HISTOLOGICAL CHARACTERISTICS OF BEEF TOP ROUND STEAKS COOKED BY DRY OR MOIST HEAT IN A CONVENTIONAL OR MICROWAVE OVEN

by

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INTRODUCTION

Since the introduction of microwave ovens for consumer use there has been a dramatic rise in their popularity. Sales of portable microwave ovens increased 60% from 1975 to 1976 (Thomas, 1977). Because cooking time for meat in the microwave oven is four to five times faster than cooking by conventional methods (Apgar et al., 1958; Headley and Jacobson, 1960; Marshall, 1960; Wooldridge, 1974) consumers are using this appliance increasingly. Armbruster and Haefele (1975) reported that greater time saving in achieved when foods cooked by microwaves are covered with plastic film that when they are left uncovered. Murray (1977) reported that 26% of microwave oven owners use the appliance for cooking intact cuts of red meat.

Histological characteristics of skeletal muscle explain, in part, some sensory characteristics of meat (Carpenter et al., 1963). Comparison of microwave and conventional heating of meat has shown differences in sensory, physical or chemical characteristics of the muscle depending on the cooking atmosphere (Marshall, 1960; Pollak and Foin, 1960; Kylen et al., 1964). Little research was found where histological characteristics of red meat cooked in the microwave oven were studied, but Bowers and Heier (1970) reported no differences in the fragmentation of turkey muscle cooked conventionally or by microwaves.

Histological characteristics of meat cooked by dry heat conventional ovens have been studied (beef - Wang et al., 1954; Skelton et al., 1963; Buck and Black, 1968; Paul et al., 1970; lamb - Cross et al., 1972). Some researchers (Ramsbottom et al., 1945; Cover et al., 1962; Ritchey et al., 1963; Ritchey and Hostetler, 1964) found that collagenous connective tissue was the most important structural component affecting tenderness

in "less tender" muscles such as the semimembranosus or the adductor. Reid and Harrison (1971) found no significant differences in histological characteristics of collagenous tissue in semimembranosus muscle cooked by four conventional methods (two moist and two dry heat treatments). McCrae and Paul (1974) reported that greater histological changes occured in collagenous tissue when beef was cooked by microwaves than when it was cooked in a conventional oven by either moist or dry heat. Although it is known that elastic tissue is affected little by conventional heating in either a moist or a dry environment, information on the effects of microwaves on elastic tissue is not available.

Several researchers have reported that muscle fiber width (or diameter) was related to tenderness (Satorius and Child, 1938; Hiner et al., 1953; Herring et al., 1965). Reid and Harrison (1971) and Reid (1971) reported no significant differences in muscle fiber width of beef cooked in moist or dry heat to 70°C.

Marbling (visible flecks of fat) may play only a relatively small part in the tenderness of meat (Ramsbottom et al., 1945; Paul, 1962; Norris et al., 1971). However, fat may influence juiciness of meat (Cover et al., 1956; McBee and Wiles, 1967; Campion and Crouse, 1975). Because microwave heating is faster than conventional cooking, differences may be noted in the mobility of the fat in meat, which could be observed in a histological study.

The objectives of this study were: (1) to compare histological changes in muscle fiber width, connective tissue and quantity and distribution of fat in top round steaks cooked by four oven-heat treatment combinations, and (2) to study relationships of selected histological characteristics of bovine muscle to sensory, chemical and physical properties of the muscle.

REVIEW OF LITERATURE

Gross structure and compostition of muscle

The striated, voluntary skeletal muscles generally called meat are composed of muscle fibers, adipose tissue and connective tissue. The muscle fibers are long, cylindrical in shape, multinucleated and are the basic contractile unit (Venable, 1963). Muscle fibers are composed mainly of myofibrils and nuclei surrounded by a network of connective tissue called endomysium. In a microscopic examination of muscle fibers cross-striations are observed. The cross-striations are categorized as A bands, I bands and Z bands (Birkner and Auerback, 1960). The space between two Z bands is called a sarcomere and is the basic structural unit (Fukuzawa and Briskey, 1970). The diameter of muscle fibers and the length of the sarcomere varies among animals, among muscles within the same animal, and within the same muscle depending on many pre- and post-mortem variables (Locker, 1960; Herring et al., 1967; Paul, 1972). Muscle fibers are gathered into bundles surrounded by perimysium. These bundles are further clustered into muscles surrounded by epimysium (Meyer, 1968; Cassens, 1971).

