/TENDERIZATION OF SPENT HEN MUSCLE USING PAPAIN, BROMELIN
OR FICIN, ALONE AND IN COMBINATION WITH SALTS/

by

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INTRODUCTION

There are approximately 250 million mature chickens marketed annually. Approximately 85% of these mature chickens are old hens culled from egg-laying flocks because they are no longer economically productive, the remainder are old birds from poultry breeder flocks. The meat from these mature chickens is generally tough, dry, and sinewy (Federal Register, 1980). As a result the market for such poultry meat is limited. These hens represent a substantial amount of ready-to-cook poultry that would appeal more readily to the consumer if they could be tenderized effectively and economically.

On October 5, 1980 the USDA approved the use of proteolytic enzymes such as bromelin, ficin, and papain as well as those from Aspergillus oryzae and Aspergillus flavus-oryzae groups to tenderize the muscle tissue of all cuts of meat including mature poultry muscle tissue.

The objectives of this project were: to determine sensory characteristics and shear press measurements of spent hen breast fillets that were soaked in solutions of papain, bromelin or ficin alone or in combination with sodium chloride and phosphate solutions.

REVIEW OF LITERATURE

Composition and Structure of Poultry Meat

Composition

Chicken is comparable to beef, lamb, and pork in composition and nutritive value and contains all the essential amino acids required for good human nutrition (Demby and Cunningham, 1980).

Vitamins and Minerals

Chicken, like all other lean meats, is a good source of B-vitamins (Demby and Cunningham, 1980). According to Charley (1970), chicken meat is rich in iron and phosphorous. It is also a good source of niacin, riboflavin, thiamin, and ascorbic acid (Mountney, 1976).

Fats

The fat content of poultry carcasses varies according to the age, sex, and species (Mountney, 1976). However, it is considered a low fat food with most of the fat being located in the skin rather than in muscle tissue (Demby and Cunningham, 1980). According to Fristrom and Weihrauch (1973), broiler meat contains only 2.5 g of fat per 100 g edible portion of meat. They also reported that broiler fat contains more unsaturated than saturated fatty acids. Poultry meat contains a higher proportion of unsaturated fatty acids than red meat but usually less than vegetable oils (Mountney, 1976). The major fatty acids in raw chicken muscle are oleic, linoleic, and palmitic; these three comprise 79% of the total and have a total unsaturation of 70% (Lee and Dawson, 1973). According to Fristorm and Weihrauch (1973), oleic is the dominant fatty acid in broiler muscle. Chicken muscle was found to contain more

phospholipids than the skin and depot fat (Katz et al., 1966). Chicken meat contains less cholesterol than many other foods of animal origin (Demby and Cunningham, 1980).

Carbohydrates

Carbohydrates in chicken, as with most meats, are a small portion of the total nutrients (Demby and Cunningham, 1980). Inositol, glucose, and fructose are the major carbohydrate constituents found in chicken muscle with ribose and mannose being minor constituents (Lilyblade and Peterson, 1962).

Structure

Birds like mammals possess three distinct types of muscles: (1) smooth, (2) cardiac, and (3) skeletal. The skeletal muscles of poultry contain three types of fibers; namely, red fibers, white fibers, and intermediate fibers. The red fibers predominate in what is commonly called "dark meat", intermediate fibers represent a type of fiber possessing some characteristics of both red and white fibers (Ensminger, 1980). Skeletal muscle is made up of fibers held together by connective tissue and surrounded by a sheath of heavier connective tissue. Each fiber is enclosed in a thin, colorless elastic membrane called the sarcolemma, and the fibers are then grouped parallel to each other in bundles called fasciculi. The perimysium is the connective tissue surrounding the fasciculous and the entire muscle is enclosed by connective tissue known as epimysium (Lowe, 1943).

The function of connective tissue is to support all the other tissues and organs in the body. Smith (1942) reported that connective tissue is most evident in the form of tendon, or gristle, but it is also

distributed in a firm state of subdivision throughout both muscle and fatty tissue. Lowe (1943) described connective tissue as follows: "Connective tissue is characterized by a small number of cells and much intercellular substance. It has many variations and transitional forms. Some are loose, like that between organs, some are compact, as in heavy connective tissue visible to the eye and some are dense, like that in tendons. It always contains fibers".

Collagenous fibers are colorless and birefrigent, but when they appear in large masses, the tissue is white and is often referred to as white connective tissue (Lowe, 1943). According to Smith (1942) the properties of collagenous tissue influence the properties of meat because of the toughness and elasticity of the collagen fiber, and its easy conversion to the tender soluble gelatin by boiling. Elastin, a second protein in connective tissue forms only a very small proportion of the diffuse connective tissue and ordinary white tendon, but it is concentrated in ligaments. Elastin according to Smith (1942) differs from collagen in that it is fractionally indigestible and is softened little during cooking. Lowe (1943) stated that the elastic fibers are thinner than the collagenous ones and that they branch readily and stretch like a fish net. The fibers forming ligaments are arranged parallel to each other and are bound together by collagenous fibers.

Factors that Affect the Tenderness of Meat

Effect of Production Factors

In 1963, Stadelman reviewed production factors affecting tenderness of poultry meat. He included age, sex, breed or strain, and feeding practices as significant considerations.

Age

According to Stadelman (1963), age and confinement are the major production factors affecting tenderness. Shrimpton (1960) showed that two month old birds would not be considered tough irrespective of processing procedures when compared with six month old or eighteen month old birds. Peterson et al. (1959) reported similar results indicating a definite increase in toughness as age increased, in this particular study, the breast muscle of the young birds were significantly tougher than the dark muscle whereas in the older birds the dark muscle was slightly tougher than the breast muscle. Dodge and Stadelman (1959) reported on factors influencing tenderness of poultry meat comparing chicken and turkeys of similar ages. They found the age of birds and the class of poultry to have an influence in tenderness. May et al. (1962) observed that the older the chickens were, the less tender and that this held at the time of slaughter and throughout aging. Wells et al. (1962) reported similar observations.

Breed and Sex

These two factors according to Stadelman (1963) have only a very slight influence on tenderness scores assigned to the meat. Gilpin et al. (1960) compared modern fast growing chickens with a slow growing old type breed, their results showed little indication that one breed produced more tender meat than the other. According to Shrimpton and Miller (1960) among birds on restricted diets or egg type chickens, the females were preferred to the males with regards to tenderness of the breast meat.

Feeding Practices

According to Stadelman (1963) turkeys fed corn were slightly more tender than those fed oats, barley, rye or wheat, but the reason could possibly be due to other differences in the diet used during 1941 as compared to 1961 or possibly due to growth rate differences. An observation by Shrimpton and Miller (1960) was that birds kept on full feed were more tender than birds kept on a restricted diet. They also observed that birds on full feed had no sex differences with respect to tenderness.

Lee et al. (1976) studied the effects of heat stress (38°C), cold stress (4°C) and extreme cold stress (-20°C) before slaughter on the tenderness and post mortem glycolysis of the excised chicken breast muscle and found that heat stress significantly increased the toughening of the breast muscle.

Effect of Processing

Several variables in processing poultry affect final muscle tenderness including struggling, slaughter method, scalding time and temperature, method of picking, chilling, pre-rigor muscle cutting and post-mortem aging (Klose et al., 1959; Stadelman, 1963). Stadelman and Pratt (1977) later added that a particular cutting method and cooking could influence tenderness. Researchers (Klose et al., 1959, Pool et al., 1959; Klose et al., 1960; and Klose et al., 1961) distinguished three phases of the poultry processing operation that could affect the tenderness of poultry meat, namely scalding, feather-plucking and aging. Scalding and feather-plucking, although separate steps in the processing line, are inter-related since very mild scalding conditions yield birds

that require more severe feather-plucking conditions and vice-versa (de
Fremery, 1963). Increasing scalding time and temperature within
commercial ranges cause small but significant increases in toughness of
cooked birds (Klose et al., 1959, de Fremery, 1963). Increasing the
severity of the feather-plucking treatment will increase the toughness of
the cooked meat (de Fremery, 1963). Klose et al. (1959) found that
hand-picked birds were more tender than those picked by machine; however,
hand-picking, he added, is not commercially feasible.

Struggling

According to Dodge and Stadelman (1959) there is no significant
difference in tenderness between birds that struggled excessively during
slaughter from those that did not struggle. Stadelman and Wise (1961)
anesthesized the birds prior to slaughter with nembutal, and found that
the anethesized birds were not as tough as the controls when determined
by shear values; however, the anethesized birds did require an extended
period of time to tenderize when compared to normal birds. Ma et al.
(1971) reported significant differences between the time course of rigor
mortis in breast muscles of turkeys that struggled compared with
restrained birds.

Other processing factors such as chilling, freezing, frozen storage
and holding after thawing have also been investigated. Lowe (1943)
demonstrated that roasters frozen within 2 hrs. after killing were less
tender than those aged 24 hrs. before freezing. Stewart et al. (1945)
found no difference in tenderness between groups given pre-freezing
chilling periods of 2 hrs. and 18 hrs. respectively. Pool et al. (1959)
stated, with regards to pre-freezing, that a 48 hr. thawing period prior

to cooking eliminated any differences that were originally present.
Koonz et al. (1954) found that freezing broiler carcasses during the
early post-mortem period fixed the state of tenderness existing at the
time and that complete tenderization was delayed until the tissues were
defrosted. They also found that cutting into the muscle immediately
after slaughter resulted in greater toughness than an uncut control at
all stages of the normal aging period.

Gainer et al. (1951) investigated the possibility that massaging
might tenderize the muscles of chicken roasters aged for short period of
time like 30 and 60 min before cooking. They found no appreciable
difference between control and massaged birds.

Aging

According to de Fremery (1963), aging is essential for the
development of tenderness although the time period for poultry is much
less (12-24 hrs) than it is for beef (10-20 days). He found that the
rapid onset of rigor and the rapid disappearance of ATP were not causes
of toughening. He observed that an acceleration of post-mortem
glycolysis in young poultry increased the toughness of fully-aged meat
and that meat from birds in which post-mortem glycolysis had been
eliminated or inhibited was initially tender and remained so during a 24
hour aging period.

In general, any treatment which resulted in more rapid loss of
glycogen induced muscle toughness (de Fremery and Pool, 1960). Mellor et
al. (1958) found that the breast muscle of birds with high glycogen
content yielded more tender meat than birds of low glycogen content.

Enzymes

Proteolytic enzymes of plant origin like papain, bromelin and ficin have been used in the past as meat tenderizers (Brandly et al., 1966; Arnon, 1970).

Papain

Papain is the most widely used proteolytic enzyme and the term papain is applied both to the crude enzyme preparation from papaya latex and to one of its distinct protease fractions (Reed, 1966). Papain is the whole dried product from the latex of the unripe fruit of the Carica papaya, the tropical melon tree (Hwang and Ivy, 1951; Kimmel and Smith, 1957; Wang and Maynard, 1955). It is found in the milky latex of the green fruit, the leaves and the trunk of the tree (Balls et al., 1940). It is a white or cream colored powder with a characteristic pungent odor (Hwang and Ivy, 1951).

The proteolytic components of papaya latex are unusually stable towards extremes of temperature and pH (Kimmel and Smith, 1957). According to Hwang and Ivy (1951), papain can retain its activity at a temperature of 105°C for 3 hours, but when in solution, it loses its activity on heating for 30 min at a temperature of 82.5°C. Remarkable activity of papain at low temperatures of about 10°C were noticed by Hwang and Ivy (1951). Crystalline papain was found to possess activity after standing at 30°C for two hours between pH 3 and pH 12 (Hwang and Ivy, 1951). The optimum stability of papain solution was in the range of pH 5 and pH 7 (Hwang and Ivy, 1951; Kimmel and Smith, 1957). Above pH 7 activity was slowly lost (Kimmel and Smith, 1957); above pH 11 and below pH 3 the enzyme was rapidly inactivated (Hwang and Ivy, 1951; Kimmel and

Smith, 1957). At pH values near neutrality, papain can withstand 50°C for 30 min without significant loss of activity, and at 75°C only about 5% of the original enzyme activity was lost in 3 min but above 75°C marked inactivation occurred. When the enzyme was exposed for longer periods of time, around 30 min, significant loss of activity at 60°C occurred (Kimmel and Smith, 1957).

According to Hwang and Ivy (1951) the most favorable temperature for the rapid digestion of ground meat by papain during a 30 min period was 80°C. The optimum temperature of papain according to Tappel et al. (1956) was 50°C, Weiner et al. (1957) reported that the optimum temperature for papain digestion of beef was 60°C to 80°C. According to Weir et al. (1958) the most rapid digestion of papain occurred between 148°F (70°C) and 185°F (85°C) with very little taking place below 86°F (30°C).

Ficin

Ficin is the proteolytic component in the latex of the fig tree, Ficus galabrata (Whitaker, 1957). Ficin was found to be effective in tenderizing meats (Miyada and Tappel, 1956), and it is considered to be superior to papain and bromelin as far as its tenderizing properties are considered (Whitaker, 1957). According to Whitaker (1957), papain and ficin are similar in their properties.

Ficin was active over a wide range of pH, pH 5.0 to pH 9.0, with greater activity at pH 5.0 and pH 7.0 at 70°C (Dawson and Wells, 1969). Maximum solubilization of all beef protein fractions occurred at pH 7.0 at a temperature of 80°C when using ficin (El-Gharbawi and Whitaker, 1963). The optimum temperature for ficin was reported as 30-50°C by Dawson and Wells (1969).

Bromelin

This proteolytic enzyme is present in the pineapple plant, _Amanas_ _comosus_. The proteases can be isolated from the juice of the fruit or from the crushed stems of the plant (Reed, 1966; Heinicke and Gortner, 1957).

The optimum temperature for bromelin was reported as 30°C-60°C by Dawson and Wells (1969). Maximum solubilization of all beef protein fractions occurred at pH 7.0 and 80°C when using bromelin (El-Gharbawi and Whitaker, 1963).

Enzyme Action

Meat tenderized with enzyme preparations is tenderized in a different way than when it is age-tenderized.

Post mortem aging according to de Fremery and Streeter (1969) can be defined as "the holding of an animal carcass for some definite period of time with the intention of producing tenderization through one or more natural processes."

It is often assumed that proteases present in muscular tissue of various animals are at least partially responsible for the tenderization occurring during aging or "ripening" of edible muscle (Whitaker, 1959). However it was concluded by Bandack and Rose (1961) that muscle proteases probably play little part in the tenderization of meat.

De Fremery and Streeter (1969) stated that the post-mortem aging of chicken meat was not caused by the breakdown or dissolution of the connective tissue proteins, nor by their increased lability to gelatinization, or solubilization during cooking.

According to Birkner and Auerbach (1960), the histological changes during aging consist mainly of disappearance of cross-striations, the

appearance of transverse breaks, kinks or waves, and decrease in the collagen. The elastin however was not affected. The connective tissue changes did not account for increased tenderness on aging.

Based on studies on bovine sternomandibularis muscles, Davey and Gilbert (1968) found that meat aging was due to the disruption and possible dissolution of the Z line material, leading to a weakening of inter-myofibrillar linkages probably located at the junctions of adjacent Z lines, and to loss of tensile strength of the myofibrils themselves. Stromer and Goll (1967a, b), Stromer et al. (1967), Davey and Gilbert (1967, 1968, 1969), Davey and Dickson (1970) and Davey et al. (1967) showed that aging reduced the strength of the myofibrillar structure while Khan and Van den Berg (1964), de Fremery and Streeter (1969) and Sayre (1968) showed that aging did not significantly affect connective tissue.

Enzyme preparations, contrary to age tenderization, act on the muscle fiber itself, breaking down the sarcolemma and the nuclei, followed by the disintegration of endomysial collagen, connective tissue collagen and elastin, and finally the complete disappearance of cross striatians (Landman, 1963; Wang et al., 1957).

Specific action of enzymes on various meat systems was studied by Wang et al. (1957) who found that Rhozyme P-11 exerted a greater affinity for the nuclei of the muscle fiber than for those of the connective tissue cells, whereas the reverse was true of papain in a commercial tenderizer. Kang and Rice (1970) studied the effect of various enzymes on meat protein fractions. Bromelin and trypsin degraded the connective tissue fraction of the muscle tissue to a greater degree than the fiber portion. However, papain and ficin degraded the myofibrillar fraction

more than the connective tissue. Wells et al. (1965) used papain at a concentration of .001% on freeze-dried chicken and found only minor breakdown of connective tissue, but extensive degradation of the muscle fibers to the extent of complete destruction of the sarcolemma, loss of striations, and disappearance of the nuclei. Wismer-Pederson (1972) used various enzyme preparations and found that bromelin, collagenase, and trypsin were extremely effective for tenderizing the connective tissue collagen. Miyada and Tappel (1956) in their studies of hydrolysis of beef proteins by enzymes found that papain and ficin digested elastin, while Rhozyme P-11, Protease 15, and Rhozyme A-4 showed slight but definite digestion of elastin. In the case of collagen they found the greatest digestion with bromelin, ficin, and trypsin, followed by rhozyme P-11 and papain. McIntosh and Carlin (1963) found that papain affected mucoprotein and collagen more than the other skeletal muscle proteins, and also the collagen suspensions were converted to thick gels by the action of papain. They concluded that the tenderizing effect of papain was due, at least in part, to the breakdown of connective tissue.

Only certain enzymes act on the collagenous and elastic fibers and ficin, bromelin, papain, viokase and trypsin attack collagenous and elastic fibers in that order of decreasing activity (Wang et al., 1957). Wang et al. (1958) in a later experiment found that of the three plant enzymes used, ficin ranked first, then papain, and finally bromelin in elastolytic activity. Miyada and Tappel (1956) also found that papain and ficin digested elastin.

According to Wang et al. (1957) the mode of collagenous action, as revealed from both colloidin sections of whole tissue and collagenous fibers treated separately, appeared to consist of two phases. First

there was a decrease in the staining capacity with acid fuchsin. This
reflected a change in the molecular structure of collagen. Then the
enzyme seemed to have reduced the sharpness of the fibrillar nature of
collagen, a change which was believed to have resulted from the
liquefaction of the ground substance or matrix which normally hold the
collagenous fibers into definitive bundles. Those observations support
the view that papain digests some nitrogen-containing components of
connective tissue without affecting the fibrillar structure. The
criterion for elastic digestion was most specific and easily
recognizable. This is partly because elastic fibers are morphologically
more discrete than collagenous fibers and partly because the mode of
action of elastase actually involves dissolution of the protein, elastin.
Papain is the weakest of the three commercial preparations in collagenase
activity and only moderate in elastase activity. Ficin out-performs, by
far, both papain and bromelin in elastase activity and equals bromelin in
collagenase activity. Miyada and Tappel (1956) used papain at 0.05%
level to digest rehydrated, ground, freeze-dried biceps femoris muscle of
beef. The insoluble protein fraction was separated into collagen and
elastin. Papain did not digest collagen, but elastin was digested to
some extent. The resistance of collagen to papain was studied by Neuman
and Tytell (1950) who extracted collagens from several sources including
chicken tendon, sheep tendon, and cattle-tail tendon. The collagens were
prepared so as not to alter their natural properties. These collagens
were shown to be resistant to the action of papain, trypsin, and
chymotrypsin.

Robbins et al (1979) used SDS (sodium dodecyl sulphate) gel
electrophoresis to show the effect of papain on bovine myofibrils.

Myofibrillar proteins, myosin, actinin and actin, were degraded and formed peptides with lower molecular weights. Lowey et al. (1969) reported that trypsin and papain initially degraded myosin to light and heavy meromyosins, and total rods and globular heads respectively; this attack was followed by more extensive breakdown with time to low molecular weight fragments.

Rattrie and Regenstein (1977) subjected actin and myosin heavy chains isolated from chicken breast muscle to crude papain at a level of 1:125 w/w. SDS electrophoresis of the myofibrillar proteins indicated that papain digested actin rapidly and produced major degradation products. The myosin was degraded slower and only 40% of the heavy chain was cleaved after 3 min. Actomyosin was less rapidly digested than the myosin.

Robbins et al. (1979) showed that cathepsin-D extracted from muscle or spleen degraded myofibrils under postmortem pH conditions (5.1-5.3) causing an alteration of Z disk structure and breakdown of myosin heavy and light chains as well as effecting changes in the tropomin-tropomyosin complex. Rattrie and Regenstein (1977) showed that the selective action of cathepsin-D on the myosin component of myofibrils was in contrast to the breakdown of that protein which would occur when myofibrils were incubated with trypsin or papain. Robbins et al. (1979) pointed out that due to the selectivity of cathepsin-D towards the proteins of the myofibrils, it could be used as an exogenous meat tenderizer and that the texture of muscle treated with cathepsin-D was similar to that of naturally aged muscles in contrast to the "mush" texture resulting from indiscriminate degradation of the myofibrillar proteins by commercial tenderizers containing papain.

Methods of Application

Early Uses of Enzymes

Lawrie (1968) and McWilliams (1966) stated that enzymatic tenderization was employed at least 500 years ago by the Mexican Indians when they wrapped meat in paw paw leaves during cooking. The natives of the tropics tenderized their meat by boiling it with the unripe fruit of papaya or with its juice or by wrapping with its leaves (Hwang and Ivy, 1951).

The usually recommended procedure for tenderizing meat is to paint the meat with a solution of papain and then cook it after an interval of a few minutes (Gottschall and Kies, 1942; Hwang and Ivy, 1951). When the meat was painted with a solution of papain and then cooked, it was found that papain would not penetrate very far in the meat under such conditions, and the papain on the surface would be inactivated by heat before much digestion could take place (Hwang and Ivy, 1951; Gottschall and Kies, 1942).

Powder Application

Tappel et al. (1956) applied a thick layer of papain to the surface of semitendinosus muscle. After 3 hours of exposure at room temperature, no histological changes were evident. Below the superficial layer of enzyme powder, all the muscle fibers were intact, with few noticeable disturbances in the striation pattern. After broiling for 10 min, the sarcolemma was destroyed at the surface and marked separation of muscle fibers were evident. Deeper in the meat, the sarcolemma was undisturbed. Wang and Maynard (1955) applied a papain preparation in powder form, spread over the sample (beef semitendinosus tissue) for a specified

length of time. They found this method to be inadequate and impractical because of limited penetration of the enzyme into the tissue. According to Weir et al. (1958) the activity of the surface coated enzyme on meat was quickly lost upon exposure to cooking above 212°F. Thus, it was necessary for the enzyme to penetrate the meat before the cooking process began. Papain, if sprinkled on the surface of meat, will penetrate a depth of about 1 min per hr. Penetration was improved, if the treated surface is forked (McWilliams, 1966). Hay et al. (1953) using round steak, sirloin tip steak, and rump roasts, applied papain by sprinkling the enzyme on the meat surface with a salt shaker and then pierced the surface with a fork to provide deeper penetration before cooking. No significant difference in cooking losses, aroma, and flavor of treated and untreated samples was found. For top round steak, bottom round steak and sirloin tip steak, all treated samples were significantly more tender.

Immersion

Application of enzyme by immersion can include immersing a sample in enzyme solution or rehydrating freeze-dried samples with enzyme solution.

Wang and Maynard (1955) immersed raw beef semitendinosus tissue in 10% solutions of a commercial papain preparation and a proteolytic enzyme of fungal origin. This method proved inadequate and impracticable because of limited penetration of the enzyme into the tissue which was less than 1 mm from the surface in 1 hr. Gottschall and Kies (1942) observed a penetration of 2.5 mm by soaking meat in papain solution for 20 min at 23°C. Tappel et al. (1955) soaked semitendinosus muscle in papain solution and found that the maximum penetration of dye was about

2 mm which clearly indicated that effective distribution of papain within the meat could not be accomplished by natural diffusion or by papain's hydrolytic activity. The histological study of beef cooked with papain also showed that papain did not affect sarcolemma, nuclei, or muscle fibers at distances greater than 0.5 mm. Youn and Yang (1974) treated beef with papain (0.01%, 0.05%, and 0.1% solution) by soaking for 40-60 min at 25°C and 10°C. Sensory evaluation indicated preference for those soaked at 10°C. Youn and Yang (1975) also treated round steaks with 0.01%, 0.05%, and 0.1% papain solutions and measured tenderness using a penetrometer. Penetration values increased proportionally with increasing papain concentrations. At the 0.05% papain level, there was significant increase in tenderness, but no further significant increase in tenderness with a 0.1% level.

Wang and Maynard (1955) immersed frozen-dried pork chops (longissimus dorsi) in a rehydrating solution containing papain. Due to the spongy nature of frozen-dried muscle tissues and the great water-absorbing capacity of the undernatured muscle fibres, the enzyme dissolved in the rehydrating liquid was quickly brought into contact with the tissue components. They found that this method of enzyme application improved tenderness. Wang et al. (1957) rehydrated freeze-dried steaks in an aqueous solution of papain. They found that the treated sample showed flavor differences and increased tenderness.

Dawson and Wells (1969) used a five-member panel and reported that 0.002% was the optimum papain concentration for tenderization and acceptability of freeze-dried chicken. At levels up to 0.05% the meat was extremely tender but not acceptable to the panel.

So far the attempts to introduce the enzyme into carcass meat prior to cooking and freeze-drying have resulted in non-uniform penetration and enzyme action and in some flavor changes (Dawson and Wells, 1969).

It was also reported that enzyme treated meat by the powder or immersion method showed over-tenderization and a mushy appearance on the exterior, but little or no effect on the interior of the sample (Birkner and Auerbach, 1960; Fry et al., 1966).

Wang and Maynard (1955) treated one set of beef semitendonosis muscle with papain powder and immersed another set in 10% papain solution. Although the enzyme solution penetrated into the meat more than the powdered preparation, both methods proved impractical because of limited penetration.

Injection

Lowe (1943) observed that if enzyme preparations are to give satisfactory results in tenderizing meat, they should be injected uniformly throughout the tissue. Rogers et al. (1965) injected dry cured country-style hams with 5 ppm papain solution. Taste panel members scored non-treated control hams significantly higher for flavor, saltiness and overall acceptability. Treated hams were significantly more tender but were generally too mushy to be acceptable.

Beuk et al. (1959) achieved a tenderizing effect by both intravenous and intraperitoneal injection of crude papain. Haleem et al. (1970) found that a concentration of 20 mg. and 40 mg. of crude papain per bird injected intravenously, produced the desired tenderness whereas 100 mg. and 300 mg. per bird resulted in "mushy" muscle tissue. Sensory evaluation of the light and dark meat by five panel members indicated an

increase in tenderness and juciness as the enzyme concentration increased, but a decrease in flavor acceptance. Sosebee et al. (1964) injected papain post mortem into poultry muscle and found that it was effective in increasing tenderness; however, at a concentration of 0.003% papain, the tenderness increased to the point of being mushy. Also there was lack of uniform tenderness with the collagen showing extensive degradation and the fibers showing little change. Fry et al. (1966) injected 3% and 6% by weight of papain solution into turkey rolls. This resulted in a tough muscle portion at the top of the roll and an overtenderized, mushy product on the bottom of the roll because the enzyme tended to migrate to the bottom of the roll during cooking. Prusa (1980) injected baking hens with 0.001% and 0.002% papain solution. He found the papain injected samples were more tender and mealier than uninjected samples. He also found that off-flavors could be detected in the papain injected samples and that these off-flavors increased as the percentage of papain injected increased.

Antemortem injection of plant proteases was introduced by Beuk et al. (1959) and was considered one of the most effective methods for tenderizing meats (Kang and Warner, 1974). This is in agreement with Huffman et al. (1961), Strandine and Koonz (1963), Fry et al. (1966), and Goodwin and Woldroup (1970), who reported that antemortem application of papain intravenously to poultry was an effective way to improve tenderness. Fry et al. (1966) showed that antemortem injection gave a more uniform and quicker distribution of the enzyme than postmortem application or injection into the peritorial cavity.

Huffman et al. (1961) found that antemortem injection of 0.35 ppm, 0.55 ppm, 35 ppm and 55 ppm of crude papain had no significant effect on

breast muscle, but 100 ppm crude papain resulted in more tender or overtenderized chicken meat. Bawa et al. (1981) injected enzyme solutions of papain and bromelin at levels of 50, 75 and 100 ppm into the vascular systems 5 min prior to slaughter. The results showed that tenderness increased with increased levels of enzyme and that papain resulted in greater tenderization than bromelin. Papain levels of 100 ppm however had an undesirable effect on flavor resulting in lower overall acceptability.

Kann et al. (1974) subjected beef neck and shoulder muscles to four different treatments. First, they sprinkled papain and salt on either side of the meat and let it stand for 10 min; next, the pieces were beaten with a meat hammer and then soaked in a solution of enzyme and salt for 10 min; next, they did the same as in treatment two but the solution was mechanically stirred; and for the final treatment, they injected the pieces with enzyme solution and let them stand for 10 min. Taste panel results indicated the last two treatments produced acceptable products.

Fry et al. (1966) compared post-mortem enzyme application with antemortem enzyme application. Their results confirm those of previous workers that post-mortem enzyme application results in lack of uniformity of penetration and action throughout the muscle, whereas antemortem application of enzymes was effective in producing a more tender product.

Effect of Salts on Tenderness of Poultry Meat

Polyphosphates

Food grade phosphates serve as additives in all phases of the meat processing industry, where they perform such useful functions as curing,

moisture retention, emulsification, chelation, preservation of color, flavor, and tenderness (Cassidy, 1977).

Phosphates have been used successfully in tenderizing meats due to their ability to increase charge, pH and subsequent hydration of proteins (Monk et al., 1964; Farr and May, 1970; Baker et al., 1972; Shults and Wierbicki, 1973; Wood and Richards, 1974).

May et al. (1962) found that chilling the carcasses in a 3% solution of polyphosphates significantly improved the tenderness of light and dark poultry meat. Klose et al. (1963) chilled poultry for 22 hrs in a 5% solution of a mixture of sodium triphosphate (STP) and tetrasodium pyrophosphate (TSPP) and found a small but significant increase in tenderness. Spencer and Smith (1962) chilled fryers in a 7.5% polyphosphate solution for 6 hrs. which significantly increased the tenderness. Baker and Darfler (1968) found that treating leg ham fowl and fryers with polyphosphates improved tenderness of the fresh frozen meat. Peterson (1977) found that the toughening effect of cutting chicken broiler breast muscle within 1 hr after slaughter could be prevented by injecting sodium polyphosphates into the muscle at 20 min postmortem.

Inorganic Salts

Studies have shown that inorganic salts increase the tenderness of red meats and poultry (Wierbicki et al., 1957; Sherman, 1961; Davey and Gilbert, 1969; Shults and Wierbicki, 1973; Wood and Richards, 1974; Hale et al., 1977; Stubblefield and Hale, 1977).

It was suggested by Hamm (1960) that the positive effects of inorganic salts on tenderness and water holding capacity were due to the increase charge of the muscle proteins introduced by the salts.

Palladino and Ball (1979) studied the effect of 15 inorganic salts on the tenderness of spent hen muscle. They found that the salts in general had a tenderizing effect on spent hen muscle, with the exception of calcium salts.

MATERIALS AND METHODS

Source of Materials

Two hundred and sixteen spent white leghorns (Babcock, 300V) were
obtained from the Avery Research Center, Kansas State University. The
birds were 12-14 months old, fed on regular layer feed and were
approximately 4.25 pounds in weight.

Processing Procedure

The birds were processed with semi-commercial equipment. They were
shackled by their feet on the processing line and were slaughtered
manually by severing the jugular vein at the ventrolateral base of the
head. They were allowed to bleed for about two minutes before they were
placed in the scalding tank where they were subscalded at a temperature
of 138°F - 140°F for about 30 seconds. The birds were defeathered
automatically by rotating drums containing long rubber projections
("fingers").

The birds were then eviscerated, washed, and allowed to age in slush
ice for about 20 hours. The breast portion together with the breast bone
and skin intact was removed, wrapped in plastic freezer bags, frozen, and
stored at -26°C until analyzed.

Samples to be used were thawed at 2°C for 18-20 hours and the breast
portions removed from the breast bone and skin. The pectoralis major was
separated from the pectoralis minor and only the pectoralis major was
used in the experiments.

Preliminary Experiments

Preliminary experiments were performed to decide what concentration
of enzyme should be used. Breast fillets were soaked in 0.002%, 0.003%

and 0.006% of solutions of papain, bromelin, and ficin for 60 min. and
then cooked and served to a three member experienced taste panel. A
randomized complete block design was used with the enzymes as treatments
and tasters as blocks. From these results, concentrations of 0.002%,
0.003% and 0.002% for papain, bromelin and ficin respectively were
selected for further study.

Statistical design

     The statistical design chosen for the main experiment was that of a
balanced incomplete block design. This design was arranged in blocks
that were smaller than a complete replication, because this experiment
involved nine treatments and it was not possible for taste panelists to
sample all nine treatments at one setting. This design had the property
that any pair of treatments appeared together equally often within some
block (in this case three times) and all comparisons among pairs of
treatments were made with equal precision. The plan of these designs was
explained in Plan 1, Appendix. The number of treatments, $t = 9$; the
number of units per block, $K = 4$; the number of replications, $r = 8$; and
the number of blocks, $b = 18$. Two blocks were done per week.

Blade-tenderization

     Uniform penetration of enzyme solutions has always been a problem
and samples in this experiment were subjected to blade-tenderization
using a Steak Master, Model 401 (Hobart-Federal Engineering Corp.,
Minneapolis, Minn.) so as to open up the structure of the meat and
facilitate penetration of the enzyme solution deeper and more uniformly
throughout the meat sample. Although blade-tenderization tenderizes meat
to a certain extent, it is still not tender enough and further enzyme
treatment is needed to produce a tender product.

Treatments

The experiment consisted of nine treatments.

Treatment 1:

This served as a control where the pectoralis major muscles were soaked in 1000 ml of distilled water for 60 minutes.

Treatment 2:

This served as a second control where the pectoralis major muscles were first blade-tenderized and then soaked in 1000 ml of distilled water for 60 minutes.

Treatment 3:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 0.002% papain[1] for 60 minutes.

Treatment 4:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 0.003% bromelin[2] for 60 minutes.

Treatment 5:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 0.002% ficin[3] for 60 minutes.

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[1] Papain (Takamine Brand papain 30,000, F.C.C., E.C. 3.4.4.10 peptide peptidohydrolase) was obtained from the Enzyme Products Division, Miles Laboratories, Inc., Elkhart, IN.

[2] Bromelin (Takamine Brand Bromelain 1:10 MS, E.C. 3.4.22.4) was obtained from the Enzyme Products Division, Miles Laboratories, Inc., Elkhart, IN.

[3] Ficin (Enzeco (R) Ficin, K-13348) was obtained from the Enzyme Development Corporation, New York, NY.

Treatment 6:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 1% sodium chloride and 6% Kena FP-28[1] for 60 minutes.

Treatment 7:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 1% sodium chloride, 6% Kena FP-28 and 0.002% papain for 60 minutes.

Treatment 8:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 1% sodium chloride, 6% Kena FP-28 and 0.003% bromelin for 60 minutes.

Treatment 9:

The pectoralis major muscles were blade-tenderized and soaked in a solution of 1% sodium chloride, 6% Kena FP-28 and 0.002% ficin for 60 minutes.

Batter and Breading Procedure:

Each breast fillet (pectoralis major muscle) was individually coated as follows: The fillet was immersed in a whole milk predip for 10 seconds; placed in a 10" by 10" plastic bag containing 70 grams of breading mix and shaken for 10 seconds. The breading mix was composed of the following ingredients: wheat flour, partially hydrogenated soybean, cottonseed and palm oils, salt, malted barley, sugar, spices, paprika, yeast, corn-starch, dried garlic, monosodium glutamate (flavor enhancer), natural hickory smoke flavor, artificial color, and dried onion.

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[1] Kena formula FP-28 was obtained from the Stauffer Chemical Company, Westport, CT.

Baking Procedure

The breast fillets (pectoralis major muscles) were placed on metal cooking pans (six fillets per pan) and baked in a commercial sized Partlow rotary oven (National Mfg. Co., Lincoln, Nebraska) which was preheated to 125°F. The fillets were allowed to bake at 125°F for 15 minutes after which the temperature was raised to 175°F for 15 minutes; it was again raised to 225°F for another 15 minutes and finally to 300°F for 10 minutes. The total cooking time was 55 minutes and the internal temperature of the breast fillets was approximately 155°F. The purpose of gradually raising the temperature of the oven was to give the enzymes a chance to act on the meat, since at a high temperature the enzymes most often are inactivated before much digestion takes place (Anonymous, 1975).

Sensory Evaluation

The taste panel was conducted in an open area in the food laboratory. Taste panelists met at the same time and at the same place every Tuesday and Thursday for nine weeks. The upper portion of the pectoralis major muscle was used for sensory evaluation. Samples were cut perpendicular to the muscle fibers. A nine point intensity scale (Form 1, Appendix) was used by the six-membered experienced taste panel for evaluation of flavor, juiciness, tenderness and overall acceptability (Larmond, 1977; IFT Sensory Evaluation Division, 1981).

Objective Measurements

The upper portion of the pectoralis major muscle was used for the objective measurements as did for sensory evaluation. Samples were cut parallel to the muscle fibers and measured 2 x 2 x 1 cm and weighed 4.5

gms. The instrument used for measuring the tenderness of the samples was an Allo-Kramer shear press (Allo Precision Metals Engineering, Inc., Rockville, Maryland). The samples were placed in the center of the standard shear-compression cell and were sheared perpendicular to the muscle fibers. A 500 pound proving ring was used to shear the sample, the chart speed was set at high and the crosshead speed was constant at all times. Only one measurement was taken from the curve, the length of the peak height. This correlates with the force required to shear a given sample and is represented in kilograms of force required to shear one gram of the sample (Cunningham and Tiede, 1981).

Soak Pick Up

The breast fillets (pectoralis major muscles) were weighed before and after soaking in the various solutions and then the percentage pick up was calculated (Table 3, Appendix).

Cooking Loss

The breast fillets were weighed before and after the cooking process and the percentage cooking loss was calculated (Table 3, Appendix).

Shrinkage

The length of the breast fillets were measured before and after cooking using stainless steel calipers. These measurements were then used as an indication of shrinkage (Table 3, Appendix).

RESULTS AND DISCUSSION

Taste panel scores, shear press measurements, moisture pickup, cooking loss, and shrinkage of spent hen breast fillets (pectoralis major muscles) treated with various enzyme and salt solutions were evaluated as shown below.

Sensory Evaluation

Results of the six-member taste-panel evaluation for flavor, juiciness, and overall acceptability using a nine-point intensity scale are presented in Table 1.

Flavor

There was no significant difference in flavor between the nine treatments (P <0.05), but there were higher mean values for the controls (treatment 1) and for breast fillets soaked in 1% sodium chloride + 6% Kena FP=28 (treatment 6). Panelists noted slight off-flavors in enzyme treated breast fillets and a slight soda like taste in breast fillets treated with 1% sodium chloride and 6% Kena FP-28. Those observations were also made by Landes (1972).

Juiciness

The controls and breast fillets that were treated with 1% sodium chloride + 6% Kena FP-28 with or without enzymes (treatments 1, 6, 7, 8, and 9) were significantly (P < 0.05) juicier than the other treatments (treatments 2, 3, 4 and 5). Breast fillets that were blade-tenderized were less juicy than the controls because they were more susceptible to moisture loss. Those fillets treated with 1% sodium chloride + 6% Kena FP-28 (treatments 6, 7, 8 and 9) were juicier because the effect of the salts compensated for the blade-tenderization.

Table 1. [*]Adjusted means for flavor, juiciness and overall
acceptability scores.[1]

Treatments[2]	Flavor	Juiciness	Overall Acceptability
1	6.20[a]	6.95[a]	6.09[a]
2	5.75[a]	5.46[b]	5.99[a]
3	5.68[a]	5.31[b]	5.71[a]
4	5.80[a]	5.40[b]	5.87[a]
5	5.62[a]	5.44[b]	6.02[a]
6	6.15[a]	5.99[a]	6.58[a]
7	5.30[a]	5.93[a]	6.17[a]
8	5.83[a]	5.81[a]	6.05[a]
9	5.88[a]	5.76[a]	6.35[a]

For juiciness LSD$_{.05}$ = .3015

[1]1, dislike extremely, extremely dry or dislike extremely to 9, like
extremely, extremely juicy or like extremely.

[2]1: Control
2: Blade-tenderized control
3: Blade-tenderized + 0.002% papain
4: Blade-tenderized + 0.003% bromelain
5: Blade-tenderized + 0.002% ficin
6: Blade-tenderized + 1% Nacl + 6% Kena FP-28
7: Blade-tenderized + 1% Nacl + 6% Iena FP-28 + 0.002% papain
8: Blade-tenderized + 1% Nacl + 6% Kena FP-28 + 0.003% bromelain
9: Blade-tenderized + 1% Nacl + 6% Kena FP-28 + 0.002% ficin.

[*]Refer Cochran and Cox (1957).

Phosphates effects on juiciness of meat was noted by Cassidy (1977), May (1962), and Spencer and Smith (1962). There were no significant differences in juiciness between breast fillets treated with 1% sodium chloride + Kena FP-28 (treatments 6, 7, 8 and 9) and controls.

Overall-acceptability

There were no significant differences in overall-acceptability between the nine treatments; however, there was a preference for the controls and for those treated with salts (treatments 6, 7, 8 and 9).

Tenderness

The blade-tenderized controls were significantly more tender ($P < 0.05$) than the regular controls. Tenderness improvement was likely due to decreased structural strength of the myofibrillar and connective tissue. Similar results were reported by Hayward (1979). Even though blade-tenderized controls were significantly more tender than the regular controls, taste panel data showed that the blade-tenderized controls were in between 'moderately tough' and 'slightly tough' on the taste panel preference scale. Breast fillets that were soaked in enzyme solutions alone (0.002% papain, 0.003% bromelin, and 0.002% ficin for treatments 3, 4 and 5, respectively) were significantly ($P < 0.05$) more tender than the blade-tenderized control (treatment 2). As shown in Table 2, the breast fillets soaked in 0.002% papain solution (treatment 3) were significantly ($P < 0.05$) more tender than those soaked in 0.003% bromelin (treatment 4) or 0.002% ficin (treatment 5).

Breast fillets soaked in 1% sodium chloride + 6% Kena FP-28 solution (treatment 6) were significantly ($P < 0.05$) more tender than the blade-tenderized control (treatment 2) but were not significantly different

from those soaked in 0.003% bromelin (treatment 4) or 0.002% ficin
(treatment 5), (Table 2).

Breast fillets soaked in 1% sodium chloride + 6% Kena FP-28 + 0.003%
bromelin (treatment 8) and 1% sodium chloride + 6% Kena FP-28 + 0.002%
ficin (treatment 9) were significantly (P < 0.05) more tender than those
soaked in 0.003% bromelin (treatment 4), 0.002% ficin (treatment 5), and
1% sodium chloride + 6% Kena FP-28 (treatment 6) but were not
significantly different from those soaked in 0.002% papain (treatment 3).

Breast fillets soaked in 1% sodium chloride + 6% Kena FP-28 + 0.002%
papain (treatment 7) were significantly (P < 0.05) more tender than all
other fillets.

The influence of salts on tenderness have been shown by Peterson
(1977), Cassidy (1977), Palladino and Ball (1979), and Baker and Darfler
(1968).

Objective Measurements

Data calculated from shear-press curves are reported in Table 2.
The shear-press values correlate well with taste panel values for
tenderness having a correlation coefficient of $r = -.984$ (Fig. 11).

The linear regression equation for the correlation is

$y = B_0 + B_1 x$

where   y = taste panel value

         x = shear-press value

         B_0 = 9.2224

         B_1 = -.0924

From this regression equation, one can calculate the expected taste panel
results by knowing the shear-press value of a sample. For example, if a

Table 2. [*]Adjusted means for tenderness from taste-panel scores[1] and shear-press values.[2]

Treatments[3]	Taste-panel Scores	Shear-press Values
1	3.45^a	62.45^a
2	4.82^b	45.70^b
3	6.82^d	31.34^c
4	6.33^c	31.83^c
5	6.09^c	33.17^c
6	6.22^c	33.76^c
7	7.44^e	18.89^e
8	$6.66^{c,d}$	24.73^d
9	6.92^d	24.32^d

Taste panel scores $LSD_{.05}$ = .462

Shear-press values $LSD_{.05}$ = 4.85

b is significantly more tender than a ($P < 0.05$)
c is significantly more tender than b ($P < 0.05$)
d is significantly more tender than c ($P < 0.05$)
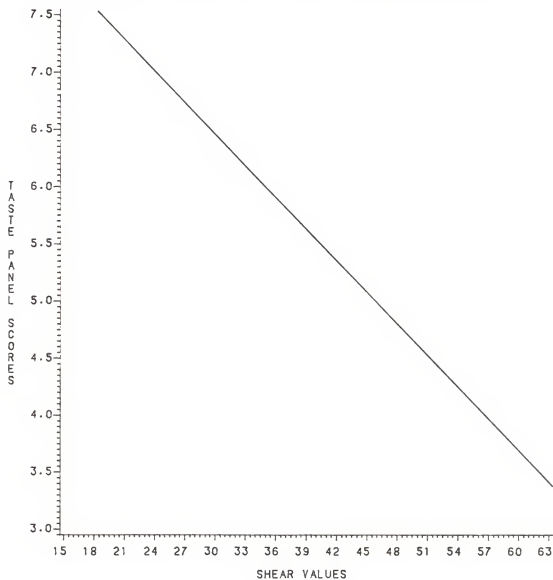e is significantly more tender than d ($P < 0.05$)

[1]1, Extremely tough to 9, Extremely tender.

[2]Measured in Kgms of force required to shear 4.5 gms of the sample.

[3]Treatments - refer Table 1.

[*]Refer Cochran and Cox (1957).

FIGURE 1
*RELATIONSHIP BETWEEN TASTE PANEL VALUES AND
SHEAR VALUES FOR TENDERNESS*

CORRELATION COEFFICIENT=-.984

fillet had a shear-value of 50, and by using the equation, $y = B_0 + B_1 x$

$y = 9.224 + (-.0923) x$

$= 9.224 - .0923 (50)$

$= 4.61$ would be the expected taste panel response.

4.61 on the score card is rated as between 'slightly tough' and 'neither tough nor tender'.

Solution Pickup, Cooking Loss, and Shrinkage

Percentage solution pickup, cooking loss, and shrinkage are shown in Table 3. Breast fillets soaked in solutions containing 1% sodium chloride and 6% Kena FP-28 (treatments 6, 7, 8 and 9) were significantly ($P < 0.05$) higher in solution pickup. May et al (1962) and Landes (1972), showed that polyphosphates increased moisture pickup.

Even though there were no significant differences between the controls and the blade-tenderized fillets (treatments 1, 2, 3, 4 and 5) regarding solution pickup, there was an increase of solution pickup for the blade-tenderized fillets compared to controls.

There was a significant increase ($P < 0.05$) in cooking loss for breast fillets that were blade-tenderized (except for two out of the four treatments that were treated with 1% sodium chloride and 6% Kena FP-28). It is understandable that blade-tenderized fillets would be more susceptible to higher cooking losses and it was hoped that the addition of sodium chloride and Kena FP-28 would reduce the cooking losses, but this was true only in two out of the four salt treatments.

There was no significant difference between any of the treatments regarding shrinkage, in contrast to findings reported by Shults and Wierbicki (1973), where the use of phosphates in meat reduced shrinkage.

Table 3. [*]Adjusted means for percent change in solution pickup, cooking loss and shrinkage.

Treatments[1]	Pickup	Cooking Loss	Shrinkage
1	1.85[a]	9.70[a]	23.38[a]
2	3.45[a]	15.18[b]	23.29[a]
3	4.64[a]	11.71[b]	22.26[a]
4	5.40[a]	13.26[b]	22.65[a]
5	4.66[a]	12.80[b]	20.79[a]
6	23.07[b]	7.02[a]	17.38[a]
7	22.54[b]	14.67[b]	20.24[a]
8	22.23[b]	11.19[b]	18.70[a]
9	29.45[b]	9.64[a]	22.85[a]

Pickup LSD$_{.05}$ = 4.86

Cooking Loss LSD$_{.05}$ = 4.20

Shrinkage NS[2]

[1]Treatments - refer Table 1, Appendix

[2]NS - Not significant

[*]Refer - Cochran and Cox (1957).

In conclusion, breast fillets that were blade-tenderized were significantly more tender (P < 0.05) than the controls, and breast-fillets soaked in solutions of 0.002% papain, 0.003% bromelin, or 0.002% ficin were significantly more tender (P < 0.05) than the blade-tenderized controls. The fillets soaked in papain were significantly more tender (P < 0.05) than those soaked in solutions of either bromelin or ficin. Breast-fillets soaked in 1% sodium chloride and 6% Kena FP-28 alone were as tender as those soaked in solutions of either bromelin or ficin. Breast-fillets soaked in solutions of 1% sodium chloride and Kena FP-28 plus either papain, bromelin or ficin were significantly more tender (P < 0.05) than all other treatments. Those treated with sodium chloride + Kena FP-28 + papain were the most tender. Taste-panel values and shear-press values for tenderness were highly correlated; the coefficient was r = -.984. There was no significant difference in flavor and overall-acceptability between the nine treatments. The control (treatment 1) and breast-fillets treated with sodium chloride and Kena (treatments 6, 7, 8 and 9) were significantly juicier (P < 0.05) than the other treatments. Breast-fillets soaked in solutions containing sodium-chloride and Kena FP-28 had a significantly higher (P < 0.05) solution pick up. Regarding cooking loss, the control, and treatments 6 and 9 had a significant decrease (P < 0.05) in cooking loss. There was no significant difference in shrinkage among the treatments.

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ACKNOWLEDGEMENTS

APPENDIX

FORM 1

SCORE SHEET FOR SENSORY EVALUATION OF SPENT HEN BREAST FILLETS

SOAKED IN ENZYME SOLUTION

SAMPLE CODE:_____ PANELISTS NAME:_____

DATE:_____TIME:_____LOCATION:_____

Please taste test these samples and check how much you like or dislike

each one. Use the appropriate scale to show your attitude by checking

at the point that best describes your feelings about the sample.

FLAVOR	JUICINESS
____Like Extremely	____Extremely Juicy
____Like Very Much	____Very Juicy
____Like Slightly	____Slightly Juicy
____Neither Like nor Dislike	____Neither Juicy nor Dry
____Dislike Slightly	____Slightly Dry
____Dislike Moderately	____Moderately Dry
____Dislike Very Much	____Very Dry
____Dislike Extremely	____Extremely Dry

TENDERNESS	OVERALL ACCEPTABILITY
____Extremely Tender	____Like Extremely
____Very Tender	____Like Very Much
____Moderately Tender	____Like Moderately
____Slightly Tender	____Like Slightly
____Neither Tender nor Tough	____Neither Like nor Dislike
____Slightly Tough	____Dislike Slightly
____Moderately Tough	____Dislike Moderately
____Very Tough	____Dislike Very Much
____Extremely Tough	____Dislike Extremely

COMMENTS:

PLAN 1. *INCOMPLETE BLOCK DESIGN

t=9, k=4, r=8, b=18, λ=3, E=.84, Type II

Block	Reps. I, II, III, and IV				Block	Reps. V, VI, VII, and VIII			
(1)	1	4	6	7	(10)	1	2	5	7
(2)	2	6	8	9	(11)	2	3	5	6
(3)	1	3	8	9	(12)	3	4	7	9
(4)	1	2	3	4	(13)	1	2	4	9
(5)	1	5	7	8	(14)	1	5	6	9
(6)	4	5	6	9	(15)	1	3	6	8
(7)	2	3	6	7	(16)	4	6	7	8
(8)	2	4	5	8	(17)	3	4	5	8
(9)	3	5	7	9	(18)	2	7	8	9

*Refer Cochran and Cox (1957).

Table 4. Analysis of variance

	Flavor D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Blocks within group (adj.)	16	1.57035
Intra-block error	46	0.82513
Total	71	
$F(8,46) = 0.6581(N.S.)$		

	Juiciness D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Blocks within group (adj.)	16	0.67173
Intra-block error	46	0.07649
Total	71	
$F(8,46) = 6.5705^{*}$		

	Overall Acceptability D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Blocks within group (adj.)	16	0.36980
Intra-block error	46	0.18043
Total	71	
$F(8,46) = 2.6806(N.S.)$		

Table 5. Analysis of variance

	% Pickup	
	D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Block within group (adj.)	16	29.22400
Intra-block error	46	22.56530
Total	71	

$F_{(8,46)} = 40.9556$[*]

	% Cooking Loss	
	D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Block within group (adj.)	16	29.61998
Intra-block error	46	16.20848
Total	71	

$F_{(8,46)} = 3.1444$[*]

	% Shrinkage	
	D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Block within group (adj.)	16	37.09450
Intra-block error	46	18.62092
Total	71	

$F_{(8,46)} = 1.8246$ (N.S.)

Table 6. Analysis of variance

	Tenderness	
	Taste-panel Scores	
	D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Blocks within group (adj.)	16	0.13429
Intra-block error	46	0.23467
Total	71	

$F(8,46) = 58.1139^{**}$

	Shear-press Values	
	D.F.	M.S.
Group	1	
Trt. (unadj.)	8	
Blocks within group (adj.)	16	45.39453
Intra-block error	46	32.12806
Total	71	

$F(8,46) = 40.4806^{*}$

TENDERIZATION OF SPENT HEN MUSCLE USING PAPAIN, BROMELIN
OR FICIN, ALONE AND IN COMBINATION WITH SALTS

by

HECTOR A. DE VITRE

B.S., Loyola College, University of Madras,
Madras, India, 1981

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AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Food Science
(Department of Animal Sciences and Industry)

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

Taste panel scores, shear-press measurements, and percentage of cooking loss, shrinkage and soak pick up of pectoralis major muscles from spent White Leghorns, soaked in solutions of papain, bromelin, and ficin alone or in combination with sodium chloride and phosphate solutions, were determined using a balanced incomplete block design with eight replications per treatment. There was no significant difference between treatments for flavor, overall-acceptability, or percentage of shrinkage. Samples that were blade-tenderized were significantly more tender ($P <$ 0.05) than the controls, and samples soaked in solutions of 0.002% papain, 0.003% bromelin, and 0.002% ficin were significantly more tender ($P < 0.05$) than the blade-tenderized controls. Those soaked in papain were significantly more tender ($P < 0.05$) than those soaked in solutions of either bromelin or ficin. Samples soaked in 1% sodium chloride and 6% Kena FP-28 alone were as tender as those soaked in solutions of either bromelin or ficin. Fillets soaked in solutions of 1% sodium chloride and Kena FP-28 plus either papain, bromelin, or ficin were significantly more tender ($P < 0.05$) than all other treatments. Those treated with sodium chloride + Kena FP-28 + papain were the most tender. Taste panel values and shear-press values for tenderness gave a correlation coefficient of $r = -.984$. The controls and samples treated with sodium chloride and Kena FP-28 were significantly juicier ($P < 0.05$) than the other treatments. Samples soaked in solutions of sodium chloride and Kena FP-28 had a significantly higher ($P < 0.05$) solution pick up.