EFFECT OF DRY AND MOIST HEAT TREATMENTS ON SELECTED HISTOLOGICAL MEASUREMENTS OF BEEF SEMIMEMBRANOSUS MUSCLE

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INTRODUCTION

Variable and often contradictory results obtained by different researchers studying the effects of similar treatments on cooked beef frequently are reported in the literature. Paul (1963) suggested that variation in type of animal, pre- and post slaughter treatments, cuts or muscles studied, cookery methods and methods of evaluating tenderness are factors that contribute to the varied results obtained by different investigators.

It is important for meat scientists to know whether or not variations in results from one laboratory to another are attributable to variations in methodology. Information of this type will be helpful in comparing results reported in the literature.

The objective of this study was to investigate the effects of dry and moist heat treatments on selected histological characteristics of cooked beef to help explain variations in results reported by different investigators studying similar problems related to cookery.

REVIEW OF LITERATURE

The Nature of Muscle Tissue

Three types of muscle are distinguishable histologically: (1) smooth, (2) cardiac and (3) skeletal. Smooth muscle lacks the striations present in the other types, is involuntary and found mainly in the internal organs. Cardiac muscle is striated

and involuntary. Skeletal muscle is striated and voluntary. It is the striated skeletal muscle of the animal that is referred to as meat. Skeletal muscle comprises approximately 40% of the live weight of the bovine animal of which approximately 75% is muscle fibers (Gillis and Henrickson, 1967).

Basically, all skeletal muscles are composed of small independent units called fibers. Each muscle fiber is composed of many myofibrils, a variable number of nuclei and inclusions such as mitochondria, glycogen granules and liposomes or fat droplets imbedded in the sarcoplasm (Birkner and Auerback, 1960). The outer membrane of the muscle fiber is known as the sarcolemma. The endomysium, a thin layer of collagenous connective tissue that may or may not contain elastic fibers, is between fibers to hold them together within a bundle or fasciculi (Hiner et al., 1953). Primary muscle fiber bundles vary from muscle to muscle in number of fibers per bundle and are surrounded by connective tissue sheaths, the perimysia. Several primary bundles are bound together into larger secondary bundles. Secondary bundles are in turn bound into tertiary bundles. A thick layer of connective tissue, the epimysium or muscle sheath, surrounds the entire muscle (Lowe, 1955).

The contractile function of the muscle fiber is made possible by the structural arrangement of the fibers. Under the light microscope both longitudinal and cross-striations can be seen on a properly fixed and stained muscle fiber. The longitudinal striations are attributed to the parallel arrangement

of the myofibrils. Alternating bands that vary in staining affinity give the appearance of cross-striations. Bands with an affinity for iron hematoxylin stain are identified as A bands, whereas the areas that will not accept the stain are designated as I bands. Each of the I bands is bisected by a narrow line, the stainable Z band. The line bisecting the A band is pale and is designated H. Within the H band there is a narrow M band. The area between two successive Z lines is called a sarcomere, which is the structural and functional unit of the muscle fiber (Copenhaver, 1964).

Electron microscopy recently revealed two types of sub-units (myofilaments) of the myofibrils. Thick myosin filaments extend from one end to the other of the A band. Thin actin filaments extend from the Z line into the A band as far as the H zone (Copenhaver, 1964). It generally is believed that during contraction the thin actin filaments are pulled over the myosin toward the center of the A band (Bendall, 1966).

The Nature of Connective Tissue

Three types of fibers (collagenous, elastic, reticular) occur in connective tissues. Collagenous or white fibers are soft, flexible and non-elastic. Small fibrils, visible only with the electron microscope, are united by a small amount of cement substance to form larger fibrils 0.3 to 0.5 u in diameter. Each collagenous fiber is composed of a number of fibrils that do not branch. However, the fibers and bundles of fibers

branch and anastomose. The fibers vary in diameter from one to 12 u and the bundles of fibers vary in diameter from 10 to 100 u. The fibers may be straight, but usually are wavy (Copenhaver, 1964).

Collagenous fibers are proteinaceous in nature. When collagen is boiled in water it is solubilized to gelatin. The fibers swell and some of the collagen is dissolved when they are placed in weak acids. Weak alkalis dissolve the interfibrilar substance and allow the fibers to separate. Both concentrated alkalis and acids destroy the fibers (Copenhaver, 1964). Collagenous fibers are digested easily by pepsin in acid solutions, but resist trypsin in alkaline solutions (Hiner et al., 1955). Humason (1962) pointed out that collagenous fibers are difficult to stain because they have no specific staining reaction and suggested staining with acid aniline dyes.

Two fractions of soluble collagen are recognized: (1) neutral salt-soluble collagen and (2) acid-soluble collagen. Both of those types of molecules are triple helices and were named tropocollagen (Goll, 1965).

Elastic fibers are stretched easily, branch and anastomose freely to form a loose network. When present in large masses in the fresh state they have a yellow appearance. Elastin, the principal constituent of elastic fibers, is extremely resistant to boiling water, acids and alkalis. The literature concerning the effects of trypsin and pepsin on elastin is conflicting.

Goll (1965) stated that elastin is not hydrolyzed by trypsin. An earlier report by Hiner et al. (1955) indicated that elastin can be digested slowly with pepsin and trypsin. Elastic fibers stain with orcein and resorcin fuchsin (Hiner, 1955).

Reticular fibers are small branching fibers that blacken intensely after silver impregnation. They occur where connective tissue is adjacent to other tissues (Birkner and Auerback, 1960). In most respects, reticular fibers appear to be chemically similar to collagenous fibers. They differ from collagenous fibers mainly by their smaller diameter and greater tendency to form a network (Copenhaver, 1964).

The Nature of Adipose Tissue

Some histologists believe that adipose tissue (fat) is a special form of connective tissue in which some cells lose their fiber forming ability and begin to store fat. Copenhaver (1964) stated that each fat cell is surrounded by strands of collagenous, elastic and reticular connective tissues. Other histologists consider adipose tissue as a specific tissue. Birkner and Auerback (1960) stated that groups of fat cells may occur anywhere in the connective tissue, and connective tissue strands group large masses of fat cells into primary, secondary and tertiary lobules similar to the organization of skeletal muscle.

Tenderness of Muscle

At least three components, muscle fibers, connective tissue and fat are involved in tenderness of skeletal muscle. The relationship of fiber diameter to tenderness has been reported by many authors. Investigators have studied the quantity, distribution and solubility of connective tissue in relationship to tenderness of muscle.

Effect of fiber diameter. Reports in the literature concerning the relationship of fiber diameter to tenderness of skeletal muscle are conflicting. Considerable variation among animals may account for some of the variability of results reported. Swanson et al. (1965) found a large and significant (P 0.01) variation in muscle fiber diameter of beef longissimus dorsi muscle from animals of the same carcass grade and weight.

Herring et al. (1965) reported that differences in fiber diameter between left and right sides of the carcass were related to differences in shear force (r = 0.73**). As fiber diameter increased tenderness decreased. Hiner et al. (1953) found a curvilinear relationship (r = 0.83) between fiber diameter and tenderness in nine cuts from animals ranging in age from 10 weeks to 9 years. Tenderness and fiber diameter were associated more closely in the mature animals than in the younger immature cattle.

A significant positive correlation between fiber diameter and shear force and a significant negative correlation between

fiber diameter and panel tenderness scores of beef longissimus dorsi and semimembranosus muscles were obtained by Tuma et al. (1962). However, when age effects were removed, essentially no relationship was noted between fiber diameter and shear force or panel scores for tenderness. Romans et al. (1965) also found that fiber diameter was not correlated significantly with tenderness of the longissimus dorsi muscle.

Effect of Connective Tissue. Collagenous fibers contribute a much larger portion of connective tissue than elastic fibers and, therefore, are considered to have a greater effect on tenderness of skeletal muscle (Hiner et al., 1955; Winegarden et al., 1952). Goll et al. (1963) cited the ratio of collagen to elastin as 3:1, on the basis of work done by Wilson et al. (1954). A recent study by Vognarova et al. (1968) indicated that the ratio of collagen to elastin in bovine muscle varied from one muscle to another. The role of reticular fibers in tenderness of meat is not clear (Miller and Kastelic, 1956).

Hiner et al. (1955) reported larger and more bunched elastic fibers in muscle where movement and/or work is required than in muscle having little or no strain. Muscles used extensively by the animals also contained larger amounts of collagenous fibers than those with less activity. Both elastic and collagenous fibers tended to increase in size with increasing maturity of the animal.

Harrison et al. (1949) and Sanderson and Vail (1963) concluded that there is a relationship between tenderness of meat

and quantity of connective tissue. Other studies have not indicated this relationship. In comparisons of collagen content of cooked beef of different grades, Griswold (1955) found that cooked beef of Commercial grade contained significantly ($P < 0.01$) more collagen than cooked beef of Prime grade. However, the quantity of collagen in the cooked meat was not correlated significantly with either shear values or taste panel scores for tenderness.

Lowe and Kastelic (1961) reported that correlations between (a) tenderness scores and collagen nitrogen scores for cooked beef muscle, (b) shear force and collagen nitrogen of raw muscle and (c) shear force and collagen nitrogen of cooked muscle were non-significant. The correlation between tenderness score for cooked muscle and the collagen nitrogen content of the corresponding raw cut was significant, but low ($r = -0.37*$).

Although Carpenter et al. (1963) found no significant relationship between tenderness measurements and the quantity of connective tissue, they obtained a significant negative correlation between coarseness of connective tissue and tenderness of longissimus dorsi muscle of pork.

Cover and Smith (1956) found that collagen content was associated with tenderness when the same muscle (biceps femoris) was cooked by different methods, but not when two different muscles (biceps femoris and longissimus dorsi) were cooked by the same method.

Effect of fat. Reports in the literature concerning the

relationship of tenderness to fat quantity and distribution are conflicting. For example, McBee and Wiles (1967) reported a close relationship between marbling score and tenderness, Gilpin et al. (1965) reported a slight relationship between marbling and tenderness and Romans et al. (1965) found a low and non-significant relationship between tenderness and marbling score. Norris (1968) recently reported that panel scores for tenderness and Warner-Bratzler shear values were not affected by level of marbling in bovine longissimus dorsi muscle. Wang et al. (1954) concluded that it is the distribution of fat rather than the quantity that affects tenderness. They found that the amount of linear fat in raw samples correlated well with tenderness scores for cooked samples.

Measurement of Muscle Fiber Diameter

Investigators have used several methods to measure muscle fiber diameter, and values reported for a given muscle vary with the method. Herring et al. (1965) measured the diameter of fibers in cross sections 12 u thick from 12 bovine muscles from animals weighing 325 to 350 kg. Fiber diameters ranged from 34.5 u for psoas major muscle to 64.5 u for semitendinosus muscle from vertically suspended carcasses. Muscle fiber diameters were measured from cross sections 10 u thick of beef longissimus dorsi muscle by Swanson et al. (1965). However, the diameters reported are not comparable with others in the literature because no correction was made for an apprximately 700X

magnification.

Several problems are associated with the measurement of cross sections of muscle. Swanson et al. (1965) pointed out that it is difficult to cut true cross sections, and any variation from the true cross section will increase the area of the fiber exposed for measurement. They also noted that there is a wide variation in the size of muscle fibers within a muscle. Therefore, large samples must be studied to obtain accurate results. In addition, muscle fibers are seldom perfectly round, which makes the selection of a representative area for measurement difficult.

Other investigators used methods that would more correctly be called measurements of muscle fiber width, because the measurements obtained are not true fiber diameters. Tuma et al. (1962) used fibers that had been separated from intact muscle by a microblendor with the blades reversed to avoid cutting the fibers. Mean fiber diameters for longissimus dorsi and semimembranosus muscle from animals ranging in age from 6 to 90 mo were 53.9 to 71.4 u and 52.4 to 64.8 u, respectively. Using this same method Romans et al. (1965) reported fiber diameters ranging from 64.02 to 69.01 u for longissimus dorsi muscles from beef carcasses from four maturity groups (A, B, C and D as defined by U.S.D.A., 1965). Although fiber diameters of the C and D maturity levels were significantly ($P < 0.05$) larger than those of the B level, none of these differed significantly from fiber diameters of the A maturity level.

Hiner et al. (1953) teased fibers from thin sections using a dissecting microscope and needles. Average fiber diameters ranged from 54.5 to 56.0 u for longissimus dorsi muscle from 900 lb steers and averaged 57.1 u for the semimembranosus, semitendinosus and biceps femoris muscles.

It is not known whether these methods for separating individual fibers from muscle result in stretching, flattening or otherwise altering the true width of the fibers.

Fibers in longitudinal sections were measured with an ocular micrometer in the eyepiece of a microscope by Ely (1967). Average diameters ranged from approximately 25 to 45 u for semimembranosus, semitendinosus and biceps femoris muscles from 16 to 25 mo old steers. Because of the shape of fibers, the area exposed for measurement depends on the plane of the fibers at which the section is cut.

Measurement of Connective Tissue

Ramsbottom et al. (1945) and Harrison et al. (1949) estimated quantity of connective tissue using a numerical histological scoring system. In addition to quantity estimates, Carpenter et al. (1963) subjectively scored the thickness of connective tissue strands on a 5-point scale.

Measurements of collagen nitrogen also have been used as an indication of the quantity of connective tissue present in muscle. Franklin (1957) reviewed several chemical methods. She explained that the greatest difficulty with the present

methods for the chemical analysis of collagen is in separating muscle fiber proteins from connective tissue proteins. Collagen is hydrolyzed to gelatin by autoclaving, and collagen nitrogen is determined by the Kjeldahl method in a majority of the methods reviewed.

Effect of Heat on the Components of Muscle

Satorius and Child (1938) heated three beef muscles, longissimus dorsi, adductor and triceps brachii to an internal temperature of 58° C at 150° C. In addition, roasts from the semitendinosus muscle were heated to 58, 67 and 75° C at 150° C. They reported fiber diameters decreased 12 to 16% during heating of the muscles to 58° C, and continued to decrease up to 67° C, but there was no difference in fiber diameter between 67° C and 75° C. They concluded that during heating hydrolysis of collagen increases tenderness, whereas coagulation of protein decreases tenderness. The effect of hydrolysis evidently is greater in heating during the first two stages (58° C and 67° C), whereas, in heating to 75° C the effect of coagulation is greater. These findings are confirmed in a recent study by Hostetler and Landmann (1968). They reported gradual but small decreases in width of bovine longissimus muscle fiber fragments heated to 45° C and rapid decreases during heating from 45° C to 62° C. The process appeared to be complete at 62° C because little further decrease in width occurred.

The properties of connective tissue are difficult to study in intact muscle because the connective tissue occurs in intimate contact with the muscle fibers. For this reason Winegarden et al. (1952) used ligaments and tendons to investigate the physical changes of connective tissue of beef during heating. They found that the extent of softening of connective tissue varied with the temperature of heating. Little or no softening occurred in tissue heated at 60° C. The critical temperature of softening seemed to be around 65° C. Both elastic and collagenous tissues were softened by heating in water at a sufficiently high temperature. The microscopic appearance of the heated fibers was altered from that of unheated fibers. After heating, fibers appeared straighter, less distinct and merged or fused in some areas. Similar changes in beef muscles heated to 70° C or higher were reported by Lowe and Kastelic (1961). In a majority of the cases muscle cooked to 90° C had more granular material than muscle cooked to 70° C. No evidence of granular material was found in muscle heated to an internal temperature of 63° C.

The longissimus dorsi muscle was examined histologically by Ramsbottom et al. (1949). They reported that collagenous fibers heated to 170° F underwent a shrinkage and disintegration after an initial swelling.

Several investigators have reported alterations in the staining characteristics of connective tissue after heating. Ramsbottom et al. (1949) reported that collagenous fibers in

cooked meat stained blue with Weigert's elastic connective tissue stain, whereas in raw samples these fibers were not colored by this stain. Paul et al. (1944) and Lowe and Kastelic (1961) noted similar changes in the affinity of beef collagenous fibers for stains. In uncooked beef the collagenous tissue was fibrous and stained a bright red with acid fuchsin. However, in the cooked meat the collagenous tissue appeared broken and granular and was colored by picric acid, but would no longer stain red with acid fuchsin.

Skelton et al. (1963) found no consistent color differences among sections of muscle cooked to temperatures of 55, 70 and 85° C. The colors of the stained cooked muscle were less brilliant than those of the raw samples. A change from fibrous to granular connective tissue was noted.

Collagen nitrogen was greater in cooked samples than in raw samples of longissimus dorsi and semimembranosus muscle, but collagen nitrogen values were not affected by degree of doneness (Skelton et al., 1963).

Cover and Smith (1956) measured collagen nitrogen content in samples of psoas major, longissimus dorsi and biceps femoris muscles. Collagen nitrogen content in the raw muscle was lowest for psoas major and highest for biceps femoris. Data reported by Lowe and Kastelic (1961) are in agreement with those findings.

Griswold (1955) found that meat braised without added water lost significantly (P 0.05) less collagen than meat

braised by the standard method. Cover and Smith (1956) reported collagen nitrogen and collagen retention were lower in braised biceps femoris than in broiled biceps femoris. They concluded that moist heat was more effective in degrading collagen in the biceps femoris than was dry heat.

The average amount of the initial collagen nitrogen remaining in psoas major, longissimus dorsi and round muscles after heating to internal temperatures of 90° C and 70° C were less than 25 and 40%, respectively (Lowe and Kastelic, 1961). Losses of collagen occurred in steaks heated to 62° C and approached 100% in steaks heated to 100° C and held for 25 min (Ritchey and Cover, 1962).

Wang et al. (1954) reported the release of fat from fat cells and dispersion of the fat among degraded collagen during heating of beef longissimus and semimembranosus muscles to 150° F. They interpreted these findings to indicate the occurrance of a process of emulsification with the collagen functioning as a dispersing agent and heat providing the source of physical agitation.

EXPERIMENTAL PROCEDURE

Samples

Samples of raw and cooked semimembranosus (SM) muscle were available for use in this study. Twelve U. S. Good beef top rounds were purchased from a local wholesale meat company. The

SM muscle was removed and cut into four pieces of comparable shape (approximately 11 X 11.5 X 6.5 cm) and weight (approximately 820 g) (Schock, 1969). Samples (1 X 1/2 X 1/2 in.) of raw muscle for histological study were removed from each piece (Fig. 1), wrapped in aluminum foil (gauge 0.0015) and stored at -17.8° C (0° F). Each piece of SM muscle was wrapped in aluminum foil, frozen and stored for 2 to 14 weeks.

Before cooking each wrapped piece was thawed to an internal temperature of 5 ± 2° C. Each piece was cooked by one of four methods: (1) deep-fat fried (DF), (2) oven-braised (OB), (3) pressure-braised (PB) or (4) oven-roasted (OR) to an internal temperature of 70° C (158° F).

Samples (1 X 1/2 X 1/2 in.) for histological study were removed from each piece of cooked SM muscle (Fig. 1), wrapped in aluminum foil (gauge 0.0015) and stored at -17.8° F (0° C).

Preparation of Slides

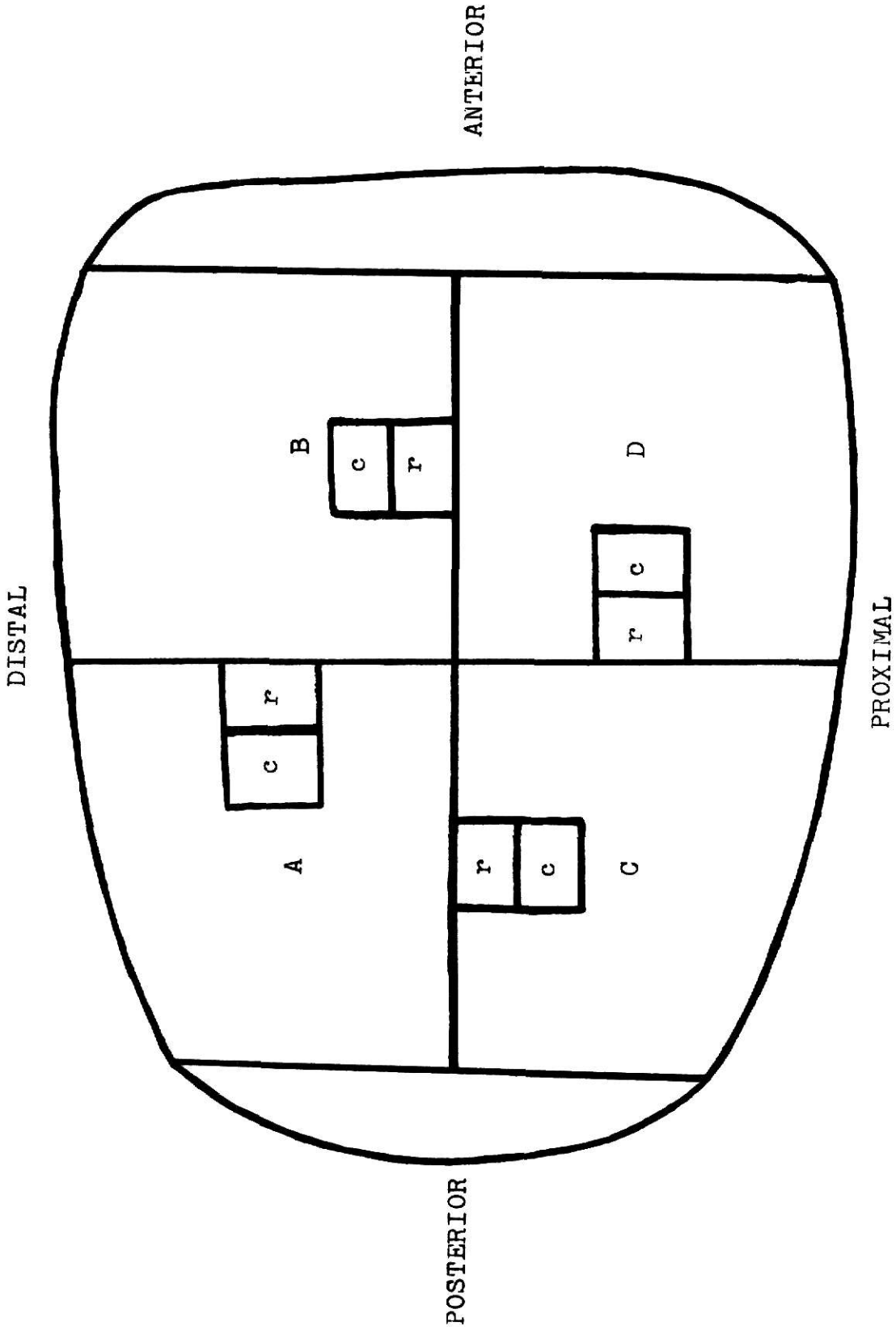
Each foil wrapped sample of SM muscle was removed from the freezer, and allowed to thaw for approximately one hour before sectioning. A CTD International Harris Cryostat Microtome was used for sectioning the muscle. Specimens approximately 1 X 1 X 1/2 cm were cut parallel with the fibers of each sample. A small amount of Cryoform, an embedding matrix, was placed on the tissue holder. The tissue to be sectioned was placed on the Cryoform and the holder inserted into the Cryostat microtome. A commercial preparation of freon gas, Cryo-Quik, was used to

Figure 1. Plan for sampling semimembranosus muscle.

A, B, C, D = Pieces of SM muscle (Schock, 1969).

r = raw samples for histological study

c = cooked samples for histological study


ANTERIOR
DISTAL
PROXIMAL
POSTERIOR
A
B
C
D
c
r
c
r
r
c
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c

rapidly freeze the muscle prior to sectioning. Sections 8-10 u thick were cut. Each section was transferred immediately to a slide by lightly touching the slide to the section while it was still on the knife blade. At least seven slides were prepared from each sample. Slides were labeled according to the code in Table 4 (Appendix).

The following staining procedure was used:

(1) Rinse in tap water

(2) Stain in a saturated solution of picric acid

(3) Rinse in tap water (2 times)

(4) Stain in picro-ponceau

(5) Dip in 70% alcohol

(6) Dip in 95% alcohol

(7) Dip in absolute alcohol

(8) Clear in xylene

(9) Mount with Permount

The formula for preparation of the picro-ponceau stain was obtained from Humason (1962):

Ponceau S, C. I. 27195, 1% aqueous	10 ml
Picric acid, saturated aqueous	86 ml
Acetic acid, 1% aqueous	4 ml

Muscle fibers stained yellow and connective tissue stained pinkish-red.

Evaluation

The slides were evaluated by three persons using a Bausch

and Lomb Dynazoom Microscope, and recorded on the score sheet, Form I, Appendix. The average scores reported were based on five slides per sample. Fiber width was measured by using an ocular micrometer in the eyepiece of the microscope. One muscle fiber from each of three microscopic fields in a section on a slide was measured. Norris (1969) recommended a magnification of 430X for measurement of fiber width as the lowest one that could be used with accuracy. Measurement of fiber width was done according to the procedure described by Norris (1969), Appendix, p. 39.

Color, type and quantity of connective tissue were noted for the section on each slide. Quantity estimates were based on the system of Ramsbottom et al. (1945). Fat distribution was recorded as clumped or scattered (Form I, Appendix, p. 41). A magnification of 35X was used for those observations, because that magnification allowed a larger field of view at one time than was possible with greater magnification.

Statistical Analysis

For each histological measurement differences between values for heated muscle and corresponding samples of raw muscle were analyzed by analysis of variance. When F-values were significant, least significant differences ($P < 0.05$) were calculated. The analysis of variance was:

Source of Variation	D/F
Treatments	3
Error	43
Total	46

RESULTS AND DISCUSSION

Mean values for histological measurements on raw and heated muscle are presented in Table 1. Detailed data are in Tables 5 and 6, Appendix. Mean values for differences between raw and heated muscle are given in Table 2.

Muscle Fiber Width

Mean muscle fiber widths for samples given all heat treatments were smaller than mean raw fiber widths (Table 1). The percentage decreases ranged from 8.4 to 11.4%, respectively, for the OR and OB treatments. The standard deviation of the DF treatment was larger than the standard deviations of the other treatments (Table 2). The relatively large values for standard deviations reflect the wide variation in the size of muscle fibers within a muscle and among the same muscle from different animals as reported by Swanson et al. (1965). Satorius and Child (1938) reported decreases in fiber diameter of 12 to 16% for muscles heated to 58° C by oven-roasting, and further decreases during heating to 67° C. Microscopic slide preparations of longissimus dorsi fibers were heated on a microscope stage by Hostetler and Landmann (1968). They observed decreases

Table 1. Mean values for selected histological measurements on raw and heat treated muscle according to heat treatment.[a]

Measurement	Raw	DF	Raw	PB	Raw	OB	Raw	OR
Muscle fiber width, u	47.9	47.3	48.9	43.8	48.3	42.8	47.7	43.7
% change	9.6		10.4		11.4		8.4	
Connective tissue characteristics[b]								
Wavy	6.5	3.6	6.3	3.7	6.5	3.8	6.4	4.0
Straight	3.7	4.3	3.7	4.2	3.6	4.3	3.6	4.5
Granular	1.1	6.4	1.1	6.3	1.2	6.4	1.2	5.8
Connective tissue quantity[c]	4.7	5.5	4.6	5.8	4.7	5.5	4.5	4.8

[a] DF - Deep-fat fried
PB - Braised, 10 p.s.i.g.
OB - Braised, atmospheric pressure
OR - Oven-roasted

[b] Panel score
7 - large proportion
5 - medium proportion
3 - small proportion
1 - none

[c] Panel score
7 - large quantity
5 - medium quantity
3 - small quantity
1 - none

Raw, DF, OB, OR
Fiber width - N = 540
Connective tissue - N = 180

PB
Fiber width - N = 495
Connective tissue - N = 165

Table 2. Mean differences between raw and heated muscle, standard deviations, F-values and LSDs attributable to heat treatment.

Measurement	Heat treatment[a] DF	PB	OB	OR	F-value	LSD[b]
Fiber width, u	4.6 (±4.1)[f]	5.1 (±2.6	5.5 (±1.8)	4.0 (±2.3)	0.64 ns	
Connective tissue characteristics[c]						
Wavy	2.9 (±0.6)	2.6 (±1.5)	2.7 (±0.8)	2.4 (±0.8)	0.56 ns	
Straight	-0.6 (±1.0)	-0.5 (±0.9)	-0.7 (±0.8)	-0.9 (±0.9)	0.34 ns	
Granular	-5.3 (±0.5)	-5.2 (±0.8)	-5.2 (±0.6)	-4.6 (±0.9)	2.87*	0.586[d] 0.599
Connective tissue quantity[e]	-0.8 (±1.3)	-1.2 (±1.1)	-0.8 (±1.3)	-0.3 (±1.1)	1.23 ns	

[a] Heat treatment
DF - Deep-fat fried
PB - Braised, 10 p.s.i.g.
OB - Braised, atmospheric pressure
OR - Oven-roasted

[b] LSD, least significant difference at 5% level

[c] Panel score
7 - large proportion
5 - medium proportion
3 - small proportion
1 - none

[d] Use LSD 0.586 for comparing values among DF, OB and OR heat treatments; use LSD 0.599 for comparing values of PB with DF, OB and OR heat treatments.

[e] Panel score
7 - large quantity
5 - medium quantity
3 - small quantity
1 - none

[f] Values in parentheses are standard deviations.

* $P < 0.05$

of approximately 20 to 25% in width of muscle fibers during heating to temperatures of 53 to 77° C. Although the rate of heat penetration in the pieces of muscle from which the histological samples were taken was affected significantly ($P < 0.01$) by the heat treatment (Schock, 1969), the differences in mean muscle fiber width among the four heat treatments were not significant (Table 2). Schock (1969) reported the order of the average rate of heat penetration was PB > DF > OB > OR, whereas the order of the percentage decrease in mean muscle fiber width was OB > PB > DR > OR (Table 1).

Mean fiber widths for cooked samples of semimembranosus muscle in this study averaged 43.4 u, with a range of 39.5 to 47.7 u for all cooked samples (Tables 1 and 3). Satorius and Child (1938) reported fiber diameters of 53.8, 57.90 and 56.72 u, respectively, for beef triceps brachii, adductor and longissimus dorsi muscles.

Mean muscle fiber widths for raw samples averaged 48.2 u, with a range of 43.7 to 57.0 u (Tables 1 and 3). Herring et al. (1965) reported values for raw bovine muscle fibers similar to those obtained in this study. They used an ocular micrometer to measure cross-sections of 12 bovine muscles from 325 to 350 kg animals. Fiber diameters varied greatly among the muscles, ranging from 34.5 to 64.5 u, respectively, for samples of psoas major and semitendinosus muscles. Mean fiber diameters were approximately 52 u for samples of longissimus dorsi muscle and 50 u for samples from semimembranosus muscle.

Table 3. Range in muscle fiber width[a] for raw samples and for samples given each heat treatment[b].

Treatment	Low	High	Difference
Raw[c]	43.7	57.0	13.3
DF[d]	39.8	46.4	4.6
OR[d]	40.5	47.7	7.2
OB[d]	39.5	47.0	5.5
PB[d]	40.6	47.7	7.1

[a] Muscle fiber width in microns.

[b] DF - Deep-fat fried
OR - Oven-roasted
OB - Braised, atmospheric pressure
PB - Braised, 10 p.s.i.g.

[c] N = 48

[d] N = 12

Slightly larger values than those found in this study were reported by Hiner et al. (1953), Tuma et al. (1962) and Romans et al. (1965). Those investigators used an ocular micrometer to measure the diameter of "teased" fibers. Hiner et al. (1953) measured fibers teased from muscle samples using a dissecting microscope and needles. The mean diameter of fibers from the longissimus dorsi muscle and the muscles of the round (semimembranosus, semitendinosus and biceps femoris) of 900 lb, 14 mo old, Good grade steers were 55.6 and 57.1 u, respectively. Tuma et al. (1962) reported muscle fiber diameters of 53.9 to 71.4 u for samples of longissimus dorsi muscle from animals ranging in age from 6 to 90 mo. Fiber diameters of the longissimus dorsi muscle of animals from four U.S.D.A. maturity groups (A, B, C and D) ranged from 64.02 to 69.01 u (Romans et al., 1965). Both Romans et al. (1965) and Tuma et al. (1962) measured fibers that had been teased from muscle samples using a microblendor.

Ely (1967) employed the same method as used in this study, but found slightly smaller average diameters (25 to 45 u) for samples from 16 to 25 mo old bovine semimembranosus, semitendinosus and biceps femoris muscles than are reported in this study.

Variations in values reported for muscle fiber diameter or width probably are the result of method of measurement; age, maturity or grade of the animal; or other pre- and post-slaughter treatments rather than heat treatment.

Connective Tissue Quantity

Subjective evaluation of collagenous connective tissue quantity indicated a greater amount was present in the cooked samples than in corresponding raw samples (Table 1). However, the differences among heat treatments were not significant (Table 2). Standard deviations of the differences between raw and heat treated muscles were similar for all four treatments (Table 2). Skelton et al. (1963) reported similar observations and attributed this to the swelling and redistribution of collagenous tissue during heating.

Connective Tissue Characteristics

Staining. Collagenous connective tissue in the raw samples stained bright pink to red. In the cooked samples some of the collagenous tissue had a granular appearance and was stained yellow by the picric acid, but would not stain red with the picro-ponceau stain. Paul et al. (1944), Lowe and Kastelic (1961) and Skelton et al. (1963) reported similar findings. It was postulated that changes in staining affinity might be evaluated by estimating the intensity of color of each stained section. However, during preparation of the slides it was noted that freshness of the stain affected the length of staining time required. It was not practical to prepare a new batch of stain for each sample. Therefore, the staining period was increased as necessary to obtain a good contrast. The sections from heated muscle stained more rapidly than sections from raw muscle.

Estimates were made of the relative quantities of wavy, granular and straight types of collagenous connective tissue present in each section (7 = large, 5 = medium, 3 = small and 1 = none).

Straight fibers made up a small to medium proportion of the total collagenous connective tissue present in the raw samples (Table 1). They comprised a slightly larger proportion of the connective tissue of the cooked sections than the raw but the differences among treatments were not significant (Tables 1 and 2). Straight fibers were present as small connective tissue strands between muscle fibers (Fig. 2a) as well as in large areas.

Wavy fibers were predominate in sections of raw muscle. In heated samples they were present in small to moderate amounts (Table 1). Differences among treatments were non-significant (Table 2). Standard deviations of the differences in the quantity of wavy fibers between raw and treatments DF, OB and OR were similar. The reason for the larger standard deviation of the PB treatment than the other treatments is not apparent. Wavy fibers were present in narrow connective tissue strands between muscle fibers, but were more common in large areas (Fig. 2b).

In most of the raw samples there was no granular connective tissue, whereas in the heated samples it was observed as a medium to large proportion of the total collagenous connective tissue (Table 1). There was a significantly ($P < 0.05$) larger

Figure 2. Examples of histological characteristics.

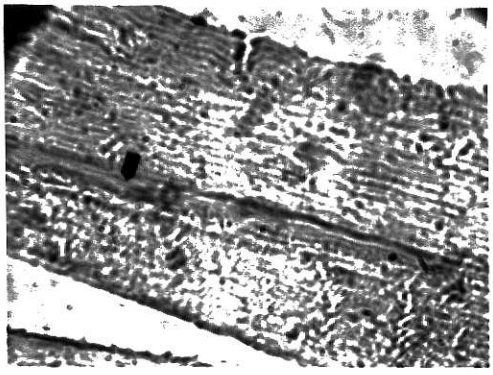
a. Arrow indicates a small area of straight connective tissue fibers between two muscle fibers. Oven-roasted; 860X.

b. A large area of wavy collagenous connective tissue fibers. Raw; 645X.

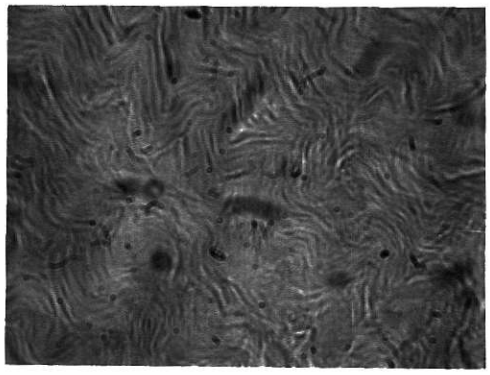
c. A mixture of straight and granular connective tissue. Oven-braised; 430X.

d. Granular collagenous connective tissue adjacent to muscle fiber. Oven-braised; 645X.

e. Clumped fat cell walls. Deep-fat fried; 645X.



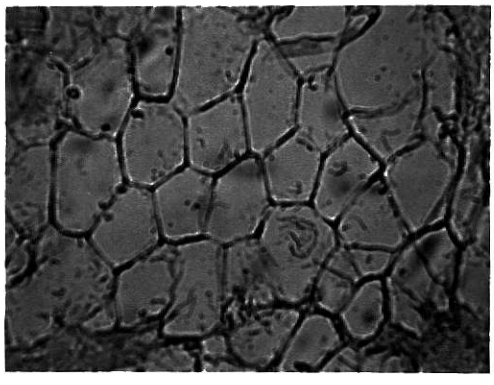
a



b

c

d



e

amount in DF, PB and OB treatments than in OR samples. In reporting cooking data and other measurements for the same pieces of semimembranosus muscle from which histological samples for this study were taken, Schock (1969) noted that OR pieces had the slowest rate of heat penetration and longest cooking time of the four treatments. Meat given the OR treatment appeared less well done than that given other treatments and had the highest values for total moisture, press fluid yield, water holding capacity and panel scores for juiciness.

In the heated samples generally granular tissue was found mingled with straight connective tissue (Fig. 2c). Narrow strands of connective tissue tended to be completely granular, whereas larger areas of connective tissue tended to contain both granular and straight fibers (Fig. 2c and Fig. 2d).

Fat Distribution

Although the solvent used in the staining technique dissolved the fat droplets from the cells, the fat cell walls were distinguishable in both raw and heated samples. Fat was present in both clumped and scattered forms in a majority of the samples. However, the predominate form was clumped (Fig. 2e). Wang et al. (1954) explained that during heating some of the fat disperses out of the fat cells without structural damage and into the degraded collagen.

SUMMARY

Samples of raw and heat treated semimembranosus muscle were examined to determine the effects of four heat treatments (DF, OR, OB and PB) on selected histological characteristics of the muscle. No significant differences occurred in the effect of the four heat treatments on muscle fiber width. Variations in values reported for muscle fiber diameter or width probably are the result of method of measurement; age, maturity or grade of the animal; or other pre- and post-slaughter treatments rather than heat treatment. Relative proportions of straight and wavy connective tissue were not affected significantly by heat treatment. However, there was a significantly ($P < 0.05$) larger quantity of granular connective tissue in DF, PB and OB samples than in OR samples. Intact fat cells were noted in both raw and heated samples.

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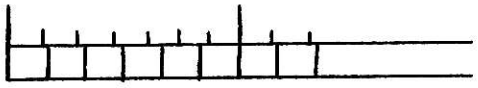
APPENDIX

MICROSCOPIC MEASUREMENT OF MUSCLE FIBER WIDTH (Norris, 1968)

An ocular micrometer is a clear glass disc on which a tiny scale is engraved. Insert this disc into the eyepiece by unscrewing the top lens and inserting the disc onto the shelf within the eyepiece. In order to measure the magnified image the units on the scale of the ocular micrometer are compared to a stage micrometer, a slide with a measurement line divided into 0.01 mm units. To calibrate the ocular micrometer, insert the stage micrometer on the stage of the microscope under high power (43X objective, 10X eyepiece and Dynazoom setting 1 = 430X magnification). Match a line of the scale on the stage with a line on the squared scale of the ocular micrometer. Count the number of ocular and stage units until another line on the ocular matches another line on the stage micrometer. To find the value of each ocular unit, the distance covered by the stage units is written in its numerical value (each stage unit = 0.01 mm) and divided by the number of ocular units.

Example:



7 stage units = 7 X 0.01 or 0.07 mm

$\frac{0.07}{6}$ = 0.012 mm/ocular unit or 1 ocular unit = 0.012 mm

Replace the stage micrometer with the slide to be studied. The width of the muscle fibers can be obtained by counting the number of units that correspond to the width of a fiber and

multiplying the number of units by the size of the unit of measure.

Example: muscle fiber width = 3 ocular units

3 X 0.012 mm = 0.036 mm for that fiber's width

Convert the mm value to u by multiplying by 1000

0.036 mm X 1000 = 36 u

For measurements of greater accuracy, through the center of the eyepiece the ocular units are further divided into 5 parts. The eyepiece can be turned in the tube, thus turning the ocular scale. In this way, fibers that do not lie in a perfectly horizontal or vertical direction can be measured.

Form I. HISTOLOGICAL SCORE SHEET

Code No. _____

Date _____

Name _____

Factor	Slide No. 1	2	3	4	5	Total Average
Muscle fiber width, u						
a)						
b)						
c)						
Total						
Connective tissue						
Color, intensity[a]						
Describe any apparent changes in hue						
Type, check appropriate characteristics[b]						
wavy						
granular						
straight						
Quantity[c]						
Fat distribution						
Check appropriate characteristics						
clumped						
scattered or beadlike						

[a] 3 - intense color; 2 - moderately intense; 1 - pale color

[b] 7 - large proportion; 5 - medium proportion; 3 - small proportion; 1 - none

[c] 7 - large quantity; 5 - medium quantity; 3 - small quantity; 1 - none

Table 4. Code for labeling slides of raw and cooked semimembranosus muscle.

Round	Treatment[b]	Piece of SM[a]	Round	Treatment[b]	Piece of SM[a]
I	OB	A	VII	OR	A
I	DF	B	VII	DF	B
I	OR	C	VII	PB	C
I	PB	D	VII	OR	D
II	DF	A	VIII	DF	A
II	PB	B	VIII	OB	B
II	OB	C	VIII	OB	C
II	OR	D	VIII	OR	D
III	OR	A	IX	DF	A
III	OB	B	IX	OR	B
III	OB	C	IX	PB	C
III	PB	D	IX	OR	D
IV	OR	A	X	PB	A
IV	PB	B	X	OB	B
IV	OR	C	X	OB	C
IV	DF	D	X	DF	D
V	OB	A	XI	DF	A
V	DF	B	XI	PB	B
V	DF	C	XI	OR	C
V	PB	D	XI	OB	D
VI	PB	A	XII	DF	A
VI	OB	B	XII	OR	B
VI	DF	C	XII	PB	C
VI	PB	D	XII	OB	D

[a] See Fig. 1.

[b] OB - Braised, atmospheric pressure
DF - Deep-fat fried
OR - Oven-roasted
PB - Braised, 10 p.s.i.g.

Table 5. Relative proportions of wavy, straight and granular connective tissue in samples of raw and heat treated muscle.

Measurement	raw	DF	raw	PB	raw	OB	raw	OR
Connective	6.2	3.8	4.3	4.1	4.6	3.5	5.4	4.3
tissue, wavy	5.9	3.7	6.9	3.1	5.7	3.7	5.7	3.7
	6.6	4.6	6.0	3.9	7.0	4.5	6.3	3.5
	6.5	3.8	6.3	5.5	6.9	4.6	6.2	5.1
	6.5	3.9	6.3	---	6.3	3.4	6.9	5.4
	7.0	4.1	4.6	4.3	6.9	4.7	6.6	3.8
	6.9	4.1	7.0	3.8	6.9	3.5	6.5	3.9
	6.5	3.0	6.3	3.0	6.9	3.1	6.7	3.1
	7.0	3.1	7.0	3.8	7.0	3.8	6.5	4.3
	6.5	3.0	7.0	3.0	6.5	3.9	6.9	3.5
	7.0	3.4	6.7	3.4	6.1	3.5	7.0	4.1
	5.8	3.0	7.0	3.0	7.0	3.0	6.3	3.3
Total	78.4	43.5	75.4	40.9	77.8	45.2	76.6	48.0
Mean	6.5	3.6	6.3	3.7	6.5	3.8	6.4	4.0
Connective	4.9	4.2	5.2	4.3	4.3	3.1	4.1	4.1
tissue,	4.3	5.7	3.4	4.2	3.9	3.7	3.9	3.0
straight	3.5	4.2	4.0	3.9	3.0	3.7	3.5	5.8
	3.9	4.3	3.2	3.7	3.1	3.7	3.8	4.3
	3.5	3.8	3.8	---	2.8	4.5	3.0	3.7
	3.4	4.1	5.1	4.7	3.3	3.9	3.4	3.9
	3.5	4.0	3.3	3.8	3.3	4.9	3.9	5.0
	3.4	4.2	3.8	3.8	3.5	4.1	3.3	5.7
	3.0	4.1	3.3	5.5	3.7	5.4	3.9	4.6
	3.5	5.0	3.0	3.4	3.5	4.3	3.5	4.3
	3.0	5.0	3.4	5.0	4.5	5.8	3.1	4.9
	4.7	3.1	3.1	3.9	3.7	4.1	3.7	4.3
Total	44.6	51.7	44.6	46.2	42.6	51.2	43.1	53.6
Mean	3.7	4.3	3.7	4.2	3.6	4.3	3.6	4.5
Connective	1.0	6.1	1.7	7.0	3.0	6.7	2.7	5.7
tissue,	1.1	5.9	1.0	6.2	1.0	6.1	1.1	6.5
granular	1.0	5.1	1.0	5.5	1.2	5.9	1.1	5.4
	1.1	6.3	1.3	4.7	1.1	6.9	1.3	4.6
	1.0	6.2	1.0	---	1.1	6.1	1.0	5.0
	1.0	6.9	1.3	5.9	1.0	6.7	1.0	6.6
	1.0	6.5	1.0	7.0	1.0	5.9	1.0	5.8
	1.1	6.9	1.0	6.9	1.1	7.0	1.0	4.9
	1.0	7.0	1.0	6.2	1.0	6.1	1.0	5.4
	1.0	6.3	1.0	7.0	1.0	6.3	1.0	6.3
	1.0	6.3	1.0	6.2	1.0	6.3	1.0	6.3
	1.3	7.0	1.0	7.0	1.0	6.6	1.0	6.3
Total	12.6	76.5	13.3	69.6	14.5	76.6	14.2	68.8
Mean	1.1	6.4	1.1	6.3	1.2	6.4	1.2	5.8

Table 6. Muscle fiber width and quantity of connective tissue in raw and heat treated muscle.

Measurement	raw	DF	raw	PB	raw	OB	raw	OR
Muscle fiber	51.0	44.4	51.0	47.7	48.0	44.9	45.7	44.1
width, u	57.0	43.8	53.8	43.3	54.8	47.0	52.1	46.0
	46.7	44.4	46.9	42.5	48.5	42.2	49.5	41.6
	49.4	42.3	50.9	47.2	46.9	40.4	46.7	40.4
	43.8	39.8	46.3	----	50.5	42.7	44.2	40.5
	49.7	45.1	50.0	43.7	51.6	45.1	49.2	44.2
	51.0	41.6	50.1	45.2	47.4	42.4	47.0	47.7
	46.2	42.5	48.0	43.5	45.4	39.5	46.6	44.1
	43.8	46.4	44.3	44.2	46.1	41.6	48.6	44.8
	46.5	42.1	47.2	40.6	43.7	41.0	45.0	41.0
	45.4	43.4	49.1	43.8	47.8	43.1	47.1	43.1
	44.7	43.8	47.9	41.4	49.0	45.0	50.2	46.8
Total	575.2	519.6	585.5	483.1	579.7	513.8	571.9	524.3
Mean	47.9	43.3	48.9	43.9	48.3	42.8	47.7	43.7
Connective	5.5	5.8	3.9	6.5	4.9	5.9	4.9	4.2
tissue,	5.1	7.0	5.4	6.7	3.5	4.9	5.3	4.2
quantity	6.1	6.5	5.4	5.7	6.3	3.8	5.7	6.9
	5.4	5.9	5.9	5.4	5.9	6.9	4.9	5.3
	4.7	4.7	4.1	---	3.8	6.3	5.9	4.9
	5.4	5.9	4.3	6.9	3.8	4.3	3.7	5.4
	4.3	6.5	5.1	5.7	4.7	6.2	3.5	5.8
	3.7	6.6	4.1	5.9	5.7	6.7	4.3	4.8
	4.2	4.1	4.7	5.9	5.8	5.7	4.6	3.7
	3.1	5.0	3.9	6.6	3.0	4.5	4.5	4.2
	4.3	4.8	4.6	5.4	5.0	6.9	3.9	5.1
	4.5	3.7	3.4	3.7	4.3	3.9	3.4	3.5
Total	56.3	66.5	54.8	64.4	56.7	66.0	54.6	58.0
Mean	4.7	5.5	4.6	5.8	4.7	5.5	4.6	4.8

EFFECT OF DRY AND MOIST HEAT TREATMENTS ON SELECTED HISTOLOGICAL MEASUREMENTS OF BEEF SEMIMEMBRANOSUS MUSCLE

by

HELEN CHARLENE BAUDER REID
B. S., Kansas State University, Manhattan, 1967

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

Samples of raw and heat treated beef semimembranosus muscles were examined histologically to determine the effect of deep-fat frying, oven-roasting, oven-braising, and pressure braising to 70° C on selected histological characteristics of the muscle. A panel of three persons used an ocular micrometer to measure muscle fiber width, observed color and type of connective tissue and distribution of fat, and estimated the quantity of connective tissue of 475 histological sections.

Muscle fiber width was not affected significantly by the four heat treatments. Variations in values reported in the literature for muscle fiber diameter or width probably are the result of method of measurement; age, maturity or grade of the animal; or other pre- and post-slaughter treatments rather than heat treatment. Relative proportions of straight and wavy connective tissue were not affected significantly by heat treatment. However, there was a significantly ($P < 0.05$) larger quantity of granular tissue in deep-fat fried, pressure-braised and oven-braised samples than in oven-roasted samples. Intact fat cells were observed in both raw and heated samples.