Collagenous and elastic fibers, along with reticular fibers and the ground substance, compose connective tissue. The collagenous tissue is a fibrous substance relatively high (5 to 13%) in hydroxyproline (Meyer, 1968). Size of the fibers and their crosslinking increase with animal age (Herring et al., 1967). The change in spatial arrangement caused by crosslinking of the collagen fibers can affect the tenderness of muscle (Cover et al., 1962; Kauffman et al., 1964; O'Shea et al., 1974). Post-mortem treatment such as stretching the muscle can cause thinning of the collagenous tissue, resulting in granulation of the tissue with heat (Buck and Black, 1968).

Elastin fibers are highly branched and elastic. They are less abundant than collagen fibers and are found largely in the tendons and ligaments (Meyer, 1968), but they occur to a lesser extent in some muscles, and are considered nonexistent in other muscles (Cassens, 1971). Intramuscular elastic tissue is observed near blood vessels (Forrest et al., 1975); it is stable and provides strength to the blood vessels (Meyer, 1968).

Adipose tissue (fat) is considered by some researchers (Forrest et al., 1975) to be a specialized form of connective tissue. It is found as an insulating material around animal organs and body tissue. Intramuscular fat generally is found in close association with other forms of connective tissue. Copenhauer (1964) stated that each fat cell is surrounded by connective tissue. Birkner and Auerback (1960) wrote that groups of fat cells are surrounded by connective tissue producing an organization similar to muscle fibers in meat.

Effects of structural components of muscle on sensory characteristics

Sensory characteristics of meat depend on many factors including degree of doneness of the cut; size, shape and serving temperature of the sensory sample and structural components. Muscle fiber width (or diameter), quantity and qualtity of connective tissue, and quantity and distribution of fat have been associated with sensory properties.

Muscle fibers play a primary role in the determination of tenderness. Researchers consider two components of the muscle fiber important, the width (or diameter) and the sarcomere length. In general, the narrower the muscle fibers, the less force is required to chew the meat and the more tender the meat seems (Satorius and Child, 1938; Hiner et al., 1953). The sarcomere length gives an indication of fiber density. The shorter the sarcomere the more dense the fiber and, therefore, the tougher the

meat, even though the fiber width (or diameter) may be small. Herring et al. (1965) found that as sarcomere length decreased tenderness also decreased. Satorius and Child (1938), Ramsbottom et al. (1945) and Hiner et al. (1953) found that bundles of thin fibers produced a smooth, fine texture.

Ramsbottom et al. (1945) reported that tenderness of muscles, such as semimembranosus and adductor, is dependent primarily on connective tissue. The collagenous connective tissue is of great importance because it composes the majority of the connective tissue (Forrest et al., 1975). Wilson et al. (1954) stated that the collagen to elastin ratio is 3:1. Cover et al. (1962) found that tenderness in biceps femoris muscle tended to increase as collagen was solubilized. Working with 50 beef muscles Strandine et al. (1949) reported a 0.70 correlation coefficient for sensory panel tenderness and histological connective tissue scores. Significant correlations between tenderness and collagenous tissue were found by Loyd and Hiner (1959, $r = -0.90^{*}$); Adams et al. (1960, $r = -0.51^{*}$); and Cross et al. (1973, $r = 0.63^{**}$). All of those researchers (including Cross et al. (1973), who used and inverted scale of 1 - abundant connective tissue to 8 - no connective tissue) found that as collagenous tissue increased, tenderness decreased. However, although all of those correlations were statistically significant only Loyd and Hiner (1959) found a "high" corrleation between tenderness and quantity of collagenous tissue. Only "moderate" correlations were found by Adams et al. (1960) and Cross et al. (1973).

Although elastic tissue is not as abundant in beef muscle as collagenous tissue is, it is an important consideration because it changes little, if any, with cooking (Winegarden et al., 1952). Hiner et al. (1955) reported that shear values increased as elastic fibers, observed

histologically, increased. Those researchers also observed that the more scattered the distribution of elastic tissue, the more tender was the beef. Cross et al. (1973) showed that quantity of elastic tissue may be related in some instances to tenderness, but that trend was not consistent enough to use elastic tissue as an indication of tenderness.

Fat cells may have a tenderizing effect on meat because they provide a barrier against excessive development of collagenous tissue webs (Hiner et al., 1955). Both the quantity and the distribution of fat may play a part in tenderness. A large quantity of intramuscular fat may suggest a tender sample (Harrison et al., 1953). Research by Wang et al. (1954) showed that distribution of fat may be more important than fat quantity. Research comparing quantity of visible fat (marbling) and tenderness has shown poor correlations for those two variates (Cover et al., 1956; Parrish, 1974). Wu (1977) observed no differences in histological estimates of fat or in tenderness of beef rib steaks graded U.S. Choice and U.S. Good.

Carpenter et al. (1963) found a significant correlation ($r = 0.48^{**}$) between high quantities of fat (observed histologically) and juiciness. Campion and Crouse (1975) reported a correlation ($r = 0.32^{**}$) between juiciness and the amount of chemically determined fat. Factors other than quantity of fat contribute to the toal sensory property of juiciness.

Limited research has been reported concerning the contribution of fat to flavor. Carpenter (1963) found a low correlation ($r = 0.38^{**}$) between histologically observed quantity of fat and flavor intensity. An increase in flavor intensity as marbling increased was observed by McBee and Wiles (1967). Their study was conducted using different grades of beef; therefore, flavor intensity may be a result of more than just increasing fat found in higher grades. Brennand and Lindsay (1978) found that lamb flavor is derived primarily from fat related components, but

beef and pork flavor come from other components. This suggests that quantity of fat in beef is not a good indicator of flavor intensity.

Effects of moist or dry heat conventional cooking on structural components

Decreases in muscle fiber width (or diameter) during cooking have been reproted for many studies using conventional cooking methods. In early studies Brady (1937) and Satorius and Child (1938) found decreases of 13% and 14%, respectively, in muscle fiber width. Hostetler and Landmann (1968) heated beef muscle fibers on the stage of a microscope and noted a rapid decrease in muscle fiber width to 58°C, then from 58°C to 67°C a more gradual decrease occured. No further decrease was observed between 67°C and 75°C. The authors noted that extreme caution must be used in extrapolating that information to intact meat cooked conventionally. Paul (1965) demonstrated that muscle fiber width and sarcomere length decreased with conventional roasting of the psoas major muscle of rabbit. Reid (1971) cooked semimembranosus and longissimus dorsi muscles to 75°C and reported fiber diameters of 37.69μ (LD muscle, dry heat), 38.57μ (LD muscle, moist heat), 39.43μ (SM muscle, dry heat) and 37.67μ (SM muscle, moist heat). Semimembranosus muscles cooked by moist heat had smaller ($P < 0.01$) fiber diameters than those cooked by dry heat, whereas, longissimus dorsi muscles cooked by dry heat had lower ($P < 0.01$) fiber diameters than did LD muscles cooked by moist heat.

Generally, it is accepted that fat begins a process of migration on heating of meat (Paul, 1972). Wang et al. (1954) stated that the distribution of fat might be dependent on the time and the temperature of cooking. They suggested that the longer the cooking time and the higher the temperature, the greater the dispersion of fat. No research was found that studied the effects of moist heat on histological

observations of fat in skeletal muscle tissue.

Numerous researchers (Winegarden et al., 1952; Griswold, 1955; Irwin and Cover, 1959; Yang and Couvillia, 1964; Bayne et al., 1971; Paul et al., 1973; Penfield and Meyer, 1975; Williams and Harrison, 1978) demonstrated that collagen is degraded by either moist or dry conventional heating. Heating in a moist atmosphere has been the traditional cooking method for "less tender" cuts, because it was believed that moisture is needed to help solubilize the collagen (Paul, 1972). Cover (1941) questioned that theory, stating that meat is approximately 70% water and probably needs no more moisture to soften the collagenous tissue. Reid (1971) demonstrated that histological characteristics of connective tissue did not differ significantly for beef LD or SM muscles cooked by modified roasting or oven braising.

Effects of moist or dry heat microwave cooking on structural components

Little research using histological techniques has been conducted to study effects of microwave cooking on the structural components of meat. McCrae and Paul (1974) found that the greatest change in the histological appearance of connective tissue occured with dry heat microwave cooking. They compared conventional roasting, braising, broiling and dry heat microwave cooking. No histological observations were found for any of the structural components of meat cooked by microwave in a moist environment.

MATERIALS AND METHODS

Samples for histological study were available from four U.S. Choice beef top rounds, approximately 9 kg., used to study the effects of

conventional and microwave cooking in a dry or moist atmosphere on selected sensory, physical and chemical measurements (Moore, 1978). There were eight replications (two replications per round) of each of four treatment combinations: (1) conventional oven, dry heat (CD); (2) conventional oven, moist heat (CM); (3) microwave oven, dry heat (MD); (4) microwave oven, moist heat (MM). Details of handling and sampling the rounds and the experimental design for cooking steaks cut from the rounds are in the Appendix, p.40.

Histological measurements

Two samples for histological study were removed from each cooked steak according to the sampling plan (Figure 1). Raw tissue samples were taken from the center of the strip of tissue removed for raw sample analysis (J - Figure 2, Appendix, p.42). Samples were fixed in physiological saline and 10% formalin solution, and were held at approximately 25°C until used (from 100 to 120 days). The order for preparing the sections for histological study was the same as that followed for cooking the steaks (Table 6, Appendix, p.43).

A specimen of muscle tissue (approximately 1.0 x 1.0 x 0.5 cm) from each sample was sectioned 10 microns thick on a CTD International Harris cryostat microtome. Twenty-four sections from each raw and each cooked specimen were mounted on microscope slides containing a thin layer of albumin fixative. Sections were placed in a warming oven (37°C) for 10 minutes to allow the albumin to dry slightly. Muscle fibers and fat in 12 sections were stained with Harris hematoxylin and Sudan IV as described by Wu (1977), Appendix, p.48. The other 12 sections were stained with Verhoff's elastica stain (Thompson, 1966) modified by using the collagenous

Figure 1 - Sampling plan for cooked top round steaks (Moore, 1978)

1 Shear cores; water-holding capacity

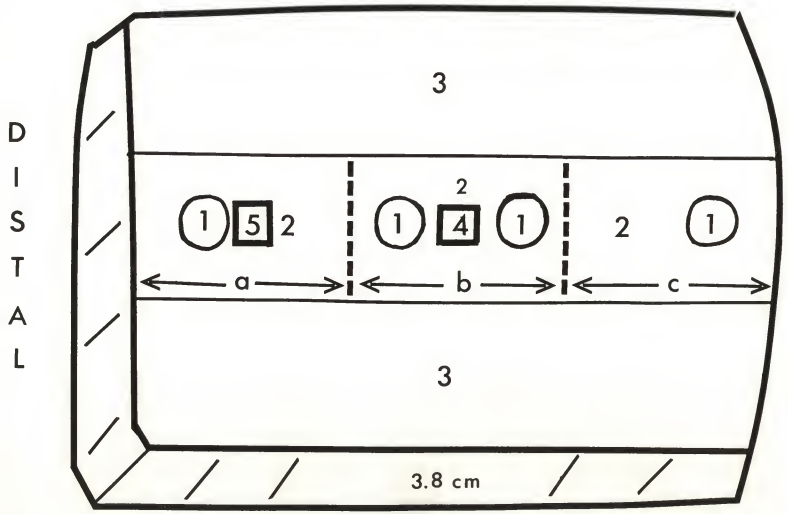
2 Total moisture; ether extract; pH; Gardner color-difference,

c) proximal b) center a) distal positions

3 Sensory evaluation

4 Histological sample - center

5 Histological sample - edge


DISTAL
PROXIMAL
3
1
5
2
2
1
4
1
2
1
a
b
c
3
3.8 cm

connective tissue staining procedure described by Reid (1971) to differentiate collagenous and elastic tissue, Appendix, p. 49. Sections from each of the two staining groups were evaluated by a three-member panel.

Each panel member, working independently, evaluated five randomly selected sections from each group of 12 sections. Thus, each person evaluated 10 sections per specimen: five for muscle fibers and fat, and five for collagenous and elastic connective tissue. Form 1 (Appendix, p. 51) was used to record muscle fiber width and the estimate of fat quantity and distribution. Form II (Appendix, p.53) was used for histological evaluation of collagenous and elastic connective tissue. For section in which mucsle fibers and fat were stained, each person obtained measurements of fiber width using a Bausch and Lomb Dynazoom microscope with a magnification of 430X. Written instructions for measuring fiber width (Form III, Appendix, p.54) and training for estimating fat and connective tissue were provided. Panelists estimated fat quantity and distribution using a Bausch and Lomb Microprojector. Each section was focused on a surface 60 cm from the slide, giving a magnification of 44X. The microprojector with the same slide-to-surface distance also was used to study the collagenous and elastic connective tissue.

Data for each measurement were analyzed by analysis of variance for a split-split plot design.

Source of Variation	D/F
Round (R)	3
Steak Position (S)	1
Error (a)	3

Source of Variation (cont.)	D/F
Type of Oven (O)	1
Type of Heat (H)	1
S X O	1
S X H	1
O X H	1
S X O X H	1
Error (b)	18
Sample Position (L)	1
S X L	1
O X L	1
H X L	1
S X O X L	1
S X H X L	1
O X H X L	1
S X O X H X L	1
Error (c)	24
Total	63

Correlation coefficients were calcualted for selected histological measurements and sensory, physical or chemical data. Data for measurements other than histological estimates were available as part of the overall project (Moore, 1978). Sensory data were obtained from a 7-to-8-member "trained" panel (Forms IV and V, Appendix, p.55).

RESULTS AND DISCUSSION

None of the histological characteristics of muscle tissue from top round steaks cooked in a conventional or a microwave oven by dry or

moist heat were affected significantly by type of oven, type of heat, steak position in the top round or sample position in the cooked steak (Table 1). Significant differences did not exist between means for the two treatment combinations comprising any main effect studied, because large variation occured among data within a given treatment (Table 8, Appendix, p.57).

Muscle fiber width

Mean muscle fiber widths for each oven/heat treatment are given in Table 2. Both dry heat treatment combinations decreased fiber width slightly more than did either of the moist heat treatments. That difference was slightly more for the conventional oven (-2.6%) than it was for the microwave oven (-1.8%). Reid and Harrison (1971), who studied effects of four conventional methods of cooking (two dry heat, two moist heat) on histological characteristics of beef semimembranosus muscle reported that differences between dry and moist heat for decreases in muscle fiber width were not significant. Width for individual muscle fibers measured for this study ranged from 30μ to 72μ for raw tissue and from 24μ to 108μ for cooked tissue. Wide variation in fiber "diameter" (28μ to 73μ) also was found by Joubert (1955) for uncooked beef longissimus dorsi muscle.

Moore (1978) found that mean initial tenderness scores (scale, 5 to 1) for top round steaks cooked in a conventional oven (4.0) or in a microwave oven (3.2) were different ($P<0.001$). Also final tenderness scores for those steaks (4.4, conventional oven; 4.0, microwave oven) were different ($P<0.001$). Data for muscle fiber width (Table 1) do not help explain differences between tenderness scores (Moore, 1978)

Table 1-Means and F-values by main effect for selected histological characteristics

Measurement	Type of oven[a]		Type of heat[b]		Steak position[c]		Sample position[d]	
	Conventional	Microwave	Dry	Moist	Inside	Outside	Center	Edge
Fiber width, μ								
$\bar{x}$, n = 2400	48.1	48.6	47.8	48.9	48.2	48.5	48.2	48.6
F-value	0.51		2.50		0.39		0.56	
Collagenous tissue:								
Total quantity score (7, large - 1, none)								
$\bar{x}$, n = 480	4.35	4.60	4.52	4.43	4.48	4.47	4.48	4.47
F-value	1.03		0.13		0.00		0.00	
Fibrous, % of total								
$\bar{x}$, n = 480	59.6	61.7	60.9	60.4	62.0	59.3	61.5	59.8
F-value	0.25		0.02		0.58		0.22	
Fibrous score (7, large - 1, none)								
$\bar{x}$, n = 480	2.55	2.68	2.66	2.58	2.50	2.73	2.64	2.60
F-value	0.29		0.13		1.81		0.05	
Elastic tissue quantity score (7, large - 1, none)								
$\bar{x}$, n = 480	1.88	1.84	1.75	1.97	1.88	1.83	1.82	1.90
F-value	0.10		2.26		0.09		0.41	
Total collagenous + elastic tissue score (14, large - 2, none)								
$\bar{x}$, n = 480	6.23	6.44	6.28	6.40	6.37	6.30	6.30	6.37
F-value	0.45		0.15		0.05		0.07	

Table 1-concluded

Measurement	Type of oven[a]		Type of heat[b]		Steak position[c]		Sample position[d]	
	Conventional	Microwave	Dry	Moist	Inside	Outside	Center	Edge
Fibrous collagenous + elastic tissue score (14, large - 1, none)								
$\bar{x}$, n = 480	4.47	4.53	4.43	4.57	4.45	4.55	4.47	4.52
F-value	0.04		0.21		0.47		0.05	
Fat quantity score (7, large - 1, none)								
$\bar{x}$, n = 480	4.37	5.09	4.65	4.81	4.84	4.62	4.64	4.82
F-value	2.69		0.13		0.88		0.45	
Fat distribution score (7, present in all areas - 1, few areas)								
$\bar{x}$, n = 480	3.29	4.21	3.75	3.74	3.76	3.74	3.59	3.90
F-value	2.72		0.00		0.01		2.35	

[a]Data combined by type of oven for all other main effects; DF = 1,18

[b]Data combined by type of heat for all other main effects, DF = 1,18

[c]Data combined by steak position for all other main effects, DF = 1,3

[d]Data combined by sample position for all other main effects, DF = 1,24

Table 2-Means for raw and cooked samples for selected histological characteristics[a]

Measurement	Treatment[b]					Change from raw to cooked, %			
	Raw	CD	MD	CM	MM	CD	MD	CM	MM
Muscle fiber width, μ	49.9	47.5	48.2	48.8	49.1	-4.8	-3.4	-2.2	-1.6
Connective tissue:									
Total collagenous tissue quantity[c]	3.5	4.3	4.7	4.4	4.5	+22.7	+34.3	+25.7	+28.5
Fibrous collagenous tissue, %	98.8	61.8	60.1	57.4	63.3	-36.8	-38.5	-41.3	-35.3
Fibrous collagenous tissue score[d]	3.4	2.6	2.7	2.5	2.7	-23.5	-20.6	-26.5	-20.6
Elastic tissue quantity[c]	1.4	1.7	1.8	2.0	1.8	+21.4	+28.6	+42.7	+28.6
Collagenous + elastic tissue score[e]	4.8	6.0	6.6	6.5	6.3	+25.0	+37.5	+35.7	+31.2
Fibrous collagenous + elastic tissue score[f]	4.9	4.3	4.6	4.6	4.5	-12.2	-6.1	-6.1	-8.2
Fat quantity score[c]	3.2	4.2	5.1	4.6	4.5	+31.2	+59.4	+43.8	+59.4
Fat distribution score[g]	2.0	3.3	4.2	3.3	4.2	+65.0	+110.0	+65.3	+110.0

[a] Data combined by oven/heat treatments (Muscle fiber width: Raw, n = 300; CD, MD, CM, MM, n = 1200
other measurements: Raw, n = 60; CD, MD, CM, MM, n = 240)

[b] CD - Conventional, dry; MD - Microwave, dry; CM - Conventional, moist; MM - Microwave, moist

[c] Range 7-1 = large quantity to none

[d] $\frac{\text{(Fibrous collagenous tissue, \%) X (Total collagenous tissue quantity)}}{100}$

[e] Total collagenous tissue quantity + Elastic tissue quantity

[f] Fibrous collagenous tissue score + Elastic tissue score

[g] Range 7-1 = Present in all areas to present in few areas

for top round steaks cooked to 65°C in conventional or microwave ovens.

Connective tissue

For all treatment combinations, mean scores for quantity of total collagenous tissue are close to the score representing a moderate amount of tissue (Table 1). Total collagenous tissue scores do not help explain differences in tenderness found by Moore (1978). Reid (1971) also found no differences among total collagenous tissue scores for longissimus dorsi or semimembranosus muscles cooked by dry or moist heat. Ramsbottom et al. (1945), using the same scoring system as the one used in this study and in the study by Reid (1971), observed that tenderness of muscles such as semimembranosus and adductor was dependent on the quantity of collagenous connective tissue. Their data were for muscles cooked in deep fat to 76.7°C.

Total collagenous connective tissue scores increased after cooking. Increases in mean scores ranged from 22.7% for CD to 34.3% for MD (Table 2). Scores for both microwave oven treatments increased more than those for conventional oven treatemtns, with the mean scores for MD increasing more than the mean score for MM. Skelton et al. (1963) also observed more total collagenous tissue in cooked than in raw muscle. They attributed the apparent increase in collagenous tissue to the swelling and redistiribtuion of the connective tissue during heating. They postulated that as the collagenous tissue swells, it becomes granualr and fills the spaces between the muscle fibers; the swollen granular tissue gives the appearance of more collagenous tissue in cooked than in raw muscle. They also stated that redistribution of the collagenous tissue during cooking may contribute to the apparent increase of that tissue in cooked muscle.

They explained that collagenous tissue appears as long, fibrous strands in sections of raw muscle and is seen as masses of granular tissue dispersed throughout the sections of cooked muscle. If redistribution of collagenous tissue occurs during cooking, it might give the illusion of a greater quantity of collagenous tissue in cooked muscle, because it appears in more parts of the sections studied. The decrease in fiber width may also contribute to the apparent increase in collagenous tissue with heating. As the muscle fibers shrink, the spaces between those fibers increase, and as the swollen collagenous tissue fills those spaces, the collagenous tissue becomes more prominent.

Paul (1972) pointed out that in numerous studies heating caused degradation of the fibrous collagenous tissue. In this study, with an end point of 65°C, degradation (decreases) of the fibrous collagenous tissue (Table 2) did not differ significantly among the four treatment combinations.

Fibrous collagenous tissue scores (which include factors for both the total collagenous tissue and the percentage of the collagenous tissue that is fibrous) are given in Table 2. Although mean fibrous collagenous tissue scores were not significantly different for the treatment combinations studied, decreases in fibrous collagenous tissue were slightly larger for both conventional oven treatments than they were for the microwave oven treatments. The smaller amounts of fibrous collagenous tissue observed in sections from the conventional oven treatments may be partially responsible for the greater tenderness of conventionally cooked samples (Moore, 1973).

Mean scores for quantity of elastic tissue ranged from 1.75 to 1.97 (Table 1), which indicates that none to trace amounts of elastic

tissue were observed in the sections of the muscle tissue studied. Those low scores suggest that elastic tissue has little influence on the tenderness of beef semimembranosus and adductor muscles. An apparent increase in elastic tissue during heating of muscle, similar to that observed for collagenous tissue, was noted (Table 2).

Ramsbottom et al. (1945) scored bovine muscle section for quantity of both collagenous and elastic tissue, then added the scores to obtain a score for "total connective tissue". That score accounted for the connective tissues that have been considered to be related to tenderness, and the authors stated that "variation in collagenous and elastic tissue, no doubt, has an important bearing on the problem of tenderness in beef". However, no correlation coefficients were reported for connective tissue scores and tenderness as measured by either shear values or sensory scores. For this study, two combined scores were obtained: 1) the elastic tissue score plus the total collagenous tissue score and 2) the elastic tissue score plus the fibrous collagenous tissue score. Those two scores did not provide any more insight into the differences in tenderness attributable to type of oven that were observed by Moore (1978) than do scores for individual components of connective tissue.

Fat

Scores for quantity of fat increased with heating; scores for the microwave oven increased 59.4%, whereas those for conventional oven treatments increased 31.2% to 43.8%. Weir et al. (1962) suggested that fat may become more extractable after cooking, because of an alteration in the muscle protein and/or breakdown of lipid-protein complexes. If such changes in the fat do occur during cooking, it is possible that

the apparent increases in fat observed histologically may be the result of the fat staining more readily in the cooked muscle than it does in the raw muscle tissue.

Scores for distribution of the fat increased after cooking 65% for both conventional heating methods and 110% for both microwave methods of cooking (Table 2). The mobility of fat increases with heating, which results in an evener distribution of fat in cooked tissue that in raw tissue.

Significant interactions of main effects

F-values were significant ($P<0.05$) for the interation of type of oven X sample position for the fibrous collagenous tissue score and for the fibrous collagenous tissue score plus the elastic tissue score (Table 3). Also, the F-value for the interaction of type of heat X steak position X sample position for fat quantity was significant ($P<0.05$), Table 4. However, LSDs did not show significant differences between mean scores for any one of those measurements. The significant F-values for the interations alert us that both positive and negative changes occured because of a given main effect interacting with other main effects. However, the LSDs also show that no real differences occured between means for the histological measurements.

The F-value for the interaction of type of oven X steak position X sample position was significant ($P<0.05$) for the fat distribution score. For that measurement one of the LSDs showed a significant ($P<0.05$) difference between means for center and edge samples of inside steaks cooked in a conventional oven. The mean score for center samples was less than the mean score for edge smaples (Table 4). Thus, the edge

Table 3-Means and F-values for significant 2-way interactions

Measurement	Type of oven	Sample position		F-value	LSD
		Center	Edge		
Fibrous collagenous tissue score (7, large - 0, none)	Conv.	2.34	2.77	6.18*	0.79[a]
	Micro.	2.94	2.42		0.91[b]
Fibrous collagenous tissue score + elastic tissue score (14, large - 1, none)	Conv.	4.16	4.77	4.70*	1.06[a]
	Micro.	4.78	4.28		1.15[b]

[a]LSD used to compare center vs edge samples in the same type of oven

[b]LSD used to compare conventional vs microwave ovens in the same sample position

*$P<0.05$

Table 4-Means and F-values for significant 3-way interactions

Measurement	Type of heat or Type of oven	Sample position: Center, Steak position: Inside	Sample position: Center, Steak position: Outside	Sample position: Edge, Steak position: Inside	Sample position: Edge, Steak position: Outside	F-value	LSD
Fat quantity score (7, large - 1, none)	Dry	5.06	4.30	4.49	4.74		2.10[a]
						4.75*	2.14[b]
	Moist	4.36	4.82	5.44	4.60		1.49[c]
Fat distribution score (7, present in all areas - 1, few areas)			*				
	Conv.	2.71	3.20	4.09	3.15		2.49[a]
						4.29*	2.62[b]
	Micro.	4.21	4.22	4.04	4.36		1.23[c]

[a]LSD used to compare inside vs outside steaks in the same type of heat or type of oven and the same sample position

[b]LSD used to compare dry vs moist heat (or conventional vs microwave oven) in the same steak position and the same sample position

[c]LSD used to compare center vs edge samples in the same type of heat or type of oven and the same steak position

*$P<0.05$

samples showed evener distribution of fat than did the center samples. Moore (1978) reported that the edges of top round steaks were more done than the centers. The greater degree of doneness in the edges may increase mobility of the fat. That agrees with the increases in scores for fat distribution with both conventional and microwave cooking (Table 2). No explanation can be given for differences being observed only in inside steaks, but not observed in steaks from the outside of the top round.

Relationships between paired variates

Correlation coefficients were calculated by oven/heat treatment combinations to study relationships between the histological characteristics studied and selected sensory, physical and chemical measurements. Relationships are discussed using the classification of Shindell (1964) who considered correlation coefficients with absolute values from 0.00 to 0.39, low correlations; from 0.40 to 0.79, moderate correlations; and from 0.80 to 1.00, high correlations. Any mention of significance refers to the statistical probability, and not to the importance of the correlation.

For the microwave oven treatments, high or moderate correlations were found between muscle fiber width and initial or final tenderness scores, but for steaks cooked in a conventional oven, low correlations occured between muscle fiber width and tenderness scores (initial and final), Table 5. For steaks cooked in the microwave oven the narrower the fibers, the tenderer the meat (both initial and final tenderness). With MD, the correlation coefficient for muscle fiber width vs initial or final tenderness was -0.80 ($P<0.05$) and -0.66 ($P<0.10$), respectively.

Table 5-Correlation coefficients for selected paired variates on the basis of oven/heat treatment combinations

Paired variates	Heat treatments[a] CD	MD	CM	MM
Muscle fiber width vs				
Initial tenderness score	0.38	-0.80*	0.06	-0.45
Final tenderness score	0.06	-0.66+	-0.14	-0.67+
Total quantity of collagenous tissue score vs				
Initial tenderness score	0.45	0.18	-0.24	-0.46
Warner-Bratzler shear value	0.48	0.46	-0.67+	-0.20
Fibrous collagenous tissue score vs				
Inital tenderness score	0.38	0.53	0.44	-0.67+
Warner-Bratzler shear value	-0.41	-0.44	-0.65+	-0.03
Quantity of elastic tissue score vs				
Initial tenderness score	0.40	0.56	-0.08	-0.23
Final tenderness score	0.13	0.67+	-0.45	0.25
Total collagenous tissue + elastic tissue score vs				
Initial tenderness score	0.64+	0.30	-0.26	-0.43
Final tenderness score	0.41	0.61	-0.41	-0.15
Warner-Bratzler shear value	0.21	0.33	-0.80*	-0.31
Fibrous collagenous tissue + elastic tissue score vs				
Initial tenderness score	0.55	0.66+	0.33	-0.52
Warner-Bratzler shear value	-0.53	-0.50	-0.53	-0.31
Fat quantity score vs				
Juiciness score	0.09	-0.50	-0.55	-0.58
Flavor score	-0.35	0.41	-0.22	-0.73*
Ether extract	-0.06	0.57	-0.09	0.83
Warner-Bratzler shear value	-0.25	-0.61	-0.55	-0.58

Table 5-concluded

Paired variates	Heat treatments[a]			
	CD	MD	CM	MM
Fat distribution score vs				
Juiciness score	0.45	0.18	-0.24	-0.46
Warner-Bratzler shear value	0.48	0.46	-0.67^{+}	-0.20

[a]CD - Conventional, dry; MD - Microwave, dry; CM - Conventional, moist; MM - Microwave, moist

DF = 6

*, $P<0.05$

$^{+}$, $P<0.10$

With MM, r values for muscle fiber width vs initial or final tenderness were -0.45 (ns) and -0.67 ($P<0.10$), respectively. Reid (1971) reported a low correlation ($r = 0.13$) between muscle fiber width and tenderness of two beef muscles (longissimus dorsi and semimembranosus) cooked conventionally by dry or moist heat. The findings of this study suggest that muscle fiber width influences the tenderness of microwave cooked beef top round steaks more than it influences the tenderness of steaks cooked conventionally.

The total quantity of collagenous tissue correlated moderately with the initial tenderness scores for CD and MM steaks and with the Warner-Bratzler (WB) shear values for CD, CM and MD steaks (Table 4). Contrary to what would be expected, as the total collagenous tissue score increased, initial tenderness increased as measured by sensory panel scores, and as would be expected tenderness decreased as measured by WB shear values for CD and MD steaks. For CM and MM steaks, the correlation coefficients suggest that a decrease in total collagenous tissue resulted in greater tenderness as measured by sensory methods and in less tenderness as measured by the WB shear. Ramsbottom et al. (1945) suggested that the greater the collagenous tissue the tougher the meat. The findings in this study do not suggest any logical trend.

The fibrous collagenous tissue scores were correlated moderately with initial tenderness scores and with WB shear values for most of the oven/heat treatment combinations (Table 5). For CD, MD and CM, initial tenderness scores increased as the fibrous collagneous tissue increased. However, for MM, the initial tenderness decreased with an increase in fibrous collagenoud tissue. For steaks cooked by all four oven/heat treatment combinations, WB shear values decreased as the fibrous

collagenous tissue increased. