and stored at a temperature of 4-6°C. Caps were placed on the bottles but they were not tightly sealed. The samples were removed and analyzed at 1, 5, 9, 13, and 17 days of storage considering the 6th sample taken immediately after spraying as control sample, day 0.

The other ground loin sample was removed from the Mason jar and placed on the same tray used for spraying the control sample. This sample was then sprayed with 50 ml of the inoculum and handled the same as the control samples. This sample was the inoculated sample.

Inoculum Preparation

The inoculum was prepared by placing 15 grams of pork trim, taken from the packing house, in 150 ml of sterilized APT broth (Difco) which was held at 4-6°C in a loosely capped, flat, 500 milliliter bottle which was laid on the flat surface. After 5 days of incubation, 50 ml of the mixture was removed and sprayed on the inoculated sample.

In an attempt to have the inoculum contain the same type and relative number of microorganisms, the remaining inoculum was held at 4-6°C in an upright position for 23 days. At that time 15 ml of the inoculated broth were removed and placed in 150 ml of freshly prepared and sterilized APT broth. This was then held 5 days in the flat position at 4-6°C and 50 ml of the resultant broth were used to inoculate a succeeding loin sample.

Bacterial Numbers

The method outlined by the American Public Health Association (1958) was used. Eleven grams of sample were blended in a sterile blender with 99 ml of sterile water. This slurry was then appropriately diluted and 1.0 ml or 0.1 ml pipetted into sterile petri dishes. Tryptone glucose extract agar was used as the incubating media. The plates were incubated 72 hours at 32°C after which the colonies were counted and reported as the number per gram of sample.

Protein Extraction

The method used for extracting the different protein fractions was similar to that used by Helander (1957) and is outlined in Figure 1. Davis (1965) and Topel (1965) also used modifications of Helander's method and provided sources of information for the method outlined.

All extractions were accomplished at 4-6°C. Ten grams of the meat sample were weighed and blended with 35 ml of 0.03M phosphate buffer, pH 7.4, for 30 seconds. The slurry was transferred to a 300 ml centrifuge bottle. The blender was then rinsed with successive 15 ml, 25 ml, and 25 ml of 0.03M phosphate buffer with all rinses being placed in the centrifuge bottle. A Magna-Stirrer bar was added to the bottle and the bottle was placed on a Magna-Stirrer where the solution was gently agitated for 30 minutes. The solution was then centrifuged 20 minutes at 1400 x G. Supernatant I was filtered through glass wool into a 250 ml flask. One hundred ml of 0.03M phosphate buffer were added to the centrifuge bottle and mixed with Residue I. This was

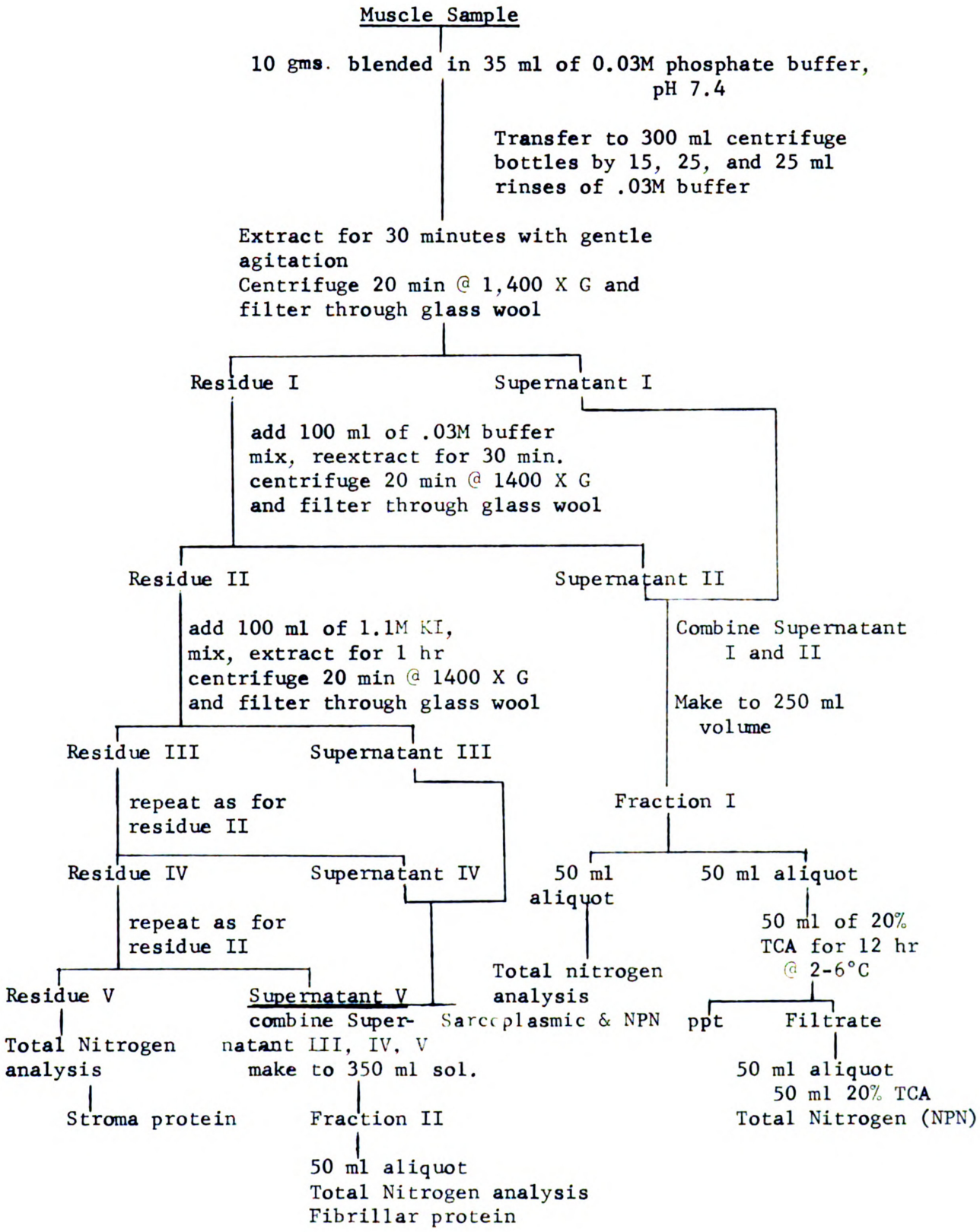



Figure 1. Outline of Protein Fractionation.

agitated for 30 minutes and again centrifuged and filtered. Supernatants I and II were combined and diluted to 250 ml. Protein analysis was then made on Fraction I and recorded as sarcoplasmic protein nitrogen and non-protein nitrogen.

Residue II was suspended in 100 ml of 1.1M KI, 0.1M phosphate buffer solution. This mixture was agitated for 1 hour and was then centrifuged for 20 minutes at 1400 X G. Supernatant III was filtered through glass wool and held in a 500 ml flask. Residue III was suspended in 100 ml of 1.1M KI, 0.1M phosphate buffer solution, agitated for 1 hour, centrifuged 20 minutes at 1400 X G, and Supernatant IV was filtered through glass wool and held with Supernatant III in a 500 ml flask. Residue IV repeated the same process as Residue III. Supernatants III, IV, and V were combined and diluted to 350 ml (Fraction II) and the fibrillar protein was determined on a 50 ml aliquot of Fraction II. Residue V was added to a Kjeldahl flask for protein determination and designated as the stroma protein.

The non-protein nitrogen (NPN) was determined by taking a 50 ml aliquot of Fraction I and adding 50 ml of 20% trichloroacetic acid solution. This was held for 12 hours at 4-6°C and was then filtered through Whatman No. 1 filter paper. A 50 ml aliquot of the filtrate was used for determining the amount of NPN. The amount of NPN was subtracted from the amount of nitrogen found in Fraction I and the remainder was designated as the sarcoplasmic protein.

Emulsion Capacity

The method used was similar to that reported by Swift (1961). Twenty-five grams of sample were blended in 100 ml of cold (2-6°C) 1.0M NaCl solution in a Waring blender for 2 minutes. 6.25 grams of the resultant slurry were placed in a Mason quart jar. Then 37.5 ml of cold 1.0M NaCl solution and 25 ml of once-refined soybean oil (from Central Soya Co.) were added to the jar. The mixture was stirred at approximately 1750 rpm with a Lightnin Model F mixer equipped with an open 3 bladed-propeller. Once-refined soybean oil was added at a rate of approximately 1 ml/sec from a 750 ml separatory funnel. An emulsion was formed which was noted by an increasing viscosity and a fine honeycomb-like appearance. The endpoint was reached when the viscosity was suddenly lost and the mixture had an oil-like appearance. The amount of oil used was then measured by pouring oil into the separatory funnel from a 500 ml graduated cylinder recording the amount required to refill the funnel plus the 25 ml added as the emulsion was started. The emulsifying capacity per 100 milligrams of total protein was then calculated.

Extract Release Volume (ERV)

The procedure followed was first reported by Jay (1964a). Twenty-five grams of sample tempered at 32°C for at least 1 hour were blended with 100 ml of distilled water for 2 minutes. The slurry was poured into a funnel equipped with a piece of Whatman No. 1 filter paper folded thrice to yield eight sides. The filtrate was collected in a 100 ml graduated cylinder by running the filtrate down the side of the graduated cylinder.

After 15 minutes, the volume of the filtrate was recorded as the Extract Release Volume (ERV). The filtration was carried out in a constant temperature chamber at 32°C.

pH Determination

The pH of the samples was determined by blending 10 grams of sample in 100 ml of distilled water for 30 seconds. The pH was then taken using a Beckman Zero-matic pH meter.

Protein Determination

The Kjeldahl method was used as outlined by the A.O.A.C. (1960). A pre-mixed package of the catalysts, called Kelpac No. 5 was used in the digestion flask. The nitrogen content was multiplied by 6.25 to get the protein content.

Moisture Determination

The A.O.A.C. (1960) method of drying 2.5-3.0 grams of sample for 3 hours at 125°C was used.

Statistical Analysis

Analysis of variance was done as outlined by Guenther (1964). The data which indicated a significant difference by analysis of variance were further analyzed by ranking and comparing means by Duncan's new multiple range test (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### Bacterial

Zender et al. (1958) obtained lamb and rabbit muscle samples which were practically sterile. Ockerman et al. (1964) processed mice so that bacteria-free carcasses were stored and studied. These two studies, however, did not compare aseptic samples with contaminated samples as did Davis (1965) in his work. He used sterile isolators to obtain aseptic beef samples and stored these samples for 35 days without evidence of bacterial contamination. The study reported here was undertaken to determine if aseptic samples could be obtained using methods not requiring isolators and to use such samples to compare with bacterially inoculated samples.

