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Rate of Heat Penetration of Microwave-Cooked Beef Semitendinosus as Related to Histological Characteristics and Tenderness

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To the Graduate Council:

I am submitting herewith a thesis written by Judy Lynn Simmons entitled "Rate of Heat Penetration of Microwave-Cooked Beef Semitendinosus as Related to Histological Characteristics and Tenderness." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Grayce E. Goertz, Major Professor

We have read this thesis and recommend its acceptance:

Jane R. Savage, Bernadine Meyer

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 4, 1970

To the Graduate Council:

I am submitting herewith a thesis written by Judy Lynn Simmons entitled "Rate of Heat Penetration of Microwave-Cooked Beef Semitendinosus as Related to Histological Characteristics and Tenderness." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

RATE OF HEAT PENETRATION OF MICROWAVE-COOKED BEEF SEMITENDINOSUS AS RELATED TO HISTOLOGICAL CHARACTERISTICS AND TENDERNESS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Judy Lynn Simmons

March 1970

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ABSTRACT

Three-inch pieces of beef semitendinosus muscles were cooked by microwaves for zero-, three-, four-, five-, six- and seven-minute intervals to internal temperatures of 1, 16-25, 26-35, 36-45, 46-55, and 56-65°C, respectively. An index to tenderness was obtained by the Warner-Bratzler shear and histological characteristics were studied.

Increase in internal temperatures of the muscle pieces was accompanied by a decrease in shear values, the greatest decrease occurring at the seven-minute time interval. Scores for muscle fiber disintegration (none to excessive), changes in fat cells (full to empty) and degradation of collagenous connective tissue (fibrous to granular) increased as internal temperature increased. Muscle fiber diameter, as estimated by the number of fibers per field, decreased slightly as cooking time and internal temperatures of the muscle pieces increased.

Low shear values were associated with decreases in muscle fiber diameter, but not muscle fiber disintegration. As shear values decreased empty fat cells became more prevalent than full fat cells. Shear values and degradation of collagenous connective tissue were unrelated in this study.

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CHAPTER I

INTRODUCTION

Microwave heating allows generation of heat in a food at speeds limited only by the character of the product. While this reduction in cooking time may be advantageous to the consumer, the rapid rate of heat penetration may result in undesirable changes in food. Several studies have indicated that microwave-cooked meats have large cooking losses and thus considerable shrinkage. These high cooking losses may be related to the low tenderness and juiciness scores often reported for microwave-cooked meats.

Several properties of muscle change markedly as meat is heated. During cooking, meat shrinks in volume and increases in density (Meyer, 1960). The shrinkage of meat tissue and the release of juice is caused by coagulation of the protein in the muscle (Hamm, 1966). The rate and degree of this coagulation could profoundly affect the meat tenderness (Cover, 1941). It is believed that tenderness is decreased as the muscle fiber proteins coagulate and increased with the softening and hydrolysis of the connective tissue (Visser et al., 1960). Early workers noted that during cooking of beef, tenderness increased with extremely slow rates of heat penetration (Cover, 1943). Visser et al. (1960) cooked pieces of beef muscle to rare, medium and well-done by oven roasting and cooking in deep fat at 100 and 110° C. Muscle pieces cooked in deep fat at both

temperatures had higher rates of heat penetration and higher shear values than those oven roasted. Possibly, differences in shear value obtained by various heat treatments were related to time-temperature-dependent protein heat coagulation processes.

Microscopic investigation of the muscle tissue may help to explain these differences in the coagulation process and their relationship to tenderness. It is assumed that water lost during cooking of meat has been contributed by intercellular spaces and muscle fibers. This released fibrillar water is attributed to the coagulation of muscle protoplasm and results in a decrease in fiber diameter (Birkner et al., 1960). Decreases in width of muscle fibers occur soon after heating is initiated and are completed by 62°C. Shortening of the muscle occurs as soon as stable cross-linkages begin to form in the fibers. Hostetler et al. (1968) noted that the greatest decrease in muscle fiber length occurred between 54 and 70°C.

Birkner et al. (1960) reported that collagenous fibers undergo swelling, shrinkage and disintegration during cooking. Histological studies indicate that fat is lost during heating of muscle. Fat from the endomysial and perimysial fat cells diffuses from structurally undamaged cells and small droplets of fat are dispersed along the paths of collagen degradation (Wang et al., 1954).

Since the rate of heat penetration may affect meat tenderness and is rapid in microwave-cooked meat, the heat penetration rate of microwave-cooked beef semitendinosus muscle as related to histological characteristics and tenderness as measured on a Warner-Bratzler shear was investigated.

CHAPTER II

REVIEW OF LITERATURE

Meat is a complex bundle of muscle fibers, connective tissue, fat and bone, containing approximately 75 percent water, 20 percent protein, 3 to 5 percent fat and a small amount of inorganic salts. In muscle tissue, there is present myofibrils, containing the contractile system of actin and myosin; sarcoplasm, the fluid and colloidal material bathing and surrounding the fibrils; and stroma, containing the connective tissues collagen, elastin and reticulin (Bendall, 1962).

The long, cylindrical and multinucleated muscle fiber makes up the contractile unit of the muscle. These fibers may range from 1 to 40 millimeters in length and from 10 to 100 microns in diameter. Each fiber is composed of myofibrils, approximately 2 to 3 microns in diameter, arranged parallel to one another, giving the appearance of longitudinal striations. Cross-striations of the myofibrils are due to alternating bands which have different affinities for iron hematoxylin stain. A bands having an affinity for the stain and I bands which will not accept the stain, lie opposite A and I bands of the adjacent myofibrils so that the entire composite muscle fiber appears cross-striated (Birkner et al., 1960).

Thin networks of connective tissue called the endomysium surround each muscle fiber. Bundles of muscle fibers are encompassed by large

sheaths of connective tissue, the perimysium. Groupings of these bundles form the entire muscle which is encased by epimysial connective tissue (Birkner et al., 1960).

Connective tissue consists of collagenous, elastic and reticular protein fibers embedded in an amorphous ground substance. Collagenous fibers ranging from 10 to 24 microns in thickness are composed of many parallel fibrils 0.3 to 0.5 microns in diameter, enveloped in a mesh of loose unoriented single fibrillae. Reticular fibers consisting of collagen and bound lipid occur as highly branched fibers wherever connective tissue adjoins other tissue. Elastin, a yellow, homogeneous, albumoid substance, forms thin elastic fibers that branch freely. Adipose tissue may be found distributed in groups of cells embedded in the connective tissue. Droplets of fat coalesce to form large globules which enlarge until the cytoplasm and flattened nucleus constitute only a thin peripheral of the fully developed fat cell. A thin membrane of reticular fibers surrounds each fat cell (Birkner et al., 1960).

I. HEAT INDUCED CHANGES IN MUSCLE

The influence of heat on the components of a muscle encompasses many alterations. The most drastic changes during the heating of meat are those involving the muscle proteins. Changes in the fibrillar proteins result in shrinkage of the tissue and the release of juice; denaturation of the sarcoplasmic proteins accounts for the discoloration of muscle and the loss of enzyme activity (Hamm, 1966). Of the stroma proteins, collagen undergoes heat denaturation to form gelatin during

heating, whereas elastin and reticulin undergo little change (Bendall, 1964).

General chemical and physical changes. The chemical and physical changes of muscle during heating have been summarized by Hamm (1966). Few changes occur in the colloidal-chemical properties of muscle tissue or in the solubility of the muscle proteins between 20 and 30°C. As the temperature is increased from 30 to 50°C, some changes in the myofibrillar proteins occur, influencing the water holding capacity and rigidity of the tissue. An unfolding of peptide chains and the formation of stable cross-linkages result in a rigid protein structure. Some of the sarcoplasmic proteins also are denatured in this temperature range. A structural rearrangement of the myofibrillar proteins, causing a delay in the changes of water holding capacity, occurs between 50 and 55°C and the denaturation of the sarcoplasmic proteins continues. Most of the changes occurring between 40 and 60°C are continued to a lesser extent in the 55 to 80°C range. The myofibrillar and globular proteins are coagulated at 65°C. Collagenous fibers undergo swelling, shrinkage and disintegration during heating. Shrinkage of the collagen beings at 63°C and at temperatures above 80°C, collagen is converted to gelatin.

