THE EFFECTS OF FOUR SPECIES OF BACTERIA ON SOME PROPERTIES OF PORCINE MUSCLE PROTEINS

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ABSTRACT

THE EFFECTS OF FOUR SPECIES OF BACTERIA

ON SOME PROPERTIES OF PORCINE MUSCLE PROTEIN

by Ronald James Borton

In recent years a few studies have been undertaken to determine the effect of microbial spoilage on some of the properties of muscle tissue or the food product, meat. These studies generally used an unspecified mixture of microorganisms which were not controlled with respect to the type and/or ratio of types of microorganisms present. The objective of this study was to determine the effect of four species of bacteria on some of the properties of porcine proteins.

Porcine longissimus dorsi muscle was excised and ground as aseptically as possible to obtain a control sample with little or no contamination. Portions of the excised muscle were inoculated with either Pediococcus cerevisiae, Leuconostoc mesenteroides, Micrococcus luteus or Pseudomonas fragi organisms. The control and inoculated samples were divided so that portions of each were stored at 2° and 10°C and analysis for bacterial growth, protein solubility (water-soluble, salt-soluble and insoluble proteins plus non protein nitrogen (NPN)), pH and emulsifying capacity were accomplished after 0, 2, 4, 8, 12, 16 and 20 days of storage. Electrophoretic studies of water and salt-soluble protein extracts were completed after 0, 8 and 20 days of storage with emulsion stability tested after 0 and 12 days of storage.

Results of the studies involving the control samples indicated storage and storage temperature had some effects on the porcine proteins. The control samples were not free of microorganisms, however, the number of

organisms per gram were generally below 10,000 which is quite low for fresh ground meat. The samples stored at 10°C evidenced more growth than those stored at 2°C. The amount of water-soluble protein decreased with increasing storage time, while the amount of salt-soluble protein increased during the first 8 days of storage and then decreased or remained relatively constant. The quantity of insoluble protein and the quantity of NPN increased as length of storage increased. The amount of NPN found was also higher in the samples stored at 10°C than in those stored at 2°C. Electrophoresis of the water- and salt-soluble protein extracts revealed little change in the types of protein present during the storage period. Other properties studied were not influenced by storage or temperature.

Pediococcus cerevisiae was used in this study as one representative of the acid producing group of organisms or ' lactics, found in fresh and processed meat spoilages. This organism grew at 10°C but did not grow at 2°C under the conditions of this study. The pH of the inoculated samples stored at 10°C decreased with growth of the organism but other properties of the samples were not affected.

Leuconostoc mesenteroides was also chosen for this study as a representative of the lactics group of organisms. This organism grew at 10° and 2°C but growth at 2°C was slower than that at 10°C. The growth at 2°C did not influence the protein properties studied. However, growth of these organisms at 10°C lowered the pH to the lowest values obtained in this entire study. The low pH seemed to cause a decrease in the extractability of the water- and salt-soluble proteins and thus an increase in insoluble protein. The loss of protein solubility in turn decreased the emulsifying capacity. Electrophoresis of the water extracts of

inoculated samples stored 20 days at 10°C resulted in fewer protein bands present in the gel than the number of protein bands found in the extract from control samples stored 20 days at 10°C.

Micrococcus luteus organisms were used as representative of the salt-tolerant micrococci organisms which are found on fresh meat and the primary spoilage organisms of cured meat products, such as ham and bacon. These organisms only grew at 10°C and their growth increased the pH. However, the porcine protein properties were not altered by their growth.

The psycrophilic, proteolytic pseudomonads, a group of organisms associated with spoilage of fresh refrigerated meats, was represented by Pseudomonas fragi organisms in this study. These organisms grew more rapidly and to a higher number of organisms per gram of sample than any of the other organisms used in this study. The growth of the organisms at 2°C was about 4 days slower than that at 10°C, with a similar relationship in other changes found. These organisms altered the properties of the porcine proteins more than any other organism studied. The pH of the inoculated samples greatly increased. There was an increase or no change in the amount of water-soluble protein as compared to the decrease found in control samples. However, electrophoretic study of the water extracts revealed a loss of protein bands for the inoculated samples indicating the type of protein present had been altered. There was an increase in the amount of salt-soluble protein for the first 4-8 days of storage, then a decrease was noted, especially in the extracts from the samples stored at 10°C. Electrophoresis of 0.6 M KCl extracts of the samples by starch-urea and disc gel electrophoresis revealed a loss in the number of salt-soluble proteins after 20 days of storage. There was a decrease

in the amount of insoluble protein and a marked increase in the amount of NPN for inoculated samples. The results of the protein solubility and electrophoretic studies indicated proleotysis of the porcine proteins was accomplished by the organisms. The emulsifying capacity of the inoculated samples increased during the first 8 days of storage, then decreased, but it was always greater than the emulsifying capacity of the control samples. The high emulsifying capacity appeared to be related to the larger amount of soluble proteins. The stability of the emulsions from inoculated samples was much less than that of the control emulsions.

The results of a short study of disc and starch-urea gel electrophoresis of 0.6 M KCl extracts of muscles from different species (pork, beef, lamb, turkey, chicken and fish) revealed differences in the number of protein bands found.

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INTRODUCTION

Since the beginning of the meat packing industry, microorganisms have caused spoilage problems. These spoilage microorganisms have been identified and their origins on meat and meat products have been determined. The effects of microorganisms on meat properties such as odor, texture, flavor, water binding capacity, and sliminess have been established. Also, their effects on protein solubility and emulsifying capacity have been studied. The results of the latter studies were inconclusive as the types of microorganisms present were not controlled or determined.

Therefore, the major portion of this study was directed toward determining the effect of four bacterial species generally found on meat or meat products on the properties of porcine proteins. The four bacterial species used were Pediococcus cerevisiae, Leuconostoc mesenteroides, Micrococcus luteus, and Pseudomonas fragi. Pediococcus cerevisiae and Leuconostoc mesenteroides were chosen as representative of the family Lactobacillaceae. This family of organisms has been found on fresh and processed meats and has been implicated in souring spoilages. Micrococcus luteus was chosen to represent the Micrococcaceae family. Micrococci have been isolated frequently from fresh meats and are known as the primary spoilage organisms on processed meat products with high salt contents such as cured hams. Pseudomonas fragi was chosen to represent the genus Pseudomonas. Species of this genus have been isolated from fresh meat and identified as the major spoilage organisms of fresh meat. Meat under

normal refrigerated storage is an excellent medium for growth of most species of this genus as they are psycrophilic and proteolytic. Temperature has been shown to influence the growth of microorganisms, so storage temperatures of 2° and 10°C were used in this study as representative of the normal range of refrigerator temperatures. A 20 day storage period was used as a maximum expected shelf-life for fresh meat handled normally. The properties studied were protein extractability (water-soluble, salt-soluble, non protein nitrogen, and insoluble fractions), electrophoretic properties of sarcoplasmic and myofibrillar fractions, emulsifying capacity, emulsion stability, and pH.

In addition, a very limited electrophoretic study was conducted on the myofibrillar protein fractions from various animal species.

LITERATURE REVIEW

Meat Spoilage

Microbiology of Refrigerated Meat

Many studies have been conducted to determine the various species of microorganisms present on fresh beef, pork, and lamb, and also on packaged, processed meat products and cured meat items such as ham and bacon. Haines (1933a) reported that Achromobacter was the primary bacterial spoilage genus of beef stored at 0-2°C. Achromobacter was later classified as Pseudomonas in most cases (Ayres, 1960b). Sulzbacher and McLean (1951) reported 75% of the isolates from fresh pork sausage were classified into six genera which were Pseudomonas, Microbacterium, Achromobacter, Bacterium, Bacillus, and Proteus. There were also some Micrococcus species isolated and some yeasts. Kirsh et al. (1952) found ground beef which had been purchased from retail stores had total aerobic counts which ranged from 1-95 million organisms per gram of tissue. The non pigmented Pseudomonas-Aerobacter group dominated the flora, with Lactobacillus and cocci organisms present. These findings were similar to those reported by Ayres (1955), Wolin et al. (1957), and Halleck et al. (1958). The latter group also reported that during storage at 1-3°C the Pseudomonas species became dominant after two weeks of storage, and lactobacilli represented approximately 5% of the total population throughout the storage period.

Eddy and Kitchell (1959) isolated 28 strains of coli-aerogenes bacteria from chilled meat, the predominant species being of the Aerobacter genus. Ayres (1960a) isolated many bacterial genera from refrigerated beef but found that at low storage temperatures (0-10°C) only the pseudomonads increased in population and were responsible for slime production. Micrococci grew as well as the pseudomonads at 15°C. Allen and Foster (1960) reported the slime production on vacuum packed refrigerated sliced processed meats was caused by lactic acid bacteria. Jaye et al. (1962) found the composition of the bacterial flora of refrigerated ground beef was 34% pseudomonads, 34% lactic acid bacteria, 23% micrococci, and 9% microbacteria. Kitchell (1962), in a review, indicated 39% of the microorganisms on fresh pork were micrococci while in products with high salt contents such as ham or bacon the micrococci represented 89-100% of the isolated organisms. Shank and Lundquist (1963) indicated that the lactic acid bacteria were the primary spoilage agents of vacuum packaged table ready meats while under aerobic conditions the same organisms were involved but yeasts and molds plus some micrococci organisms were also found. Adams et al. (1964) indicated fish were contaminated with various genera of bacteria but the pseudomonads were the primary cause of spoilage. This was confirmed by the work of Lerke et al. (1965). Patterson (1966) isolated staphylococci and micrococci organisms from fresh and cured bacon sides and the brine used for curing. Gardner et al. (1966) reported the most important bacteria in pork stored aerobically was the Pseudomonas-Achromobacter group which represented 96% and 49% of the isolates on pork stored at 2° and 16°C, respectively. Kurthia species represented 27% of the isolates at 16°C. Jay (1967) reported the

predominant organisms found on beef were the pseudomonads. Stringer et al. (1969) found Pseudomonas species and Micrococcus luteus represented almost 80% of the microorganisms isolated from beef carcasses in a packing plant while the organisms found on the retail cuts were primarily Pseudomonas species.

Contamination of Meat

Ayres (1955), in a review of meat microbial studies, indicated that muscle tissue from or on bovine, porcine, and ovine carcasses provides most of the nutrients required by microorganisms for growth. Before slaughter animals normally have heavy contamination of microorganisms on the hide, skin, hair, and hooves and in the intestines. For example, two studies, which Ayres (1955) reviewed, reported 3.91 million aerobes, 100 million anaerobes, and 100 yeasts and molds per sq. cm. of skin surface. He also indicated that the animals lose their normal defenses to microbial infection upon death. These normal defenses include: skin and mucous membranes, hair and cilia, gastric juices, digestion, and localization of an infection if it begins.

Haines (1933b) found that during slaughter operations the number of microbes in the air increased. He also found the walls, floors, and water were contaminated with microorganisms the majority of which were pseudomonads and micrococci. Ayres (1955) indicated carcasses were contaminated by the workmen and the equipment used in processing. He also found carelessness during evisceration of the carcasses caused contamination by the organisms in the intestinal contents. Jensen and Hess (1941) reported the sticking knife carried bacteria into the blood stream either

by contamination of the knife or by forcing microorganisms on the sticking area into the animal. They also reported the scalding tank as a source of contamination for pork, especially if the heart was still beating when scalding started.

Ayres (1955) implicated sawdust on cooler floors as a source of contamination. Patterson (1966) found curing brines to contain microorganisms, especially micrococci, which then contaminated bacon or ham. Stringer et al. (1969) found microbial counts of approximately 10 million per sq. in. of wall and floor surfaces of a beef packing house. They also found air contamination increased during operations such as slaughter and decreased during chilling. They found equipment such as the saw had microbial counts of approximately 10,000 per sq. in. of surface. Also, the shrouds used to cover the beef carcasses had 50,000 microorganisms per sq. in. of surface.

Effects of Bacterial Spoilage on Meat

Meat spoilage is usually noted by either odors or slime. The odor of spoiled meat varies with the organism responsible for spoilage. Ayres (1960a) reported meat spoiled by Pseudomonas organisms had a putrid odor while Jaye et al. (1962) reported sour odors with lactic acid bacteria. Castell and Greenough (1957) claimed fish spoilage odors were due to the Pseudomonas organisms. Haines (1933a) reported Achromobacter to be responsible for slime on meat. Allen and Foster (1960) found lactic acid bacteria were the primary slime producers on processed meats. Ayres (1960a) reported Pseudomonas to be the primary slime producer on fresh beef. Eddy and Kitchell (1959) isolated Aerobacter species from the

slime found on pork. Ayres (1960a) reported slime was noted at a bacterial count of approximately 6 x 10^7 microorganisms per gram of tissue.

The pH of spoiled meat is also influenced by the type of microorganisms present. Jaye et al. (1962) found pH decreased when lactic acid bacteria predominated and increased when pseudomonads were the principal spoilage organisms. Shank and Lundquist (1963) reported pork sausage with high bacterial contamination lost flavor more rapidly than sausage with lower contamination.

Beatty and Collins (1940) reported spoilage of fish took place due to oxidation of lactic acid and sugars followed by hydrolysis of amino acids and proteins. Kitchell (1962) reviewed the work of others concerning the micrococci organisms and reported many of these organisms were proteolytic but only 13% of them were proteolytic when meat proteins were used as the incubating media. Jay (1967) found that 80% of the Pseudomonas species which were isolated from beef hydrolyzed beef proteins. Lerke et al. (1967) found fish spoilage organisms of the genus Pseudomonas were proteolytic when grown on a water extract of fish muscle. However, no proteolysis took place if the non protein portion of the extract was removed, indicating that this portion was necessary for growth of the organisms. Lipolytic activity has been reported, especially by the micrococci organisms (Kitchell, 1962; and Patterson, 1966).

Characteristics of the Bacterial Species Used in This Study

Pediococcus cerevisiae. This is a member of the Eubacteriales order, Lactobacillaceae family, Streptococceae tribe, and Pediococcus genus (Breed et al., 1957). This gram-positive, non motile, microaerophilic

species is found as spheres, 1.0-1.3 microns in diameter occurring singly, paired, or in tetrads. It produces acids, primarily lactic acid, from most sugars, but does not liquefy gelatin. It has a growth range of 7-45°C with optimum growth at 25-30°C. Its primary habitat is fermenting materials, especially beer, sauerkraut, and pickles.

Leuconostoc mesenteroides. This is a member of the Eubacteriales order, Lactobacillaceae family, Streptococceae tribe, and the Leuconostoc genus (Breed et al., 1957). This species is gram-positive, non motile, microaerophilic to facultatively anaerobic, and is found as spheres 0.9 to 1.2 microns in diameter which occur in pairs or chains. It produces acid from most sugars but does not liquefy gelatin. This species produces slime, especially when sucrose is present. It has an optimum temperature range of 21-25°C. It has been found in fermenting vegetables and prepared meat products.

Micrococcus luteus. This is a member of the Eubacteriales order, Micrococcaceae family, and Micrococcus genus (Breed et al., 1957). This species is gram-positive, non motile, aerobic and spherical, being 1.0-1.2 microns in diameter and occurring in pairs or tetrads. It produces acid from glucose, sucrose, and mannitol but not from lactose. It does not liquefy gelatin but produces ammonia from peptone. It has an optimum temperature for growth of 25°C. It is normally found in milk and dairy products. Stringer et al. (1969) found Micrococcus luteus to be one of the predominant microorganisms on beef.

Pseudomonas fragi. This is a member of the Pseudomonadales order, Pseudomonadineae suborder, Pseudomonadaceae family, and Pseudomonas genus (Breed et al., 1957). This species is made up of gram-negative, aerobic, motile rods with a polar flagellum and dimensions of 0.5-1.0 x 0.75-4.0 microns and occurs singly, paired, and in chains. It produces acid from glucose and galactose but not from most other sugars. It liquefies gelatin, produces ammonia from peptone and partially digests litmus milk. It grows at temperatures of 10-30°C but will not grow at 37°C. It has been isolated from milk and other dairy products, soil, and water. Ayres (1960) and Castell et al. (1959) have found Pseudomonas fragi to be one of the spoilage organisms on beef and fish, respectively.

Aseptic Muscle Sampling

Weiser et al. (1954) infused aureomyocin into beef rounds to prevent deep spoilage by reducing bacterial numbers. They reduced the number of organisms found in the lymph nodes from the 100 million recorded in the controls to 10 thousand found in the infused rounds. A method of infusing the antibiotic into the live animal via the jugular vein was also very successful. Davey and Gilbert (1967) sprayed beef muscle with antibiotics at certain time intervals to keep bacterial counts below 100 per cm^2 while they studied microscopic changes in the muscle associated with aging.

Zender et al. (1958) obtained rabbit and lamb muscles which were practically void of bacterial contamination by following a very precise and aseptic means of excision. Ockerman et al. (1964) slaughtered and eviscerated germ-free mice in a sterilized isolator with sterilized equipment. The carcasses were stored in sealed tubes and no bacterial

contamination was found. Davis (1965) combined the methods of Zender et al. (1958) and Ockerman et al. (1964) to obtain aseptic beef muscle. Davis (1965) used sterilized equipment for slaughter and he scrubbed and shaved the animal's neck before sticking and then eviscerated and dehided normally except for a large patch of the hide which was left over the short loin. This area was scrubbed thoroughly and rinsed with alcohol. After chilling, the whole rough loin was removed from the carcass and placed on a cart so that a surgical isolator could be attached. The isolator was slit as well as the hide to expose the muscle which was excised, ground and placed in sterile containers with all operations taking place in the isolator. Such samples could be stored at 2-5°C for 35 days without evident bacterial contamination. This technique was also described by Ockerman et al. (1969).

The method outlined by Davis (1965) was modified by Borton et al. (1968a) and Miller (1968) to obtain porcine muscle with relatively little bacterial contamination. Their method did not require the surgical isolators but excision of the sample took place in a room with limited air movement. Their controls had bacterial counts ranging from 0 to 1000 per gram for up to 20 days of storage at 2-10°C.

Bothast et al. (1968) used a 90 sec dip in 90°C water to obtain rabbit carcasses with no bacterial contamination. The rabbits were slaughtered, skinned, and eviscerated by conventional methods without any special sanitary treatment before being passed through a V tube, which contained the hot water, into a surgical isolator. Following this procedure, no contamination was noted on the treated carcasses while the non-treated carcasses had bacterial counts of 1000-10,000 per gram of tissue during a 38 day storage period at 3°C.

Muscle Proteins

Skeletal muscle consists of fibers enclosed in a sheath of perimysium permeated with fat deposits and connective tissue. The contractile element is the fibrous 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 stroma protein. The intercellular material, or sarcoplasma, is a liquid 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, and myoglobin, but Whitaker (1959) also included most of the muscle enzymes. Hill (1962) reported that sarcoplasmic proteins accounted for 15-20% of the total nitrogen in bovine muscle, 20-25% in porcine, and 25% in ovine muscle. These results have been substantiated by numerous other reports. The amount of sarcoplasmic proteins extracted from various tissues depended on many environmental and physiological conditions, including storage temperature, age of carcass, pH, degree of rigor mortis, etc.

The amount of extractable sarcoplasmic protein nitrogen decreases in muscle tissue during storage or aging. Aberle and Merkel (1966) reported a decrease in the amount of sarcoplasmic protein in bovine semitendinosus muscle but not in the longissimus dorsi muscle. Fujimaki (1962), Goll et al. (1964), and Davis (1965) found beef muscle sarcoplasmic proteins decreased during aging. Thompson et al. (1968) held beef ribs at 30°C for 24 hrs and then stored them up to 10 days at 3°C. Ribs stored in this manner had a higher quantity of extractable sarcoplasmic proteins for the first three days of storage and had less extractable sarcoplasmic proteins for the remaining days of storage than control beef ribs held at 3°C for the entire storage period. The amount of sarcoplasmic protein extracted from porcine muscle also decreased with the length of aging (Sayre and Briskey, 1963; and McLoughlin, 1963). This decrease was also noted in poultry sarcoplasmic proteins (Khan and Van Den Berg, 1964; and Sharpf and Marion, 1964) and fish sarcoplasmic proteins (Baliga et al., 1962).

The pH of the sample influenced the amount of sarcoplasmic protein extracted (Scopes, 1964). As the pH decreased, less sarcoplasmic protein was extracted. Scopes and Lawrie (1963) found fewer electrophoretic bands at lower pH values for beef and pork sarcoplasmic proteins. They reported that the pH fall associated with rigor mortis could account for part of the decrease in the sarcoplasmic fraction during aging. Scopes (1964) reported storage temperatures near 37°C caused denaturation of some of the proteins and thus a decrease in extractability. Khan and Van Den Berg (1964) found no variation in extractability of the

sarcoplasmic fraction from chicken muscle at storage temperatures ranging from 0-5°C. Borchert and Briskey (1965) found partial freezing of porcine muscle in liquid nitrogen decreased the loss of sarcoplasmic protein fraction due to aging but did not eliminate it.

Ockerman et al. (1969) found that beef muscle which had been inoculated with bacteria had a higher amount of extractable sarcoplasmic protein than did an aseptic sample through a 35 day storage period at 3°C. Borton (1966) found a lower amount of extractable sarcoplasmic protein in pork muscle inoculated with bacteria when compared to an aseptic control through a 17 day storage period at 5°C.

Using various buffers and extraction procedures, the sarcoplasmic fractions of many animal species have been subjected to electrophoresis (Giles, 1962; Scopes and Lawrie, 1963; Scopes, 1964; Neelin and Rose, 1964; MacRae and Randall, 1965; Aberle and Merkel, 1966; and Awad et al., 1968). Scopes (1968) reported a means of identifying at least 20 glycolytic enzymes which were found in the sarcoplasmic fraction plus some of the pigments which were also found. Scopes and Lawrie (1963) reported 35 distinct bands after electrophoresis of the sarcoplasmic fraction of beef muscle. They found fewer bands from the sarcoplasmic fraction of pork muscle. Scopes (1963) found differences in the electrophoretic patterns of the sarcoplasmic fractions from different breeds of hogs. Giles (1962) found distinct differences in the electrophoretic patterns of the sarcoplasmic fractions of various species. Scopes (1968) reported differences in the patterns from two muscles ("psoas" and semitendinosus) from the same rabbit. Awad et al. (1968) found differences in the electrophoretic patterns of sarcoplasmic fractions from beef muscle stored

up to two weeks at -4°C. There was a loss in the number and intensity of the bands as the storage time increased.

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 them as fibrous, highly viscous, high molecular weight proteins. Whitaker (1959) identified the myofibrillar proteins as the contractile proteins such as myosin, actin, actomyosin, tropomyosin, and some less abundant proteins. Hill (1962) reported the myofibrillar fraction comprised approximately 56% of the nitrogen in procine muscle, 55% in bovine, and 53% in ovine muscle. These results have been substantiated by numerous other reports.

The extractability of myofibrillar proteins from muscle tissue is influenced by environmental and physiological conditions. McIntosh (1967) studied the extractability of proteins from beef, pork, and chicken muscle and found an increase in the total amount of protein extracted after two weeks of storage for pork and beef and one week for chicken when stored at a temperature of 4°C. The myofibrillar extractability did not change during further storage up to 4 weeks. The increase in total extractability was accompanied by an increase in the amount of actomyosin, indicating that two weeks aging was necessary for complete post-mortem changes to take place in muscle proteins. Aberle and Merkel (1966) found the fibrillar fraction of two muscles decreased from 0 hrs to 24 hrs post-mortem and then increased to the highest value at two weeks of storage at 4°C.

Sayre (1968) found a rapid decrease in myosin extractability with aging up to 4 hrs for chicken muscle. After this time there was an increase in the extractability of actomyosin. In both instances there occurred an opposite effect in the alkali soluble protein which is the fraction which contains fibrillar proteins which are not soluble in other salt solutions due to various interactions. He postulated that myosin was initially bound to the nonextractable thin filaments which in turn disintegrated or detached from the Z membrane. Davey and Gilbert (1968) found 52% of the myofibrillar protein was extractable in 40 min from unaged beef and rabbit muscle as compared to 78% from aged muscle. They thought these results indicated either a progressive weakening of the fibrous protein linkage with the stroma or a disintegration of the stroma itself.

They also found the rate and extent of such changes were determined by the ultimate pH with samples having a lower pH showing less extractability even after aging. Scopes (1964) also reported pH influenced solubility and strength of myofibrils.

Robson et al. (1967) and Goll and Robson (1967) found the nucleoside triphosphatase activity of the myofibrillar proteins of beef was not markedly changed by aging or rigor mortis, indicating that the proteins were not proteolytically altered during post-mortem changes. Fukazawa et al. (1961) reported that phosphates increased binding capacity of sausages because the myofibrillar proteins were more easily extracted from the intact fibril when phosphates were present.

Partmann (1963) reported freezing and thawing did not disrupt the structure of actin and myosin filaments. However, Khan et al. (1963)

indicated a loss in the extractability of chicken myofibrillar proteins after freezing. Borchert and Briskey (1965) reported freezing pork in liquid nitrogen prevented the decrease in myofibrillar extractability which normally took place during the first 24 hrs post-mortem. Yasui and Hashimoto (1966) found that freeze-drying denatured the myofibrillar proteins of rabbit muscle. At storage temperatures of 0-5°C, Khan and Van Den Berg (1963) found little difference in the extractability of myofibrillar proteins. Sayre and Briskey (1963) found severe loss of the myofibrillar fraction when beef muscle was stored at a temperature above 35°C.

Ockerman et al. (1969) found inoculated beef muscle had a slightly larger myofibrillar fraction than did the aseptic control during most of a 35 day storage period at 3°C. However, by the 35th day the myofibrillar fraction of the inoculated sample had decreased to a lower value than the control. Borton (1966) found little difference in the extractability of the myofibrillar fraction of bacterially inoculated pork and an aseptic control during 17 days of storage at 5°C.

Biochemists have been attempting to purify and study the myofibrillar proteins for many years. However, considerable difficulty has been encountered in separating the proteins without disrupting the structure and/or enzyme activity. Electrophoresis has been used in this type of work. Small et al. (1961) used urea acrylamide disc gel electrophoresis to study the homogeneity of three myosin fractions. Locker and Hagyard (1967) used polyacrylamide disc gel electrophoresis to study differences in myosins from different animal species. Actin homogeneity has been

studied by starch gel electrophoresis by Krans et al. (1962) and Carstein and Mommaerts (1963). Mair and Fisher (1966) used acrylamide disc gel electrophoresis to study changes in the salt-soluble proteins during post-mortem aging but found no evident difference. Neelin and Rose (1964) found no changes during a two day storage period in the myofibrillar fraction of chicken muscle when subjected to starch urea gel electrophoresis. Awad et al. (1968) used disc urea gel electrophoresis to study the effect of frozen storage at -4°C on beef muscle. They found a loss in the number and intensity of the bands during an eight week storage period. In most of the above cases urea was used which, according to Stracher (1961), changes the protein so that it loses enzyme activity.

Various methods of column chromatography and gel filtrations have been utilized for purification of the myofibrillar proteins and yet keep their enzyme activities. Richards et al. (1967) used a DEAE Sephadex A-50 chromatography column to purify myosins from rabbit, chicken, and fish muscles. Smoller and Fineberg (1964) used Sephadex G-200 gel to purify mouse myosin by gel filtration. Baril et al. (1966) used DEAE cellulose and gel filtration to purify chicken myosin. Gel filtration with Sephadex G-200 has also been used to purify actin (Adelstein et al., 1963; and Rees and Young, 1967).

Stroma Protein

The stroma proteins are the connective tissues, nerve tissues, and vascular systems of muscle tissue (Helander, 1957). He identified the stroma 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 in bovine muscle, 8-12% in ovine muscle, and 7-10% in porcine muscle.