Figure 2 shows the control samples were found to be slightly contaminated using the methods outlined previously. However, the controls had a bacterial count of approximately 10 on day 0 and day 1 which is a very low count for any meat sample. By day 9 the control had a bacterial count of slightly more than 100 which was much less than the 100 million counted in the inoculated samples. From the 9th day of storage, the bacterial numbers of the control increased to a count of 1 million at the 17th day of storage. This count was about the same as or less than the 1-95 million bacteria per gram reported by Kirsh et al. (1952) in fresh ground beef purchased at retail stores. Also the 1 million bacteria per gram of the control was less than the critical slime or rejection values of 60-500

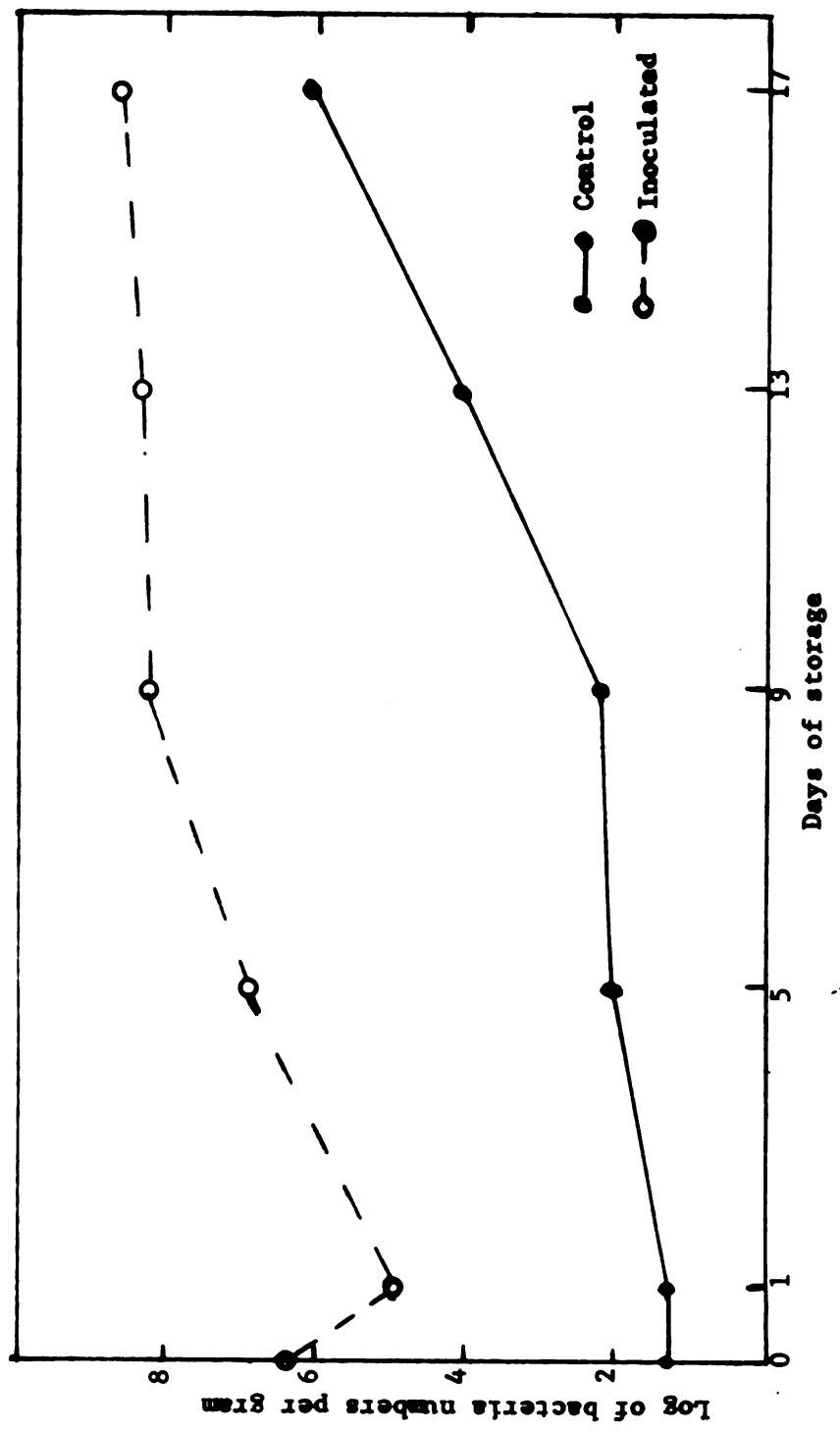



Figure 2. Relationship of the log of bacterial numbers/gram of control and inoculated samples.

million reported by Kirsh et al. (1952), Ayres (1960), and Jay and Kontou (1964). The value was also less than the 450 million recorded in the inoculated sample. Figure 2 shows the number of bacteria per gram of tissue in the inoculated sample started at approximately 5 million on day 0 but then decreased to approximately 100,000 on day 1. This decrease probably resulted from the need for the bacteria in the inoculum to become acclimated to the pork tissue. After the decrease at day 1, the bacterial numbers increased rapidly until day 9 which corresponds to a bacterial count of slightly more than 150 million. The bacterial load continued to increase but at a much slower rate as the count at day 17 was approximately 450 million. An off-odor, slimy, off-color condition was noted between the 5th and 9th days in the inoculated sample, which corresponds to the critical slime value of 60 million reported by Ayres (1960) and Jay and Kontou (1964). Davis (1965) reported slime and off-odor at 15 days of storage with a bacterial count of 35 million. However, his initial contamination of 30,000 was less than the 5 million obtained in this study, which could cause a slower onset of spoilage.

Pseudomonas and Aerobacter bacteria have been reported as the primary meat spoilage organisms (Ayres, 1960). In order to determine what organisms were added to the inoculated sample in this study, a portion of an inoculum handled similarly to the ones used on the pork loin samples was sent to Hilltop Milk and Food Laboratories, Cincinnati, Ohio, for gross identification of the microorganisms present. The inoculum was sampled at 5 days, then 3 additional samples were taken at 4 week intervals, or in other words, a culture was sent at the same time a sample

would have been inoculated if this inoculum would have been used. The ratio of the different bacteria types are shown in Table 1. The Gram-positive diplococci were identified grossly as bacteria of the genera Leuconostoc and Lactobacillus. Non-pathogenic bacteria of the Clostridium genus were the primary Gram-positive spore-forming bacillus organisms. The genera found in the Gram-negative rods were Escherichia and Aerobacter. Therefore, only a small portion of the bacteria added to the inoculated sample was of the genus Aerobacter and none of the genus Pseudomonas. Kirsh et al. (1952) reported that Lactobacillus was present in meat samples and Ayres (1960) found Clostridium organisms were also present. The ratio of the different types of organisms changed during storage of the inoculum but the Gram-positive diplococci were the predominant organism throughout the storage time. One cause of this predominance was that the APT broth used for preparing the inoculum was for the cultivation of heterofermentative Lactobacillus and other organisms requiring a high thiamine content.

Table 1. Ratio of bacterial types in inoculum cultures

| Bacteria / Culture No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Gram-positive diplococci | 9 | 18 | 2000 | 1500 |
| Gram-positive spore-forming bacillus | 5 | 2 | 80 | 280 |
| Gram-negative rods | 1 | 1 | 1 | 1 |

Extract Release Volume (ERV)

The extract release volume (ERV) of the control and inoculated samples are compared in Figure 3. The ERV of the control decreased from 55.0 ml at day 0 to 47.0 ml at day 17. However, the decrease was very slight when compared to the decrease in the ERV of the inoculated sample from 53.0 ml at day 0 to 30.0 ml at day 17. The ERV of the control gradually decreased to approximately 51.0 ml at day 5, then was constant until day 13 at which time it decreased moderately to 47.0 ml at day 17. The ERV of the inoculated sample changed very little from day 0 to day 1. However, from day 1 to day 9 there was a very fast decrease from 53.0 ml to 29.0 ml. A slight increase was noted at day 13 but at day 17 the ERV was again near 29.0 ml indicating a steadying trend. Jay and Kontou (1964) found a high correlation between the ERV and the number of bacteria per gram. Jay (1964b) reported the ERV decreased in a straight line relationship as the bacteria numbers increased. This relationship can be seen by comparing Figures 2 and 3. As the bacteria numbers increased rapidly in the inoculated sample from day 1 to day 9, the ERV decreased rapidly. This relationship was also found in the control sample at day 17. Also, as the bacterial growth of the inoculated sample equilibrated from day 9 to day 17, the ERV also tended to hold constant.

An analysis of variance was completed on the ERV data and the results are summarized in Table 2. A significant difference was found between the treatments of samples or between the inoculated and control samples at the significance level of 1%. Also, the results indicated that time

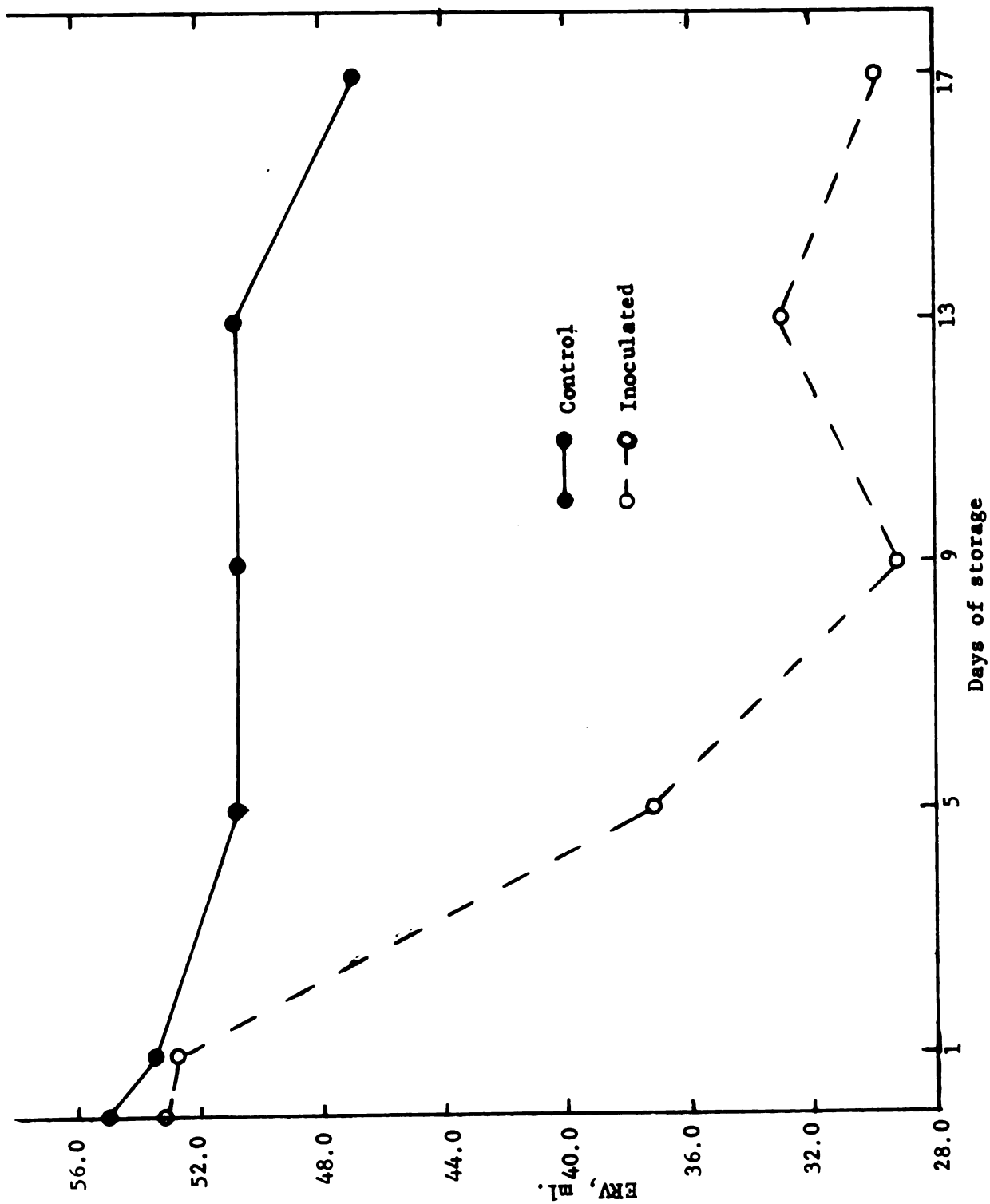



Figure 3. Relationship of the ERV of control and inoculated samples.

Table 2. Analysis of variance of ERV among two treatments and 6 time periods

| Source | Sum of squares | Degrees of freedom | Mean square | F value |
|---|---|---|---|---|
| Treatments | 3078.2 | 1 | 3078.2 | 37.73** |
| periods | 3224.4 | 5 | 644.9 | 7.90** |
| interaction | 1337.3 | 5 | 267.5 | 3.28* |
| error | 5874.8 | 72 | 81.6 | |
| Total | 13,514.7 | 83 | | |

**significant at $P < .01$
*significant at $P < .05$

or periods caused a significant variation at the 1% level while a bacteria and time interaction was significant at the 5% level. However, when the data were analyzed by Duncan's New Multiple Range Test (Steel and Lawrie, 1960), it was found to be divided into two groups (Table 3). Except for control mean, day 17, and inoculated mean, day 5, all of the control means and the inoculated means from day 0 and day 1 are significantly different from the inoculated means day 5 to day 17. These results indicate the number of bacteria present had the greatest influence on the ERV. The addition of a high number of bacteria to the inoculated sample at day 0 did not alter the ERV very much. This indicates the ERV decreased due to the action of the growing bacteria rather than the presence of the bacteria on the sample. This may also partially account for the variation due to time and the time-bacteria interaction.

Table 3. Ranking of ERV means with indication of significance by Duncan's New Multiple Range Test

| Rank | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample[+] | C-0 | C-1 | I-0 | I-1 | C-5 | C-13 | C-9 | C-17 | I-5 | I-13 | I-17 | I-9 |
| Mean, ml | 53.1 | 53.6 | 53.2 | 52.9 | 50.9 | 50.9 | 50.8 | 46.9 | 37.3 | 32.9 | 29.9 | 29.2 |

+C = control
I = inoculated
Number = days of storage
Those means underlined by the same line are not significantly different at the 1% level.

An off-odor, slimy condition was noted between day 5 and day 9 in the inoculated sample which corresponded to an ERV value of 37.3 ml to 29.2 ml. Price et al. (1965) found the onset of spoilage occurred when the ERV value ranged between 30 and 40 ml and the sample was definitely spoiled when the ERV was below 30 ml. Jay and Kontou (1964) reported a rejection of meat samples by a trained panel when the mean ERV value was 30.4 ml. Therefore, the results of this study were in close agreement with those of other workers in this area.

## Proteins

### Sarcoplasmic

In this study the amount of sarcoplasmic protein nitrogen in the control and inoculated samples of pork loin decreased with storage time (Figure 4), which agreed with the results reported by Sayre and Briskey (1963) and McLoughlin (1963). The inoculated sample lost sarcoplasmic protein nitrogen at a more rapid rate than the control sample similar

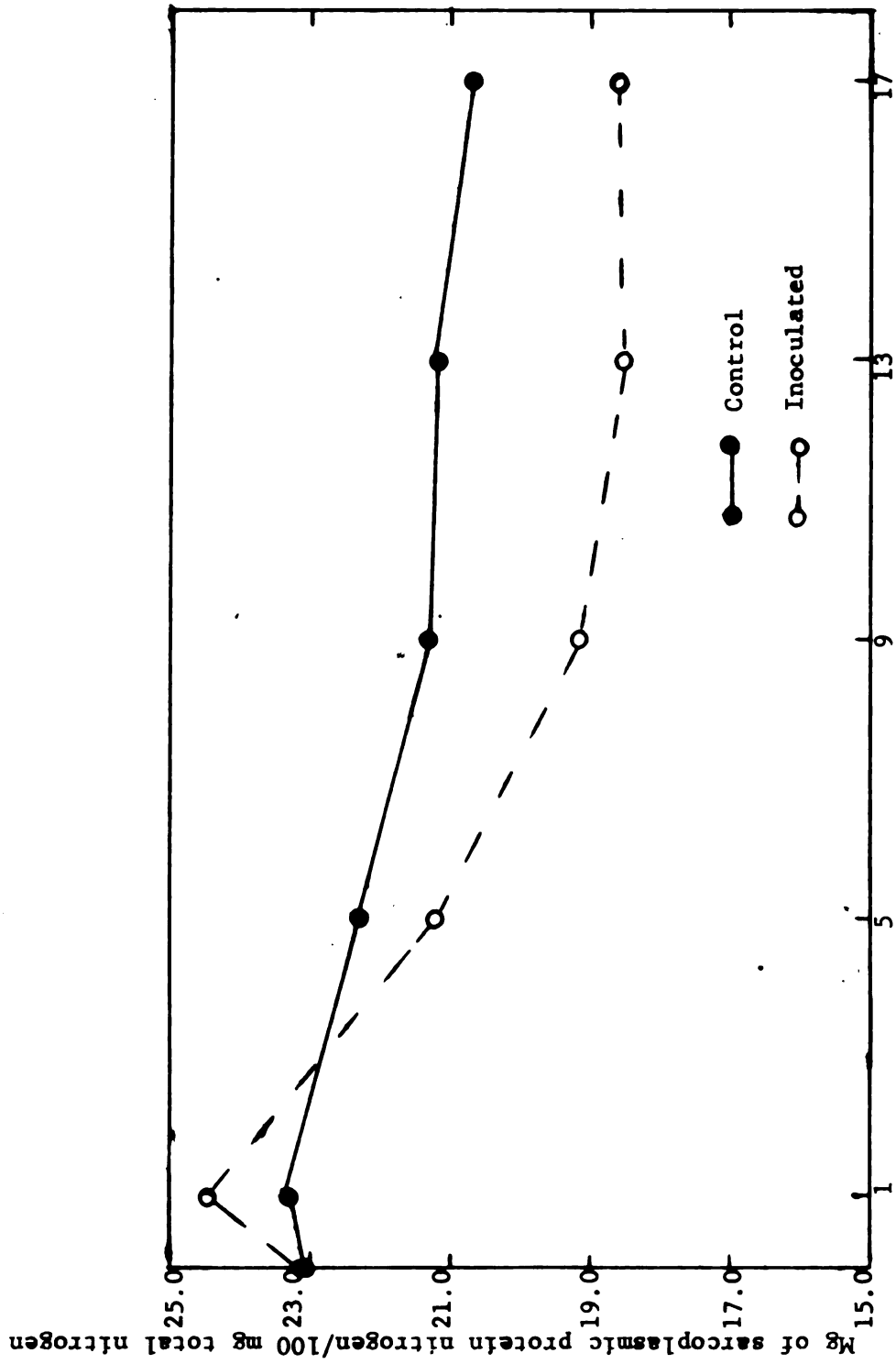



Figure 4. Relationship of the sarcoplasmic protein nitrogen of control and inoculated samples.

to the results reported by Davis (1965). However, statistical analysis did not indicate any significant difference due to the inoculation. The loss of sarcoplasmic protein nitrogen due to aging was significant at the 1% level (Table 4). The loss of sarcoplasmic protein nitrogen in the inoculated sample appeared to be most rapid during the period of greatest bacterial growth, day 1 - day 9. After day 9, the sarcoplasmic protein nitrogen remained relatively constant as did the bacterial numbers. In both samples there was an increase in the sarcoplasmic protein at day 1, with a greater increase in the inoculated sample.

Table 4. Analysis of variance of sarcoplasmic protein nitrogen among two treatments and six time periods

| Source | Sum of squares | Degrees of freedom | Mean square | F value |
|---|---|---|---|---|
| treatments | 24.9 | 1 | 24.90 | 1.80 |
| periods | 224.3 | 5 | 44.86 | 3.34** |
| interaction | 39.1 | 5 | 7.82 | .58 |
| error | 953.0 | 71 | | |
| Total | 1241.3 | 82 | | |

**significant at $P < .01$

The sarcoplasmic protein nitrogen values ranged from 18-23% which were the same as the 21-23% reported by Hill (1962).

Myofibrillar

The myofibrillar protein nitrogen of the control and inoculated samples followed a similar pattern until day 17 at which time the myofibrillar protein nitrogen of the controls increased as the inoculated samples decreased. Figure 5 shows the myofibrillar protein nitrogen was lowest at day 0 then increased until day 9 at which time it decreased to day 13. The low myofibrillar extractability at day 0 or 1 day post-mortem could be due to the state of rigor of the sample as Baliga et al. (1962) found a decrease in the salt soluble protein fraction during the onset of rigor, then as the resolution of rigor took place an increase was noted. As this study started 1 day post-mortem, the sample should have been in rigor and thus the low extractability on day 0, then as the resolution of rigor took place the myofibrillar protein nitrogen increased for a period of time. This general pattern was followed by the inoculated and control samples. At day 9 a second decrease was noted in both samples which was similar to results found by Baliga et al. (1962) which they reported was caused by denaturation of the proteins and was not recoverable. However, the control sample did show another increase at day 17 indicating the decrease in myofibrillar protein was recoverable. The inoculated sample had a lower myofibrillar protein nitrogen extractability initially but reached a higher peak than the control. However, by the 17th day of storage the control again had a higher myofibrillar protein nitrogen fraction. Davis (1965) found the inoculated tissue in his study had a higher myofibrillar protein fraction for the first 10-15 days of storage