Hostetler et al. (1968) also found the changes in muscles during heating to be closely related to the water holding property of muscle protein. Muscle fibers were heated from room temperature to 80°C on the stage of a microscope. Small decreases in width began soon after heating was initiated and continued to 45°C. A rapid decrease in muscle fiber diameter occurred at 45°C and was essentially completed at 62°C. Little

or no shortening of the muscle fibers was observed until a temperature of 55°C was reached. Fibers shortened suddenly between 55 and 65°C to about 80 percent of their original length. Changes in the fiber lengths seemed to coincide with the loss of acidic groups in the muscle proteins reported by Hamm et al. (1960). The muscle proteins were more rapidly denatured in this temperature range.

Microscopic study has revealed that fat is lost from beef muscles during heating. In longissimus dorsi and semitendinosus broiled to 150°F, fat diffused from structurally undamaged cells and small droplets were dispersed along the paths of collagen degradation (Wang et al., 1954).

Effect of rate and extent of heating on tenderness. The reactions that take place during cooking are not well understood in their relationship to the final tenderness of meat. The coagulation of the muscle fiber proteins and the partial hydrolysis of the connective tissue are two important factors influencing meat tenderness. The coagulation of the muscle fiber protein is believed to result in a decrease in tenderness which may be affected by the rate and degree of this protein coagulation. The degree of connective tissue tendering may depend on the meat temperature reached and the time required to reach this temperature (Cover et al., 1960).

Smith (1957) measured the changes in tenderness of single muscle fibers during heating with a special tensiometer. Five small pieces of meat cut from beef round steak and fillet mignon, and heated at different temperatures, were found to increase in tensile strength during cooking,

which was interpreted as a decrease in tenderness. The changes in tenderness were thought to be associated with protein denaturation.

Ritchey et al. (1965) found juiciness and softness of longissimus dorsi and biceps femoris steaks cooked to internal temperatures of 61, 68, 74 and 80°C to decrease as the temperature increased, except between 61 and 68°C. Changes in panel scores for fragmentation of the muscle fibers were small and not significant between adjacent temperatures in either muscle, but were significant between extreme treatments in the biceps femoris. Shear values increased in the longissimus dorsi steaks as the end point temperature increased, but were not changed in the biceps femoris steaks.

The effect of heating time and temperature on the shear values of U. S. Choice beef semitendinosus muscle pieces heated in test tubes in a steam bath for up to five hours at temperatures with 1°C increments from 50 to 90°C was observed (Machlik et al., 1963). The semitendinosus pieces underwent a marked decrease in shear at 58°C, reflecting a dependence upon the time and temperature rate, especially temperature. Lowest shear values occurred in the 60 to 64°C range after 30 to 60 minutes of heating. In this temperature range, collagen degradation was completed quickly, whereas the hardening of muscle protein associated with higher temperatures was avoided.

In early studies slow rates of heat penetration were found to increase meat tenderness. Cover (1943) cooked paired roasts from three cuts to rare and well-done at oven temperatures of 80 and 125°C. When the rate of heat penetration was slow enough to require 30 hours or more

for the loss of the pink color, the roasts were nearly always tender. The long cooking time was believed to be necessary to allow the conversion of collagen to gelatin.

Increasing the rate of heat penetration was noted to toughen meat. Cover (1941) compared paired round, arm bone and standing rib roasts cooked at an oven temperature of 125°C to an end point of 80°C, with and without skewers. Skewers decreased the cooking time, but increased the toughness of the roasts.

In three experiments by Visser et al. (1960), semitendinosus muscle pieces from paired U. S. Good hind quarters were cooked to rare, medium and well-done by oven roasting at 300°F and cooking in fat at 100 and 110°C. Cooking in fat resulted in higher rates of heat penetration and higher shear values than oven roasting. As the internal temperatures increased, average cooking losses of the semitendinosus muscle pieces cooked in fat at both temperatures increased significantly, but tenderness scores were similar, regardless of end point temperatures. However, tenderness and juiciness of the oven-roasted semitendinosus muscle pieces decreased significantly as the end point temperature increased.

II. PRINCIPLES OF MICROWAVE HEATING OF FOOD

Microwaves are high frequency oscillations of small wave length generated by radio frequency power tubes from high voltage direct current (Copson, 1960). In the microwave region of the spectrum, the frequencies of 915 and 2450 megahertz are utilized most often for heating of foodstuffs. Microwave heating differs from conventional methods

of cooking which involve heat transfer by conduction, radiation and convection or a combination of these methods. Microwave heating is primarily a radiant process in which microwaves are dissipated from a power or magnetron tube (Goldblith, 1966). In the magnetron tube, a number of resonant cavities are arranged around a common cathode. Electrons spiral across the cavity by transfer of magnetic field, thus setting up the required oscillations. The electron beams transfer power from the microwave generator into the food (Copson, 1960).

Microwaves impinging upon a food may be reflected, transmitted or absorbed, depending upon the molecular structure of the food. Food is composed of both negatively and positively charged particles. When a food is placed in an electromagnetic field, its charged asymmetric particles attempt to align themselves with the rapidly changing alternating current. As the molecules oscillate in an attempt to go toward their proper poles, intermolecular friction is created producing heat (Goldblith, 1966). Once heat is produced, it behaves as heat from conventional cooking (Fenton, 1957).

III. MICROWAVE HEATING OF MEAT

Microwave heating of meat has had limited investigation. Most studies reported have compared the effects of microwave heating with conventional methods of cooking. Headley et al. (1960) reported greater cooking loss and shrinkage with microwave heating of lamb roasts than with conventional cooking. Shrinkage of the roasts was greatest in the dimension of length. Total cooking losses of beef roasts were higher

and tenderness and juiciness scores lower with microwave than with conventional cooking (Marshall, 1960 and Carpenter et al., 1968).

Pork roasts and chops cooked in an institutional microwave oven, a home microwave oven and a conventional oven were not different in shear values, but the microwave-cooked meat tended to have slightly lower tenderness scores (Apgar et al., 1959).

The effects of conventional broiling and roasting, microwave heating and deep fat cooking of beef steaks from the semitendinosus and longissimus dorsi muscles were investigated by Fielder et al. (1962). Deep fat and microwave heating decreased the collagen content more than oven roasting or broiling. Moisture loss was great and juice content low in the microwave-cooked steaks. No apparent effect on elastin or fat was observed that was related to the method of cooking.

CHAPTER III

PROCEDURE

I. PLAN OF STUDY

The effect of microwave heating upon the histological characteristics and tenderness of beef semitendinosus was investigated. Three-inch pieces of beef semitendinosus muscle were cooked by microwaves in an institutional Raytheon Radarange, Mark IV, operating at 2450 megahertz, for zero-, three-, four-, five-, six- and seven-minute intervals to internal temperatures of 1, 16-25, 26-35, 36-45, 46-55 and 56-65°C, respectively. Voltage into the range was regulated at 220 volts by Variac.

II. SOURCE OF MEAT

Six paired bottom rounds from U. S. Choice steer carcasses (600 to 650 lb) were procured from the Missouri Beef Packers in Rockford, Missouri. Since only the semitendinosus muscles were to be used in this study, these were dissected from the bottom rounds and trimmed of most external fat and connective tissue. From each muscle (1272 to 1740 g), three three-inch pieces were cut across the grain, coded, wrapped in heavy duty aluminum foil and stored at -18°C in an institutional freezer.

III. STATISTICAL DESIGN AND ANALYSES OF DATA

Each of the six cooking treatments was represented once within a pair of semitendinosus muscles from a carcass, occurred twice in each location of the muscle and was replicated six times (Table I). The six treatments were assigned according to a table of random numbers (Cochran et al., 1957).