Very few reports have been concerned with the effect of post-mortem conditions on the stroma fraction of muscle. Ockerman et al. (1969) found the stroma fraction decreased during a 35 day storage period at 3°C when beef muscle was inoculated with an unspecified inoculum. The aseptic control had an increasing stroma fraction during the same storage period. The samples inoculated with Pseudomonas and Achromobacter organisms also had a decrease in the stroma fraction during storage at refrigerator temperatures. Borton (1966) found a slight increase in the stroma protein fraction of porcine muscle inoculated with an unspecified inoculum when stored 17 days at 5°C.

Non Protein Nitrogen (NPN)

Some of the nitrogen present in a muscle sample is not part of the protein material. This nitrogen is present as amino acids, ammonia, peptides, nucleic acids, and related materials. Hill (1962) found that NPN accounted for 11-13% of the total nitrogen in beef, pork, and lamb muscle. NPN is primarily influenced by proteolytic activity which can come from two sources, proteolytic enzymes in the muscle, and proteolytic enzymes from sources external to the muscle such as bacteria. Chen and Bradley (1924) reported an increase in the NPN content of muscle during storage but concluded that intercellular protease was incapable of completely digesting muscle tissues. Kahn et al. (1963) reported that the

NPN content of chicken muscle increased during a storage time of 5 weeks with bacterial growth kept at a minimum with chemical treatment. Scharpf and Marion (1964) obtained similar 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 an increase in beef. Borton (1966) found a slight increase in the NPN content of porcine tissue stored 17 days at 5°C but no difference between inoculated and aseptic tissue. Ockerman et al. (1969) found a slight increase in the amount of NPN in aseptic beef sample held at 3°C for 35 days. However, three beef samples, one inoculated with an unspecified culture, a second with Pseudomonas organisms, and a third with Achromobacter organisms held under the same storage conditions had a large increase in the amount of NPN.

Emulsifying Capacity and Emulsion Stability

An emulsion is a dispersion of one liquid into another, the liquids being immiscible (Jirgensons and Straumanis, 1962). For an emulsion to remain stable, emulsifying agents are needed in most instances. Such agents lower interfacial tension and aid in the formation of stable droplets which are surrounded by the continuous phase of the emulsion.

A meat emulsion may not be a true emulsion as some solid materials are present in the muscle tissues. 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. He substantiated this with microscopic examination. Borchert et al. (1967) also have shown

this with electron micrographs. Pearson et al. (1965) found that protein extenders such as nonfat dry milk, soy sodium proteinate, and potassium caseinate did have some emulsifying capacity with nonfat dry milk giving the best results at the approximate pH of meat (5.4). Inklaar and Fortuin (1969) found isolated soy protein to be an adequate emulsifier in meat emulsions.

Since Hansen (1960) has reported that meat proteins are the primary emulsifying agents in meat emulsions, others have studied this aspect of sausage emulsions. Swift et al. (1961) found salt-soluble proteins were efficient emulsifying agents. Trautman (1964) reported pre-rigor meat had a higher emulsifying capacity than post-rigor meat. He found the pre-rigor meat had a higher amount of salt-soluble protein than the post-rigor meat which accounted for the greater emulsifying capacity. His results have been substantiated by Acton and Saffle (1969). Trautman (1964) found the water soluble proteins formed weak emulsions which were readily dispersed. These results were in disagreement with those of Hegarty et al. (1963) who found that at the pH of normal fresh meat (5.6 -5.8) the sarcoplasmic proteins formed the most stable emulsions. Maurer and Baker (1966) found collagen to be a detrimental factor in forming emulsions with poultry meat. Hudspeth and May (1967) found light colored poultry meat to have a greater amount of salt-soluble proteins but that the dark colored meat had a greater emulsifying capacity per unit of protein so that the types of meat had similar emulsifying capacity per unit of tissue.

Borton et al. (1968b) found that meat products with a higher amount of protein normally had a higher emulsifying capacity on a unit weight basis. However, on a unit of protein basis the meat products with lower amounts of protein were more efficient emulsifying agents. They also found that pre-blending a meat product with 3% salt and allowing it to set 24 hrs increased the emulsifying efficiency of the protein without much loss in overall emulsifying capacity on a weight basis. Acton and Saffle (1969) substantiated the pre-blending results on post-rigor samples. They found pre-blending pre-rigor meat tissue did not enhance emulsification properties.

The emulsification properties of muscle tissue are affected by various conditions. Hansen (1960), using a slow chop procedure, found the chopping time must be sufficient to enclose the fat globules in the protein matrix. However, chopping too long would increase the temperature sufficiently to denature the proteins and cause breakdown of the emulsion upon cooking. A chopping temperature between 15-19°C formed the most desirable emulsion. Helmer and Saffle (1963) found similar results using a high speed chopper. Saffle et al. (1967) studied the effects of processing temperatures and humidity on the stability of emulsions and found the greater the temperature and humidity the greater the possibility of emulsion breakdown. However, lower humidity and temperature resulted in the most shrinkage. Townsend et al. (1968) used a method of differential thermal analysis and found the lower the emulsion temperature the more stable the emulsion in the temperature range 0-38°C.

Swift et al. (1961) found the emulsifying capacity of meat increased with an increasing concentration of salt. Swift and Sulzbacher (1963) found salt increased the emulsifying capacity of sarcoplasmic proteins. They also found that pH influenced the emulsifying capacity of muscle proteins. They found that at a pH of 4.8-5.5 sarcoplasmic proteins had the greatest emulsification potential but the salt-soluble proteins reached their greatest emulsification potential at a pH of 6.0 or above. The emulsifying capacity of meat increased from pH 5.0 to 8.0 but had the greatest increase from pH 5.0 to 6.0. Hegarty et al. (1963) also found pH to influence emulsification properties of the various meat proteins.

Christian and Saffle (1967) found differences in the amounts of various animal fats which could be emulsified, but from a practical standpoint these differences were not significant. They also found differences in the ability of other fats and oils to be emulsified but the differences did not correlate with differences in iodine values, acid values, or specific gravities.

Borton et al. (1968a) found that porcine tissue inoculated with an unspecified bacterial culture had a lower emulsifying capacity than the aseptic control when stored 17 days at 5°C. Ockerman et al. (1969) found that a bacterially inoculated beef sample had a greater emulsifying capacity than the aseptic control during a 35 day storage period at 3°C. They also found samples inoculated with Pseudomonas organisms had a lower emulsifying capacity than the control up to 20 days of storage at 3°C. Thereafter the control had the lower emulsifying capacity until termination of the storage period at 35 days. Samples inoculated with

Achromobacter organisms had a higher emulsifying capacity than the aseptic control after 10 days of storage.

EXPERIMENTAL METHODS

Slaughter

The eight 180-230 lb hogs used in this study were either produced by the Michigan State University Farms or bought locally at an auction. The animals were brought to the Meat Laboratory 12 hrs before slaughter and were given water but no feed. One hog was slaughtered every four weeks. The animal was stunned with an electric stunner and hoisted by one rear leg. The sticking area of the neck was scrubbed thoroughly with a warm solution of pHisoHex bacteriocidal soap. A knife which had been held in a steam-heated knife sterilizer for 10 min was used to stick the hog which then died by exsanguination. The hog was scalded, dehaired, eviscerated, and cleaned following normal procedures, except the carcass was not split. Before placing the carcass in the 1°C cooler, it was thoroughly rinsed with alcohol which was removed by flaming. The above method was similar to that reported by Borton et al. (1968a).

Excision and Inoculation of the Muscle Samples

All equipment used in the following procedures was sterilized for 15 min at 121°C and 15 lb pressure in an autoclave.

The carcass was chilled in the cooler for approximately 24 hrs after which the shoulders were removed. The carcass was placed on a kraft-paper covered table so that the midline of the backfat cover was easily accessible. The carcass was rinsed with alcohol. At this time the two

persons who were to excise and inoculate the samples donned sterile, disposable rubber gloves. One knife was used to make three cuts in the external fat cover. The first cut was along the midline with the other two cuts being made perpendicular to it, one about 5-8 cm posterior to the cut surface of the shoulder end and the second over the tuber coxae. The backfat was stripped and rolled back to expose the longissimus dorsi muscle. The muscle was sliced in approximately 3 cm slices using a second knife. The slices were transferred to containers using hemostats. The opposite muscle was removed following the same procedure using a third knife. Each container contained approximately 900-1000 gm of sample which was a composite of every third slice excised.

The sample from one container was placed on the feeding tray of a grinder. The slices were fed through the grinder and as the ground sample emerged from the two mm grinder plate 10 ml of sterilized water were added. The sample was reground and designated as the control. The muscle slices from a second container were treated the same as the control except 10 ml of a 1/100 dilution of a 48 hr culture of either Pediococcus cerevisae, Leuconostoc mesenteroides, Micrococcus luteus, or Pseudomonas fragi were used to inoculate the sample. After regrinding the first inoculated sample, the grinder was disassembled, washed, reassembled, sterilized, and cooled before repeating the above procedure using a second bacterial species to inoculate the third sample. After grinding and inoculation, each of the samples was aseptically divided into thirteen jars with each jar containing 60-70 g of sample. One jar of each sample was used as the 0 day sample. Six of the remaining jars of each sample were stored

at 2°C and the other six were stored at 10°C. The samples were then analyzed after 2, 4, 8, 12, 16, and 20 days of storage.

The bacterial cultures were prepared by taking one ml of a refrigerated culture and placing it in a tube containing 10 ml of APT broth and incubating at room temperature (approximately 22°C) for 72 hr. One ml of this culture was transferred to a second tube containing 10 ml of APT broth and allowed to incubate 48 hr at room temperature.

Bacterial Numbers

The method outlined by the American Public Health Association (1958) was used for determination of the number of bacteria per gram of sample. Eleven grams of sample were blended in a sterile blender with 99 ml of sterilized water. This slurry was appropriately diluted and 1.0 or 0.1 ml pipetted into sterile disposable petri dishes. APT agar was used as the plating medium. The plates were incubated 48-72 hr at 25°C after which the colonies were counted and recorded 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. All extractions were done at 2-6°C. Five grams of the meat sample were weighed into a VirTis jar and blended with 35 ml of 0.03 M phosphate buffer, pH 7.4, for 1 minute. The slurry was transferred to a 125 ml Erlenmeyer flask. The jar was rinsed with 15 ml of the 0.03 M PO_4 buffer

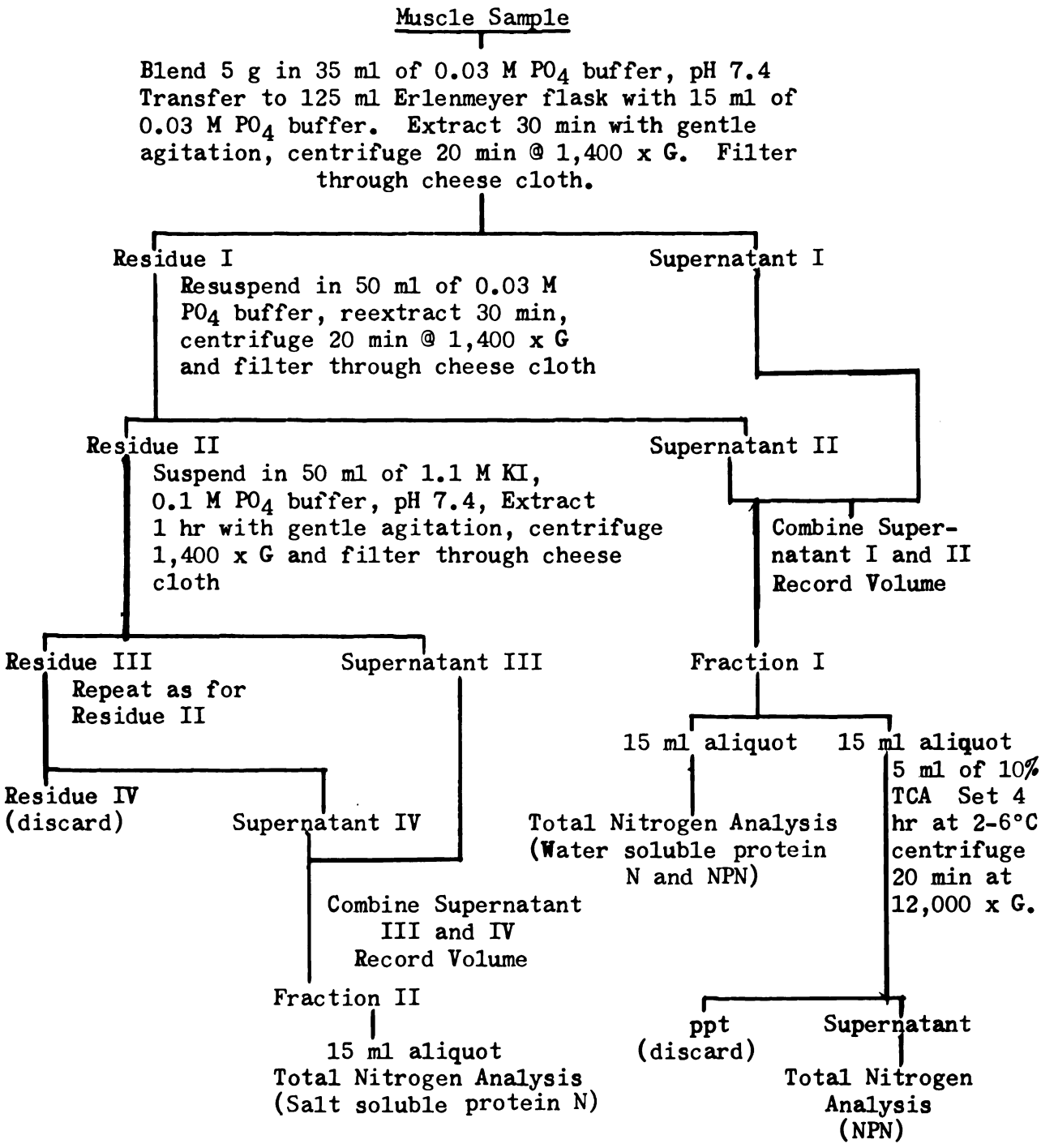


Figure 1. Outline of protein fractionation.

and the rinse added to the flask. The flask was placed on a magnetic stirrer and gently agitated for 30 minutes. The mixture was transferred to a 200 ml centrifuge cup and centrifuged at 1400 x G for 20 min in a Sorvall superspeed RC2-B automatic refrigerated centrifuge set at 2°C. The supernatant (Supernatant I) was filtered through cheese cloth into a 100 ml graduated cylinder. The residue was reextracted with 50 ml of 0.03 M PO_4 buffer centrifuged and filtered, with the supernatant (Supernatant II) being added to Supernatant I to form Fraction I. The volume of Fraction I was recorded with a 15 ml aliquot used for nitrogen analysis and the result recorded as the amount of water soluble protein and non protein nitrogen.

The residue was extracted twice for an hour each time with 50 ml of 1.1 M KI, 0.1 M PO_4 buffer, pH 7.4. After centrifugation, the supernatants (III and IV) were combined to form Fraction II. The volume was recorded with a 15 ml aliquot used for nitrogen analysis and the result recorded as the amount of salt soluble protein nitrogen.

To determine the non protein nitrogen (NPN) fraction, a 15 ml aliquot of Fraction I was added to 5 ml of 10% trichloracetic acid (TCA) with this mixture being held for 4 hr at 2-6°C. The mixture was centrifuged at 12,000 x G for 20 min, with the supernatant used for nitrogen analysis and the result recorded as the NPN. The amount of NPN was subtracted from the amount of nitrogen in Fraction I with the remainder designated as the water soluble protein nitrogen.

Total nitrogen was found by subjecting approximately one-half gram of sample, weighed on nitrogen-free paper to the nearest 0.0001 g, to nitrogen analysis.

Stroma nitrogen was found by subtracting the amount of nitrogen in Fractions I and II from the total nitrogen.

Nitrogen and Protein Analysis

The micro Kjeldahl method outlined by the American Instrument Company (1961) was used. The sample (meat sample or extract aliquot) was placed in a Kjeldahl flask with approximately 0.5 g of sodium sulfate, 1 ml of 10% copper sulfate, and 7 ml of concentrated sulfuric acid. Two glass beads were added and the flask was placed over heat for digestion. Digestion was continued with occasional swirling until the solution cleared (2-4 hr). The flask and its contents were cooled, and approximately 10 ml of distilled water were added. For distillation, 10 ml of 2% boric acid and 3 drops of bromocresol-green indicator solution were added to a 125 ml Erlenmeyer flask. This flask was positioned on the distillation apparatus to collect the distillate. The Kjeldahl flask was positioned for distillation, then approximately 15 ml of 40% sodium hydroxide were added, and by addition of steam from a boiling water flask, distillation took place for six minutes. The distillate-boric acid solution was titrated to the green end point of the bromocresol-green with 0.1 N sulfuric acid. A factor of 6.25 was used to determine the percent protein from the nitrogen analysis.

Protein Electrophoresis

Water Soluble Proteins

1. Extraction. Ten grams of meat sample were weighed into a VirTis blender jar and blended in 25 ml of deionized distilled water for 1 min.

The slurry was transferred to a 125 ml Erlenmeyer flask and the jar rinsed with 5 ml of deionized distilled water and the rinse added to the flask. The flask was placed on a magnetic stirrer and gently agitated for 30 minutes. The slurry was transferred to a 200 ml centrifuge cup and centrifuged 20 min at 10,000 x G. The supernatant was filtered through cheese cloth and in most cases filtered through Whatman No. 1 filter paper. The samples inoculated with Pseudomonas fragi would not filter through filter paper after 8 and 20 days of storage. The solution was dialyzed against 1.0 M sucrose for 12-16 hours after which it was ready for electrophoresis. An extraction of this type was done on all samples after 0, 8, and 20 days of storage.

2. Electrophoresis. The method for horizontal starch gel electrophoresis described by McRae and Randall (1965) was used. The gel was formed by adding and heating 24 g of hydrolized starch (Connaught) in 200 ml of a solution, pH 7.5, made up of 5.5 ml of 0.2 N HCl and 30 ml of 0.19 M Tris (hydroxymethyl aminomethane) diluted to 250 ml with deionized distilled water. After heating to 86°C, the gel was deaerated under vacuum and poured into a two layered gel tray. A slot former which made six slots was positioned about 6 cm from one end of the gel tray. The gel was allowed to harden and then covered with a polyvinyl film to prevent drying while setting overnight. After removal of the film and slot former, sample extracts were placed in the slots and covered with vaseline. The gel tray was laid horizontally between two buffer tanks with each end of the tray resting on the inside edge of the buffer tank. The buffer tanks contained a solution made up of 0.6 M boric acid and

0.2 M sodium hydroxide, pH 8.9. Filter paper bridges served as conductors between the tank solutions and the gel. The slots were positioned nearest the cathode as most proteins moved toward the anode. For the first 15 min, 165 volts were applied and then the voltage was increased to 350 volts. Electrophoresis was done at 2-6°C and continued for a total time of 6 hr at which time the leading boundary had moved approximately 10 cm.

After electrophoresis, one of the gel tray layers was removed and the gel sliced in half with a very thin, taut piano wire. The lower half was stained with a 1% Buffalo Black NBR dye in a 5:4:1 solution of methanol, water, and acetic acid. After staining 20 min, the gel was destained in a fresh solution of methanol, water, and acetic acid with the solution being changed twice in 48 hours.

Salt Soluble Proteins

1. Extraction. The extraction of the salt soluble proteins was described by Rampton (1969). After removing the water soluble proteins as described previously in this section, the residue was washed with 80 ml of deionized distilled water for 1 hr, centrifuged at 10,000 x G for 20 min, the wash discarded, and the residue again washed with 80 ml of deionized distilled water and centrifuged. The residue was suspended in 60 ml of Weber-Edsall solution (0.6 M KCl, carbonate buffer, pH 9.2) and gently agitated for 20-24 hours. The mixture was centrifuged 1 hr at 25,000 x G and the supernatant filtered through cheese cloth. The filtrate was dialyzed against 8.0 M urea for 16-18 hr with gentle swirling of the urea solution by a magnetic stirrer. After dialysis, the salt soluble extract was ready for starch urea gel and disc gel electrophoresis.

2. Starch Urea Gel Electrophoresis. The method used was a modification of one reported by Neelin and Rose (1964) for myogen extracts. The gel was formed by adding 30 g of starch to 200 ml of a buffer composed of 0.076 M Tris and 0.005 M citric acid, pH 8.6. This mixture was heated to a temperature of approximately 60°C, then 72 g of urea were added and the gel heated to approximately 86°C. Immediately after heating, the mixture was deaerated under vacuum and poured into a two layered gel tray. The slot former which formed six slots was positioned about 6 cm from one end of the tray. After sufficient hardening, a polyvinyl film was placed over the gel to prevent dehydration while the gel set overnight. After removal of the film and slot former, the salt soluble extracts were placed in the slots and then covered with vaseline. The gel was then electrophoresed, sliced, stained, and destained the same as the water soluble protein gel except 350 volts were applied throughout the electrophoresis period.

3. Disc Gel Electrophoresis. The method outlined by Davis (1964) was used with modification. The running gel contained a final concentration of 6.5% cyanogum which replaced the acrylimide-bis-acrylimide used by Davis (1964). The spacer gel contained 5.0% cyanogum. Both the running and spacer gels contained 7.0 M urea purified over MB-3 resin. The gels were placed in glass tubes and polymerized by fluorescent light for 20 minutes. The tank buffer used for electrophoresis was a Tris-glycine buffer, pH 8.5. Three drops of bromthymol blue were added to the buffer and 0.05 ml of the salt soluble extract was applied with a pipette on the surface of the gel beneath the tank buffer. A current of 2 ma per gel

was maintained for protein electrophoresis. Electrophoresis was completed when the leading bromothymol blue band reached the end of the gel. The gel was removed from the glass tube by sliding a hypodermic needle along the internal surface and forcing water along the side of the gel. The gel was placed in a test tube and stained 20 min in a 0.4% Buffalo Black NBR dye solution of water, methanol, and acetic acid (5:5:1). The gel was held overnight in a destaining solution of water, methanol, acetic acid, and glycerol (5:5:1:1). This solution was decanted and discarded and the gel was electrophoretically destained in more of the same destaining solution at 200 volts for approximately 4 hours. After destaining, the gel was stored in fresh destaining solution or 7.5% acetic acid.

Thin Layer Gel Filtration

The procedure followed was similar to that reported by Andrews (1964) and Johansson and Rymo (1964). Sephadex G-200 superfine gel was allowed to swell in the various buffers used for at least 48 hours. This was spread on either a 20 x 20 cm or a 20 x 40 cm glass plate at a thickness of 0.5 mm. The plate was placed in a chromatography cabinet and bridged to an elevated buffer tank with filter paper. The elevation (10-20°) was such that the buffer flow was maintained for about 16 hr on the 40 cm plate and 6-8 hr on the 20 cm plate. The plate was allowed to equilibrate for 1 hr at which time it was spotted with salt soluble protein extracts. After the desired time, a piece of filter paper the same size as the plate was placed over the gel. The plate with paper attached was dried at

100°C for 15 minutes. After drying, the plates were stained with a 0.1% Nigrosin Black dye in a solution of methanol, water, and acetic acid (5:4:1). The plates were then destained for 48 hrs in a destaining solution of methanol, water, and acetic acid (5:4:1). During destaining, the stained filter paper loosened from the plate and was removed.

Emulsifying Capacity

The method used was similar to the method reported by Borton et al. (1968b). Twelve and one-half grams of sample were blended in 50 ml of cold (2-6°C), 1.0 M NaCl solution in a VirTis jar for 1 minute. A 6.25 g portion of the resultant slurry was placed in a quart jar. Then 37.5 ml of cold, 1.0 M NaCl solution and 25 ml of cottonseed oil (Kraft) were added to the jar. The mixture was stirred at approximately 1750 rpm with a Lightnin Model F stirrer equipped with 5 open 3-bladed propellers spaced 1 cm apart. Cottonseed oil was added at a rate of approximately 1 ml per sec from a 500 ml separatory funnel. An emulsion was formed as indicated by an increasing viscosity and a fine honeycomb-like appearance. The end point was noted by a sudden decrease in viscosity and an oily appearance. The amount of oil used was measured by pouring oil into the separatory funnel from a 500 ml graduated cylinder and recording the amount required to refill the funnel plus the 25 ml added at the beginning of emulsification. The emulsifying capacity per 10 mg of total nitrogen was calculated.

Emulsion Stability

Emulsion stability was determined by the method outlined by Borton et al. (1968b). The procedure was the same as that for emulsifying capacity except only 200 ml of oil were added rather than adding oil to the emulsion endpoint. A 50 ml aliquot of the resultant emulsion was transferred to a 50 ml graduated cylinder and allowed to sit at room temperature for 48 hours. The amount of separation of water at the bottom and oil at the top of the cylinder was recorded at time intervals of 0, 0.25, 0.50, 0.75, 1, 2, 24, and 48 hours.

pH

The pH of the samples was determined by blending 5 g of sample in 50 ml of deionized distilled water for 1 minute. The pH was read with a Corning Model 12 pH meter.

Moisture Determination

The A.O.A.C. (1965) method of drying 2.5-3.0 g of sample for 16-18 hr at 100-105°C was used.

Fat Determination

The A.O.A.C. (1965) ether extraction of the dried sample was used.

Statistical Analysis

Analysis of variance was completed at the Michigan State University Computer Laboratory according to the procedures outlined in Michigan

State University Agricultural Experiment Station STAT Series Description No. 14 (1967). The data which were significantly different by analysis of variance were further analyzed by ranking and comparing means by Duncan's new multiple range test (Steel and Torrie, 1960).

Samples for Species Comparison

The samples used for comparison of electrophoretic patterns of the salt soluble proteins were treated in the following manner. About 20 g samples of pork, beef, and lamb longissimus dorsi and beef and pork semimembranosus muscles were taken from three carcasses and frozen and stored at -30°C for about 2 weeks. The fish samples were received frozen and were held at -30°C until thawed for use. The poultry samples were held at -30°C for about 4 weeks before thawing. All samples were thawed and then extracted and subjected to electrophoresis as described previously in Electrophoresis of Salt Soluble Proteins.

RESULTS AND DISCUSSION

General Chemical Composition

The percent protein, moisture, and fat was not influenced by any of the treatments. There were differences in the general composition of some of the replicates. For example, the replicate % protein means for all four inoculum treatments ranged from 18.02 to 21.28%. The water and fat content of the samples varied according to the amount of protein present. Higher protein content was accompanied by higher water content and lower fat content. The differences noted were due to two factors which were variability between the pigs used and the amount of fat trimmed from the muscle sample prior to excision. The differences due to replication were noted in most of the other properties studied but did not influence the general trends in which this study was primarily interested.

Bacterial Growth

The control samples were not sterile as shown in figures 2-5. However, the amount of contamination was quite low, generally being less than 10,000 organisms per gram of sample which was much lower than the 1-95 million bacteria per gram of fresh ground beef reported by Kirsh et al. (1952). The control samples held at 10°C had a greater increase in microbial numbers than those held at 2°C. Also, it should be noted that mold seemed to be the prevalent contaminant of the control samples,

especially those stored at 10°C. Very little mold was noted on any of the bacterially inoculated samples. The mean logarithims of microbial numbers for the control samples in figures 4 and 5 were somewhat higher than the others due to an oversight in the excision of one set of sample tissue. The oversight involved improper sterilization of the plunger used to force the muscle tissue through the grinder. As can be noted in Appendix B, one control sample had initial counts in the range of 10,000 whereas most of the others did not have enough organisms to count (<30) at that point. It should be pointed out that the log number 0.00 really should be read as <30 organisms/g of tissue as the lowest dilution plated was a 1/10 dilution. The 0.00 was used only when there was no growth from the 1/10 dilution and because it was more convenient than using <1.48.

The growth curves of Pediococcus cerevisiae when grown on porcine muscle tissue and incubated at 2° and 10°C are noted in figure 2. These organisms did not increase in numbers when incubated at 2°C but they did remain viable. At 10°C the organisms increased in numbers from about 100,000 to 100 million per gram and had almost reached the highest number by the 8th day of storage after which there was little increase or decrease. The growth of these organisms produced a slight sour odor which was more noticeable toward the end of the storage period.

The relationship of the growth of Leuconostoc mesenteroides organisms on porcine muscle stored 20 days at 2° and 10°C is depicted on figure 3. These organisms grew at both 2° and 10°C with less growth being noted at 2°C. The initial bacterial count was in the low ten thousands and increased to a peak of slightly over 10 million organisms per gram at 8 days

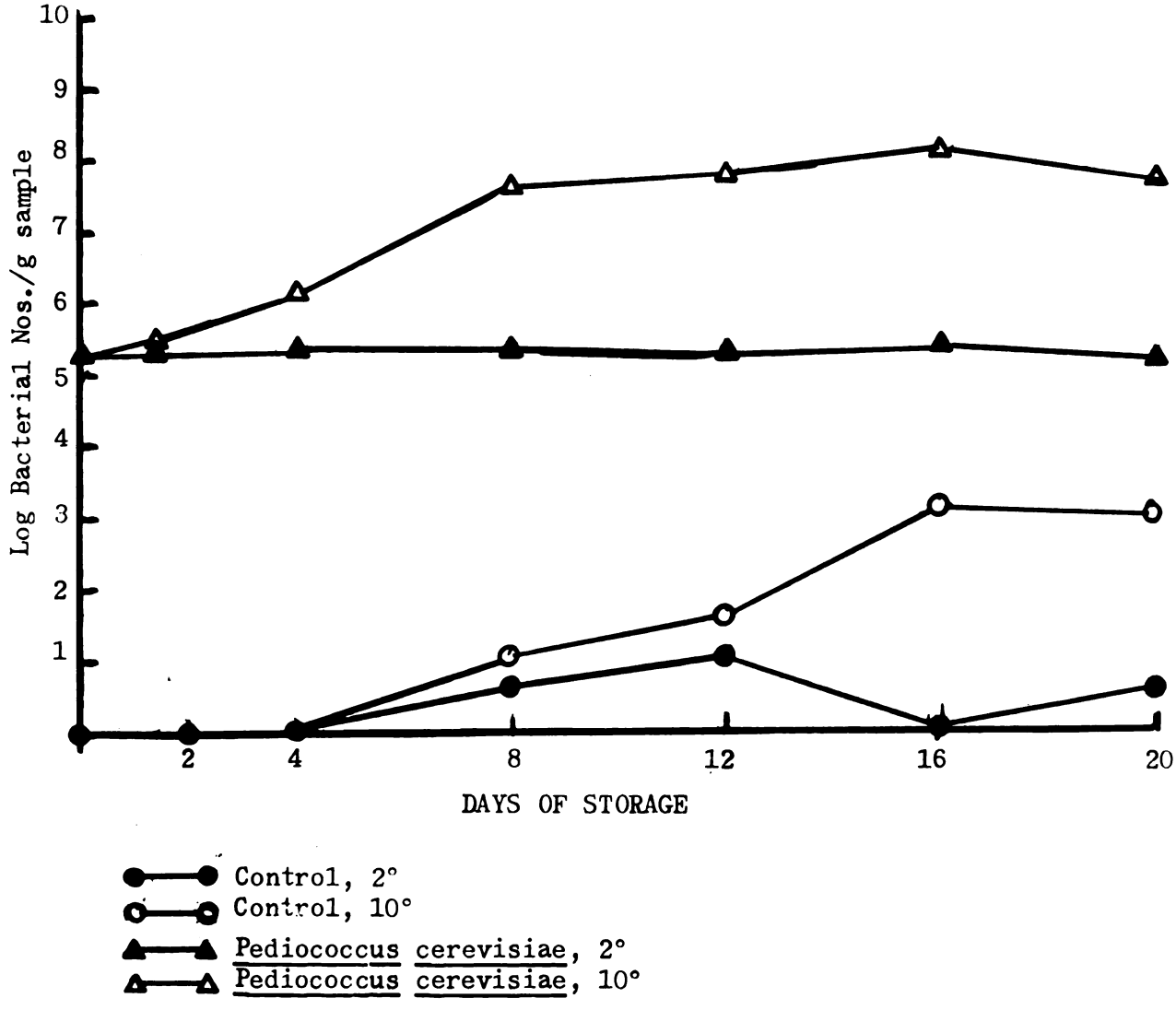


Figure 2. Relationship of the log of bacterial numbers per gram of control and Pediococcus cerevisiae inoculated porcine samples stored at 2° and 10°C for 20 days.

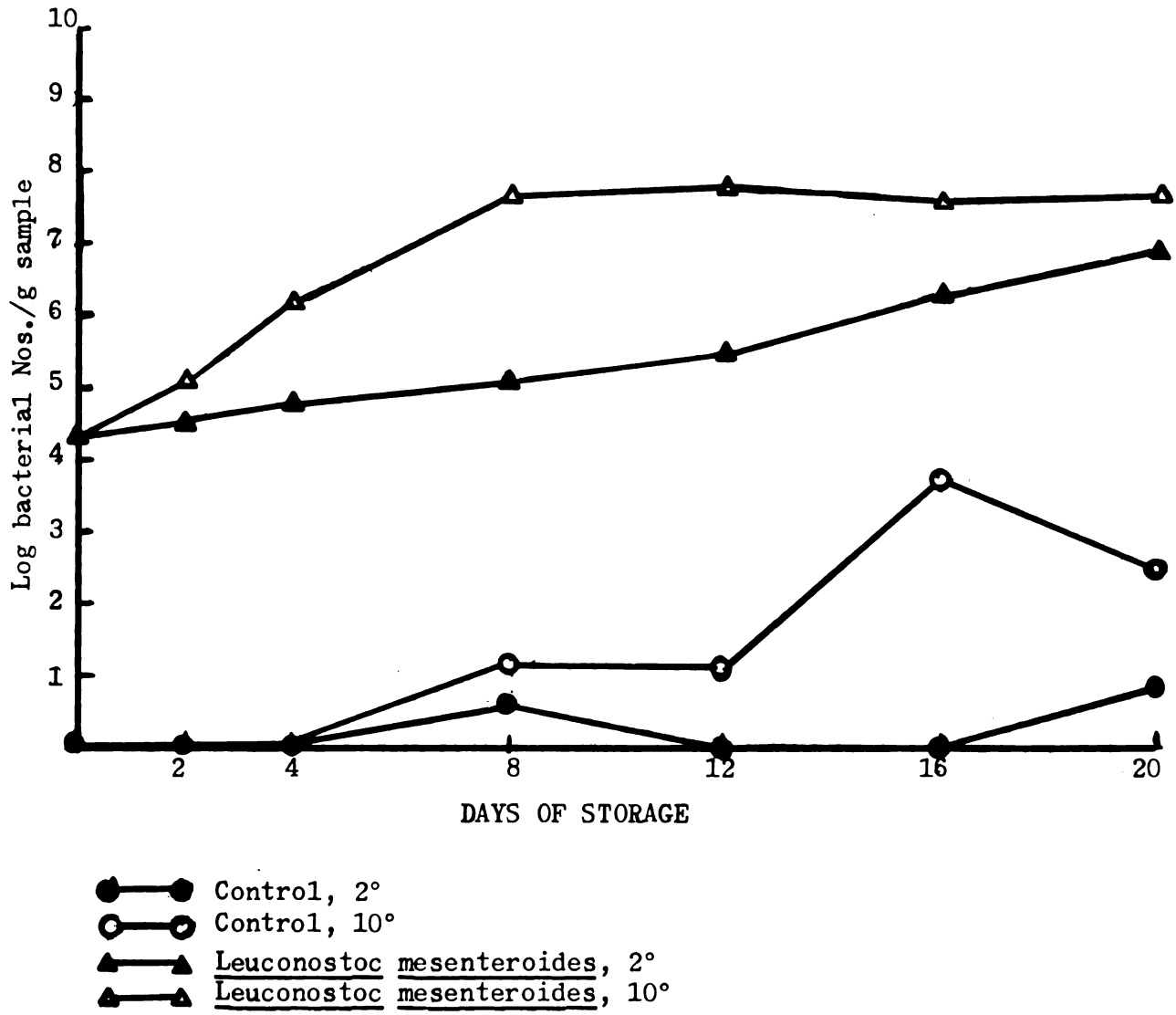


Figure 3. Relationship of the log of bacterial numbers per gram of control and Leuconostoc mesenteroides inoculated porcine samples stored at 2° and 10°C for 20 days.

of storage at 10°C. After day 8, there was a slight decrease in the number of organisms throughout the remaining storage period. However, the sample stored at 2°C exhibited a continual increase in the number of organisms throughout the 20 day storage period. At day 20 the count was slightly over 1 million organisms per gram of tissue. The samples stored at 10°C had a noticeable sour odor at day 8 which was very evident by day 20. However, the samples stored at 2°C only had a very slight sour odor by day 20 even though growth occurred.

The growth curves of Micrococcus luteus incubated on porcine muscle tissue at 2° and 10°C for 20 days are shown in figure 4. As was the case with Pediococcus cerevisiae, Micrococcus luteus did not increase in numbers at 2°C but did remain viable. At 10°C these organisms grew from an initial count of around 70,000 to a peak of 100 million at day 16 even though the greatest increase in numbers occurred between days 4 and 8. After 12 days of storage, samples held at 10°C had an orange-yellow slime on the meat surface and a distinctive odor which was described as a "sweat sox" or "sweaty-feet" odor.

The relationship of the growth of Pseudomonas fragi organisms incubated at 2° and 10°C for 20 days on porcine muscle tissue is noted in figure 5. These organisms grew more readily at 2° and 10°C and to a higher count than any of the other organisms used in this study. When incubated at 10°C, the Pseudomonas fragi organisms increased in numbers from an initial count of around 10,000 to a peak of 1 billion per gram of sample at day 8. From that point there was a decrease to about 100 million organisms per gram at day 20. When incubated at 2°C, the same peak was

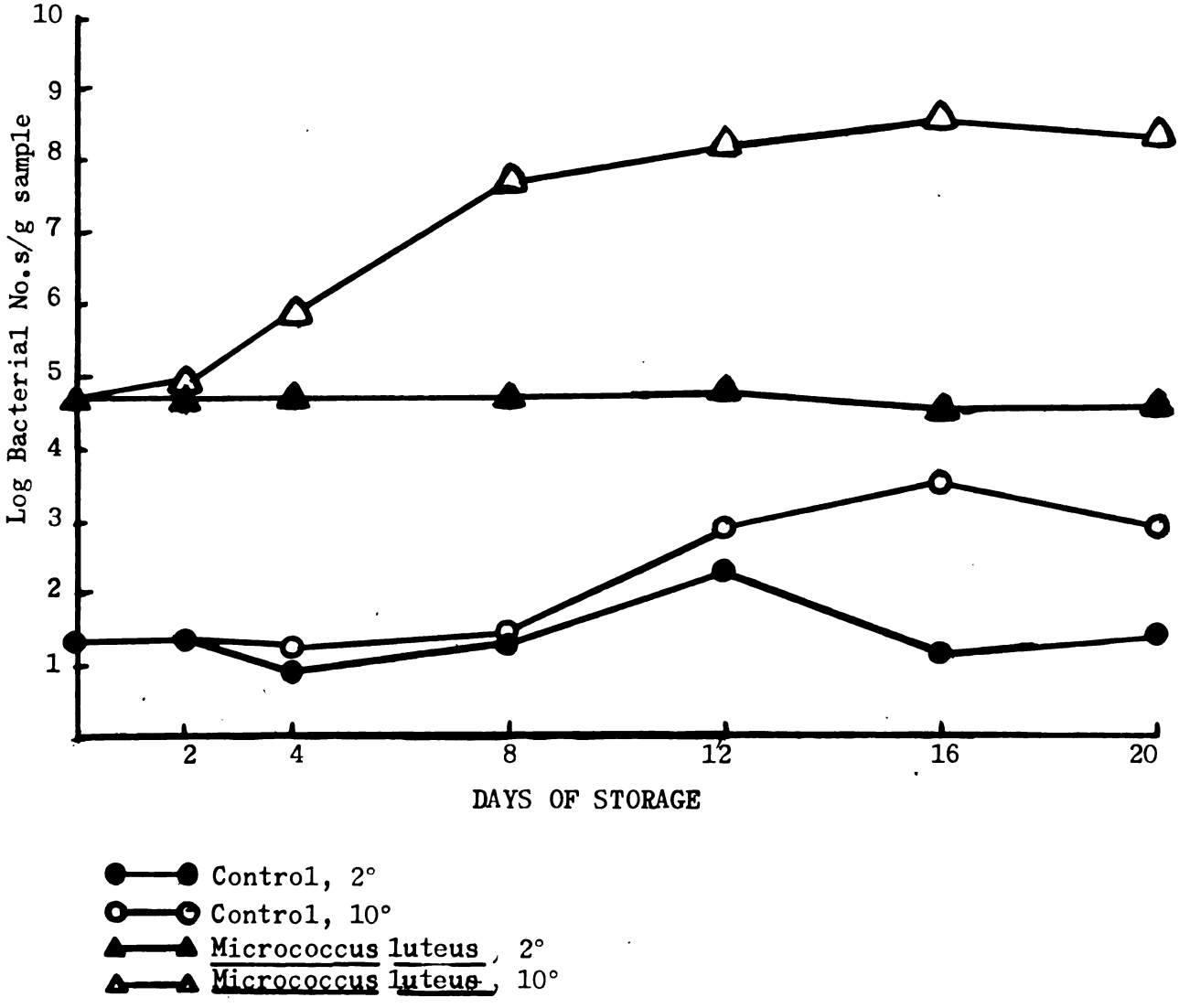


Figure 4. Relationship of the log of bacterial numbers per gram of control and Micrococcus luteus inoculated porcine samples stored at 2° and 10°C for 20 days.

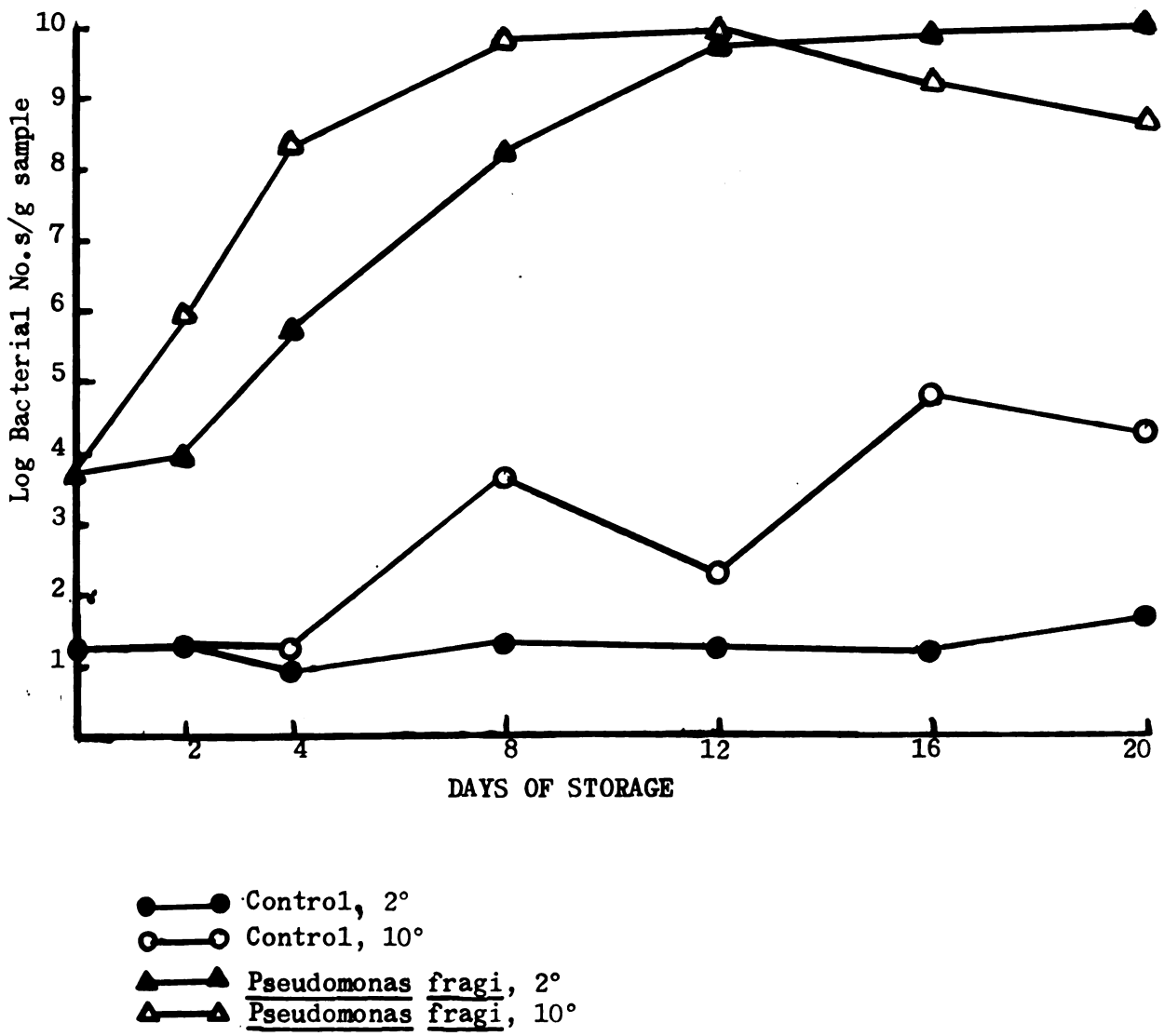


Figure 5. Relationship of the log of bacterial numbers per gram of control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

reached but it was not attained until day 12 or day 16 and there was no evident decrease in numbers during the 20 day storage period. At both temperatures, a slimy condition was noted, at day 4 for the samples stored at 10°C and at day 8 for those stored at 2°C. Also accompanying the slimy condition was a putrid odor which became very strong by the end of the storage period. Ockerman et al. (1969) found a general inoculum of beef stored at 3° had an increase in numbers similar to that of the Pseudomonas fragi inoculated samples held at 2°C. The Pseudomonas organisms they used in another study did not increase as rapidly or to the same extent as did the Pseudomonas fragi species used in this study.

pH

Since the pH of the sample influences protein solubility and electrophoretic properties, the pH changes associated with the growth of each of the four organisms should be noted. Pediococcus cerevisiae is an acid producing species and thus a decrease in pH was expected and found as noted in figure 6. The pH of the control and inoculated samples stored at 2°C and the control samples stored at 10°C did not change significantly. However, the pH of the control samples stored at 10°C did increase slightly after 8 days of storage. There was a highly significant difference ($P \leq .01$) between the overall pH mean of Pediococcus cerevisiae inoculated samples, 5.34 and the overall pH mean of control samples, 5.40. Also, there was a significant difference ($P \leq .05$) between the pH means due to a treatment X temperature X storage time interaction. The pH means of inoculated samples stored at 10°C for 16 and 20 days (5.19 and 5.13) were different

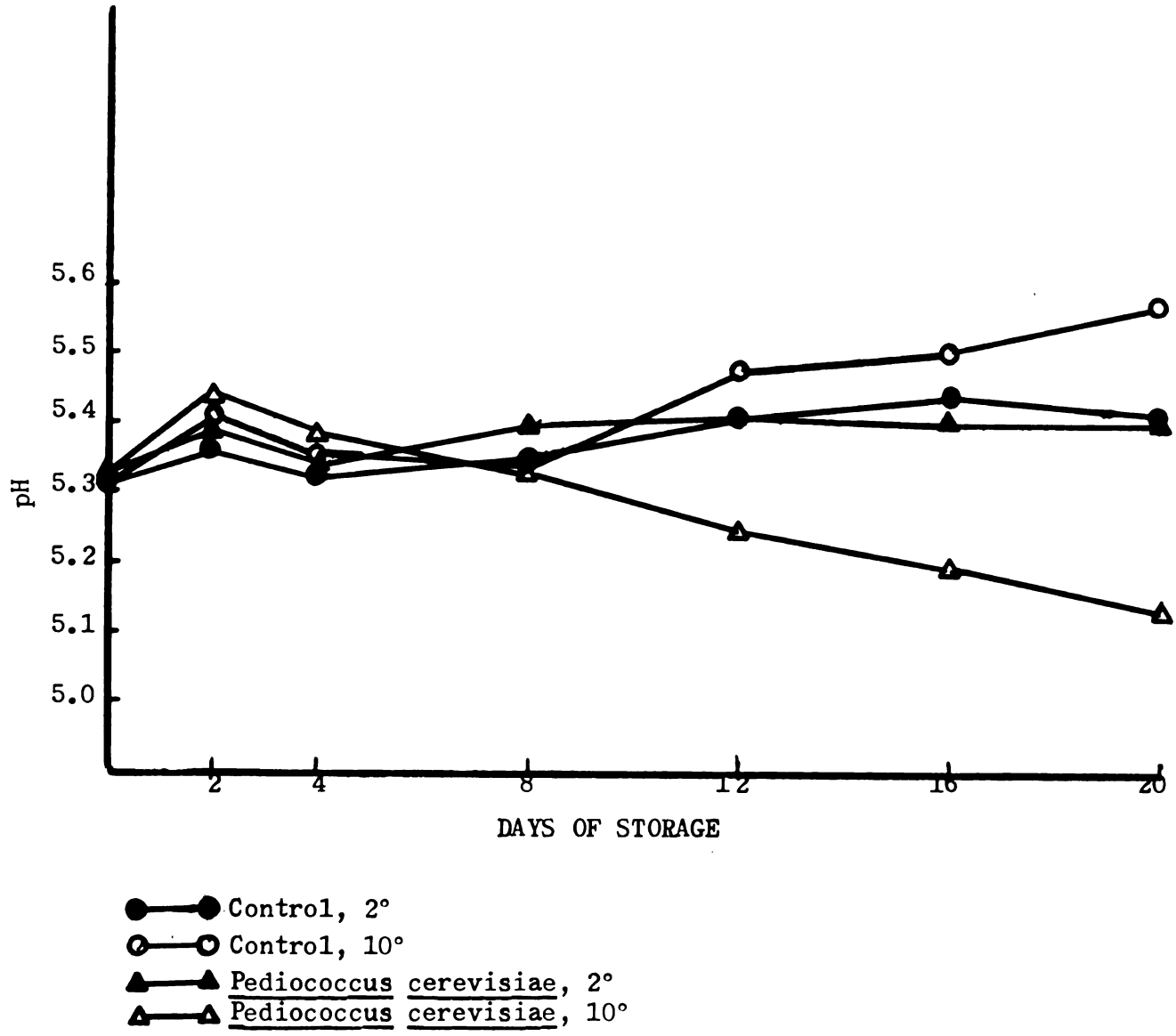


Figure 6. Relationship of the pH of control and Pediococcus cerevisiae inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

($P \leq .01$) from the pH means of control and treated samples stored at 2°C and control samples stored at 10°C for 12, 16 and 20 days as revealed by the multiple range test.

Leuconostoc mesenteroides is also an acid producer and in figure 7 it is shown that samples inoculated with this organism and incubated at 10°C did have a decreasing pH. The pH of control and Leuconostoc mesenteroides treated samples stored at 2°C changed very little during the 20 day storage period while the pH of the control samples stored at 10°C increased. There was a highly significant difference ($P \leq .01$) between the overall pH mean, 5.31, of the Leuconostoc mesenteroides treated samples and the overall pH mean, 5.46, of the control samples. There was a highly significant difference ($P \leq .01$) between pH means of a treatment X temperature X storage time interaction. A multiple range test indicated that the pH means of Leuconostoc mesenteroides inoculated samples stored at 10°C for 12, 20 and 16 days (5.04, 5.04, and 5.01, respectively) were different ($P \leq .01$) from all other pH means except pH 5.14 of the inoculated sample stored at 10°C for 8 days. Also, the pH mean, 5.76, of the control sample stored at 10°C for 20 days was different ($P \leq .01$) from all other pH means except the pH, 5.69, of the control sample stored at 10°C for 16 days.

The relationship between the pH of control and Micrococcus luteus inoculated porcine muscle tissue stored at 2° and 10°C for 20 days is indicated in figure 8. The inoculated samples stored at 10°C had an evident increase in pH while there was little change in the pH of the other

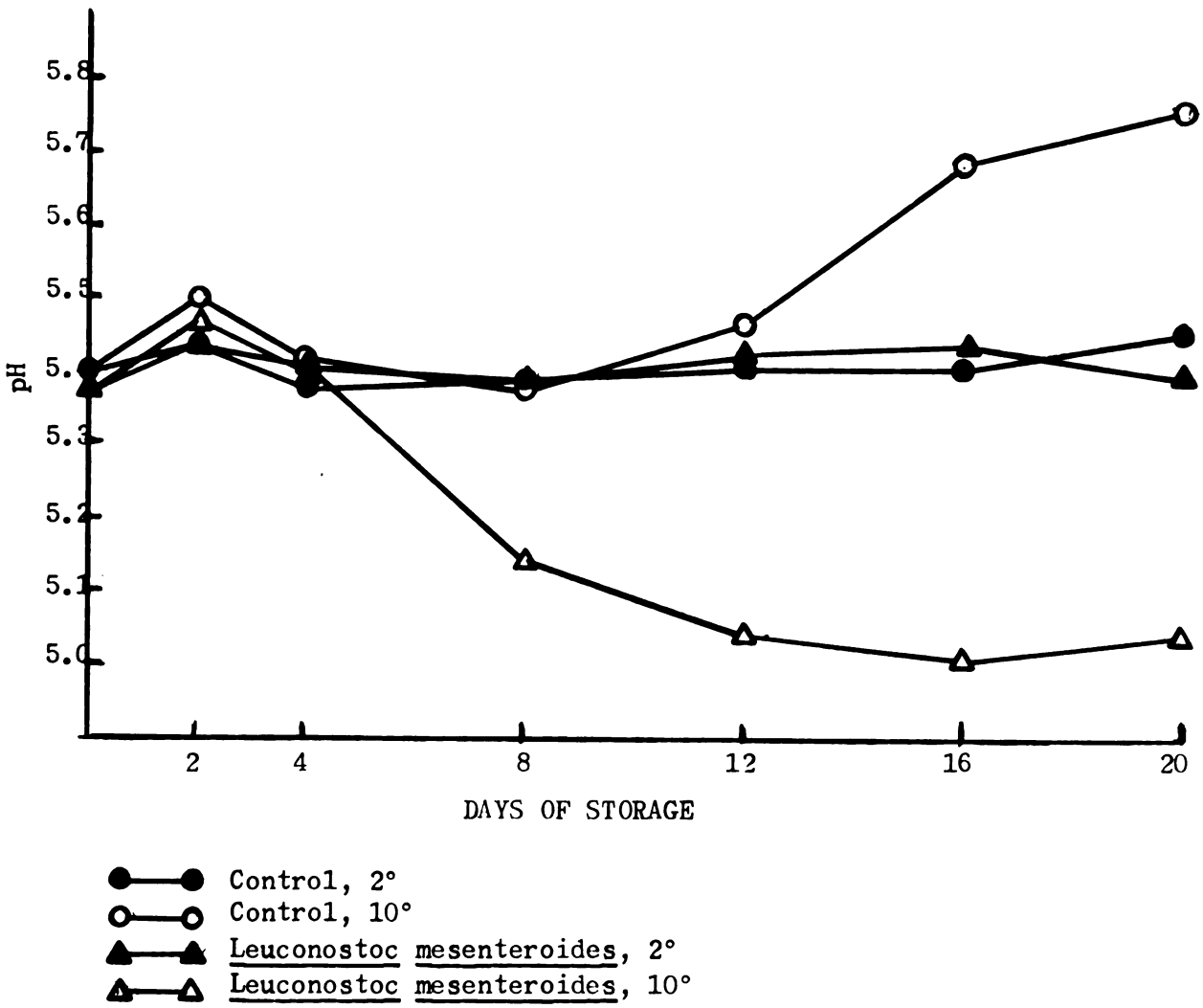


Figure 7. Relationship of the pH of control and Leuconostoc mesenteroides inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

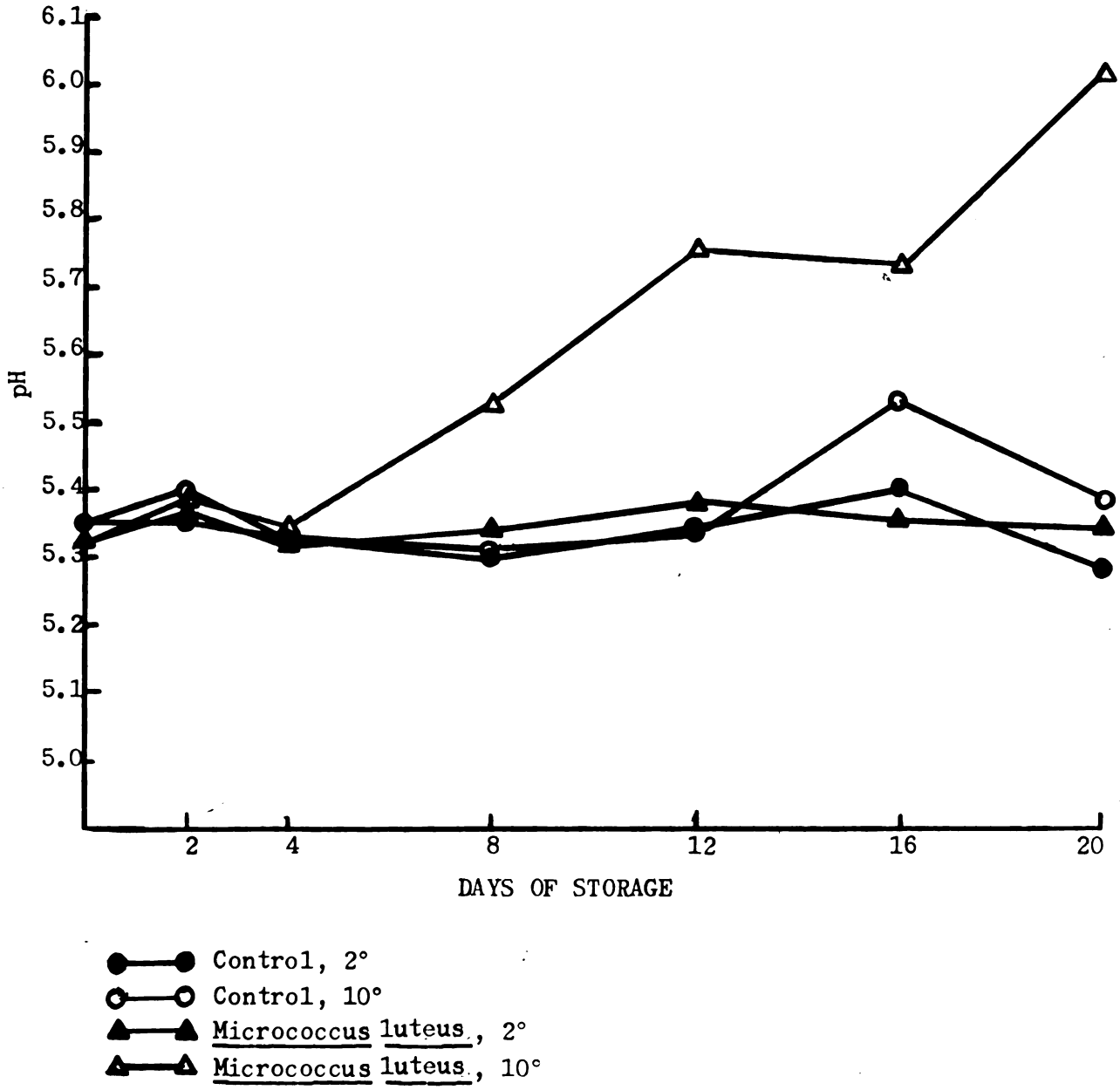


Figure 8. Relationship of the pH of control and Micrococcus luteus inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

samples. There was a highly significant difference ($P \leq .01$) between the overall pH mean, 5.47, of the Micrococcus luteus inoculated samples and the overall pH mean, 5.37, of the control samples.

The pH of Pseudomonas fragi inoculated samples increased very rapidly and to the highest value of any of the control or other treated samples used in this study. This is shown in figure 9 which depicts the relationship of the pH of Pseudomonas fragi inoculated samples and related controls stored at 2° and 10°C for 20 days. The pH of the treated samples stored at 2°C reached the same level as that of the treated samples stored at 10°C after an additional 4 days of storage. This relationship is similar to that exhibited in figure 5 which depicts the growth of these organisms. The overall pH mean, 6.48, of the Pseudomonas fragi treated samples was significantly different ($P \leq .01$) from the overall pH mean, 5.36, of the control samples. There was a highly significant difference ($P \leq .01$) between the pH means of the samples due to a treatment X temperature X storage time interaction. The pH means, 6.84 and higher, were significantly higher ($P \leq .01$) than the pH means of all the other samples in this group according to the multiple range test. The pH means which were 6.84 or higher included Pseudomonas fragi inoculated samples stored at 10°C for 8, 12, 16 and 20 days and inoculated samples stored at 2°C for 12, 16 and 20 days. Ockerman et al. (1969) reported increased pH values for beef samples inoculated with each of the following: a general inoculum, Pseudomonas organisms, and Achromobacter organisms.

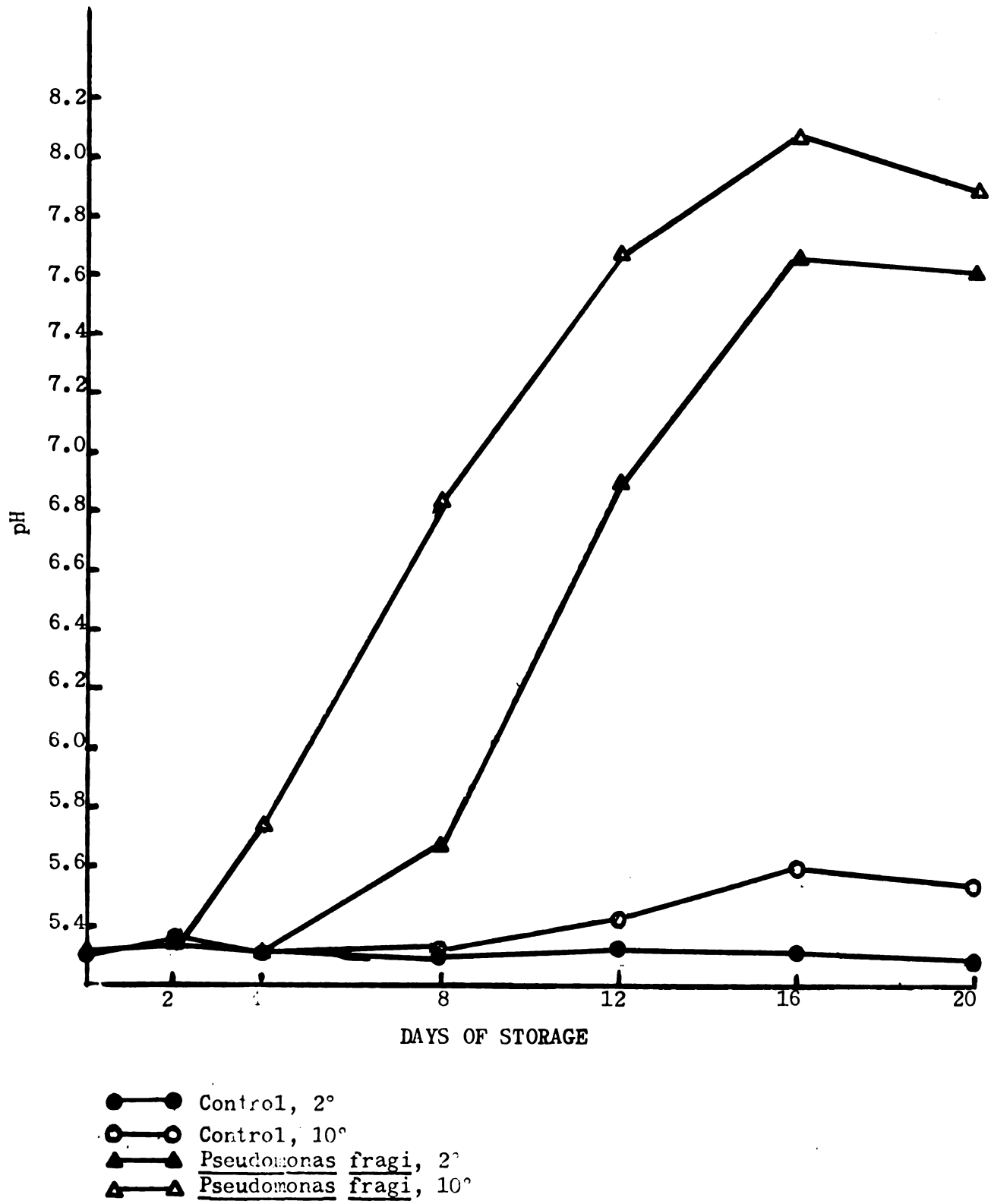


Figure 9. Relationship of the pH of control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

Water-Soluble Proteins

Solubility. In this study the amount of extractable water-soluble protein nitrogen decreased with increasing storage time in all of the control samples and in all of the treated samples except those inoculated with Pseudomonas fragi. The rank of the water-soluble protein nitrogen means of various days of storage is shown in table 1 as an example of the decrease found. The means in this table were obtained from control and Micrococcus luteus inoculated samples stored at 2° and 10°C for the length of time shown. Similar data were obtained from samples inoculated with Leuconostoc mesenteroides and Pediococcus cerevisiae. In the example shown, the amount of water-soluble protein nitrogen decreased approximately 4% from day 0 to day 8. After that time there was little change throughout

Table 1. Rank of mg of water-soluble protein nitrogen/100 mg total nitrogen means for various storage times which include control and Micrococcus luteus inoculated samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7
Days of storage	0	2	4	12	8	20	16
Mean*	22.45	20.93	20.75	18.44	18.16	18.10	18.01

*Those means not underlined by the same line are significantly different ($P \leq .01$) from each other.

the remainder of the storage period. Also, there was a 1 1/2% decrease from day 0 to day 2 with a slight decrease from day 2 to day 4. The loss of water-soluble protein nitrogen due to increasing storage time was in agreement with the results reported by Sayre and Briskey (1963) and Mc Loughlin (1963).

The Pediococcus cerevisiae and Micrococcus luteus inoculated samples exhibited no differences from the controls in the amount of water-soluble protein nitrogen which could be extracted. However, there were differences noted between the amount of water-soluble protein nitrogen extracted from controls and from samples inoculated with Leuconostoc mesenteroides or Pseudomonas fragi.

The relationship between the amount of water-soluble protein nitrogen found in control and Leuconostoc mesenteroides inoculated porcine samples stored at 2° and 10°C for 20 days is shown in figure 10. The general downward trend noted in the control samples stored at 2° and 10°C and the treated samples stored at 2°C gives an indication of the decrease attributed to storage time. However, the amount of water-soluble protein nitrogen extracted from the inoculated samples stored at 10°C decreased to a greater extent. When the means were ranked, it was found that the three means 14.59, 14.68 and 14.83 of the treated samples stored at 10°C for 12, 16 and 20 days, respectively, were different ($P \leq .05$) from all other means except the means for the control and inoculated samples stored 20 days at 2°C. The greater decrease noted in the treated samples stored at 10°C was probably associated with the lower pH which was shown in figure 7, since Scopes (1964) has reported that the lower the pH the smaller the amount of extractable water-soluble protein.

The water-soluble protein nitrogen means for control and Pseudomonas fragi inoculated samples stored 20 days at 2° and 10°C are presented in figure 11. There was a difference ($P \leq .01$) between the overall mean amount of water-soluble protein nitrogen found in the control samples,

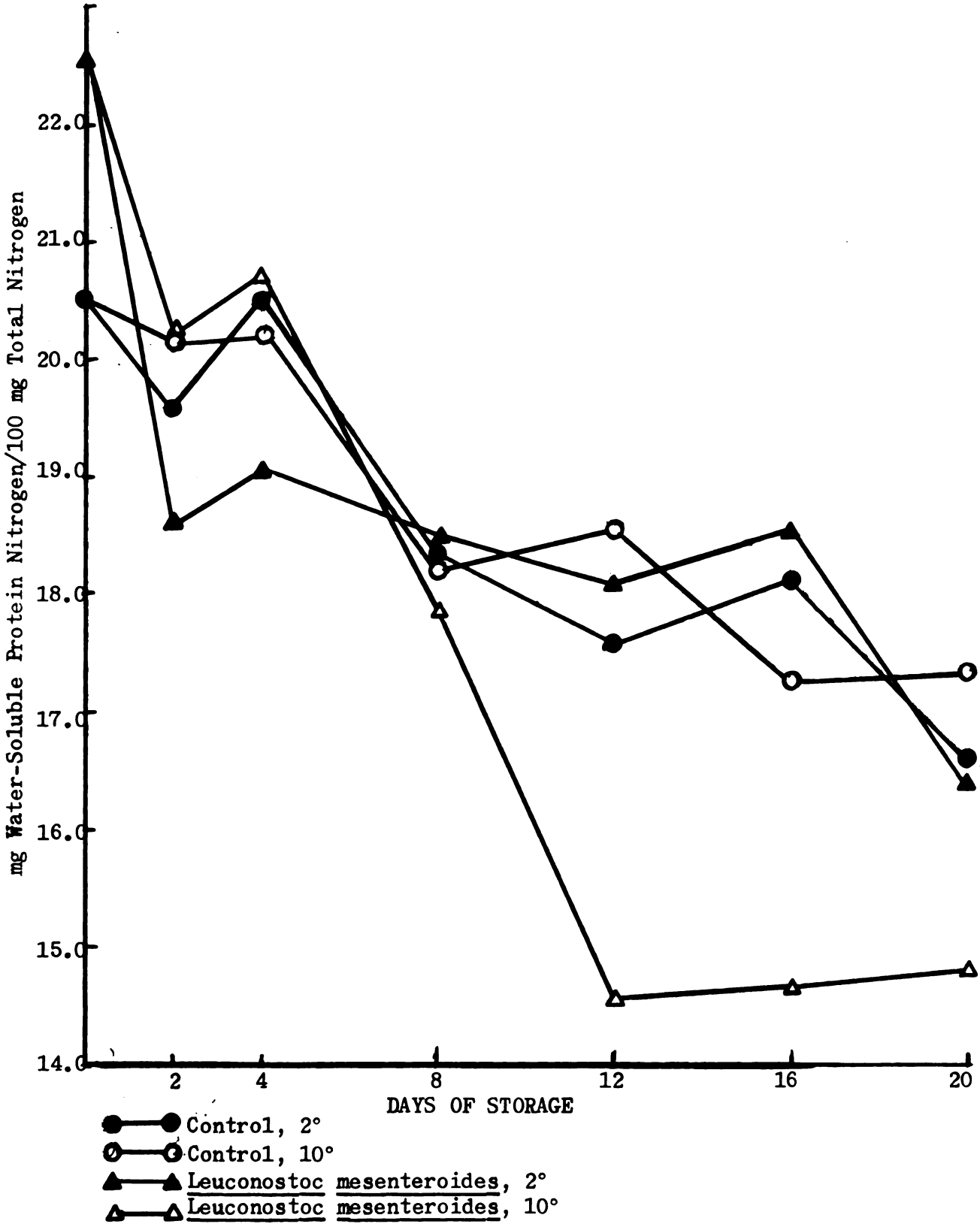


Figure 10. Relationship of water-soluble protein nitrogen from control and Leuconostoc mesenteroides inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

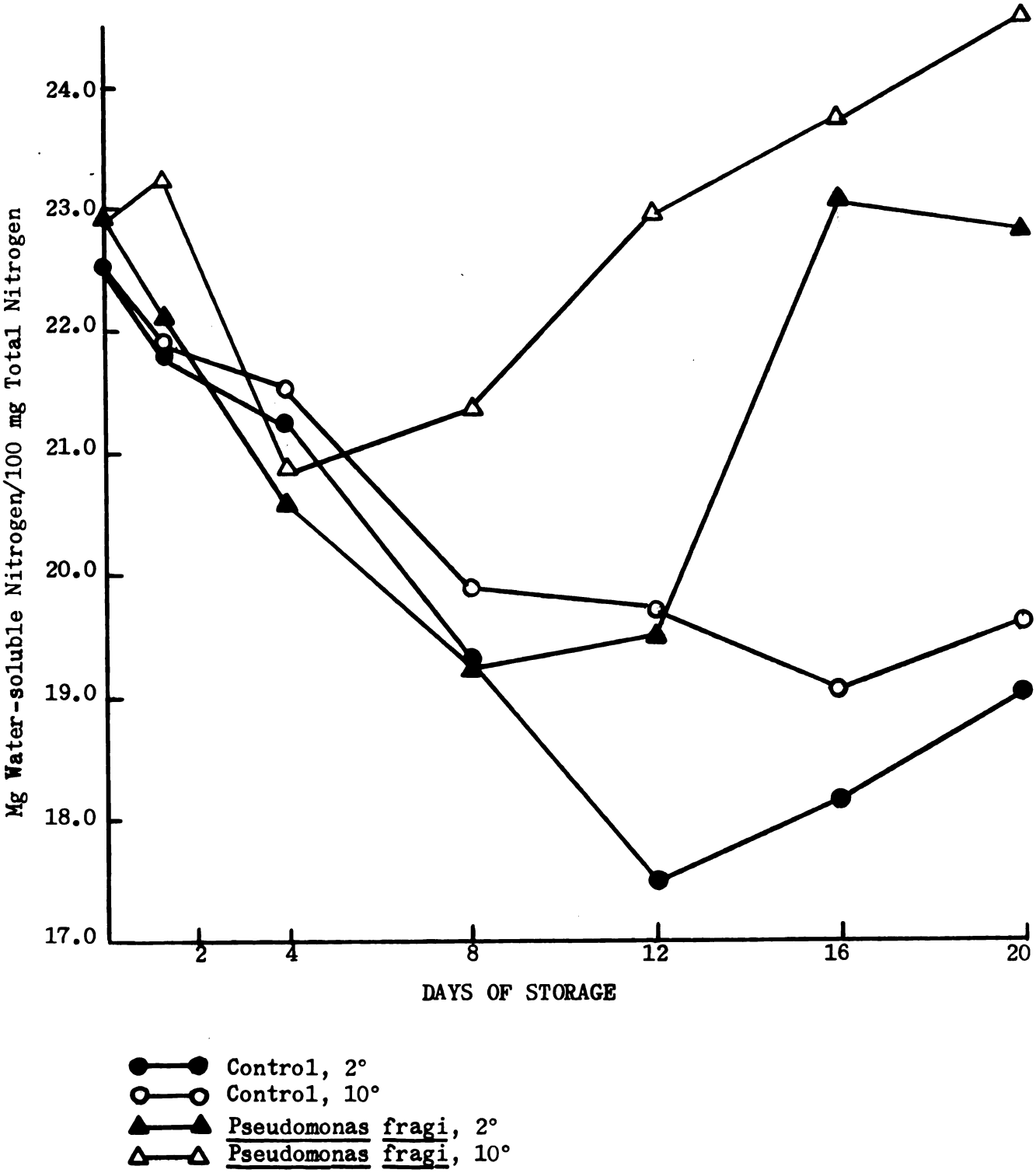


Figure 11. Relationship of water-soluble protein nitrogen from control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

20.28, and that found in the Pseudomonas fragi inoculated samples, 22.14. The control samples exhibited the normal decrease associated with length of storage except the control samples stored at 2°C did have an unexplainable increase from day 12 to day 20. The inoculated samples started to decrease but then increased. The inoculated samples stored at 10°C had an increasing amount of water-soluble protein nitrogen from day 4 to day 20 while the inoculated samples stored at 2°C did not exhibit a similar increase until day 8. The amount of water-soluble protein nitrogen from the treated samples stored 16 and 20 days at 2°C reached the same amount as was extracted at day 0 while the amount extracted from the treated samples stored 12, 16 and 20 days at 10°C was actually more than extracted after day 0. The increase may have been associated with an increasing pH, however, as will be shown later, it is more likely due to proteolytic action by the organisms on the insoluble and salt-soluble proteins.

Starch Gel Electrophoresis. Diagramatic electrophoretograms of water extracts from control samples are shown in figure 12. It can be seen that very little change took place in the 15 bands between day 0 and day 8. By day 20 there was an evident loss of one or two bands and a loss in the intensity of the stain in some others. However, it would appear that the loss in the amount of extractable water-soluble protein was related more to a general loss of water-soluble proteins rather than a loss of any specific protein or proteins. Water extracts from samples inoculated with Micrococcus luteus and Pediococcus cerevisiae had electrophoretograms similar to those shown of the control samples (Figure 12).

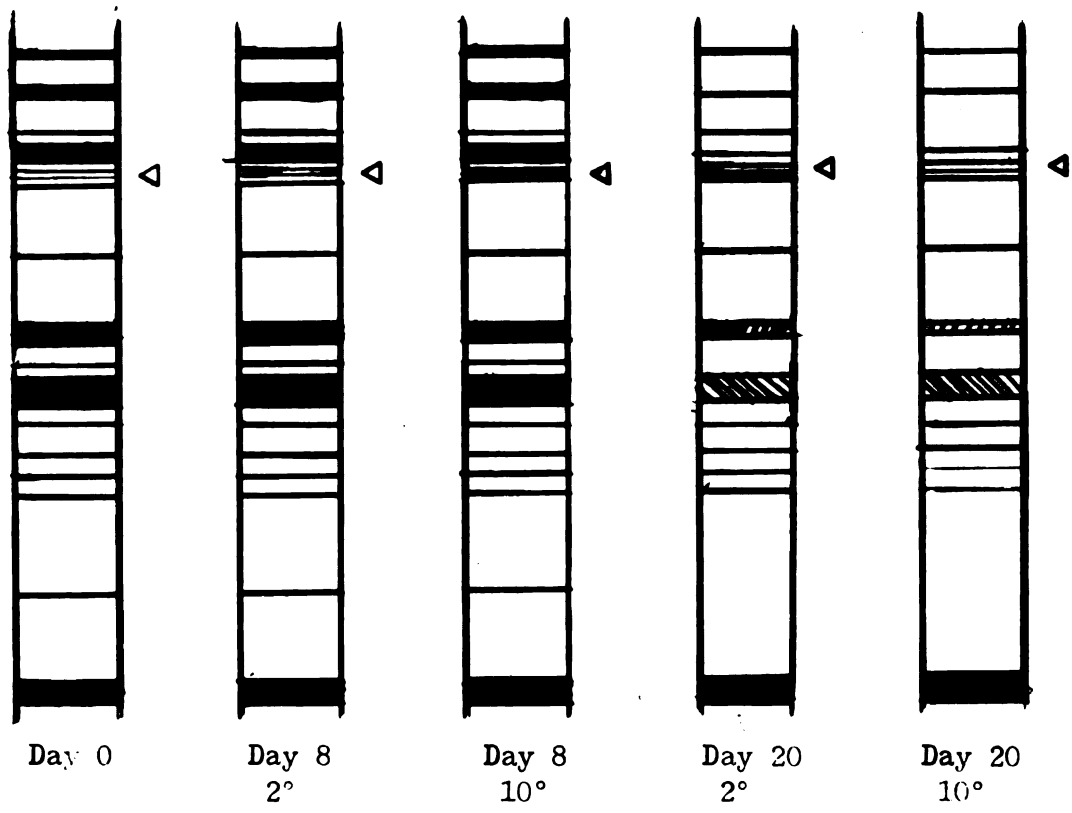


Figure 12. Electrophoretograms of water extracts of control porcine muscle samples stored for 0, 8 and 20 days at 2° and 10°C.

N.B. ◀ indicates the point of sample application in figures 12, 13 and 14.

Water extracts from samples inoculated with Leuconostoc mesenteroides had the diagramatic electrophoretograms depicted in figure 13. These electrophoretograms exhibit the same trends as those of the controls except the one from the sample stored at 10°C for 20 days. In this case more protein bands were lost which was probably the result of denaturation due to the low pH.

Electrophoretograms of water extracts of porcine samples inoculated with Pseudomonas fragi are diagrammed in figure 14. In this case the banding patterns were the same as those of the controls until day 20 when only 6 or 7 of the original 15 bands were found. The loss of bands was probably due to proteolytic action of the Pseudomonas fragi organisms.

Salt-Soluble Proteins

Solubility. The amount of salt-soluble protein nitrogen which could be extracted seemed to increase during the first 8 days of storage after which it tended to remain constant or decrease slightly. This trend was noted in all groups of samples except the samples inoculated with Micrococcus luteus and related controls. An example of the changes in the amount of salt-soluble protein nitrogen associated with length of storage is given in table 2. The means reported in the table were from data obtained from Pseudomonas fragi inoculated samples and related controls but similar results were obtained with samples inoculated with Pediococcus cerevisiae and Leuconostoc mesenteroides and their controls. The lowest amount of salt-soluble protein nitrogen was obtained at day 0 and this increased to the highest amount by day 8. After day 8 there was a gradual

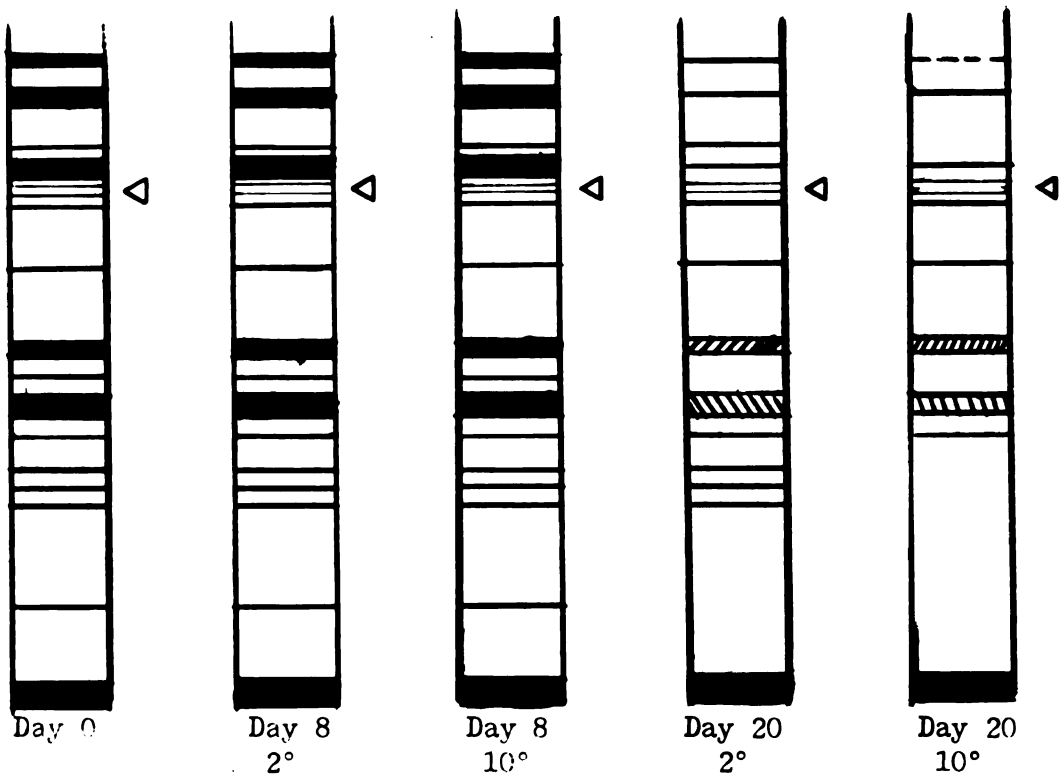


Figure 13. Electrophoretograms of water extracts of Leuconostoc mesenteroides inoculated porcine muscle samples stored for 0, 8 and 20 days at 2° and 10°C.

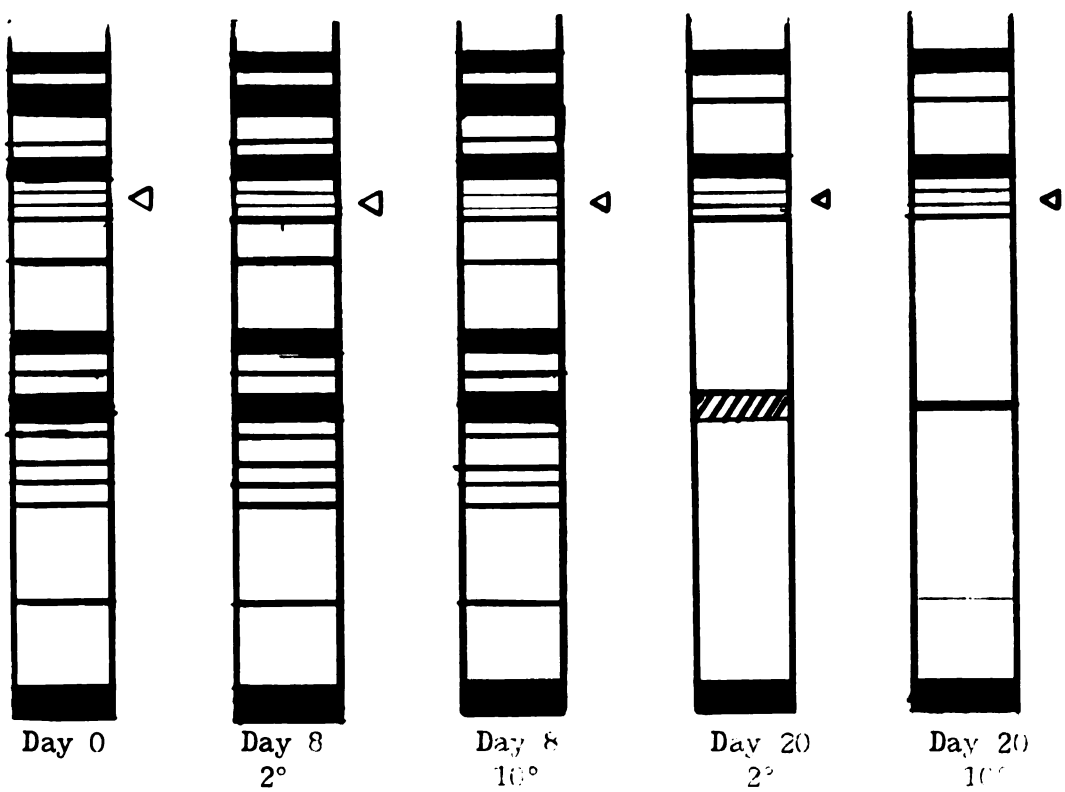


Figure 14. Electrophoretograms of water extracts of Pseudomonas fragi inoculated porcine muscle samples stored for 0, 8 and 20 days at 2° and 10°C.

Table 2. Rank of mg of salt-soluble protein nitrogen/100 mg total nitrogen means for various storage times which include control and Pseudomonas fragi inoculated samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7
Days of storage	8	12	4	20	16	2	0
Mean*	46.16	44.47	43.83	42.16	41.95	41.70	38.68

*Those means not underlined by the same line are significantly different ($P \leq .01$) from each other.

decrease to a relatively constant amount of salt-soluble protein nitrogen. This table also shows that the mean at day 0, 38.68, was significantly lower ($P \leq .01$) than the means at days 8, 12 and 4 which were 46.16, 44.17 and 43.83, respectively. These results were in general agreement with those reported by McIntosh (1967).

There were no differences in the amount of salt-soluble protein nitrogen found due to inoculation with any of the organisms used in this study. However, there were some differences due to interactions which merit consideration.

Analysis of variance revealed a significant difference ($P \leq .05$) between the temperature X storage interaction means of salt-soluble protein nitrogen of control and Leuconostoc mesenteroides inoculated pork samples. Early in the storage period (days 2 and 4) little difference was noted between any of the samples but as the storage period progressed (day 8-16) the control and inoculated samples stored at 10°C had higher amounts of extractable salt-soluble protein nitrogen than those stored at 2°C (figure 15). At day 20 the control samples maintained the relationship

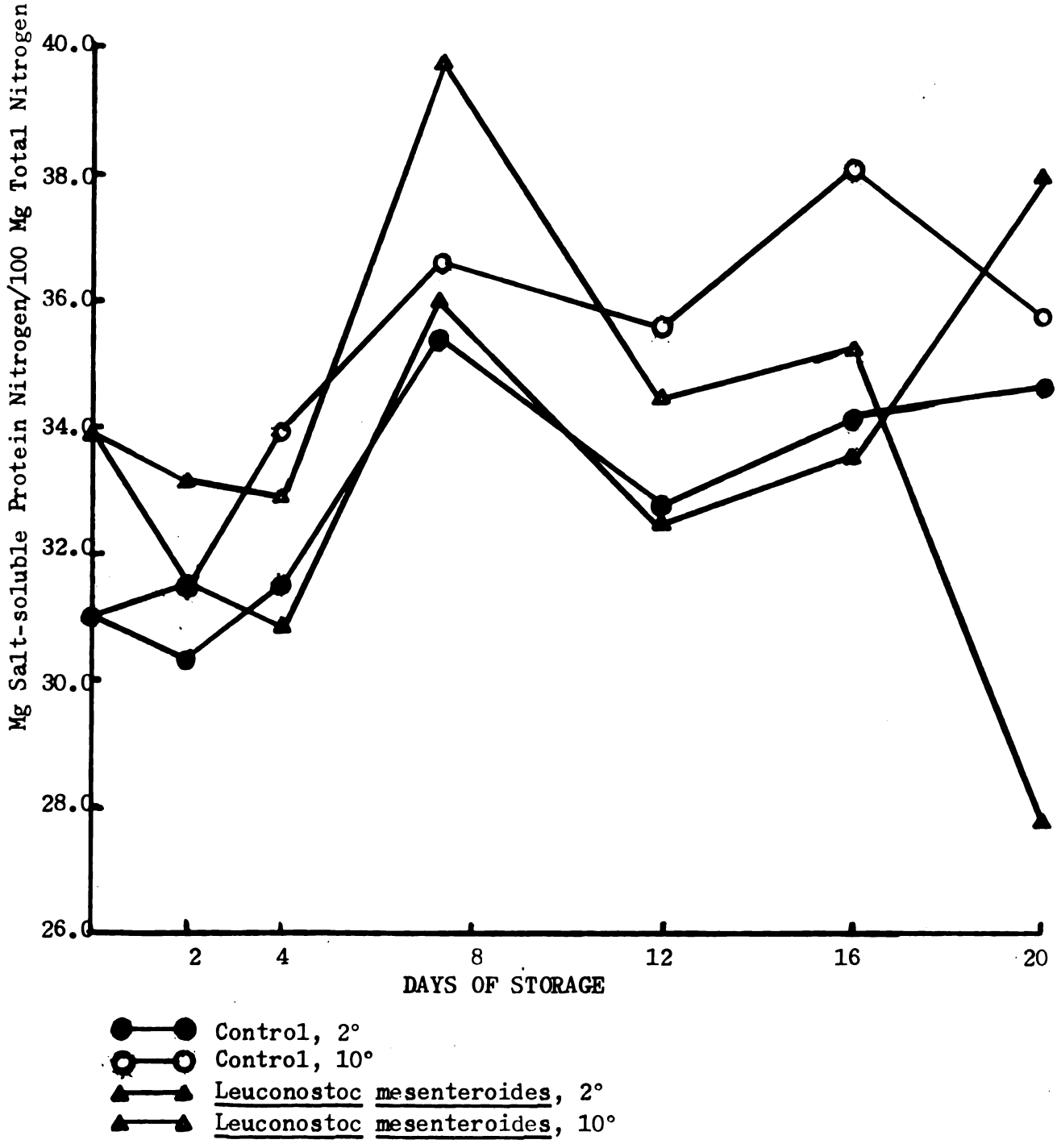


Figure 15. Relationship of the salt-soluble protein nitrogen of control and Leuconostoc mesenteroides inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

of having a higher amount of salt-soluble protein nitrogen when stored at 10°C as compared to storage at 2°C. However, at the same time the inoculated samples had opposite results, that is, those stored at 2°C had a higher amount of salt-soluble protein nitrogen than those stored at 10°C. The Leuconostoc mesenteroides inoculated sample stored at 10°C for 20 days had the lowest amount of salt-soluble protein nitrogen, 27.76 mg/100 mg total nitrogen, shown in figure 15. This low value was probably associated with denaturation of the proteins due to continuous storage (day 12-20) at low pH (figure 7). The reason for the increase in the amount of salt-soluble protein nitrogen of the inoculated samples stored at 2°C is unknown except that it was approximately the same amount as that recorded at day 8 for the inoculated samples stored at 10°C. In both instances the number of organisms was 1-10 million per g (figure 3) and the possibility of a physiological condition suitable to greater extractability of the salt-soluble proteins existed.

There was a significant temperature X storage time interaction for control and Pseudomonas fragi inoculated samples. In general, the samples stored at 10°C had a higher amount of salt-soluble protein nitrogen than those stored at 2°C. There was a treatment X storage time interaction difference which was approaching significance ($P \leq .084$). The ranking of the means of salt-soluble protein nitrogen for this interaction is shown in table 3. The mean salt-soluble protein nitrogen of the inoculated samples at day 8 was significantly higher ($P \leq .01$) than the means of control and treated samples at day 0 and the mean of the treated samples at day 20. Also, it can be seen in figure 16 that the highest amount of

Table 3. Rank of mg of salt-soluble protein nitrogen/100 mg total nitrogen means for control and Pseudomonas fragi inoculated samples at various storage times, the means including samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Treatment-storage time[1]	P-8	P-12	C-20	C-8	P-4	C-12	C-16	P-2	C-4	P-16	C-2	C-0	P-20	P-0
Mean*	47.43	45.22	45.10	44.89	44.88	43.73	43.45	43.39	42.77	40.44	40.00	39.36	39.21	37.99

[1]P = Pseudomonas fragi inoculated samples, C = control samples, 0, 2, 4, 8, 12, 16, 20 - days of storage.
*Those means not underlined by the same line are significantly different ($P \leq .01$) from each other.

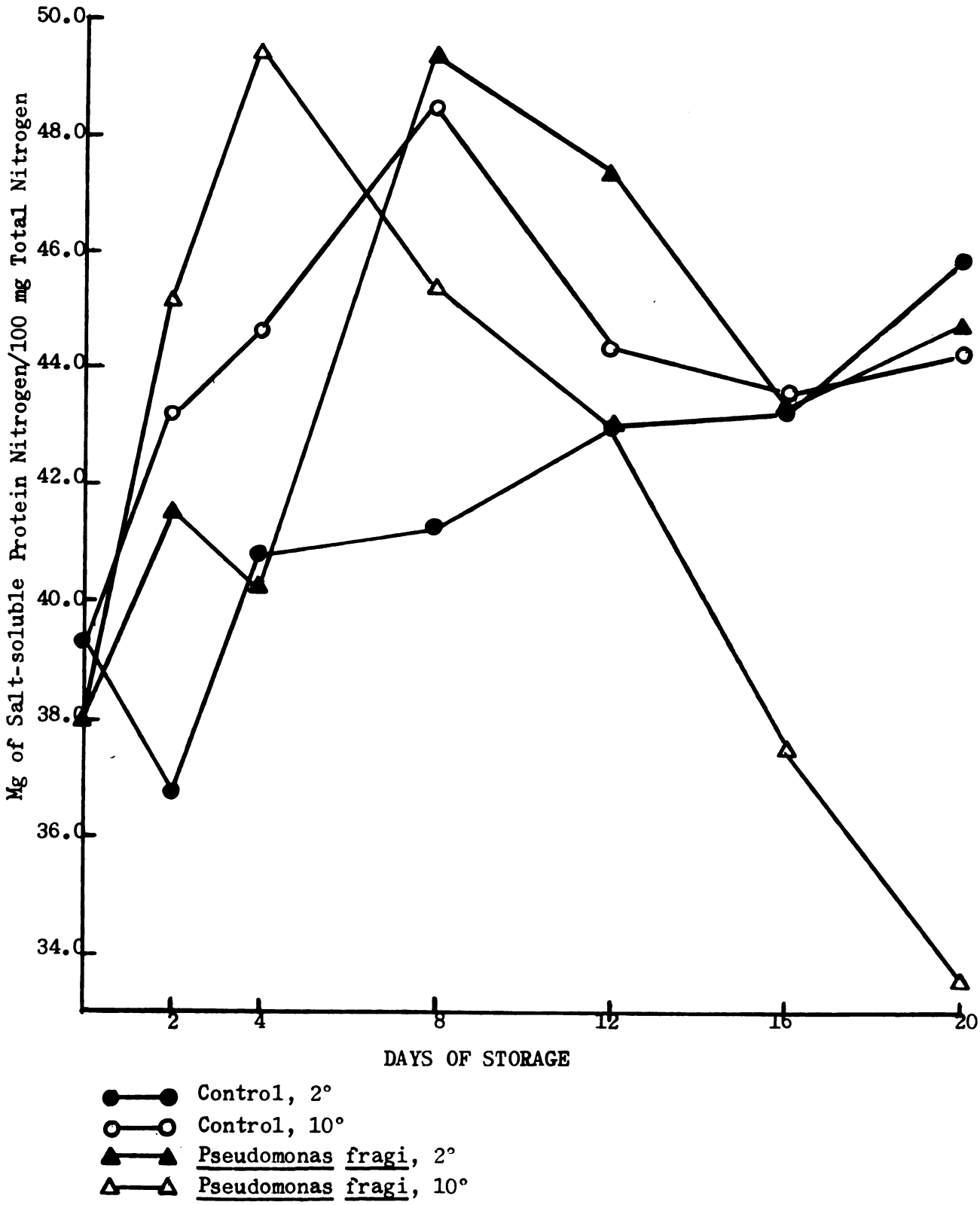


Figure 16. Relationship of salt-soluble protein nitrogen from control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

salt-soluble protein extracted from the inoculated samples was reached about 4 days apart, at day 4 for the samples stored at 10°C and at day 8 for samples stored at 2°C. The 49.5 mg of salt-soluble protein nitrogen/ 100 mg total nitrogen reached by each of these samples coincides with a bacterial count of approximately 100 million (figure 5) and a pH of approximately 5.7 (figure 9) recorded at day 4 for the sample stored at 10°C and at day 8 for the sample stored at 2°C. Thus, the higher amount of extractable salt-soluble protein nitrogen was probably associated with an increasing pH plus the effect of storage. From day 4 through day 20 a decreasing amount of salt-soluble protein nitrogen was noted for the inoculated samples stored at 10°C even though the pH of the samples increased (figure 9). This decrease seemed to be associated with the proteolytic action of this organism. The results of the extractability of the salt-soluble protein nitrogen from the Pseudomonas fragi inoculated samples were similar to those reported by Ockerman et al. (1969).

Starch-Urea Gel Electrophoresis. The results of starch-urea gel electrophoresis of 0.6 M KCl extracts of control porcine samples and samples inoculated with Pediococcus cerevisiae and Leuconostoc mesenteroides stored for 8 and 20 days at 2° and 10°C are shown in figure 17. The results are from one trial but are representative of all four replications. At day 0 there was no difference between these samples, and 6-9 bands were generally evident with 7 bands present in the results shown. In this group of samples there were no differences at day 8 or day 20 between the electrophoretograms of the inoculated and control samples nor were there any differences due to storage temperature. There

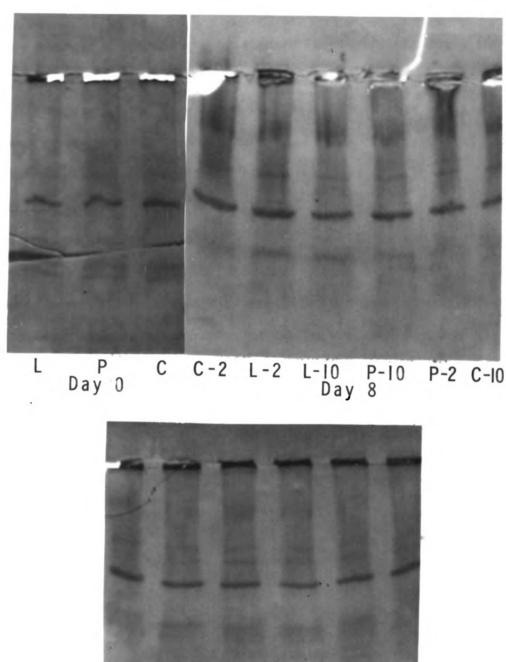


Figure 17. Starch-urea gel electrophoretograms of 0.6 M KCl extracts of control porcine muscle samples (C) and samples inoculated with Pediococcus cerevisiae (P) and Leuconostoc mesenteroides (L) stored 0, 8 and 20 days at 2° and 10°C.

were some changes as the length of storage increased. At day 8 the first, second and third bands from the top of the gel were more distinct than at day 0. At day 20 the first band was less diffuse than at day 8. These differences were probably associated with changes occurring with the resolution of rigor and/or storage time.

The electrophoretograms of 0.6 M KCl extracts of control porcine samples and samples inoculated with Micrococcus leutus and Pseudomonas fragi stored 8 and 20 days at 2° and 10°C are shown in figure 18. At day 0, 9 bands were evident but no differences were found between the control and inoculated samples. The number of bands increased to 14 by day 8. These electrophoretograms exhibited the largest number of bands of any samples studied. However, the general trend shown was typical of the various replicates of samples inoculated with these organisms. At day 8 as at day 0 there were no differences in the number or pattern of the bands due to treatment nor due to storage temperature. One or two bands disappeared by day 20 for all of the samples but those inoculated with Pseudomonas fragi had the least number of bands. This loss of protein bands was probably due to proteolytic action by the organisms, however, as will be explained in the discussion of disc gel electrophoresis, some problems were encountered in washing the water-soluble proteins out of the samples.

Disc Gel Electrophoresis. The results of disc gel electrophoresis of 0.6 M KCl extracts of control samples and samples inoculated with Pediococcus cerevisiae and Leuconostoc mesenteroides and stored for 8 and

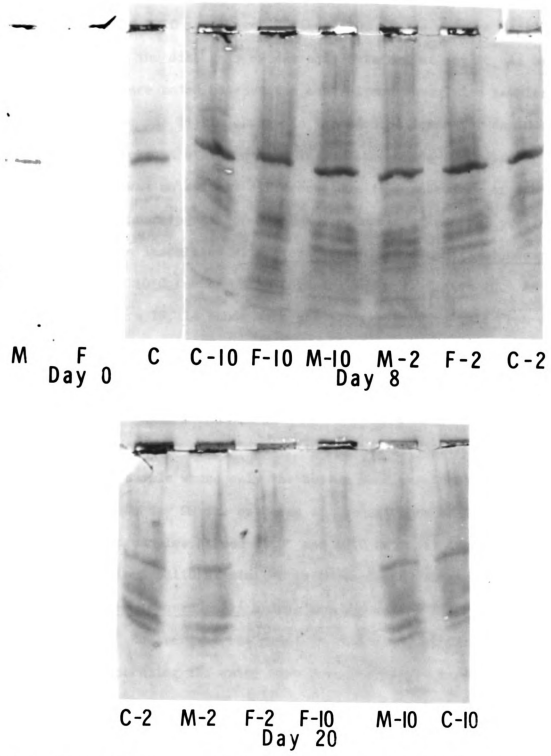


Figure 18. Starch-urea gel electrophoretograms of 0.6 M KCl extracts of control porcine muscle samples and samples inoculated with Micrococcus luteus (M) and Pseudomonas fragi (F) stored 0, 8 and 20 days at 2° and 10°C.

20 days at 2° and 10°C are shown in figure 19. There were at least 14 bands present on the disc gels of extracts obtained at day 0. As expected, no differences were noted between the control and inoculated samples at that time. There was little change in the band patterns for day 8 and day 20 extracts except stain intensities of some bands increased or decreased. There was no evident difference due to temperature of storage.

The electrophoretograms of 0.6 M KCl extracts of control porcine samples and those inoculated with Micrococcus luteus and Pseudomonas fragi stored at 2° and 10°C for 8 and 20 days are shown in figure 20. As was the case in figure 19, 14 bands were present on the disc gels from extracts obtained at day 0. At day 8 band patterns similar to those found at day 0 were evident except the bands seemed to be more distinct. The pattern of the extract of Pseudomonas fragi inoculated samples stored 8 days at 10°C was different from the others. It appeared that three bands were present in this sample where only the bottom band was located for the other samples. By day 20 the patterns of the extracts of the Pseudomonas fragi inoculated samples stored at 2° and 10°C had lost many bands while the others retained patterns similar to those found at day 0. Thus, it appeared that Pseudomonas fragi hydrolyzed the salt-soluble proteins of porcine tissue. However, at one point in this study difficulty was encountered in separating the water wash from the sample tissue due to the higher water holding capacity of the sample associated with a higher pH. This difficulty indicated that possibly the proteins were being discarded. However, when the wash was collected, recentrifuged at a higher speed, the residue extracted with 0.6 M KCl, and subjected to disc gel electrophoresis, the same pattern as shown in figure 20 for the Pseudomonas fragi

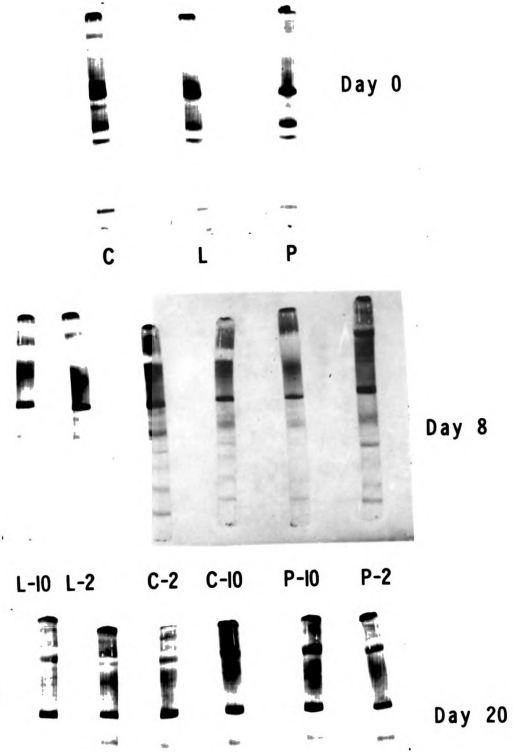


Figure 19. Disc gel electrophoretograms of 0.6 M KCl extracts of control porcine muscle samples (C) and samples inoculated with Pediococcus cerevisiae (P) and Leuconostoc mesenteroides (L) stored 0, 8 and 20 days at 2° and 10°C.

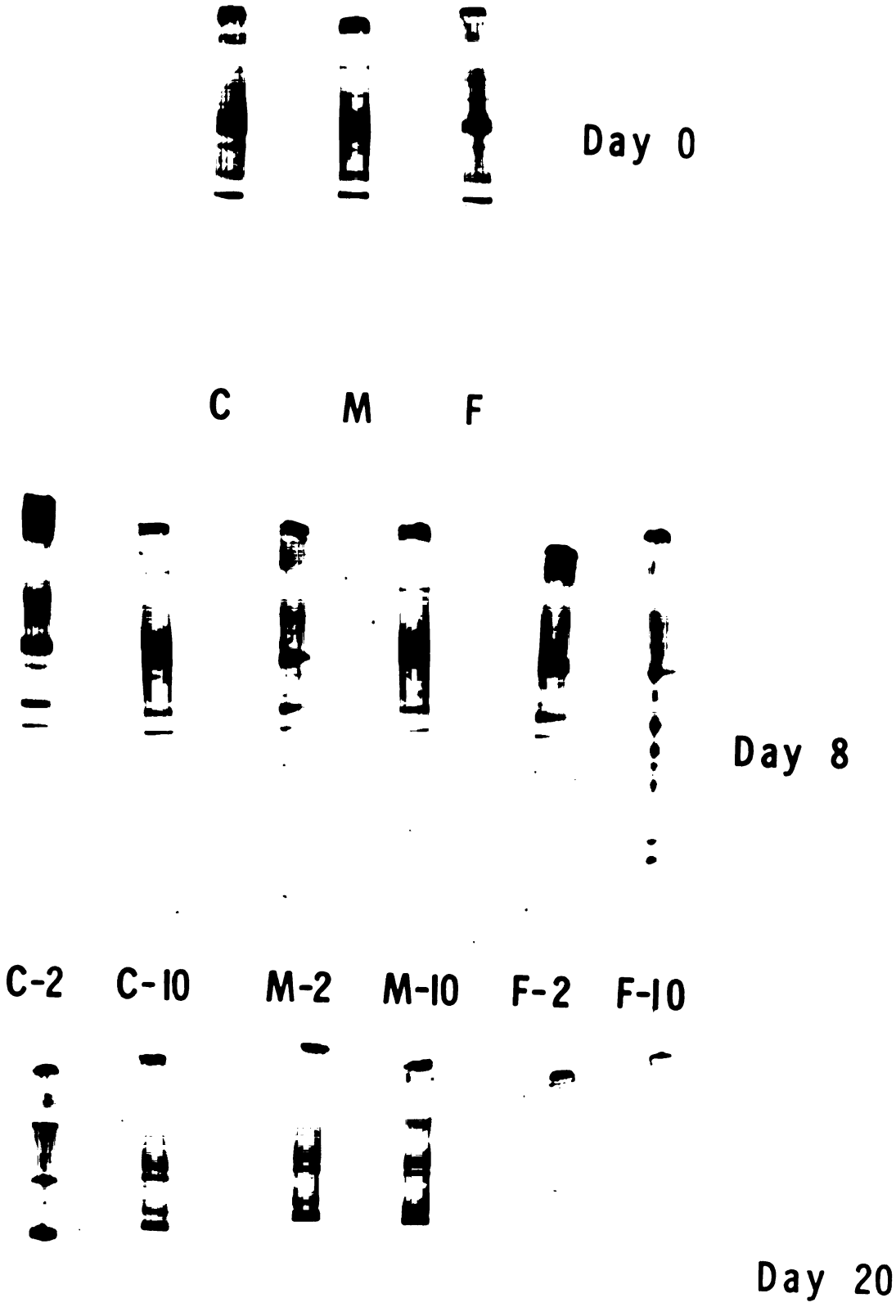


Figure 20. Disc gel electrophoretograms of 0.6 M KCl extracts of control porcine muscle samples (C) and samples inoculated with Micrococcus luteus (M) and Pseudomonas fragi (F) stored 0, 8 and 20 days at 2° and 10°C.

inoculated samples was found. This indicated some proteins may have been washed out, but they were the same proteins as were present in the extracts from inoculated samples (F-2, F-10, Day 20), so all that was lost was stain intensity of the protein bands. Thus, proteolysis did take place which was expected for organisms of this type.

Thin Layer Gel Filtration. Thin layer gel filtration of different extracts of the salt-soluble proteins was attempted. The porcine muscle tissue was extracted with 1.1 M KI, PO_4 buffer used in the solubility studies, 0.6 M KCl, CO_3 buffer used in the electrophoretic studies, 1.0 M NaCl used in the emulsifying capacity studies, and a 0.4 M KCl, PO_4 buffer. The same buffers (4) were used to form the Sephadex superfine G-200 gels. Varied amounts of extracts were applied to the gels with similar results. In all cases the proteins moved through the gel but there was no protein separation regardless of the system used. The KI extract usually resulted in a streak of nigrosin stained material while the other extracts resulted in one large spot which had moved some distance depending on the size of the plate (20 cm or 40 cm) and the length of time buffer flow was maintained. The salt-soluble proteins are known to have high molecular weights which may have been responsible for the lack of separation. Thus, the use of a Bio Gel A-50 gel was attempted but the particle size of the gel was too large for successful thin-layer gel filtration.

Insoluble Proteins

The amount of insoluble protein nitrogen was determined by subtracting the amount of soluble nitrogen/100 mg total nitrogen (water-soluble protein nitrogen, salt-soluble protein nitrogen, and nonprotein nitrogen) from 100. Thus, as there was a significant difference due to storage time for water-soluble and salt-soluble proteins, it would be expected that such a difference should be found for the insoluble protein. An example of the changes associated with storage is given in table 4.

Table 4. Rank of mg of insoluble protein nitrogen/100 mg total nitrogen means for various storage times which include control and Pediococcus cerevisiae inoculated samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7
Days of storage	20	16	12	2	8	4	0
Mean*	32.34	30.29	27.76	27.53	27.29	26.89	25.71

*Those means not underlined by the same line were significantly different ($P \leq .01$) from each other.

The Pediococcus cerevisiae inoculated samples and related controls were chosen as other differences, such as treatment and the various interactions were not complicating the effect of storage. As shown in table 4, the amount of insoluble protein nitrogen increased from day 0 to day 20 with those two means being significantly different ($P \leq .01$) from each other. This relationship was the reciprocal of that found for the water-soluble proteins (table 1).

The relationship of the amount of insoluble protein nitrogen from control and Leuconostoc mesenteroides inoculated samples stored at 2° and 10°C for 20 days is shown in figure 21. Although it may not be readily apparent, there was a significant difference ($P \leq .05$) due to a treatment X storage time interaction. Also a difference ($P \leq .064$) due to a treatment X temperature X storage time interaction was noted. There was a large increase in the amount of insoluble protein nitrogen at day 20 for the inoculated sample stored at 10°C. This was probably due to the lower pH which caused a decrease in the extractability of the water and salt-soluble proteins as shown in figures 10 and 15 and thus increased the amount of insoluble protein.

The amount of insoluble protein nitrogen found in control and Pseudomonas fragi inoculated porcine samples stored at 2° and 10°C for 20 days is depicted in figure 22. In this case the differences due to a treatment X storage time interaction were approaching significance ($P \leq .067$). This type of difference may be more readily apparent than was the case with the Leuconostoc mesenteroides group of samples as the means of the Pseudomonas fragi inoculated samples were lower than those of the control after 12 days of storage. As the storage time progressed, the amount of insoluble protein nitrogen found in the Pseudomonas fragi inoculated samples stored at 10°C decreased up to 12 days of storage and then increased slightly. The amount of insoluble protein nitrogen found in the inoculated sample stored at 2°C decreased after 4 days of storage. The decreasing amounts of insoluble protein nitrogen were associated with the increased extractability of the water and salt-soluble proteins

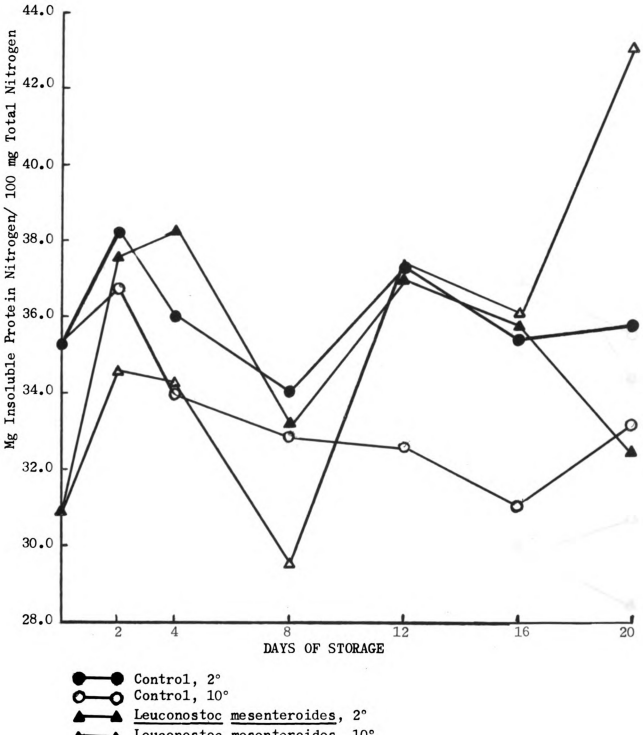


Figure 21. Relationship of insoluble protein nitrogen of control and Leuconostoc mesenteroides inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

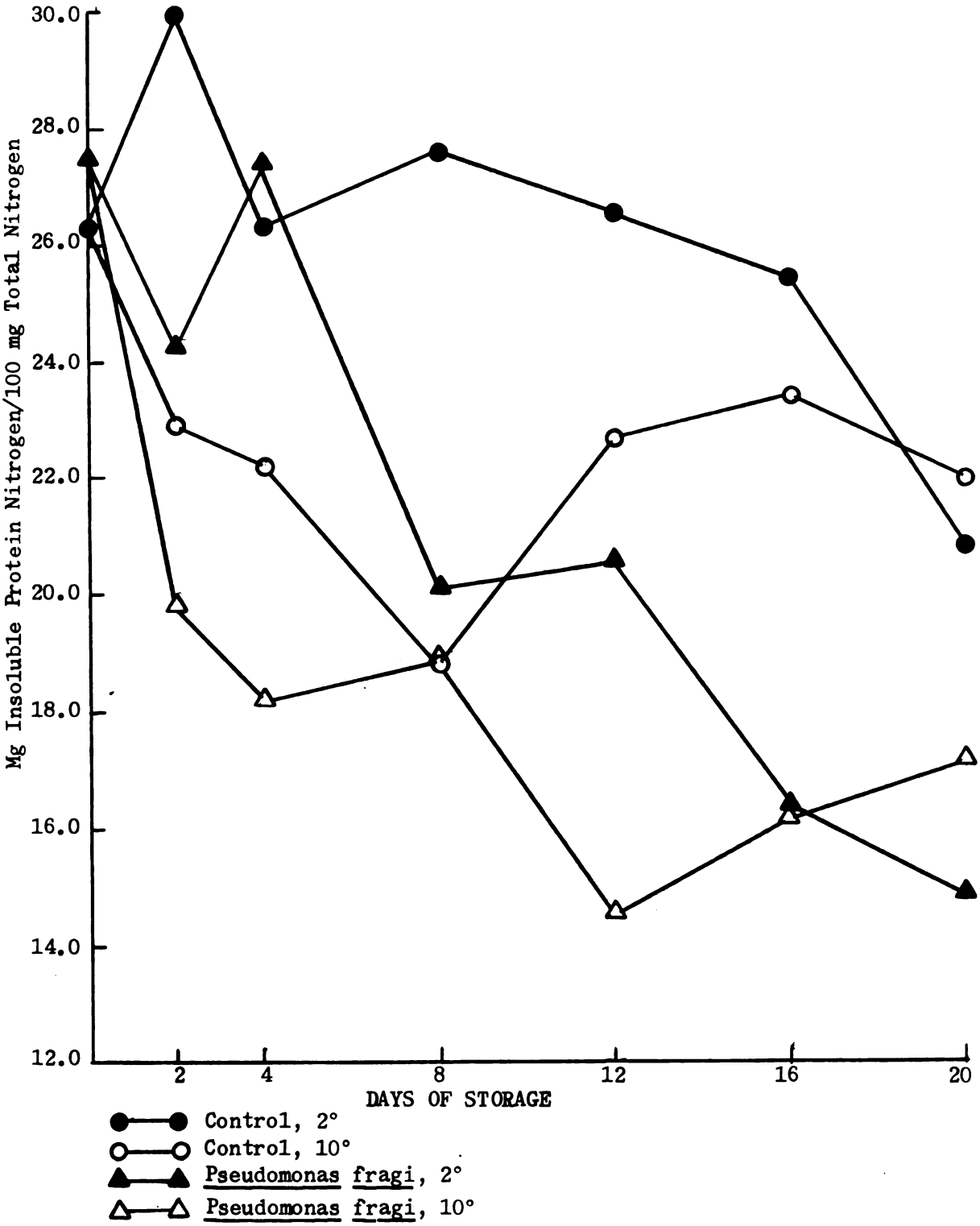


Figure 22. Relationship of insoluble protein nitrogen of control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

as shown in figures 11 and 16 which again seemed to follow pH changes and proteolytic action of the microorganisms. Ockerman et al. (1969) reported similar results when they inoculated three beef samples with a general inoculum, Pseudomonas and Achromobacter, respectively.

Non Protein Nitrogen (NPN)

There was a highly significant difference ($P \leq .01$) between the overall means of the NPN found in samples stored at 10°C and that found in samples stored at 2°C in all of the sample groups except the Micrococcus luteus group which had overall mean differences approaching significance ($P \leq .121$). In all cases, except the Pseudomonas fragi group, the overall mean of the NPN extracted from the samples stored at 10°C was higher than the overall mean of NPN extracted from the samples stored at 2°C by approximately 0.5 mg NPN/100 mg total nitrogen. The difference between the amount of NPN found in Pseudomonas fragi inoculated samples and related controls stored at 10° and the same group of samples stored at 2°C was about 1.5 mg NPN/100 mg total nitrogen.

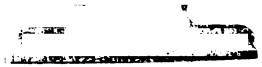
There was a highly significant difference ($P \leq .01$) due to the length of storage in all composite groupings of inoculated and control samples. An illustration of the effect of storage time on the amount of NPN found is given in table 5. The amount of NPN extracted from samples stored longer periods of time was higher than the amount extracted from samples stored shorter periods of time. The amount of NPN found in the samples at days 20, 12 and 16 was significantly higher ($P \leq .01$) than the amount of NPN found in the samples at days 4 and 2. The amount of NPN extracted

Table 5. Rank of mg of NPN/100 mg total nitrogen means for various storage times which include control and Pediococcus cerevisiae inoculated samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7
Days of storage	20	12	16	0	8	4	2
Mean*	13.45	13.20	12.96	12.55	12.48	11.63	11.50

*Those means underlined by the same line are significantly different ($P \leq .01$) from each other.

at day 0 was higher ($P \leq .01$) than the amount extracted at day 2. The reason for this difference could not be ascertained by this study but the disruption of intact cells by the excision and grinding procedures used on day 0 may have caused considerable autolysis which then subsided during storage. Also, those samples inoculated with bacteria may have evidenced a decrease in the amount of NPN in the early days of storage (2 and 4) as the bacteria could have readily used the NPN components as a source of nitrogen for growth of the organisms. The increase in the amount of NPN recorded at the longer periods of storage was similar to that reported by Chen and Bradley (1924). The example (table 5) used was Pediococcus cerevisiae inoculated samples and related controls. However, similar results were found for the Micrococcus luteus and Leuconostoc mesenteroides inoculated samples and related controls. The Pseudomonas fragi inoculated samples and related controls also had a significant difference ($P \leq .01$) due to storage time but the inoculated samples had a greater increase than that attributable to storage alone.



The relationship of the amount of NPN found in Pseudomonas fragi inoculated samples and related controls stored for 20 days at 2° and 10°C is presented in figure 23. The graph of the amount of NPN recorded for the control samples (2° and 10°C) shows the general pattern of increasing amounts of NPN throughout the storage period which was typical of other sets of data. However, a large increase was found in the amount of NPN extracted from the Pseudomonas fragi inoculated samples stored at 2° and 10°C. There was a highly significant difference ($P \leq .01$) in this group of samples due to a treatment X temperature X storage time interaction. The NPN means of the Pseudomonas fragi inoculated samples stored at 10°C for 12, 16 and 20 days (19.55, 22.54 and 24.67, respectively) were significantly higher ($P \leq .01$) than all of the other means except those of the inoculated samples stored at 2°C for 16 and 20 days (17.13 and 17.51). The latter samples NPN means (17.13 and 17.51) were significantly higher ($P \leq .01$) than the remaining means except those of the inoculated samples stored at 10°C for 8 days (14.36) and the control samples stored at 10°C for 16 and 20 days (13.75 and 14.12) and at 2°C for 20 days (14.19). The reason for the large increase in the amount of NPN extracted from Pseudomonas fragi inoculated samples appeared to be due to proteolytic action. It can be seen in figure 23 that even though the same amount of growth took place in the inoculated samples stored at 2° and 10°C (figure 5), there was a higher amount of NPN found in the inoculated samples stored at 10°C than in those stored at 2°C. This indicates that temperature may be involved in the ability of the organisms to hydrolyze proteins even though growth was not inhibited very much at

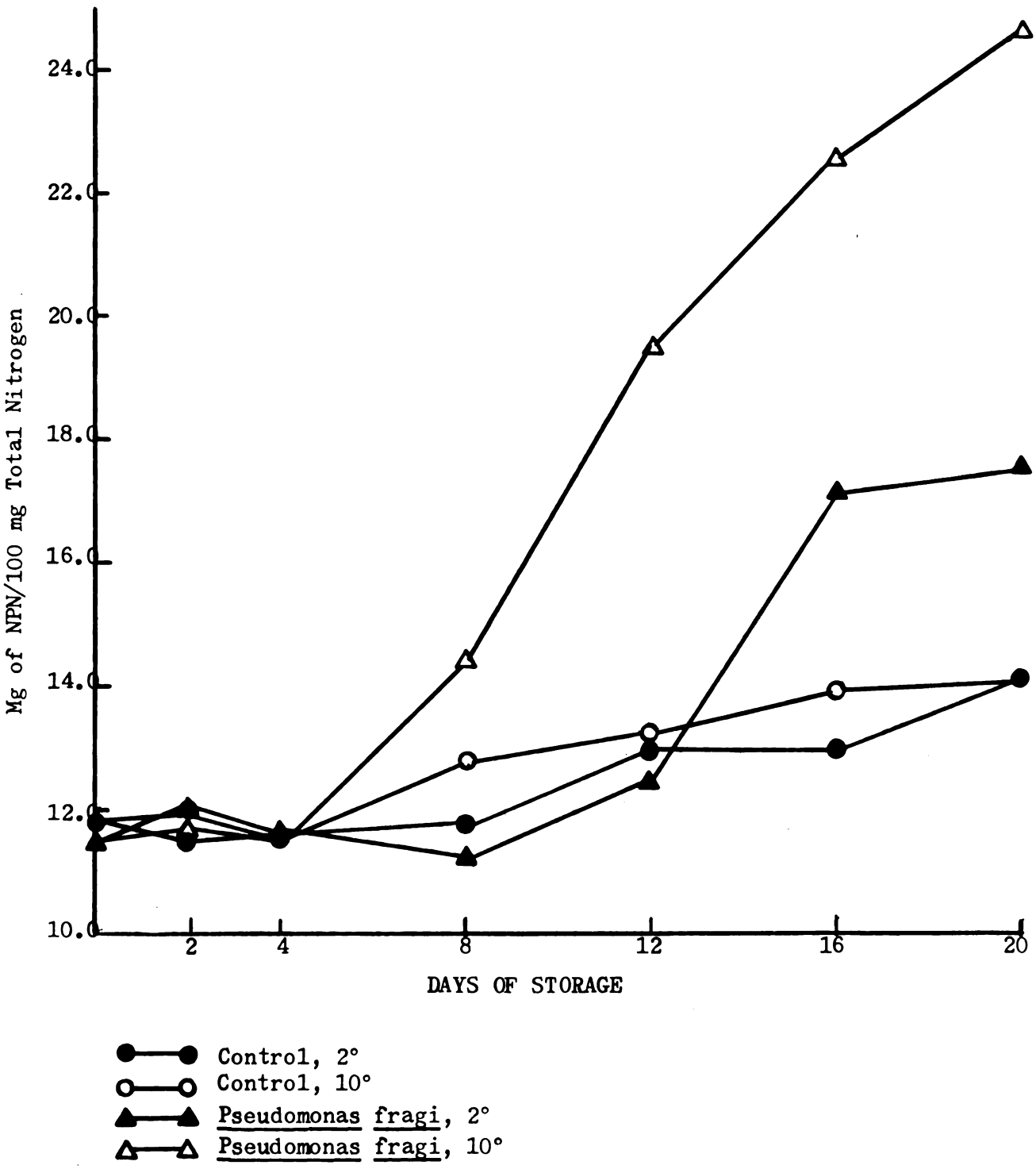


Figure 23. Relationship of non protein nitrogen (NPN) of control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

2°C. Also by comparing figures 23 and 5, it can be noted that proteolysis did not take place until after the peak number of organisms was reached. At least, there was no evidence of proteolysis until that time. Ockerman et al. (1969) also reported an increase in the amount of NPN found in three beef samples inoculated with Pseudomonas, Achromobacter, and an unspecified culture, respectively, when stored for 35 days at 3°C.

Emulsifying Capacity

The emulsifying capacity did not seem to be influenced by the factors of temperature or storage as was the case with protein extractability. The samples inoculated with Micrococcus luteus and Pediococcus cerevisiae had almost the same emulsifying capacity means as the related controls throughout the storage period and at both storage temperatures (2° and 10°C). The Pseudomonas fragi inoculated samples overall emulsifying capacity mean of 103.9 ml oil/10 mg total nitrogen was higher ($P \leq .01$) than the control samples overall emulsifying capacity mean of 98.5 ml oil/10 mg total nitrogen. There were some interactions approaching significance in the Leuconostoc mesenteroides inoculated samples and related controls.

The emulsifying capacities of control and Leuconostoc mesenteroides inoculated porcine samples stored at 2° and 10°C for 20 days are depicted in figure 24. Except for the emulsifying capacity of the inoculated sample stored at 2°C for 12 days, the emulsifying capacities of the inoculated samples stored at 2°C and control samples stored at 2° and 10°C for various storage periods were within the same general range. The low emulsifying capacity recorded at day 12 for inoculated samples stored at 2°C seemed to be the result of one very low value recorded in the first

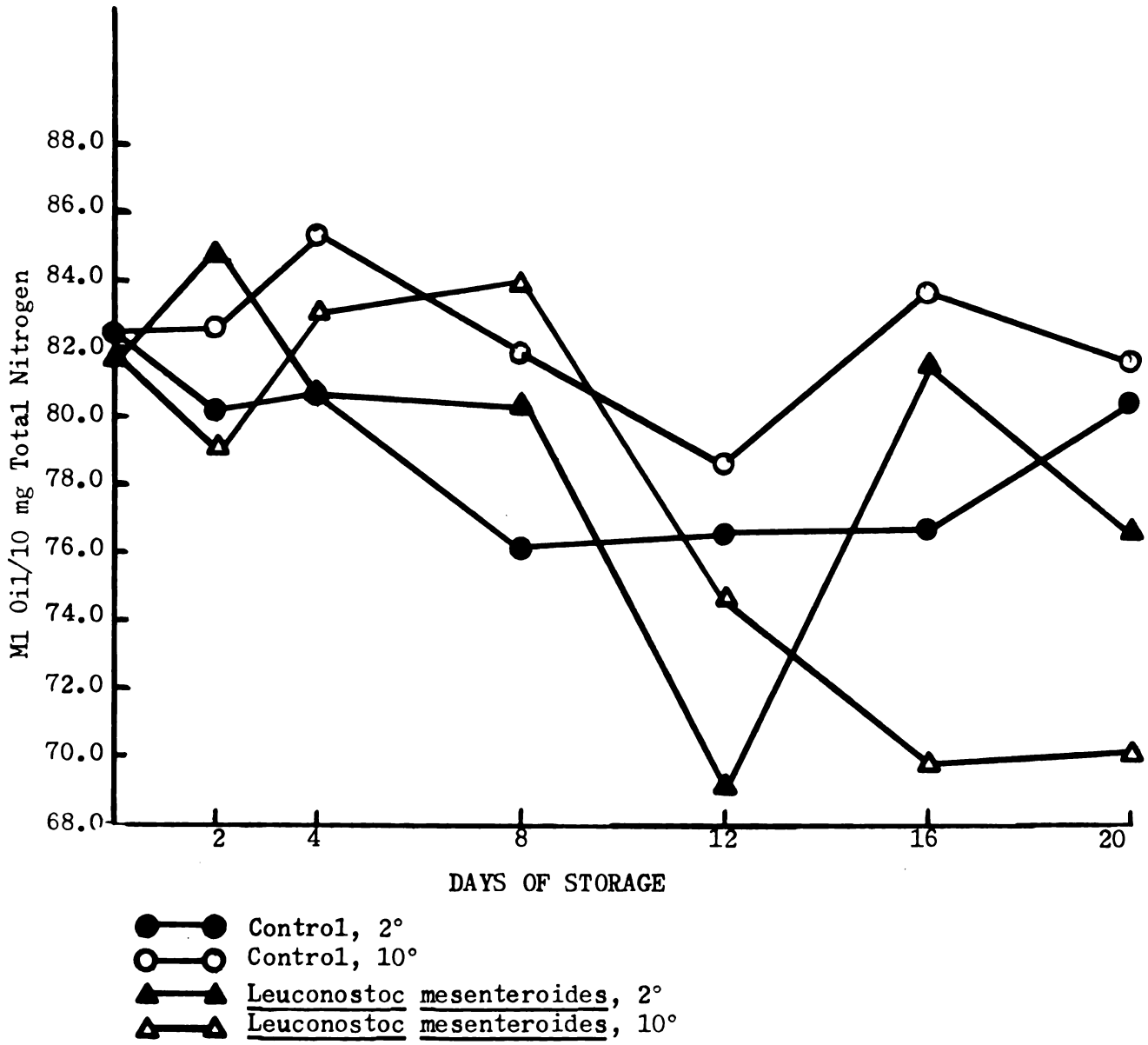


Figure 24. Relationship of the emulsifying capacity of control and Leuconostoc mesenteroides inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

replicate when compared to other values within the replicate (Appendix D). Disregarding the aforementioned mean, it can be seen that the emulsifying capacity of the inoculated samples stored at 10°C exhibited a general decreasing trend from day 8 to days 16 and 20. Analysis of variance revealed that a temperature X treatment interaction was approaching significance ($P = .099$). Also, the differences due to length of storage were approaching significance ($P = .057$) with the emulsifying capacities of the earlier days of storage (days 0-8) being higher than those of the later days of storage (days 12-20). However, the greatest difference shown in figure 23, except for the one mean which was discussed previously, was due to the decrease in emulsifying capacity noted in the inoculated samples stored at 10°C at days 12 to 20. The decrease in emulsifying capacity followed the trends evidenced for lower pH (figure 7), the loss of water-soluble protein nitrogen extractability (figure 10), and the loss of salt-soluble protein nitrogen (figure 15). Thus, it would seem that the emulsifying capacity decreased due to a loss of soluble protein which in turn was caused by a lower pH. The decrease in emulsifying capacity due to inoculation with Leuconostoc mesenteroides was similar to the results reported by Borton et al. (1968a) when using a unspecified culture.

The relationship of the emulsifying capacities of control and Pseudomonas fragi inoculated porcine samples stored at 2° and 10°C for 20 days is shown in figure 25. As mentioned earlier, there was a highly significant difference ($P \leq .01$) between the overall emulsifying capacity mean of the treated samples and that of the control samples which is evident

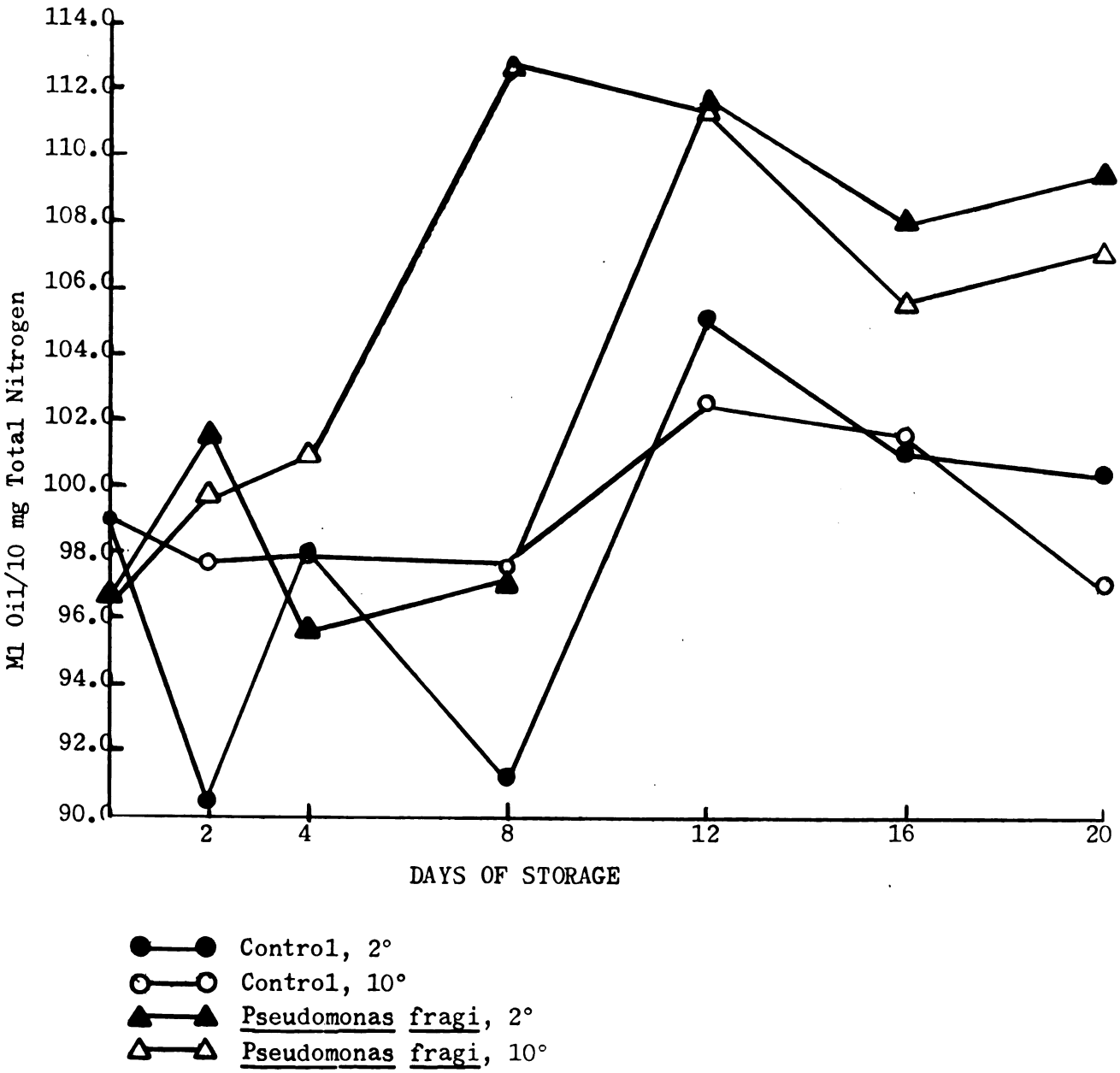


Figure 25. Relationship of the emulsifying capacity of control and Pseudomonas fragi inoculated porcine muscle samples stored at 2° and 10°C for 20 days.

from day 8 through day 20. Also, there was a significant difference due to the length of storage as shown in table 6. The emulsifying capacity of the samples at day 12 was higher ($P \leq .01$) than the emulsifying capacities of the samples at days 8, 4, 0 and 2. The increase in the emulsifying capacities of the inoculated samples was the primary reason for

Table 6. Rank of emulsifying capacity means (ml oil/10 mg total nitrogen) for various storage times which include control and Pseudomonas fragi inoculated samples stored at 2° and 10°C.

Rank	1	2	3	4	5	6	7
Days of storage	12	16	20	8	4	0	2
Mean*	107.7	104.1	103.5	99.7	98.2	97.9	97.4

*Those means not underlined by the same line are significantly different ($P \leq .01$) from each other.

the higher emulsifying capacities at the later storage periods. Also, it can be shown from figure 25 that the greatest emulsifying capacity of the inoculated samples stored at 10° and 2°C was reached at days 8 and 12, respectively. This type of pattern was similar to that shown by the growth curves (figure 5), the pH curves (figure 9), and the salt-soluble protein nitrogen extractability curves (figure 16) of the inoculated samples stored at 2° and 10°C. Thus, it appeared that emulsifying capacity increased due to increasing solubility of the proteins which in turn was influenced by increasing pH. The results of this portion of the emulsifying capacity study were similar to those reported by Ockerman et al. (1969) for three beef samples inoculated with Pseudomonas, Achromobacter, and an unspecified culture.

Emulsion Stability

The results of the emulsion stability studies are shown in table 7. It should be pointed out that the values in the table are averages for different numbers of replicates. The control sample values included 4 replicates, Pediococcus cerevisiae, 2 replicates, Leuconostoc mesenteroides, 1 sample, Micrococcus luteus, 2 replicates, and Pseudomonas fragi, 3 replicates. The samples were only examined for emulsion stability at days 0 and 12 because of the time involved in completing such studies. In general, there was little difference between the emulsion stability of the control samples and those samples inoculated with Pediococcus cerevisiae, Leuconostoc mesenteroides, and Micrococcus luteus. Also, there was little if any difference between the emulsion stability of the above samples stored 12 days and the day 0 samples. There was no difference due to the storage temperature (2° and 10°C) between the emulsion stability of samples inoculated with the same organism when stored 12 days. There was a general trend for each emulsion to separate slightly as it was held for 48 hrs at room temperature, however, the amount of separation was negligible in all cases except for the Pseudomonas fragi inoculated samples. The latter samples evidenced considerable water separation, indicating the emulsions lacked stability. The reason for the separation may have been due to the results of proteolysis by the organisms. That is, if the proteins were hydrolyzed into smaller molecules they were probably not capable of holding as much water. Thus, even though the emulsifying capacity was greater, the stability of the emulsion was not as good as the other samples, thus such a meat item would not be beneficial in a processed meat product.

Table 7. Emulsion stability as measured by oil and water separation of control and inoculated samples at day 0 and after 12 days of storage at 2° and 10°C.

Sample Time, hrs emulsion held	Control		Pediococcus cerevisiae		Leuconostoc mesenteroides		Micrococcus luteus		Pseudomonas fragi	
	Oil*	Water*	Oil*	Water*	Oil*	Water*	Oil*	Water*	Oil*	Water*
					Day 0					
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	T	0	0	0	0	0
.75	0	0	0	0	T	0	0	0	0	0
1	T	0	T	0	1	0	0	0	0	0
2	T	0	T	0	1	0	0	0	T	0
24	T	0	T	0	1	0	T	T	T	0
48	1	T	1	0	2	0	T	T	T	T
					Day 12 - 2°C					
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	0	0	0	0	0	0
.75	0	0	0	0	T	0	0	0	0	T
1	T	0	0	0	T	0	0	0	T	T
2	T	0	T	0	1	0	0	0	T	2
24	T	1	1	0	1	0	T	T	T	4
48	T	1	1	0	1	0	T	T	1	5
					Day 12 - 10°C					
0	0	0	0	0	0	0	0	0	0	0
.25	0	0	0	0	0	0	0	0	0	0
.50	0	0	0	0	0	0	0	0	T	0
.75	T	0	0	0	T	0	0	0	T	T
1	T	0	0	0	T	0	T	0	T	T
2	T	0	T	0	1	0	T	0	T	1
24	1	0	1	0	1	0	T	0	1	4
48	1	0	1	0	1	0	T	0	1	5

*ml of separation - oil at top of graduated cylinder, water at bottom
T = trace amount (definite separation but not 1 ml)

Electrophoretic Study of Salt-Soluble Proteins from Various Species and Muscles within a Species

Starch-urea gel electrophoretograms of 0.6 M KCl extracts of porcine and bovine semimembranosus and porcine, bovine and ovine longissimus dorsi muscles are shown in figure 26. The patterns for the extracts of the bovine and porcine muscles were similar even though the pattern for the bovine semimembranosus muscle (D) was not very distinct. There were fewer bands for the extract of the ovine longissimus dorsi muscle (E). There were about 11 protein bands for the bovine and porcine muscles and 8 for the ovine muscle.

The disc gel electrophoretograms of the same muscles used in figure 26 are shown in figure 27. The patterns were very similar for the extracts of all of the muscles in this case except some of the extracts exhibited a slightly slower rate of migration which resulted in the bands on some gels being closer together than bands on other gels. For example the pattern for the ovine longissimus dorsi muscle extract had the same general pattern as the other extracts except the bands did not migrate as far.

The results of starch-urea gel electrophoresis of 0.6 M KCl extracts of chicken, turkey and coho salmon are presented in figure 28. The chicken breast muscle (F) and turkey breast muscle (I) extracts have very similar patterns as do the chicken and turkey thigh muscle extracts (G and H). There was a difference between the breast and thigh muscle extracts which can be seen by comparing the largest or darkest band of the breast muscle electrophoretograms with the two bands which appear at the same position in the electrophoretograms of the thigh muscles. The two fish extracts

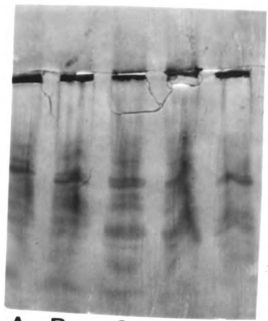


Figure 26. Starch-urea gel electrophoretograms of 0.6 M KCl extracts of porcine, bovine and ovine muscles.

Figure 27. Disc gel electrophoretograms of 0.6 M KCl extracts of porcine, bovine and ovine muscles.

N.B. In figures 26 and 27, A = porcine longissimus dorsi, B = porcine semimembranosus, C = bovine semimembranosus, D = bovine longissimus dorsi, and E = ovine longissimus dorsi.

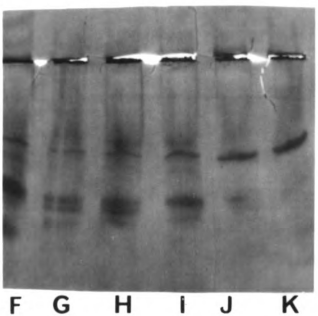


Figure 28. Starch-urea gel electrophoretograms of 0.6 M KCl extracts of chicken, turkey and fish muscle.

Figure 29. Disc gel electrophoretograms of 0.6 M KCl extracts of chicken, turkey and fish muscle.

N.B. In figures 28 and 29 F = chicken breast muscle, G = chicken thigh muscle, H = turkey thigh muscle, I = turkey breast muscle, J = fish muscle-coho grade No. 1, K = fish muscle-coho grade No. 2.

have the fewest bands in their electrophoretic patterns when compared to the others but there was no difference between grades No. 1 and No. 2 coho salmon. Also, the chicken and turkey muscle extracts had fewer bands than those found in the red meat species muscle extracts.

The disc gel electrophoretograms of 0.6 M KCl extracts of chicken, turkey and fish muscles are shown in figure 29. The differences evident in the breast and thigh muscle extracts found with starch-urea gel electrophoresis were not evident after disc gel electrophoresis. More bands were recorded on the disc gels of the fish extracts than on the starch-urea gel electrophoretograms. There also appeared to be more bands for the grade No. 2 coho extracts than for the grade No. 1 extracts. However, though the figure does not show it, the patterns were the same, only the intensity of the stain was different.

The results of this short study indicated that there was little difference between the electrophoretograms from bovine, porcine and ovine muscle extracts but differences were evident between them and poultry and fish muscle electrophoretograms. There were also differences noted between the electrophoretograms of poultry and fish muscle extracts. The results of this study were similar to those reported by Locker and Hagyard (1967).

SUMMARY AND CONCLUSIONS

The procedure used to obtain porcine muscle samples as aseptically as possible did not result in tissue entirely free of microorganisms. However, contamination of the control samples was quite low, being 10,000 organisms per gram or less throughout the 20 day storage period with samples stored at 10°C showing more growth than those stored at 2°C. The storage conditions used in this study did effect some of the studied properties of the control samples. The amount of water-soluble protein decreased significantly ($P \leq .01$) with increasing length of storage. The amount of salt-soluble protein also increased up to 8 days of storage and then decreased slightly or remained relatively constant. The quantity of insoluble protein nitrogen increased during the storage period as did the quantity of non protein nitrogen (NPN). The quantity of NPN found was also higher in the samples stored at 10°C than in those stored at 2°C. Electrophoresis of the water- and salt-soluble extracts of control samples revealed little change in the types of proteins present during the storage period. The pH, emulsifying capacity and emulsion stability of the control samples was not influenced by the storage time or temperature.

The Pediococcus cerevisiae organisms grew when incubated at 10°C for 20 days in porcine muscle tissue but did not grow when incubated at 2°C. The growth of these organisms at 10°C decreased the pH significantly ($P \leq .01$) but did not influence any of the other properties studied.

The Leuconostoc mesenteroides organisms grew when incubated at 2° and 10°C for 20 days in porcine muscle tissue with growth at 2°C being somewhat slower than that at 10°C. Even though growth took place at 2°C, the growth did not seem to influence any of the properties studied. Growth of this organism at 10°C did alter some of the properties of the samples studied. The pH of such samples was lower than any of the other pH values obtained in this study. The lower pH appeared to cause a decreased extractability of the water and salt-soluble proteins and an increase in amount of insoluble protein which caused a decrease in the emulsifying capacity. Also, electrophoresis of the water-extracts of Leuconostoc mesenteroides inoculated samples stored 20 days at 10°C resulted in fewer protein bands than were present in the control sample extracts.

The Micrococcus luteus organisms grew when incubated at 10°C for 20 days in porcine muscle tissue but did not grow when incubated at 2°C. The growth of these organisms at 10°C increased the pH significantly ($P \leq .01$) but did not influence any of the other properties studied.

The Pseudomonas fragi organisms grew when incubated at 2° and 10°C for 20 days in porcine muscle tissue with the amount of growth recorded at any one day for samples stored at 10°C being reached about 4 days later by the samples stored at 2°C. Such a relationship seemed to exist for all of the properties studied. These organisms influenced the properties of the samples more than any of the other organisms used in this study. The pH of samples inoculated with these organisms increased greatly. There was an increase or no change, rather than a decrease as found in the controls,

in the quantity of water-soluble proteins. However, electrophoresis of the water extracts of these samples revealed a loss in the number of protein bands when compared to electrophoretic results of control samples indicating that the type of protein present in the samples had been altered. There was an increase in the amount of salt-soluble protein for the first 4-8 days of storage and then a decrease, especially in the amount extracted from the inoculated samples stored at 10°C. Electrophoresis of the 0.6 M KCl extracts by both starch-urea and disc gel methods indicated a loss of many of the salt-soluble proteins after 20 days of storage. There was a decrease in the amount of insoluble protein and a marked increase in the amount of NPN. The results of the protein solubility and electrophoretic studies indicated that proteolysis of the porcine proteins was accomplished by these organisms. However, due to the increased protein solubility in the earlier periods of storage, emulsifying capacity was increased, then decreased to a relatively constant value but the emulsifying capacity of the inoculated samples remained larger than that of the controls. Even though the emulsifying capacity was greater, the stability of the emulsion formed was markedly lower than those of the controls.

The results of the short study of 0.6 M KCl extracts of muscle from various species (beef, pork, lamb, chicken, turkey and fish) by disc gel and starch-urea gel electrophoresis indicated that differences existed in the number of proteins found.

The results of this study indicated that the number of bacteria present in porcine muscle tissue was involved in altering the properties of the proteins. However, the type of organism present in the samples was as important than the number of organisms.

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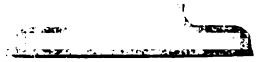
APPENDIX



Appendix A. Composition of solutions used in this study.

I. Protein solubilities

A. 0.03 M phosphate buffer, pH 7.4

0.602 g of KH_2PO_4 and 4.391 g of K_2HPO_4 were dissolved in 1 liter of deionized distilled water.

B. 1.1 M KI, 0.1 M phosphate buffer, pH 7.4

182.6 g of KI, 2.178 g of KH_2PO_4, and 14.631 g of K_2HPO_4 were dissolved in 1 liter of deionized distilled water.

II. Electrophoretic solutions

A. Salt-soluble protein extraction solution

89.5 g of KCl, 8.01 g $KHCO_3$, and 2.76 g of K_2CO_3 were dissolved in 2 liters of deionized distilled water.

B. Starch-urea gel solution, pH 8.6

9.204 g of Tris and 1.052 g of citric acid were dissolved in 1 liter of deionized distilled water.

C. Starch and starch-urea gel tank buffer, pH 8.9

16.0 g of NaOH and 74.2 g of citric acid were dissolved in 2 liters of deionized distilled water.

D. Disc gel solutions

1. Running gel - made by mixing 6.4 ml of solution 1, 1.6 ml of solution 2, and 2.67 ml of solution 3 for 8 tubes.

a. Solution 1

5 ml of 2 N HCl, 7.62 g of Tris, 0.10 ml TMED. 81.25 ml of 10 M urea were mixed and then diluted to 100 ml with deionized distilled water.

b. Solution 2

43.3 g of cyanogum were dissolved in 25 ml of 10 M urea and then diluted to 100 ml with deionized distilled water.

Appendix A. Composition of solutions used in this study (continued)

c. Solution 3

1 mg of riboflavin was dissolved in 35 ml 10 M urea and then diluted to 50 ml with deionized distilled water.

2. Spacer gel - made by mixing 1.6 ml of solution 1, 0.4 ml solution 2, and 0.67 ml of solution 3 for 8 tubes.

a. Solution 1

5 ml of 2 N HCl, 1.25 g of Tris, 0.075 ml TMED and 81.25 ml of 10 M urea were mixed and then diluted to 100 ml with deionized distilled water.

b. Solution 2

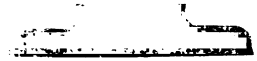
33.3 g cyanogum were dissolved in 25 ml of 10 M urea and then diluted to 100 ml with deionized distilled water.

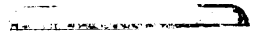
c. Solution 3

This solution was identical to solution 3 used in the running gel.

3. Tank buffer

6.0 g Tris and 28.8 g of glycine were dissolved in 1 liter of distilled deionized water. 100 ml of this buffer was diluted to 1 liter with distilled deionized water to provide the buffer used for each electrophoretic run.





Appendix B. Log of bacteria nos/g of sample for control and inoculated samples.

	Control								Pediococcus cerevisiae							
Temp. °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	0.00	0.00	0.00	0.00					5.38	5.34	5.18	5.34				
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.38	5.26	5.30	5.36	5.56	5.36	5.18	5.58
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.52	5.42	5.34	5.30	5.98	5.87	6.51	6.28
8	2.48	0.00	0.00	0.00	0.00	0.00	4.34	0.00	5.32	5.18	5.32	5.53	7.78	7.04	7.97	7.75
12	0.00	4.36	0.00	0.00	4.30	2.42	0.00	0.00	5.34	5.30	5.38	5.26	7.15	7.83	8.11	8.00
16	0.00	0.00	0.00	0.00	4.11	2.00	6.48	0.00	5.48	5.30	5.30	5.51	8.30	7.92	8.11	8.11
20	2.48	0.00	0.00	0.00	4.18	4.00	4.04	0.00	5.30	5.15	5.18	5.26	7.15	7.88	8.00	7.89
	Control								Leuconostoc mesenteroides							
0	0.00	0.00	0.00	0.00					4.20	4.28	4.73	4.36				
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.18	4.36	5.04	4.51	4.95	4.70	5.90	5.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.43	4.48	5.95	4.56	6.30	4.70	7.49	6.53
8	2.48	0.00	0.00	0.00	0.00	0.00	4.60	0.00	5.08	4.78	5.75	4.70	7.91	7.26	7.85	7.68
12	0.00	0.00	0.00	0.00	4.30	0.00	0.00	0.00	5.83	5.11	5.78	5.78	7.83	7.89	7.73	7.92
16	0.00	0.00	0.00	0.00	4.11	5.10	5.74	0.00	6.54	5.79	6.78	6.20	8.04	7.58	6.83	8.04
20	2.48	0.00	1.00	0.00	4.18	0.00	5.81	0.00	7.32	6.20	7.58	6.71	7.68	7.34	7.77	8.08

Appendix B. Log of bacteria nos/g of sample for control and inoculated samples (continued).

	Control								Micrococcus luteus							
Temp °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	0.00	0.00	5.34	0.00					4.76	4.70	4.69	4.59				
2	0.00	0.00	5.30	0.00	0.00	0.00	5.28	0.00	4.71	4.76	4.81	5.64	4.93	4.90	5.00	4.89
4	0.00	0.00	3.85	0.00	0.00	0.00	5.00	0.00	4.53	4.72	4.81	4.72	6.40	4.78	6.61	5.89
8	0.00	0.00	5.30	0.00	0.00	0.00	5.68	0.00	4.69	4.82	4.83	4.59	8.28	5.08	9.04	8.60
12	4.36	0.00	5.00	0.00	2.42	0.00	6.00	3.30	4.66	4.63	5.30	4.62	9.56	5.51	9.04	8.72
16	0.00	0.00	4.78	0.00	2.00	5.10	7.23	0.00	4.60	4.36	4.70	4.56	9.43	7.28	8.96	8.73
20	0.00	0.00	5.80	0.00	4.00	0.00	7.60	0.00	4.51	5.00	4.48	4.32	9.11	7.20	8.56	8.72

	Control								Pseudomonas fragi							
0	0.00	5.34	0.00	0.00					3.28	4.59	3.34	3.28				
2	0.00	5.30	0.00	0.00	0.00	5.28	0.00	0.00	4.48	4.49	3.60	3.23	5.93	6.54	6.86	4.38
4	0.00	3.85	0.00	0.00	0.00	5.00	0.00	0.00	6.30	5.71	7.68	3.15	8.95	8.48	9.48	6.48
8	0.00	5.30	0.00	0.00	4.60	5.68	4.34	0.00	8.75	9.23	8.51	6.48	9.98	10.15	10.08	9.11
12	0.00	5.00	0.00	0.00	0.00	6.00	0.00	3.30	9.68	9.52	10.08	9.71	9.62	10.20	10.08	9.73
16	0.00	4.78	0.00	0.00	5.74	7.23	6.48	0.00	10.20	9.88	10.15	9.32	8.70	9.75	8.48	10.00
20	1.00	5.80	0.00	0.00	5.81	7.60	4.04	0.00	10.34	10.15	9.84	9.64	9.04	9.66	7.58	8.48

Appendix C. pH of control and inoculated samples.

	Control								Pediococcus cerevisiae							
Temp °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	5.52	5.18	5.35	5.20					5.54	5.16	5.37	5.26				
2	5.70	5.20	5.32	5.22	5.79	5.24	5.35	5.26	5.72	5.22	5.35	5.27	5.82	5.26	5.38	5.30
4	5.56	5.24	5.26	5.21	5.68	5.21	5.29	5.23	5.64	5.16	5.29	5.25	5.69	5.20	5.36	5.25
8	5.66	5.17	5.34	5.23	5.60	5.16	5.36	5.25	5.76	5.20	5.36	5.28	5.68	5.22	5.24	5.21
12	5.68	5.33	5.39	5.23	5.82	5.38	5.43	5.25	5.62	5.35	5.39	5.26	5.65	5.16	5.13	5.00
16	5.74	5.49	5.28	5.24	5.92	5.51	5.36	5.22	5.72	5.28	5.29	5.30	5.76	5.17	4.91	4.91
20	5.87	5.23	5.25	5.28	6.45	5.14	5.39	5.28	4.76	5.26	5.31	5.25	5.73	5.00	4.90	4.88

	Control								Leuconostoc mesenteroides							
0	5.52	5.63	5.25	5.20					5.55	5.46	5.30	5.22				
2	5.70	5.49	5.35	5.22	5.79	5.60	5.35	5.26	5.66	5.51	5.33	5.26	5.73	5.53	5.34	5.27
4	5.56	5.45	5.29	5.21	5.68	5.45	5.31	5.23	5.67	5.43	5.29	5.23	5.66	5.45	5.25	5.25
8	5.66	5.41	5.26	5.23	5.60	5.41	5.25	5.25	5.59	5.41	5.27	5.27	5.55	5.12	4.95	4.92
12	5.68	5.42	5.29	5.23	5.82	5.48	5.34	5.25	5.72	5.44	5.33	5.24	5.59	4.91	4.87	4.78
16	5.74	5.43	5.24	5.24	5.92	5.63	5.99	5.22	5.81	5.46	5.24	5.26	5.61	4.94	4.73	4.76
20	5.87	5.40	5.29	5.28	6.45	5.49	5.83	5.28	5.81	5.40	5.17	5.23	5.73	4.87	4.79	4.77

Appendix C. pH of control and inoculated samples (continued)

	Control								Micrococcus luteus							
Temp °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	5.18	5.63	5.27	5.33					5.20	5.50	5.30	5.31				
2	5.20	5.49	5.40	5.31	5.24	5.60	5.42	5.32	5.28	5.53	5.39	5.28	5.33	5.53	5.41	5.28
4	5.24	5.45	5.33	5.30	5.21	5.45	5.32	5.30	5.21	5.43	5.40	5.25	5.23	5.46	5.38	5.28
8	5.17	5.41	5.29	5.32	5.16	5.41	5.37	5.30	5.24	5.42	5.39	5.30	5.44	5.47	5.70	5.51
12	5.33	5.42	5.31	5.33	5.38	5.48	5.39	5.51	5.34	5.45	5.41	5.30	6.16	5.46	5.69	5.73
16	5.49	5.43	5.26	5.45	5.51	5.63	5.72	5.31	5.32	5.46	5.37	5.28	6.24	5.63	5.43	5.67
20	5.23	5.40	5.30	5.23	5.14	5.49	5.67	5.25	5.32	5.54	5.31	5.21	6.39	6.98	5.29	5.43
	Control								Pseudomonas fragi							
0	5.25	5.27	5.35	5.33					5.24	5.32	5.36	5.33				
2	5.35	5.40	5.32	5.31	5.35	5.42	5.35	5.32	5.32	5.37	5.34	5.31	5.31	5.43	5.37	5.28
4	5.29	5.33	5.26	5.30	5.31	5.32	5.29	5.30	5.30	5.35	5.30	5.30	6.10	5.66	5.85	5.35
8	5.26	5.29	5.34	5.32	5.25	5.37	5.36	5.30	5.43	5.68	6.24	5.38	7.12	6.74	7.30	6.19
12	5.29	5.31	5.39	5.33	5.34	5.39	5.43	5.51	7.20	6.64	7.36	6.38	8.26	7.34	8.07	7.03
16	5.24	5.26	5.28	5.45	5.99	5.72	5.36	5.31	7.91	7.28	8.13	7.30	8.30	7.80	8.23	8.00
20	5.29	5.30	5.25	5.23	5.83	5.67	5.39	5.25	7.68	7.48	8.10	7.23	8.12	7.50	8.20	7.73

Appendix D. Emulsifying capacity (ml oil/10 mg total nitrogen) for control and inoculated samples.

	Control								Pediococcus cerevisiae							
Temp °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	96.5	83.3	88.2	68.8					99.3	82.9	87.6	73.6				
2	98.8	94.8	82.0	73.8	92.0	101.1	90.0	69.9	107.3	91.9	91.3	68.5	94.3	95.1	88.6	72.0
4	81.4	97.9	81.0	70.6	105.4	98.6	90.7	73.9	109.3	99.6	79.6	70.4	99.3	99.4	89.0	71.6
8	83.4	100.4	85.7	74.9	94.6	96.2	83.4	71.2	107.1	88.2	84.1	70.9	97.1	95.1	82.3	87.9
12	79.5	112.8	123.1	72.4	78.3	94.8	115.8	74.9	81.5	105.6	115.9	72.3	76.1	79.9	114.0	69.4
16	67.9	105.0	92.0	70.8	94.9	91.6	88.8	72.8	84.9	100.9	102.6	70.7	81.1	83.2	87.6	74.3
20	95.6	104.8	90.6	68.9	90.3	104.9	91.1	73.1	80.5	100.9	97.7	67.1	87.4	71.3	72.4	66.1

	Control								Leuconostoc mesenteroides							
0	96.5	71.4	97.3	68.8					83.2	70.4	100.3	73.1				
2	98.8	73.8	74.0	73.8	92.0	75.3	97.4	69.9	97.9	73.8	94.9	72.6	80.4	71.3	96.9	68.1
4	81.4	67.6	103.1	70.6	105.4	64.3	97.8	73.9	86.6	72.9	96.1	67.4	93.4	66.0	101.3	71.8
8	83.4	65.2	81.0	74.9	94.6	66.3	95.3	71.2	83.8	66.9	98.9	72.4	105.7	67.3	88.8	73.9
12	79.5	63.0	90.0	72.4	78.3	67.1	94.1	74.9	54.1	63.8	89.6	69.1	104.3	60.8	63.5	69.9
16	67.9	64.3	103.9	70.8	94.9	63.6	102.9	72.8	97.8	60.3	99.1	68.9	86.2	56.5	68.9	67.0
20	95.5	60.1	97.2	68.9	90.3	65.1	98.1	73.1	77.4	63.6	97.4	68.4	89.2	60.6	65.1	65.0

Appendix D. Emulsifying capacity (ml oil/10 mg total nitrogen) for control and inoculated samples (continued).

	Control								Micrococcus luteus							
Temp °C	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	83.3	71.4	124.6	86.1					82.8	73.1	116.9	93.4				
2	94.8	73.8	115.9	90.1	101.1	75.3	114.0	89.1	94.3	73.8	112.5	92.3	91.5	73.2	107.8	93.6
4	97.9	67.6	116.2	92.1	98.6	64.3	113.1	89.8	95.8	68.2	122.0	97.1	92.3	71.5	116.7	93.1
8	100.4	65.2	114.3	84.2	96.2	66.3	119.8	91.9	96.1	67.3	111.3	91.3	95.2	65.0	121.3	90.3
12	112.8	63.0	117.4	89.1	94.8	67.1	114.4	85.9	108.3	61.9	112.6	88.6	77.8	72.8	130.0	88.9
16	105.0	64.3	115.9	92.4	91.6	63.6	125.0	89.5	100.9	55.9	119.1	93.4	102.2	69.9	117.3	90.4
20	104.8	60.1	128.1	85.6	104.8	65.1	116.3	82.6	103.8	63.8	121.8	85.5	107.5	71.8	118.5	86.7

	Control								Pseudomonas fragi							
0	97.3	124.6	88.2	86.1					98.6	114.5	83.6	90.1				
2	74.0	115.9	82.0	90.1	97.4	114.0	90.0	89.1	105.3	114.8	92.0	94.4	99.9	113.1	94.4	91.9
4	103.1	116.2	81.0	92.1	97.8	113.1	90.7	89.8	99.6	113.6	78.7	90.9	104.1	117.8	90.5	91.8
8	81.0	114.3	85.7	84.2	95.3	119.8	83.4	91.9	88.7	114.9	94.1	90.7	103.8	128.8	120.8	97.1
12	90.9	117.4	123.1	89.1	94.1	114.4	115.8	85.9	106.8	126.2	119.8	93.8	106.0	124.1	116.2	99.3
16	103.9	115.9	92.0	92.4	102.9	125.0	88.8	89.5	115.5	120.9	100.3	95.4	102.9	114.1	91.8	113.8
20	97.2	128.1	90.6	85.6	98.1	116.3	91.1	82.6	110.9	125.0	94.7	107.5	103.5	118.2	96.3	110.8

Appendix E. % protein for control and inoculated samples.

Temp °C	Control								Pediococcus cereviseae							
	2°				10°				2°				10°			
Day/ Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	20.89	19.67	18.56	18.31					20.47	20.80	18.55	18.36				
2	21.40	20.45	20.74	18.12	21.49	20.03	19.59	18.07	21.86	20.55	19.45	19.16	21.72	21.16	19.04	18.23
4	20.84	20.56	19.59	18.61	20.94	20.66	19.30	18.11	21.28	20.33	19.01	18.82	20.52	20.74	19.20	18.34
8	21.56	20.18	20.41	18.37	21.55	20.28	20.08	18.61	21.12	20.69	19.32	18.17	21.14	20.64	19.90	18.21
12	21.26	17.73	19.20	17.62	21.26	19.78	20.18	17.85	21.64	18.36	19.95	17.82	21.35	18.76	18.86	18.02
16	21.36	19.05	20.66	18.01	21.64	19.21	20.14	18.38	21.33	18.82	18.28	18.04	21.76	18.03	20.40	18.34
20	21.36	19.33	19.37	18.50	21.60	19.69	19.77	18.66	22.16	20.20	19.84	18.62	20.93	18.95	19.85	18.91

	Control								Leuconostoc mesenteroides							
0	20.89	18.02	20.55	18.31					20.75	18.11	19.70	17.78				
2	21.40	18.31	20.44	18.12	21.49	18.26	20.80	18.07	20.10	18.64	20.81	18.06	21.55	18.41	20.89	19.28
4	20.84	18.86	19.53	18.61	20.94	19.07	21.36	18.11	21.43	18.16	20.69	19.28	21.60	19.32	20.24	18.10
8	21.56	18.80	19.76	18.37	21.55	19.05	21.24	18.61	21.73	18.86	20.47	18.30	21.34	18.58	20.84	17.94
12	21.26	18.85	21.05	17.62	21.26	18.43	21.25	17.85	21.72	18.43	20.24	18.27	21.44	19.32	21.46	18.07
16	21.36	18.86	19.85	18.01	21.64	20.64	20.66	18.38	22.09	18.46	20.55	18.50	21.35	19.46	20.32	17.92
20	21.36	18.11	21.10	18.50	21.60	19.58	21.65	18.66	20.83	18.89	21.16	18.64	21.35	19.59	21.51	18.33

Appendix E. % protein for control and inoculated samples (continued).

Temp °C / Day/ Rep	Control								Micrococcus luteus							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	19.67	18.02	17.66	19.31					21.28	17.97	18.40	19.40				
2	20.45	18.31	18.22	19.85	20.03	18.26	18.42	19.93	21.22	18.82	18.44	19.90	19.81	18.11	19.26	19.50
4	20.56	18.86	17.97	19.68	20.66	19.07	18.35	20.18	19.98	18.52	17.21	18.42	21.67	18.54	18.64	19.88
8	20.18	18.80	18.16	19.89	20.28	19.05	17.95	19.46	20.56	19.34	18.76	19.72	21.28	19.42	18.04	20.23
12	17.73	18.85	18.21	19.77	19.78	18.43	18.68	19.93	18.59	19.39	18.43	20.18	19.30	18.56	16.44	19.96
16	19.05	18.86	18.12	20.15	19.21	20.64	18.40	19.69	19.74	19.01	17.73	19.42	19.45	19.68	18.22	20.18
20	19.33	18.11	16.50	19.56	19.69	19.58	17.45	19.98	19.39	19.59	17.35	19.59	18.72	20.01	17.51	19.47
	Control								Pseudomonas fragi							
0	20.55	17.66	18.56	19.31					20.55	18.34	19.00	19.71				
2	20.44	18.22	20.74	19.85	20.80	18.42	19.59	19.93	20.27	18.40	19.09	20.00	20.71	19.01	18.93	19.18
4	19.53	17.97	19.59	19.68	21.36	18.35	19.30	20.18	20.09	18.15	19.07	19.95	20.41	18.34	19.21	19.75
8	19.76	18.16	20.41	19.89	21.24	17.95	20.08	19.46	21.71	18.60	17.80	19.85	21.44	18.25	19.56	19.95
12	21.05	18.21	19.20	19.77	21.25	18.68	20.18	19.93	21.07	18.92	20.45	20.41	20.76	18.44	19.47	19.77
16	19.85	18.12	20.66	20.15	20.66	18.40	20.14	19.69	20.02	18.20	18.69	19.79	20.53	18.74	20.15	20.21
20	21.10	16.50	19.37	19.56	21.65	17.45	19.77	19.98	20.73	16.90	20.60	19.77	21.26	18.51	18.83	19.41

Appendix F. Mg of water-soluble protein nitrogen per 100 mg total nitrogen for control and inoculated samples.

Temp °C / Day / Rep	Control								Pediococcus cerevisiae							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	20.21	26.72	26.11	22.15					24.26	26.22	23.04	21.13				
2	20.26	23.86	23.95	18.81	23.02	24.14	22.70	20.60	21.54	24.41	25.81	16.67	22.03	22.02	24.05	20.64
4	21.94	22.85	22.05	19.07	24.29	24.09	22.92	17.06	23.61	25.42	19.56	19.36	24.85	23.57	20.86	19.55
8	21.64	18.77	19.29	17.59	21.90	19.96	20.53	17.54	24.17	21.11	21.08	18.07	22.60	19.49	19.60	19.25
12	19.98	20.71	19.47	18.99	22.43	19.37	20.32	18.78	20.25	21.63	16.90	16.72	19.71	21.09	20.55	17.38
16	20.08	19.17	17.54	18.24	20.65	19.16	20.11	16.13	19.29	19.37	20.12	16.77	21.78	20.53	13.22	15.83
20	21.93	19.11	20.76	15.22	21.43	17.06	21.10	16.20	20.24	19.48	18.93	15.12	19.95	19.32	17.85	14.99

	Control								Leuconostoc mesenteroides							
0	20.21	20.28	19.62	22.15					24.87	20.80	22.85	21.65				
2	20.26	18.51	20.76	18.81	23.02	15.50	21.53	20.60	18.26	15.29	19.88	21.05	23.17	18.09	19.78	20.00
4	21.94	19.01	22.11	19.07	24.29	17.38	22.18	17.06	20.78	18.36	20.70	16.19	23.21	18.01	21.24	20.29
8	21.64	15.02	19.11	17.59	21.90	14.52	18.84	17.54	21.35	14.13	20.09	18.38	23.44	15.73	14.88	17.36
12	19.98	14.81	16.49	18.99	22.43	15.12	17.97	18.78	21.25	13.93	18.69	18.48	18.48	12.63	12.29	14.95
16	20.08	15.31	18.82	18.24	20.65	14.69	17.66	16.13	20.67	16.41	19.09	18.05	18.19	14.45	12.31	13.76
20	21.93	14.25	15.05	15.22	21.43	13.68	18.04	16.20	22.01	13.14	14.36	16.24	19.13	12.47	14.20	13.53

Appendix F. Mg of water-soluble protein nitrogen per 100 mg total nitrogen for control and inoculated samples. (continued)

Temp °C Day/ Rep	Control								Micrococcus luteus							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	26.72	20.28	21.50	22.90					24.67	20.37	20.23	22.94				
2	23.86	18.51	21.26	21.19	24.14	15.50	20.83	22.44	21.46	17.06	20.92	21.49	26.69	18.00	17.68	23.79
4	22.85	19.01	21.08	19.72	24.09	17.38	20.09	20.89	25.51	17.54	22.87	20.70	23.45	16.13	20.20	20.46
8	18.77	15.02	19.89	18.91	19.96	14.52	20.15	20.02	18.18	14.91	18.27	19.35	18.35	15.47	20.89	17.89
12	20.71	14.81	16.11	17.87	19.37	15.12	20.02	20.55	21.05	13.88	17.38	19.81	21.47	15.74	20.39	20.72
16	19.17	15.31	19.13	17.63	19.16	14.69	19.72	18.73	19.27	13.16	17.88	19.16	21.73	14.64	18.90	19.82
20	19.11	14.25	21.31	19.13	17.06	13.68	21.49	17.86	19.46	12.99	19.30	19.60	20.87	13.39	21.62	18.52
	Control								Pseudomonas fragi							
0	19.62	21.50	26.11	22.90					24.10	20.22	25.40	22.16				
2	20.76	21.26	23.95	21.19	21.53	20.83	22.70	22.44	22.66	20.24	24.20	21.39	23.78	20.14	25.24	23.87
4	22.11	21.08	22.05	19.72	22.18	20.09	22.92	20.89	20.38	18.75	21.78	21.67	21.55	19.07	21.76	21.06
8	19.11	19.89	19.29	18.91	18.84	20.15	20.53	20.02	16.90	18.41	22.08	19.59	20.19	21.36	23.53	20.31
12	16.49	16.11	19.47	17.87	17.97	20.02	20.32	20.55	19.10	19.86	21.65	17.40	21.57	20.89	26.23	23.08
16	18.82	19.13	17.54	17.63	17.66	19.72	20.11	18.73	21.16	20.25	28.75	22.10	26.26	23.15	25.10	20.33
20	15.05	21.31	20.76	19.13	18.04	21.49	21.10	17.86	19.42	26.07	23.00	22.63	22.23	28.96	25.02	22.03

Appendix G. Mg of salt-soluble protein nitrogen per 100 mg of total nitrogen for control and inoculated samples.

Temp °C / Day/ Rep	Control								Pediococcus cerevisiae							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	35.38	44.36	41.81	29.24					42.81	40.02	42.32	28.24				
2	39.88	42.72	36.05	29.62	38.75	38.71	48.16	30.09	42.34	45.85	41.77	26.61	40.11	40.15	46.22	35.97
4	42.29	39.23	42.90	27.71	49.19	36.87	49.47	30.99	43.90	36.83	42.51	26.67	49.89	36.81	48.09	29.32
8	47.44	34.10	42.25	30.39	47.93	34.97	48.74	30.95	45.36	37.08	45.78	31.57	48.19	32.18	45.52	38.86
12	44.72	36.55	49.11	28.94	47.10	29.72	46.70	33.99	44.76	39.57	44.75	29.56	42.64	26.82	49.40	36.08
16	42.68	35.41	41.73	30.66	44.69	30.84	45.82	34.46	45.98	38.89	47.45	28.08	36.49	32.63	41.28	33.13
20	40.36	26.74	44.12	30.10	45.25	28.60	42.60	35.23	41.62	27.64	42.76	33.68	30.94	25.38	41.71	31.97

	Control								Leuconostoc mesenteroides							
0	35.38	24.48	35.03	29.24					49.26	22.29	36.19	29.79				
2	39.88	22.75	30.92	27.62	38.75	22.03	34.98	30.09	42.79	23.86	31.96	27.53	46.77	22.38	33.06	30.56
4	42.29	20.35	35.57	27.71	49.18	23.17	32.45	30.99	46.25	20.41	33.29	23.50	48.74	21.83	31.58	29.37
8	47.44	24.32	39.45	30.39	47.93	23.11	44.58	30.95	50.00	23.71	40.43	29.98	52.04	22.06	46.65	38.44
12	44.72	24.29	33.17	28.94	47.10	23.43	38.02	33.99	45.43	26.64	30.98	27.01	48.35	20.52	33.66	35.56
16	42.68	23.72	39.50	30.66	44.69	32.63	40.78	34.46	49.73	23.44	33.07	28.20	45.23	29.93	34.96	31.06
20	40.36	24.96	43.10	30.10	45.25	21.94	40.72	35.23	53.17	24.00	44.30	30.56	31.52	20.92	27.22	31.37

Appendix G. Mg of salt-soluble protein nitrogen per 100 mg of total nitrogen for control and inoculated samples. (continued)

Temp °C / Day/Rep	Control								Micrococcus luteus							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	44.36	24.48	38.51	42.10					42.99	23.99	39.80	38.84				
2	42.72	22.75	41.49	38.53	38.71	22.03	49.14	40.74	38.46	24.14	41.71	42.53	37.69	22.86	43.95	43.97
4	39.23	20.35	41.42	43.55	36.87	23.17	49.03	47.77	38.14	22.25	43.44	44.63	37.00	25.09	44.97	45.95
8	34.10	24.32	46.12	37.31	34.97	23.11	53.22	47.42	36.45	25.55	49.12	44.59	32.31	21.21	51.63	47.12
12	36.55	24.29	45.97	43.83	29.72	23.43	49.86	43.17	35.52	22.96	45.10	42.52	31.98	20.89	57.25	45.01
16	35.41	23.72	48.01	43.96	30.84	32.63	46.67	41.15	35.11	23.26	45.87	42.88	33.57	30.59	47.96	44.14
20	26.74	24.96	50.26	46.20	28.60	21.94	46.82	47.05	26.76	23.07	52.47	48.35	31.60	23.25	50.06	43.50
	Control								Pseudomonas fragi							
0	35.03	38.51	41.81	42.10					34.30	37.05	42.29	38.31				
2	30.92	41.49	36.05	38.53	34.98	49.14	48.16	40.74	33.17	47.55	44.14	41.32	38.43	49.79	48.78	43.97
4	35.57	41.42	42.90	43.55	32.45	49.03	49.47	47.77	36.69	42.64	43.26	38.70	47.93	52.63	51.24	45.95
8	39.45	46.12	42.25	37.31	44.58	53.22	48.74	47.42	42.19	55.74	52.53	47.18	46.71	45.04	42.93	47.12
12	33.17	45.97	49.11	43.83	38.02	49.86	46.70	43.17	47.33	45.24	43.76	53.39	46.07	46.15	34.77	45.01
16	39.50	48.01	41.73	43.96	40.78	46.67	45.82	41.15	50.37	44.81	28.33	50.19	37.79	37.31	30.60	44.14
20	43.10	50.26	44.72	46.20	40.72	46.82	42.60	47.05	49.29	45.63	35.21	49.25	38.05	18.90	33.88	43.50

Appendix H. Mg of insoluble protein nitrogen per 100 mg of total nitrogen for control and inoculated samples.

Temp °C	Control								Pediococcus cerevisiae							
	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	28.06	16.63	21.00	37.37					19.40	21.90	22.84	38.47				
2	27.36	20.72	29.86	42.24	26.98	25.59	16.27	37.75	25.12	17.46	22.60	45.73	25.89	26.74	18.25	31.97
4	22.77	25.98	24.19	41.68	14.26	29.14	15.86	39.77	21.71	26.86	26.66	42.17	13.09	27.49	19.06	39.61
8	17.92	33.40	27.63	39.87	17.34	33.29	17.77	38.83	17.75	29.86	21.58	37.75	16.21	36.20	22.43	28.76
12	23.01	28.72	17.39	38.73	17.54	37.45	19.84	33.53	22.85	25.94	26.12	40.34	23.95	39.53	16.41	32.83
16	25.25	34.51	28.79	37.11	21.48	38.17	21.38	34.59	21.70	29.12	19.44	41.68	30.06	31.25	32.96	37.15
20	25.56	41.97	21.77	41.63	18.41	38.21	23.06	34.92	25.57	40.77	26.08	38.60	34.42	42.06	26.39	38.09
	Control								Leuconostoc mesenteroides							
0	28.06	42.03	33.48	37.37					14.53	44.76	29.41	34.86				
2	27.37	45.98	37.27	42.24	26.99	50.32	31.90	37.75	25.65	48.44	36.53	39.78	18.11	46.87	35.52	38.08
4	22.78	48.92	30.63	41.68	14.27	46.57	35.37	39.77	21.08	48.11	35.37	48.61	15.11	48.83	35.74	37.58
8	17.93	48.81	29.71	39.87	17.35	49.51	25.75	38.83	15.29	49.77	28.78	38.95	11.03	50.32	26.78	29.76
12	23.03	48.05	39.26	38.73	17.55	47.24	31.95	33.53	21.63	46.15	38.42	41.73	19.09	53.07	41.82	35.57
16	25.26	49.62	29.60	37.11	21.49	40.48	27.62	34.59	17.91	49.32	36.17	39.93	22.67	42.85	38.93	40.12
20	25.57	46.88	28.93	41.63	18.42	50.71	28.72	34.92	12.15	50.01	28.13	39.90	34.89	52.80	45.49	39.33

Appendix H. Mg of insoluble protein nitrogen per 100 mg of total nitrogen for control and inoculated samples (continued).

Temp °C / Day/Rep	Control								Micrococcus luteus							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	16.63	42.03	28.47	22.19					20.82	43.17	28.08	24.52				
2	20.72	45.98	26.03	26.75	25.59	50.32	18.56	24.86	27.91	47.15	26.16	23.57	23.77	46.65	25.59	15.20
4	25.98	48.92	26.07	24.44	29.14	46.57	18.83	18.81	23.84	46.93	21.60	20.75	28.50	45.58	22.83	16.55
8	33.40	48.81	21.25	32.01	33.29	49.51	12.76	18.93	33.14	47.74	20.04	22.36	37.28	51.49	13.87	20.29
12	28.72	48.05	25.22	24.15	37.45	47.24	16.64	22.29	30.86	50.96	24.72	24.47	33.98	50.88	8.08	17.71
16	34.51	49.62	19.21	24.15	38.17	40.48	19.04	25.59	34.55	52.72	22.31	24.99	33.30	42.35	18.77	17.53
20	41.97	46.88	12.70	19.96	38.21	50.71	16.51	19.54	42.44	51.52	14.46	16.62	34.65	51.90	14.60	16.56
	Control								Pseudomonas fragi							
0	33.48	28.47	21.00	22.19					30.69	30.48	21.30	27.57				
2	37.27	26.03	29.86	26.75	31.90	18.56	16.27	24.86	32.34	20.01	19.83	24.94	26.70	18.49	14.55	19.67
4	30.63	26.07	24.19	24.44	35.37	18.83	15.86	18.81	31.07	26.49	23.78	28.22	18.79	16.11	16.13	21.73
8	29.71	21.25	27.63	32.01	25.75	12.76	17.77	18.93	30.99	14.35	14.36	20.65	19.64	19.47	16.83	19.46
12	39.26	25.22	17.39	24.15	31.95	16.64	19.84	22.29	22.07	23.34	19.61	17.35	11.49	15.39	15.60	15.56
16	29.60	19.21	28.79	24.15	27.62	19.04	21.38	25.59	11.10	17.94	20.97	15.53	9.72	18.74	19.91	16.82
20	28.93	12.70	21.77	19.96	28.72	16.51	23.06	19.54	20.84	8.59	20.95	14.09	17.27	25.43	13.02	13.06

Appendix I. Mg of nonprotein nitrogen (NPN) per 100 mg of total nitrogen for control and inoculated samples.

Temp °C	Control								Pediococcus cerevisiae							
	2°				10°				2°				10°			
Day/ Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	16.36	12.30	11.10	11.26					13.53	11.86	11.80	12.16				
2	12.50	12.71	10.15	11.34	11.25	11.56	12.87	11.58	11.00	12.29	9.82	11.00	11.97	11.09	11.47	11.43
4	13.00	11.96	10.86	11.56	12.27	9.92	11.76	12.19	10.78	10.90	11.28	11.80	12.17	12.14	12.00	11.53
8	13.00	13.73	10.84	12.15	12.83	11.79	12.97	12.69	12.72	11.96	11.58	12.62	13.00	12.14	12.46	13.14
12	12.29	14.03	14.05	13.34	12.93	13.47	13.17	13.71	12.14	12.86	12.24	13.38	13.70	12.56	13.65	13.72
16	11.99	10.93	11.94	14.00	13.18	11.84	12.70	14.83	13.03	12.64	13.00	13.48	11.67	15.60	12.55	13.90
20	12.15	12.19	13.35	13.07	14.91	16.14	13.26	13.66	12.57	12.12	12.24	12.61	14.69	13.26	14.05	14.97

	Control								Leuconostoc mesenteroides							
0	16.36	13.21	11.87	11.26					13.35	12.18	11.56	13.70				
2	12.50	12.78	11.05	11.34	11.25	12.17	11.60	11.58	13.32	12.42	11.65	11.66	11.96	12.67	11.64	11.36
4	13.00	11.72	11.71	11.56	12.27	12.90	10.01	12.19	11.90	13.12	10.65	11.71	12.96	11.36	11.43	12.77
8	13.00	11.86	11.74	12.15	12.83	12.87	10.84	12.69	13.37	12.41	10.71	12.69	13.51	11.90	11.70	14.45
12	12.29	12.86	11.09	13.34	12.93	14.22	12.12	13.71	11.70	13.29	11.92	12.78	14.08	13.79	12.25	13.93
16	11.99	11.37	12.10	14.00	13.18	12.20	13.95	14.83	11.69	10.84	11.68	13.82	13.91	12.78	13.80	15.06
20	12.15	13.93	12.93	13.07	14.91	13.67	12.53	13.66	12.68	12.86	13.21	13.31	14.48	13.82	13.16	15.78

Appendix I. Mg of nonprotein nitrogen (NPN) per 100 mg of total nitrogen for control and inoculated samples (continued).

Temp °C / Day/Rep	Control								Micrococcus luteus							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	12.30	13.21	11.52	12.82					11.53	12.48	11.90	13.70				
2	12.71	12.78	11.23	13.55	11.56	12.17	11.47	11.96	12.17	11.67	11.22	12.41	11.86	12.51	12.78	12.48
4	11.96	11.72	11.45	12.30	9.92	12.90	12.06	12.53	12.52	13.29	12.08	13.94	11.06	13.21	12.01	12.86
8	13.73	11.86	12.76	11.78	11.79	12.87	13.87	13.65	12.22	11.81	12.59	13.71	12.07	11.83	13.61	14.14
12	14.03	12.86	12.71	14.17	13.47	14.22	13.50	14.00	12.58	12.20	12.81	13.20	12.58	12.50	14.30	13.89
16	10.93	11.37	13.66	14.26	11.84	12.20	14.59	14.55	11.08	10.86	13.96	12.99	11.41	12.43	14.40	14.49
20	12.19	13.93	15.74	14.72	16.14	13.67	15.11	15.56	11.35	12.43	13.79	15.44	12.90	11.43	13.75	15.46
	Control								Pseudomonas fragi							
0	11.87	11.52	11.10	12.82					10.93	12.25	11.02	11.97				
2	11.05	11.23	10.15	13.55	11.60	11.47	12.87	11.96	11.84	12.20	11.84	12.37	11.09	11.59	11.63	12.51
4	11.71	11.45	10.86	12.30	10.01	12.06	11.76	12.53	11.88	12.13	11.22	11.41	11.74	12.20	10.88	11.28
8	11.74	12.76	10.84	11.78	10.84	13.87	12.97	13.65	9.94	11.51	11.04	12.60	13.46	14.13	16.73	13.13
12	11.09	12.71	14.05	14.17	12.12	13.50	13.17	14.00	11.51	11.57	14.99	11.87	20.88	17.57	23.39	16.37
16	12.10	13.66	11.94	14.26	13.95	14.59	12.70	14.55	17.38	17.00	21.96	12.18	26.24	20.81	24.40	18.72
20	12.93	15.74	13.35	14.72	12.53	15.11	13.26	15.56	15.43	19.73	20.84	14.04	22.45	26.71	28.10	21.43

Appendix J. % water in control and inoculated samples.

Temp °C	Control								Pediococcus cereviseae							
	2°				10°				2°				10°			
Day/Rep	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	69.86	65.51	60.94	63.94					71.34	62.45	61.56	64.00				
8	68.86	62.19	59.06	64.73	68.40	62.56	59.08	65.14	69.34	62.78	60.04	65.37	69.39	62.75	60.06	64.24
20	69.68	62.57	60.63	63.80	68.38	60.60	59.68	63.92	69.52	62.54	60.18	64.62	68.66	62.50	60.38	65.08
	Control								Leuconostoc mesenteroides							
0	69.86	65.54	65.82	63.94					71.45	64.43	65.70	64.94				
8	68.86	65.80	66.52	64.73	68.40	66.76	64.04	65.14	70.32	64.11	65.98	65.37	69.44	63.68	66.49	64.24
20	69.68	65.70	64.27	63.80	68.38	64.18	63.10	63.92	71.12	64.58	65.78	64.62	69.44	64.18	---	65.08
	Control								Micrococcus luteus							
0	65.51	65.54	62.22	67.78					64.54	65.11	63.78	67.67				
8	62.19	65.80	61.52	67.87	62.56	66.76	63.38	69.04	65.34	64.78	61.92	68.08	64.06	65.97	61.50	67.41
20	62.57	65.70	61.80	67.82	60.60	64.18	63.32	67.43	64.44	66.04	62.84	67.50	64.18	63.61	63.50	67.80
	Control								Pseudomonas fragi							
0	65.82	62.22	60.94	67.78					65.06	64.10	60.16	66.62				
8	66.52	61.52	59.06	67.87	64.04	63.38	59.08	69.04	65.96	61.52	58.88	67.96	66.34	59.92	59.82	68.40
20	64.27	61.80	60.63	67.82	63.10	63.32	59.68	67.43	66.12	64.10	61.63	68.80	65.65	62.14	61.21	68.56

Appendix K. % fat in control and inoculated samples.

Temp °C / Day/Rep	Control								Pediococcus cereviseae							
	2°				10°				2°				10°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	8.98	13.95	19.62	16.98					6.63	17.56	19.06	16.02				
8	8.71	17.66	20.98	16.24	8.88	16.56	21.30	16.28	8.30	16.78	20.26	15.88	8.02	16.38	20.42	15.70
20	8.20	17.15	19.67	17.62	8.06	16.56	21.74	16.42	8.16	16.06	20.30	16.55	8.68	16.82	19.56	16.70
	Control								Leuconostoc mesenteroides							
0	8.98	13.78	13.12	16.98					6.59	15.30	13.44	16.86				
8	8.71	14.54	13.08	16.24	8.88	13.37	15.66	16.28	8.10	15.83	13.15	15.82	7.26	16.54	12.51	16.80
20	8.20	14.18	15.08	17.62	8.06	14.08	15.86	16.42	7.88	15.36	13.30	16.34	7.28	15.74	---	16.10
	Control								Micrococcus luteus							
0	13.95	13.78	18.60	11.74					15.07	14.62	16.55	11.24				
8	17.66	14.54	19.13	11.34	16.56	13.37	16.82	10.28	14.12	15.64	19.58	10.84	14.89	14.43	18.95	11.70
20	17.15	14.18	19.03	11.16	16.56	14.08	18.11	11.52	14.56	14.54	18.21	11.28	15.23	16.41	17.14	11.07
	Control								Pseudomonas fragi							
0	13.12	18.60	19.62	11.74					14.39	16.41	20.70	12.74				
8	13.08	19.13	20.98	11.34	15.66	16.82	21.30	10.28	13.71	19.63	21.92	11.77	12.74	20.14	19.59	10.98
20	15.08	19.03	19.67	11.16	15.86	18.11	21.74	11.52	12.92	17.19	18.01	9.98	13.64	18.60	18.89	10.60

Appendix L. Log of the number of organisms per ml of undiluted culture used for the inoculation of pork samples.

Organism / Replicate	1	2	3	4
Pediococcus cereviseae	9.45	8.54	9.52	9.51
Leuconostoc mesenteroides	8.00	8.11	7.84	8.08
Micrococcus luteus	7.70	8.64	8.72	8.70
Pseudomonas fragi	9.11	7.15	7.32	7.78