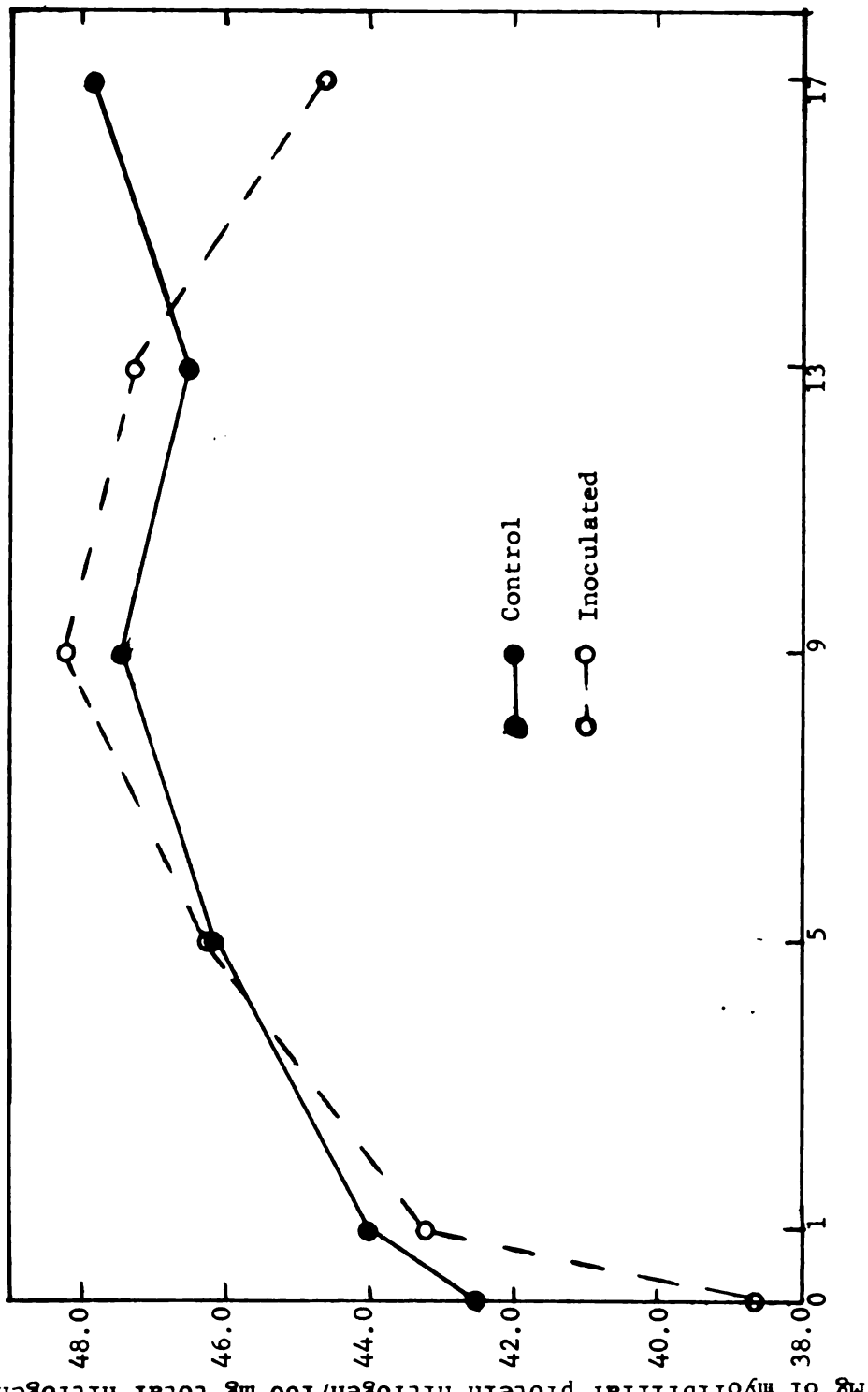



Figure 5. Relationship of the myofibrillar protein nitrogen of control and inoculated samples.

than the aseptic tissue but by the 35th day of storage the aseptic tissue had a higher myofibrillar protein nitrogen fraction than the inoculated sample.

The myofibrillar protein nitrogen values averaged between 38-48% for 7 trials of control and inoculated samples which were much lower than the 56% reported by Hill (1962). Appendix C gives the results from all trials and it can be noted that most results were in the range of 45-55 mg of myofibrillar protein nitrogen per 100 mg of total nitrogen except trial 3 which ranged from 26-31. Samples from trials 6 and 7 also had low myofibrillar protein nitrogen fractions.

Stroma

The stroma protein nitrogen of the control and inoculated samples followed the same general pattern except for day 0 and day 17 (Figure 6). The stroma protein nitrogen at day 0 was higher in the control sample than in the inoculated sample. However, the control decreased sharply while the inoculated increased very slightly, thus at day 1 the inoculated sample had a stroma protein nitrogen extractability which was approximately 1 mg stroma protein nitrogen/100 mg total protein nitrogen higher than the control sample which remained constant until day 17 at which time the inoculated sample had a much higher stroma value than the control. These results are not in agreement with those of Davis (1965) who found the stroma fraction decreased during storage in the bacterially inoculated samples. By comparing figures 5 and 6, it appears that the stroma and

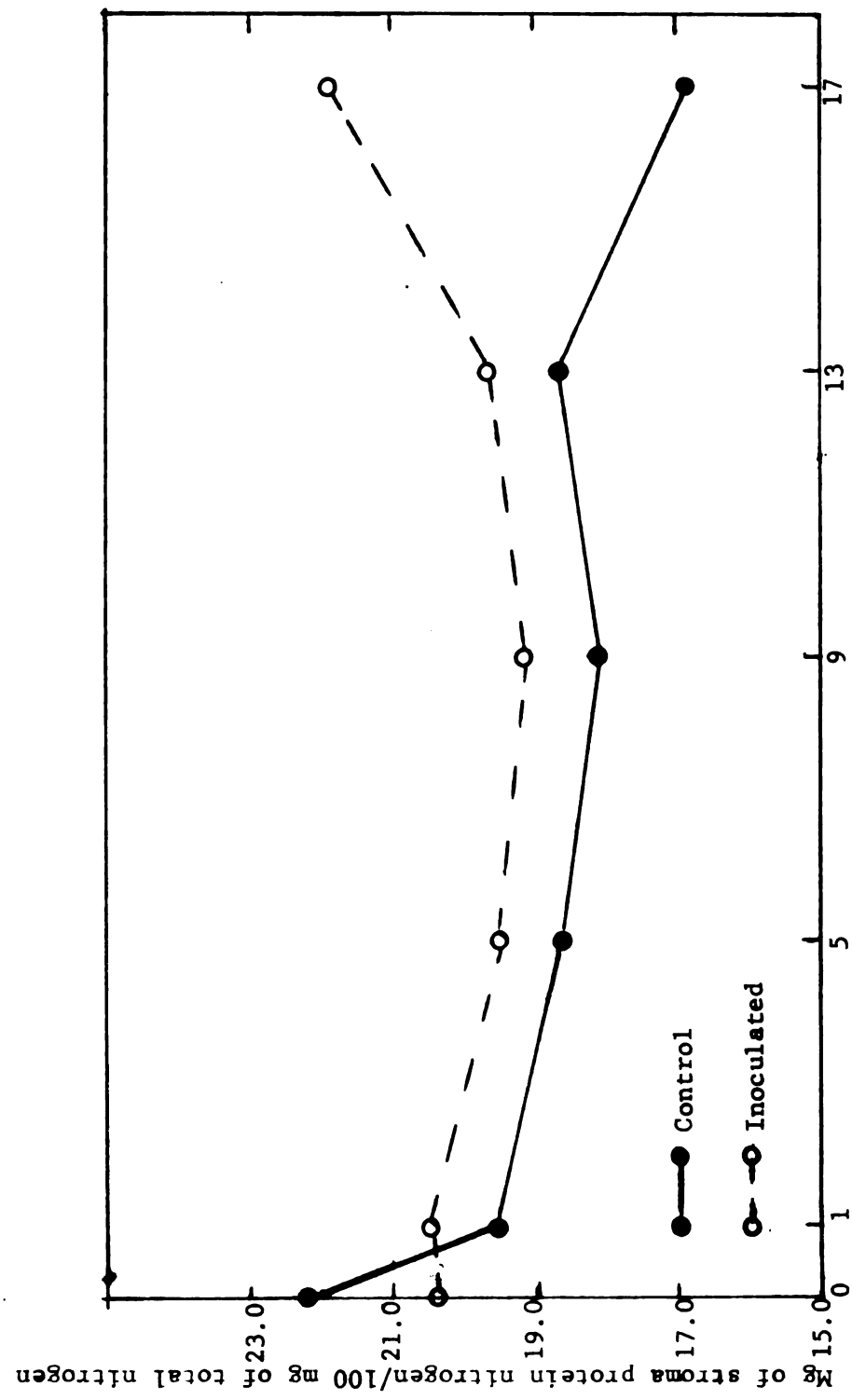



Figure 6. Relationship of the stroma protein nitrogen of control and inoculated samples.

myofibrillar fractions were inversely dependent on each other. This was especially evident at day 17 when the greatest divergence between the control and inoculated samples was noted. In the control sample the myofibrillar fraction increased while stroma fraction decreased while the opposite results were noted in the inoculated samples. The average value of 19.66 mg of stroma protein nitrogen per 100 mg of total nitrogen was much higher than the average of 7-10% found by Hill (1962) in porcine muscle. Appendix D lists the results from each trial and it can be seen that trial 3 had an extremely high stroma fraction and trials 6 and 7 are also rather high. Those 3 runs can account for the high average of stroma protein nitrogen obtained in this study. It also stressed the dependence of the stroma and myofibrillar fractions on each other as the opposite was true of the myofibrillar fraction.

Nonprotein Nitrogen

Except for day 1 the NPN values of the control and inoculated samples were relatively constant (Figure 7). The increase of NPN found at day 1 in the control sample may be the result of the decrease noted in the stroma fraction. The NPN fraction of both samples increased gradually during the storage period indicating that proteolytic activity was taking place due to aging as reported by many other workers. There was little difference between the control and inoculated samples during the 17 day storage period. As there was little difference, it indicated that proteolytic bacteria were not present in the inoculum which had been substantiated previously. Therefore, these results do not agree with those of

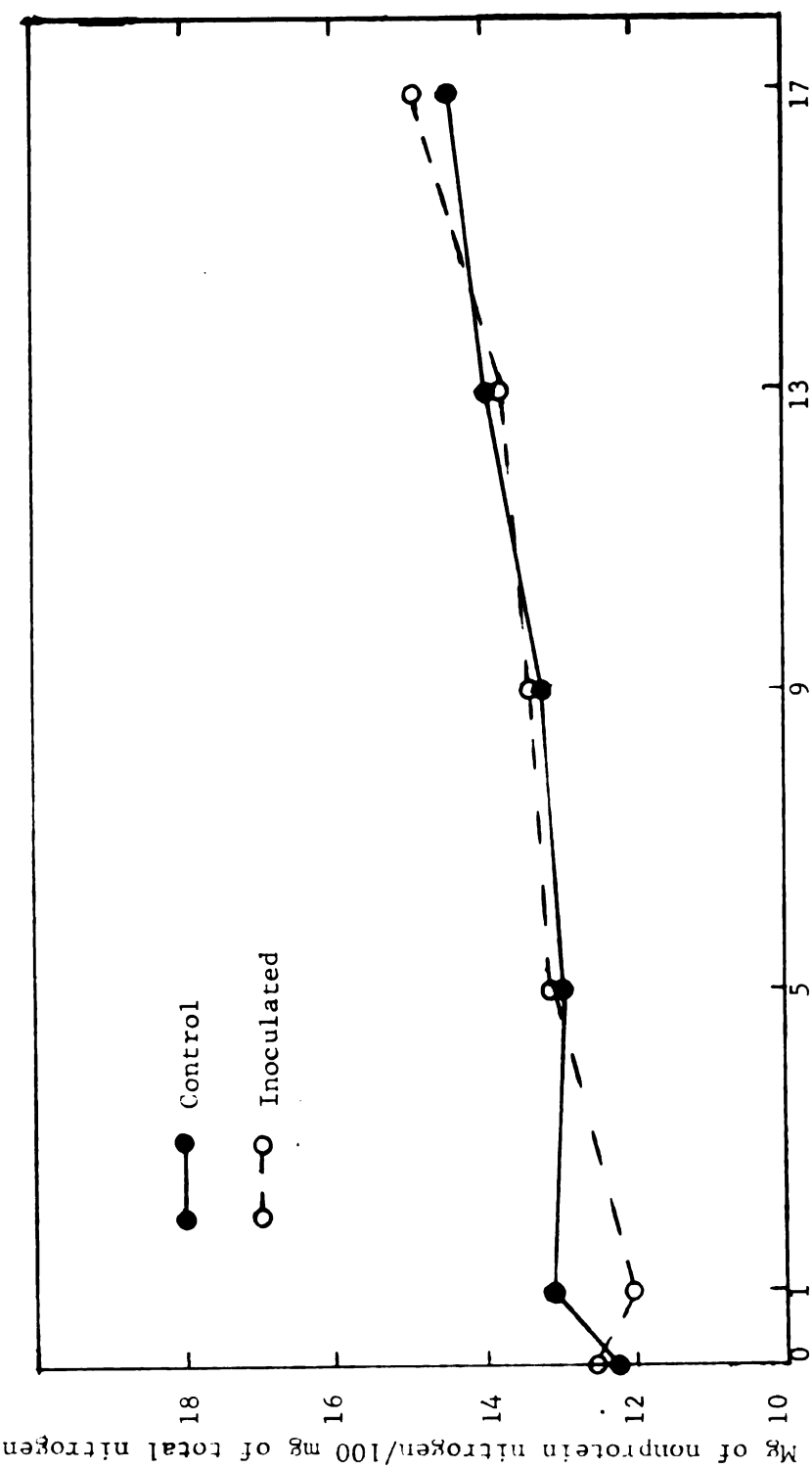



Figure 7. Relationship of the nonprotein nitrogen of control and inoculated samples.

Davis (1965) who found a greater NPN fraction in the inoculated sample than in the aseptic sample. The average NPN value in this study was 13.32 mg of NPN per 100 mg of total nitrogen which was close to the 11-13% NPN reported for pork by Hill (1962).

Emulsifying Capacity

Figure 8 indicates the relationship of the emulsifying capacities of the control samples to those of the inoculated samples. Both types of samples have increasing emulsifying capacities from day 0 to day 1. From day 1 to day 5 the emulsifying capacity of the control remained relatively the same while the emulsifying capacity of the inoculated sample decreased rather sharply. The sharp decrease in the emulsifying capacity of the inoculated sample continued until day 9 as the control decreased sharply from day 5 to day 9. From day 9 the control sample continued to gradually lose emulsifying capacity until day 17. However, during the same time period the emulsifying capacity of the inoculated sample became constant and then increased sharply. Swift et al. (1961) found the salt-soluble proteins were the primary emulsifying proteins. By comparing Figures 5 and 8 it can be seen that the emulsifying capacity did increase as the salt-soluble or myofibrillar protein fraction increased between day 0 and day 1. However, from that point the two curves do not follow each other. As the myofibrillar protein nitrogen fraction reached a peak at day 9, the emulsifying capacities were at the lowest point of a sharp decrease. Then at day 17 when the control myofibrillar protein

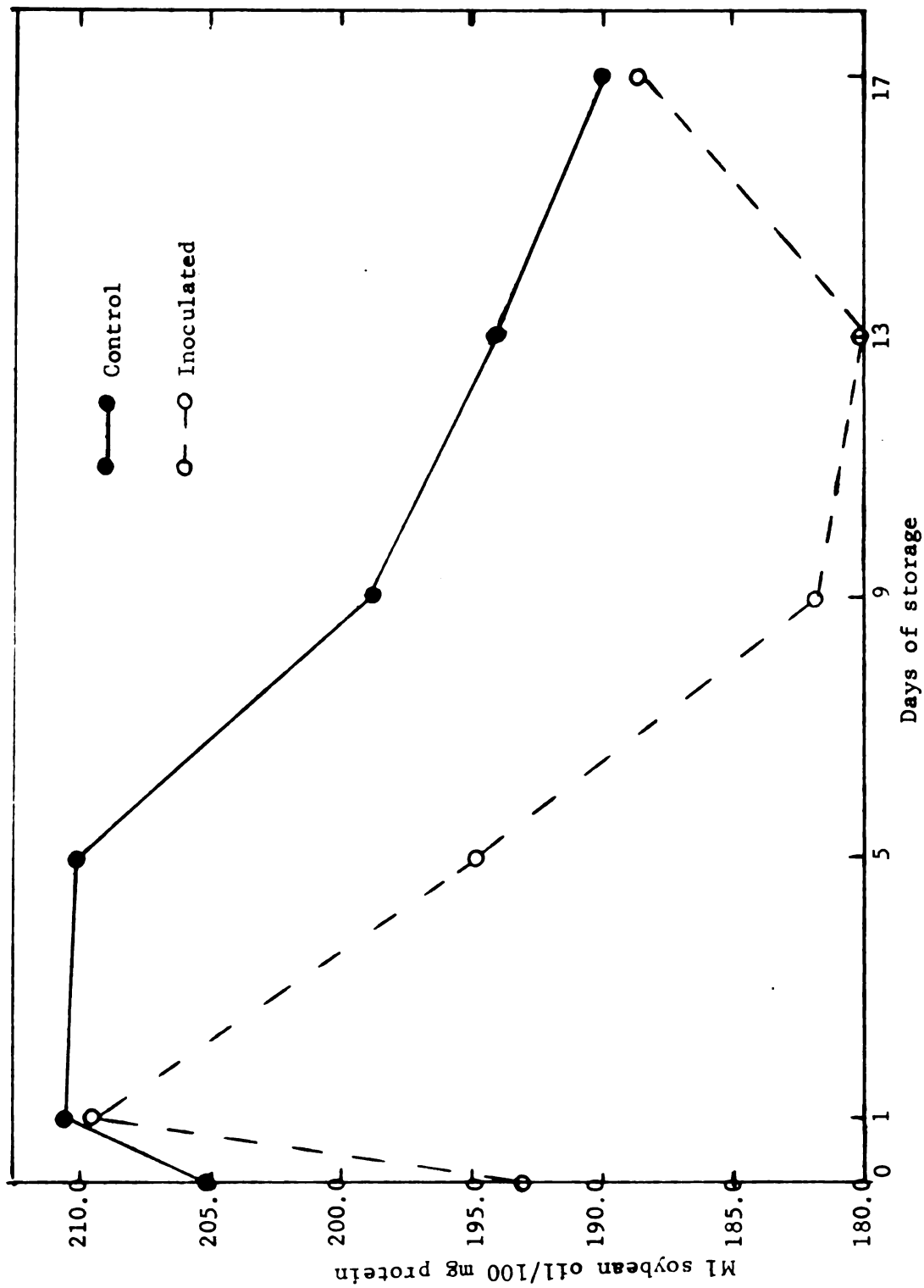



Figure 8. Relationship of emulsifying capacities of control and inoculated samples.

nitrogen increased, the control emulsifying capacity continued to decrease, and as the inoculated myofibrillar protein nitrogen decreased, the inoculated emulsifying capacity increased. Hegarty et al. (1963) reported that emulsifying capacities were not entirely dependent on the myofibrillar proteins as they formed emulsions with sarcoplasmic proteins also. The results of this study also do not agree with those of Davis (1965) who found inoculated tissue had a slight increase in emulsifying capacity during storage while the aseptic sample remained the same. The results of this study indicate that during the time the bacteria increased in numbers most rapidly the emulsifying capacity decreased most rapidly. However this relationship was not significant statistically.

pH

As many of the previously discussed subjects, such as protein extractability, emulsifying capacities, and ERV have been reported to be dependent on pH, the pH results shown in Figure 9 will be discussed. The average pH changed slightly until day 9 at which time the inoculated tissue had a slight increase in pH while the control remained constant. The change was about 0.7 pH units which should not have influenced the results as the protein extraction was buffered and the ERV and emulsifying capacities are not that sensitive to pH changes. However, Appendix H gives the results of the individual trials and the changes were not as small as Figure 9 portrays. All of the control samples remained relatively constant except Trial 7 which varied almost 1 pH unit. The inoculated samples varied, however. Trials 1, 5, 6, 7 tended to have an increasing pH, while Trials 2 and 3 decreased in pH and Trial 4 remained

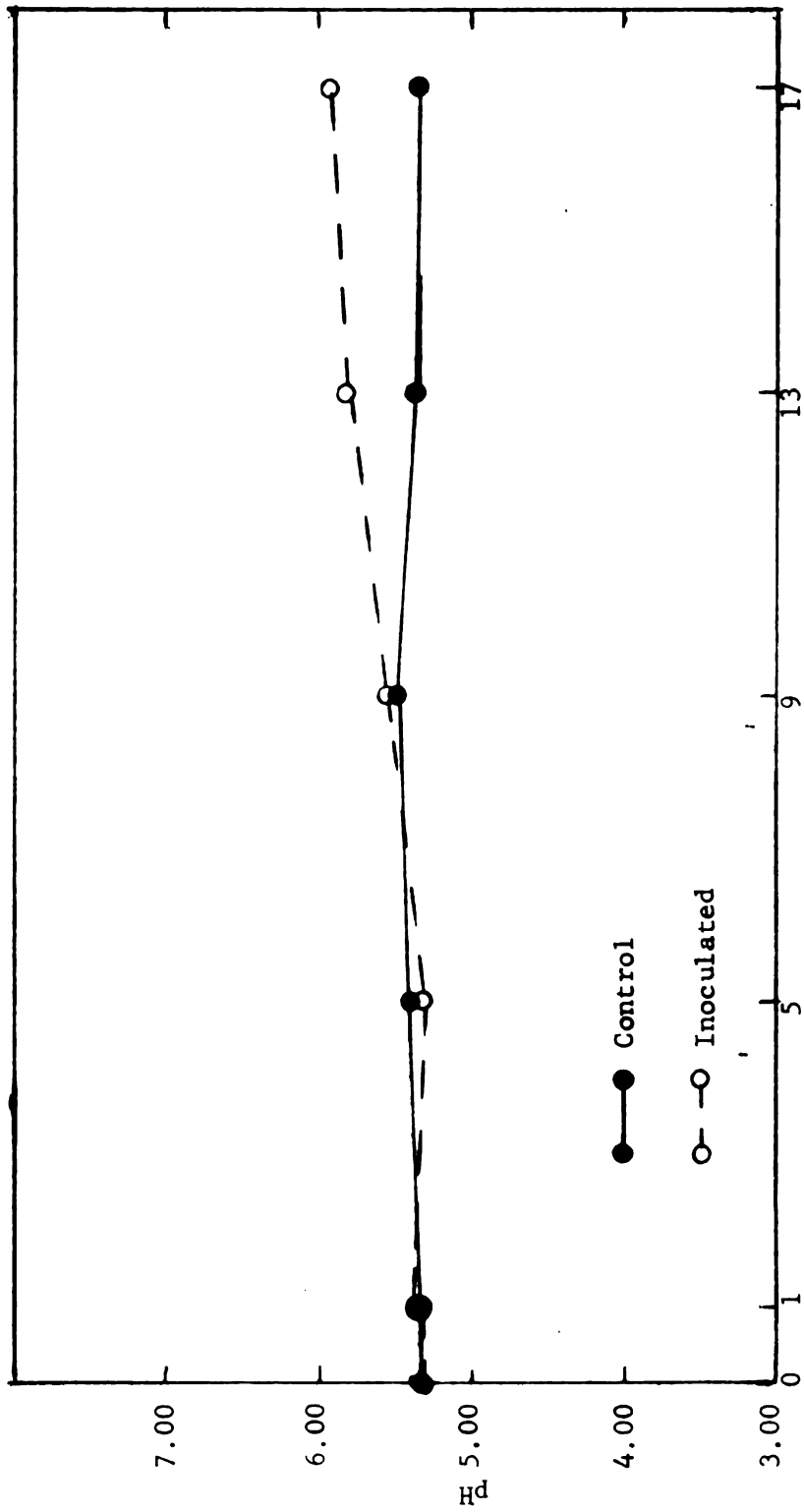



Figure 9. Relationship of pH of control and inoculated samples.

relatively constant. The variation in pH is due to the predominant bacteria genera. It has been previously reported in this manuscript that Lactobacillus was one of the predominant genera in the inoculum. If these bacteria grew rapidly on the sample, the pH would decrease. Thus, the primary growing organisms in Trials 2 and 3 were probably the Lactobacillus bacteria. Most other bacteria reported would cause an increase in the pH of the sample and would be the predominant organisms in Trials 1, 5, 6, and 7. The variation in pH noted in Appendix H could influence the results of protein extractability, ERV and emulsifying capacity. This was especially true of the two trials with the ultimate low pH. The buffering power of the protein extract buffers may have been overcome. The ERV was non-existent or very low with pH below 4.9 (Jay, 1964a), and emulsifying capacity was affected by the insolubility of the protein. Davis (1965) found an increasing pH in his inoculated sample which increased 1.5 pH units by the 35th day of storage.

## SUMMARY AND CONCLUSIONS

A procedure for slaughtering and processing pork was used to obtain muscle samples relatively free of bacteria. The procedure did not provide sterile samples but they had very low counts of 10-100 at the time the samples were placed in storage. The samples had counts of 10,000 bacteria per gram or less until the 17th day of storage when the count was over 1 million.

By comparing the samples obtained by this method with a duplicate sample which was inoculated with a bacterial culture such that the initial bacterial count was 5 million per gram at day 0, it was found bacteria had a great influence on the ERV. As the bacteria grew in numbers, the ERV decreased rapidly. Also, the ERV of the control and inoculated samples were similar at day 0 and day 1 indicating that the growth of the bacteria rather than the number of bacteria caused the change in the ERV value.

The protein extractability changed with storage time but the bacteria present in the inoculated tissue altered the protein extractability slightly. The sarcoplasmic protein fraction had a noticeable difference between the control and inoculated samples but the difference was not significant statistically.

The emulsifying capacity was lower for the inoculated tissue than for the control tissue throughout the 17 day storage period. The reason for this is not known as there was no evidence of protein breakdown in the inoculated sample by a greater NPN content.

The results of this study are inconclusive in many areas, such as the bacterial influence on protein extractability and emulsifying capacity which exhibited trends but no definite results. More study should be directed in the area of controlled bacterial types. It is the contention of this author that if the predominant organism in a meat sample was Pseudomonas there would be protein breakdown by proteolytic activity. Also, Lactobacillus as the predominant organism would yield different changes such as a souring or decrease in pH. This study lacked control in this area and probably caused the varied results especially noticeable when examining the pH of the inoculated trials as given in Appendix H.

BIBLIOGRAPHY

Aberle, E. D. and R. A. Merkel. 1966. Solubility and electrophoretic behavior of some proteins of post-mortem aged bovine muscle. J. Food Sci., 31, p 151.

American Public Health Association, Inc. 1958. Recommended Methods for the Microbial Examination of Foods. American Public Health Assn., New York, N.Y.

A.O.A.C. 1960. Official Methods of Analysis. Assn. of Official Agricultural Chemists, Washington, D.C. 9th edition.

Ayres, J. C. 1955. Microbiological implications in the handling, slaughtering, and dressing of meat animals. Advances in Food Research, 6, p.109.

Ayres, J. C. 1956. The source and significance of microorganisms on meats. Proceedings Ninth Annual Reciprocal Meat Conference, 9, p 164.

Ayres, J. C. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Research, 25, p 1.

Baliga, B. R., M. N. Moorjani, and N. L. Lahiry. 1962. Changes with pyrophosphate-containing buffer and precipitation of protein fraction at $\Gamma/2 = 0.225$ during storage of fresh-water fish in ice. Food Technol., 16, p 84.

Camp, B. J. and W. C. Van Der Zant. 1957. Proteolytic enzymes from Pseudomonas Putrefaciens. II. Characteristics of a endocellular proteolytic enzyme system. Food Research, 22, p 158.

Connell, J. J. 1962. Changes in the amount of myosin extractable from cod flesh during storage at -14°, J. Sci. Food and Agri. 13, p 607.

Davis, Carl E. 1965. A procedure for obtaining and comparing aseptic and bacterially inoculated bovine muscle tissue. M.S. Thesis. The Ohio State Univ.

Fujimaki, M. 1962. Post-mortem changes in muscle proteins. Proceedings 15th Annual Reciprocal Meat Conference, 15, p 220.

Fujimaki, M. and F. E. Deatherage. 1964. Chromatographic fractionation of sarcoplasmic proteins of beef skeletal muscle on ion exchange cellulose. J. Food Sci., 29, p 316.

Fukazawa, T., Y. Hashimoto, T. Yasui. 1961. Effect of some proteins on the binding quality of an experimental sausage. J. Food Sci., 26, p 541.

Goll, D. E., D. W. Henderson and E. A. Kline. 1961. Post mortem changes in physical and chemical properties of bovine muscle. J. Food Sci., 29, p 590.

Guenther, William C. 1964. Analysis of Variance. Prentice-Hall, Inc. Englewood Cliffs, N.J.

Halleck, F. E., C. O. Ball and E. F. Stier. 1958. Factors affecting quality of prepackaged meat. IV. Microbiological studies A. Culture studies on bacterial flora of fresh meat, classification by genera. Food Technol., 12, p 197.

Hansen, L. J. 1960. Emulsion formulation of finely comminuted sausage. Food Technol., 14, p 565.

Hegarty, G. R. 1963. Solubility and emulsifying characteristics of intracellular beef muscle proteins. Ph.D. Thesis, Michigan State Univ.

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, p 663.

Helander, Einar. 1957. On quantitative muscle protein determination. Acta Physiologica Scandinavica, 41, Supplementum 41.

Helmer, R. L. and R. L. Saffle. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17, p 1194.

Hill, Fergus. 1962. Fibre composition of tough and tender muscles of meat animals. Nature, 196, p 65.

Hurley, J. C., F. A. Gardner and C. Vanderzant. 1963a. Some characteristics of a proteolytic enzyme system of Pseudomonas fluorescens. J. Food Sci., 28, p 47.

Hurley, W. C., F. A. Gardner and C. Vanderzant. 1963b. Growth and proteolytic activity of Pseudomonas fluorescens in eggs and egg products. J. Food Sci., 28, p 114.

Jay, J. M. 1964a. Release of aqueous extracts by beef homogenates, and factors affecting release volume. Food Technol., 18, p 129.

Jay, J. M. 1964b. Beef microbial quality determined by extract release volume (ERV). Food Technol., 18, p 133.

Jay, J. M. and K. S. Kontou. 1964. Evaluation of the extract-release volume phenomenon as a rapid test for detecting spoilage in beef. Applied Microbiol., 12, p 378.

Jensen, L. B. and W. R. Hess. 1941. A study of ham souring. Food Research, 6, p 273.

Jirgensons, B. and M. E. Straumanis. 1962. A Short Textbook of Colloid Chemistry. 2nd revised edition, The MacMillan Company, New York.

Khan, A. W., L. Van Den Berg and C. P. Lentz. 1963. Effect of frozen storage on chicken muscle proteins. J. Food Sci., 28, p 425.

Khan, A. W. and L. Van Den Berg. 1964a. Changes in chicken muscle proteins during aseptic storage at above freezing temperatures. J. Food Sci., 29, p 49.

Khan, A. W. and L. Van Den Berg. 1964b. Some protein changes during post mortem tenderization in poultry meat. J. Food Sci., 29, p 597.

Kirsh, R. H., F. H. Berry, C. L. Baldwin and E. M. Foster. 1952. The bacteriology of refrigerated ground beef. Food Research, 17, p 495.

Kronman, M. J. and R. J. Winterbottom. 1960. Post-mortem changes in the water-soluble proteins of bovine skeletal muscle during aging and freezing. J. Agric. and Food Chem., 8, p 67.

Landy, J. J., J. H. Growdon and R. L. Sandberg. 1961. Use of large, germ-free animals in medical research. J. Am. Med. Assn., 178, p 1084.

Lepovetsky, B. C., H. H. Weiser and F. E. Deatherage. 1953. A microbiological study of lymph nodes, bone marrow and muscle tissue obtained from slaughtered cattle. Applied Microbiology, 1, p 57.

McLoughlin, J. V. 1963. Studies on pig muscle. 2. The effect of rapid post-mortem pH fall on the extraction of sarcoplasmic and myofibrillar proteins of post-rigor muscle. Irish J. Agri. Research, 2, p 115.

Meyer, J. A., W. L. Brown, N. E. Giltner and J. R. Guinn. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18, p 138.

Meyer, R. C., E. H. Bohl and E. M. Kohler. 1964. Procurement and maintenance of germ-free swine for microbiological investigations. Applied Microbiology, 12, p 295.

Moorjani, M. N., B. R. Baliga, B. Vijayaranga, and N. L. Lahiry. 1962. Post-rigor changes in nitrogen distribution and texture of fish during storage in crushed ice. Food Technol., 16, p 80.

Ockerman, H. W., V. R. Cahill, K. E. Davis and C. E. Davis. 1964. Tissue storage without contaminants. J. An. Sci., 23, p 142.

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 nonfat dry milk. Food Technol., 19, p 103.

Peterson, A. C. and M. F. Gunderson. 1960. Some characteristics of proteolytic enzymes from Pseudomonas fluorescens. Applied Microbiology, 8, p 98.

Price, J. F., C. Wisidigama and L. J. Bratzler. 1965. Water release by meats as a rapid method of assessing spoilage and microbial growth. Unpublished.

Saffle, R. L. and J. W. Galbreath. 1964. Quantitative determination of salt-soluble protein in various types of meat. Food Technol., 18, p 119.

Sayre, R. N. and E. J. Briskey. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci., 23, p 675.

Scharpf, Jr., L. G. and W. W. Marion. 1964. Extraction of fibrillar and sarcoplasmic proteins of turkey muscle. J. Food Sci., 29, p 303.

Scopes, R. K. and R. A. Lawrie. 1963. Post-mortem lability of skeletal muscle proteins. Nature, 197, p 1202.

Scopes, R. K. 1964. The influence of post-mortem conditions on the solubilities of muscle proteins. Biochemical J., 91, p 201.

Sharp, J. G. 1963. Aseptic autolysis in rabbit and bovine muscle during storage at 37°. J. Sci. Food and Agri., 14, p 468.

Steel, R. G. D. and J. H. Torrie. 1960. Principles And Procedures of Statistics. McGraw Hill Book Co., Inc., New York. p 107.

Swift, C. E., C. Lockett and A. J. Fryar. 1961. Comminuted meat emulsions: The capacity of meats for emulsifying fats. Food Technol., 15, p 468.

Swift, C. E. and W. L. Sulzbacher. 1963. Comminuted meat emulsions: Factors affecting meat proteins as emulsion stabilizers. Food Technol., 17, p 224.

Topel, D. G. 1965. Role of adrenal cortex and thyroid gland function upon some physical and chemical properties of porcine muscle. Ph.D. Thesis, Michigan State Univ.

Trautman, J. C. 1964. Fat emulsifying properties of prerigor and post-rigor pork proteins. Food Technol., 18, 1065.

Van Den Berg, L., A. W. Khan, and C. P. Lentz. 1963. Biochemical and quality changes in chicken meat during storage at above-freezing temperatures. Food Technol., 17, p 91.

Van Der Zant, W. C. 1957. Proteolytic enzymes from Pseudomonas Putrefaciens I. Characteristics of an extracellular proteolytic enzyme system. Food Research, 22, p 151.

Vold, R. C. and R. C. Groot. 1962. An ultracentrifugal method for the quantitative determination of emulsion stability. J. Physical Chem., 66, p 1962.

Vold, R. D. and R. C. Groot. 1964. The effects of electrolytes on the ultracentrifugal stability of emulsions. J. Colloid Sci., 19, p 384.

Weiser, H. H., L. E. Kunkle and F. E. Deatherage. 1954. The use of antibiotics in meat processing. Applied Microbiology, 2, p 88.

Whitaker, J. R. 1959. Chemical changes associated with aging of meat with emphasis on the proteins. Advances in Food Research, 9, p 1.

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 and diethylstilbestrol treated bulls and steers. Food Technol., 10, p 80.

Witter, L. D. 1961. Psychrophilic Bacteria - A Review. J. Dairy Sci., 44, p 983.

Wolin, E. F., J. B. Evans and C. F. Niven, Jr. 1957. The microbiology of fresh and irradiated beef. Food Research, 22, p 682.

Zender, R., C.-Lataste-Dorolle, R. A. Collet, P. Rowinski and R. F. Mouton. 1958. Aseptic autolysis of muscle: Biochemical and microscopic modifications occurring in rabbit and lamb during aseptic and anaerobic storage. Food Research, 23, p 305.

# APPENDIX

Appendix A. Log of the bacterial numbers per gram of sample for control and inoculated samples plus log of bacterial numbers per ml of inoculum

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Control - Day 0 | 0.00 | 1.18 | 0.00 | -- | 0.00 | 3.49 | 2.92 |
| Control - Day 1 | 0.00 | 0.00 | 2.00 | 0.00 | 0.00 | 3.58 | 3.51 |
| Control - Day 5 | 0.00 | -- | 0.00 | 3.70 | 0.00 | 5.70 | 3.02 |
| Control - Day 9 | 0.00 | 0.00 | 2.80 | 3.95 | 1.98 | 3.95 | 2.67 |
| Control - Day 13 | 4.53 | 3.30 | 3.06 | 4.71 | 3.73 | 3.40 | 5.70 |
| Control - Day 17 | 8.70 | 4.44 | 4.50 | 5.41 | 5.70 | 6.70 | 7.70 |
| Pre-inoculated - Day 0 | 0.00 | 0.00 | 2.02 | | 0.00 | | |
| Inoculated - Day 0 | -- | 5.70 | 6.73 | -- | 5.03 | 7.51 | 7.20 |
| Inoculated - Day 1 | 3.48 | 6.67 | 5.37 | 0.00 | 6.13 | 5.60 | 7.60 |
| Inoculated - Day 5 | 5.20 | -- | 7.28 | 3.59 | 7.70 | 7.99 | 9.70 |
| Inoculated - Day 9 | 9.70 | 6.81 | 7.57 | 5.41 | 9.70 | 9.40 | 9.30 |
| Inoculated - Day 13 | 9.46 | 7.19 | 6.62 | 5.70 | 10.39 | 9.74 | 9.33 |
| Inoculated - Day 17 | 9.96 | 7.53 | 7.74 | 7.03 | 9.94 | 8.00 | 10.06 |
| Inoculum | 4.98 | 8.70 | 8.33 | <3.00 | 6.98 | 9.31 | 10.17 |

Appendix B. Milligrams of sarcoplasmic protein nitrogen per 100 milligrams total protein nitrogen for control and inoculated samples.

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 25.06 | 23.46 | 16.21 | 25.86 | 26.49 | 20.99 | 23.23 | 23.04 |
| Control - Day 1 | --- | 24.84 | 17.83 | 29.46 | 27.53 | 18.56 | 21.60 | 23.30 |
| Control - Day 5 | 23.85 | 20.69 | 17.27 | 27.73 | 23.85 | 20.06 | 22.56 | 22.29 |
| Control - Day 9 | 23.78 | 21.59 | 17.52 | 24.53 | 21.43 | 19.98 | 20.16 | 21.28 |
| Control - Day 13 | 21.49 | 21.30 | 15.01 | 24.91 | 22.46 | 20.26 | 22.97 | 21.20 |
| Control - Day 17 | 21.87 | 23.33 | 14.78 | 23.90 | 21.78 | 20.26 | 19.01 | 20.70 |
| Inoculated - Day 0 | 25.62 | 22.67 | 18.62 | 27.83 | 23.21 | 21.91 | 22.36 | 23.17 |
| Inoculated - Day 1 | 28.96 | 21.71 | 19.16 | 30.48 | 26.66 | 23.44 | 21.19 | 24.51 |
| Inoculated - Day 5 | 25.83 | 18.94 | 14.91 | 25.31 | 21.93 | 22.07 | 19.58 | 21.22 |
| Inoculated - Day 9 | 22.32 | 16.71 | 13.14 | 24.33 | 22.35 | 18.58 | 16.99 | 19.20 |
| Inoculated - Day 13 | 20.67 | 15.04 | 12.87 | 23.43 | 22.71 | 16.96 | 18.19 | 18.55 |
| Inoculated - Day 17 | 20.83 | 15.74 | 11.91 | 24.02 | 23.40 | 17.28 | 17.15 | 18.62 |

Appendix C. Milligrams of myofibrillar protein nitrogen per 100 milligrams of total nitrogen for control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 50.24 | 49.87 | 26.04 | 49.99 | 50.49 | 30.54 | 39.99 | 42.45 |
| Control - Day 1 | --- | 51.79 | 26.93 | 51.38 | 50.52 | 40.79 | 42.66 | 44.01 |
| Control - Day 5 | 49.56 | 54.83 | 29.37 | 51.91 | 53.53 | 43.03 | 41.16 | 46.20 |
| Control - Day 9 | 53.53 | 56.89 | 28.81 | 52.95 | 54.49 | 39.23 | 46.05 | 47.42 |
| Control - Day 13 | 48.77 | 54.00 | 26.40 | 53.27 | 55.28 | 41.65 | 46.68 | 46.58 |
| Control - Day 17 | 51.02 | 51.43 | 28.21 | 55.22 | 56.01 | 44.62 | 48.55 | 47.87 |
| Inoculated - Day 0 | 51.26 | 51.98 | 28.73 | 52.49 | 49.72 | 36.52 | 37.81 | 38.67 |
| Inoculated - Day 1 | 46.49 | 52.20 | 31.06 | 51.95 | 48.39 | 32.70 | 40.41 | 43.31 |
| Inoculated - Day 5 | 49.38 | 55.04 | 29.26 | 54.01 | 52.10 | 40.20 | 44.55 | 46.36 |
| Inoculated - Day 9 | 54.29 | 41.87 | 28.00 | 51.71 | 55.91 | 54.31 | 51.46 | 48.22 |
| Inoculated - Day 13 | 56.19 | 33.56 | 22.46 | 53.48 | 57.35 | 52.88 | 55.13 | 47.29 |
| Inoculated - Day 17 | 54.89 | 23.17 | 26.23 | 51.06 | 55.60 | 51.37 | 49.92 | 44.61 |

Appendix D. Milligrams of stroma protein nitrogen per 100 milligrams of total nitrogen for control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 12.33 | 14.24 | 46.50 | 10.62 | 10.74 | 37.64 | 23.49 | 22.27 |
| Control - Day 1 | --- | 10.07 | 41.50 | 7.21 | 10.33 | 25.03 | 23.16 | 19.55 |
| Control - Day 5 | 14.77 | 11.15 | 40.89 | 6.46 | 11.26 | 23.70 | 22.04 | 18.62 |
| Control - Day 9 | 8.19 | 8.54 | 40.45 | 9.81 | 11.42 | 27.59 | 20.67 | 18.10 |
| Control - Day 13 | 16.42 | 10.44 | 44.30 | 10.16 | 9.18 | 23.74 | 16.55 | 18.68 |
| Control - Day 17 | 13.18 | 10.60 | 41.91 | 7.91 | 8.79 | 19.20 | 16.72 | 16.90 |
| Inoculated - Day 0 | 10.59 | 12.67 | 40.51 | 7.34 | 13.24 | 30.46 | 26.53 | 20.19 |
| Inoculated - Day 1 | 12.66 | 13.55 | 37.62 | 7.17 | 13.90 | 31.59 | 25.78 | 20.32 |
| Inoculated - Day 5 | 12.29 | 11.88 | 43.77 | 8.02 | 13.54 | 23.80 | 23.17 | 19.50 |
| Inoculated - Day 9 | 9.82 | 27.02 | 44.24 | 9.08 | 9.89 | 14.95 | 19.29 | 19.18 |
| Inoculated - Day 13 | 9.12 | 37.90 | 50.89 | 10.35 | 8.01 | 15.29 | 13.19 | 20.67 |
| Inoculated - Day 17 | 9.51 | 45.14 | 46.62 | 11.27 | 8.79 | 14.72 | 17.20 | 21.89 |

Appendix E. Milligrams of nonprotein nitrogen (NPN) per 100 milligrams of total nitrogen for control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 12.34 | 12.45 | 11.26 | 13.22 | 12.30 | 10.83 | 13.30 | 12.24 |
| Control - Day 1 | --- | 12.81 | 14.18 | 11.96 | 11.63 | 15.63 | 12.59 | 13.13 |
| Control - Day 5 | 12.33 | 13.33 | 12.47 | 13.91 | 11.37 | 13.20 | 14.25 | 12.98 |
| Control - Day 9 | 14.48 | 12.50 | 13.23 | 12.72 | 13.18 | 13.21 | 13.13 | 13.21 |
| Control - Day 13 | 16.42 | 14.27 | 14.30 | 11.67 | 13.09 | 14.36 | 13.82 | 13.99 |
| Control - Day 17 | 13.18 | 14.66 | 15.17 | 12.97 | 13.43 | 15.95 | 15.79 | 14.45 |
| Inoculated - Day 0 | 12.53 | 12.69 | 12.16 | 12.35 | 13.85 | 11.10 | 13.31 | 12.59 |
| Inoculated - Day 1 | 11.90 | 12.55 | 12.17 | 11.79 | 11.05 | 12.27 | 12.63 | 12.05 |
| Inoculated - Day 5 | 12.50 | 14.15 | 12.06 | 12.69 | 12.93 | 14.44 | 13.16 | 13.13 |
| Inoculated - Day 9 | 13.58 | 14.40 | 14.64 | 14.90 | 11.86 | 12.17 | 12.28 | 13.40 |
| Inoculated - Day 13 | 14.03 | 14.99 | 13.78 | 12.76 | 11.94 | 15.38 | 13.51 | 13.77 |
| Inoculated - Day 17 | 14.77 | 15.96 | 15.26 | 13.71 | 12.23 | 16.65 | 15.74 | 14.90 |

Appendix F. Emulsifying capacity of control and inoculated samples ml of soybean oil per 100 mg of protein

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 288.1 | 211.7 | 147.0 | 179.4 | 224.1 | 208.1 | 177.9 | 205.2 |
| Control - Day 1 | 273.1 | 185.0 | 143.6 | 249.6 | 239.9 | 203.5 | 179.9 | 210.7 |
| Control - Day 5 | 216.0 | 238.9 | 143.4 | 177.1 | 240.7 | 230.2 | 225.2 | 210.2 |
| Control - Day 9 | 199.4 | 187.2 | 191.2 | 171.1 | 210.5 | 201.8 | 230.5 | 198.8 |
| Control - Day 13 | 193.3 | 171.0 | 174.4 | 159.7 | 205.8 | 205.5 | 259.7 | 194.1 |
| Control - Day 17 | 165.4 | 165.4 | 150.3 | 226.5 | 214.5 | 192.9 | 215.7 | 190.1 |
| Inoculated - Day 0 | --- | 154.8 | 191.2 | 206.7 | 237.5 | 173.8 | 195.3 | 193.2 |
| Inoculated - Day 1 | 280.3 | 156.0 | 169.6 | 226.7 | 246.6 | 200.3 | 187.3 | 209.5 |
| Inoculated - Day 5 | 253.8 | 140.5 | 163.7 | 170.9 | 210.5 | 211.4 | 213.4 | 194.9 |
| Inoculated - Day 9 | 141.9 | 162.2 | 158.0 | 170.6 | 197.2 | 197.0 | 245.7 | 181.8 |
| Inoculated - Day 13 | 159.1 | 162.3 | 159.6 | 160.8 | 232.6 | 209.4 | 177.5 | 180.2 |
| Inoculated - Day 17 | 173.3 | 161.7 | 146.0 | 169.6 | 231.6 | 218.6 | 211.3 | 188.7 |

Appendix G. Milliliters of extract release volume (ERV) for control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 60.5 | 50.0 | 60.0 | 48.5 | 54.5 | 57.5 | 54.5 | 55.1 |
| Control - Day 1 | 58.5 | 51.0 | 55.0 | 53.5 | 50.0 | 55.0 | 52.5 | 53.6 |
| Control - Day 5 | 52.0 | 48.5 | 61.5 | 42.0 | 43.5 | 53.5 | 55.0 | 50.9 |
| Control - Day 9 | 50.0 | 48.0 | 56.2 | 46.8 | 50.5 | 53.5 | 50.5 | 50.8 |
| Control - Day 13 | 48.0 | 49.0 | 58.0 | 46.5 | 51.5 | 52.5 | 50.5 | 50.9 |
| Control - Day 17 | 44.0 | 39.0 | 52.0 | 39.5 | 51.0 | 48.5 | 54.0 | 46.9 |
| Inoculated - Day 0 | 55.0 | 46.0 | 52.5 | 48.0 | 54.5 | 57.5 | 59.0 | 53.2 |
| Inoculated - Day 1 | 55.5 | 52.0 | 52.0 | 47.0 | 55.5 | 51.0 | 57.0 | 52.9 |
| Inoculated - Day 5 | 48.5 | 8.5 | 15.0 | 48.0 | 49.0 | 48.0 | 44.0 | 37.3 |
| Inoculated - Day 9 | 32.0 | 9.0 | 11.5 | 54.0 | 20.5 | 34.0 | 43.5 | 29.2 |
| Inoculated - Day 13 | 37.0 | 8.0 | 43.5 | 47.0 | 23.0 | 22.0 | 50.0 | 32.9 |
| Inoculated - Day 17 | 16.5 | 19.5 | 12.0 | 51.5 | 9.0 | 53.5 | 47.5 | 29.9 |

Appendix H. pH of control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 5.5 | 5.4 | 5.3 | 5.5 | 5.6 | 5.3 | 4.8 | 5.34 |
| Control - Day 1 | 5.5 | 5.3 | 5.3 | 5.5 | 5.4 | 5.4 | 5.1 | 5.36 |
| Control - Day 5 | 5.5 | 5.5 | 5.1 | 5.4 | 5.4 | 5.3 | 5.5 | 5.39 |
| Control - Day 9 | 5.7 | 5.4 | 5.4 | 5.4 | 5.5 | 5.3 | 5.7 | 5.49 |
| Control - Day 13 | 5.6 | 5.4 | 5.2 | 5.3 | 5.5 | 5.3 | 5.4 | 5.39 |
| Control - Day 17 | 5.6 | - | 5.3 | 5.3 | 5.4 | 5.2 | 5.5 | 5.38 |
| Inoculated - Day 0 | - | 5.4 | 5.4 | 5.6 | 5.5 | 5.3 | 4.8 | 5.33 |
| Inoculated - Day 1 | 5.5 | 5.4 | 5.1 | 5.4 | 5.4 | 5.4 | 5.3 | 5.36 |
| Inoculated - Day 5 | 5.5 | 5.0 | 4.8 | 5.5 | 6.0 | 5.0 | 5.4 | 5.31 |
| Inoculated - Day 9 | 6.2 | 4.7 | 5.0 | 5.5 | 6.8 | 5.2 | 5.2 | 5.51 |
| Inoculated - Day 13 | 5.8 | 4.7 | 5.0 | 5.3 | 7.1 | 6.1 | 6.8 | 5.83 |
| Inoculated - Day 17 | 6.8 | - | 4.8 | 5.3 | 7.3 | 6.3 | 5.1 | 5.93 |

Appendix J. The percent moisture in the control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 66.20 | 68.40 | 57.98 | 62.22 | 61.68 | 64.58 | 63.36 | 63.49 |
| Control - Day 1 | 66.00 | 70.00 | 59.58 | 60.20 | 64.54 | 62.15 | 63.23 | 63.67 |
| Control - Day 5 | 66.58 | 70.06 | 60.76 | 64.42 | 59.68 | 63.24 | 64.88 | 64.23 |
| Control - Day 9 | 65.93 | 68.11 | 58.88 | 62.37 | 64.95 | 65.41 | 62.76 | 64.06 |
| Control - Day 13 | 66.37 | 69.09 | 61.99 | 62.38 | 66.05 | 63.67 | 64.77 | 64.90 |
| Control - Day 17 | 66.09 | 68.29 | 61.10 | 62.73 | 67.31 | 64.54 | 66.34 | 65.20 |
| Inoculated - Day 0 | --- | 69.30 | 64.98 | 65.91 | 60.33 | 65.09 | 64.96 | 65.10 |
| Inoculated - Day 1 | 65.18 | 69.45 | 62.04 | 63.77 | 60.36 | 63.30 | 66.52 | 64.37 |
| Inoculated - Day 5 | 65.42 | 70.24 | 63.01 | 65.87 | 58.00 | 64.69 | 65.75 | 64.71 |
| Inoculated - Day 9 | 64.15 | 69.02 | 62.43 | 64.97 | 65.20 | 63.34 | 66.70 | 65.12 |
| Inoculated - Day 13 | 63.24 | 69.16 | 61.21 | 63.56 | 63.78 | 64.70 | 68.27 | 64.85 |
| Inoculated - Day 17 | 65.48 | 69.16 | 62.55 | 64.91 | 63.65 | 66.82 | 66.98 | 65.65 |

Appendix K. The percent protein in the control and inoculated samples

| Sample / Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Average |
|---|---|---|---|---|---|---|---|---|
| Control - Day 0 | 19.30 | 18.29 | 16.54 | 19.96 | 18.86 | 17.49 | 18.46 | 18.41 |
| Control - Day 1 | 18.60 | 16.65 | 17.77 | 18.19 | 19.59 | 17.89 | 18.12 | 18.12 |
| Control - Day 5 | 18.85 | 18.07 | 17.16 | 19.65 | 18.87 | 17.38 | 18.03 | 18.29 |
| Control - Day 9 | 18.96 | 18.16 | 16.53 | 19.29 | 18.15 | 17.84 | 18.31 | 18.18 |
| Control - Day 13 | 18.98 | 17.78 | 17.48 | 19.41 | 19.05 | 17.52 | 18.10 | 18.33 |
| Control - Day 17 | 19.10 | 17.78 | 17.83 | 18.19 | 19.02 | 17.00 | 18.17 | 18.16 |
| Inoculated - Day 0 | --- | 18.43 | 17.99 | 20.22 | 19.03 | 18.30 | 19.15 | 18.85 |
| Inoculated - Day 1 | 18.48 | 17.31 | 17.76 | 20.07 | 18.41 | 17.67 | 19.22 | 18.15 |
| Inoculated - Day 5 | 18.44 | 18.59 | 17.35 | 19.54 | 18.05 | 17.98 | 19.49 | 18.49 |
| Inoculated - Day 9 | 18.60 | 18.38 | 19.72 | 19.58 | 18.36 | 17.16 | 19.86 | 18.52 |
| Inoculated - Day 13 | 18.93 | 18.44 | 18.04 | 20.28 | 18.49 | 17.29 | 19.04 | 18.64 |
| Inoculated - Day 17 | 18.93 | 18.18 | 16.71 | 20.13 | 17.96 | 16.65 | 19.59 | 18.31 |