AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECTS OF ULTRA-HYDROSTATIC PRESSURIZATION OF PRE-RIGOR

MUSCLE ON CHARACTERISTICS OF ECONOMIC IMPORTANCE

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This study was designed to confirm or refute the tenderizing
effect of ultra-hydrostatic pressure on warm excised pre-rigor
muscle, plus to take a thorough look at its effect on some meat
characteristics of economic importance.

Samples for the experiment were obtained from animals (cattle
and sheep) slaughtered at Oregon State University, Meat Science
Laboratory. Two muscles from ewe [Longissimus dorsi (L.D) and semi-
membranosus (S.M)] and four muscles from steer [semitendinosus (S.T),
sternomandibularis, supra spinatus (S.S) and Longissimus dorsi (L.D)]
were excised from carcasses immediately after slaughtering, skinning
and washing. They were vacuum packed and subjected to a hydrostatic
pressure of 103.5 MNm^{-2} at a temperature of 39°C for two minutes.

Parameters measured in both control and treatment samples include
pH, purge loss, water-holding capacity (WHC), sarcomere length, shear

force value, over all shortening percentage and taste panel test. Immediately after treatment the mean pH of the treated samples was found to be significantly ($P<.05$) lower than the pH from the control samples, however the difference was not significant at 24 hour post mortem. Pressure treatment produced a significant reduction in the water-holding capacity of muscle. Treated _Longissimus dorsi_ (L.D) shortened by 36 percent while control muscle showed a 25 percent over all shortening. Sarcomere length of the treated samples was always significantly ($P<.01$) shorter than that of controls. Shear force value and taste panel scores indicated a significant tenderizing effect resulting from pressure treatment.

The Effects of Ultra-hydrostatic Pressurization
of Pre-rigor Muscle on Characteristics of
Economic Importance

by

Elgasim Elgasim Ali

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INTRODUCTION

Tenderness is a very variable meat characteristic, yet it is indispensible if meat is to be readily accepted by the consumers. It is therefore becoming increasingly important for meat scientists to elucidate the causes of variation in tenderness and find a way to maintain it at a consistently desirable level.

The discovery of organisms living at a depth of 6,000 meters at a hydrostatic pressure of 600 atm has intrigued investigators and led to the study of the effect of pressure on biological activity. High hydrostatic pressure (5,000 - 15,000 atm) at room temperature denatured protein. F-actin was irreversibly denatured at a pressure of 1,500 - 3,000 kg/cm^2 however this denaturation was found to be ATP dependent (Ikkai and Ooi, 1966). Erying and Johnson (1970) have shown that pressure influences the development of tension in muscle. Bruege and Strauffer (1974) reported that the contractile state of muscle at the time it enters rigor mortis has a marked effect on meat tenderness.

Pressure treated pre-rigor warm excised muscle was found to have low pH, short sarcomere length and to be more tender than the control samples (MacFarlane, 1973). Water-holding capacity has been related to some organoleptic properties such as juiciness and tenderness (Hamm, 1960). On the other hand, a high correlation between water-holding capacity and ultimate pH was reported by Bouton et al. (1971).

Under extensive systems of animal production (e.g., Sudan, my home country), the nutritive value of the pasture increases in the rainy season and animals gain weight for four to six months of the year. Upon onset of the dry season the nutritive value of the pastures drop and animals lose weight for six to eight months, consequently it requires a long time for animals to reach market weight (400 - 420 kg). It has been shown that the number of cross links in the connective tissue increases with age. Even under intensive systems of animal production, in the future, there is a great possibility of marketing cattle directly off grass and hence a high proportion of the lower grades of beef going into the market. A lack of dependable tenderness could create a marketing problem with this class of beef.

The traditional method (aging) used to bring about some tenderizing effect is not an appropriate technique at this time of energy crises. The use of pressure as a tenderizing technique seems very possible and offers an excellent alternative to the expensive and time consuming aging techniques.

The objective of the current study was to confirm or refute the tenderizing effect of ultra-hydrostatic pressure and at the same time shed light on its effect on some meat characteristic of economic importance.

LITERATURE REVIEW

Chemical Composition of Muscle

To understand the chemical and biochemical changes that take

place in muscles post mortem, a knowledge of the chemical composition

of muscle is important. However, the chemical composition varies from

animal to animal and from one species to another. The figures in

Table 1 give the approximate chemical composition of muscles.

It is very apparent from Table 1 that the main components of

muscle are water, protein and fat. Water, protein and fat constitute

more than 96 percent of the muscle. Since neither water nor fat can

contribute to meat toughness in any significant way, therefore any

variation in meat tenderness should be due to muscle proteins.

Goll et al. (1970) classified muscle proteins into three classes

according to their solubilities:

1) Sarcoplasmic proteins which are soluble at low

ionic strength of 0.05 or less.

2) Myofibrillar proteins are soluble at high ionic

strength 0.3 or greater however some of them

are water soluble.

3) Stroma proteins which are insoluble at any ionic

strength. Collagen and elastin constitutes a

large portion of the stromal proteins.

Sarcoplasmic proteins are globular and form solutions with low viscosity

and little resistance to shearing therefore they do not contribute to

the physical strength of the muscle. On the other hand, myofibrillar

Table 1. CHEMICAL COMPOSITION OF MUSCLE.

Component		Percent
Water		75
Protein		18
Actomyosin in the myofilament	10%	
Enzymes		
Soluble protein in the sarcoplasm		
Insoluble particles: mitochondria	6%	
Lyosomes		
Myoglobin		
Collagen		
Lipo-proteins of sarcolemma	2%	
and sarcoplasmic reticulum		
Fat		3
Extra cellular fat, lipo-proteins,		
phospholipids, fatty acids		
Non-protein organic nitrogenous substances,		2.1
creatine, amino acids, nucleotides		
Carbohydrate and its metabolites		1.2
Inorganic salts, traces of vitamins, etc.		.7

(Goll et al., 1974)

and stromal proteins are fibrous and form viscous solutions with high resistance to shearing and hence are responsible for the physical strength of muscles.

Investigators resolved meat toughness into background and actomysin toughness (Locker, 1960; Marsh, 1972; Marsh and Leet, 1966). Background toughness is due to connective tissue and other stromal proteins while actomyosin toughness is attributed to myofibrillar proteins. According to Purchas (1972) myofibrillar proteins are the major contributor to differences in meat tenderness. Myosin and actin are the major myofibrillar proteins (Table 2).

Muscle Structure

Meat is mainly muscle plus variable quantities of connective tissues as well as some epithelial, nervous tissues and blood vessels (Figure 1, a & b). The skeletal muscle is the principal source of muscle tissue in meat, it constitutes 35 - 65 percent by weight. The structural unit of skeletal muscle is the muscle fiber which is surrounded by an elastic membrane known as sarcolemma which itself is surrounded by a delicate connective tissue called endomysium. Sarcolemma is a lipid-protein unit while endomysium is a protein-polysaccharide coating. Each 20 - 40 muscle fibers grouped together forms what is known as primary bundle. Groups of primary muscle bundles ensheathed in perimysial connective tissue are known as secondary bundles which in turn when grouped together and ensheathed with epimysium form a muscle (Figure 1, b).

Table 2. APPROXIMATE AMOUNT OF EACH MYOFIBRILLAR PROTEIN IN THE
          MYOFIBRIL.

Protein	% of myofibril by weight
Myosin	50 to 55
Actin	15 to 20
Tropomysin	5 to 8
Troponin	5 to 8
α-actinin	2 to 3
β-actinin	0.5 to 1
Component C	2 to 3
M-line protein	3 to 5

(Goll et al., 1974)

(a)

MUSCLE

Vein
Nerve   Artery

Tendon

(b)

Epimysium

Perimysium

Endomysium

Blood Vessel

Nerve

Muscle Fiber

Myofibril

(c) MUSCLE
BUNDLE

(d) MUSCLE
FIBER
Myofibril

(e) MYOFIBRIL

I-band   A-band

Sarcomere   Z-line

(f) MYOFILA-
MENTS

Cross-bridge   Actin Filaments

Myosin Filament
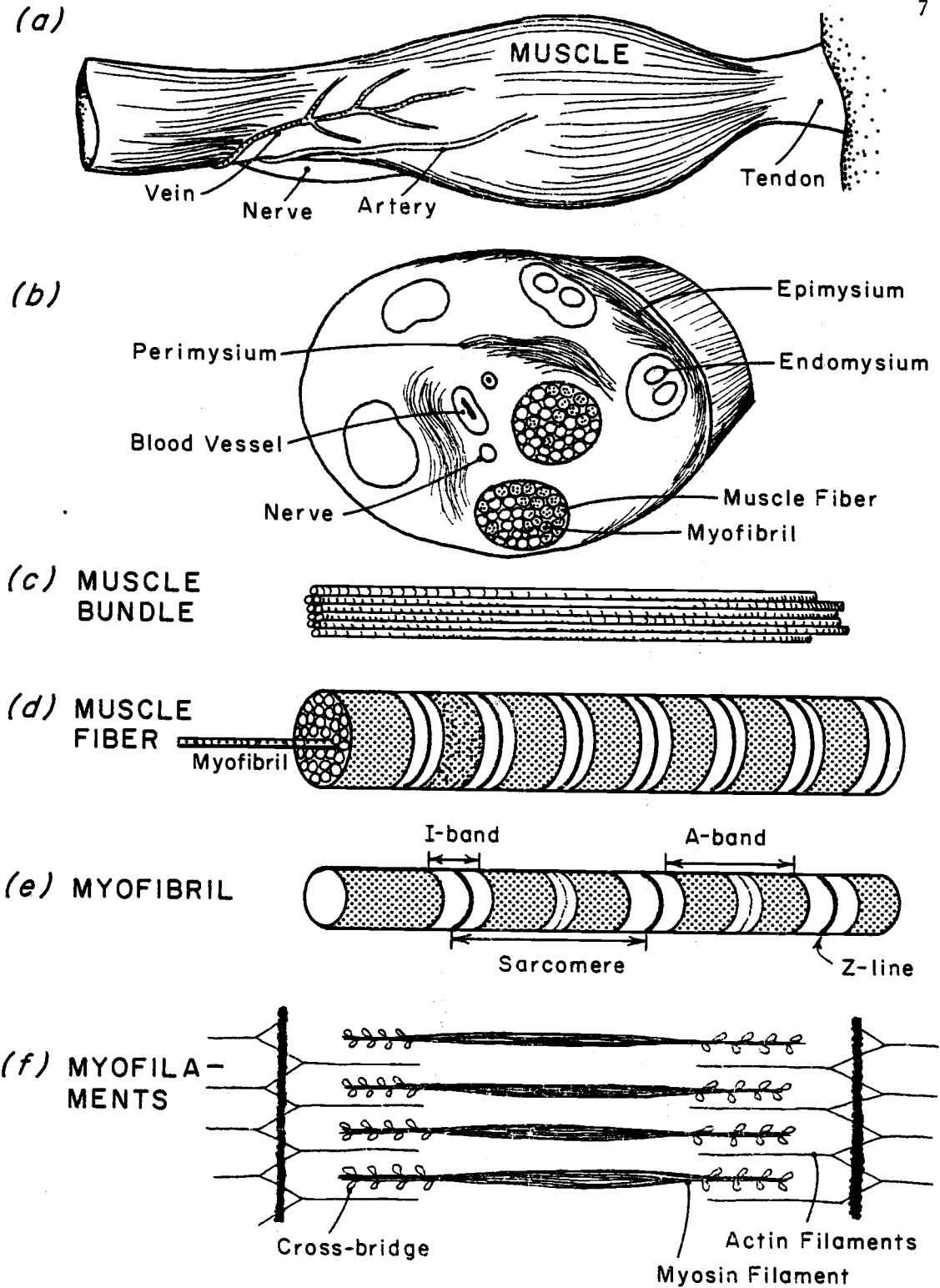
Figure 1.   THE STRUCTURE OF SKELETAL MUSCLE AT DIFFERENT LEVELS
OF ORGANIZATION.

The structural unit of muscle fiber is the myofibril. Myofibrils are long thin cylindrical rods (Figure 1, e) composed of light bands called I-bands, dark bands called A-bands and Z-lines. The repeating structural unit of myofibril is the sarcomere which is terminated at each end by a Z-line. Sarcomeres are the basic units in which the events of muscle contraction-relaxation occur.

In 1954, A. F. Huxley and Jean Hanson suggested an interdigitating filament array of myofibril. The main features of it are shown in Figure 2. Goll et al. (1974) considered the discovery of this structure as the most important finding ever made in meat science. Two kinds of filament were shown in myofibril structures, thick filament composed mainly of myosin protein and thin filament composed mainly of actin protein. The diameter of the thick filaments is twice that of the thin filaments.

How Muscles Contract

Two conditions for the muscle to remain relaxed:

1) Should have low ionic $Ca^{++}(<10^{-7}$ moles/litre)

in the sarcoplasmic fluid (Martonosi et al., 1972).

2) High ATP concentration in the presence of Mg^{++}.

Under these conditions thick and thin filaments slide freely over each other. Actin filaments are pushed 10 nm toward the center of sarcomere whenever the cross bridges on myosin rotate.

The role of ATP in muscle contraction is a complicated one. It is needed as a source of energy for contraction events, at the same time it has been shown that ATP in physiological concentration is
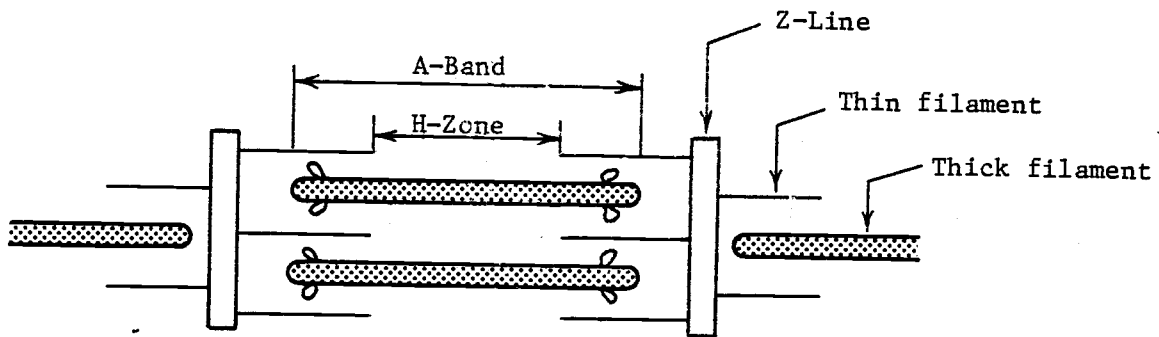
Figure 2. THE INTERDIGITATING STRUCTURE OF MYOFIBRIL.
(modified from Goll et al., 1974)
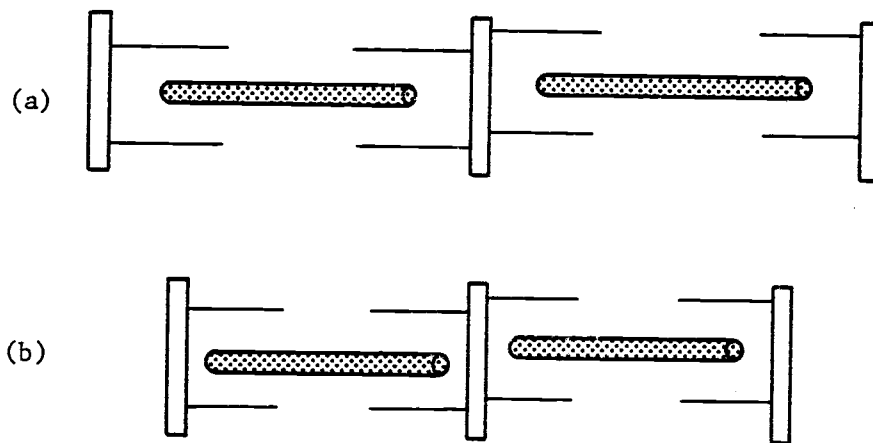
Figure 3. (a) RELAXED MUSCLE FIBER AND

(b) CONTRACTED MUSCLE.

(modified from Goll et al., 1974)

necessary for the disassociation of the cross-linkages between actin and myosin. After the death of the animal the capacity of a muscle to contract continues as long as glycolysis is able to maintain the supply of ATP. ATP acts as a sort of lubricant for the relative motion of the myofilaments. However as soon as glycolysis slows down ATP is lost and the muscle goes into rigor. Scope (1971) reported that the level of ATP in muscle showed more variation but followed a steadily declining course with pH, falling from 6.0 μmol/g at pH 7 to near zero at pH 5.6 - 6.0.

It is worth mentioning that the filaments themselves are not shortened, they just slide over each other (Figure 3).

Causes of Variation in Tenderness

For convenience, the factors that affect meat tenderness can be divided into ante-mortem and post-mortem factors. The ante-mortem ones include animal age, species, stress, inheritance and the degree of finish of the animal.

Age and the degree of finish have been intensively studied by numerous investigators. Webb et al. (1964) designed an experiment to investigate the effect of age, ante-mortem stress and methods of aging upon carcass tenderness. Sixty-six cattle carcasses were tested in their experiment. The animals were divided into one year, two year, and five year old groups. The whole experiment was subdivided into four experiments. Adrenaline was used as an ante-mortem stress factor. Their finding was in line with what Tuma et al. had reported earlier in 1962 and 1963. They concluded that tenderness and juiciness

decreases with increase in animal age. Consumers prefer meat from younger cattle because it is tender and juicy (Dunsing, 1959). Meat from old animals is tougher than that from young animals because with increasing age the number of cross links between collagen molecules in the fibers increases (Verzar, 1964).

The biosynthesis and nature of the cross links have been studied by many investigators. These studies show two types of cross links:

1) Intramolecular cross links within the tropo-collagen molecule (Bornstein and Piez, 1966).

2) Intermolecular cross link -- this is the one responsible for stabilization of collagen fibers.

Shimokomaki et al. (1972) studied changes in bovine intermuscular collagen with age. Age of animals investigated ranged from pre-natal fetus to 15 years old. The samples were analyzed for the presence of reducible cross links by amino acid analysis. Five reducible components (Figure 4) were found in intramuscular collagen and tendon collagen. These components are:

a) di hydroxy lysinonorleucine

b) hydroxy lysinonorleucine

c) Fraction C

d) A_1

e) A_2

The first three are the major reducible components and found to be of aldimine-type bonds (Bailey, 1969), two of them were characterized (Figures 5 and 6) but not the third. Shimokomaki et al. (1972) found large numbers of cross links in the one year old steer than in the
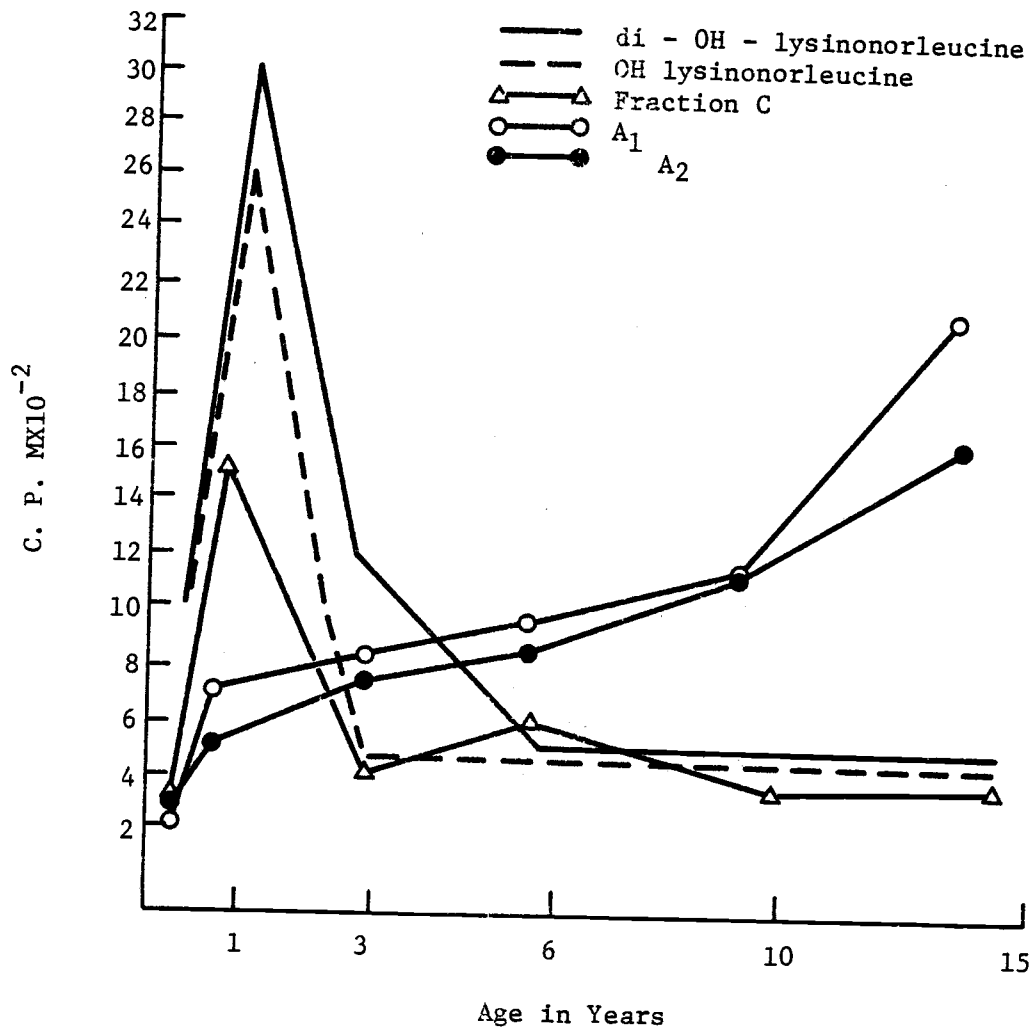
Figure 4. EFFECT OF AGE ON THE RELATIVE AMOUNTS OF THE VARIOUS
REDUCIBLE COMPONENTS IN INTRAMUSCULAR COLLAGEN FROM
EXTENSOR CARPI RADIALIS. (Shimokomaki et al., 1972)

α-chain - NH - CH - CO-   NH - CH - CO-   -NH - CH - CO-

Left column:

α-chain - NH - CH - CO-
|
$(CH_2)_3$
|
CHO
|
NH_2
|
CH_2
|
H-C-OH
|
$(CH_2)_2$
|
α-chain - NH - CH - CO-

$\longrightarrow$

Middle column:

NH - CH - CO-
|
$(CH_2)_3$
|
CH
||
N
|
CH_2
|
H-C-OH
|
$(CH_2)_2$
|
-HN - CH - CO-

dehydrohydroxy -
lysinonorleucine

$\xrightarrow{\text{NaBH}_4}$

Right column:

-NH - CH - CO-
|
$(CH_2)_3$
|
CH_2
|
NH
|
CH_2
|
H C-OH
|
$(CH_2)_2$
|
HN - CH - CO-

hydroxy
lysinonor
leucine

Figure 5. BIOSYNTHESIS AND BOROHYDRIDE STABILIZATION OF ONE OF THE
MAJOR ALDIMINE-TYPE INTERMOLECULAR CROSS LINKS IN COLLAGEN.
(Shimokomaki et al., 1972)

α-chain – NH – CH – CO
|
(CH$_2$)$_2$
|
CH – OH
|
CHO
|
NH$_2$
|
CH$_2$
|
H–C–OH
|
(CH$_2$)$_2$
|
α-chain NH – CH – CO–

$\longrightarrow$

–NH – CH – CO–
|
(CH$_2$)$_2$
|
HC – OH
|
CH
|
N
‖
CH$_2$
|
HCOH
|
(CH$_2$)$_2$
|
–HN – CH – CO–

dehydro – hydroxy
lysino hydroxy
norleucine

$\xrightarrow{\text{NaBH}_4}$

–NH – CH – CO–
|
(CH$_2$)$_2$
|
HCOH
|
CH$_2$
|
NH
|
CH$_2$
|
HCOH
|
(CH$_2$)$_2$
|
–HN – CH – CO–

hydroxy lysino-
hydroxynor-
leucine

Figure 6.   BIOSYNTHESIS AND BOROHYDRIDE STABILIZATION OF A SECOND
MAJOR ALDIMINE-TYPE CROSS LINK IN COLLAGEN.
(Shimokomaki et al., 1972)

fetal, however rate of increase in the number decreased after this age up to five years. In ten year old animals these cross links disappeared, at the same time there was an increase in other two components named Al and A2. They were suspected of being the major reducible component at the age of five to six years but Robins and Bailey (1972) proved that they were not cross links.

Another factor that causes variation in tenderness is fat content. Fat acts as an insulator against chilling effects. The effect of degree of fatness on meat tenderness was studied by Smith et al. (1976). Forty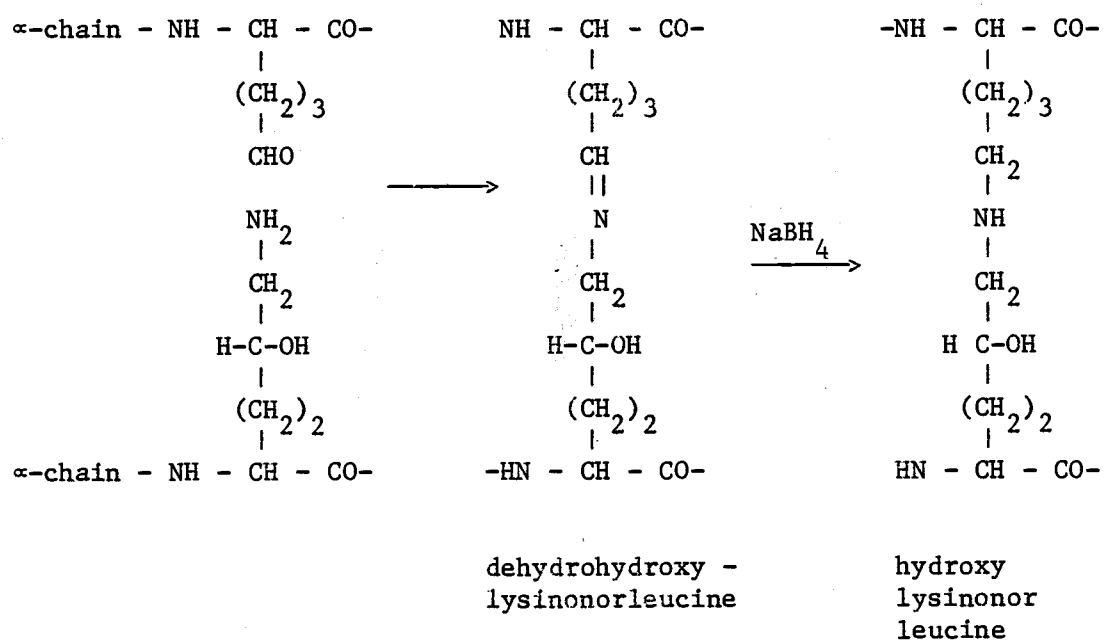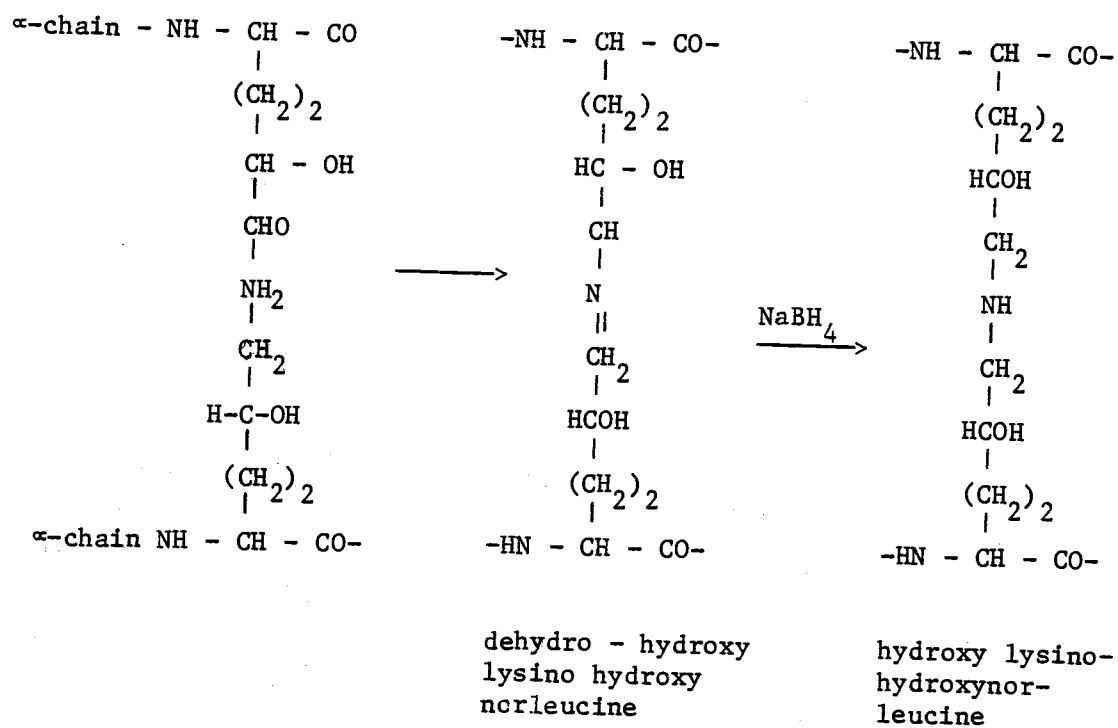 lambs of different degrees of finish (thick, intermediate and thin) were used. Carcasses from groups with thick finish chilled more slowly than the other two groups, attaining an internal temperature of 1°C in 8.1 hours, whereas carcasses from intermediate and thin finish reached 1°C in 5.9 hours and 5.6 hours, respectively. The pH of the carcass from the thick group was lower than that from the intermediate or thin group. Sarcomeres from the thick group were longer than sarcomeres from the intermediate or thin groups. They concluded that meat from carcasses with thick finish was more tender than that from carcasses with thin finish. Similar findings were reported by Wenham et al. (1973), Smith and Carpenter (1975). With regard to the effect of marbling score on tenderness, Smith et al. (1976) found that it was important when the fat covering is thin. Cross et al. (1972) reported significant (P<.01) correlation between intramuscular fat and sarcomere length in lamb.

Kennick et al. (1971) found a significant correlation (r = .771) between marbling and chemically determined intramuscular fat. The

interest in marbling results from its profound effect on beef grading and its debatable effect on tenderness and overall acceptability. Lowe (1955) reported that fat retarded the rate of heat penetration while Heldman (1975) found meat with high fat content to heat more rapidly than meat with low fat. Intramuscular fat was found to have no effect on heat penetration (Weir, 1960). Many investigators have found high correlations between internal temperature of the muscle post mortem and shear force requirement of the cooked muscle. Correlations of -.61 and -.67 were reported by Field et al. (1970) and Smith et al. (1976).

Meat is a collection of muscle fibers bound together by a network of connective tissue composed primarily of collagen fibers. Post mortem aging has been shown to produce a significant change in the contractile tissue (Davey and Dickson, 1970) but it has a non-significant effect on connective tissue toughness (Bouton and Harris, 1972). Webb et al. (1967) conducted an experiment to determine the effect of aging on tenderness, water-holding capacity and juiciness and also to determine whether there is relationship between them. They concluded that aging tenderizes meat and they found a significant correlation (r = -.35) between panel tenderness and water-holding capacity. Some peptide bonds are thought to be broken as a result of aging (Hamm, 1960). Some cracks were observed in S.T muscle fibers (Paul et al., 1962) which were stored for longer periods. These cracks developed into breaks.

Depending on the position of the muscle in the skeletal structure during the onset of rigor mortis, some muscles shorten more than others

and hence differences in tenderness occur (Herring et al., 1965). Sarcomere length accounts for 12 percent of the variation in tenderness. A correlation of -.34 between sarcomere length and tenderness was found by Hostetler et al. (1972).

Five carcass suspension methods namely vertical, horizontal, neck-tied, hip-free and hip-tied were studied by Hostetler et al. (1972) to determine their effect on tenderness. They investigated the effect of these methods on the sarcomere length and shear force. requirement of triceps brachii (TB), LD, Psoas major (PM), adductor (AD), gluteus medius (GM), rectus femoris (RF), SM, ST and biceps femoris (BF). The vertical, neck-tied and horizontal treatments were found to shorten the sarcomere length of BF, GM, AD and RF whereas the hip-free method has increased the sarcomere length of all muscles tested except PM. From these findings and the shear force results Hostetler et al. concluded that hip-free method was the most effective one on tenderness.

Herring et al. (1967) have shown that shorten sarcomeres are highly related to actomyosin toughness as measured by shear force. The shortening in fiber length is produced by sliding of the thin filament over thick filament when the muscle contract.

When considering the effect of cooking on meat properties, time of cooking and temperature, especially the internal temperature of the cooked muscle, are very important. Cooking softens connective tissues of the muscle, collagen is converted to gelatin (Lowrie, 1966). Hill (1960), Carmichael and Lowrie (1967) and Herring et al. (1967) have shown that the effect of temperature on the rate and degree of

solubilization of collagenous connective tissue was animal age dependent.

Bouton and Harris (1972) studied the effect of cooking temperature and time on meat characteristics. Twenty-nine steers were divided into three groups according to age. They measured changes in water-holding capacity, adhesion (tensile strength), chewiness, instron compression (Kg/cm^2) and shear value using Warner-Bratzler shear device. Both chewiness and adhesion decreases with increase in cooking time (Figure 7). Samples cooked at 60°C for one hour showed a significant decrease in shear values when compared to those cooked at 40°C. Similar results were reported by Machlik and Draudt (1963). Samples cooked at 50°C for one hour showed lower cooking loss values than those cooked at 60°C. Changes in water-holding capacity were attributed to changes in temperatures. High temperature short time heat produced higher weight loss than low temperature long time heat. A slow heating rate causes less contraction of muscle fibers than a fast heating rate. Sarcomeres shortened less at 60°C than at 70°C.

Water-holding capacity is an index of palatability (Miller and Harrison, 1965), microbial activity (Jay, 1967) and manufacturing potential (Saffle, 1968). Moisture in the muscle gives consumers the satisfaction of juiciness.

Hamm (1960) related water-holding capacity of meat to some important organoleptic properties such as juiciness and tenderness. Weir (1960) stated that the more tender the meat, the more quickly the juices are released by chewing and the more juicy the meat appears.
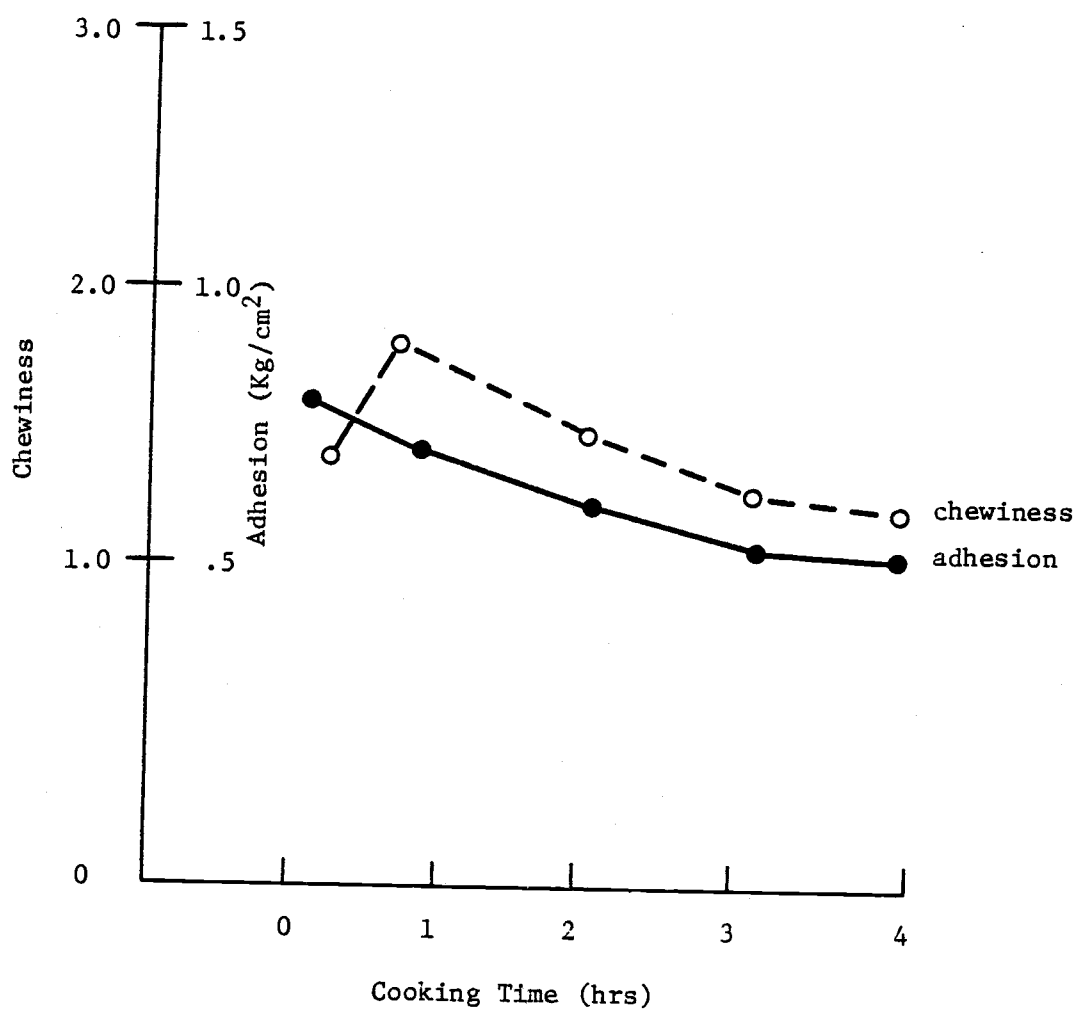
Figure 7. EFFECT UPON INSTRON COMPRESSION (CHEWINESS) AND ADHESION OF
COOKING DEEP PECTORAL MUSCLE FROM A ONE YEAR OLD STEER AT
90°C FOR UP TO FOUR HOURS. (Bouton and Harris, 1972)

Moisture in the muscle can be classified as free water or bound water. The free water would be that adsorbed by the tissue and held there by secondary forces such as dipole - dipole induction, capillary action, surface attraction and hydrogen bonding. Bound water does not flow freely from tissues when a moderate force is applied and all of it is to some degree under the influence of biological structure or solute. It is electrostatically bound to minerals and various protein molecules in the tissues.

Hamm and Deatherage (1960) studied the effect of pH on water-holding capacity of muscles at different temperatures and found that muscles have the lowest holding capacity at pH of 4.5 to 5.0 (Figure 8). This can be explained by the fact that this is the isoelectric point of the major contractile proteins hence they will have a net charge of zero, so less polar groups of proteins are available for binding water.

Swift and Berman (1959) investigated the effect of pH on water retention of eight major beef muscles. They found a positive correlation between water retention and pH values. Water retention increases with increasing pH values (r = 0.947 ± .028, P<0.01). pH affects binding of ions to proteins, calcium, magnesium and potassium content varies inversely with water retention but increased with increasing protein content. Iron, sodium and chloride content increased with increasing water retention.

According to Hamm (1959) water-holding capacity of muscle homogenates has been shown to be closely related to pH and can be
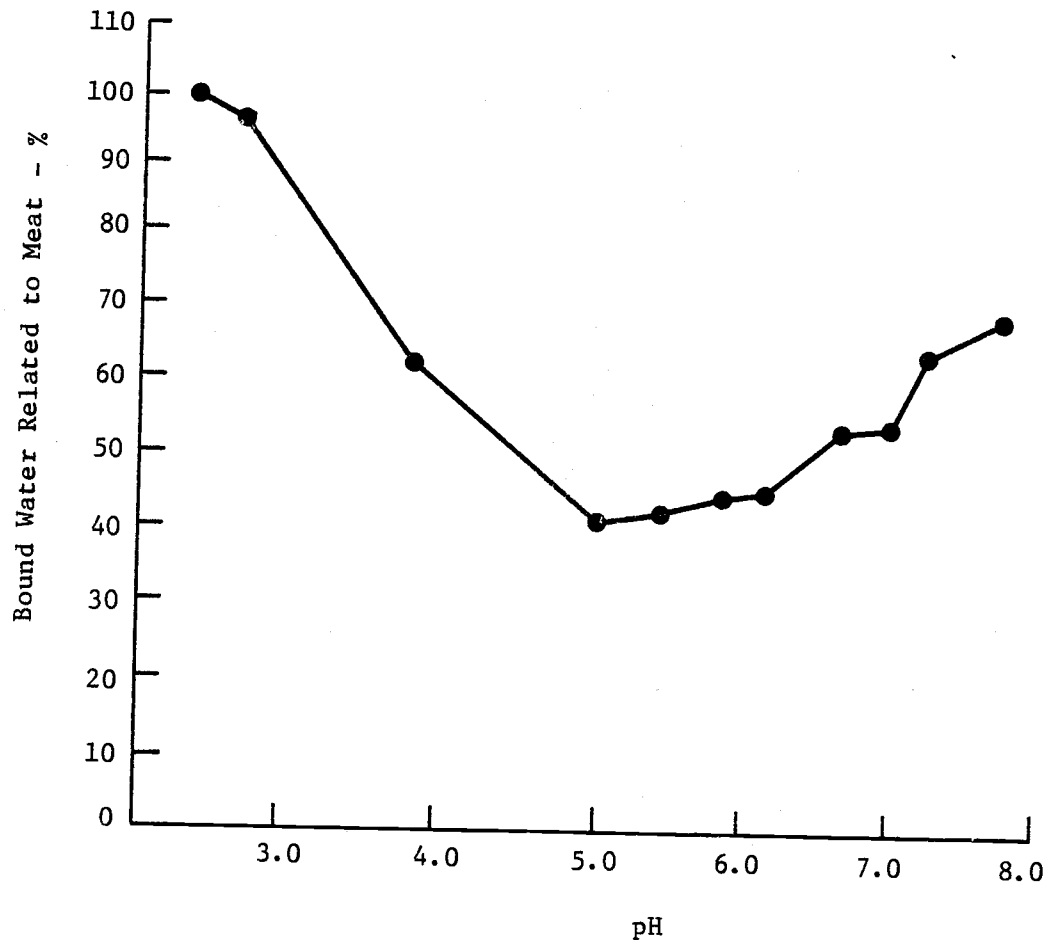
Figure 8. INFLUENCE OF pH ON WATER-HOLDING CAPACITY OF FRESH BEEF
MUSCLE AT 20°C. (Hamm and Deatherage, 1960)

used as indicators of variation in the charges and structure of muscle proteins.

Different techniques were used to vary muscle pH, Grau et al. (1953) added acid/alkali to adjust muscle homogenates, Winkler (1939) injected lactic acid or ammonia, Bouton et al. (1957) used pre-slaughter stress or drug administration, Lewis et al. (1967) stressed cattle before slaughtering, DeFremery (1963) and Penny et al. (1963) used epinephrine and/or iodoacetate and found that meat with a high pH were more tender and juicy than those with low pH.

Bouton et al. (1971) investigated the relationship between pH and WHC on 16 sheep (3 - 4½ years old). Animals were injected with adrenaline chloride and 3- (2-methyl phenoxy) propane -1:2 diol to produce muscles with pH range of 5.6 - 7.0. They reported that WHC is highly correlated with ultimate pH of the muscle.

Since Talisman 1882-1883 found living organisms at a depth of 6,000 meters at a hydrostatic pressure of 600 atm several investigators have studied the effect of pressure on biological activity.

A pressure of 5,000 - 15,000 atms was found to cause protein denaturation (Bridgman and Conant, 1928; Basset et al., 1938; Lauffer and Dow, 1941). Experiments conducted on myofibrillar components have shown that myosin, actin and actomyosin were influenced by pressure (Marsland and Brown, 1942; Josephs and Harrington, 1967, 1968; Ikkai and Ooi, 1966, 1969). F-actin under the influence of pressure (147 MNm^{-2}) started to undergo irreversible denaturation. Ikkai and Ooi (1966) also found that F-actin was

transformed to G-actin. Actomyosin was shown to disassociate into actin and myosin. MacFarlane (1973) reported that pressure weakens actin filament.

Further work has shown that the effect of pressure on a given muscle is a function of pressure level, time and temperature during pressurization. Mathews and Anderson (1940) investigated the influence of temperature and found that the effect of pressure has a small positive temperature coefficient between 0°C and 35.5°C. At 0°C a 25 percent decrease in peptic activity under 5,000 atm took place in five hours; however, under similar conditions at 35.5°C the decrease in activity was 60 percent.

Brown (1934) showed clearly that the contraction of muscle could be increased, decreased or unaffected by pressure according to the temperature and source of the muscle. Brown (1934-1935) reported a decrease in the amount of contraction of pectoral fin muscle in the red grouper (Epinephelus morio) at temperatures below 14 - 16°C and an increase at temperatures above 14 - 16°C and no change at a temperature of 14 - 16°C. He observed similar results on frog muscles.

MacFarlane (1973) found that the effect of pressure on pre-rigor warm excised muscles from sheep and ox carcasses to be a function of time, temperature and pressure. He investigated different levels of pressure (69, 103.5 and 138 MNm^{-2}), a time duration of 1 to 16 minutes and a temperature range of 0° to 40°C. He found a small drop in pH of muscle pressurized at 0°C, when the temperature was increased to 30°C the drop in pH increased. pH change resulting from pressurization

increased with increase in pressure. A pressurization time of two to four minutes was found to be enough to cause most of the drop in pH.

In sheep muscle the maximum change in pH was achieved by a pressure of 103.5 MNm^{-2} while ox muscles showed a continuous drop with increase in pressure level up to 138 MNm^{-2}. With the exception of semitendinosus all muscles investigated showed similar pH drop. At all levels of pressure (90 to 138 MNm^{-2}) the shear force value of the treated samples was found to be lower than the control, suggesting that the treatment improved tenderness but a decrease in juiciness was observed by panel tasters. One disadvantage of this technique was that muscle has to be pressurized before the onset of rigor mortis. Very recently Bouton et al. (1977) suggested a technique which is a combination of pressure and heat treatment. They investigated the effect of different levels of pressure (20, 40, 60, 100, 140 and 150 MNm^{-2}), and pressurization time of 0, 2½, 5, 10, 20, 30 and 60 minutes, on post rigor muscle heated before pressurization to 10, 25, 35, 40, 55 and 60°C. They compared this technique with pre-rigor pressurization suggested by MacFarlane (1973) and found that both techniques tenderize meat significantly. The maximum effect was produced by a pressure of 100 MNm^{-2} applied for two to five minutes on a post-rigor muscle heated to 40 - 60°C.

MATERIALS AND METHODS

Samples for the experiment were obtained from sheep and cattle slaughtered at Oregon State University Meat Science Laboratory, for other purposes, over a period of several months. The animals were slaughtered, skinned, eviscerated and washed. One side of the animal was assigned at random as control and the other as treatment. Immediately after washing (25 - 30 minutes post mortem in the case of steers, 20 - 25 minutes in the case of ewes) the muscles to be treated were excised from the side assigned for treatment. The control side was left intact and chilled according to normal commercial practices. After 48 hours chill, the control muscles were excised, vacuum packed and held under refrigeration $1.0 \pm 1°C$ along with the treated samples, for further study. Longissimus dorsi (L.D) and semimembranosus (S.M) from ewe and supra spinatus (S.S), sternomandibularis, Longissimus dorsi (L.D) and semitendinosus (S.T) from cattle were the muscles investigated in this study. Muscles to be treated were vacuum packed in Cry-o-vac bags, then placed in a water bath at 39.0°C to raise the temperature of the muscle to that of the treatment (39.0°C) because it has been shown that the effect of pressure is both temperature and time dependent. The pressure chamber was filled with water at 39.0°C. The vacuum packed muscle was inserted in the chamber which was then tightly closed and a pressure of 103.5 MNm^{-2} (15,000 lb/sq.in) was electrically pumped and maintained for two minutes. The pressure chamber (10.16 cm in diameter and 30.48 cm long) used in this study

was designed by W. Kennick of the OSU Meat Science Laboratory and constructed by the OSU Physics Shop.

pH Measurement

For the pH measurement, a technique similar to that of Bouton and Harris (1971) was used. The pH was measured immediately after removal of muscle from the pressure chamber, using Orion Research Model 801/digital pH meter with a probe type combined electrode, Model 605, at the same time the pH of the control sample was recorded. Then the samples were stored at 1 ± 1°C. The pH was recorded for both control and treated samples at 1 hour, 2 hours, 4 hours, 6 hours and 24 hours post mortem. Muscles used for pH measurement were L.D, S.M from ewe and S.T from cattle.

Shortening Percentage Measurement

The muscles were marked with a measuring device, having sharp points with 5 cm gaps between the points, immersed in staining liquid prior to usage. For the treated samples shortening was measured immediately after removal of the muscle from the pressure chamber while for the control samples it was measured 72 hours post mortem. Seven samples (S.T and L.D) were used for this measurement.

Sarcomere Length Measurement

Sarcomere lengths were taken three days post mortem on uncooked samples. Approximately 5 gm of muscle were blended with 40 ml of 0.25 M physiological sucrose solution (85.57 gm sucrose in 1000 ml

dist. H_2O) for 30 seconds in a Waring (Customer solid state 750) blender at low speed. A few drops of the solution containing myofibrillar fragments were transfered to a glass slide and covered with a cover slip. Measurements were taken immediately after blending using a phase contrast microscope (500X magnification) equipped with filarmicrometer. The filarmicrometer was focused on myofibrillar filaments selected at random then the number of sarcomere per 20 division of filarmicrometer were counted and the length of each sarcomere was obtained from the following formula:

$$\text{sarcomere length } \mu = \frac{20 \times 1.2}{\text{number of sarcomere}}$$

1.2 = length of each division

Shear Force Measurement

Shear forces were taken seven days post mortem. The muscles (all of them are approximately of equal weight) were removed from the cold storage, repacked in Kordite (multi deck pack) bags and cooked in a water bath at $80 \pm 1°C$ for 40 minutes ± 2.5 minutes, to an internal temperature of $70.5 \pm 1°C$ then allowed to cool to 45°C. A rectangular piece (1.25 x .8 cm) with a cross sectional area of 1 cm^2 and fiber lying parallel to the long axis of the sample were consistently removed and sheared at right angles to the fiber axis using Warner-Bratzler (W.B) shear device.

Taste Panel Test

The S.S muscle was used for this test. Samples were cooked in similar ways as previously described for shear force measurement. Guests and the staff of the OSU Meat Science Laboratory were used as panel members and they were asked to evaluate each sample for tenderness on a nine-point scale ranging from 1.0 (very tough) to 9.0 (very tender).

Purge Loss

To determine the amount of purge loss both control and treated samples were weighed on a Toledo scale, packed in Cry-o-vac bags and stored at 1.0 ± 1°C for seven days. Then the samples were removed from the cold storage, dried with a paper towel, at the same time the Cry-o-vac bag was cleaned and dried. Then both sample and bag were weighed. The difference between the initial weight and weight after storage is the purge loss. It was expressed as a percentage of the initial weight.

Water-Holding Capacity Measurement

A press method similar to that used by Grau and Hamm (1953) was used. Approximately 3 - 5 gm of meat tissue was placed on filter paper (Whatman No. 1) covered with Aquabee acetate pad - 730, then placed between two plates of Carver laboratory press No. 10719-81 and a pressure of 5000 lb/sq.in. was applied for four minutes. Immediately after release of pressure water squeezed out on the filter paper was

traced on acetate paper, similarly the area of pressed meat on filter paper was traced. The area (in square inches) of the outer ring (water absorbed by filter paper) and the inner ring (pressed meat) was measured by planimeter No. 80-510. The area of the ring of expressed juice absorbed by filter paper is proportional to the amount of loose water. This technique has the advantages of simplicity and the result is fixed on acetate paper permitting evaluation at any time.

The WHC was calculated from the following equation:

$$\% \text{ WHC} = (1 - EJ/TW) \ 100$$

$$EJ = \text{expressed juice}$$

$$TW = \text{total moisture content.}$$

STATISTICAL ANALYSIS OF RESULTS

Analysis of variance was used to test treatment differences.

RESULTS AND DISCUSSION

The mean pH of the treated muscles (L.D and S.M from sheep and S.T from cattle) following treatment was 5.81 while the mean pH of the control samples was 6.54 at the same time post mortem. The pH of the treated samples was found to differ significantly (P<.01) from the control immediately after treatment. At 2, 4 and 6 hours post mortem the difference was still significant but at the five percent level; however, at 24 hours post mortem the difference was not significant (Table 3). It is also noted that the rate of pH fall after treatment was greater in control samples than in the treated ones (Figures 9, 10 and 11). This suggested that the conditions of treatment increased the rate of glycolysis converting most of the glycogen stored in muscle to lactic acid and energy while it is under pressure. Lactic acid brought about the immediate drop observed in pH while the energy, which is in the form of ATP, was utilized by the muscle for the events of contraction. Although all treated muscles showed a greater pH drop than the control during the first time period, some of them responded more than others, for example L.D showed the highest induced pH drop (.81) while S.M showed an induced pH drop of .68.

The relationship between meat tenderness and pH has been studied by many investigators, some found a relationship while others did not. Bouton et al. (1957) found a curvilinear relationship between ultimate pH of beef (5.5 - 6.48) and tenderness, however Mackey et al. (1952) found no relationship between pH (5.57 - 6.39) and tenderness in pork.

Table 3. EFFECT OF PRESSURE ON pH OF LONGISSIMUS DORSI, SEMIMEMBRANO-
SUS (FROM SHEEP) AND SEMITENDINOSUS (FROM CATTLE)

| Muscle | n | Treatment | pH hours post mortem | | | | |
			1**	2*	4*	6*	24
Longissimus dorsi	10	C	6.63	6.22	5.97	5.86	5.66
∿	10	T	5.82	5.76	5.70	5.67	5.65
Semimembranosus	10	C	6.48	6.16	5.92	5.84	5.69
∿	10	T	5.80	5.78	5.72	5.69	5.63
Semitendinosus	15	C	6.54	6.27	6.00	5.90	5.74
∿	15	T	5.81	5.75	5.73	5.73	5.64

C = control

T = treat

** Significant at the 1% level.
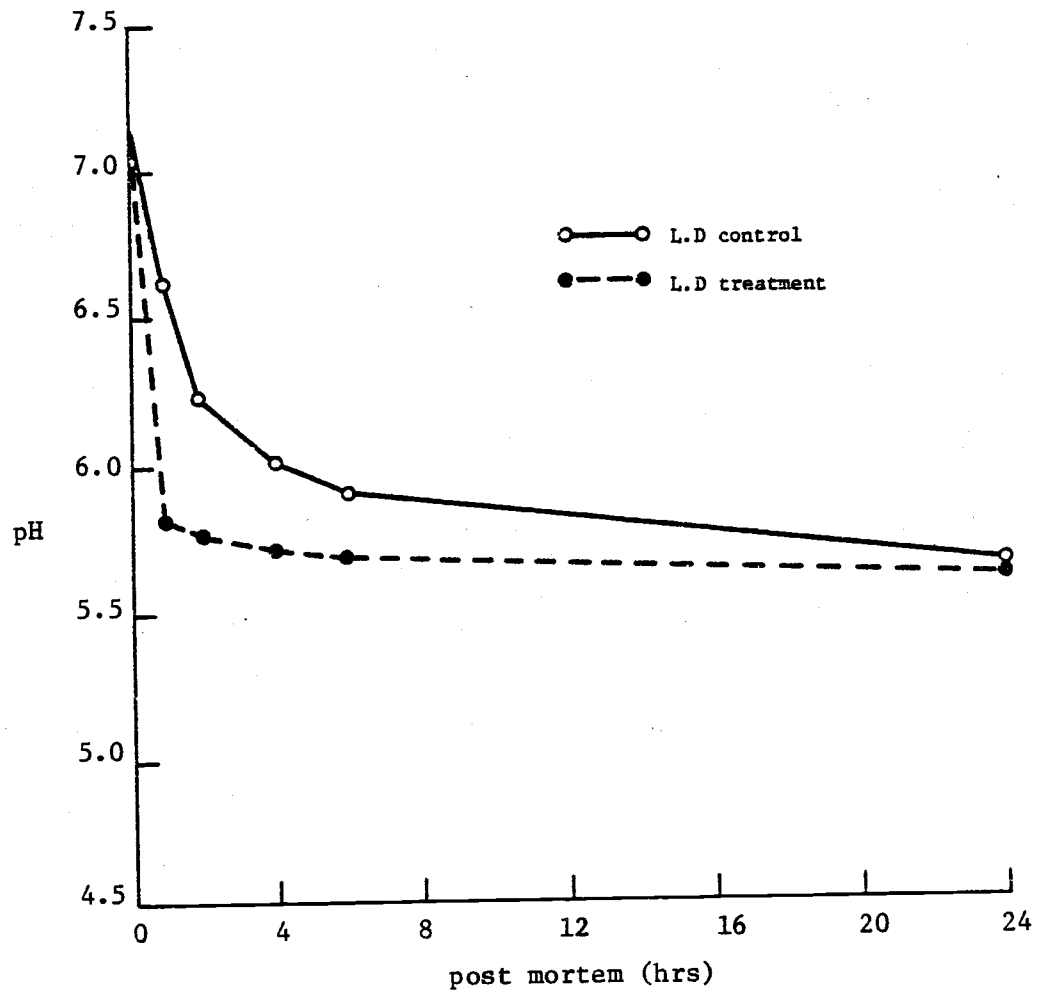
 * Significant at the 5% level.

Figure 9. EFFECT OF PRESSURE ON THE POST MORTEM CHANGES IN pH OF
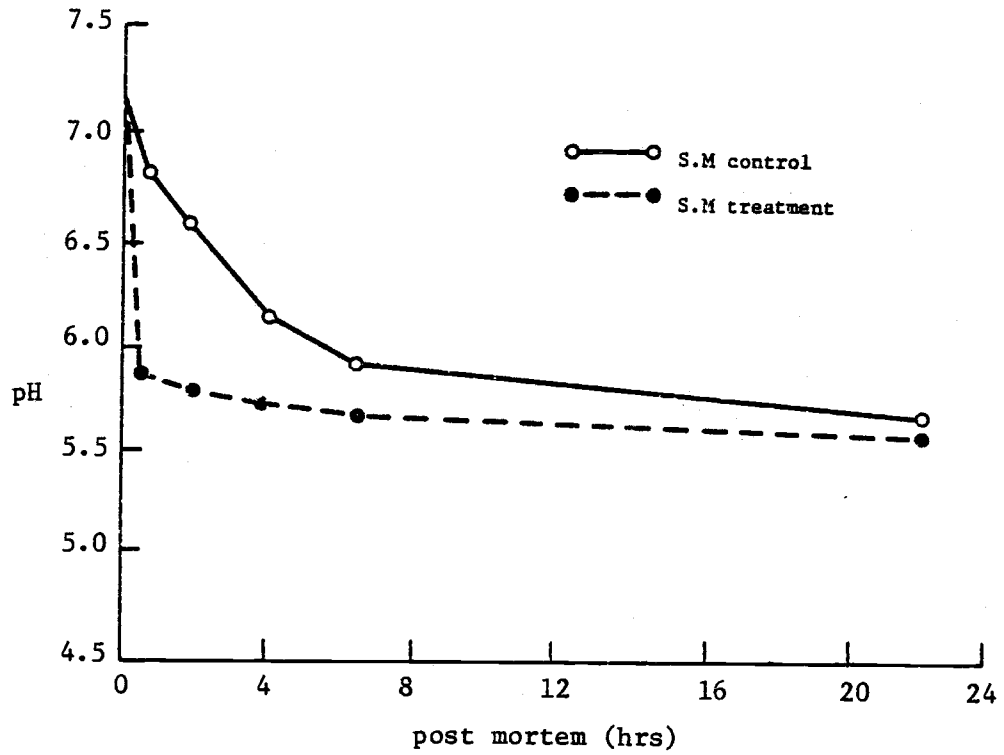LONGISSIMUS DORSI MUSCLE FROM SHEEP.

Figure 10.   THE EFFECT OF PRESSURE ON THE POST MORTEM CHANGES IN pH OF
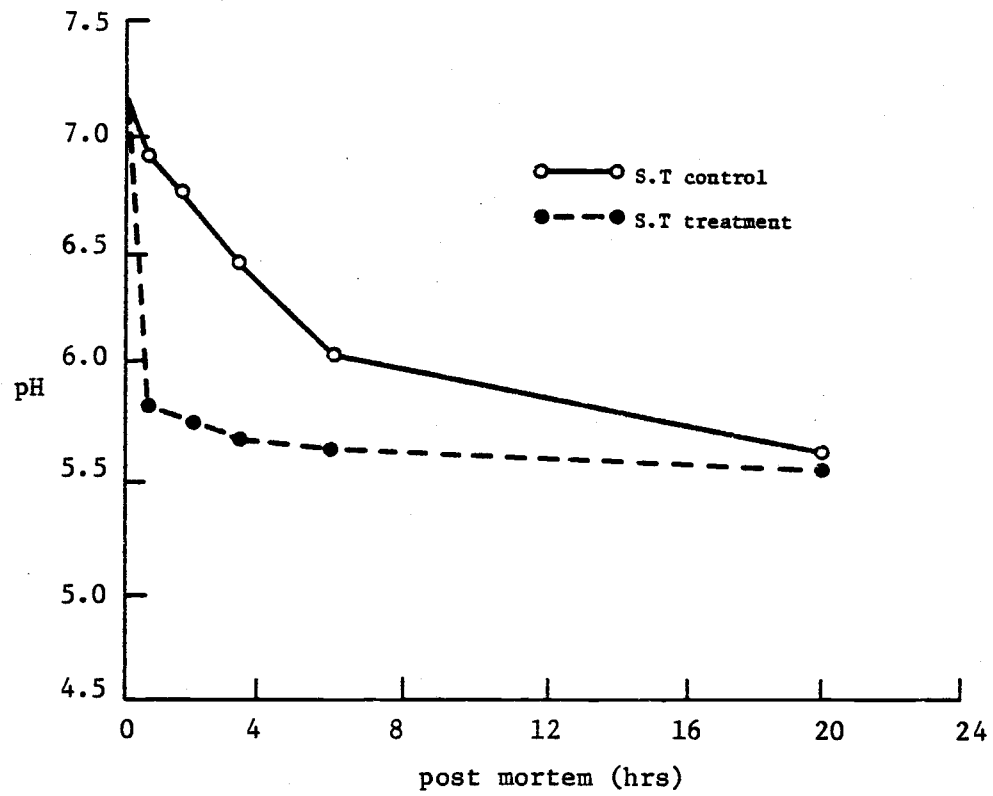SEMIMEMBRANOSUS MUSCLE FROM SHEEP.

Figure 11. THE EFFECT OF PRESSURE ON THE POST MORTEM CHANGES IN pH OF SEMITENDINOSUS MUSCLE FROM CATTLE.

Apparently there are two schools of thought with regard to the relationship between tenderness and pH of the muscle. One school believes that tenderness is associated with high pH. Bouton et al. (1971) have shown that the tenderness of sheep muscle was associated with high pH. Peterson (1977) reported a similar finding in chicken. The other school believes that tenderness is associated with low pH. Field et al. (1970) found a positive correlation between shear force and pH (r = .73). Smith et al. (1976) found that less tender muscles had a higher pH than tender muscles. It seems that the conditions that regulate the course of pH post mortem are very important. In this study the pH of the treated and untreated samples did not differ significantly at the time of tenderness measurement as such one would expect the degree of tenderness not to be affected by pH.

Water-holding capacity (WHC) of control and treated L.D and S.M were determined three days post mortem and the results are shown in Table 4. The treated samples retained significantly (P<.05) less moisture than the control. When the results were combined WHC was significantly correlated with pH for control (r = 0.576) and for treated samples (r = 0.467).

The sarcolemma that surround the muscle fiber adds to the physical strength of the muscle. The scanning electromicrograph (Figure 12) showed the control muscle samples to be structurally intact without any damage to the sarcolemma while the micrograph of the treated samples has shown considerable damage to the sarcolemma and the structure of the treated muscle is more open than that of the control. These observations may account for the observed decrease

Table 4.   THE EFFECT OF PRESSURE ON WATER-HOLDING CAPACITY OF
           LONGISSIMUS DORSI AND SEMIMEMBRANOSUS MUSCLES FROM SHEEP.

Muscle	n	Treatment	WHC
Longissimus dorsi	9	C	38.41^a
∿	9	P	33.60^a
Semimembranosus	9	C	41.91^b
∿	9	P	37.15^b

C = control

P = pressure treated, conditions of treatment

pressure of 103.5 MNm^{-2}, temperature 39°C and time of two minutes.

a, b, Values bearing the same superscript are significantly different
     (P<.05).

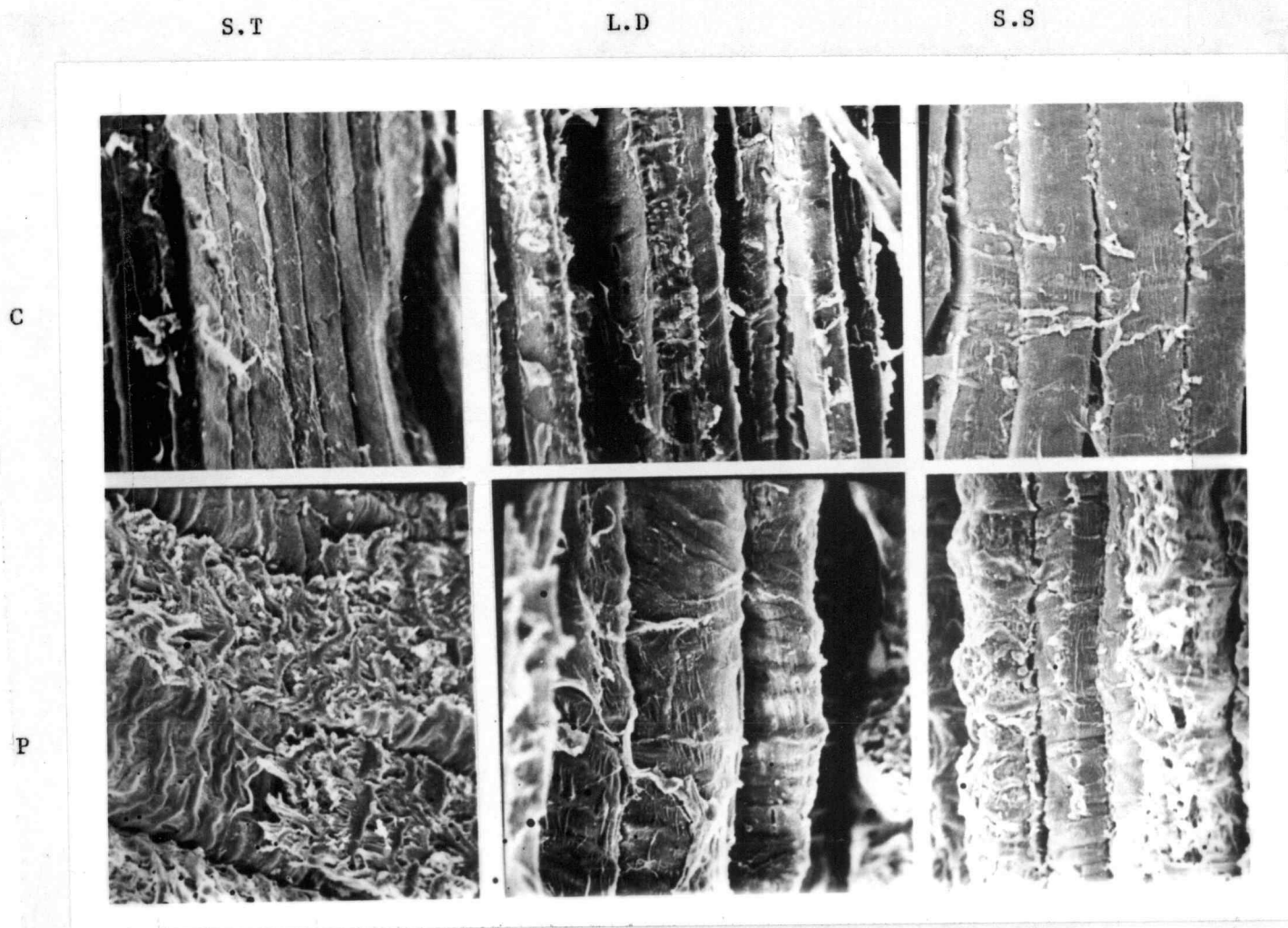S.T   L.D   S.S

C

P

Figure 12.   SCANNING ELECTROMICROGRAPH (400X MAGNIFICATION) OF SEMITENDINOSUS, LONGISSIMUS DORSI AND SUPRASPINATUS MUSCLES CONTROL AND PRESSURE TREATED.

in the WHC and increase in the tenderness of the treated samples.
Weir (1960) stated that the more tender the meat the easier the juices
are released giving consumers the satisfaction of juiciness.

From Table 4 it is very clear that L.D retained less moisture
than S.M. Such differences may be characteristics of different
muscles. Webb et al. (1967) found a significant correlation between
water-holding capacity and panel tenderness (r = -.35) and this is
exactly in agreement with that reported in this study. A significant
correlation (P<.05) between water-holding capacity and tenderness
(measured by W-B shear device) was found (r = -.408). A close
association was established between subjective evaluation of tenderness
(panel tenderness) and instrumental measurement of tenderness using
W-B shear force device. Bouton et al. (1971) found that Warner-
Bratzler shear values correlate well with taste panel evaluations of
meat tenderness.

The percentage of purge loss (Table 5) was found to be
significantly (P<.05) higher in the pressurized sample (5.34 ± .77%)
than that of the control (3.80 ± .57%). This is in line with that
reported by MacFarlane (1973).

The results of tenderness evaluation measured by W-B shear device
are summarized in Table 6. For sheep, the mean shear force values of
the treated muscles were 4.07 lb and 5.08 lb for L.D and S.M respec-
tively while the control of the same muscles had a shear force value
of 12.65 lb and 13.81 lb. The control samples, whether from sheep or
cattle, had consistantly higher shear force values than the pressure
treated samples. When the data were subjected to analysis of variance

Table 5. THE EFFECT OF PRESSURE ON THE AMOUNT OF PURGE LOSS FROM
SEMITENDINOSUS MUSCLE FROM CATTLE.

Animal No.	Treatment	Gross Wt.(Lb)	Purge Wt.	% Purge Loss
R-533	C	2.00	.07	3.50
	P	2.96	.16	5.40
580	C	2.84	.13	4.58
	P	3.16	.20	6.33
B5	C	2.34	.09	3.85
	P	2.47	.11	4.45
565	C	2.63	.09	3.27
	P	3.09	.17	5.18
Mean control		--	--	3.80(.57)*
Mean treatment		--	--	5.34(.77)*

C = control

P = pressure treatment

* Significantly different at 5% level

Figures between brackets are the st. dev.

Table 6. EFFECT OF PRESSURE ON W-B SHEAR FORCE VALUES OF TWO MUSCLES FROM SHEEP AND FOUR MUSCLES FROM CATTLE.

Muscle	Treatment	n	Shear force values lb/cm^2	S.E
Longissimus dorsi (sheep)	C	7	12.65^a	1.49
∿	P	7	4.07^a	1.18
Semimembranosus (sheep)	C	7	13.81^b	1.96
∿	P	7	5.08^b	1.08
Longissimus dorsi (cattle)	C	4	18.50^c	.82
∿	P	4	6.60^c	.59
Semitendinosus (cattle)	C	4	13.60^d	.56
∿	P	4	9.95^d	.34
Supraspinatus (cattle)	C	4	15.72^e	1.53
∿	P	4	9.60^e	1.16
Sternomandibularis (cattle)	C	3	33.00^f	1.41
∿	P	3	12.80^f	.41

C = control

P = pressure treated

S.E = standard error

a,b,c,d,e,f, Values bearing the same superscript are significantly different (P<.01).

to test the significance of observed differences between control and treated samples, a highly significant difference (P<.01) was found. The standard error for the shear force values of the treated samples was found to be always lower than that for the control samples (Table 6) indicating consistency of tenderness.

Cover (1959) found a significant (P<.05) correlation between juiciness scores and shear force values of the L.D. A positive correlation (r = 0.84) between tenderness and amount of expressable juice was found in this study. However, MacFarlane (1973) found a decrease in juiciness scores with increase in panel score tenderness.

The data in Table 7 presents a comparison of mean sarcomere length of treated and control muscles from sheep and shortening percentage of S.T from cattle. The over all shortening percentage of S.T treated muscle from cows was found to be 36 ± 2.5, which was at least ten percent greater than control. The percentage of sarcomere length shortening was found to be less than the over all shortening. This may mean that some of the muscle fiber escaped contraction or contract to a lesser degree only.

Locker (1960) observed that M.P which has a low content of connective tissues is very tender, however when it was excised before rigor onset it became very tough. He found that tender M.P has a sarcomere length of 2.4 - 3.7 microns. When Marsh and Leet (1966) extended this work on neck muscle of beef animals, they found that a decrease in length of up to about 20 percent causes little or no toughness but decreases from 20 to 40 percent increased toughness, beyond 40 percent meat became rapidly more tender and at 60 percent

Table 7. EFFECT OF PRESSURE ON SARCOMERE LENGTH OF LONGISSIMUS DORSI
AND SEMIMEMBRANOSUS (FROM SHEEP) AND OVER ALL SHORTENING
PERCENTAGE OF SEMITENDINOSUS FROM BEEF.

Muscle	Treatment	Sarcomere length, μ	Shortening percentage
Longissimus dorsi (sheep)	C	1.798^a	--
∿	T	1.638^a	--
Semimembranosus (sheep)	C	1.857^b	--
∿	T	1.674^b	--
Semitendinosus (beef)	C	--	25 ± 2.1^c
∿	T	--	36 ± 4.0^c

C = control

T = treated

a,b,c, Values bearing the same superscript are significantly different
(P<.01).

shortening it was again as tender as if no contraction of the muscle had occurred. In this study only one out of six samples (L.D) taken for shortening percentage measurement exceeded 40 percent. MacFarlane (1973) has reported 35 ± 3.2 over all shortening percentage before cooking. Hence one would speculate that the tenderizing effect of pressure is due to the sudden enormous contraction of the muscle fiber in a very short time (two minutes) rather than super contraction (>40 percent shortening). The shortening percentage reported by MacFarlane and in this study did not exceed 40 percent.

The difference between the mean sarcomere length of the treated samples and mean sarcomere length of the control is highly significant (P<.01). Under the phase contrast microscope, the sarcomere length of the control samples was measured much easier than that of the treated sample due to appearance of some globular material, the nature of which is unknown.

A very significant (P<.05) correlation (r = .83) between sarcomere length and shear force values was found. Herring et al. (1965, 1966), Berry et al. (1974), Smith and Carpenter (1970), Cross et al. (1972) and Jeremiah and Martin (1976) have observed a significant relationship between histological measures of the myofibrillar components and meat tenderness.

The results of the taste panel assessment were presented in Table 8. The treated samples according to this subjective evaluation were found to be significantly (P<.05) more tender than the control. This is in agreement with the objective evaluation using W-B shear device.

Table 8. TASTE PANEL SCORES FOR TENDERNESS OF PRESSURIZED AND CONTROL SUPRA SPINATUS MUSCLE OF STEER.

Animal No.	n	C	T
475	3	5.33	7
406	3	4.67	6.17
W4	3	6.33	7.67
483	3	5.33	6
430	3	5.5	8.5
mean	15	5.43(.59)*	7.07(1.04)*

C = control

T = treatment. The treatment consisted of a pressure of 103.5 MNm^{-2} at 39°C and two minutes duration.

* Significantly different (P<.05)

This study confirms the tenderizing effect of pre-rigor pressurization reported by MacFarlane (1973), however, the mechanism by which pressure tenderizes meat is not known. Is it physical or chemical effect or a combination of both? According to MacFarlane (1973) it is a mechanical one (break down of myofibrillar structure). As it is very evident from the scanning electronmicrograph, the treatment had caused a severe disruption to the sarcolemma of the muscle fiber. The conditions of severe decline in pH during treatment and high temperature of the experiments are very conducive to lysosomal enzymes release, so the action of these enzymes especially cathepsin might produce some tenderizing effect.

SUMMARY AND CONCLUSIONS

The increased interest of slaughtering forage finished and short-fed animals plus increased energy crises necessitated a search for an alternative to the traditional methods of tenderization.

The samples were obtained from sheep and cattle slaughtered at the OSU Meat Science Laboratory over a period of several months. Six muscles were tested L.D and S.M from ewes and S.T, L.D, P.M and sternomandibularis from cattle. They were excised from one side of the carcass leaving the other side as a control, vacuum packed, pressure treated and stored at $1 \pm 1°C$. The treatment consisted of 103.5 MNm^{-2} pressure at a temperature of $39°C$ for two minutes. The pH of both control and treatment was recorded at 1, 2, 4, 6 and 24 hours post mortem. Sarcomere length was measured microscopically using phase contrast microscope equipped with filarmicrometer. Tenderness was measured objectively using Warner-Bratzler shear device and subjectively by a taste panel. The water-holding capacity of muscles was measured three days post mortem. Collected data was subjected to analysis of variance to test for significant differences.

After pressure was released, the pH of the treated samples was found to be significantly lower than the pH of the control samples. Twenty-four hours post mortem pH control and treated samples were approximately equal. Water-holding capacity of the treated samples was reduced significantly $(P<.05)$. Sarcomere length of L.D treated shortened to 1.64 μ while the control shortened to 1.80 μ. This difference in length was found to be highly significant $(P<.001)$.

A difference of at least 8 lb/cm^2 shear force was reported between control and treatment. This difference was significant at the five percent level. The correlation between the above mentioned parameters was also discussed and in most cases was found to be significant.

Three possible explanations for the tenderizing effect of pressure are:

1) Breakdown of myofibrillar structures.

2) Lysosomal enzymes especially cathepsin were released during pressurization.

3) Pressure might create some breaking points in the fibers or aggravate already existing breaking points.

However, more research will be needed to elucidate exactly what happened during pressurization and how pressurization tenderizes meat.

Among the factors responsible for high meat prices is energy expenditures required for extended refrigerated storage which necessitates more handling of the carcass and input of man hours. Such pre-rigor pressurization techniques will permit rapid processing of carcass and hence reduction of input of man hours. It has been estimated that about 40 percent of carcass weight is trimmed as fat and bone when processed. Therefore, rapid processing, besides reducing the input of man hours, will make available more space in the cold storage for the edible portion of the carcass. This in turn reduces shipping costs from areas of production to areas of consumption. The feasibility of commercial application of this pressurization technique, after the determination of optimum conditions, for commercial use, seems to be very great.

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