Average end point temperatures, shear values and scores for muscle fiber disintegration and diameter, collagen degradation and changes in fat cells for each muscle piece were subjected to an analysis of variance. Means of each of the factors were analyzed by Duncan's multiple range test and correlation coefficients were obtained between each of the six factors studied. Standard deviations from the means were calculated for the moisture and fat determinations for the six muscle pairs.

IV. CHEMICAL ANALYSES

Moisture and fat determinations were made in duplicate on the ground raw semitendinosus pieces, representing each of the six muscle pairs. The samples were dried overnight (15.5 hr), in a vacuum oven at 65°C, weighed, redried in a vacuum oven for two hours and reweighed. The crude fat was determined by extracting the dried samples with petroleum ether on a Goldfisch apparatus for eight hours, drying in a vacuum oven for one hour and reextracting with petroleum ether for two hours (AOAC, 1955).

TABLE I

STATISTICAL DESIGN FOR TREATMENT OF SEMITENDINOSUS MUSCLE PIECES

Replication	Muscle	Treatments		
		Anterior (min)	Middle (min)	Posterior (min)
1	I Left	6	0	7
	I Right	5	4	3
2	II Left	3	6	0
	II Right	7	5	4
3	III Left	4	3	6
	III Right	0	7	5
4	IV Left	5	4	3
	IV Right	6	0	7
5	V Left	7	5	4
	V Right	3	6	0
6	VI Left	0	7	5
	VI Right	4	3	6

V. RATE OF HEAT PENETRATION

On the day prior to cooking, the frozen muscle pieces were defrosted to an internal temperature of 1 ± 0.5°C in an institutional refrigerator (5°C) and maintained at this temperature until cooking. In preparation for cooking, samples were weighed and three spirit-filled thermometers were positioned into each piece equidistant from each other and to a depth of one and one-half inches (Figure 1). Five pieces from each muscle pair then were cooked for three-, four-, five-, six- and seven-minute intervals, respectively. The internal temperatures of each sample were recorded immediately after removal from the range. In a preliminary study, it was determined that internal temperatures of the muscle pieces did not increase following removal from the microwave range.

VI. SHEAR VALUES

The Warner-Bratzler shear was used to obtain an index to tenderness. Upon removal from the microwave range, a one-inch core was taken immediately along the grain of the meat and three shears made as near the position of each of the thermometers as possible. An average shear for each muscle piece was determined.

VII. HISTOLOGICAL STUDY

Small sections from each semitendinosus muscle sample, approximately three-fourths inch square, were cut near each thermometer position and preserved in a physiological salt and formalin solution (Appendix A).

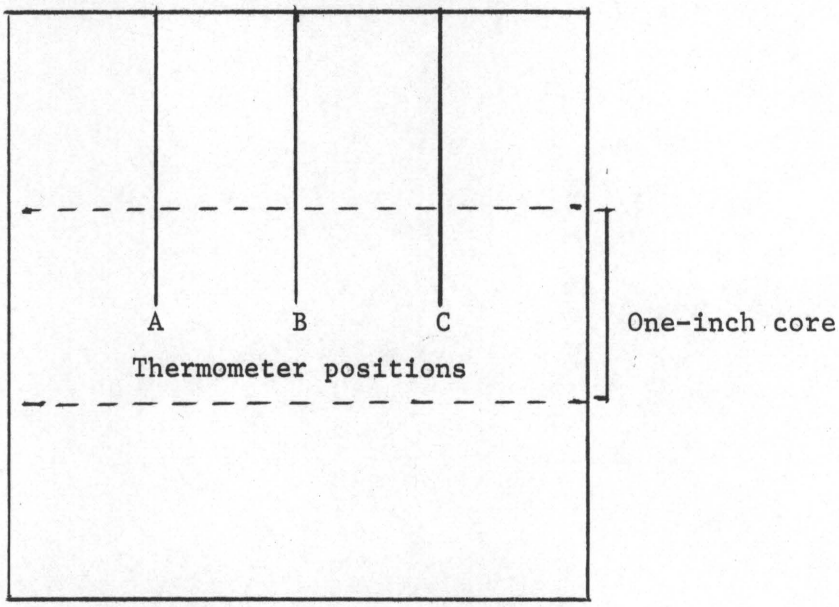


Figure 1. Position of thermometers in each three-inch slice of semitendinosus muscle.

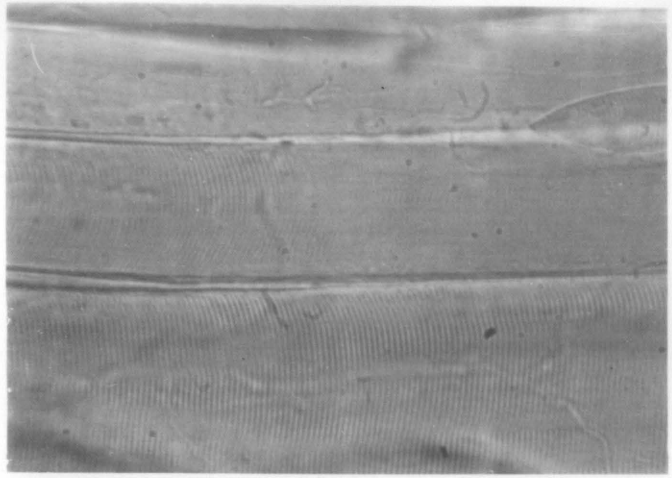
In preparation for sectioning, small blocks of preserved tissue were placed on the platform of a Freezing Clinical Microtome. Longitudinal sections then were cut 25 microns thick. The cut sections were placed in a copper wire mesh basket and into a 70 percent ethyl alcohol for 30 seconds, followed by Sudan III fat stain (Appendix A) for three to five minutes. The tissues were next passed through 70, 50 and 30 percent ethyl alcohol for 30 seconds each and distilled water for 60 seconds. After staining with Harris' hematoxylin muscle stain (Appendix A) for one to three minutes followed by two one-minute rinses in tap water, the stained sections were teased onto slides and mounted in glycerine jelly. Sudan III stain imparted a bright red to fat cells and Harris' hematoxylin, a bluish purple to muscle fibers. Fibrous collagenous connective tissue stained purple, whereas granular collagenous connective tissue appeared gray.

In a preliminary study, the two side temperatures were determined by a Student's t test to be similar, thus only slides prepared from sections near one of the side thermometers from each pieces were observed. The side positions of the samples of each treatment to be studied were selected at random (Cochran et al., 1957). Although a difference ($P < .05$) between the side and center thermometer temperatures was found by a Student's t test, this difference was so consistent that slides from near the side and center thermometers of each muscle piece were combined during observation and analysis of scores.

Five slides prepared from the center and five slides prepared from one of the side positions were studied by two observers using a Dynazoom

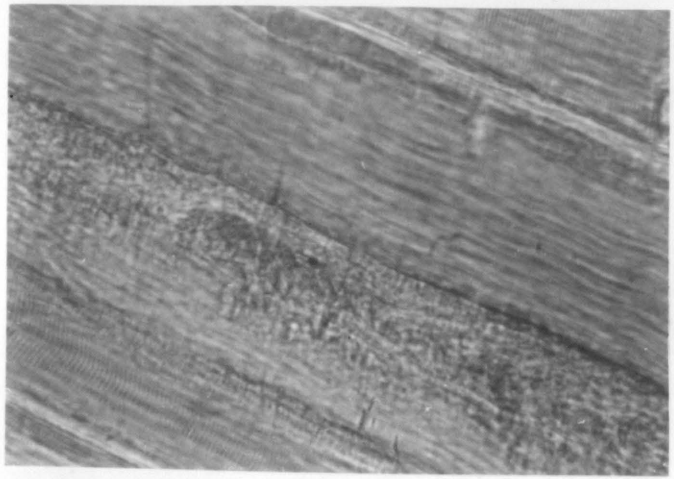
microscope equipped with phase contrast objectives. Each observer independently studied the slides at a magnification of x430, noting the degree of muscle fiber disintegration and fragmentation, occurrence of empty and full fat cells and the breakdown of fibrous collagenous connective tissue to the granular state. To facilitate studying and increase accuracy between observers, photomicrographs corresponding to weighted adjectives and representative of the various degrees of fiber disintegration (1, none to 7, excessive, Figure 2), changes in fat cells (1, full to 5, empty, Figure 3) and degradation of collagenous connective tissue (1, fibrous to 5, granular, Figure 4) were developed. Observations were recorded on a checksheet (Appendix A).

The diameter of the muscle fibers was estimated by counting the number of fibers per field at a magnification of x430 in two random areas of a given slide. For each slide, the average number of fibers per field and the average numerical weight for each of the factors studied was determined. Photomicrographs of representative areas were taken with a Graphic Polaroid black and white Polapan Land film 200, speed type 40.

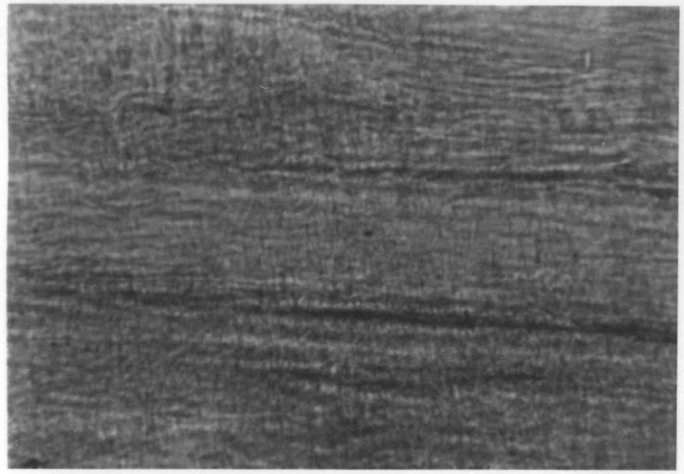


1. No disintegration or fragmentation

2. Slight disintegration, no fragmentation



3. Moderate disintegration, no fragmentation

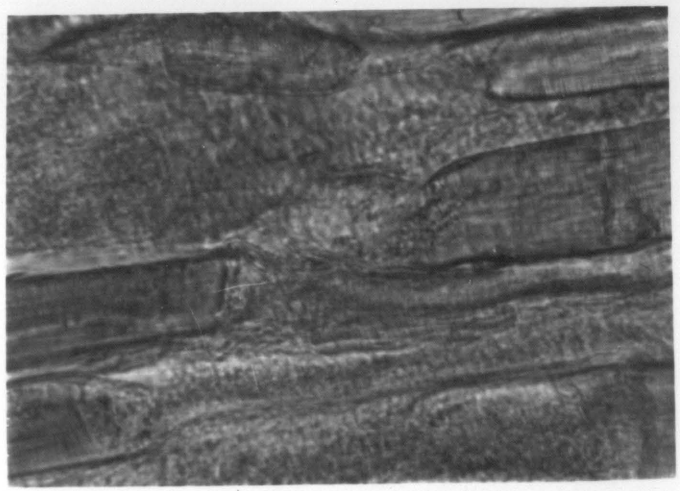


4. Excessive disintegration, no fragmentation

Figure 2. Muscle fiber disintegration x430.

5. Slight disintegration and fragmentation

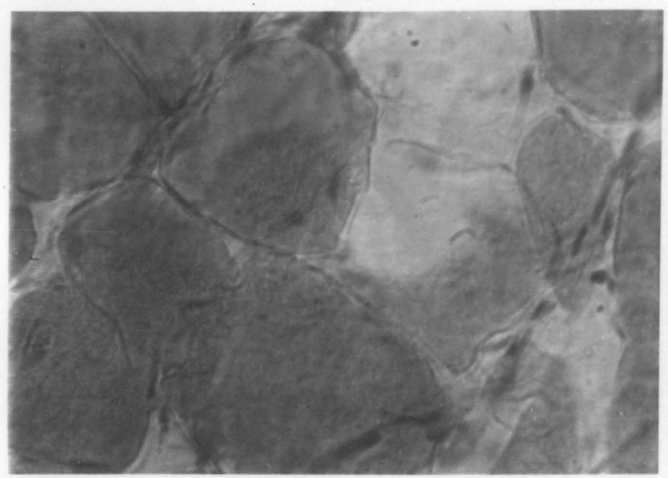
6. Moderate disintegration and fragmentation



7. Excessive disintegration and fragmentation

Figure 2 (continued).

1. Full fat cells

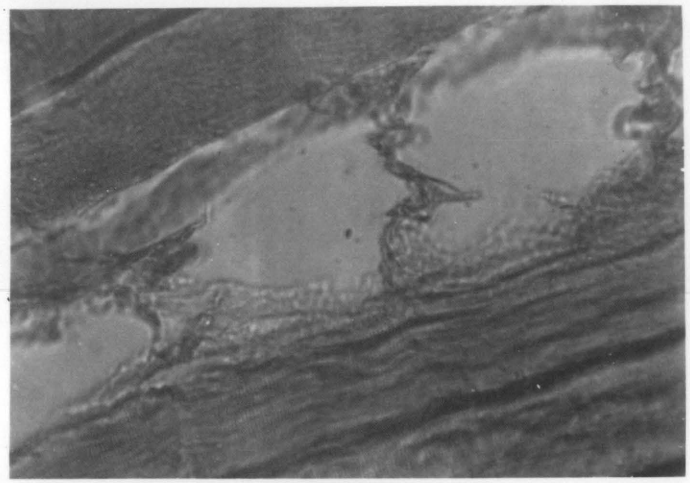


2. Number of full > number of empty fat cells

3. Number of full = number of empty fat cells

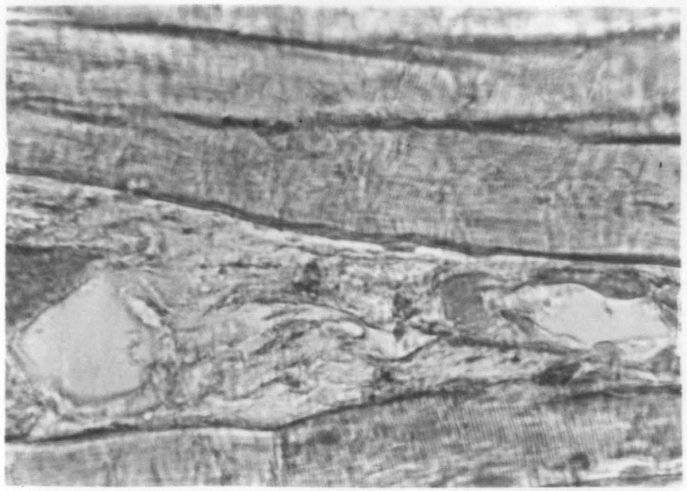
Figure 3. Changes in fat cells x430.

4. Number of full < number of empty fat cells

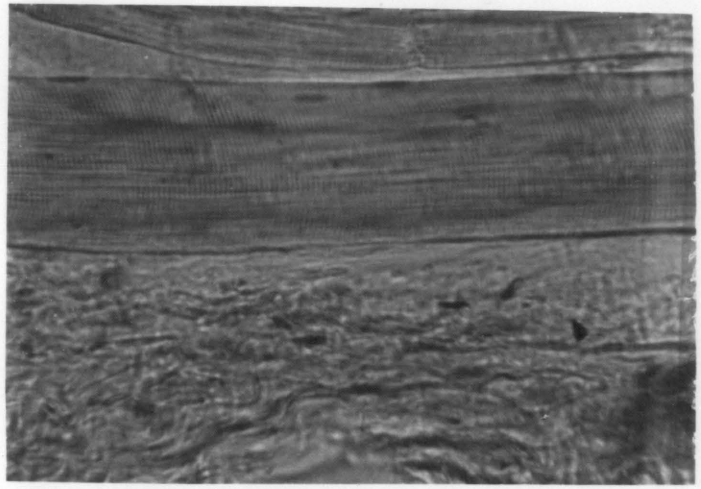


5. Empty fat cells

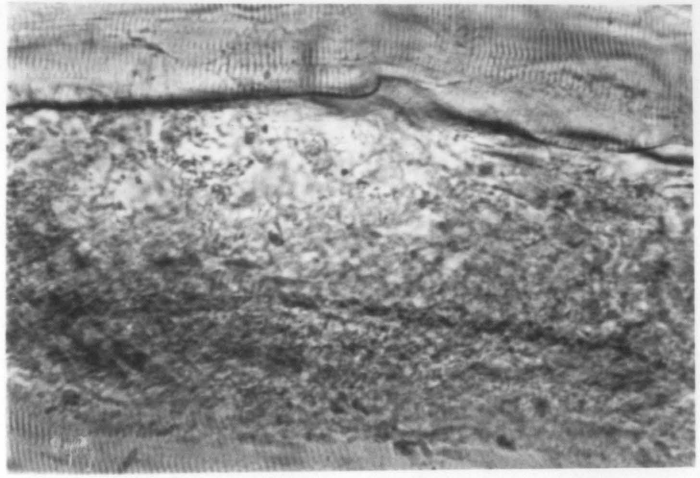
Figure 3 (continued).



1. Fibrous

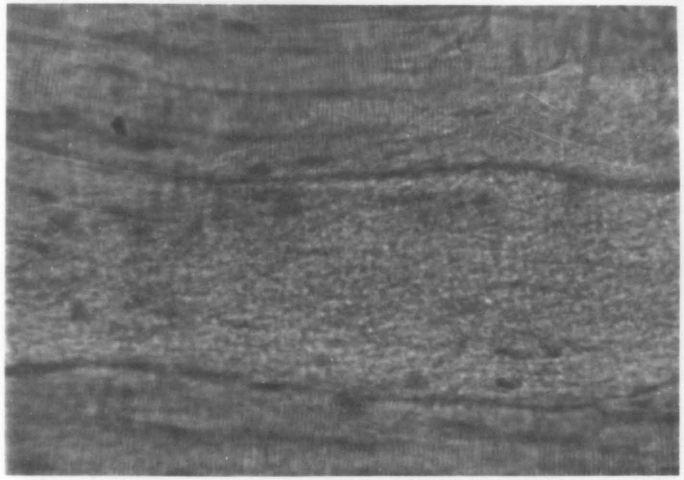


2. Fibrous > granular

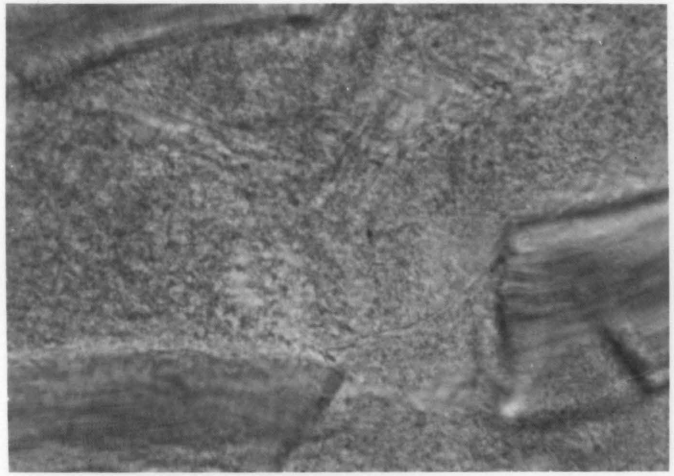


3. Fibrous = granular

Figure 4. Degradation of collagenous connective tissue x430.



4. Fibrous < granular



5. Granular

Figure 4 (continued).

CHAPTER IV

RESULTS AND DISCUSSION

The effects of microwave heating on tenderness as measured by a Warner-Bratzler shear and on histological characteristics of small pieces of beef semitendinosus muscle heated for zero-, three-, four-, five-, six- and seven-minute intervals to internal temperatures of 1, 16-25, 26-35, 26-45, 46-55 and 56-65°C, respectively, were studied.

I. EFFECT OF COOKING TIME ON INTERNAL TEMPERATURE

The internal temperatures of the semitendinosus muscle pieces fell within the predicted ten degree range slightly more than one-half of the time (Table VI, Appendix B). Mean internal temperatures for the three-, four- and five-minute cooking times were slightly higher than the midpoint of their respective temperature ranges, whereas the six-minute temperature was lower and the seven-minute higher (Tables II and III). The difference between the mean internal temperatures of the five- and six-minute intervals was therefore only 4.7°C, whereas that between the six- and seven-minute intervals was 17.3°C. These variations in the range between the mean internal temperatures may help to explain histological changes of the muscle pieces discussed later. The variation in the mean internal temperature may have been attributable to inherent sample differences. Although the muscle pieces used were as uniform as possible, even slight

TABLE II

MEAN INTERNAL TEMPERATURES, SHEAR VALUES, MUSCLE FIBERS PER FIELD, AND SCORES FOR MUSCLE FIBER DISINTEGRATION, CHANGES IN FAT CELLS AND DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

Factors	Cooking Time (min) 0	3	4	5	6	7	Sign. of F
Internal temperature (°C)	1.0	22.2	31.7	43.8	48.5	65.8	**
Shear value (lbs)	26.6	29.3	25.3	24.6	25.6	15.9	*
Muscle fibers/field (x430)	6.3	6.5	6.6	6.6	6.9	7.2	ns
Muscle fiber disintegration[a]	2.5	3.7	3.7	2.8	3.4	4.2	*
Changes in fat cells[b]	1.9	2.2	2.2	2.1	2.3	2.6	ns
Degradation of collagenous connective tissue[c]	1.1	1.4	1.6	1.8	1.9	3.2	**

[a]Scoring range 1, none to 7, excessive.

[b]Scoring range 1, full to 5, empty.

[c]Scoring range 1, fibrous to 5, granular.

*Significant $P < .05$. **Significant $P < .01$. ns, nonsignificant.

TABLE III

DUNCAN'S MULTIPLE RANGE TEST FOR MEAN INTERNAL TEMPERATURES, SHEAR VALUES, MUSCLE FIBERS PER FIELD, AND SCORES FOR MUSCLE FIBER DISINTEGRATION, CHANGES IN FAT CELLS AND DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

Factors	Cooking Time[a] (min)					
Internal temperature	0	3	4	5	6	7
Shear value	3	0	6	4	5	7
Muscle fibers/field	0	3	4	5	6	7
Muscle fiber disintegration	0	5	6	3	4	7
Changes in fat cells	0	5	3	4	6	7
Degradation of collagenous connective tissue	0	3	4	5	6	7

[a]Data for cooking times ranked from smallest to largest mean, except shear values, largest to smallest. Cooking times not underscored by the same line are different $P < .05$.

variations in size, shape and weight may have affected the cooking time and internal temperature. According to Van Zante et al. (1967) cooking time and internal temperature in the microwave range are related to the weight, shape and composition of the food. The semitendinosus muscle was selected for this study, since it was reported as one of the muscles most uniform in composition and consistent in tenderness (Taylor et al., 1961). Fat and moisture analysis of raw muscle samples from each of the six animals, presented in Table IV, showed little difference in the percent of these constituents present.

TABLE IV

FAT AND MOISTURE IN RAW SEMITENDINOSUS MUSCLES

	Animal						Mean	Std. Dev.
	I	II	III	IV	V	VI		
Moisture (%)	73.5	74.2	73.6	74.4	74.0	72.2	73.6	± 0.71
Fat (%)	3.4	2.5	3.2	2.1	2.7	3.7	2.9	± 0.60

II. EFFECT OF COOKING TIME AND INTERNAL TEMPERATURE ON SHEAR VALUES

The mean shear values obtained on a Warner-Bratzler shear and mean internal temperatures for each cooking time are shown in Table II. Shear values of the muscle pieces were similar for the first five cooking time intervals, but as the cooking time increased from six to seven minutes, the mean shear values decreased ($P < .05$, Table III). Between the six-

and seven-minute cooking time intervals, the greatest increase in mean internal temperature also occurred. Shear values were related to internal temperatures ($r = -0.370$, $P < .05$, Table V) in that shear values tended to decrease as temperature increased. Internal temperatures recorded for

TABLE V

WITHIN TREATMENTS CORRELATION COEFFICIENTS FOR INTERNAL TEMPERATURES, SHEAR VALUES, MUSCLE FIBERS PER FIELD, MUSCLE FIBER DISINTEGRATION, CHANGES IN FAT CELLS AND DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

	Correlation Coefficients				
	Shear Values	Muscle f./ Field	Muscle f. Disintegr.	Changes in Fat Cells	Degradation of CCT
Internal temperature	-0.370*	0.381*	0.388*	0.408*	0.447*
Shear values		-0.657**	-0.214	-0.563**	-0.342
Muscle fibers/ field			0.326	0.492**	0.450*
Muscle fiber disintegration				0.572**	0.320
Changes in fat cells					0.669**

*Significant $P < .05$.

**Significant $P < .01$.

muscle pieces cooked seven minutes were near the temperatures reported by Machlik et al. (1963) that resulted in a decrease in the shear of semitendinosus muscle. Muscle pieces heated in a steam bath at 58°C underwent

a marked decrease in shear after 11 minutes of heating. Minimum shear values occurred during the 60 to 64°C range after heating 30 to 60 minutes.

III. EFFECT OF COOKING TIME AND INTERNAL TEMPERATURE ON MUSCLE FIBERS

Mean muscle fiber diameter, as estimated by the number of fibers per field at a magnification of x430, tended to decrease slightly as cooking time of the muscle pieces increased (Table II, p. 25). However, cooking time affected only the muscle fiber diameter of pieces cooked seven minutes as compared with that of raw tissue (Table III, p. 25), even though a low, but significant r value (0.381, $P < .05$) was noted between internal temperature and muscle fibers per field (Table V). Sartorius et al. (1938) also observed a decrease in the muscle fiber diameter of longissimus dorsi from raw to the cooked state. Muscle fiber diameter of longissimus dorsi was noted by Bley (1968) to decrease as end point temperature increased from 140 to 158°F at oven temperatures of 225 and 325°F. Hostetler et al. (1968), however, heated muscle fibers on the stage of a microscope and observed a decrease in the diameter as soon as heating was initiated which was completed by 62°C. Probably no significant difference in muscle fiber diameter was noted in this study until seven minutes of cooking because of the 64.8°C temperature range from the raw to the seven-minute cooked as compared to 47.3°C or less from the raw to the other time intervals.

Mean muscle fiber disintegration scores showed no consistent trend from three to six minutes of cooking time (Table II), but the mean

scores for the raw and five-minute cooked muscle pieces were different ($P < .05$) from the mean scores for the seven-minute interval. The difference between the raw and seven-minute mean muscle fiber disintegration scores is probably attributable to the wide temperature range between these two time intervals. Muscle fiber disintegration scores and internal temperature were related ($r = 0.388$, $P < .05$, Table V). No explanation can be given, however, for the low mean muscle fiber disintegration score for the five-minute time interval. It should be pointed out that histological study is subjective and observations were made on a relatively minute portion of the meat which may not be entirely representative of the sample.

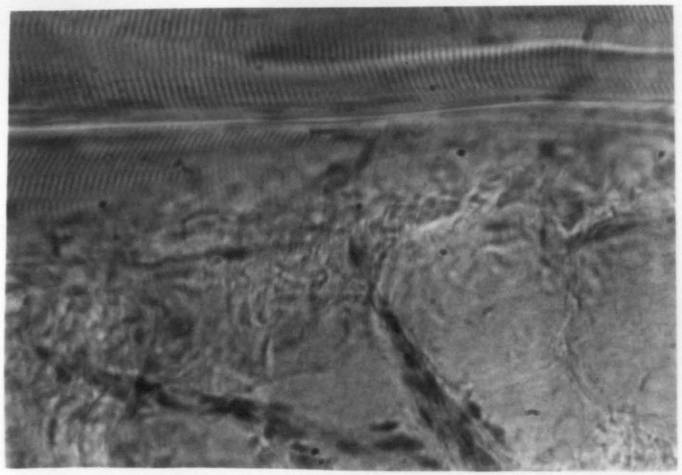
Muscle fibers underwent disintegration in the range of slight disintegration with no fragmentation to slight disintegration and fragmentation (Figure 2, p. 18). Excessive disintegration and fragmentation of the muscle fiber rarely was noted even in those pieces cooked for seven minutes. Upon heating, Paul et al. (1945) noted an increase in the breakage and granulation of muscle fibers.

IV. EFFECT OF COOKING TIME AND INTERNAL TEMPERATURE ON FAT CELLS AND COLLAGENOUS CONNECTIVE TISSUE

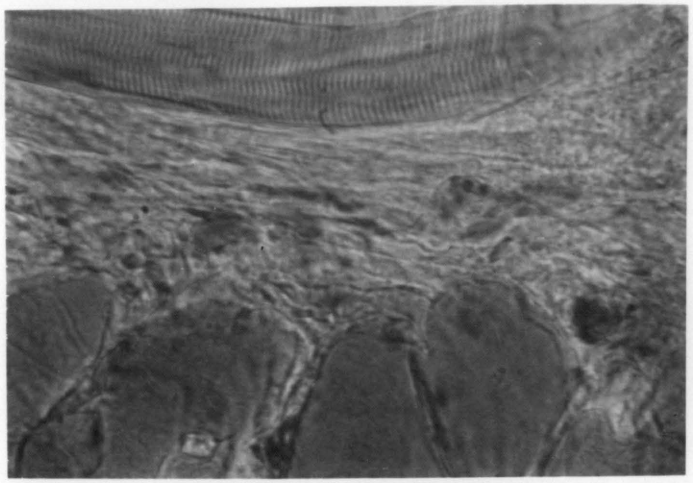
As cooking time of the muscle pieces increased, there was a slight, but nonsignificant, trend for fat cells to change from being full to appearing empty (Table II, p. 25). Fat cells of tissues cooked at all time intervals were similar and only those of raw were different from ($P < .05$) those cooked seven minutes (Table III, p. 25). Changes in the

fat cells during cooking appeared to be so gradual that the difference was not significant until a wide range in cooking time and temperature occurred. Scores for degradation of collagenous connective tissue increased as the cooking time increased ($P < .01$, Table II, p. 25). The greatest increase occurred between the six- and seven-minute intervals, when the internal temperatures of the muscles reached 60°C and above. Collagen which undergoes swelling, shrinkage and disintegration during heating is reported to be converted to gelatin at these temperatures (Hamm, 1966). In this study, collagen degradation increased ($r = 0.447$, $P < .05$, Table V, p. 27) as internal temperature increased. The change from fibrous collagenous connective tissue in the raw tissue, to fibrous and granular in the five-minute cooked and all granular in the seven-minute cooked may be seen in Figure 5.

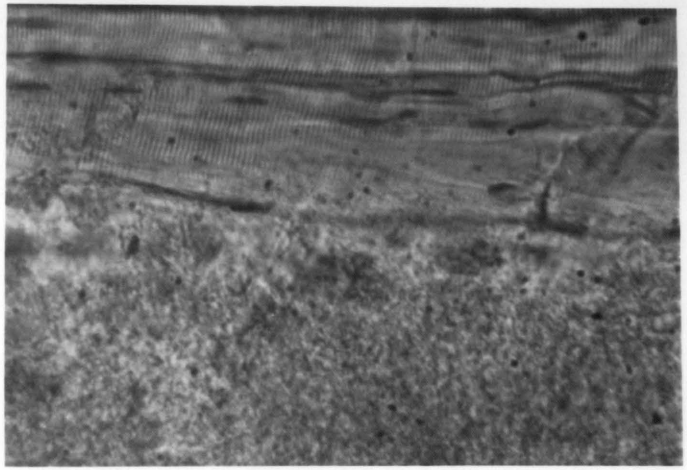
Scores for changes in fat cells were related ($r = 0.408$, $P < .05$, Table V) to internal temperature in that increased internal temperatures were accompanied by an increase in the amount of empty fat cells. Fat droplets as shown by the darker spots in the photomicrograph of the seven-minute cooked muscle tissue (Figure 5), were dispersed along areas of granular collagenous connective tissue. Wang *et al.* (1954) reported that longissimus dorsi muscle steaks cooked to 150°F had a translocation and dispersion of fat cells along the path of collagen degradation. The degradated collagen was suggested to function as a dispersing agent and the heat provided the necessary physical agitation. Bley (1968) observed fibrous collagenous connective tissue present in all raw longissimus dorsi muscles studied, but as the end point and oven temperature increased,



1. Raw



2. Five-minute cooked



3. Seven-minute cooked

Figure 5. Representative areas of microwave-cooked beef semitendinosus x430.

there was a slow change from banded, which occurred with intermediate heating, to the granulated form. Melted fat was found dispersed from the fat cells into the granulated collagenous connective tissue.

V. RELATIONSHIP OF SHEAR VALUES TO CHANGES IN MUSCLE FIBERS, FAT CELLS AND COLLAGENOUS CONNECTIVE TISSUE

Shear values were related to muscle fibers per field ($r = -0.657$, $P < .01$) and to changes in fat cells ($r = -0.563$, $P < .01$), but were unrelated to degradation of collagenous connective tissue (CCT) and muscle fiber disintegration (Table V). A decrease in shear values was accompanied by an increase in muscle fibers per field or a decrease in muscle fiber diameter. Raw muscle fiber diameter has been related to tenderness in that tenderness increased as diameter decreased (Hiner et al., 1953). Smith (1957), however, reported an increase in the tensile strength, thus indicating a decrease in the tenderness of individual muscle fibers upon heating. Possibly the decrease in tenderness usually associated with the hardening and shrinkage of the muscle fibers was masked by changes in fat during heating which increased the tenderness. Fat cells progressed from full to empty as shear values decreased.

Degradation of collagenous connective tissue and shear values were unrelated, yet tenderness of meat is thought to increase with hydrolysis of the connective tissue. The correlation coefficient for shear values and collagen degradation between treatments ($r = -0.936$, $P < .01$) shows an almost linear relationship (Table XVIII, Appendix B) possibly attributable to less variation in scoring between than within

treatments. This variation may be accounted for partially by the difficulty encountered by the observers in discerning granular connective tissue. Mean shear values decreased and scores for collagen degradation increased at the seven-minute interval, when the greatest increase in mean internal temperature occurred (Table II, p. 25). As stated previously, this mean internal temperature was 65.8°C and collagen is converted to gelatin near this temperature (Hamm, 1966).

Muscle fiber disintegration scores and shear values were unrelated ($r = -0.214$) for within treatments, yet were highly significantly related ($r = -0.466$, $P < .01$) between treatments (Table XVIII, Appendix B).

VI. RECOMMENDATIONS

The variation in temperature of the semitendinosus muscle pieces heated for the same time interval, indicates a need for more work regarding the relationship of time and temperature in the microwave range. Investigation of the relationship of meat composition to cooking time also is needed as well as an accurate means of measuring temperature during microwave heating. Since the temperatures of the side thermometers were similar and the difference between the side and center thermometers was consistent, the use of one thermometer is suggested. This would permit small muscle pieces, uniform in size, to be used and thus allow more replications.

The photomicrographs and checksheets used in this study proved to be a valuable aid in studying the slides; however, greater standardization, especially of the collagenous connective tissue photomicrographs is needed.

CHAPTER V

SUMMARY

The internal temperatures of three-inch pieces of beef semitendinosus muscle heated by microwaves for zero-, three-, four-, five-, six- and seven-minute intervals tended to increase as cooking time increased. Tenderness, as measured by a Warner-Bratzler shear, was related to internal temperature ($r = -0.370$, $P < .05$) in that shear values decreased as temperatures increased, the most significant decrease ($P < .05$) occurring after seven minutes of cooking when most temperatures reached 60°C and above. Scores for muscle fiber disintegration, changes in fat cells and degradation of collagenous connective tissue increased as internal temperatures of the muscle pieces increased. Muscle fiber diameter, as estimated from the number of fibers per field, tended to decrease as cooking time and internal temperature of the muscle pieces increased.

Shear values were related to muscle fibers per field ($r = -0.657$), $P < .01$), but not to muscle fiber disintegration. Changes in fat cells from full to empty were noted as shear value decreased. Shear values and degradation of collagenous connective tissue were unrelated, but the greatest decrease in shear value and the largest areas of granular collagen both were noted at the seven-minute cooking time interval.

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APPENDIXES

APPENDIX A

METHODS FOR PREPARATION OF STAINS AND SOLUTIONS

Physiological Salt and Formalin Solution

Seventeen g sodium chloride were dissolved in 200 ml formalin and made up to the volume of 2000 ml with distilled water.

Sudan III Red Fat Stain

One g Sudan III was mixed in 50 ml acetone, C.P., then added to 50 ml of 70 percent ethyl alcohol. The mixture was kept in a tightly stoppered bottle.

Harris' Hematoxylin Muscle Tissue Stain

One g of hematoxylin was dissolved in 10 ml 95 percent alcohol and added to a warm solution of 20.0 g alum and 200 ml distilled water. The mixture was brought to a boil and 0.5 g mercuric oxide added.

After an additional two minutes of boiling, the flask was plunged into cold water and cooling was continued by placing the flask under running water. A few drops of glacial acetic acid were added before use. The mercuric oxide ripened the stain ready for immediate use.

Date_____

Rep_____

Observer_____

Sample_____

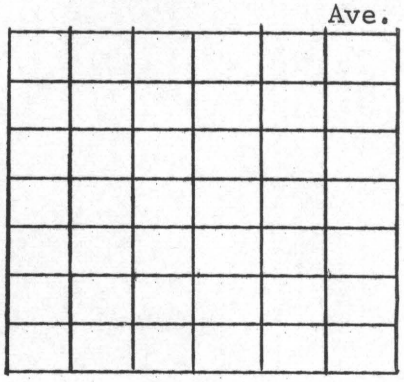
Check Sheet for Slides
Microscope--Bausch & Lomb Dynazoom Phase Contrast 430X

Slide Numbers

Ave.

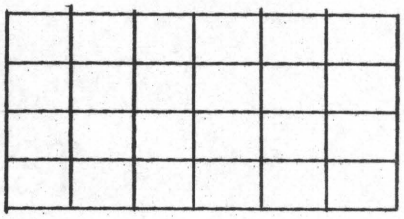
Muscle Fibers

1. No disintegration, no fragmentation
2. Slight disintegration, no fragmentation
3. Moderate disintegration, no fragmentation
4. Excessive disintegration, no fragmentation
5. Slight disintegration and fragmentation
6. Moderate disintegration and fragmentation
7. Excessive disintegration and fragmentation



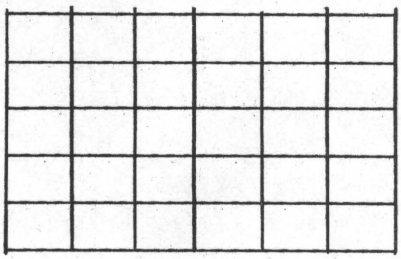
Fat Cells

1. Full fat cells only
2. Number of full > number of empty fat cells
3. Number of full = number of empty fat cells
4. Number of full < number of empty fat cells
5. Empty fat cells only



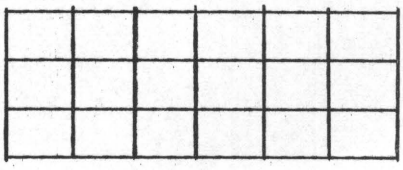
Connective Tissue

1. Fibrous collagenous connective tissue
2. Fibrous > granular connective tissue
3. Fibrous = granular connective tissue
4. Fibrous < granular connective tissue
5. Granular collagenous connective tissue



Fibers Per Field

1. Area 1
2. Area 2
3. Ave.



Comments

APPENDIX B

TABLE VI

INTERNAL TEMPERATURES[a] OF MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	1.0	20.3	27.6	49.3	38.3	46.0
II	1.0	19.3	29.0	43.6	53.6	77.3
III	1.0	19.0	30.0	39.0	45.3	73.0
IV	1.0	21.0	32.0	47.3	58.6	57.6
V	1.0	26.0	31.6	36.6	52.6	62.0
VI	1.0	27.3	40.0	47.3	45.6	78.6
Mean	1.0	22.2	31.7	43.8	48.5	65.8

[a]Temperatures in °C.

TABLE VII

ANALYSIS OF VARIANCE FOR INTERNAL TEMPERATURES

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	15291.80	3058.3	66.82**
Error	30	1373.11	45.77	
Total	35	16664.91		

**Significant $P < .01$.

TABLE VIII

SHEAR VALUES[a] OF MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	29.0	33.6	28.3	38.3	40.6	27.0
II	22.8	26.7	24.7	22.0	21.0	12.4
III	33.0	33.6	37.7	22.0	18.4	16.1
IV	21.8	32.6	29.8	24.9	30.2	19.3
V	24.2	28.2	13.9	20.3	24.2	11.1
VI	28.6	20.9	17.6	20.3	19.1	9.3
Mean	26.6	29.3	25.3	24.6	25.6	15.9

[a]Shear (lbs).

TABLE IX

ANALYSIS OF VARIANCE FOR SHEAR VALUES

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	620.41	124.08	2.64*
Error	30	1408.26	46.94	
Total	35	2028.67		

*Significant $P < .05$.

TABLE X

MUSCLE FIBERS PER FIELD[a] OF MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	5.6	6.1	5.7	6.0	6.0	5.9
II	7.0	6.5	7.1	6.7	6.7	7.2
III	5.8	6.0	6.5	6.6	7.6	7.2
IV	6.5	7.1	6.8	6.8	6.4	7.4
V	6.8	6.2	6.6	6.4	7.5	7.3
VI	6.2	7.0	7.2	7.4	7.3	8.0
Mean	6.3	6.5	6.6	6.6	6.9	7.2

[a]Fibers per field x430.

TABLE XI

ANALYSIS OF VARIANCE FOR SEMITENDINOSUS MUSCLE FIBERS PER FIELD

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	2.78	0.56	1.70 ns
Error	30	9.88	0.33	
Total	35	12.66		

ns, nonsignificant $P < .05$.

TABLE XII

SCORES[a] FOR MUSCLE FIBER DISINTEGRATION OF MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	1.3	3.8	2.9	2.0	1.8	3.0
II	3.2	4.8	4.6	2.3	3.4	5.1
III	1.4	3.0	5.0	3.2	3.2	4.9
IV	3.6	2.6	3.0	3.8	5.2	4.7
V	2.2	4.5	2.4	2.4	3.5	3.8
VI	3.1	3.7	4.3	2.9	3.5	3.8
Mean	2.5	3.7	3.7	2.8	3.4	4.2

[a]Scoring range 1, none to 7, excessive.

TABLE XIII

ANALYSIS OF VARIANCE FOR SCORES FOR MUSCLE FIBER DISINTEGRATION

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	12.84	2.57	3.02*
Error	30	25.41	0.85	
Total	35			

*Significant $P < .05$.

TABLE XIV

SCORES[a] FOR CHANGES IN FAT CELLS OF MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	1.5	2.0	2.0	1.4	2.0	1.2
II	1.6	2.8	2.2	2.0	2.0	3.0
III	2.2	1.9	2.8	2.1	2.3	2.8
IV	1.8	1.8	1.6	2.4	3.0	2.9
V	1.8	2.3	2.4	2.6	1.9	2.7
VI	2.3	2.4	2.2	2.3	2.4	3.0
Mean	1.9	2.2	2.2	2.1	2.3	2.6

[a]Scoring range 1, full to 5, empty.

TABLE XV

ANALYSIS OF VARIANCE FOR SCORES FOR CHANGES IN FAT CELLS

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	1.68	0.34	1.70 ns
Error	30	6.14	0.20	
Total	35	7.82		

ns, nonsignificant $P < .05$.

TABLE XVI

SCORES[a] FOR DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE IN MICROWAVE-COOKED SEMITENDINOSUS MUSCLE PIECES

Replication	Cooking Time (min)					
	0	3	4	5	6	7
I	1.0	1.8	1.7	1.6	1.7	1.4
II	1.0	1.6	1.4	1.4	1.8	4.3
III	1.2	1.1	1.7	1.4	1.9	2.9
IV	1.2	1.4	1.2	2.2	2.0	3.3
V	1.2	1.4	1.4	2.0	2.0	2.8
VI	1.0	1.2	1.8	2.4	2.0	4.6
Mean	1.1	1.4	1.6	1.8	1.9	3.2

[a]Scoring range 1, fibrous to 5, granular.

TABLE XVII

ANALYSIS OF VARIANCE FOR SCORES FOR DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

Source of Variation	D.F.	S.S.	M.S.	F
Cooking time	5	16.32	3.26	11.64**
Error	30	8.32	0.28	
Total	35	24.64		

**Significant $P < .01$.

TABLE XVIII

CORRELATION COEFFICIENTS FOR INTERNAL TEMPERATURES, SHEAR VALUES, MUSCLE FIBERS PER FIELD, MUSCLE FIBER DISINTEGRATION, CHANGES IN FAT CELLS AND DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

	Correlation Coefficients		
	Between	Within	Total
Internal temperature x shear value	-0.745	-0.370	-0.483
Internal temperature x muscle fibers per field	0.958	0.381	0.527
Internal temperature x muscle fiber disintegration	0.629	0.388	0.440
Internal temperature x changes in fat cells	0.896	0.408	0.501
Internal temperature x degradation of CCT	0.099	0.447	0.774
Shear value x muscle fibers per field	-0.819	-0.657	-0.696
Shear value x muscle fiber disintegration	-0.466	-0.214	-0.295
Shear value x changes in fat cells	0.148	-0.563	-0.453
Shear value x collagen degradation	-0.936	-0.342	-0.587
Muscle fibers per field x muscle fiber disintegration	0.692	0.326	0.423
Muscle fibers per field x changes in fat cells	0.925	0.492	0.586
Muscle fibers per field x degradation of CCT	0.934	0.450	0.587
Muscle fiber disintegration x changes in fat cells	0.882	0.572	0.650
Muscle fiber disintegration x degradation of CCT	0.658	0.320	0.462
Changes in fat cells x degradation of CCT	0.921	0.669	0.692

Between: 4 degrees of freedom $r = 0.811$, $P < .05$; $r = 0.917$, $P < .01$.

Within: 29 degrees of freedom $r = 0.355$, $P < .05$; $r = 0.456$, $P < .01$.

Total: 34 degrees of freedom $r = 0.325$, $P < .05$; $r = 0.418$, $P < .01$.

TABLE XIX

DUNCAN'S MULTIPLE RANGES[a] FOR DIFFERENCE BETWEEN MEANS OF INTERNAL TEMPERATURE, SHEAR VALUES, MUSCLE FIBERS PER FIELD, AND SCORES FOR MUSCLE FIBER DISINTEGRATION, CHANGES IN FAT CELLS AND DEGRADATION OF COLLAGENOUS CONNECTIVE TISSUE

Factors	Range Between Means				
	2	3	4	5	6
Internal temperature	8.7	9.2	9.2	9.7	9.8
Shear value	8.8	9.3	9.6	9.8	10.0
Muscle fibers/field	0.7	0.8	0.8	0.8	0.8
Muscle fiber disintegration	1.2	1.2	1.3	1.3	1.3
Changes in fat cells	0.6	0.6	0.6	0.6	0.7
Degradation of collagenous connective tissue	0.7	0.7	0.7	0.7	0.8

[a]30 degrees of freedom, $P < .05$.

VITA

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