ABSTRACT

TENDERNESS OF FREEZE-DRIED CHICKEN
WITH EMPHASIS ON ENZYME TREATMENTS

By Gordon H. Wells, Jr.

The effects of age on the tenderness of freeze-dried chicken breast muscle were determined, using chickens 11, 20 and 52 weeks of age. The old birds (toughest) were selected for proteolytic enzyme treatments and evaluation of muscle tissues.

Tenderness was determined with a Warner-Bratzler shear by relating shear force to area of breast muscle sheared. A correlation coefficient of 0.59 was obtained between sensory panel scores and Warner-Bratzler shear values, using cooked and rehydrated freeze-dried muscle, and a correlation coefficient of 0.80 was calculated for non-freeze-dried muscle. Tenderness of muscles was inversely related to age of birds. Sensory evaluations indicated juiciness to be directly related to tenderness. Percentage water uptake during rehydration was directly related to tenderness (as measured by the panel and Warner-Bratzler shear) and juiciness (as measured by the panel). More significant differences were noted when breast muscles were measured by the sensory panel than when measured by the Warner-Bratzler shear.

Papain, ficin, bromelin and Rhozyme P-11 were incorporated directly into the rehydration solutions. All samples were rehydrated in the enzyme solutions for five minutes. A three-minute heating time at 100°C was used to inactivate the enzymes immediately after rehydration. Inactivation was complete when no increases were found in non-protein nitrogen with time.

A sensory panel and an Allo-Kramer shear press were used to determine optimum tenderness of breast meat treated with various enzyme concentrations by using several pH and temperatures. Enzyme concentrations of 0.02%, 0.0008%, 0.002% and 0.002% (calculated as weight of pure enzyme/ volume of buffer) were most suitable for Rhozyme P-11, ficin, bromelin and papain, respectively. Rhozyme P-11, ficin and bromelin were most active at pH 5.0, while papain had maximum activity at pH 7.0. Optimum reaction temperatures were 50°, 50°, 60° and 70°C for Rhozyme P-11, papain, bromelin and ficin, respectively.

Control samples were significantly more tender when rehydrated at pH 7.0 than at higher or lower pH values. This may have been due to an increase in water uptake at pH 7.0 during rehydration. The percentage of water uptake of the control samples also increased with decreasing rehydration temperatures.

After chicken breast samples were rehydrated in enzyme solutions under optimum conditions for tenderization, they were studied histologically. Masson's trichrome stain was

modified for use on the cooked and rehydrated tissue. Ficin was most active on muscle fibers, while bromelin was least active. The effects of Rhozyme P-11 and papain were intermediate between those two extremes. Ficin produced the most activity on connective tissue, papain showed some activity, but bromelin and Rhozyme P-11 demonstrated little or no activity. Enzyme-induced tenderness seemed to be more related to muscle fiber destruction than to dissolution of the connective tissue.

Muscle fibers affected by enzymes showed a distinct swelling, dissolution of the sarcolemma, extensive granulation, disappearance of nuclei and loss of cross striations.

TENDERNESS OF FREEZE-DRIED CHICKEN WITH EMPHASIS ON ENZYME TREATMENTS

By

Gordon H. Wells, Jr.

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Food Science

1965

ACKNOWLEDGMENT

The author is deeply indebted to Dr. Lawrence E. Dawson for his assistance in conducting this study and for his counsel and understanding in the preparation of the manuscript. The author also wishes to express his appreciation for the assistance rendered him by Dr. Robert K. Ringer and his laboratory assistants, Mrs. Ellen Sanders and Mrs. Sandra Pangborn.

The author's appreication is extended to Dr. Lawrence Dawson, Dr. James Price, Mr. John Steinhauer, Mr. Raleigh Wilkinson, Mr. Carter Crigler, Mr. Julius Miller and Mrs. Maurice Ritchey for their interest and participation in sensory evaluations.

An expression of thanks is extended to Dr. W. D. Baten for his aid and advice in analyzing the data and to Professor James Davidson for his assistance in the procurement of chickens.

A sincere expression of appreciation is reserved for my wife, Nora, for her proof reading, helpful suggestions and typing assistance in the preparation of the manuscript and for her patience throughout this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Freeze-drying, drying by sublimation, or lyophilization is the process of removing moisture from a substance while in the frozen state. The frozen product is placed in a vacuum chamber and a controlled amount of heat is applied. Heat is used to keep the temperature of the product higher than the temperature of the ice at the chamber condenser, but not high enough to melt the product. The condenser is used to collect the moisture vapor by condensing and freezing it and thus preventing moisture from being drawn into the vacuum pump. Dehydration depends on the difference in water vapor pressure between the dry immediate environment of the product and the ice in the frozen interior of the product. When a proper relationship exists, water vapor is continuously transported away from the substance, but the ice within never melts. The rapid sublimation of moisture cools the product sufficiently to prevent thawing. Thus, product shrinkage is minimized. The structure of the dried material permits a sometimes slow but practically complete reconstitution. Conventional freeze-drying, in which meat remains frozen throughout the drying cycle, permits a reduction in moisture content to less than 2% without an appreciable change in product appearance.

Burke and Decareau (1964) revealed that at least 20 major food processors market freeze-dried foods, while in 1960 there were only two. Despite this rapid growth, in

some respects the process is still in its infancy as a means of food preservation.

The main commercial use for freeze-dried chicken is in the manufacture of dehydrated soups, although chicken in serving-size pieces is now marketed for consumption in other prepared foods. The primary advantage of using freeze-dried chicken in dehydrated foods is its highly acceptable flavor. It has excellent color and storage stability, but a dry texture often remains after rehydration. Even more serious is the toughness of rehydrated meat, and this factor has been an important limiting factor in successful commercial production of freeze-dried meats and poultry. Not only freeze-drying but all methods of drying are known to toughen meat and poultry.

One method of increasing the tenderness of meat and poultry involves the use of proteolytic enzymes. Commercial tenderizers are not completely effective in tenderizing meat. Many problems exist, including those associated with penetration of the tenderizers, uniform action, and flavor changes. Although penetration of enzymes into meat is usually limited, penetration into freeze-dried meat is quite satisfactory when the meat is rehydrated in enzyme solutions.

Most of the research on freeze-dried foods--particularly meats--has been published within the past ten years. Practically all of the meat research has been concerned with freeze-dried beef, while research on freeze-dried

poultry meat has been neglected.

The following study was undertaken in an effort to provide basic information on some of the factors affecting the tenderness of freeze-dried poultry. Attention has been directed to both preslaughter and post-rigor conditions with emphasis on the latter. Preslaughter conditions were concerned with the ultimate tenderness of reconstituted freeze-dried chicken as affected by age of birds.

The Warner-Bratzler and Allo-Kramer shear presses were used to evaluate tenderness objectively, while a sensory panel was used for subjective evaluation of tenderness. The Warner-Bratzler shear press requires the use of a core of meat taken at right angles to the muscle fiber plane. Since many birds have a very shallow breast muscle, it is difficult to obtain a satisfactory core for evaluation. Thus, a method for adapting the Warner-Bratzler press to chicken breast muscle was a necessary objective.

Post-rigor conditions were concerned with the effects of commercial proteolytic enzymes on tenderness. Optimum conditions for enzyme activity were desirable for this study. Thus pH, concentration of enzyme, and temperature were necessary factors to control. The rehydration times were planned to be of equal duration.

Microscopic observation of histological sections is an excellent method for studying the physical structure of muscle and the structural alterations caused by various treatments. Since microscopic examination of structural

changes brought about by these enzymes has been utilized to determine the site(s) and mode of enzyme action, histological sections were planned for the present study.

LITERATURE REVIEW

Low-temperature evaporation of water under vacuum to produce freezing, followed by sublimation of the ice was known before 1813, when William Hyde Wallaston apologetically discussed the process before the Royal Society of London (Flosdorf, 1945). The freeze-drying method of studying tissue structure was introduced by Altmann (1890). The first clearly recorded use of sublimation for preserving biological substances was reported by Shackell (1909). However, it was not until 1935 that foods were preserved by such a method (Flosdorf, 1945).

Freeze-dried foods have been commercially produced and marketed for consumption during the past several years. Nair (1963) estimated that one billion pounds of freeze-dried foods would be produced on four million square feet of freeze-dryer shelf area by 1970.

Freeze-Drying of Meats and Its Relationship to Meat Quality

Precooked, freeze-dried chicken with good functional and organoleptic properties was prepared by Yao *et al.* (1956), Tappel *et al.* (1957), Seltzer (1961), Rowe (1961) and Wells *et al.* (1962).

Yao *et al.* (1956) found that precooked chicken meat samples dried faster than uncooked samples, due to a lower initial moisture content. They also reported that the usual mode of heat transfer to chicken was predominately by radiation. When heat transfer by conduction was increased,

the drying rate was noticeably increased. Optimum drying cycles for diced and cooked chicken meat were later prepared (Anonymous, 1962a).

Toumy et al. (1962) reported that platen temperatures of 65.5°, 80.0° and 93.3°C did not significantly affect tenderness, juiciness or cutting ease of precooked, sliced beef. They also stated that overdrying by two hours was not critical.

The advantages of freeze-dried chicken as reported by Bird (1963) were: (1) When the best known drying techniques were used, most flavor constituents remained in the food; (2) the physical structure remained the same, so that rehydration was easy and rapid; (3) the product was relatively stable at room temperature; and (4) the product could be shipped more economically than frozen or canned food. He stated that the general impression of freeze-dried chicken was that it was not as good as the processed standard. However, the addition of other ingredients (as in creamed chicken, soups, or salads) greatly improved palatability. The USDA Marketing Economics Division (1963) substantiated these findings. Flosdorf (1945) found that the biological activity of labile components was generally unaffected by freeze-drying. Even vitamin C was reported to be completely stable. Rowe (1961) reported substantiating results.

According to Hunt and Matheson (1958), long before muscle consumption, the actin and myosin combined to form actomyosin, which was present in greater quantity than any

other protein. Since actomyosin was rather labile, they suggested its survival of any particular treatment meant that many other proteins and enzymes also remained undamaged. Hopkins (1955) reported that the contractile system of living frog muscle was not destroyed by freeze-drying although the rate of contraction was slower.

Harper and Tappel (1957) stated that the texture of freeze-dried meat was drier than the frozen control and that this dry texture was one of the principal problems which remained to be solved in the field of lyophilization. This deficiency in texture has been attributed to the loss of water-holding capacity by the muscle proteins, and protein denaturation during drying has been suggested as the cause of this loss (Brooks, 1958).

The effects of fast and slow freezing on freeze-dried beef were reported by Luyet (1960, 1961). Water penetrated more freely through solid parts than through cavities. Fast-frozen tissues did not shrink as much as slowly frozen tissues which swelled back to their original size when rehydrated. Smithies (1961) related that rapid freezing in a dry ice-acetone mixture yielded a product which rehydrated more slowly than was typical of slowly-frozen meat. After cooking, the fast-frozen meat was tougher and drier than more slowly frozen meat. Meryman (1961) stated that mammalian tissues could not be frozen rapidly without mechanical injury from intracellular crystal formation. Neither could they be frozen slowly without chemical injury

from the concentration of solutes. Auerbach (1960) found that a much better product resulted when meat was frozen slowly before drying, while meat that was frozen rapidly by evaporative cooling yielded a product that reconstituted poorly. Luyet and MacKenzie (1960) found that meat frozen rapidly had smaller channels and absorbed less fluid than material having larger channels. They found that meat rehydrated in NaCl solutions gave higher absorption values than when rehydrated in water. A procedure was also described for rehydrating freeze-dried meat in a vacuum to speed up absorption. In general, they referred to fast freezing as intra-fiber freezing and slow freezing as extra-fiber freezing, when considering fiber-contained water.

Worland and Urbin (1960) reported that some water in meat remained unfrozen even at very low temperatures. Luyet (1961) suggested several reasons why this water remained unfrozen: (1) because its temperature had not been lowered enough to cause the crystallization of all freezable water; (2) because it contained bound water, which does not freeze at any temperature; or (3) because it was cooled so rapidly that some of its water remained amorphous or in a state of incomplete crystallization. The binding energy for electrostatic bonds was not high according to Meryman (1961). He stated that this water was probably removed during the latter stages of freeze-drying. Also, since many organisms survived the loss of at least one-half their bound water, the removal of this weakly-bound water was apparently not

entirely irreversible.

According to Hamm and Deatherage (1960a), the decreased ability of meat proteins to rehydrate was usually due to the formation of an excessive amount of electrostatic and hydrogen bonds between actin and myosin in the myofibrillar filaments. According to the authors, formation of these bonds might be counteracted by relaxation of the muscle as well as by an increase of pH away from the isoelectric point. Thus, the least possible overlapping of the actin and myosin threads should occur.

Connell (1957) suggested that increases in toughness and a loss of gel-forming ability in dehydrated fish might be due to increased cross-linking of protein chains. He proposed that this could also cause dryness, due to poor water-binding capacity. Connell (1962) stated that even small amounts of cross-linkages could produce large textural changes.

According to Ziemba (1960), chicken meat tends to deteriorate very rapidly after drying when exposed to oxygen and/or elevated temperatures. Therefore, an inert gas or a high vacuum must be present for the successful storage of freeze-dried chicken, to prevent oxidative deterioration of the protein. The shelf life for freeze-dried chicken products packaged in polyethylene lined, lever-locked fiber drums was reported to be approximately six months at room temperature (Anonymous, 1962). The average shelf life of freeze-dried products, gas-packed in hermetically sealed

containers, was extended to about one year. According to Flosdorf (1949), freeze-dried products were stable for five years when dried to a minimal moisture content and stored at about 5°C.

Connell (1962) suggested that the carbonyl-amine browning or Maillard reaction was not entirely inhibited by freeze-drying to low moisture levels. Olcott (1961) reported that oxidative changes usually occurred more rapidly at low moisture content (1 - 2%) than when higher amounts of water were present. When water was absent, the most important single deteriorative reaction in freeze-dried meat or fish was the browning reaction. The brown reaction products resulted from the reaction of reducing sugars with proteins and probably accounted for losses in solubility and rehydratability. Sidwell et al. (1962) substantiated the results of Connell (1962) and in turn found that the oxygen content of freeze-dried chicken was lower when it was precooked.

According to Connell (1957), many freeze-dried foods showed high ratios of rehydration, or more accurately, water uptake, but much of this water was easily expelled, presumably because it was held only by weak capillary forces.

Auerbach et al. (1954) stated that freeze-dried meat generally rehydrated rather well. He reported that one-inch-thick samples of freeze-dried beef rehydrated from 80 to 90% of the original water content. Wang (1954a) stated that freeze-dried muscle tissues from beef rehydrated from 85 to

90% of their original moisture content. The muscle fibers also returned to 95% or more of their original diameter.

Norman and Auerbach (1963) found that the level of rehydration in 72°F water was in the 90 to 95 percentile range, while in 180°F water the rehydration level was in the 70 to 80 percentile range. According to Steinberg (1960a), the rehydration ratio for beef samples precooked to a center temperature of 140°F to 150°F was slightly higher than for samples precooked to a center temperature of 180°F to 198°F. Suden et al. (1964) reported that the percentage rehydration of freeze-dried pork fillets ranged from 48.5 to 92.4% with a mean of 73.8%. Rehydration was not influenced significantly by either pH of the rehydrating solution or pH of the rehydrated meat. However, the fillets were rehydrated for 4½ hours at 4°C.

To detect small changes in texture, Smithies (1961) found it useful to rehydrate samples for only five minutes before cooking and before presentation to a panel. Ground poultry breast meat rehydrated in 30 seconds in 180°F to 200°F water, while dark poultry meats required from 1½ to 2 minutes (Anonymous, 1965). Wang et al. (1945b) used the reappearance of distinct cross striations in the muscle fibers as a criterion for true reconstitution of muscle tissue.

Wismer-Pedersen (1965a) reported that after injection with solutions of EDTA (ethylenediaminetetraacetic acid) and pyrophosphate, freeze-dried pork samples had improved rehydration capacities and texture. The main effect of EDTA

appeared to be better penetration of water into the dried meat structure. This effect was probably associated with removal of calcium and magnesium ions from the fibrillar proteins through chelation before drying. Pyrophosphate appeared to cause swelling of the wetted areas rather than improvement of water diffusion into the dried meat.

Wismer-Pedersen (1965b) noted that when calcium and magnesium ions were added to pork myofibrils, the pH before drying influenced the water-holding capacity after rehydration. At pH 7.0, the rehydrated myofibrils had the same water holding capacity as the corresponding fresh myofibrils.

Factors Affecting the Tenderness of Poultry Meat

Miyada and Tappel (1956a) and Parrish et al. (1962) stated that tenderness was the foremost factor considered in meat acceptability.

A. Physiology and Chemistry of Muscle

The data of Blakeslee and Miller (1948) demonstrated that beef short loins were less tender at the rib end than at the porter house steak end. Ramsbottom et al. (1944) found a variation in tenderness of beef muscles in different muscles of the same commercial cut. According to White et al. (1964), tenderness differences in turkeys were smaller between inner breast muscles than between outer breast muscles. Thus when inner breast muscles were used, they accounted for greater difficulty in detecting tenderness differences. Koonz and Robinson (1946) reported that variations existed among principal muscles of the poultry carcass.

Wise (1961) found significant variations in the tenderness of poultry skeletal muscle tissues. Breast meat was reported to be significantly more tender than meat from the thigh (Goodwin et al., 1962). Wise and Stadelman (1959) reported that resistance to shear force was related, at a highly significant level, to the depth at which samples from poultry were taken.

Various components of muscle tissue have been found to contribute to tenderness. Deatherage and Harsham (1947) reported that both connective tissue and muscle plasma affected tenderness. Their results with beef indicated that initial post mortem changes involved the muscle plasma rather than connective tissue, and they postulated that changes in the plasma were more important during the initial aging period. They also proposed that in later post mortem stages, muscle plasma was less important than connective tissues in contributing to toughness. Lowe (1948) stated that meat from young birds, when aged for the same period of time, was more tender than that from older birds. In general older birds, as shown by histological sections, were found to contain more connective tissue within a given muscle than younger birds. Ramsbottom et al. (1944) found a significant correlation between shear press readings and the amounts of collagen and elastin in beef muscle, and between panel results and the amounts of collagen and elastin in the muscle. Koonz and Robinson (1946) found that elastic connective tissue was almost completely absent in poultry breast muscle.

A relationship between the amount of nitrogen extracted by buffer solution and tenderness of beef was reported by Wierbicki et al. (1954). Paul et al. (1958) found that a correlation between tenderness scores and percent nitrogen extracted by buffer solution was statistically significant, but too low to indicate a decided usefulness for measuring tenderness in chickens.

The study of meat tenderness covers the transition of muscle from the living state through the dead state, a period of time which includes the condition known as rigor mortis. Bendall (1963) related that the energy for muscle contraction came directly from the splitting of ATP (adenosine triphosphate) and that the opposite process, relaxation, occurred when certain specific conditions inhibited this splitting.

Bendall (1963) reported that when birds were slaughtered, their muscles became soft and pliable. Immediately after death, ATP was broken down and its concentration in muscle diminished. He reported that this splitting was the direct result of a sarcoplasmic ATPase which was probably associated with mitochondria. As a result of this breakdown, ADP (adenosine diphosphate) was produced and glycogen was depleted. Creatine phosphate, which served to phosphorylate ADP in muscle to ATP, continued to perform this function. At this state, blood circulation through the muscle limited its ability to maintain aerobic metabolism. Thus, the remaining metabolism of post-mortem muscle was forced to depend

completely upon anaerobic glycolysis which led to an accumulation of lactic acid. This accumulation of lactic acid caused a decrease in the muscle pH from its initial pH (7.2) to a pH of approximately 5.6 to 5.8. The decrease in pH caused a decrease in anaerobic metabolism, since the enzymes involved were no longer at their optimum pH. As the concentration of ATP fell, the muscle slowly hardened until it became quite stiff. This latter state of stiffness was called rigor mortis, although rigor mortis was really the result of the entire series of changes which started to occur at the moment of death.

The rate of development of rigor mortis and related biochemical changes in chicken muscle were studied by DeFremery and Pool (1960) in relation to ultimate tenderness of the cooked muscle. They found that correlations between the loss of ATP and the onset of rigor mortis were the same for chicken as for other species. Muscles from 10- to 16-week-old chickens, held at room temperature, passed into rigor from 2 to 4½ hours post-mortem and reached an ultimate pH of 5.8 to 5.9. They found that muscle toughness was induced with every treatment which caused a rapid loss of ATP, more rapid drop in pH, more rapid development of rigor mortis and more rapid loss of glycogen. In other words, toughness increased as the rate in the onset of rigor increased.

DeFremery and Pool (1959, 1960) postulated that the relative toughness of cooked muscle in uniform groups of chickens was directly related to the rate of development of

rigor mortis. In addition, the following pre-rigor treatments, which accelerated the onset of rigor mortis, also decreased tenderness; freezing and thawing, exhaustive electrical stimulation, and electron irradiation. Weinberg and Rose (1960) suggested that upon the resolution of rigor, the resulting tenderization was not just a random autolysis but instead resulted from a specific cleavage of the actin association responsible for the maintenance of the muscle matrix.

Lethal doses of sodium monobromoacetate were injected into chickens by DeFremery (1959). These injections accelerated the onset of rigor mortis and caused a marked increase in the rate of ATP depletion, but had little influence on pH or glycogen levels. However, the tenderness of the cooked meat was the same as the injected controls. He suggested that this ruled out the rapid loss of ATP as the determinant of increased toughness. The pH of these muscles (pH 6.5) was appreciably higher than normal. He reported that the isoelectric point of actomyosin was near pH 5.3, and a higher pH might lead to greater water-binding of actomyosin and, presumably, more tender meat.

Gawronski et al. (1964) obtained data which indicated that the oxidation of muscle sulfhydryl groups to disulfides contributed to the onset of rigor. They concluded that sulfhydryl/disulfide exchange had an important role in post-rigor-tenderization.

B. Slaughter

Goodwin et al. (1961) found that the method of slaughter

had no effect on the tenderness of breast muscles. However, humane slaughter treatments resulted in increased shear values for thigh muscles. Lineweaver (1959) stated that pre-mortem exercise, electric stunning, full feeding versus 24-hour fasting, and post-mortem delay before scalding had little or no effect on poultry tenderness.

The struggling effect has been somewhat controversial but most researchers agreed that under normal processing conditions, struggling did not exert an effect on post-mortem tenderization (Dodge, 1959 and Dodge and Stadelman, 1960a). However, Stadelman (1959) did state that excitement before slaughter should be avoided, since it altered the normal rigor pattern and caused more birds to be tough, even though others were tender. Gainer et al. (1951) previously reported that the muscles of birds which struggled during slaughter were more tender than muscles from birds of the same lot that did not struggle.

C. Scalding

Koonz et al. (1954), Stadelman and McLaren (1954), Lineweaver (1955, 1959), Klose et al. (1956a, 1959), Shannon et al. (1957), Pool et al. (1959) and Wise and Stadelman (1959) reported that chicken breast muscle was toughened by excessive scalding. Longer scald times and higher temperatures were found to significantly reduce the tenderness of poultry meat. Wise and Stadelman (1959) reported that the toughening effect of high-temperature, long-time scalds was related to the depth to which the scald heat penetrated the

muscle tissue.

Variations in scalding temperature were found by Klose and Pool (1954) to have no effect on the tenderness of roasted muscles from Broad Breasted Bronze turkeys. However, in the case of roasted skin, increased scalding temperatures produced a marked increase in toughness and wrinkling.

Wise (1961) concluded that the toughening effect of excessive scalding was a direct function of the tissue temperature during the early post-mortem period.

Stadelman and McLaren (1954) concluded that the layer of fat surrounding the breast muscle on mature birds acts as an insulator to minimize any change in muscle tone or tenderness during scalding. They also related that the time in the scald water was more important than the scald water temperature.

D. Picking

Stadelman and McLaren (1954), Wise and Stadelman (1959) and Lineweaver (1955, 1959) agreed that ultimate toughness after aging increased with the extent of beating action incurred by the carcass during feather removal. Beating was reported by Pool et al. (1959) to exert its greatest toughening effect when applied immediately after slaughter. Beating delayed from one to three hours after slaughter had less effect.

Klose et al. (1956a) reported that toughness induced in chickens and turkeys by excessive beating could not be resolved by prolonged aging. The authors found the effects of beating to be cumulative and stated that they could be reduced by limiting the beating action to the minimum required

for sufficient feather removal.

Gainer et al. (1951) found that muscles from hand picked birds were significantly more tender than those from machine picked birds. Klose et al. (1959) and DeFremery and Pool (1959) found similar results. The latter authors also noted that machine picking markedly accelerated the rate of onset of rigor mortis.

Goodwin and Stadelman (1962) reported that after two hours of muscle flexing and hand masaging of turkeys, significantly higher shear values were recorded than for controls. Massaging for shorter times affected toms and fryers more than hens.

E. Excising of Muscle

Lowe and Stewart (1948) noted that when breast muscles of chicken were cut soon after slaughter, the shock of cutting induced a turgidity and roughness of the cut surface which persisted even after 24 hours of carcass aging and subsequent cooking. In general, the sooner after slaughter the muscle was cut, the greater the effect. However, when rigor developed before the muscle was cut, turgidity did not develop. DeFremery and Pool (1960) substantiated these findings.

Koonz et al. (1954) altered the tenderness pattern by cutting into muscles of dispatched birds. Under these conditions toughness, which was presumably associated with rigor, was maintained over a relatively long period of time. Pool et al. (1959) also reported that cutting up the carcass in the early post-mortem period had a small toughening effect.

F. Aging

Perhaps the most important single factor affecting poultry tenderness is aging. Chajuss and Spencer (1962) obtained results which indicated that certain oxidation reactions played an important role in chicken meat tenderization during post-mortem aging. Muscles treated with sodium sulfite (a redox agent) were more tender than controls. They indicated that the probable action of this compound on meat protein was to reduce the disulfide bonds. The sulfhydryls thus formed were probably reoxidized so that the final products were the S-sulfonates.

At the present time practically all ready-to-cook chickens are aged for a period of time in a slush ice-water mixture in order to maintain high quality during the resolution of rigor. Stadelman (1959) stated that aging at 55°F took approximately three times as long to resolve rigor as at 32°F.

Most authorities agreed that aging for a period up to 24 hours provided maximum tenderness and that after this period no increase in tenderness was obtained (Lowe, 1948; Carlin et al., 1949; Koonz et al.; 1954; Lineweaver, 1955; Stadelman, 1956; Klose et al., 1956b; and Dawson et al., 1958.) Dawson et al. (1958) found that a holding time of between three to six hours for 10-week-old fryers was sufficient. Several investigators found that at chill temperatures, most tenderization took place within twelve hours post mortem and that very little occurred after this time (Anon., 1957; Pool et al. 1959; and Klose et al., 1959). Klose et al. (1956b)

reported that most tenderization in poultry took place in the first six hours. Pool et al. (1959) found that most tenderization took place within four hours at chill temperatures and that the rate then decreased up to about twelve hours after which no appreciable tenderization occurred. They found that no appreciable tenderization took place in hard-frozen carcasses held at 25°F to 27°F for several days.

Stadelman and Spencer (1955) indicated that turkeys packaged warm from the eviscerating line and cooled in the package for 24 hours prior to freezing resulted in a satisfactory frozen appearance. These turkeys were as tender as turkeys cooled in ice-water and then packaged and frozen.

Dodge and Stadelman (1959) stated that the temperature of the aging medium appeared to affect the pattern of rigor and the level of tenderness at a given time post-mortem. Lowe (1948) reported that the onset of rigor in chickens usually began within one to two hours post-mortem and the greatest rigidity usually occurred between six and twelve hours after death. She found a direct relationship between temperature and the onset of rigor as well as its resolution. No signs of rigor were observed in the cooked carcasses of fowl aged for three hours or longer before cooking. None of the birds aged for periods up to one hour were in rigor when they went into the oven but all were in this state when they were removed.

Effects of aging without freezing were compared with effects of aging, freezing, and thawing on the palatability

of roasters and fowl (Carlin et al., 1949). The unfrozen birds tenderized rapidly. Freezing resulted in a marked increase in tenderness of those halves aged less than six hours. When halves were aged for 24 hours, there was little difference in tenderness between frozen and unfrozen halves. Koonz et al. (1954) found that freezing interfered with the tenderness pattern and that complete tenderization was delayed until the tissues were defrosted. In another experiment these same authors immersed dispatched birds in hot water for various time periods. The muscles became significantly less tender as the time of immersion increased.

A slight increase in potassium content in poultry tissue after an eight-hour aging period at 32°F was found by Stadelman (1959). A slight increase in tenderness and juiciness was also detected. Chicken stags (12-month-old cross-breds) aged in 2% KCl were as tender after two hours of aging as the controls were after eight hours.

Dodge and Stadelman (1960b) showed that water uptake and rates of cooling during aging did not affect tenderness. Tenderization was closely associated with pH. Total moisture content of the tissue was not associated with water uptake, nor was it related to tenderness. Dodge (1959) showed similar results.

Pippen and Klose (1955) reported that aging of poultry in ice water, although beneficial from the tenderness standpoint, caused a leaching-out of flavoring components when the holding period was prolonged.

pH adjustment of intact meat to pH 7.0 to 7.4 with phosphate salts enabled meat fibers to take up and hold their normal water content (Swift and Ellis, 1956; Morse, 1955, and Kamstra and Saffle, 1959). An increase in tenderness was associated with this water-holding capacity. Carpenter et al. (1961) found that tenderness in beef was improved by pre-rigor infusions of sodium hexametaphosphate. May et al. (1962a) and Spencer and Smith (1962) reported that chilling chickens in polyphosphate solutions resulted in significant increases in tenderness. In contrast, Klose et al. (1963) found that shear force values of cooked fryer meat, after either three-hour or 22-hour chilling periods, did not show a significant effect of polyphosphates on tenderness. They stated that polyphosphates controlled cooking shrink and preserved quality. Mountney and Arganosa (1962) and Schermerhorn and Stadelman (1962) reported that phosphates in the aging solutions increased water retention. Swift and Berman (1959) reported that increased pH values in beef were closely correlated with increased water retention.

G. Freezing

Koonz and Ramsbottom (1939) found that the rate of freezing affected the size, number and location of ice formations. Nearly instantaneous freezing produced minute, evenly distributed ice columns within the fibers. When the rate of freezing was slower, the ice columns within the fibers were larger in diameter and fewer in number. The importance of ice crystal size was emphasized by Birdseye (1946). He

proposed that large ice crystals, as a result of slow freezing, resulted in physical damage to the cell (cell rupture) or in a physio-chemical change which he termed "salt dehydration." Structural changes in muscle tissue upon repeated freezing and thawing were observed by Nichols and McIntosh (1952). Repeated freezing and thawing caused an increased amount of drip loss. As the number of broken muscle fibers increased, more fluid was released. Both the intra- and inter-cellular ice formations contributed to the fragmentation of fibers.

Dubois et al. (1942) stated that by normal observation, it was difficult to differentiate between rapidly and slowly frozen chickens. However, they noted that birds frozen by these two methods could be differentiated through the use of histological cross-sections of tissue viewed microscopically.

Early investigations showed that freezing allowed for the continuation of the aging process with a resultant increase in tenderization (Carlin, 1949; Carlin et al., 1949; Hepburn, 1960; Monzini, 1953; and Swanson and Sloan, 1953). However, more recent research has shown that the tenderizing process was arrested and that complete tenderization was delayed until the tissues were defrosted (Koonz et al., 1954; Spencer et al., 1956; and Klose et al., 1956a, 1959). Klose et al., (1959) reported that holding inadequately aged, frozen turkey fryers for as long as nine months at 0°F had no effect on tenderness.

Marion and Stadelman (1958) evaluated tenderness of poultry breast muscle by four different freezing methods. Method of freezing did not significantly affect tenderness.

Deatherage (1959) reported that the freezing rate affected the water-holding capacity of meat; tenderness of meat was related to the ability of meat proteins to hold water. Deatherage and Hamm (1960) substantiated these results. They reported that quick freezing and thawing of beef resulted in no appreciable denaturation of muscle protein. However, quick freezing caused a significant increase in the water-holding capacity of the meat, probably by a mechanical loosening of tissue structure due to the formation of tiny ice crystals within the cells. Slow freezing caused a significant decrease in the water-holding capacity, probably due to some destruction of protein structure by formation of large ice crystals between the cells.

H. Cooking

Hamm and Deatherage (1960b) detected a mild denaturation in muscle after the temperature reached 30°C to 40°C. This denaturation resulted in an unfolding of protein chains with the formation of new salt and/or hydrogen bonds. The denaturation and formation of new cross-linkages in muscle continued until about 65°C at which temperature the denaturation was almost complete. The step-wise change in the water-holding capacity of meat and in pH during heating was determined by following a corresponding decrease in the acidic groups of muscle proteins. Heat denaturation did not cause a significant decrease in the amount of basic groups in muscle proteins.

Kahn and van den Berg (1965) recently reported that the

sulfhydryl group content and tenderness of chicken muscle decreased simultaneously during cooking and frozen storage. They suggested that the sulfhydryl groups which survived heat denaturation in the muscle tissue contributed in some way to maintaining the eating quality of meat. They proposed that the loss of this sulfhydryl group content during storage might serve as an index of tenderness. Tenderness changes became apparent when the sulfhydryl group content of muscle tissue had decreased to about 50% of its value in the fresh cooked meat.

Mickelberry and Stadelman (1962) reported that pre-cooked, frozen chicken meat was significantly less tender than chicken cooked after freezing. Goodwin et al. (1962) found that all turkey muscles became more tender when cooked. Koonz and Robinson (1946) found similar results with chickens. However, they also found that moderate cooking of beef caused many muscles to become tougher. Although Goodwin et al. (1962) found that the rate of cooking had no significant effect on shear values, there appeared to be a trend toward lower average shear values for the chicken breasts cooked at the lower temperatures. May et al. (1962b) observed that broilers and roasters cooked in an electronic range had slightly higher shear values than similar birds cooked by a moist heat method. Dawson et al. (1959) found that in general, dry heat methods yielded more tender beef than moist heat methods. Mickelberry and Stadelman (1962) found that birds fried in deep fat were less tender than birds cooked by other methods.

Shear values for pressure cooked breast meat were reported by Kahlenberg and Funk (1961) to be significantly lower than shear values for either boiled or simmered breast muscle. Tenderness of old fowl cooked in various salt solutions was similar to tenderness of birds cooked in plain water. However, Goodwin et al. (1962) reported that the method of cooking had no statistical effect on the shear values of turkey meat. The methods used included cooking by microwave oven, deep fat frying, steam pressure, rotary reel oven and combinations of steam and deep fat frying and of microwave heating and deep fat frying.

For a comprehensive review of the literature concerning cooking and tenderness of meat other than poultry, the reader is referred to a discussion by Paul (1963).

Objective and Subjective Evaluation of Tenderness

A. Objective Measurements (Mechanical and/or Chemico-Physical Methods)

Pearson (1963) related that mechanical methods were more widely accepted for measuring meat tenderness objectively than were chemical and histological methods. Of the mechanical methods, the Warner-Bratzler shear was most widely used (Deatherage, 1951). However, the Warner-Bratzler machine necessitates the use of a cross-sectional core of meat for evaluation (Bratzler, 1932). In chicken breast, it is often difficult to obtain such a core due to the relative thinness of this muscle. Thus, the Kramer shear press has proven to be the most satisfactory device for measuring tenderness of

chickens (Cameron and Ryan, 1955).

The Warner-Bratzler shear has been fully described by Bratzler (1932) after his adaptation of the original instrument developed by Warner in 1928. The Kramer shear press is an instrument which was originally developed for fruits and vegetables by Kramer et al. (1951).

Paul et al. (1958) reported a negative correlation of -0.71 between the average tenderness scores of a taste panel and the Warner-Bratzler shear values from chicken meat. Deatherage and Garnatz (1952) also compared sensory panel scores to Warner-Bratzler shear values. Although shear values measured a property of meat fairly satisfactorily, a poor relationship existed between shear press values and sensory panel scores when broiled steaks were evaluated.

Although the Warner-Bratzler shear has several shortcomings, results have revealed that correlation coefficients between shear values and sensory evaluations generally lie in the range of 0.60 to 0.85, with an average value of about 0.75 (Pearson, 1963).

Shannon et al. (1957) reported a correlation coefficient of 0.86 between Kramer shear press values and taste panel scores. Dodge and Stadelman (1960c) obtained a correlation coefficient of 0.97 when cooked meat was evaluated by the same two methods. Bailey et al. (1962) measured 258 beef steaks and found a correlation coefficient of -0.89 between taste panel scores and Kramer shear press values for all steaks evaluated within grades and cuts. Disregarding grade

or cut, a -0.74 correlation coefficient was calculated.

Dodge and Stadelman (1960c) found significant correlations between Kramer shear values on raw meat and panel evaluation of cooked samples from the same poultry carcass. However, the relationship was not as high as that found between shear values and panel scores of cooked samples.

Cameron and Ryan (1955) reported that sample size affected tenderness as measured by the Kramer shear press.

Wells et al. (1962) found a poor relationship between Kramer shear values and taste panel scores when tenderness of freeze-dried poultry breast muscle was evaluated. Low correlations were obtained by Steinberg (1960a) between objective and subjective texture measurements of freeze-dried beef.

B. Subjective Measurements (Sensory Methods)

Sensory methods for tenderness evaluation approximate the actual sensation realized by consumers. Two types of panels have been used in sensory evaluations (Pearson, 1963): (1) the large consumer or acceptance panel and (2) the smaller expert or difference panel.

Consumer panels have been more expensive to conduct and have not always been applicable due to sample size and availability of personnel. The reactions of a large, untrained panel of 355 people and of a small, trained panel of seven judges were reported by White et al. (1964) while evaluating toughness differences in turkeys. The small panel used a triangle test method and distinguished differences in

tenderness of light meat which varied in shear resistance by 4 pounds in a 9 to 22 pound range. The small panel distinguished differences more accurately than the consumer panel. Lowe (1949) stated that four judges were a minimum number for a trained panel. She proposed that a small, sensitive panel was preferable to a larger, less sensitive one for measuring textural differences.

According to Pearson (1963), selection of a panel was best achieved by use of a triangle testing procedure, whereby each judge was given three samples of meat, two of which were alike. He stated that the chew count was the most objective of the sensory procedures for studying meat tenderness. The chew count method consisted of the number of chews required to completely masticate a sample before it was swallowed.

Lowe (1949) proposed that the triangle test was an accurate and reliable method for tenderness evaluation. Peryam and Pilgrim (1957), in turn, preferred the hedonic scale method. A numerical rating was used and each panel member selected a description best fitting the sample involved. The hedonic scale method was designed for use with subjects having little experience in food tasting. These authors stated that the hedonic scale method was developed on the assumption that direct responses, which were assumed to be based considerably on feelings, were more valid for predicting actual behavior toward food than were responses which depended more on reasoning. The authors stated that

long or short lines, vertical or horizontal orientation, or terminology such as "like" or "dislike", did not appear to be significant.

Cover et al. (1962) identified six separate components of tenderness and related them to shear force and fiber extensibility. These six components included connective tissue, juiciness, mealiness, softness to tongue and cheek, softness to tooth pressure and ease of fragmentation and adhesion.

Sartorius and Child (1938) and Deatherage (1951) reported significant positive correlation coefficients between tenderness and juiciness scores in meat.

Structure of Muscle and Connective Tissue

A. Muscle Structure

Maximow and Bloom (1954) presented a comprehensive outline of tissue structure. They classified muscle in vertebrates as smooth and striated muscle. In general, smooth muscles contracted independently of voluntary control, while striated muscles were of voluntary control. Cardiac muscle, although striated, was involuntary. Smooth muscle displayed a close relationship to ordinary connective tissue and was found primarily in the internal organs. The muscles attached to the mammalian skeleton consisted of striated muscular tissue. The authors stated that the individual muscle fiber was the functional unit of a muscle. In striated muscle where these fibers were large, multinucleated cells, the

thickness of the individual fiber varied from 10 to 100 microns. This depended on the type and age of the animal and on the particular muscle. The fibers were relatively long, some of which extended the full length of the muscle. An average-sized skeletal muscle fiber contained several hundred nuclei. The striated fiber was covered with the sarcolemma, a thin, elastic, transparent and stuctureless membrane which completely enveloped the fiber. Muscles were formed of parallel muscle fibers cemented together by networks of connective tissue. The muscle fibers combined to form the so-called primary bundles, and several primary bundles combined to form secondary bundles.

According to Copenhaver (1964), the sarcolemma encompassed the nuclei and a cross-striated substance composed principally of the myofibrillae. Surrounding the fibrillae and accumulated near the nuclei was the sarcoplasm, the more fluid portion of the fiber. The myofibrillae imparted to the muscle fiber as a whole the appearance of longitudinal striation. Each of the myofibrils was composed of a number of thinner, thread-like elements known as myofilaments. The striations appeared as alternating light and dark bands. The dark band was labeled the A band or Q band. The light band was designated by the letter I or J. Each of these bands was bisected by a narrow line, which stained deeply in the I band and was designated Z; the line bisecting the A band was pale and was designated H. Within the H line or disc was a narrow stripe designated by the letter M. In these various bands, actin and myosin filaments combined to form

actomyosin during contraction.

B. Connective Tissue Structure

Ham and Leeson (1961) classified the connective tissue into certain main types: loose, dense fibrous, adipose, cartilage, bone, dentin and hemopoietic. Only the loose connective tissue was of concern in the present study.

According to Ham and Leeson (1961), loose, irregularly-arranged connective tissue bound structures together loosely and held them in position. It acted as a pathway for nerves and blood vessels and served as a padding. Loose connective tissue, like all other connective tissues, was composed of cells, intercellular fibers, and ground substance which was the material forming the background. It contained most of the cell types and all of the kinds of fibers found in the other varieties of connective tissue.

Birkner and Auerbach (1960) stated that individual muscle fibers were separated by very thin networks of connective tissue called the endomysium. Primary muscle fiber bundles varied in the number of fibers per bundle, depending on the muscle, and were encompassed by larger sheets of connective tissue, the perimysium. The epimysium was the large outer layer of connective tissue which surrounded the entire muscle.

Copenhaver (1964) reported three types of fibers in adult connective tissue: white or collagenous fibers, reticular fibers and elastic fibers. The collagenous fibers were in bundles of indefinite length and variable thickness ranging

from 10 to 100 microns or more. Each collagenous fiber was composed of fibrillae. The fibrillae lay parallel to one another and imparted a longitudinally striated appearance to the fiber. They, themselves, did not branch but the fibers and bundles did. The course of the fibers was usually wavy. They consisted of collagen, a substance which stained easily with most acid dyes and yielded gelatin upon cooking.

Reticular fibers were small fibers which branched to form a supporting framework or reticulum. Their magnitude was so small that they were masked by surrounding structures in ordinary stained preparations. Reticular fibers were often found to be continuous with collagenous fibers and had a very close resemblance to the latter. They were particularly sparse in the loose connective tissues except for regions around muscle fibers.

According to Copenhaver (1964), the elastic fibers were usually thinner than white fibers. They branched freely and were a distinct yellow when seen in the fresh state. Chemically, these fibers consisted of elastin, which had a remarkable resistance to most agents. Elastin was not affected by boiling. The elastic fibers reacted very poorly with most stains, but were colored deeply with certain specific dyes such as orcein and resorcin-fuchsin.

Tenderization by Commercial Proteolytic Enzymes

In June, 1955, the Meat Inspection Division (MID) of the USDA officially permitted the use of enzymatic tenderizers in MID-inspected meats (Bavisotto, 1958).

A serious problem associated with the use of these proteolytic enzymes was that of penetration, especially in cooked meat (Auerbach, 1960). Enzyme-treated meat often showed overtenderization and a mushy appearance on the outside but little or no effect on the inside. The problem in raw meat was partially solved by injecting enzyme tenderizers into the animal prior to slaughter. This problem was also minimized with freeze-dried meat, when the enzyme was incorporated directly into the rehydration media.

Hamm (1960) stated that freeze-dried meat showed a better rehydration than meat dried by other methods. Penny (1960) reported that when meat was first dried by the accelerated freeze-drying method and then reconstituted with proteolytic enzymes, a resultant tenderized product was achieved. Sosebee et al. (1963) reported similar results with freeze-dried chicken breast muscle. They also stated that much lower concentrations of enzyme were required to produce tenderness in chicken than those reported necessary for tenderizing beef. According to Schweigert (1960), much higher concentrations of enzyme preparations were needed to show histological changes than for differences to be detected by a taste panel. Weiner et al. (1957) reported that it was possible for tenderization to occur before the proteolytic effect was measurable. Thus, organoleptic testing should be used for the determination of tenderization.

Wang et al. (1957) studied the relative potencies of twelve enzyme preparations on the tenderness and muscle

structure of beef. They reported that the amount of enzyme needed to produce meat of desirable tenderness varied with the initial tenderness of the meat. The same amount of enzyme made a tender steak mushy, whereas it improved a very tough steak. They classified the enzymes into three categories, depending on their origin: those of plant origin, such as papain, bromelin and ficin; those of bacterial or fungal origin, such as Rhozyme P-11; and those of animal origin, such as trypsin and Viokase which were not used in the present study.

According to Bavisotto (1958), papain was the dried latex of the fruit of the Carica papaya which was cultivated extensively in Ceylon and in British East Africa. The fig tree of the genus Ficus was the source of the fig latex from which ficin was isolated. It was grown in Central and South America. Bromelin was produced commercially from the stem of the pineapple, Ananas comosus, and was imported from Hawaii. Rhozyme P-11 was obtained by isolation from a selected species of fungus in the Aspergillus flavus-oryzae group.

Weir (1959) rehydrated freeze-dried beef steaks in the above-mentioned enzyme solutions for five minutes at 130°F. An extension of the holding time at 130°F after rehydration from 5 to 30 minutes did not affect the tenderness of, or amounts of residue from, the steaks. Greater enzyme concentrations were needed to produce measurable changes in cooked beef than in raw beef.

The optimum temperature for an enzymatic reaction, according to Weiner et al. (1957), was closely related to the time which the reaction covered. In general, the shorter the digestion time, the higher was the optimum temperature for that reaction.

Tsen and Tappel (1959) reported that the heat stability of papain generally permitted a maximum hydrolysis of proteins at 60°C. Tappel et al. (1956a) found the optimum temperature for papain digestion to be between 60°C and 80°C. Optimum temperature ranges for ficin, bromelin and papain were 30°C to 50°C, 30°C to 60°C, and 60°C to 85°C, respectively (Anonymous, 1963). At the same time, the optimum range for Rhozyme P-11 was reported to be 43°C to 60°C. Laboratory tests indicated that most of the tenderization took place during cooking. Maximum solubilization of all beef protein fractions occurred at pH 7.0 and 80°C with ficin and bromelin, according to El-Gharbawi and Whitaker (1963). These workers also stated that it was not practical to add buffer to influence the pH of raw, fresh meat, but that this could be done readily during the rehydration of freeze-dried meat.

According to Kimmel and Smith (1957), the pH optimum for the digestion of fibrin by papain was 7.0. Cohen (1958) determined the optimum activity of ficin using 0.01 M cysteine as an activator. He observed a broad optimum pH from pH 6.5 to pH 9.5. Wang and Birkner (1957) stated that ficin was active on beef muscle over a pH range from 5.0 to 9.0 with an optimum at around pH 5.0 to 6.0. The optimum pH for

Rhozyme P-11 was reported to be between pH 5.5 and 6.0 (Anonymous, 1963). Yatco-Manzo and Whitaker (1962) found ficin-catalyzed hydrolysis of elastin to be optimum at a pH 5.0 to 5.5 and at a temperature of 55°C.

Sosebee et al. (1963) obtained sufficient tenderization of poultry breast muscle with concentrations of papain and Rhozyme P-11 equal to 0.003% and 0.02%, respectively. A 30-minute rehydration time was used. Wang et al. (1958) used ficin, bromelin, papain, and Rhozyme P-11 on beef at concentrations of 0.0002%, for all enzymes except Rhozyme P-11 whose concentration was 100 times stronger. These investigators stated that hemoglobin and gelatin assay methods of expressing enzyme activity might not reflect the meat tenderizing properties of the enzymes used.

According to Thomas and Partridge (1960), the plant enzymes required a reducing agent such as cysteine for activation. In the absence of cysteine, there was a marked decrease in activity towards elastin and gelatin. Kimmel and Smith (1957) reported that all activators of papain were capable of reducing disulfide bonds; they included compounds such as H_2S, HCN, and other reducing agents. Free thiol groups were considered essential for papain activity. They stated that papain contained eight atoms of sulfur per mole of papain but that only six of these could be accounted for as half-cysteine. It was further acknowledged that removal of heavy metals was essential for maximal papain activity.

Liener (1961a, 1961b) concluded that ficin contained at

least two sulfhydryl groups, only one of which was directly involved in the catalytic site of the enzyme. Ficin contained at least one disulfide bond which appeared to be unessential for the maintenance of activity. Hammond and Gutfreund (1959) concluded that three reactive groups were necessary for the catalytic action of ficin; - SH, NH_3^+, and CO_2^-. These investigators also proposed a reaction sequence between enzyme and substrate. The sequence involved the rapid formation of a loose enzyme-substrate compound, a subsequent acylation of the enzymic sulfhydryl group by the carbonyl of the substrate, and finally the decomposition of the acyl enzyme.

Histological Considerations

For a brief review of muscle histology, the reader is referred to an article by Venable (1963).

Histologically, freeze-dried muscle tissue was characterized by a system of interfibral spaces (Wang, 1954). These spaces were found to arise, in most cases, as a result of muscle fiber shrinkage without a corresponding alteration of the tissue volume. Wang et al. (1953) found similar results with the additional finding that conventional drying resulted in a gradual loss of both longitudinal and transverse striations. Also, the nuclei were reported to have stained poorly, and there was a merging of individual muscle fibers. Similar findings were reported by Doty et al. (1953) when slices of raw beef were dehydrated at 70°C in an air oven. These investigators found that the histological

appearance of freeze-dried meat was almost indistinguishable from that of fresh raw meat. Sosebee et al. (1963) found that freeze-drying of chicken did not significantly affect the histological appearance.

Ramsbottom and Strandine (1949) described the presence of granular protein material between the muscle fibers. During cooking, both longitudinal and transverse breaks occurred in the muscle fibers, and at these points greater breakdown of the muscle fibers resulted. Collagenous fibers, when cooked, underwent first a swelling and then a shrinkage and disintegration. Chemical changes in collagenous fibers were reported to have occurred during cooking as evidenced by changes in the affinity of the fibers for dyes.

For years cooking was known to cause a decrease in muscle fiber diameter due to shrinkage (Sartorius and Child, 1938). Doty and Pierce (1961) referred to the granulation which occurred during cooking as the "erosion" of muscle fibers. They stated that this "erosion" or granulation started at the edges of muscle fibers and, when heating was continued, progressed to complete granulation of the fiber. The endomysial reticulum remained relatively intact. Although collagenous fibers were affected by heating, cooking did not appreciably alter the structure, staining affinities or physical properties of elastic fibers (Birkner and Auerbach, 1960; Winegarden et al., 1952; and Weir et al., 1958). Paul (1963) summarized the effects of cooking and the influence of cooking methods on tenderness. Photographs of several

histological sections of cooked tissue were included.

Bavisotto (1958) stated that proteolytic enzymes of microbiological origin exhibited potent activity on muscle fibers and in some cases slight activity on collagenous fibers. Wang and Maynard (1955a) reported that Rhozyme P-11 had no effect on collagenous or elastic fibers from freeze-dried pork muscle. Miyada and Tappel (1956b) found that papain and ficin hydrolyzed elastin and that bromelin, ficin, trypsin, papain and Rhozyme P-11 hydrolyzed collagen. Thomas and Partridge (1960) found that bromelin also had elastolytic activity. Wang and Maynard (1955b) found that papain and Rhozyme P-11 had very similar effects on muscle tissue components. Both attacked muscle fiber protein, the nuclei of muscle fibers and of cells located in the endomysia, but the enzymes were inactive on collagenous and elastic fibers at room temperature.

Wang et al. (1957) conducted a comprehensive study on the influence of enzyme tenderizers on the microscopic structure of freeze-dried beef. Among the twelve enzymes used were ficin, papain, bromelin, and Rhozyme P-11. Steaks were rehydrated in an enzyme solution of known concentration for 15 minutes. The earliest change in the muscle fibers was the dissolution of the sarcolemma followed by the disintegration of the connective cell nuclei (mostly fibrocytes). Continued enzyme action resulted in the complete disappearance of cross-striations. Since the fibers had lost their sarcolemma, they tended to merge. This merging was accentuated

by the swelling caused by the enzyme treatment. Enzymatic collagenase activity was manifested by a decrease in staining capacity with acid fuchsin and a decreasing discreteness in the fibrillar nature of collagen. The latter change was believed to have resulted from the liquefaction of ground substance which normally holds the collagenous fibers into definite bundles. In the presence of elastase, the elastic fibers underwent a process of segmentation (linear breakage), which made the fibers appear beaded. When the enzyme activity continued, complete digestion of elastin was noted. This point was reached when the fibers were no longer stained. Sometimes a trail of "ghosts" was detected after complete elastin digestion, which indicated the presence of fibers prior to the treatment. Wang and Maynard (1955a, 1955b), Tappel et al. (1956b), Wang and Birkner (1957) and Sosebee et al. (1963) reported similar results.

Tappel et al. (1956b) reported that papain hydrolyzed the sarcolemma and muscle cell nuclei before there was any apparent digestion of the muscle fibers themselves. They postulated that the heat labile muscle proteins denatured before the relatively heat-stable papain, and that papain then hydrolyzed these denatured proteins with maximum effect. Tenderization by papain was not ascribed to one specific reaction but rather to a general hydrolysis of all the structural components of beef muscle. According to McIntosh and Carlin (1963), papain affected the mucoprotein and collagen more than the other skeletal muscle proteins. Collagen suspensions were converted to thick gels by the action of papain.

Weir et al. (1958) rehydrated freeze-dried beef in solutions of commercial tenderizers for 30 minutes. They found that the granulation invariably occurred in the interfibral spaces. However, this was the only manifestation of the treatment noted. In all other respects the tissue appeared indistinguishable from normal tissues. The granulated material was probably derived from endomysial collagen and the muscle fiber envelopes. Both structures made early contact with the liquid tenderizer and were disintegrated. A relationship between sarcolemma destruction and an increase in tenderness was demonstrated.

Studies by Wang and Maynard (1955a) showed the effects of papain and Rhozyme P-11 on freeze-dried pork. Rhozyme manifested a greater sensitivity on the muscle fiber nuclei than on those nuclei of the connective tissue cells, while the reverse was true of papain (in the form of Adolph's Meat Tenderizer).

At present, the only published research found by this researcher concerning the effects of proteolytic enzymes on freeze-dried poultry was published by Sosebee et al. (1963). Solutions of papain and Rhozyme P-11 were used in rehydration. Rhozyme P-11 was used at a concentration of 0.02% and papain at 0.003%. Both enzymes altered the appearance of skeletal muscle and collagen. Papain caused extensive degradation and loss of staining ability of collagen and some granulation of muscle fibers. Rhozyme P-11 caused granulation of muscle fibers and some vacuolation and loss of staining ability of collagen.

MATERIALS AND METHODS

Selection of Chickens

A total of 111 White Leghorn females of three different ages (11, 20 and 52 weeks) were used in Part I of the study (effects of age on tenderness). All birds were from the same brood and were raised under identical management practices.

Two hundred twenty-six White Leghorn females (17 months of age) were processed for Part II of the study (effects of enzyme treatments on chicken breast muscle). The chickens used in this latter study were from three different broods. However, all of the birds used in each treatment were from the same brood.

Processing and Sample Preparation

All birds were slaughtered on a killing wheel by means of a semi-Kosher cut which severed the jugular vein and carotid artery on one side of the neck. They were bled and then scalded in a Rotomatic (basket-type) scalder at 59°C for 70 seconds. Feathers were removed by an Ashley automatic rubber-fingered picker. Seven birds were placed in the picker at a time for a period of 45 seconds. Following the picking operation, the birds were eviscerated and placed in tanks containing slush ice and water for a 24-hour aging period.

The ready-to-cook birds, in lots of 15, were simmered in a steam-jacketed kettle. They were cooked to center breast temperatures of 82°C. The temperatures were registered by a

Brown recording potentiometer. Thermocouples were inserted in six of the 15 birds in each lot. The thermocouples were connected by a series circuit. Thus, an average temperature from the six birds was obtained.

After cooking, the birds were cooled in water for five minutes. The two pectoralis major muscles from each bird were removed, packaged in polyvinylidene-chloride bags, frozen at -35°C for four hours, and transferred to -18°C for storage.

After 24 hours of storage at -18°C, each breast in Part I was cut into a single uniform piece on a band saw. A 3 1/2- by 1 3/16-inch wooden block was used as a guide to cut standard samples from the center of each breast. The block was placed on the breast, and a scalpel was used to trace the outline of the block on the breast. The samples were cut with the band saw along the tracing. The samples were freeze-dried for 18 to 20 hours in a Stokes freeze-dryer, Laboratory Model 2003 F-2, using a vacuum of 100 to 150 microns of mercury and a plate temperature of 30°C. The freeze-dried meat was packaged under partial vacuum in polyvinylidene-chloride bags and stored at -18°C until used.

The chicken breasts used in Part II were handled in the same manner, with one exception. Just prior to freeze-drying, the frozen breasts were removed from the polyvinylidene-chloride bags and diced into 3/8-inch cubes by a Toledo one-horsepower meat saw.

Non-freeze-dried control birds were prepared by the same methods. To maintain constant conditions, these breast

samples were subjected to the same rehydration procedure as the freeze-dried breasts.

Rehydration

The muscles used in Part I were rehydrated in 100°C distilled water at a water-to-sample ratio of 6:1. The samples were rehydrated for 15 minutes. After rehydration, the samples were emptied into a 20-mesh sieve and drained for five minutes before Warner-Bratzler shear press and sensory evaluations were conducted.

In Part II the diced, freeze-dried samples were rehydrated in various buffer solutions to provide the desired pH. The various buffers used in this study with their respective pH values were as follows:

(1) pH 4.0 -- 0.2 M acetic acid and
(2) pH 5.0 0.2 M sodium acetate

(3) pH 6.0 -- 0.2 M monobasic sodium phosphate
(4) pH 7.0 and 0.2 M dibasic sodium phosphate

(5) pH 8.0 -- 0.2 M tris (hydroxymethyl) amino
(6) pH 9.0 methane and 0.2 M HCl

The above solutions were made up according to the specifications of Gomori (1955). A total of 200 ml of each buffer was placed in a 500 ml wide-mouthed Erlenmeyer flask. A 40-inch air condenser was inserted into the drilled hole in a #10 rubber stopper and fitted into the neck of the reaction flask. The condenser was used to prevent water evaporation from the reaction flask. Thus, it prevented changes in pH due to evaporation, or changes in the water-to-sample ratio which would affect rehydration.

The entire apparatus containing the buffer was then placed in a Magni Whirl constant-temperature water bath and held at the desired temperature until the buffer solution and water bath temperatures equilibrated. The temperatures used in this study were 40°, 50°, 60°, 70°, and 80°C.

Just prior to the addition of the enzyme and substrate, five ml of a stock solution of 0.5M l-cysteine hydrochloride was added to the 200 ml of buffer solution. This gave the buffer solution an actual concentration of 0.0125 M cysteine. The cysteine was added to activate the enzyme.

The enzymes used in this study were papain, ficin, bromelin and Rhozyme P-11.[1] The first three enzymes were obtained from the Nutritional Biochemical Corporation and the latter one from the Rohm and Haas Company. All enzymes were stored at 2°C and low relative humidity.

Immediately after the addition of cysteine to the buffer, a predetermined amount of enzyme was added with a pre-weighed freeze-dried meat sample. The enzymes were weighed accurately to the third decimal place on a Mettler analytical balance. The freeze-dried chicken samples were weighed accurately to the first decimal place on a Torsion balance.

The reaction mixture was allowed to incubate at the desired temperature for five minutes. After incubation, the reaction flask was removed from the bath and heated to boiling over a hot flame to stop the reaction. The reaction

---

[1]Hemoglobin assay units of these enzymes as received from the suppliers were: Rhozyme P-11 3,200; papain 10,100; bromelin 15,000 and ficin 27,200.

mixture was allowed to boil gently for 3 1/4 minutes. Immediately after removal from the water bath, a water-cooled condenser was inserted into the neck of the reaction flask to prevent evaporation, which would be greatly accelerated at the higher temperatures.

The meat sample was weighed prior to and following rehydration to determine the amount of water absorbed. Immediately following enzyme inactivation, the reaction mixture was emptied into a 20-mesh sieve and the buffer collected for pH determination. The meat sample was weighed and a constant weight was then subjected to Kramer shear press analysis.

Chemical Determinations

A. Protein Nitrogen

The commercial proteolytic enzymes used were sold as a crude mixture which was diluted with a filler to a specific activity. Previous workers, when dealing with meat tenderization through the use of proteolytic enzymes, expressed the enzymes used in terms of percentage concentration (weight/volume). As a result it was necessary to conduct protein-nitrogen determinations on the commercial preparations to specify the concentrations of enzyme used. For each enzyme, six protein-nitrogen determinations were made. All determinations were carried out by using the micro-Kjeldahl procedure (Ogg, 1960). Boric acid was used as the receiving agent.

Non-protein nitrogen determinations were also used as

a method of determining enzyme inactivation. Sosebee et al. (1963), after reconstituting freeze-dried chicken in proteolytic enzyme solutions, used an enzyme inactivation time of ten minutes at 100°C. It was believed that the added effects of heat on tenderness could be reduced by reducing the inactivation time. Thus, it was reasoned that if after the heating period the enzyme was not destroyed, then there should be continued proteolysis with a resultant increase in non-protein nitrogen. This non-protein nitrogen could then be determined by the micro-Kjeldahl method.

A procedure was developed which involved heating the reaction mixture at 100°C for three minutes. The reaction mixture was transferred from the reaction flask to a Waring blendor and blended for 60 seconds. Two 10-ml aliquots were pipetted from the blended mixture and deposited in two large test tubes. Twenty ml of 20% trichloroacetic acid was added to each of the test tubes, which were then shaken vigorously. This resulted in the precipitation of all of the protein. The samples were held for five minutes and filtered twice through Whatman #3 filter papaer. Ten ml of the filtrate was pipetted directly into a micro-Kjeldahl flask for nitrogen determination.

During the five-minute time interval mentioned, the remaining blended mixture was transferred to a clean 500 ml wide-mouthed Erlenmeyer flask and returned to the thermostatically controlled water bath. Additional, duplicate aliquots of this mixture were taken at intervals of 30 and 60 minutes.

B. Moisture

Moisture determinations were made immediately after freeze-drying on samples selected at random. Such determinations were also conducted on the crude enzyme preparations to calculate their initial moisture content.

Moisture contents were determined according to the procedure outlined by the A.O.A.C. (1960). This method involved drying a ground sample for 16 to 18 hours in a dry-air oven at 100°C.

Physical Determinations

A. pH Measurements

All pH determinations were made with a Beckman Zeromatic pH meter. pH of rehydration solutions were measured 60 minutes after reconstitution in enzyme solutions. The pH of each freeze-dried chicken breast was measured after first rehydrating for five minutes in distilled water at a water-to-sample ratio of 6:1 and then blending in a Waring blendor for 60 seconds. A 25-ml aliquot of the blended mixture was used to determine pH.

B. Heat Penetration

Several control samples from Part II were rehydrated as usual, except without enzymes. Before rehydration, six thermocouples from a Brown recording potentiometer were connected in series and one thermocouple inserted into each of six pieces of freeze-dried chicken breast samples. One thermocouple measured the temperature of the reaction mixture.

The meat containing the thermocouples was immersed in the buffer-cysteine rehydration solution; the condenser was attached; the reaction flask was placed in the controlled water bath; and the meat was rehydrated for five minutes. A multipoint potentiometer, recording every 30 seconds, provided data for the time-course curve which consisted of the temperatures required to bring the reaction mixture to water-bath temperature. Similar data were obtained for all five temperatures used in the study.

Samples were taken directly from the water bath and placed on a hot flame without removal of the thermocouples and heated to 100°C. The air condenser was replaced by the water condenser during the "come-up" time. The time-course curve obtained showed the time involved at the various reaction temperatures to bring the reaction mixture up to 100°C. The data obtained showed that the individual meat cubes reached 100°C 15 seconds after the buffer-cysteine solution. Thus, 3¼ minutes was the actual time used for enzyme inactivation rather than 3 minutes--the excess 15 seconds allowed for the delay in heat transfer.

Measurements of Tenderness

A. Warner-Bratzler Shear

Tenderness of rehydrated freeze-dried chicken breast muscle from birds in Part I was measured with a Warner-Bratzler shear. This shear press consists of a steel blade 1/32 of an inch thick, with a triangular-shaped aperture

slightly larger than the sample of meat. Each sample was placed in the aperture, and the blade pulled through a rigidly supported opening formed by two steel plates. The steel plates were just wide enough to allow free passage of the blade. The gear system was powered by a constant-speed, 1/12-horsepower motor. The shear readings were taken from a spring scale calibrated in pounds.

Each sample was sheared four times at intervals of approximately 11/16 inches. The first shear was obtained at the anterior end of the muscle.

Since a cross-sectional core of breast muscle was very difficult to obtain from chicken, a different method was used to express the shear force from the chicken meat. After each shear, the muscle section was pressed lightly against an ink blotter (a piece of coarse Whatman filter paper would be satisfactory). The moist outline remaining was traced with an ink pen, and the area was measured by taking duplicate readings with a Keuffel and Esser compensating polar planimeter. The force was thus ultimately expressed in pounds of force to shear each square inch of cross-sectional muscle area. An average of the four shear values was used as the tenderness value for each individual sample.

B. Sensory Evaluation

The "triangle test", as recommended by Pearson (1963), was used to select the panel. Five panel members, as recommended by Ohlson (1955), were used.

Panel members were selected from the staff of the Food

Science Department, who had previous experience on other taste panels. The project, objective, score sheet, and tasting procedure were explained to panel members during training. Trial practice sessions were held using dehydrated freeze-dried chicken several times before the actual project began.

A seven-point hedonic scale was used in this study. Although space was provided on the score card for the evaluation of four factors--initial tenderness, residual tenderness, juiciness and flavor--only the first three factors were considered. (Initial tenderness was defined as the sensation realized after completing the first chew through the sample, while residual tenderness referred to the sensation realized after the complete mastication of the sample.) Even though more than one factor was evaluated, the panelists were asked to evaluate each sample independently from other samples. Descriptions for initial and residual tenderness ranged from "extremely tender" to "very tough" (Appendix Table 12). The word descriptions for juiciness ranged from "very juicy" to "very dry".

When the tenderness of breasts from birds of different ages were evaluated by this panel and calculated statistically, the initial and residual tenderness factors were not significantly different. Therefore, only the residual tenderness was used for the evaluation of enzyme-treated chicken.

For statistical purposes each description was assigned a point value; i.e., the toughest category was assigned

seven points and the most tender category, one point.

Since the Warner-Bratzler shear was used to shear each sample of chicken breast four times, this provided five individual sections of breast muscle for immediate panel evaluation. The first shear was obtained from the anterior end of the sample and the remaining three shears in succession. Each panel member obtained approximately the same section of muscle every time. Five samples were rated by each panelist at each sitting.

Panel members were provided coded plates with five randomly selected numbers marked on each plate. At each sitting, each panel member was provided a plate with a different code. Samples were rotated so that at no two successive sittings were samples of the same age on the same number.

The panel members were asked to chew each sample across the grain of the meat. All samples were evaluated at room temperature. The performance of panel members was checked periodically by providing identical samples for evaluation.

C. Kramer Shear Press

An Allo-Kramer shear press with a Model SP-12 recording attachment was used to measure the tenderness of rehydrated chicken breast muscles in Part II.

The Kramer shear press measured the maximum pressure required to force the shearing ram through the material. The instrument contains an electronically sensitive pressure plate, which registers through a proving ring. The pressure-

sensitive plate was connected through an amplifier to a recording chart. As the force was applied to the sample in the shearing cell, the proving ring compressed (deflected) by an amount proportional to the force. This compression was detected by an electrical transducer, whose output was an electrical signal of amplitude proportional to the deflection. The output signal of the proving ring was amplified to the recording mechanism. It was possible to record continuous pressure as the press ram completed its downward stroke. The shearing head was located on the end of the press and contained several thin, rectangular-shaped blades which passed through the product. A pressure-time curve was obtained from the recorder, and this curve was used to calculate the work required to penetrate the product. Wells et al. (1962) found that the peak of the pressure-time curve was as accurate as the area under the curve for measuring tenderness of rehydrated freeze-dried chicken.

All of the samples were placed in a single layer in the shearing cell with the grain of the meat perpendicular to the shearing blades. Measurements were based on the pounds of force required to shear the sample. In order to simplify comparisons, all of the results were expressed in pounds of force necessary to shear each gram of sample. A proving ring setting of 1,000 pounds was used, which required that each shear value be multiplied by a factor of 10. Since the recorder chart paper was divided into single-unit increments from one to 100, this multiplication factor allowed the 1,000

pounds to be equally dispersed over the entire scale. A 3,000-pound proving ring was used and the shear press speed was standardized so that each downward stroke of the ram was completed in 15 seconds.

Tissue Preparation for Microscopic Examination

Samples of cooked, rehydrated, freeze-dried chicken breast muscle were placed in tissue buttons and subjected to the following treatment in an Autotechnicon as suggested by the Armed Forces Institute of Pathology (1960):

Step	Procedure	Time
1	Dehydrated in 70% ethyl alcohol	Holding point
2	" " 80% ethyl alcohol	1 hour
3	" " 95% ethyl alcohol	1 hour
4	" " 95% ethyl alcohol	1 hour
5	" " 100% ethyl alcohol	1 hour
6	" " 100% ethyl alcohol	2 hours
7	Cleared in 100% ethyl alcohol + xylene (50:50)	1 hour
8	Cleared in Methyl benzoate	1 hour
9	Cleared in Xylene	1 hour
10	Infiltrated in Paraffin (50-52°C)	1½ hours
11	Infiltrated in Paraffin (56-58°C)	1½ hours

Since the above samples were from cooked muscle, the samples were not fixed in 10% Formalin. Cooking, by itself, is a fixing procedure. However, raw samples were placed in 10% Formalin for six hours preceding the above procedure.

After the above cycle was completed, the container of paraffin and tissue samples was removed from the Autotechnicon and placed in a vacuum oven at 60°C for 20 minutes. The tissue samples were then immersed in new paraffin and returned to the vacuum oven for 20 minutes. They were then imbedded in paraffin (56-58°C), which was allowed to solidify. Six

individual tissue samples were placed in each embedding mold.

After solidifying the mold in running tap water, tissue blocks were cut, trimmed and labeled. A 12-hour period of soaking in distilled water was required prior to sectioning, because of the extremely brittle nature of the cooked tissues. Tissues were sectioned at six microns on a Spencer #820 microtome. Slides were coated with Mayer's egg albumin so tissues would adhere. This solution was composed of blended egg white and glycerine in a 1:1 ratio. The ribbons of sections were floated on water at 48°C. The coated slides were then immersed in the bath and the sections floated onto the slides. The slides were placed in staining racks and air dried for at least 12 hours.

The racks containing the slides were placed in a 56°C oven for one hour. This melted the paraffin so that it would not interfere with staining.

All slides prepared in this experiment were stained with a modification of Masson's trichrome stain (Masson, 1929) according to the following procedure:

Step 1. Deparaffinized in Xylene for 5 minutes
2. Deparaffinized in Xylene for 5 minutes
3. Deparaffinized in 100% ethyl alcohol for 3 minutes.
4. Rehydrated in 95% ethyl alcohol for 3 minutes
5. Rehydrated in distilled water for 3 minutes
6. Stained in Weigert's iron hematoxylin solution for 10 minutes

7. Rinsed in running tap water for 10 minutes
8. Rinsed in distilled water
9. Stained in Biebrich scarlet-acid fuchsin solution for 10 minutes when raw tissues were used or for 5 minutes in the case of cooked tissues. The solution was saved.
10. Rinsed in distilled water
11. Differentiated in phosphomolybdic acid-phosphotungstic acid solution for 5 minutes. The solution was discarded.
12. Stained in aniline blue solution for 40 seconds in the case of raw tissue or for 10-15 seconds if cooked tissue was used. The solution was saved.
13. Rinsed in distilled water
14. The excess aniline blue stain was removed by placing in 1% acetic acid solution for 4 minutes. The solution was discarded.
15. Dehydrated in 95% ethyl alcohol for 3 minutes
16. Dehydrated in 100% ethyl alcohol for 3 minutes
17. Dehydrated in 100% ethyl alcohol for 3 minutes
18. Clearing in Xylene for 5 minutes
19. Clearing in Xylene for 5 minutes

The tissue sections were mounted in Permount, dried for two hours and labeled.

According to the procedure of Masson (1929), the preparation was stained with Biebrich scarlet-acid fuchsin solution.

Differentiation was accomplished with the phosphomolybdic-phosphotungstic acid solution, which discolored the collagen and fixed the stain to the cytoplasm. Collagen was toned with aniline blue.

In the modified procedure used in this study, the nuclei stained black; the cytoplasm, muscle fibers and intercellular fibers, red; and the collagen, blue.

RESULTS AND DISCUSSION

Part I. Effects of Age on Tenderness

The initial phase of this study was conducted to evaluate the relationship between age of birds and the tenderness of freeze-dried and reconstituted breast muscle. Cooked pectoralis major muscles from White Leghorn hens 11, 20 and 52 weeks of age were freeze-dried in pieces measuring 3 1/2 inches long, 1 3/16 inches wide and normal muscle thickness. Each muscle sample was sheared four times with a Warner-Bratzler shear. Shear values were calculated in pounds of force to shear each square inch of sample. Water uptake during rehydration of freeze-dried samples was obtained and calculated as a percentage increase of the dried weight.

Shear values for freeze-dried and control samples of breast muscle from different aged birds are reported in Table 1. Shear values from freeze-dried samples were higher than from control samples. Differences in tenderness due to freeze-drying were accentuated in samples from 11-week-old birds, and shear force was directly related to age of birds. The volume of water absorbed during rehydration was inversely related to shear force and age of birds.

Shown in Table 2 are results from panel evaluations of tenderness and juiciness of freeze-dried chicken breast muscle from different aged birds. Tenderness and juiciness decreased with increasing numerical scores. Control samples were more tender than freeze-dried samples, and breast meat from young birds was more tender than from 20- and 52-week-

TABLE 1. Effects of freeze-drying on tenderness of chicken breast muscle as determined by shear values

Age of Bird	No. of Samples	Treatment	Water Uptake		Shear Force	
Weeks			%		lbs/sq in	lbs/gm
11	14	control			17.3	
	60	freeze-dried	212.5		48.6	0.68
				diff.	31.3	
20	14	control			33.3	
	60	freeze-dried	173.5		43.8	0.67
				diff.	10.5	
52	14	control			47.6	
	60	freeze-dried	142.7		56.8	0.94
				diff.	9.2	

TABLE 2. Effects of freeze-drying on tenderness of chicken breast muscle as determined by panel scores[1]

Age of Bird	No. of Samples	Treatment	Tenderness		Juiciness
Weeks			initial	residual	residual
11	14	control	2.5	2.3	3.3
	60	freeze-dried	3.5	3.4	3.2
		diff.	+1.0	+1.1	+0.1
20	14	control	3.3	3.0	3.3
	60	freeze-dried	4.5	4.3	3.9
		diff.	+1.2	+1.3	+0.6
52	14	control	5.0	3.9	3.8
	60	freeze-dried	5.7	5.4	4.9
		diff.	+0.7	+1.5	+1.1

[1]Larger numbers represent less tender or less juicy meat.

old birds.

Juiciness scores of freeze-dried and control breast samples from 11-week-old birds were similar. However, juiciness scores of breast samples from 20- and 52-week-old birds decreased with increasing age of birds.

The data were evaluated by analyses of variance to determine significance between treatments. These analyses were conducted according to Snedecor (1956) and are presented in Tables 3-8. The mean panel scores and mean shear press values were compared by Duncan's Multiple Range Test (Duncan, 1955). Significance was determined by calculating the variance ratio (F). In each of the analyses significance was indicated by * (5%) and ** (1%) levels of probability. Two correlation coefficients were computed and are presented along with their parameters in Table 9.

Tenderness was measured objectively by the Warner-Bratzler shear and an analysis of variance is reported in Table 3. Differences in tenderness due to age of birds were significant at the 1% probability level. Results obtained by using Duncan's Multiple Range Test showed that 52-week-old birds were toughest. Unlike the panel scores, shear press values were not significantly different between breasts from birds of the two younger age groups.

In the above analysis, no significant difference in tenderness was noted between right and left pectoralis major muscles, whether tenderness was evaluated by the panel or shear, or using freeze-dried or control breast meat samples.

TABLE 3. Analysis of variance and Duncan's Multiple Range Test for Warner-Bratzler shear values of freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	179	62381.48		
Ages	2	5179.34	2589.67	7.75**
Breasts	1	101.55	101.55	0.30
Age x Breasts	2	664.37	332.19	0.99
Replications	29	7985.65	275.37	0.82
Error	145	48450.57	334.14	

Duncan's Multiple Range Test[1]

Age of birds	20 Weeks	11 Weeks	52 Weeks
Mean shear value	43.8	48.6	56.8

[1]Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

As mentioned previously, two tenderness scores were recorded by each panel member for each sample. One score was based on the sensation realized after the first chew, whereas the other score was based on evaluation after mastication of the sample. Differences in tenderness due to age of birds were highly significant (1% level) as shown in Table 4. The younger birds were more tender. However, no significant difference was found between initial and residual tenderness scores (indicated as treatments) nor between the interaction of these tenderness scores and the differences due to age. Therefore, residual tenderness scores were used in the final analysis of tenderness.

The statistical analysis of residual tenderness scores of the panel is presented in Table 5. Significant differences (1% level) were found among birds of different ages, among panel members and among replications.

A significant F value for replications was anticipated. Individual birds vary considerably in tenderness, and replications represent individual breasts. Although tenderness scores varied significantly among panel members, individual tenderness scores were fairly consistent.

Since there were significant differences in tenderness between age groups, the Multiple Range Test was used to evaluate significant differences between means. It was found that 11-week-old birds were significantly more tender than the 20-week-old birds, and the latter were significantly more tender than the 52-week-old hens. The lower numerical ratings

TABLE 4. Analysis of variance between initial and residual panel tenderness scores of freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	179	324.76		
Ages	2	134.68	67.34	60.67**
Treatments	1	2.22	2.22	2.00
Age x Treatments	2	0.81	0.41	0.37
Replications	29	26.44	0.91	0.81
Error	145	160.61	1.11	

TABLE 5. Analysis of variance and Duncan's Multiple Range Test for panel tenderness scores of freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	899	2090.38		
Ages	2	581.73	290.87	192.63**
Breasts	1	1.77	1.77	1.17
Age x Breasts	2	0.52	0.26	0.17
Panel	4	129.79	32.45	21.49**
Age X Panel	8	12.29	1.54	1.02
Breasts x Panel	4	0.22	0.06	0.04
Age x Breasts x Panel	8	8.39	1.01	0.67
Replications	29	87.78	3.03	2.01**
Error	841	1267.89	1.51	

Duncan's Multiple Range Test

Age of birds	11 Weeks	20 Weeks	52 Weeks
Mean Tenderness Score	3.4	4.2	5.4

represent more tender samples.

Similar results were obtained by an analysis of variance of panel tenderness scores from non-freeze-dried control samples (Table 6). Although no significant difference was found among replications, a significant F value was noted among mean scores of panel members and age of birds. Duncan's Multiple Range Test indicated that panel tenderness scores decreased with increasing age of birds.

Panel members were asked to evaluate juiciness of breast meat samples. The analysis of variance of juiciness scores is presented in Table 7. Differences in juiciness scores due to age of birds were highly significant, as were differences among scores of panel members and replications (individual breasts). Results from Duncan's Multiple Range Test showed that juiciness scores of samples from all three age groups were significantly different. Juiciness and tenderness of breast meat decreased with an increase in age of birds. Steinberg (1960b) found no significant correlation between objective tests and sensory tenderness and juiciness scores of freeze-dried, cooked beef. The results of this study did not confirm his findings but instead supported those of Deatherage (1951) who reported a positive correlation between tenderness and juiciness scores.

In the analysis of variance of the percentage of water uptake (Table 8), breast meat samples from birds of different ages absorbed significantly different amounts of water. Percentage of water uptake was inversely related to age of birds.

TABLE 6. Analysis of variance and Duncan's Multiple Range Test for panel tenderness scores of non-freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	209	336.20		
Ages	2	90.20	45.10	43.37**
Breasts	1	0.04	0.04	0.04
Age x Breasts	2	5.61	2.81	2.70
Panel	4	34.55	8.64	8.31**
Age x Panel	8	13.52	1.69	1.63
Breasts x Panel	4	3.70	0.93	0.89
Age x Breasts x Panel	8	3.38	0.42	0.40
Replications	6	3.43	0.57	0.55
Error	174	181.77	1.04	

Duncan's Multiple Range Test

Age of Birds	11 Weeks	20 Weeks	52 Weeks
Mean Tenderness Score	2.3	3.0	3.9

TABLE 7. Analysis of variance and Duncan's Multiple Range Test for panel juiciness scores of freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	899	1674.60		
Ages	2	467.90	233.95	184.21**
Breasts	1	1.69	1.69	1.33
Age x Breasts	2	0.78	0.39	0.31
Panel	4	55.59	13.90	10.94**
Age x Panel	8	16.39	2.05	1.61
Breasts x Panel	4	0.71	0.18	0.14
Age x Breasts x Panel	8	3.64	0.46	0.36
Replications	29	62.90	2.17	1.70*
Error	841	1065.00	1.27	

Duncan's Multiple Range Test

Age of Birds	11 Weeks	20 Weeks	52 Weeks
Mean Juiciness Score	3.2	3.9	4.9

Since tenderness also decreased with increasing age (Table 2), a direct relationship was found between percentage rehydration and tenderness.

Two correlation coefficients were obtained from the data summarized in the analysis of variance tables and are included in Table 9. The panel tenderness scores for chicken breast meat from birds of each age group studied were correlated with the Warner-Bratzler shear values obtained from the same meat samples. A correlation coefficient $r = 0.59$ was calculated between panel tenderness scores and Warner-Bratzler shear values using freeze-dried chicken, as compared to a correlation coefficient $r = 0.80$ for similar control samples. Freeze-dried breast meat was noticeably tougher than control samples.

Unlike the results of Wells et al. (1962), shear values for freeze-dried chicken breast in this study agreed with panel scores. However, more significant differences were noted when breast muscles were measured by the sensory panel than when measured by the Warner-Bratzler shear. With the shear, only the older birds were significantly different in tenderness, whereas the panel found all three age groups to differ significantly.

Seltzer (1961) found that older, more mature birds produced the most tender freeze-dried chicken meat. The results of this study do not support his findings but instead agree with the sensory results of Wells et al. (1962).

Tenderness varied greatly between individual birds; most

TABLE 8. Analysis of variance and Duncan's Multiple Range Test for percentage water uptake by freeze-dried breast meat from birds of three different ages

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	179	280294.1		
Ages	2	146930.9	73465.4	100.4**
Breasts	1	760.9	760.9	1.0
Age x Breasts	2	260.3	130.2	0.2
Replications	29	26262.8	905.6	1.2
Error	145	106079.2	731.6	

Duncan's Multiple Range Test

Age of Birds	52 Weeks	20 Weeks	11 Weeks
Mean % Water Uptake	142.7	173.5	212.5

TABLE 9. Correlation analyses for panel tenderness scores and Warner-Bratzler shear values

Sample	$\bar{x}$	Sx	$\bar{y}$	Sy	b	$Sy \cdot x^2$	r
Freeze-Dried Chicken	4.28	1.27	49.24	18.39	8.49	150.77	0.59
Non-Freeze-Dried Chicken	3.06	0.89	32.71	18.25	16.46	123.56	0.80

of this variation was accounted for in the replications of the analyses. The remaining variation was present as part of the error term.

In many instances, tenderness scores of individual panel members differed significantly. This significant variation was the result of lower-than-average scores from one panel member and higher-than-average scores from another. However, this should not imply that any one panel member was inconsistent in evaluating samples; actually, a very high degree of consistency was noted.

Part II. Effects of Enzyme Treatments on Chicken Breast Muscle

Proteolytic enzymes were incorporated in rehydration solutions to cause proteolytic breakdown and increased tenderness in the meat.

Wang et al. (1958) found that Rhozyme P-11 and papain were similar in their ability to hydrolyze gelatin, but 150 times as much Rhozyme P-11 as papain was needed to affect significantly the initial tenderness of meat. Therefore, in the present study, it was decided to base the concentration of enzyme on percentage rather than on activity. Preliminary trials led to the establishment of an effective concentration range for each enzyme for optimum tenderization. Different enzyme concentrations were placed in the rehydration solutions, and after the samples were rehydrated, they were evaluated for tenderness by the sensory panel. Table 10 shows the results of these evaluations. The sample was considered

TABLE 10. Effects of proteolytic enzyme concentration on tenderness and acceptability of freeze-dried chicken breast meat as determined by a panel

Enzyme	Concentration (Weight/Volume) (%)	Average Tenderness Score	Acceptable ?
Rhozyme P-11	0.010	3.2	yes
"	0.015	2.9	yes
"	0.020	2.6	yes
"	0.025	2.2	?
"	0.030	1.7	no
Ficin	0.0001	4.2	yes
"	0.0005	3.3	yes
"	0.0010	2.4	no
"	0.0020	---[a]	no
Bromelin	0.0005	4.1	yes
"	0.0010	3.2	yes
"	0.0020	2.7	yes
"	0.0030	1.8	no
Papain	0.001	3.3	yes
"	0.002	2.6	yes
"	0.003	2.3	no
"	0.005	1.3	no

[a]Too "mushy" to give to the panel and was given an automatic unacceptable rating.

acceptable when four of the five members agreed that it was not too soft or "mushy". The sample was rated as questionable when two panel members thought the sample was unacceptably tender. When more than two members found that a sample was too soft or "mushy", it was given an unacceptable rating. Thus, it was possible to find an enzyme concentration of optimum strength for producing the most tender yet acceptable product.

An enzyme concentration of 0.02% was found optimum for Rhozyme P-11, whereas considerably smaller concentrations were most desirable for the other three enzymes. Ficin was most desirable at a concentration of between 0.0005% and 0.001%. A value of 0.0008% was used. Both bromelin and papain exerted maximum acceptable effectiveness at a concentration of 0.002%. In most cases, average panel scores of 2.0 or below were not acceptable. Enzyme concentrations were inversely related to tenderness values.

The most desirable concentration for each enzyme was used to find an optimum pH. Also considered was the phenomenon of water uptake. These two factors could not really be separated, since an increase in the amount of solution absorbed by the meat would result in more enzyme being absorbed into the structure of the meat, and it was conceivable that rehydration might be affected by pH.

Effects of pH on tenderness of breast meat are presented in Figures 1 and 2. Each point plotted, except at pH 4.0, represents average tenderness values of five replicate samples

as determined by the Kramer shear press. The same data were plotted for controls in each figure. The most desirable enzyme concentrations (from Table 8) were selected for use. Temperatures of rehydration solutions were 50°C for Rhozyme P-11 and 70°C for the other three proteases.

Two important observations are: (1) Ficin, bromelin and Rhozyme P-11 all produced the most tender samples at pH 5.0 while pH 7.0 was optimum for the most tender papain-treated samples. (2) The non-enzyme-treated control samples were noticeably more tender at pH 7.0 than at the other pH values. The enzyme treated samples also showed a marked increase in tenderness at pH 7.0.

Since pH 5.0 was the lowest pH originally selected for study and preliminary results indicated that maximum tenderness occurred at this pH, it was considered desirable to test the results of the tenderizing action at pH 4.0. As expected, the shear values for bromelin and Rhozyme P-11 were higher at pH 4.0 than at pH 5.0. At pH 4.0, these enzymes were inactivated, since the shear force required to penetrate the meat samples was of the same magnitude as that required for the controls. Samples were not rehydrated in ficin solutions at pH 4.0 because of the lack of birds with the same background.

All enzymes appeared to possess some activity over the pH range 5.0 to 9.0. However, ficin activity was greatly reduced at pH 9.0.

It is not entirely clear why the controls were more

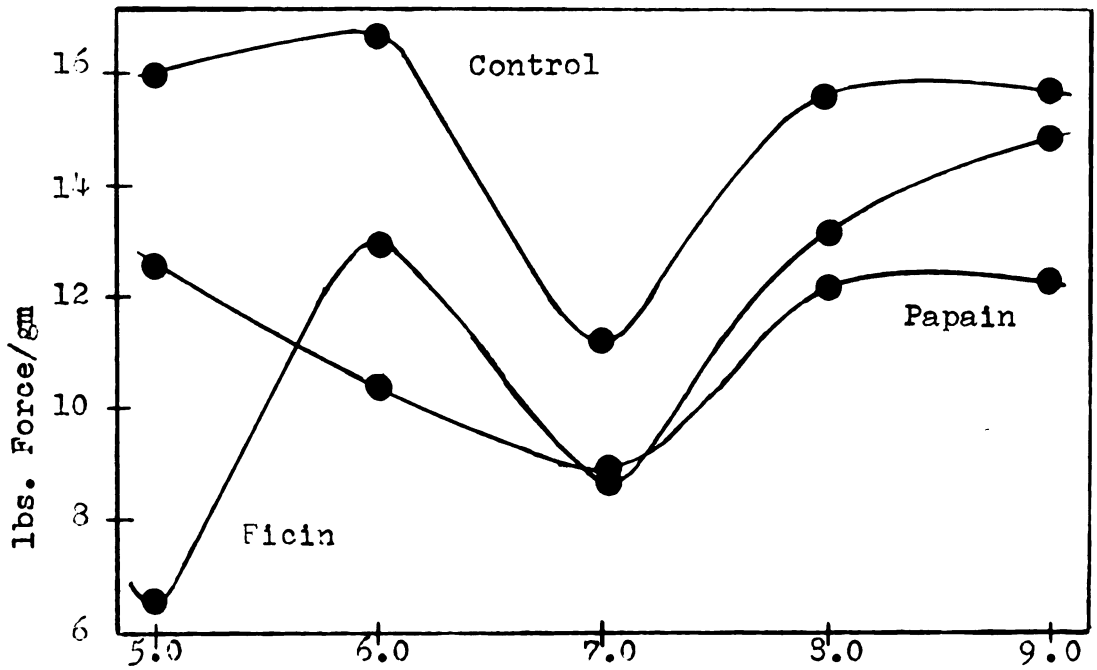


Figure 1. Shear press values of freeze-dried chicken rehydrated in papain and ficin solutions at various pH values.

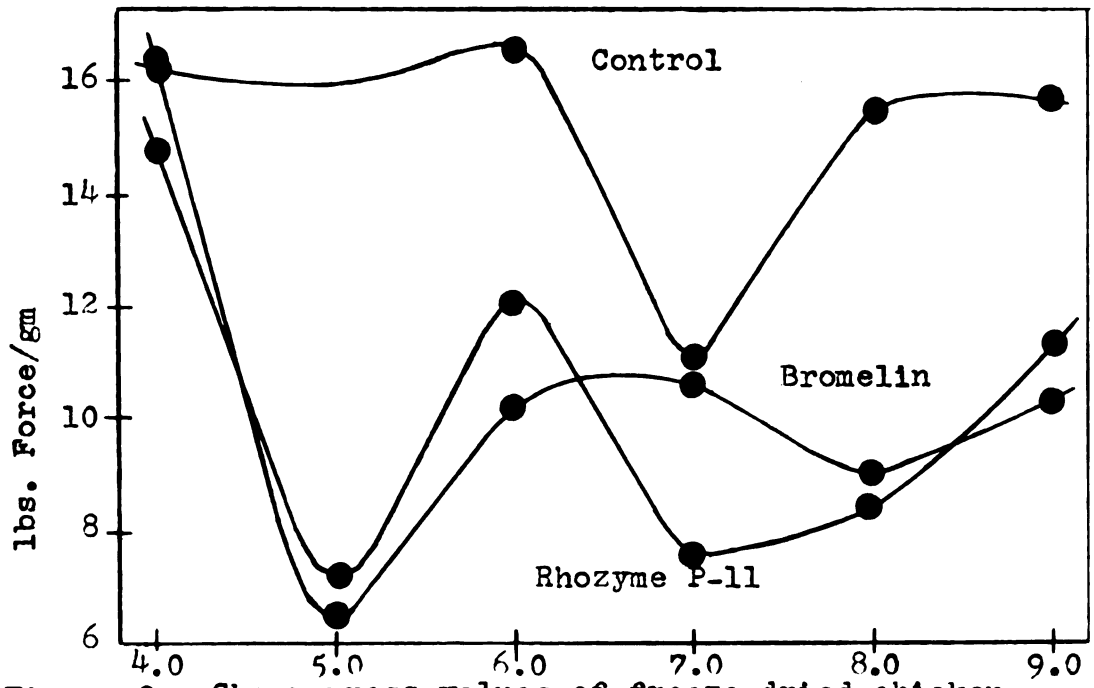


Figure 2. Shear press values of freeze-dried chicken rehydrated in bromelin and Rhozyme P-11 solutions at various pH values.

tender at pH 7.0, but it is probably due somewhat to the extent of the rehydration. Auerbach et al. (1954) found that the highest level of rehydration of freeze-dried beef occurred in solutions in which the pH was near 7.0, regardless of the osmotic pressure. Similar results were obtained in the present study with the controls and are presented in Figure 3. (The scale for % water uptake was reversed for ease of plotting.) The greatest amount of water uptake occurred at pH 7.0 as did the lowest shear values. The lower tenderness scores of enzyme-treated meat at or near pH 7.0 may be attributed in part to the relationship of water uptake to shear force. Although water uptake may have accounted for the increased tenderness produced by papain at pH 7.0, this pH was optimum since no other depression in that curve was obtained.

In the present study, the determination of optimum pH for enzymatic action on freeze-dried meat actually includes the consideration of both pH and rehydratability. This optimum pH for activity is not necessarily the same as that found by gelatin or hemoglobin assay (Wang et al., 1958).

Although Sosebee et al. (1963) used papain and Rhozyme P-11 during rehydration of freeze-dried chicken, no attempt was made to control the pH of the solutions. Also, the reaction temperatures selected were not necessarily optimum for the enzymes used for tenderization.

The pH values of rehydration solutions were determined before and after reconstitution (Table 11). When pH was

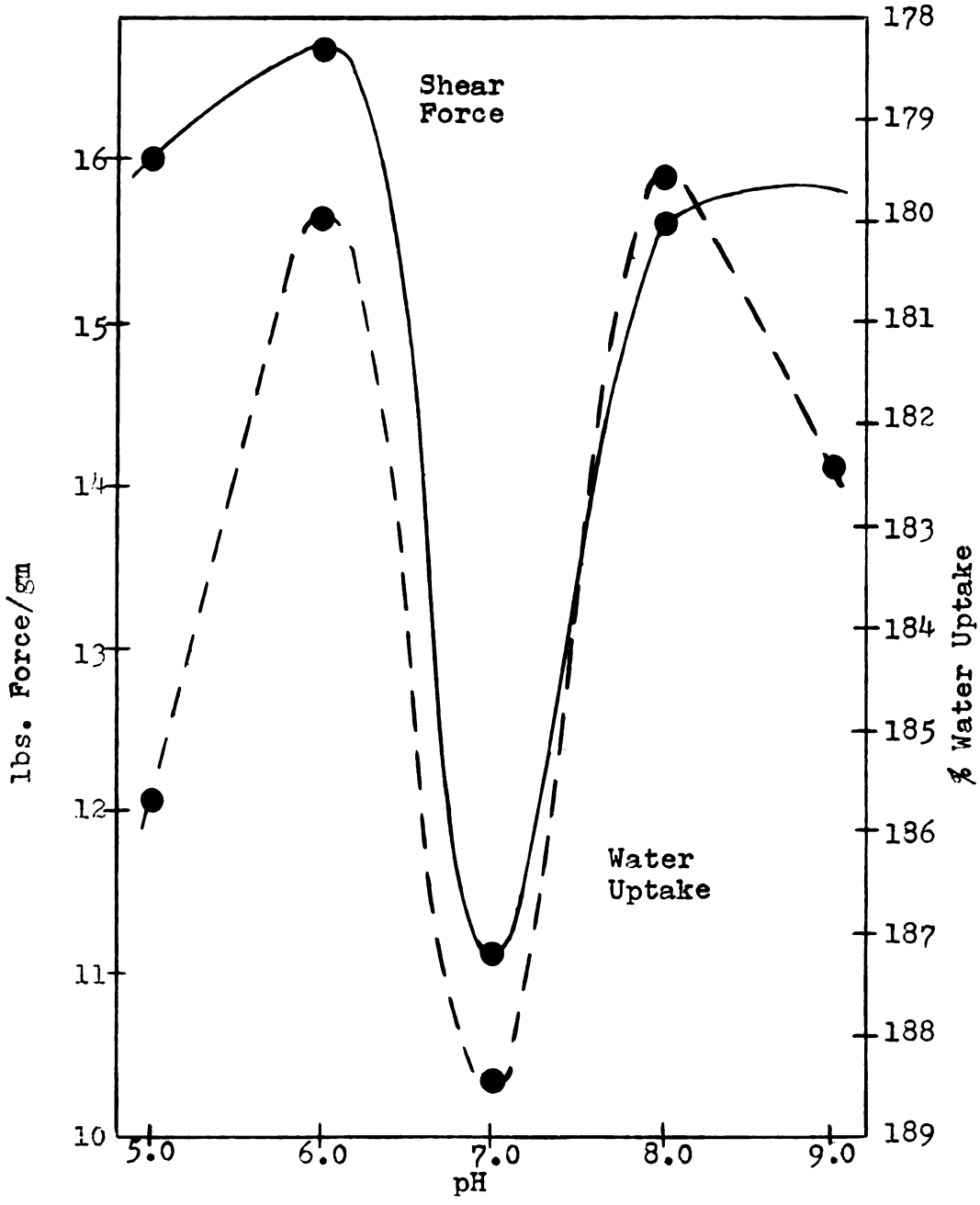


Figure 3. Relationship between water uptake of freeze-dried chicken and shear force values.

TABLE 11. Change in pH of rehydration solutions during reconstitution

Initial pH	Average pH after Reconstitution	Difference
4.0	3.9	-0.1
5.0	5.0	0.0
6.0	5.6	-0.4
7.0	6.7	-0.3
8.0	7.5	-0.5
9.0	8.4	-0.6

measured after the normal five minutes of rehydration time but without the addition of meat, there was no change in pH. Therefore, when there were changes in pH of the solutions, they were attributed solely to the meat samples. All changes in pH were to the acid side of the initial pH. This was believed due to the lower pH of the freeze-dried meat (pH 5.8) and to the increase in free acidic groups resulting from proteolysis.

Figures 4 and 5 show the pounds of force to shear each gram of sample, when samples of freeze-dried breast muscle were rehydrated at temperatures from 40° to 80°C. Optimum rehydration temperatures of 50°, 50°, 60° and 70°C were selected for papain, Rhozyme P-11, bromelin and ficin, respectively. Each point recorded in these figures represents the average force obtained from five replicate samples. The same control data were used in each figure.

Unlike the pH-shear force curves, temperature-shear force curves showed no sharp peaks. Variations in the temperature of the rehydrating solution did not appear to be as critical as variations in pH in the evaluation of tenderness. Ficin and bromelin were quite active at all the temperatures used. Papain and Rhozyme P-11 possessed little or no activity at 80°C. In general, papain was less active than the other enzymes. The tenderness of control samples was not affected by rehydration temperature.

The percentage of water uptake by the control samples was plotted against temperature (Figure 6). The percentage

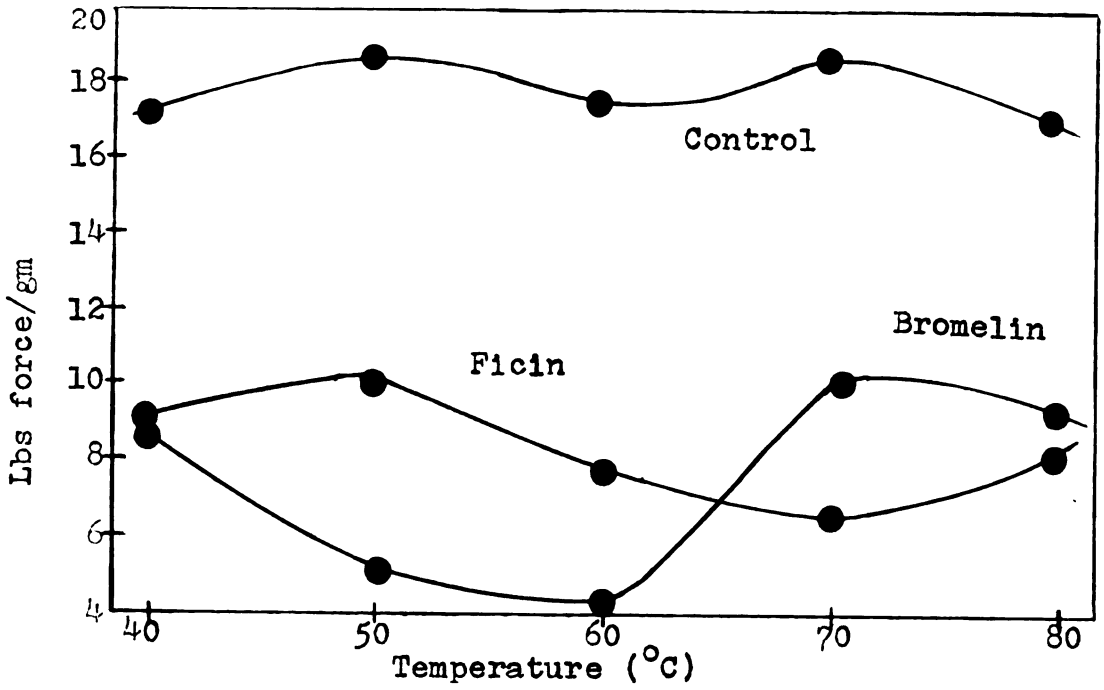


Figure 4. Shear press values of freeze-dried chicken rehydrated in ficin and bromelin solutions at various temperatures.

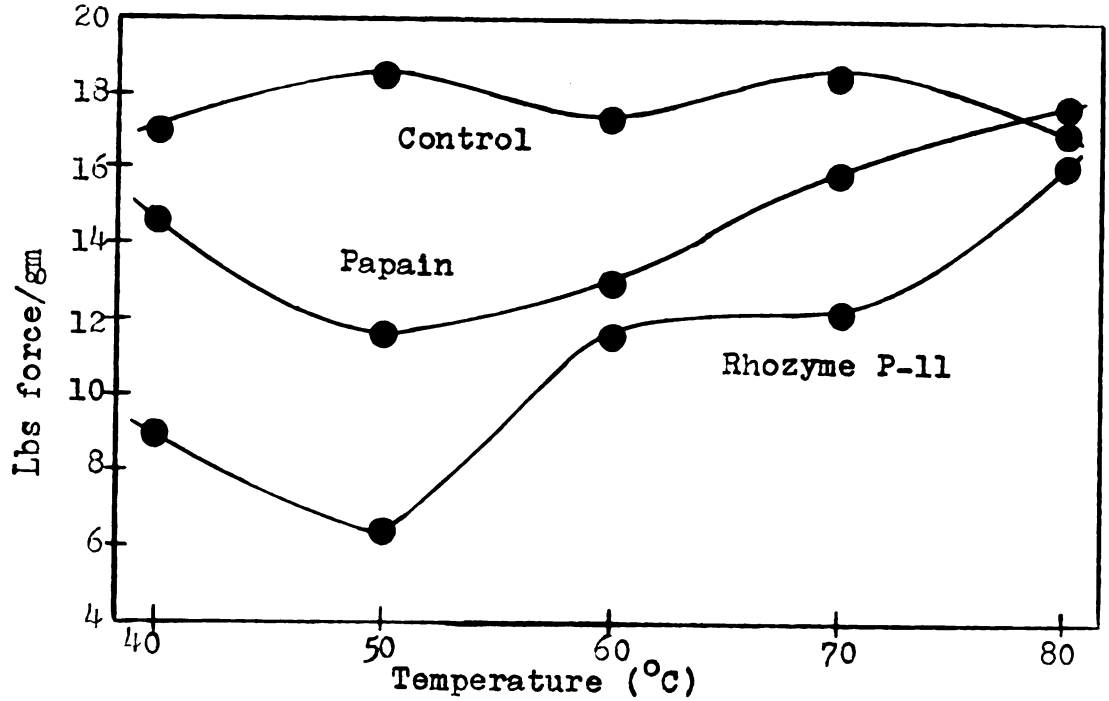


Figure 5. Shear press values of freeze-dried chicken rehydrated in papain and Rhozyme P-11 solutions at various temperatures.

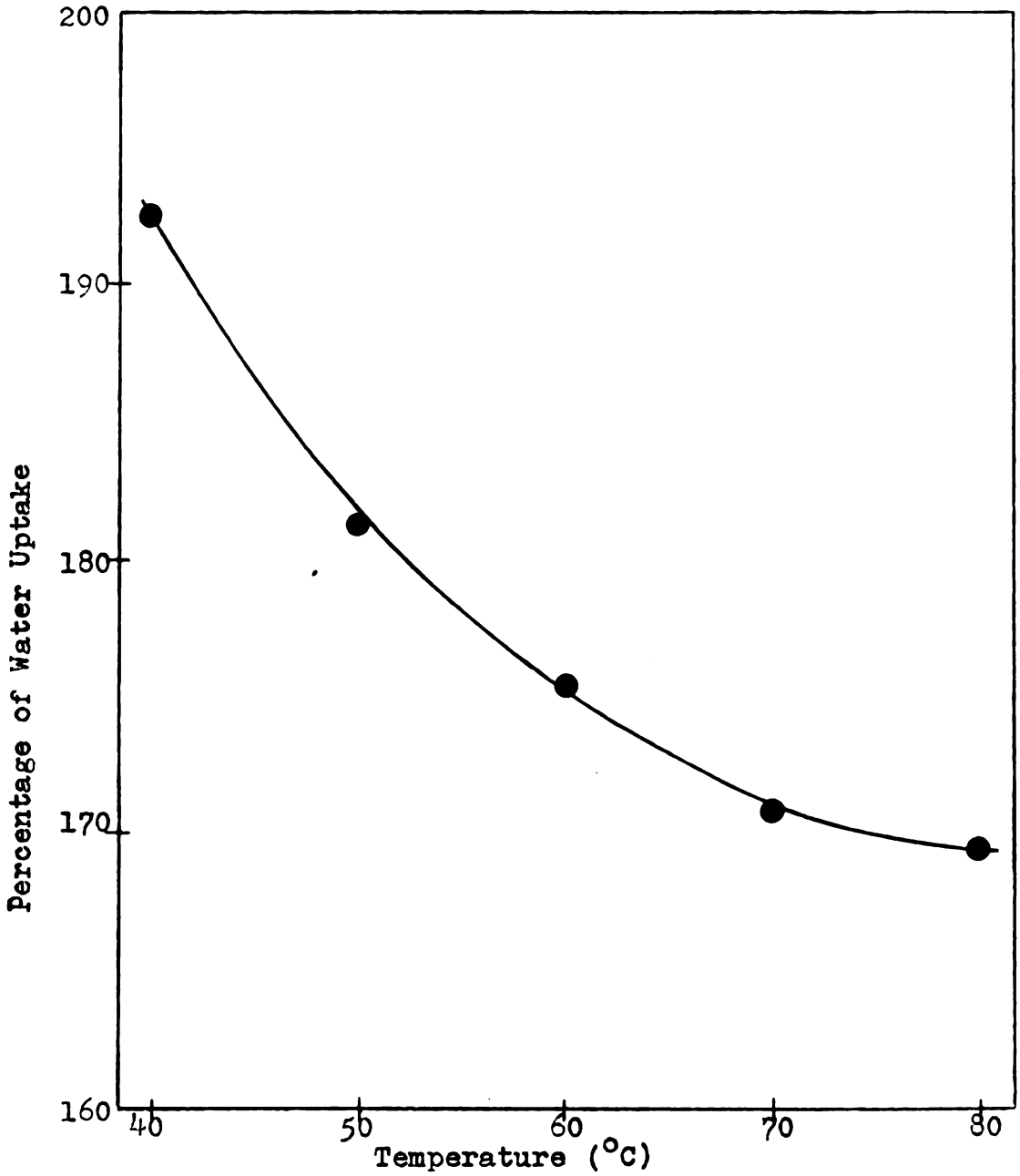


Figure 6. Percentage water uptake of freeze-dried chicken at various temperatures.

of absorbed water (based on the dry weight) decreased as the temperature of the rehydration solution increased. Steinberg (1960a, 1960c) and Norman and Auerbach (1963) reported similar results with freeze-dried beef.

Although water uptake by control samples was higher at the lower temperatures used, tenderness remained fairly constant (Figures 4 and 5). Thus, shear force (tenderness) is probably not directly related to water uptake. However, Deatherage (1959) reported that tenderness of meat was related to the ability of meat proteins to hold water. He also found that water-binding capacity decreased with increasing temperatures of the rehydrating solution.

Kimmel and Smith (1957) reported that the pH optimum for digestion of fibrin was pH 7.0, and results of the present work agree. Tappel et al. (1956a) and Weiner et al. (1957) reported that the optimum temperature for papain digestion of beef was 60° to 80°C. An optimum temperature of 50°C was reported for papain in the present study. Tappel et al. (1956a) and Weiner et al. (1957) also stated that the optimum temperature for any enzymatic reaction was closely related to the length of time which that reaction covered. In general, the shorter the digestion time, the higher the optimal temperature for that reaction. Weiner et al. (1957) used a three-minute digestion period. Since a five-minute digestion period was used in the present study, it was expected that the optimum temperature would be comparatively lower.

Wang (1957) stated that ficin showed a wide range of pH

activity (pH 5.0 to 9.0) on beef connective tissue with an optimum at around pH 5.0 to 6.0. Results from the present study with chicken muscle agree. Yatco-Manzo and Whitaker (1962) found that ficin-catalyzed hydrolysis of elastin was optimum at a pH 5.0 to 5.5 and at a temperature of 55°C. In the present study, the optimum pH was 5.0 while the optimum temperature was 70°C. However, ficin activity was not greatly affected by the temperature differences investigated.

The optimum pH for Rhozyme P-11 was reported to be between pH 5.5 and 6.0 with an optimum temperature range of 43° to 60°C (Anonymous, 1963). The results of the present study agree, since a 50°C optimum was obtained, although a slightly lower pH optimum of 5.0 was determined.

The optimum temperature range for bromelin was reported to be between 30° and 60°C (Anonymous, 1963). An optimum temperature of between 50° and 60°C was found in the present study.

With optimum concentrations, pH, and temperatures established, chicken samples were rehydrated under these conditions and the resulting proteolysis was examined histologically.

It was necessary to inactivate the enzymes after rehydration. If the enzymes were not completely inactivated, continued proteolysis, after rehydration, would invalidate conclusions based on histological observations.

As stated in the Procedure, preliminary results showed

that a three-minute heating period at 100°C was sufficient for enzyme inactivation. An increase in non-protein nitrogen would occur in the rehydrating solution when proteolysis continued after the three-minute destruction time. Any increase due to continued proteolytic breakdown could be measured by calculation of this non-protein nitrogen.

Non-protein nitrogen was measured in the present study by the micro-Kjeldahl method. The results are reported in Figure 7. Two replicate samples and a control were used for each of the four enzymes. The control samples were not heat treated, and proteolysis was allowed to continue to provide a basis for comparing the heat-treated replicates.

In all heat-treated samples, there were no increases in non-protein nitrogen with time after heat treatment. Slight differences which did exist were attributed to experimental error, since the differences were always less than 0.5 ml of HCl titrated.

A direct linear increase in non-protein nitrogen occurred in control samples held up to 60 minutes of incubation for ficin, bromelin, papain and Rhozyme P-11, respectively. This was probably due to the reaction rates of the individual enzymes used. When the five-minute rehydration period was extended to 30 and 60 minutes, the reaction rate of ficin was greater than the reaction rate of Rhozyme P-11.

Histological sections were prepared from the freeze-dried chicken samples treated with enzymes under optimum conditions for tenderness.

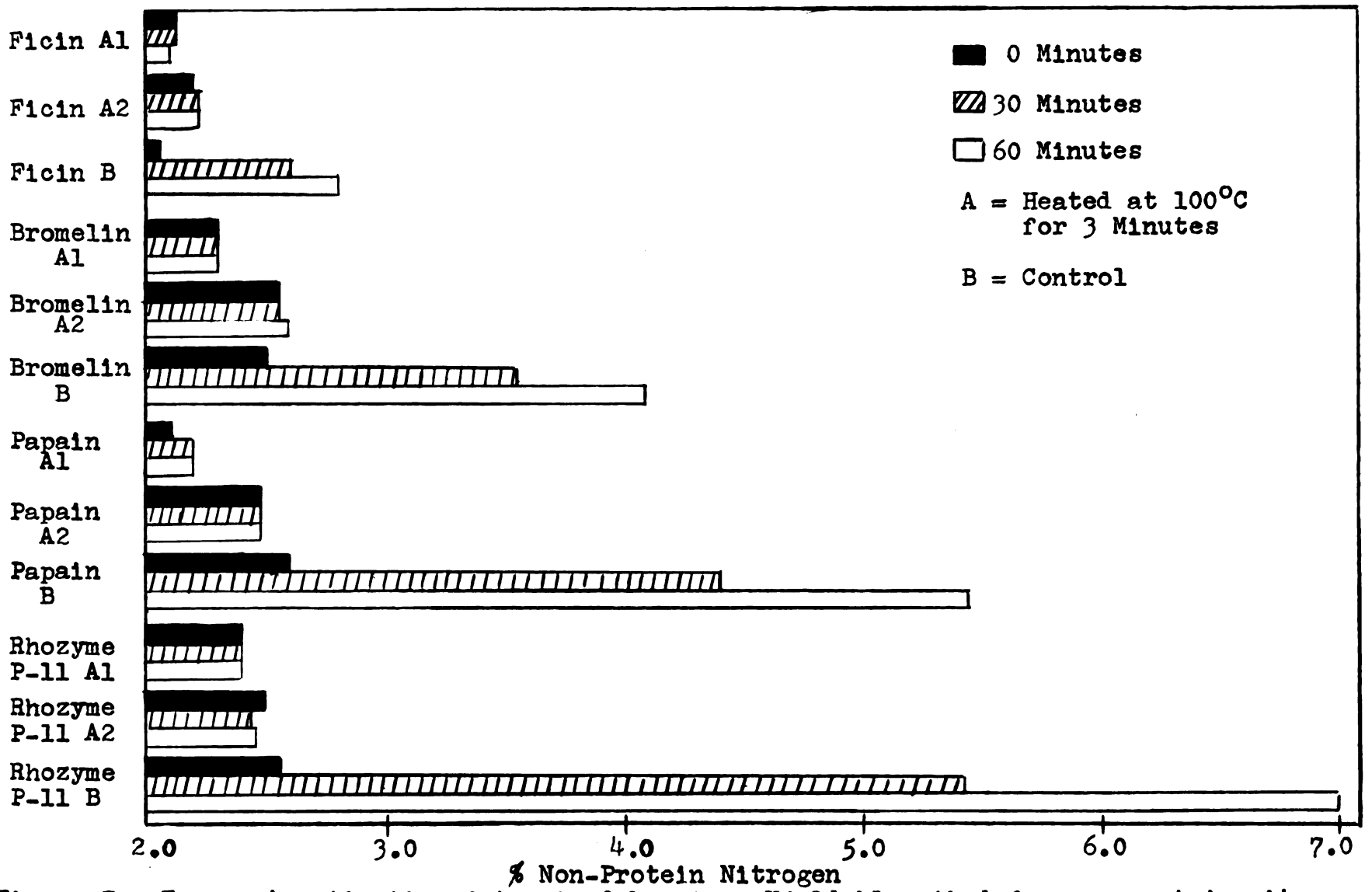


Figure 7. Enzyme inactivation determined by micro-Kjeldahl method for non-protein nitrogen

Many preliminary trials were necessary to perfect a procedure for staining the cooked and freeze-dried sections. A staining technique was needed which, in one operation, would differentiate nuclei, connective tissue and muscle fibers.

A differential stain for elastic fibers and collagen (Margolena, 1951) was first attempted. Microscopic observations showed a minute amount of elastic tissue in breast muscle. Thus, a stain to discern elastic tissue was of little value. Therefore, a general stain for connective tissue was used with the knowledge that it was mostly collagen. Preliminary results indicated that Masson's trichrome stain (Masson, 1929) could be used after considerable modification. After many preliminary trials, it was found necessary to reduce the recommended staining times and concentrations. This was probably a result of staining cooked muscle tissue.

Staining results indicated that cooked muscle tissue was very receptive to both acid and basic dyes and that the differential staining ability of cooked tissue was very poor.

Tissues were stained unevenly and excessive staining with the counter-stain(s) occurred readily. Thus, extreme care was necessary at this step in the procedure.

Many of the histological sections showed similar effects of enzymatic breakdown of the tissue. There were considerable variations in this degree of breakdown, however. Four longitudinal sections of muscle, two horizontal sections of muscle and two horizontal sections of connective tissue are presented

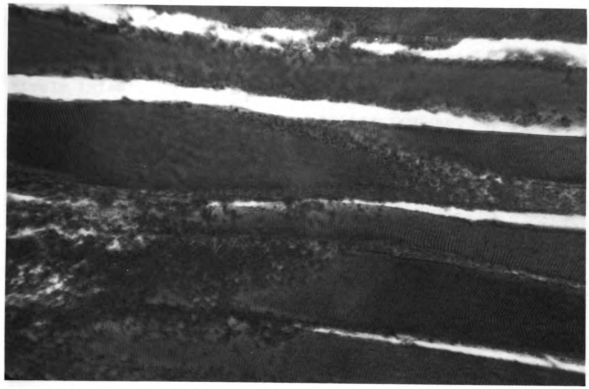
in Figures 8 through 15, respectively.

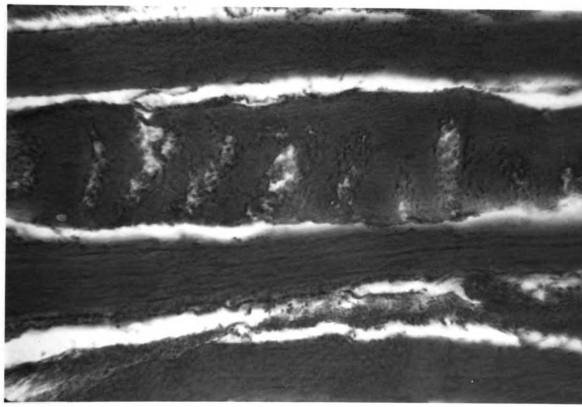
Figure 8 shows a longitudinal section of muscle that was exposed to the action of ficin. This enzyme exerted the greatest effect on the muscle fibers of any of the four used. Disintegration of the sarcolemma was apparent, and complete dissolution is shown in a portion of the bottom two fibers. The extensive granulation was caused by the dissolution of the sarcolemma. A complete absence of nuclei and a gradual disappearance in cross striations was observed. Overall, there was a slight swelling of muscle fibers as compared to controls which were rehydrated in buffer without the addition of enzyme.

The effects of bromelin on chicken muscle are shown in Figure 9. Bromelin was the enzyme least reactive on muscle fibers. Although bromelin definitely affected connective tissue (Wang et al., 1957), it is controversial whether or not bromelin activity can be detected on muscle fibers. Figure 9 shows that there was some action on the muscle fiber. The large, swelled fiber in the center of the photograph shows proteolysis of the sarcolemma with the disappearance of nuclei and cross striations. Nuclei, although poorly stained, are evident in the intact fibers. The fiber was hydrolyzed at specific sites. These results indicate that bromelin did not hydrolyze the fiber in a progressive step-by-step manner, since it attacked various exposed sites along the fiber simultaneously. Although fibers, such as the one shown, were relatively uncommon in chicken muscle treated

Figure 8: Longitudinal section of cooked freeze-dried chicken breast muscle rehydrated in 0.0008% ficin solution at pH 5.0 and 70°C. 430X

Figure 9: Longitudinal section of cooked freeze-dried chicken breast muscle rehydrated in 0.002% bromelin solution at pH 5.0 and 60°C. 430X





with bromelin, several did exist. However, most of the fibers and the remaining structures in the figure were left relatively intact.

The effects of bromelin activity discussed here support the data of Wang et al. (1957) who also found that bromelin had a trace of activity on muscle fibers.

Figure 10 shows the action of papain on muscle fibers. The actions of Rhozyme P-11 and papain were similar--both possessed more activity than bromelin but less than ficin.

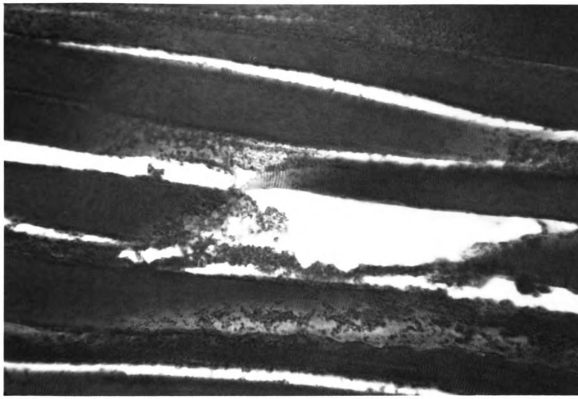
Granular cytoplasmic material may be found throughout the photograph. As in the previous figures, some of this was due to the cooking process, although further granulation occurred with enzymatic activity. A sarcoplasmic breakdown was evidenced by the broken fiber in the center of the figure. There was a gradual loss of cross striations in other fibers. No nuclei are evident since their disappearance was usually the next step in proteolysis after the loss of the sarcolemma.

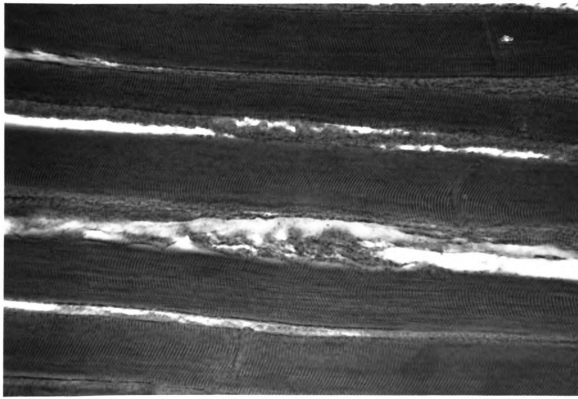
A micro-photograph of a section of cooked, non-freeze-dried and non-enzymatically treated tissue is presented in Figure 11. Cooked, freeze-dried tissue not treated with enzymes had similar structural characteristics and therefore this photograph represents both types of tissue.

The cooked tissue had a certain amount of fiber shrinkage (not as evident here as in cross sections) when compared to raw tissue. The granular material, evident between the muscle fibers, was derived only from the periphery of the protoplasm of the muscle fiber and is distinct from that

Figure 10: Longitudinal section of cooked freeze-dried chicken breast muscle rehydrated in 0.002% papain solution at pH 7.0 and 50°C. 430X.

Figure 11: Longitudinal section of cooked non-freeze-dried enzymatically treated chicken breast muscle. 430X.





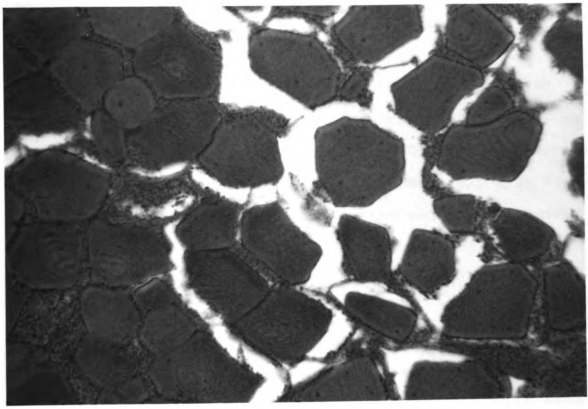
caused by proteolysis. Wang (1957) reported that the sarcolemma itself was not hydrolyzed during the cooking process. The muscle fibers appeared quite intact with the presence of very distinct cross striations. Although the nuclei were visible as small, dark, elongated areas in the fibers, they were not clear. In the center of Figure 11 is a small amount of greyish connective tissue. It is mostly collagen which was coagulated into a gel-like mass by the cooking process.

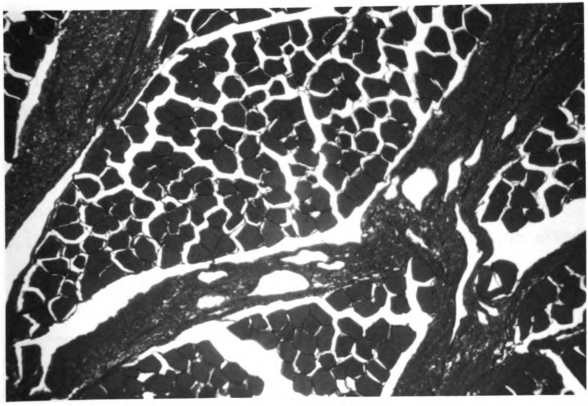
Enzymatic action on the muscle fibers was best observed in longitudinal sections. The four enzymes had marked similarity in their activity when muscle was observed in cross section. (Figure 12 is representative of activity by all four enzymes.) Again, ficin was most active, Rhozyme P-11 and papain were intermediate in activity, and bromelin was least active.

The cooking process resulted in a shrinkage of the main body of the fiber away from the endomysium. The intact endomysium surrounding some of the individual fibers is distinct in the lower central portion of Figure 12. However, the enzymes destroyed the endomysium and extensive fragmentation is visible around the remainder of the exposed fibers. The nuclei are present in some of the fibers, and granular material is evident throughout the compact tissue area and along the endomysium and its fragments, where the granules tend to collect. As evidenced by the more compact cross-sectional area, the enzymes do not completely separate the individual fibers. If they did, there would be no forces to

Figure 12: Cross section of cooked freeze-dried chicken breast muscle rehydrated in 0.0002% ficin solution at pH 5.0 and 70°C. 430X

Figure 13: Cross section of cooked freeze-dried chicken breast muscle rehydrated in 0.002% bromelin solution at pH 5.0 and 60°C. 100X





hold the tissue together, and the muscle would become "mushy" in appearance.

A micro-photograph of a cross section observed under 100X power is shown in Figure 13. The nuclei are evident as is the intact and fragmented endomysium. Of primary interest is the vast granular material, which "eroded" away from the periferal protoplasm and collected in the connective tissue of the perimysial spaces, completely camouflaging the perimysium. A distinct fiber shrinkage is also evident, resulting in rather large endomysial spaces.

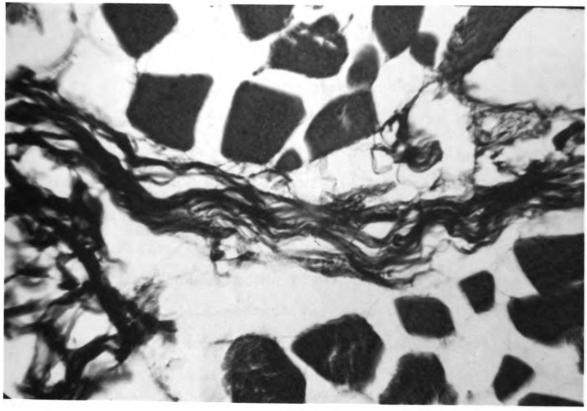
A micro-photograph of a section of raw, once-frozen connective tissue is presented in Figure 14. The connective tissue (collagen) is evident as dark fibers with a wave-like appearance. The fibers were stained with aniline blue, which is an acid aniline dye.

When this raw collagen or connective tissue was heated during the cooking process, it formed a compact gelaceous substance (Figure 15). The connective tissue on the left side of the figure is representative of this heat-coagulated material. No longer do the individual fibers appear wavy. Although elastic tissue was not considered in this study, Weir et al. (1958) stated that cooking did not visibly affect elastic fibers.

During cooking, chemical changes occurred in the collagenous fibers as evidenced by the changes in their affinity for the aniline blue dye. Raw collagenous fibers did stain blue with this dye, but the cooked fibers solidified into a

Figure 14: Horizontal section of uncooked and once-frozen connective tissue. 430X

Figure 15: Horizontal section of cooked freeze-dried connective tissue rehydrated in 0.0008% ficin solution at pH 5.0 and 70°C. 430X



substance which appeared grey.

The enzymes did not possess very much collagenase activity at the concentrations used in this study. Ficin produced the greatest breakdown of connective tissue. Bromelin showed some activity, but Rhozyme P-11 and papain demonstrated little or no activity.

Enzymatic action on cooked connective tissue is demonstrated in Figure 15. There is a gradual dissolution of the connective tissue on the right from the compact mass on the left. This would account for some of the increased tenderness due to enzymatic proteolysis. However, at the concentrations used in this study, the increased tenderness in chicken was probably due to dissolution of the muscle fibers. Since there is much more connective tissue in beef, proteolytic action on this tissue may be of greater importance.

SUMMARY

This study was designed to investigate tenderness of freeze-dried chicken breast muscle, as affected by age of bird and applications of commercial proteolytic enzymes. Proteolysis was observed in histological sections from enzyme-treated samples.

Chickens 11, 20 and 52 weeks of age were selected for tenderness evaluations. A procedure was developed to determine tenderness with the Warner-Bratzler shear press by relating shear force to cross-sectional area sheared. Tenderness data obtained from freeze-dried meat resulted in a correlation coefficient of 0.59 between mean panel scores and Warner-Bratzler shear press values. A correlation coefficient of 0.80 was obtained between similar data from non-freeze-dried muscle. Freeze-dried meat was less tender and more variable in texture than control samples.

Tenderness of muscles was inversely related to age of birds. As age increased, tenderness decreased. Although results were similar, panel scores were better indices of tenderness than were shear press values.

Panel scores indicated that juiciness was directly related to tenderness. The percentage of water uptake was calculated for each sample, and it was directly related to both tenderness and juiciness of the rehydrated samples.

Papain, ficin, bromelin and Rhozyme P-11 were incorporated directly into the rehydration solutions. All freeze-dried samples were rehydrated in the enzyme solutions for

five minutes. A three-minute heating time at 100°C was used to inactivate the enzymes. Inactivation was determined by a micro-Kjeldahl method; non-protein nitrogen did not increase after three minutes of heating.

Panel scores and Allo-Kramer shear press values were obtained to detect conditions of enzyme concentration, pH and temperature, which would produce the most tender yet acceptable chicken. Panel results indicated that enzyme concentrations (weight/volume) of 0.02%, 0.0008%, 0.002% and 0.002% were suitable for Rhozyme P-11, ficin, bromelin, and papain, respectively. Various buffers were used to control the pH of rehydration solutions. Shear press values showed that Rhozyme P-11, ficin and bromelin were most active at pH 5.0. Papain was most active at pH 7.0. Optimum reaction temperatures were 50°, 50°, 60° and 70°C for Rhozyme P-11, papain, bromelin and ficin, respectively.

The optimum conditions for tenderization by the enzymes used were found to be affected by a combination of water uptake and pH or temperature. Control samples were significantly more tender when rehydrated at pH 7.0 than at pH values higher or lower than pH 7.0. This may have been due to a simultaneous increase in water uptake at pH 7.0 during rehydration. In control samples, significant increases in water uptake were found with decreasing rehydration temperatures.

Histological sections were obtained from chicken breast muscles after they were rehydrated in enzyme solutions under the most optimum conditions for tenderization. Masson's

trichrome stain was modified for use on the cooked and rehydrated tissues.

Ficin was most active on muscle fibers, while bromelin was least active. Rhozyme P-11 and papain both produced effects which were intermediate between the above two extremes. Ficin produced the most activity on connective tissue, papain showed some activity, but bromelin and Rhozyme P-11 showed little or no activity. Enzyme-induced tenderness seemed to be more related to muscle fiber destruction than to dissolution of the connective tissue in chicken breast muscle.

Muscle fibers which were attacked by enzymes showed a distinct swelling, dissolution of the sarcolemma, extensive granulation, the disappearance of nuclei and the loss of cross striations. Some of the granulation present was due to "erosion" of the periferal protoplasm caused by cooking, rather than to enzymatic action.

LITERATURE CITED

Altmann, R., 1890. Die Elementarorganismen und ihre Beziehungen zur den Zellen. Veit, Leipsig.

Anonymous, 1957. Research on tenderizing frozen poultry. Ind. Ref. 133:26.

Anonymous, 1962. Stable, flavorful, dehydrated chicken. Food Process. 23:107.

Anonymous, 1963. Enzyme Topics. Special Products Department of Rohm and Haas Company, Philadelphia.

Anonymous, 1965. Take pulse of freeze-drying. Food Eng. 37(4): 45.

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

Armed Forces Institute of Pathology, 1960. Manual of Histologic and Special Staining Techniques, 2nd ed. McGraw-Hill Book Company, Inc., New York.

Auerbach, E., 1960. Meat preservation: dehydration. The Science of Meat and Meat Products. The American Meat Institute Foundation, ed. W. H. Freeman and Company, San Francisco, p. 295.

Auerbach, E., H. Wang, N. Maynard, D. M. Doty and H. R. Kraybill, 1954. A histological and histochemical study of beef dehydration. V. Some factors influencing the rehydration level of frozen-dried muscle tissue. Food Res. 19:557.

Bailey, M. E., H. B. Hedrick, F. C. Parrish and H. D. Naumann, 1962. L.E.E.-Kramer shear force as a tenderness measure of beef steak. Food Technol. 16:99.

Bavisotto, V. S., 1958. Meat tenderizing by enzymes. Proceedings 10th Research Conference. American Meat Institute Foundation Circular No. 45, p. 67.

Bendall, J. R., 1963. Physiology and chemistry of muscle. Proceedings Meat Tenderness Symposium. Campbell Soup Company, Camden, New Jersey, p. 33.

Bird, K., 1963. Freeze-drying poultry: Problems, prospects, listed by researcher. Southeastern Poultry Times 3:7.

Birdseye, C., 1946. The preservation of foods by freezing--R. E. Application Data Section 22. Ref. Eng. 51: following page 176.

Birkner, M. L. and E. Auerbach, 1960. Microscopic structure of animal tissues. The Science of Meat and Meat Products. American Meat Institute Foundation, ed. W. H. Freeman and Company, San Francisco, p. 10.

Blakeslee, L. H. and J. I. Miller, 1948. Shear tenderness tests on beef short loins (abstract). J. Animal Sci. 7:517.

Bratzler, L. J., 1932. Measuring the tenderness of meat by means of a mechanical shear (M.S. Thesis). Kansas State College, Manhattan, Kansas.

Brooks, J., 1958. The structure of the animal tissues and dehydration (abstract). Food Mfr. 33:204.

Burke, R. F. and R. V. Decareau, 1964. Recent advances in the freeze-drying of food products. Advances in Food Research 13. Academic Press Inc., New York, p. 1.

Cameron, J. K. and E. A. Ryan, 1955. Tenderness in poultry meat. I. An improved method of measuring. Food Technol. 9:29.

Carlin, F., 1949. The effect of freezing on tenderness and on ice crystal formation in poultry after various periods of aging. J. Home Econ. 41:516.

Carlin, F., B. Lowe and G. F. Stewart, 1949. The effect of aging versus aging, freezing and thawing on the palatability of eviscerated poultry. Food Technol. 3:156.

Carpenter, J. A., R. L. Saffle and L. D. Kamstra, 1961. Tenderization of beef by pre-rigor infusion of a chelating agent. Food Technol. 15:197.

Chajuss, D. and J. V. Spencer, 1962. The effect of oxidizing and reducing aging media on the tenderness of excised chicken muscle. J. Food Sci. 27:303.

Cohen, W., 1958. Characterization of ficin. Nature 182:659.

Connell, J. J., 1957. Some aspects of the texture of dehydrated fish. J. Sci. Food Agr. 8:526.

Connell, J. J., 1962. The effects of freeze-drying and subsequent storage on the proteins of flesh foods. Freeze-Drying of Foods. F. R. Fisher, ed. Nat. Acad. Sci. -- Nat. Res. Council, Washington, D. C., p. 50.

Copenhaver, W. M., 1964. Bailey's Textbook of Histology, 15th ed. Williams and Wilkins Company, Baltimore.

Cover, S., R. L. Hostetler and S. J. Ritchey, 1962. Tenderness of beef. IV. Relations of shear force and fiber extensibility to juiciness and six components of tenderness. J. Food Sci. 27:527.

Dawson, E. H., G. S. Linton, A. M. Harkin and C. Miller, 1959. Factors influencing the palatability, vitamin content, and yield of cooked beef. U.S.D.A., Home Economics Research Report No. 9.

Dawson, L. E., J. A. Davidson, M. Frang and S. Walters, 1958. The effects of time interval between slaughter and freezing on the toughness of fryers. Poultry Sci. 37: 231.

Deatherage, F. E., 1951. A survey of the organoleptic testing methods used in meats research. Proceedings 4th Annual Reciprocal Meats Conference. Nat. Live Stock and Meat Board, Chicago, p. 184.

Deatherage, F. E., 1959. Ion-protein interrelationships affecting the quality of dehydrated meat. Quartermaster Food and Container Institute for the Armed Forces. Report A-316 No. 17.

Deatherage, F. E. and G. Garnatz, 1952. A comparative study of tenderness determination by a sensory panel and by shear strength measurements. Food Technol. 6:260.

Deatherage, F. E. and R. Hamm, 1960. Influence of freezing and thawing on hydration and changes of muscle proteins. Food Res. 25:623.

Deatherage, F. E. and A. Harsham, 1947. Relation of tenderness of beef to aging time at 33°-35°F. Food Res. 12: 164.

DeFremery, D., 1959. Fundamental biochemical studies of tenderization in chicken muscle. Proceedings Conference on Eggs and Poultry. U.S.D.A. Res. Service Report ARS-74-12, p. 6.

DeFremery, D. and M. F. Pool, 1959. Rate of rigor mortis development in relation to tenderness of chicken muscle. Poultry Sci. 38:1180.

DeFremery, D. and M. F. Pool, 1960. Biochemistry of chicken muscle as related to rigor mortis and tenderization. Food Res. 25:73.

Dodge, J.W., 1959. A study of selected factors involved with early post-mortem tenderness of poultry meat. Dissertation Absts. 20:1501.

Dodge, J. W. and W. J. Stadelman, 1959. Post mortem aging of poultry meat and its effect on the tenderness of the breast muscles. Food Technol. 13:81.

Dodge, J. W. and W. J. Stadelman, 1960a. Variability in tenderness due to struggling. Poultry Sci. 39:672.

Dodge, J. W. and W. J. Stadelman, 1960b. Relationship between pH, tenderness, and moisture levels during early post-mortem aging of turkey meat. Food Technol. 14:43.

Dodge, J. W. and W. J. Stadelman, 1960c. Studies on tenderness evaluation. Poultry Sci. 39:184.

Doty, D. M. and J. C. Pierce, 1961. Beef muscle characteristics as related to carcass grade, carcass weight, and degree of aging. U.S.D.A. Agr. Marketing Serv. Tech. Bull. No. 1231.

Doty, D. M., H. Wang and E. Auerbach, 1953. Dehydrated foods. Chemical and histological properties of dehydrated meat. J. Agr. Food Chem. 1:664.

Dubois, C. W., D. K. Tressler and F. Fenton, 1942. The effect of the rate of freezing and temperature of storage on the quality of frozen poultry. Ref. Eng. 44:93.

Duncan, D. R., 1955. Multiple range and multiple F tests. Biometrics 11:1.

El-Gharbawi, M. and J. R. Whitaker, 1963. Factors affecting enzymatic solubilization of beef proteins. J. Food Sci. 28:168.

Flosdorf, E. W., 1945. Drying by sublimation. Food Ind. 17:22.

Flosdorf, E. W., 1949. Freeze-Drying. Reinhold Publishing Company, New York.

Gainer, J. M., G. F. Stewart and B. Lowe, 1951. Effect of hand and machine massage on the tenderness of poultry muscles aged for short time periods. Food Res. 16:469.

Gawronski, T. H., J. V. Spencer and M. H. Pubols, 1964. The role of sulfhydryl groups in rigor mortis and post rigor tenderization of chicken muscle (abstract). Poultry Sci. 43:1321.

Gomori, G., 1955. Preparation of buffers for use in enzyme studies. Methods in Enzymology 1. S. P. Colowick and N. O. Kaplan, ed. Academic Press, Inc., New York, p. 138.

Goodwin, T. L., V. D. Bramblett, G. E. Vail and W. J. Stadelman, 1962. Effects of end-point temperature and cooking rate on turkey meat tenderness. Food Technol. 16:101.

Goodwin, T. L., W. C. Mickelberry and W. J. Stadelman, 1961. The influence of humane slaughter on the tenderness of turkey meat. Poultry Sci. 40:921.

Goodwin, T. L. and W. J. Stadelman, 1962. The effect of precooling before processing and hand massaging of turkeys and their effects on tenderness (abstract). Poultry Sci. 41:1646.

Ham, A. W. and T. S. Leeson, 1961. Histology, 4th ed. J. B. Lippincott Company, Philadelphia.

Hamm, R., 1960. Biochemistry of meat hydration. Freeze dehydration. Advances in Food Research 10. Academic Press, Inc., New York, p. 411.

Hamm, R. and F. E. Deatherage, 1960a. Changes in hydration and charges of muscle proteins, during freeze-dehydration of meat. Food Res. 25:573.

Hamm, R. and F. E. Deatherage, 1960b. Changes in hydration, solubility and charges of muscle proteins during heating of meat. Food Res. 25:587.

Hammond, B. R. and H. Gutfreund, 1959. The mechanism of ficin-catalyzed reactions. Biochem. J. 72:349.

Harper, J. C. and A. L. Tappel, 1957. Freeze-drying of food products. Advances in Food Research 7. Academic Press Inc., New York, p. 172.

Hepburn, J. S., 1960. Influence of the temperature and the period of keeping upon the biochemical changes in the common fowl, "Gallus domesticus". J. Franklin Inst. 249:393. Abstr. in Chem. Abstr. 1950. 44:8013i.

Hopkins, A. L., 1955. Effects of lyophilization on the contractile mechanism of muscle. Fed. Proc. 14:75.

Hunt, S. M. V. and N. A. Matheson, 1958. The effects of dehydration on actomyosin in fish and beef muscle. Food Technol. 12:410.

Kahlenberg, O. J. and E. M. Funk, 1961. The cooking of fowl with various salts for precooked poultry products. Poultry Sci. 40:668.

Kamstra, L. D. and R. L. Saffle, 1959. The effects of a prerigor infusion of sodium hexametaphosphate on tenderness

and certain chemical characteristics of meat. Food Technol. 13:652.

Khan, A. W. and L. van den Berg, 1965. Changes in chicken muscle proteins during cooking and subsequent frozen storage, and their significance in quality. J. Food Sci. 30:151.

Kimmel, J. R. and E. L. Smith, 1957. The properties of papain. Advances in Enzymology and Related Subjects of Biochemistry 19. F. F. Nord, ed. Interscience Publishers, Inc., New York, p. 267.

Klose, A. A., A. A. Campbell and H. L. Hanson, 1963. Influence of polyphosphates in chilling water on quality of poultry meat. Poultry Sci. 42:743.

Klose, A. A., M. F. Pool and M. B. Wiele, 1956a. Effect of processing factors on the tenderization of poultry (abstract). Poultry Sci. 35:1152.

Klose, A. A., H. L. Hanson, M. F. Pool and H. Lineweaver, 1956b. Poultry tenderness improved by holding before freezing. Quick Frozen Foods 18:95.

Klose, A. A. and M. F. Pool, 1954. Effect of scalding temperature on quality of stored frozen turkeys. Poultry Sci. 33:280.

Klose, A. A., M. F. Pool, M. B. Wiele, H. L. Hanson and H. Lineweaver, 1959. Poultry tenderness. I. Influence of processing on tenderness of turkeys. Food Technol. 13:20.

Koonz, C. H., M. I. Darrow and E. O. Essary, 1954. Factors influencing tenderness of principal muscles composing the poultry carcass. Food Technol. 8:97.

Koonz, C. H. and J. H. Ramsbottom, 1939. A method for studying the histological structure of frozen products. I. Poultry. Food Res. 4:117.

Koonz, C. H. and J. E. Robinson, 1946. Variations existing within the principal muscles composing the poultry carcass (abstract). Poultry Sci. 25:405.

Kramer, A., K. Aamlid, R. B. Guyer and H. P. Rodgers, Jr., 1951. New shear press predicts quality of canned lima beans. Food Eng. 23:112.

Liener, I. E., 1961a. A study of the number and reactivity of the sulfhydryl groups of ficin. Biochim. et Biophys. Acta 53:332.

Liener, I. E., 1961b. The thiol groups of ficin. Fed. Proc. 20:220.

Lineweaver, H., 1955. The toughness problem -- a progress report. Turkey World 30:11.

Lineweaver, H., 1959. Flavor and tenderness in poultry meat. Proceedings 30th Annual Fact Finding Conference, Inst. Am. Poultry Ind.

Lowe, B., 1948. Factors affecting the palatability of poultry with emphasis on histological post mortem changes. Advances in Food Research 1. Academic Press, Inc., New York, p. 203.

Lowe, B., 1949. Organoleptic tests developed for measuring the palatability of meat. Proceedings 2nd Reciprocal Meat Conference. Nat. Live Stock and Meat Board, Chicago, p. 111.

Lowe, B. and G. F. Stewart, 1948. The cutting of the breast muscles of poultry soon after killing and its effect on tenderness after subsequent storage and cooking. Unpublished data. Iowa State College, Ames, Iowa.

Luyet, B. J., 1960. Rehydration of freeze-dried meat. Quartermaster Food and Container Institute for the Armed Forces. Res. and Eng. Command. Report A-334 No. 5.

Luyet, B. J., 1961. Effect of freezing rates on the structure of freeze-dried materials and on the mechanism of rehydration. Freeze-Drying of Foods. F. R. Fisher, ed. Nat. Acad. Sci. -- Nat. Res. Council, Washington, D. C., p. 194.

Luyet, B. J. and A. P. MacKenzie, 1960. Rehydration of freeze-dried meat. Quartermaster Food and Container Institute for the Armed Forces. Res. and Eng. Command. Report A-334 No. 4.

Margolena, L. A. and E. H. Dolnick, 1951. A differential staining method for elastic fibers, collagenic fibers and keratin. Stain Technol. 26, 119.

Marion, W. W. and W. J. Stadelman, 1958. Effect of various freezing methods on the quality of poultry meat. Food Technol. 12:367.

Masson, P., 1929. Some histological methods. Trichrome stainings and their preliminary technique. J. Tech. Methods 12:75.

Maximow, A. A. and W. Bloom, 1954. A Textbook of Histology,

7th ed. W. B. Saunders Company, Philadelphia.

May, K. N., R. L. Helmer and R. L. Saffle, 1962a. Effect of phosphate treatment on carcass weight changes and organoleptic quality of cut-up chicken. Poultry Sci. 42:24.

May, K. N., R. L. Saffle, D. L. Downing and J. J. Powers, 1962b. Interrelations of post-mortem changes with tenderness of chicken and pork. Food Technol. 16:72.

McIntosh, E. N. and A. F. Carlin, 1963. The effect of papain preparations on beef skeletal muscle proteins. J. Food Sci. 28:283.

Meryman, H. T., 1961. Introductory survey of biophysical and biochemical aspects of freeze-drying. Freeze-Drying of Foods. F. R. Fisher, ed. Nat. Acad. Sci. -- Nat. Res. Council, Washington, D. C., p. 1.

Mickelberry, W. C. and W. J. Stadelman, 1962. Effect of cooking methods on shear-press values and weight changes of frozen chicken meat. Food Techno. 16:94.

Miyada, D. S. and A. L. Tappel, 1956a. Meat tenderization. I. Two mechanical devices for measuring texture. Food Technol. 10:142.

Miyada, D. S. and A. L. Tappel, 1956b. The hydrolysis of beef proteins by various proteolytic enzymes. Food Res. 21:217.

Monzini, A., 1953. Quick freezing applied to meat preservation. I. Influence on proteolysis. Ann. Sper. Agrar. 7:1067. Abstr. in Chem. Abstr. 1954. 48:4143d.

Morse, R., 1955. How phosphates can benefit meats. Food Eng. 27:84.

Mountney, G. J. and F. C. Arganosa, 1962. The effect of phosphates on moisture absorption, retention and cooking losses of broiler carcasses. Poultry Sci. 43:384.

Nair, J., 1963. Predict 400-fold increase in freeze-drying production facilities by 1970. Food Process. 24:84.

Nichols, J. B. and D. L. MacIntosh, 1952. Structural changes occurring in muscle tissue during repeated freezing and thawing. Food Technol. 6:170.

Norman, W. and E. Auerbach, 1963. Enhancement of rehydration of precooked freeze-dried meat. Proceedings 15th Research Conference. American Meat Institute Foundation, p. 18.

Ogg, C. L., 1960. Determination of nitrogen by the micro-Kjeldahl method. J. Assoc. Offic. Agr. Chemists 43:689.

Ohlson, M. A., 1955. Unpublished data. Michigan State University, East Lansing, Michigan.

Olcott, H. S., 1961. Deteriorative reactions in stored freeze-dried meat and fish. Freeze-Drying of Foods. F. R. Fisher, ed. Nat. Acad. Sci. -- Nat. Res. Council, Washington, D. C., p. 74.

Parrish, F. C., M. E. Bailey and H. D. Naumann, 1962. Hydroxyproline as a measure of beef tenderness. Food Technol. 16:68.

Paul, P. C., 1963. Influence of methods of cooking on meat tenderness. Proceedings Meat Tenderness Symposium. Campbell Soup Company, Camden, New Jersey, p. 225.

Paul, P. C., C. I. Sorenson and H. Abplanalp, 1958. Variability in tenderness of chicken. Food Res. 24:205.

Pearson, A. M., 1963. Objective and subjective measurements for meat tenderness. Proceedings Meat Tenderness Symposium. Campbell Soup Company, Camden, New Jersey, p. 135.

Penny, I. F., 1960. Up-grading of low-grade meat. Chem. and Ind. 11:288.

Peryam, D. R. and F. J. Pilgrim, 1957. Hedonic scale method of measuring food preferences. Food Technol. 11:9.

Pippen, E. L. and A. A. Klose, 1955. Effects of ice water chilling on flavor of chicken. Poultry Sci. 34:1139.

Pool, M. F., D. DeFremery, A. A. Campbell and A. A. Klose, 1959. Poultry tenderness. II. Influence of processing on tenderness of chickens. Food Technol. 13:25.

Ramsbottom, J. M. and E. J. Strandine, 1949. Initial physical and chemical changes in beef as related to tenderness. J. Animal Sci. 8:398.

Ramsbottom, J. M., E. J. Strandine and C. H. Koonz, 1944. The comparative tenderness of representative beef muscles (abstract). J. Animal Sci. 3:445.

Rowe, D. M., 1961. The thiamine, riboflavin, and niacin content of chicken muscle as affected by freeze-drying (Ph.D. thesis). Ohio State University, Columbus, Ohio.

Sartorius, M. J. and A. M. Child, 1938. Problems in meat research. I. Four comparable cuts from one animal.

II. Reliability of judges scores. Food Res. 3:627.

Schermerhorn, E. P. and W. J. Stadelman, 1962. Effects of polyphosphates on water uptake, moisture retention and cooking loss in broilers (abstract). Poultry Sci. 41:1680.

Schweigert, B. S., 1960. Food aspects of enzymes affecting proteins. Food Enzymes. The Avi Publishing Company, Westport, Connecticut, p. 97.

Seltzer, E., 1961. Importance of selection and processing method for successful freeze-drying of chicken. Food Technol. 15:18.

Shackell, L. F., 1909. An improved method of dessication, with some applications to biological problems. Am. J. Physiol. 24:325.

Shannon, W. G., W. W. Marion and W. J. Stadelman, 1957. Effect of temperature and time of scalding on the tenderness of breast meat of chicken. Food Technol. 11:284.

Sidwell, C. G., H. Salwin and R. B. Koch, 1962. The molecular oxygen content of dehydrated foods. Food Sci. 27:255.

Smithies, W. R., 1961. The influence of processing conditions on the rehydration of freeze-dried foods. Freeze-Drying of Foods. F. R. Fisher, ed. Nat. Acad. Sci. -- Nat. Res. Council, Washington, D. C., p. 191.

Snedecor, G. W., 1956. Statistical Methods. Iowa State College Press, Ames, Iowa.

Sosebee, M. E., K. N. May and S. C. Schmittle, 1963. The histological effects of proteolytic enzyme addition on freeze-dehydrated chicken meat. Poultry Sci. 43:553.

Spencer, J. V., W. E. Matson and W. J. Stadelman, 1956. The effect of cooking and freezing on consumer acceptability factors of turkey meat. Food Technol. 10:16.

Spencer, J. V. and L. E. Smith, 1962. The effect of chilling chicken fryers in a solution of polyphosphates upon moisture uptake, microbial spoilage, tenderness, juiciness, and flavor. Poultry Sci. 41:1685.

Stadelman, W. J., 1956. How to avoid toughness in turkeys. Turkey World 31:14.

Stadelman, W. J., 1959. Tenderness of poultry meat -- technological studies and industry status. U.S.D.A. Res. Serv. Report ARS-74-12, p. 3.

Stadelman, W. J. and B. A. McLaren, 1954. Scalding affects tenderness. Poultry Process. and Mktng. 60:26.

Stadelman, W. J. and J. V. Spencer, 1955. Here is a stop-gap solution to the aging problem. Turkey World 30:60.

Steinberg, M. P., 1960a. Development of techniques for the objective description of freeze dehydrated, cooked beef. Quartermaster Food and Container Institute for the Armed Forces. Res. and Eng. Command. Report A-333 No. 7.

Steinberg, M. P., 1960b. Development of techniques for the objective description of freeze dehydrated, cooked beef. Quartermaster Food and Container Institute for the Armed Forces. Res. and Eng. Command. Report A-333 No. 6.

Steinberg, M. P., 1960c. Development of techniques for the objective description of freeze dehydrated, cooked beef. Quartermaster Food and Container Institute for the Armed Forces. Res. and Eng. Command. Report A-333 No. 8.

Suden, J. R., A. M. Pearson and L. R. Dugan, 1964. Rehydration of freeze-dried pork as related to pH and protein denaturation. J. Food Sci. 29:192.

Swanson, M. H. and H. J. Sloan, 1953. Some protein changes in stored frozen poultry. Poultry Sci. 32:643.

Swift, C. E. and M. D. Berman, 1959. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. Food Technol. 13:365.

Swift, C. E. and R. Ellis, 1956. The action of phosphates in sausage products. I. Factors affecting the water retention of phosphate-treated ground meat. Food Technol. 10:546.

Tappel, A. L., R. Martin and E. Plocher, 1957. Freeze-dried meat. V. Preparation, properties, and storage stability of precooked freeze-dried meats, poultry, and seafoods. Food Technol. 11:599.

Tappel, A. L., D. S. Miyada, C. Sterling and V. P. Maier, 1956a. Application of meat tenderizer. Calif. Agr. 10:10.

Tappel, A. L., D. S. Miyada and V. P. Maier, 1956b. Meat tenderization. II. Factors affecting the tenderization of beef by papain. Food Res. 21:375.

Thomas, J. and S. M. Partridge, 1960. The chemistry of connective tissues. 5. The elastase activity of proteolytic enzymes. Biochem. J. 74:600.

Toumy, J. M., R. J. Lechnir and T. Miller, 1962. Effect of temperature on the tenderness of cooked beef. Quartermaster Food and Container Institute for the Armed Forces. Report No. 28-62.

Tsen, C. C. and A. L. Tappel, 1959. Meat tenderization. III. Hydrolysis of actomyosin, actin and collagen by papain. Food Res. 24:362.

U.S.D.A., Marketing Economics Division, 1963. Freeze-dried foods. Marketing Res. Report No. 617.

Venable, J. H., 1963. The histology of muscle. Proceedings Meat Tenderness Symposium. Campbell Soup Company, Camden, New Jersey, p. 7.

Wang, H., 1954. Dehydration of meat. American Meat Institute Foundation. Circular No. 12, p. 59.

Wang, H., 1957. Beef tenderness. Histological and enzymatic aspects. Proceedings 10th Annual Reciprocal Meats Conference. Nat. Live Stock and Meat Board, Chicago, p. 29.

Wang, H., F. Andrews, E. Rasch, D. M. Doty and H. R. Kraybill, 1953. A histological and histochemical study of beef dehydration. I. Rate of dehydration and structural changes in raw and cooked meat. Food Res. 18:351.

Wang, H., E. Auerbach, V. Bates, D. M. Doty and H. R. Kraybill, 1954a. A histological and histochemical study of beef dehydration. IV. Characteristics of muscle tissues dehydrated by freeze-drying techniques. Food Res. 19:543.

Wang, H., E. Auerbach, V. Bates, F. Andrews, D. M. Doty and H. R. Kraybill, 1954b. A histological and histochemical study of beef dehydration. II. Influence of carcass grade, aging, muscle and electrolysis pre-treatment. Food Res. 19:154.

Wang, H. and M. Birkner, 1957. Action of proteolytic enzyme preparations on elastic fibers in beef muscles. Anat. Rec. 127:384.

Wang, H. and N. Maynard, 1955a. Histological observations of the action of proteolytic enzyme preparations on striated muscle. Anat. Rec. 121:379.

Wang, H. and N. Maynard, 1955b. Studies on enzymatic tenderization of meat. I. Basic Technique and histological observations of enzymatic action. Food Res. 20:587.

Wang, H., C. E. Weir, M. L. Birkner and B. Ginger, 1957. The influence of enzyme tenderizers on the structure and

tenderness of beef. Proceedings of 9th Research Conference. Am. Meat Inst. Foundation, p. 69.

Wang, H., C. E. Weir, M. L. Birkner and B. Ginger, 1958. Studies on enzymatic tenderization of meat. III. Histological panel analyses of enzyme preparations from three distinct sources. Food Res. 23:423.

Weinberg, B. and D. Rose, 1960. Changes in protein extractability during post-rigor tenderization of chicken breast muscle. Food Technol. 14:376.

Weiner, S., M. Mangel, L. Maharg and G. G. Kelley, 1957. Effectiveness of commercial papain in meat tenderization. Food Technol. 12:248.

Weir, C. E., 1959. Effect of proteolytic enzymes upon tenderness of dehydrated cooked beef. American Meat Institute Foundation. Circular No. 51, p. 52.

Weir, C. E., H. Wang, M. L. Birkner, J. Parsons and B. Ginger, 1958. Studies on enzymatic tenderization of meat. II. Panel and histological analyses of meat treated with liquid tenderizers containing papain. Food Res. 23:411.

Wells, G. H., K. N. May and J. J. Powers, 1962. Taste-panel and shear press evaluation of tenderness of freeze-dried chicken as affected by age and pre-slaughter feeding of ions. Food Technol. 16:137.

White, E. D., H. L. Hanson, A. A. Klose and H. Lineweaver, 1964. Evaluation of toughness differences in turkeys. J. Food Sci. 29:673.

Wierbicki, E., L. E. Kunkle, V. R. Cahill and F. E. Deatherage, 1954. The relationship of tenderness to protein alterations during post mortem aging. Food Technol. 8:506.

Winegarden, M. W., B. Lowe, J. Kastelic, E. A. Kline, A. R. Plagge and P. S. Shearer, 1952. Physical changes of connective tissues of beef during heating. Food Res. 17:172.

Wise, R. G., 1961. Some factors affecting tenderness of poultry meat. Dissertation Absts. 21:1688.

Wise, R. G. and W. J. Stadelman, 1959. Tenderness at various muscle depths associated with poultry processing techniques. Food Technol. 13:689.

Wismer-Pedersen, J., 1965a. Effect of EDTA and pH on properties of freeze-dried pork muscle. II. Effect of injection of EDTA and NaOH before drying. J. Food Sci. 30:91.

Wismer-Pedersen, J., 1965b. Effect of EDTA and pH on properties of freeze-dried pork muscle. I. Effect of pH and magnesium and calcium ions on freeze-dried myofibrils. J. Food Sci. 30:85.

Worland, M. C. and M. C. Urbin, 1960. Some physical-chemical aspects of frozen and freeze-dried muscle. American Meat Institute Foundation Circular No. 59, p. 25.

Yao, A., A. I. Nelson and M. P. Steinberg, 1956. Factors affecting the rate of chicken meat dehydration under vacuum. Food Technol. 10:145.

Yatco-Manzo, E. and J. R. Whitaker, 1962. Ficin-catalyzed hydrolysis of elastin. Arch. Biochem. and Biophys. 97:122.

Ziemba, J. V., 1960. Freeze-drying. Food Eng. 32:57.

APPENDIX

APPENDIX TABLE 1

Effects of age on the rehydration of freeze-dried chicken breast meat

Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake	Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake
			11-Week-Old Birds				
1	7.4	22.1	198.6	31	6.0	20.0	233.3
2	5.6	16.6	196.4	32	7.5	22.8	204.0
3	8.3	25.5	207.2	33	6.9	20.0	189.8
4	7.5	22.9	205.3	34	7.5	24.6	228.0
5	5.5	17.8	223.6	35	7.1	20.6	190.1
6	5.6	15.9	183.9	36	7.7	24.2	214.3
7	8.4	25.8	207.1	37	8.1	24.6	203.7
8	7.8	25.8	230.8	38	6.9	21.6	213.0
9	7.3	22.7	210.9	39	6.6	21.0	218.1
10	6.4	29.9	367.2	40	7.4	21.5	190.5
11	7.4	22.5	204.1	41	7.9	25.7	225.3
12	6.2	19.9	221.0	42	7.3	22.7	211.0
13	7.5	22.8	204.0	43	7.0	21.6	208.6
14	6.8	20.1	195.6	44	10.0	30.0	200.0
15	7.0	22.7	224.3	45	9.0	26.6	195.6
16	7.9	24.2	206.3	46	9.0	28.4	215.6
17	8.2	24.4	197.6	47	7.4	24.8	235.1
18	7.1	22.2	212.7	48	7.7	23.7	207.8
19	7.4	21.6	191.9	49	7.7	23.0	198.7
20	6.1	19.1	213.1	50	7.2	22.9	218.1
21	6.5	19.2	195.4	51	6.9	23.0	233.3
22	7.8	24.2	210.3	52	7.6	22.7	198.7
23	7.5	23.0	206.7	53	8.7	27.2	212.6
24	7.7	24.7	220.8	54	7.3	23.4	220.5
25	6.7	22.8	240.3	55	6.6	20.2	206.1
26	6.7	21.4	219.4	56	6.7	20.6	207.5
27	7.2	23.0	219.4	57	6.5	21.4	229.2
28	6.4	19.1	198.4	58	5.9	20.0	239.0
29	6.0	19.0	216.7	59	6.6	21.6	227.3
30	9.9	26.3	165.7	60	7.9	22.3	182.3
						Average:	212.5
			20-Week-Old Birds				
1	11.3	27.9	146.9	31	9.5	28.1	195.8
2	10.4	30.1	189.4	32	10.1	29.5	195.0
3	11.0	33.1	200.9	33	12.0	33.2	176.7
4	9.9	26.9	171.7	34	10.8	32.4	200.0
5	10.8	32.4	200.0	35	10.8	32.8	203.7
6	9.2	27.4	197.8	36	12.5	36.2	189.6
7	10.2	31.3	206.9	37	10.0	29.4	194.0

Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake	Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake
8	16.0	28.0	75.0	38	9.7	27.7	185.6
9	9.7	25.8	165.9	39	10.9	30.8	182.6
10	8.4	25.2	200.0	40	10.0	28.5	185.0
11	10.0	29.8	198.0	41	9.3	27.2	192.5
12	9.3	27.4	194.6	42	9.7	27.2	180.4
13	8.9	24.7	177.5	43	10.0	30.1	201.0
14	12.1	35.8	195.9	44	12.1	32.8	171.1
15	10.5	30.9	194.3	45	9.6	22.6	135.4
16	10.9	29.5	170.6	46	8.7	24.2	178.2
17	10.5	30.0	185.7	47	10.5	29.8	183.8
18	10.0	27.2	172.0	48	10.2	29.7	191.2
19	12.1	36.2	199.2	49	11.3	28.9	155.8
20	11.1	30.1	171.2	50	8.7	24.8	185.1
21	10.5	24.3	131.4	51	11.2	25.1	124.1
22	9.8	28.6	191.8	52	10.3	27.6	168.0
23	13.1	29.2	122.9	53	12.4	27.5	121.8
24	10.5	25.7	144.8	54	9.7	29.5	204.1
25	11.3	26.0	130.1	55	8.6	24.2	181.4
26	11.3	33.2	193.8	56	10.5	29.6	181.9
27	10.2	27.8	172.5	57	12.3	33.8	174.8
28	10.7	29.3	173.8	58	12.6	33.7	167.5
29	10.2	23.0	125.5	59	13.0	31.5	142.3
30	13.8	34.0	146.4	60	12.0	26.1	117.5
						Average	173.5
			52-Week-Old Birds				
1	10.0	20.8	108.0	31	12.9	28.5	120.9
2	9.2	24.4	165.2	32	13.4	30.0	123.9
3	12.2	25.6	109.8	33	10.6	24.7	133.0
4	13.4	27.6	106.0	34	11.1	22.8	105.4
5	15.1	30.0	98.7	35	14.0	33.7	140.7
6	14.3	30.5	113.3	36	11.1	27.2	145.0
7	10.8	21.8	101.9	37	10.5	29.8	183.8
8	10.2	31.6	209.8	38	11.8	28.8	144.1
9	10.4	23.9	129.8	39	13.0	31.0	138.5
10	11.6	28.5	145.7	40	11.2	27.4	144.6
11	14.9	31.5	125.0	41	14.6	32.3	233.0
12	11.1	26.3	136.9	42	7.1	19.0	167.6
13	12.3	26.8	117.9	43	10.3	21.5	108.7
14	11.8	28.1	138.1	44	10.8	25.2	133.3
15	15.0	36.0	140.0	45	9.2	26.6	189.1
16	11.5	23.6	105.2	46	6.9	20.0	190.0
17	12.5	28.5	128.0	47	10.0	28.4	184.0
18	9.0	26.5	194.4	48	11.4	27.1	137.7
19	9.7	25.1	158.8	49	8.8	25.9	194.3
20	9.1	19.5	114.3	50	11.1	25.7	131.5
21	14.5	31.0	113.8	51	14.1	33.7	139.0
22	8.9	17.4	95.0	52	9.7	29.8	207.2

Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake	Sample	Weight Before Rehyd. (gms)	Weight After Rehyd. (gms)	% Water Uptake
23	11.2	27.8	148.2	53	10.1	30.0	197.0
24	10.9	32.2	195.4	54	8.1	20.1	148.1
25	9.7	27.2	180.4	55	9.0	29.0	222.2
26	9.7	26.4	172.2	56	12.0	26.0	116.7
27	12.3	29.0	135.8	57	8.5	16.4	92.9
28	10.2	23.1	126.5	58	9.1	20.0	119.8
29	10.7	24.1	125.2	59	12.1	26.4	118.2
30	11.7	24.2	106.8	60	12.0	24.7	106.0
						Average:	142.7

APPENDIX TABLE 2

Effects of age on the tenderness of freeze-dried chicken breast muscle as measured by the Warner-Bratzler shear

Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force/ Gram (lb/gm)	Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force/ Gram (lb/gm)
			11-Week-Old Birds				
1	0.28	39.6	0.54	31	0.31	34.1	0.45
2	0.28	41.5	0.52	32	0.31	45.0	0.63
3	0.37	45.0	0.56	33	0.33	46.5	0.71
4	0.34	47.7	0.72	34	0.38	42.4	0.67
5	0.37	35.5	0.47	35	0.35	39.9	0.53
6	0.39	44.7	0.58	36	0.31	52.2	0.71
7	0.30	64.3	0.83	37	0.34	56.4	0.78
8	0.41	27.3	0.62	38	0.34	48.3	0.77
9	0.26	34.0	0.47	39	0.25	72.8	0.91
10	0.23	77.6	0.96	40	0.36	38.6	0.57
11	0.27	57.7	0.63	41	0.31	43.1	0.65
12	0.27	49.2	0.60	42	0.34	41.7	0.62
13	0.26	71.4	0.80	43	0.37	31.4	0.43
14	0.36	51.6	0.75	44	0.33	36.1	0.53
15	0.34	36.8	0.54	45	0.31	41.3	0.64
16	0.37	35.2	0.51	46	0.29	55.7	0.97
17	0.37	49.8	0.86	47	0.38	34.2	0.53
18	0.32	31.9	0.46	48	0.21	82.5	0.98
19	0.28	57.2	0.69	49	0.33	40.4	0.61
20	0.32	50.7	0.72	50	0.30	42.6	0.43
21	0.28	61.6	0.81	51	0.33	48.4	0.71
22	0.23	40.5	0.48	52	0.33	48.4	0.69
23	0.26	55.5	0.82	53	0.31	55.4	0.83
24	0.22	63.9	0.90	54	0.29	61.3	0.90
25	0.34	50.0	0.74	55	0.36	50.1	0.69
26	0.35	68.0	0.98	56	0.26	57.0	0.74
27	0.37	50.8	0.83	57	0.38	47.3	0.70
28	0.36	58.4	0.80	58	0.35	36.3	0.57
29	0.26	59.2	0.79	59	0.37	37.9	0.55
30	0.32	54.8	0.81	60	0.30	39.7	0.52
				Average:	0.32	48.6	0.68
			20-Week-Old Birds				
1	0.35	73.9	1.06	31	0.46	57.7	0.84
2	0.35	46.2	0.65	32	0.47	51.3	0.98
3	0.54	23.8	0.40	33	0.49	72.9	0.98
4	0.44	37.8	0.61	34	0.45	27.3	0.41
5	0.46	23.8	0.36	35	0.43	39.1	0.57
6	0.35	45.7	0.67	36	0.49	41.9	1.79
7	0.35	67.2	0.79	37	0.44	31.2	0.49

Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force/ Gram (lb/gm)	Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force/ Gram (lb/gm)
8	0.53	21.9	0.35	38	0.54	43.4	0.69
9	0.40	45.5	0.70	39	0.44	56.0	0.86
10	0.45	42.4	0.63	40	0.42	70.8	0.98
11	0.52	41.5	0.62	41	0.48	24.7	0.35
12	0.43	48.4	0.76	42	0.51	27.5	0.43
13	0.43	46.6	0.68	43	0.50	44.3	0.75
14	0.37	41.1	0.62	44	0.40	40.3	0.58
15	0.47	39.6	0.64	45	0.40	46.0	0.66
16	0.40	41.5	0.64	46	0.54	24.8	0.37
17	0.47	33.7	0.51	47	0.45	33.0	0.54
18	0.37	34.4	0.47	48	0.61	45.5	0.93
19	0.39	42.0	0.60	49	0.45	25.6	0.41
20	0.51	40.5	0.68	50	0.40	39.3	0.63
21	0.48	24.0	0.35	51	0.44	79.1	1.27
22	0.34	67.8	0.91	52	0.40	76.7	1.19
23	0.44	30.8	0.48	53	0.48	33.9	0.53
24	0.47	34.3	0.53	54	0.46	87.1	1.36
25	0.41	37.4	0.52	55	0.59	20.9	0.34
26	0.39	45.2	0.72	56	0.51	40.4	0.68
27	0.47	25.9	0.38	57	0.41	38.1	0.58
28	0.38	60.9	0.87	58	0.44	40.2	0.54
29	0.46	35.4	0.55	59	0.40	96.8	1.54
30	0.40	43.4	0.62	60	0.42	29.2	0.44
				Average:	0.45	43.8	0.67

52-Week-Old Birds

1	0.38	86.0	1.44	31	0.51	87.0	1.48
2	0.48	40.8	0.72	32	0.34	45.3	0.62
3	0.42	30.5	0.45	33	0.35	46.1	0.61
4	0.45	63.7	1.01	34	0.46	32.0	0.50
5	0.43	42.5	0.67	35	0.40	45.1	0.80
6	0.51	25.6	0.41	36	0.34	55.3	0.74
7	0.34	66.5	0.86	37	0.40	34.2	0.51
8	0.39	44.5	0.61	38	0.51	45.5	0.72
9	0.31	49.6	0.77	39	0.43	37.2	0.53
10	0.41	103.6	1.79	40	0.25	61.8	0.79
11	0.50	27.7	0.47	41	0.38	60.8	0.91
12	0.49	35.2	0.48	42	0.59	46.6	1.15
13	0.55	50.6	0.98	43	0.38	44.9	0.82
14	0.37	66.7	0.92	44	0.37	38.9	0.62
15	0.33	87.3	1.31	45	0.35	85.0	1.40
16	0.39	57.9	0.88	46	0.57	48.4	0.89
17	0.56	37.9	0.66	47	0.55	53.1	1.07
18	0.51	31.7	1.01	48	0.42	63.2	0.94
19	0.40	52.7	0.86	49	0.43	82.8	1.24
20	0.47	42.3	0.75	50	0.40	40.0	0.61
21	0.39	86.0	1.54	51	0.37	79.3	1.36
22	0.50	46.1	0.82	52	0.52	25.0	0.48

Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force Gram (lb/gm)	Sample	Sample Area Sheared (in^2)	Shear Force/ Area (lb/in^2)	Shear Force/ Gram (lb/gm)
23	0.37	142.3	2.41	53	0.43	70.8	1.15
24	0.29	38.0	0.59	54	0.56	88.2	1.47
25	0.51	82.9	1.37	55	0.42	71.1	1.08
26	0.53	65.7	1.40	56	0.50	34.0	0.56
27	0.42	101.7	1.66	57	0.48	36.0	0.67
28	0.40	41.1	0.57	58	0.34	69.4	1.43
29	0.38	53.5	0.84	59	0.39	63.6	1.02
30	0.50	28.4	0.50	60	0.40	88.0	1.50
				Average:	0.43	56.8	0.94

APPENDIX TABLE 3

Effects of age on the tenderness of freeze-dried chicken breast muscle as measured by a sensory panel

Sample	Tenderness Initial	Tenderness Residual	Juiciness	Sample	Tenderness Initial	Tenderness Residual	Juiciness
			11-Week-Old Birds				
1	3.4	4.4	3.4	31	4.4	4.4	3.4
2	3.8	3.4	2.8	32	3.0	3.4	3.0
3	4.4	4.0	3.0	33	2.6	2.6	4.0
4	3.0	2.6	3.0	34	3.4	3.4	3.2
5	3.4	3.2	2.8	35	3.6	3.6	3.2
6	4.0	3.6	3.8	36	3.0	2.4	3.0
7	3.8	3.4	3.4	37	2.8	2.6	2.8
8	2.8	2.8	3.0	38	3.8	3.2	3.0
9	4.0	3.4	3.4	39	2.6	2.4	3.4
10	2.0	2.2	3.6	40	3.6	3.8	2.8
11	3.0	2.8	3.2	41	2,8	2.8	3.2
12	3.0	3.2	3.4	42	3.6	4.2	3.2
13	4.0	4.2	3.2	43	4.6	4.4	3.2
14	4.0	4.0	3.6	44	4.0	4.0	3.2
15	3.4	3.2	3.0	45	4.2	4.0	3.0
16	4.0	4.2	3.0	46	4.4	4.0	3.4
17	2.6	2.4	3.4	47	3.0	2.8	3.0
18	3.6	3.6	3.4	48	2.8	3.2	3.2
19	2.4	2.4	3.4	49	4.2	4.2	3.0
20	3.8	3.6	3.0	50	3.4	3.4	3.2
21	3.8	3.8	2.6	51	2.8	2.8	3.2
22	4.4	4.6	3.4	52	3.0	3.4	3.2
23	2.8	2.8	3.0	53	3.6	3.6	3.0
24	3.2	3.2	2.6	54	3.2	2.8	3.6
25	4.2	4.0	2.6	55	4.0	4.0	2.8
26	2.6	2.6	3.2	56	2.6	2.6	2.6
27	3.0	3.0	3.0	57	3.8	3.6	2.8
28	3.4	3.6	3.2	58	4.4	4.4	3.4
29	4.0	3.8	3.0	59	4.6	4.4	4.0
30	3.6	3.4	3.4	60	2.2	2.4	2.4
				Average:	3.5	3.4	3.2
			20-Week-Old Birds				
1	6.0	5.8	4.8	31	7.0	6.6	4.2
2	6.6	6.4	5.6	32	6.4	6.4	5.6
3	6.2	5.4	4.6	33	6.8	6.4	5.4
4	4.4	3.4	3.0	34	2.8	2.8	3.8
5	4.6	4.2	3.2	35	4.0	4.2	3.2
6	4.6	4.6	4.0	36	4.2	3.8	5.2
7	4.0	4.0	4.2	37	4.4	4.2	2.8
8	3.2	2.8	4.2	38	5.4	5.0	4.4

Sample	Tenderness Initial	Tenderness Residual	Juiciness	Sample	Tenderness Initial	Tenderness Residual	Juiciness
9	4.0	3.8	4.2	39	6.0	6.0	3.4
10	4.0	4.0	3.4	40	3.2	3.0	4.6
11	4.2	4.4	3.2	41	5.0	4.4	4.4
12	4.6	4.4	3.8	42	2.8	2.8	3.2
13	2.6	2.4	2.4	43	3.0	3.0	3.4
14	2.6	2.8	3.8	44	4.2	4.0	3.6
15	3.6	3.8	3.2	45	3.8	4.0	3.6
16	6.0	5.8	5.0	46	7.0	6.6	6.2
17	6.8	6.2	5.0	47	6.4	6.4	6.2
18	6.2	5.4	5.6	48	6.8	6.4	5.6
19	4.4	3.4	3.2	49	2.8	2.8	3.6
20	4.6	4.2	4.2	50	4.0	4.2	3.2
21	4.6	4.6	3.4	51	4.2	3.8	3.4
22	4.0	4.0	4.4	52	4.4	4.2	3.4
23	3.2	3.8	3.4	53	5.4	5.0	3.2
24	3.2	3.4	4.0	54	6.0	6.0	3.4
25	4.0	3.8	3.6	55	3.2	3.0	3.8
26	4.2	4.4	3.0	56	5.0	4.4	3.2
27	4.6	4.4	3.8	57	2.8	2.8	2.6
28	2.6	2.4	2.6	58	3.0	3.0	2.8
29	2.6	2.8	3.0	59	4.2	4.0	3.0
30	3.6	3.8	3.2	60	3.8	4.0	3.0
			Average:		4.5	4.3	3.9

52-Week-Old Birds

1	3.8	3.6	2.8	31	3.6	3.0	2.8
2	4.0	4.0	3.4	32	4.0	4.2	3.4
3	3.4	3.2	4.4	33	4.6	3.8	4.0
4	3.6	3.6	3.4	34	6.2	6.0	5.4
5	4.4	4.2	5.0	35	5.6	4.8	4.4
6	5.6	4.4	4.6	36	6.0	5.8	5.2
7	6.0	5.8	5.0	37	5.8	5.8	5.2
8	6.8	6.2	5.2	38	6.2	5.8	5.0
9	7.0	6.4	5.8	39	6.6	6.0	5.4
10	5.0	4.8	4.6	40	6.4	3.6	4.8
11	6.6	6.4	6.0	41	6.4	6.0	5.6
12	7.0	7.0	6.4	42	7.0	6.6	5.0
13	6.6	6.2	6.0	43	6.0	5.8	4.4
14	6.8	6.4	6.0	44	6.6	6.4	6.2
15	6.8	6.4	6.4	45	6.6	5.8	5.6
16	3.2	3.4	2.8	46	3.2	3.0	4.0
17	5.0	4.8	3.6	47	5.0	4.6	3.2
18	3.4	3.2	3.6	48	3.4	3.0	3.2
19	4.4	4.2	3.2	49	6.4	5.6	5.4
20	5.4	5.8	5.6	50	6.2	6.0	4.6
21	6.6	6.2	4.4	51	6.4	5.8	6.0
22	6.0	5.6	5.2	52	6.0	6.0	5.8
23	6.4	5.8	5.2	53	7.0	6.4	6.2
24	6.8	6.4	5.6	54	6.4	6.2	5.4

Sample	Tenderness Initial	Tenderness Residual	Juiciness	Sample	Tenderness Initial	Tenderness Residual	Juiciness
25	4.6	4.2	3.6	55	6.4	6.0	4.8
26	6.6	6.4	6.2	56	6.4	6.0	5.2
27	7.0	7.0	6.2	57	7.0	6.6	6.0
28	6.6	6.2	6.2	58	5.8	5.4	4.8
29	6.4	6.2	5.0	59	6.8	6.4	6.2
30	6.2	5.4	5.8	60	6.2	5.0	4.4
			Average:		5.7	5.4	4.9

APPENDIX TABLE 4

Effects of age on the tenderness of breast muscle from control chickens as measured by the Warner-Bratzler shear and sensory panel

Sample	Shear Press Values: Sample Area Sheared (in^2)	Shear Press Values: Shear Force/ Area (lb/in^2)	Panel Scores: Initial Tenderness	Panel Scores: Residual Tenderness	Panel Scores: Juiciness
		11-Week-Old Birds			
1	0.29	19.3	2.2	2.2	3.4
2	0.47	22.7	2.4	2.4	2.4
3	0.51	11.5	1.8	1.8	2.8
4	0.30	22.0	2.2	1.6	2.4
5	0.58	16.1	2.4	2.0	3.8
6	0.63	15.6	2.4	2.0	3.2
7	0.42	14.5	2.2	2.2	3.8
8	0.50	29.6	3.6	3.4	2.8
9	0.49	16.9	2.6	2.0	3.0
10	0.59	14.6	2.6	3.0	4.0
11	0.47	15.3	3.2	2.6	4.4
12	0.61	11.6	2.8	2.0	3.0
13	0.57	16.6	2.8	2.6	3.8
14	0.44	16.2	2.2	2.4	3.6
Average:	0.49	17.3	2.5	2.3	3.3
		20-Week-Old Birds			
1	0.56	15.2	2.2	2.2	2.4
2	0.40	18.6	2.6	2.6	3.2
3	0.42	25.6	3.2	2.6	3.0
4	0.40	28.9	3.4	2.8	3.0
5	0.44	28.8	3.4	3.0	3.0
6	0.24	23.5	2.4	2.4	4.8
7	0.36	40.9	4.2	3.8	3.0
8	0.51	23.3	4.0	3.6	3.4
9	0.36	46.8	4.8	3.8	3.2
10	0.30	52.1	2.6	2.6	2.6
11	0.31	46.8	2.6	2.4	2.2
12	0.35	76.5	5.4	4.6	4.0
13	0.39	17.8	2.4	2.6	3.8
14	0.26	21.2	2.6	2.6	4.4
Average:	0.38	33.3	3.3	3.0	3.3
		52-Week-Old Birds			
1	0.38	33.8	4.6	4.6	3.2
2	0.29	30.6	3.0	3.0	4.0
3	0.30	46.2	4.4	4.8	4.0

Sample	Shear Press Values: Sample Area Sheared (in^2)	Shear Press Values: Shear Force/ Area (lb/in^2)	Panel Scores: Initial Tenderness	Panel Scores: Residual Tenderness	Panel Scores: Juiciness
4	0.44	31.4	4.8	3.8	3.0
5	0.40	77.3	4.8	5.2	3.6
6	0.23	68.0	3.2	3.4	3.0
7	0.37	33.2	3.6	2.8	3.8
8	0.33	42.1	3.6	3.4	4.0
9	0.27	70.6	4.4	4.6	3.4
10	0.27	53.9	4.0	3.6	3.6
11	0.39	33.2	3.2	3.2	3.8
12	0.27	56.1	5.0	5.0	4.8
13	0.32	39.3	3.6	3.4	4.4
14	0.28	50.8	4.4	4.2	4.6
Average:	0.32	47.6	5.0	3.9	3.8

APPENDIX TABLE 5

Effects of proteolytic enzyme concentration on water uptake, tenderness and acceptability of freeze-dried chicken breast meat

Sample	Enzyme Conc.[1] (%)	Water Uptake (%)	Shear Weight (gms)	Shear Force/ Gram (lb/gm)	Ave. Panel Score	Acceptable to Panel ?
			Rhozyme P-11			
1	0.010	202.1	17.7	7.1	3.2	yes
2	0.010	173.7	20.0	4.0	2.8	yes
3	0.010	166.4	17.8	5.3	3.0	yes
4	0.015	179.7	26.2	1.5	3.0	yes
5	0.015	210.0	22.0	7.0	2.4	yes
6	0.015	213.3	18.2	6.6	2.8	yes
7	0.015	201.9	20.0	4.5	2.6	yes
8	0.020	180.3	17.4	5.2	2.8	yes
9	0.020	211.7	18.4	5.4	2.0	yes
10	0.020	180.7	16.3	4.6	2.0	no
11	0.020	194.3	23.2	8.0	4.2	yes
12	0.020	205.3	23.1	3.5	2.2	yes
13	0.025	185.2	14.0	8.6	2.0	?
14	0.025	214.9	19.5	3.1	2.2	?
15	0.025	202.8	17.8	3.4	2.2	?
16	0.025	204.5	21.4	4.2	3.2	yes
17	0.030	194.1	19.3	2.3	3.0	no
18	0.030	242.0	20.0	1.8	1.2	no
19	0.030	178.3	17.8	1.7	2.0	no
20	0.030	222.8	25.5	2.5	1.0	no
21	0.030	207.4	24.1	1.7	1.4	no
			Ficin			
1	0.0001	161.0	24.7	16.1	3.8	yes
2	0.0001	166.5	21.0	19.5	4.6	yes
3	0.0001	167.0	20.3	14.5	3.8	yes
4	0.0003	172.5	29.6	10.6	3.4	yes
5	0.0005	163.3	23.8	14.5	3.4	yes
6	0.0005	176.0	21.7	15.2	3.2	yes
7	0.0005	203.4	21.3	7.3	2.8	yes
8	0.0005	189.0	21.5	16.7	4.0	yes
9	0.0007	142.9	20.7	14.8	3.0	yes
10	0.0010	162.3	26.9	4.1	1.2	no
11	0.0010	205.1	20.0	3.5	1.4	no
12	0.0010	162.0	20.5	11.7	4.4	yes
13	0.0010	254.1	22.8	10.1	2.8	?
14	0.0010	207.3	21.8	3.9	2.2	?
15	0.0010	205.0	20.4	3.5	---	no
16	0.0020	190.1	25.0	3.3	---	no

Sample	Enzyme Conc.[1] (%)	Water Uptake (%)	Shear Weight (gms)	Shear Force/ Gram (lb/gm)	Ave. Panel Score	Acceptable to Panel ?
17	0.0030	200.0	20.0	3.2	---	no
18	0.0050	-----	----	---	---	no
			Bromelin			
1	0.0005	166.5	24.0	22.1	5.0	yes
2	0.0005	163.5	15.5	9.7	3.2	yes
3	0.0005	185.1	15.8	6.3	4.2	yes
4	0.0005	184.0	17.2	20.9	3.6	yes
5	0.0005	161.6	23.7	14.3	4.6	yes
6	0.0010	194.0	25.2	8.7	2.8	yes
7	0.0010	190.3	18.3	11.7	3.6	yes
8	0.0010	180.7	16.4	7.3	3.4	yes
9	0.0010	206.7	15.8	12.7	3.6	yes
10	0.0010	193.5	25.6	6.8	2.6	yes
11	0.0020	212.1	25.2	16.1	3.8	yes
12	0.0020	234.0	18.2	6.0	1.6	yes
13	0.0020	205.5	18.1	4.1	3.0	yes
14	0.0020	254.0	24.1	14.9	3.0	yes
15	0.0020	237.5	23.0	5.0	2.2	yes
16	0.0030	279.0	24.7	4.5	2.0	no
17	0.0030	243.0	26.5	12.1	2.4	yes
18	0.0030	225.5	18.7	7.8	1.6	no
19	0.0030	246.7	20.0	4.3	1.6	no
20	0.0030	260.5	24.0	2.5	1.6	no
			Papain			
1	0.001	159.8	36.2	11.0	3.2	yes
2	0.001	240.3	27.7	11.1	2.6	yes
3	0.001	271.3	27.4	9.8	3.4	yes
4	0.001	193.7	27.3	19.5	4.0	yes
5	0.001	149.0	37.5	9.3	3.4	yes
6	0.002	182.5	35.5	8.2	3.0	yes
7	0.002	244.1	39.8	5.7	1.8	no
8	0.002	201.0	37.2	8.7	3.6	yes
9	0.002	238.4	35.3	12.6	1.4	yes
10	0.002	224.8	31.5	5.0	2.6	yes
11	0.002	212.7	35.0	6.9	3.2	yes
12	0.003	208.7	35.8	3.9	2.0	no
13	0.003	260.6	30.0	3.5	---	no
14	0.003	231.5	37.9	10.8	3.4	yes
15	0.003	228.3	36.7	3.8	1.6	no
16	0.005	247.6	21.8	3.5	1.4	no
17	0.005	221.6	36.0	5.3	1.2	no

Sample	Enzyme Conc.[1] (%)	Water Uptake (%)	Shear Weight (gms)	Shear Force/ Gram (lb.gm)	Ave. Panel Score	Acceptable to Panel ?
			Control			
1	0.000	175.3	36.1	17.5	4.6	yes
2	0.000	205.6	38.6	19.3	5.0	yes
3	0.000	189.0	15.5	12.9	2.2	yes
4	0.000	256.3	28.4	14.3	4.0	yes
5	0.000	208.9	36.2	8.3	3.4	yes
6	0.000	166.5	37.0	13.8	4.2	yes
7	0.000	178.2	28.1	20.3	---	---
8	0.000	171.9	26.1	19.2	---	---
9	0.000	171.0	33.6	13.4	---	---

[1]Rhozyme P-11 was used at pH 7.0 and 50°C. Bromelin, ficin and papain were employed at pH 7.0 and 70°C. All samples were rehydrated for five minutes.

APPENDIX TABLE 6

Water uptake and tenderness of freeze-dried chicken breast muscle rehydrated in enzyme solutions[1] at various pH

Sample	pH	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
		Rhozyme P-11		
1	4.0	187.4	22.6	15.2
2	4.0	187.7	29.7	17.2
3	5.0	200.0	34.9	4.9
4	5.0	188.3	25.4	5.7
5	5.0	188.1	28.6	10.5
6	5.0	199.1	27.3	5.5
7	5.0	196.3	35.5	5.9
8	6.0	203.6	35.0	12.0
9	6.0	188.2	25.2	9.7
10	6.0	202.5	28.4	8.6
11	6.0	162.6	23.8	13.0
12	6.0	199.3	35.0	8.4
13	7.0	195.7	34.8	9.5
14	7.0	179.6	23.3	13.7
15	7.0	177.8	26.7	16.1
16	7.0	211.0	26.6	6.8
17	7.0	191.9	34.2	8.3
18	8.0	178.1	30.2	9.9
19	8.0	181.9	24.8	9.3
20	8.0	189.0	26.8	8.9
21	8.0	208.3	28.3	6.7
22	8.0	190.4	33.9	10.1
23	9.0	201.4	34.2	8.2
24	9.0	200.0	25.4	9.3
25	9.0	205.1	28.3	8.7
26	9.0	214.7	29.7	7.4
27	9.0	206.7	36.0	10.4
		Ficin		
1	4.0	218.6	22.3	16.1
2	4.0	237.2	24.2	16.9
3	5.0	195.4	31.0	6.6
4	5.0	201.9	36.6	5.5
5	5.0	253.0	35.9	5.2
6	5.0	189.3	26.5	10.6
7	5.0	197.9	36.2	5.2
8	6.0	166.7	31.6	8.2
9	6.0	83.9	23.4	15.2
10	6.0	150.0	31.2	12.2
11	6.0	152.7	26.0	17.3

Sample	pH	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
12	6.0	193.7	34.0	10.6
13	7.0	208.5	31.5	9.0
14	7.0	201.9	36.2	4.7
15	7.0	176.7	31.2	10.7
16	7.0	198.0	25.9	12.0
17	7.0	190.3	35.1	7.7
18	8.0	149.0	31.4	14.3
19	8.0	140.0	32.4	11.1
20	8.0	158.9	32.1	11.7
21	8.0	168.7	26.3	6.3
22	8.0	160.8	30.8	22.2
23	9.0	159.9	31.5	15.7
24	9.0	161.7	34.2	13.5
25	9.0	161.9	31.1	12.1
26	9.0	173.3	26.7	14.2
27	9.0	155.5	29.9	19.1
		Bromelin		
1	5.0	260.6	45.8	4.6
2	5.0	226.8	30.3	5.9
3	5.0	209.9	26.8	5.4
4	5.0	233.9	31.8	10.8
5	5.0	215.0	26.1	9.2
6	6.0	207.1	39.0	14.5
7	6.0	201.8	26.9	12.3
8	6.0	202.0	24.6	7.2
9	6.0	191.7	26.3	8.7
10	6.0	162.7	27.6	18.1
11	7.0	233.3	43.0	6.3
12	7.0	238.9	29.9	7.7
13	7.0	247.4	26.5	7.7
14	7.0	221.8	30.4	8.2
15	7.0	216.7	30.6	8.0
16	8.0	208.7	39.2	9.5
17	8.0	228.8	28.3	4.1
18	8.0	168.3	22.3	8.9
19	8.0	178.2	25.2	8.9
20	8.0	173.8	28.6	11.0
21	9.0	191.4	37.3	9.8
22	9.0	176.6	25.5	8.4
23	9.0	191.0	24.7	7.7
24	9.0	176.4	25.3	19.4
25	9.0	182.5	29.7	11.8
		Papain		
1	5.0	162.1	30.6	15.8
2	5.0	248.4	35.8	12.8
3	5.0	172.1	35.4	9.9

Sample	pH	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
4	5.0	161.2	36.0	14.0
5	5.0	186.0	37.9	11.2
6	6.0	161.8	31.2	16.3
7	6.0	255.5	35.9	5.0
8	6.0	180.1	34.1	14.2
9	6.0	163.6	36.3	9.2
10	6.0	202.4	40.8	7.3
11	7.0	176.6	31.5	12.7
12	7.0	205.2	30.1	6.8
13	7.0	177.9	33.8	8.3
14	7.0	173.2	36.0	9.8
15	7.0	212.0	41.3	6.7
16	8.0	156.2	31.6	14.0
17	8.0	200.0	36.0	11.3
18	8.0	154.7	30.4	12.4
19	8.0	156.4	36.4	8.8
20	8.0	183.5	39.6	14.4
21	9.0	150.0	30.0	10.1
22	9.0	211.9	35.6	12.2
23	9.0	182.1	36.9	7.5
24	9.0	153.3	36.3	16.7
25	9.0	158.1	37.2	15.2
		Control		
1	5.0	187.2	28.2	15.2
2	5.0	182.4	30.4	15.3
3	5.0	209.9	26.0	17.5
4	5.0	192.2	28.7	15.0
5	5.0	156.9	28.8	17.0
6	6.0	163.5	25.2	18.8
7	6.0	167.4	28.9	12.6
8	6.0	198.0	23.6	16.7
9	6.0	181.6	27.5	15.5
10	6.0	181.2	30.0	20.7
11	7.0	188.8	26.7	8.2
12	7.0	175.2	30.2	12.1
13	7.0	206.9	25.1	12.0
14	7.0	187.0	28.4	10.3
15	7.0	180.4	29.2	12.7
16	8.0	170.7	26.2	13.5
17	8.0	153.8	28.2	16.8
18	8.0	196.0	24.1	16.8
19	8.0	197.4	29.5	14.0
20	8.0	179.3	29.0	16.9
21	9.0	176.7	26.9	13.9
22	9.0	161.5	26.8	15.7
23	9.0	195.0	24.0	16.9

Sample	pH	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
24	9.0	198.3	30.1	16.1
25	9.0	189.1	30.1	16.4

[1]Concentrations of enzymes used were 0.02%, 0.0008%, 0.002% and 0.002% for Rhozyme P-11, bromelin, ficin and papain, respectively. Rhozyme P-11 was allowed to react at 50°C while the other enzymes reacted at 70°C.

APPENDIX TABLE 7

Water uptake and tenderness of freeze-dried chicken breast muscle rehydrated in enzyme[1] solutions at various temperatures

Sample	Temperature (°C)	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
		Rhozyme P-11		
1	40	195.5	29.0	11.6
2	40	200.9	27.9	5.4
3	40	206.3	29.1	11.7
4	40	215.9	28.4	9.3
5	40	234.2	29.2	7.4
6	50	200.0	34.9	4.9
7	50	188.3	25.4	5.7
8	50	188.1	28.6	10.5
9	50	199.1	27.3	5.5
10	50	196.3	35.5	5.9
11	60	161.5	25.9	13.9
12	60	198.3	26.2	7.3
13	60	176.9	27.1	7.6
14	60	159.3	25.3	13.2
15	60	203.4	29.7	16.5
16	70	192.6	22.4	8.3
17	70	197.9	22.5	9.6
18	70	188.7	21.5	15.8
19	70	188.7	22.5	8.4
20	70	153.2	19.6	19.4
21	80	200.0	23.8	11.8
22	80	172.1	23.7	22.6
23	80	177.8	24.8	12.3
24	80	177.8	24.2	11.6
25	80	199.1	24.1	22.8
		Ficin		
1	40	211.8	33.1	12.4
2	40	228.0	34.5	7.0
3	40	217.5	33.0	6.6
4	40	168.0	29.5	12.0
5	40	229.4	31.1	6.3
6	50	260.0	35.1	3.1
7	50	236.6	35.7	9.4
8	50	218.5	31.2	4.2
9	50	195.9	31.6	12.0
10	50	202.4	31.3	22.2
11	60	231.8	29.0	4.3
12	60	205.7	26.0	6.9

Sample	Temperature (°C)	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb/gm)
13	60	200.0	26.0	13.5
14	60	239.6	26.0	7.3
15	60	217.1	26.4	7.0
16	70	195.4	31.0	6.6
17	70	201.9	36.6	5.5
18	70	253.0	35.9	5.2
19	70	189.3	26.5	10.6
20	70	197.9	36.2	5.2
21	80	235.9	31.5	5.1
22	80	200.9	28.9	4.7
23	80	172.6	27.1	14.6
24	80	199.1	29.6	7.1
25	80	199.1	29.3	9.7
		Bromelin		
1	40	240.9	41.4	6.3
2	40	289.5	42.4	4.5
3	40	219.8	39.5	4.3
4	40	187.8	29.2	12.7
5	40	236.6	39.4	15.8
6	50	286.9	41.4	7.7
7	50	267.9	38.8	5.7
8	50	257.0	32.2	4.7
9	50	240.7	36.8	5.7
10	50	297.2	40.1	2.0
11	60	317.2	42.5	3.5
12	60	355.4	42.3	2.4
13	60	291.9	42.4	7.2
14	60	267.2	42.4	4.8
15	60	266.4	40.4	3.5
16	70	213.2	29.3	10.2
17	70	259.0	29.9	3.5
18	70	247.9	31.1	10.8
19	70	223.8	29.0	12.1
20	70	221.6	30.0	14.0
21	80	218.4	27.5	8.7
22	80	193.2	25.4	9.4
23	80	196.1	25.7	8.9
24	80	218.6	26.1	9.4
25	80	202.0	24.1	10.8
		Papain		
1	40	167.8	32.4	15.1
2	40	189.0	34.4	16.0
3	40	174.4	33.2	13.6
4	40	168.6	32.5	13.8
5	40	191.5	34.4	14.8

Sample	Temperature (°C)	Water Uptake (%)	Shear Weight (gm)	Shear Force/ Gram (lb.gm)
6	50	168.4	35.7	13.4
7	50	200.8	40.0	12.0
8	50	199.2	37.2	12.4
9	50	177.4	36.9	10.0
10	50	197.0	39.5	10.9
11	60	181.3	39.0	9.7
12	60	156.2	35.1	12.8
13	60	186.8	39.0	19.0
14	60	152.8	35.9	11.4
15	60	181.6	38.3	12.6
16	70	178.0	35.3	12.2
17	70	193.7	37.3	26.8
18	70	191.3	37.0	16.8
19	70	189.0	36.7	10.1
20	70	184.9	35.9	14.2
21	80	180.9	36.8	21.2
22	80	178.0	36.7	12.3
23	80	174.4	36.5	15.9
24	80	176.6	35.4	27.4
25	80	187.9	36.0	11.9
		Control		
1	40	176.1	26.2	13.4
2	40	200.9	26.2	20.0
3	40	192.9	26.7	17.2
4	40	184.2	27.5	13.3
5	40	207.9	27.8	22.3
6	50	165.8	27.1	24.4
7	50	188.3	27.9	26.2
8	50	184.3	27.6	13.4
9	50	186.8	26.9	14.3
10	50	181.0	27.0	15.1
11	60	189.0	30.6	16.0
12	60	175.8	28.6	16.1
13	60	169.5	27.2	16.7
14	60	167.2	27.9	21.1
15	60	175.8	29.2	17.5
16	70	177.4	29.8	15.6
17	70	165.7	30.3	24.9
18	70	172.0	30.7	22.8
19	70	168.4	29.7	14.1
20	70	170.7	30.2	15.4
21	80	173.9	26.3	15.6
22	80	166.4	25.9	15.3
23	80	168.7	26.3	19.0
24	80	168.9	25.9	18.1
25	80	169.0	26.5	17.1

[1]Concentrations of enzymes used were the same as for Table 6. Papain was allowed to react at pH 7.0 whereas all other enzymes reacted at pH 5.0.

APPENDIX TABLE 8

Moisture content of freeze-dried chicken breasts

Sample	Initial Frozen Weight (gm)	Weight After Freeze-Drying (gm)	Weight After Dry Air Oven (gm)	% Moisture
		1 3/16" X 3 1/2" Samples		
A1	29.38	8.24	8.02	0.75
A2	22.18	7.59	7.45	0.61
A3	25.09	7.05	7.00	0.17
A4	25.34	7.49	7.20	1.16
A5	30.06	8.57	8.31	0.85
B1	33.48	9.77	9.54	0.69
B2	33.49	9.44	9.27	0.51
B3	36.55	9.92	9.21	1.94
B4	38.53	11.53	11.29	0.62
B5	31.71	8.99	8.63	1.13
C1	62.48	18.71	18.32	0.63
C2	38.62	11.74	11.53	0.55
C3	44.96	13.81	13.49	0.71
C4	36.50	10.12	9.88	0.67
C5	40.11	12.49	12.20	0.72
		Diced Samples		
1	41.14	12.53	12.16	0.89
2	40.98	12.41	12.11	0.74
3	38.73	11.33	10.98	0.92
4	36.46	9.83	9.53	0.80
5	38.71	11.33	10.94	1.01
6	45.63	13.97	13.66	0.67
7	42.11	12.89	12.42	1.11
8	35.80	9.90	9.55	0.97
9	39.93	12.40	12.03	0.93
10	38.45	11.09	10.79	0.78
11	43.64	13.32	12.95	0.86
12	40.14	11.87	11.34	1.32

Average Percent Moisture in Freeze-Dried Samples:
Group A = 0.71 Diced Samples = 0.92
Group B = 0.98
Group C = 0.66
Total Average = 0.78

APPENDIX TABLE 9

Protein content of proteolytic enzyme preparations

Enzyme	Sample	Weight Of Enzyme (gm)	HCl Titrated (ml)	HCl Normality	% Nitrogen	% Protein
Ficin	1	0.3029	23.45	0.0971	10.52	65.75
	2	0.3027	23.20	0.0971	10.42	65.13
	3	0.3057	23.60	0.0971	10.49	65.56
	4	0.3015	22.15	0.1018	10.47	65.44
	5	0.3087	23.55	0.0971	10.37	64.81
Papain	1	0.3144	3.38	0.0971	1.46	9.12
	2	0.3028	3.10	0.0971	1.39	8.69
	3	0.2990	3.30	0.0971	1.50	9.38
	4	0.3086	3.50	0.0971	1.54	9.63
	5	0.2996	3.43	0.0971	1.55	9.71
Bromelin	1	0.3017	11.43	0.1418	5.40	33.73
	2	0.3001	11.70	0.1018	5.56	34.73
	3	0.3002	12.60	0.1018	5.98	37.39
	4	0.3037	13.23	0.1018	6.21	38.81
	5	0.3010	13.42	0.1018	6.35	39.69
Rhozyme P-11	1	0.3019	2.65	0.1018	1.25	7.81
	2	0.3018	2.85	0.1018	1.37	8.43
	3	0.3025	2.78	0.1018	1.31	8.19
	4	0.3027	2.60	0.1018	1.22	7.77
	5	0.30000	2.80	0.1018	1.33	8.31

Averages

Enzyme	% Protein
Ficin	65.34
Papain	9.31
Bromelin	36.87
Rhozyme P-11	8.10

Sample Calculations

(Ficin--1)

$$\% \text{ Nitrogen} = \frac{(\text{Meq.}N_2)(\text{ml HCl})(\text{Normality HCl})(100)}{\text{Sample Weight}}$$

$$= \frac{(0.014)(23.45)(0.0971)(100)}{0.3029}$$

$$= 10.52\%$$

$$\% \text{ Protein} = \% \text{ Nitrogen } (6.25)$$

$$= 10.52\ (6.25)$$

$$= 65.75\%$$

APPENDIX TABLE 10

Moisture content of proteolytic enzyme preparations

Enzyme	Sample	Weight Before Drying (gm)	Weight After Drying (gm)	Percent
Papain	1	2.0026	1.8920	5.52
"	2	2.0025	1.8922	5.51
"	3	2.0008	1.8908	5.50
Ficin	1	2.0009	1.8310	8.49
"	2	2.0000	1.8327	8.37
"	3	2.0013	1.8316	8.48
Bromelin	1	2.0129	1.8750	6.85
"	2	1.9998	1.8617	6.91
"	3	2.0127	1.8749	6.85
Rhozyme P-11	1	2.0113	1.9810	1.51
"	2	2.0042	1.9747	1.47
"	3	1.9997	1.9718	1.40

Averages

Enzyme	% Moisture
Papain	5.51
Ficin	8.45
Bromelin	6.87
Rhozyme P-11	1.46

APPENDIX TABLE 11

pH of buffer solutions before and after rehydration

Sample	Initial pH	pH After Rehydration	Sample	Initial pH	pH After Rehydration
1	4.0	3.9	40	7.0	6.7
2	4.0	3.9	41	7.0	6.7
3	4.0	3.9	42	7.0	6.7
4	4.0	3.8	43	7.0	6.7
5	5.0	5.0	44	7.0	6.7
6	5.0	5.1	45	7.0	6.8
7	5.0	5.1	46	7.0	6.8
8	5.0	5.0	47	7.0	6.6
9	5.0	5.1	48	7.0	6.7
10	5.0	5.1	49	7.0	6.6
11	5.0	5.1	50	7.0	6.7
12	5.0	5.0	51	8.0	7.5
13	5.0	5.0	52	8.0	7.5
14	5.0	5.0	53	8.0	7.5
15	5.0	5.0	54	8.0	7.5
16	5.0	4.9	55	8.0	7.5
17	5.0	5.0	56	8.0	7.8
18	5.0	5.0	57	8.0	7.6
19	6.0	5.5	58	8.0	7.5
20	6.0	5.9	59	8.0	7.5
21	6.0	5.5	60	8.0	7.5
22	6.0	5.6	61	8.0	7.5
23	6.0	6.0	62	8.0	7.5
24	6.0	5.7	63	8.0	7.5
25	6.0	5.6	64	8.0	7.5
26	6.0	5.6	65	9.0	8.4
27	6.0	5.6	66	9.0	8.4
28	6.0	5.5	67	9.0	8.5
29	6.0	5.6	68	9.0	8.4
30	6.0	5.7	69	9.0	8.4
31	6.0	5.6	70	9.0	8.4
32	6.0	5.6	71	9.0	8.4
33	7.0	6.7	72	9.0	8.4
34	7.0	6.8	73	9.0	8.4
35	7.0	6.7	74	9.0	8.4
36	7.0	6.7	75	9.0	8.4
37	7.0	6.8	76	9.0	8.5
38	7.0	6.7	77	9.0	8.4
39	7.0	6.8	78	9.0	8.4

Averages

Initial pH of Buffer	pH After Rehyd.	Initial pH of Buffer	pH After Rehyd.
4.0	3.9	7.0	6.7
5.0	5.0	8.0	7.5
6.0	5.6	9.0	8.4

APPENDIX TABLE 12

Panel score card

Date: _____

Judge: _____ Code No.: _____

Table 1:

FACTOR	1	2	3	4	5	6	7
Initial Tenderness	Extremely Tender	Very Tender	Tender	Slightly Tender	Slightly Tough	Tough	Very Tough
Residual Tenderness	Extremely Tender	Very Tender	Tender	Slightly Tender	Slightly Tough	Tough	Very Tough
Juiciness	Very Juicy	Juicy	Slightly Juicy	Neither dry nor Juicy	Slightly Dry	Dry	Very Dry
Flavor	Very Desirable Flavor	Desirable Flavor	Slightly Desirable Flavor	Neither Desirable Nor Undesirable	Slightly Undesirable Flavor	Undesirable Flavor	Very Undesirable

Table 2:

	Sample No.				
FACTOR					
Initial Tenderness					
Residual Tenderness					
Juiciness					
Flavor					

1) Taste, evaluate, and score each sample independently from other samples.

2) Chew each sample the same way initially.. across the grain of the meat.

3) Score in Table 2 the number from Table 1 best representing the sample.

Comments: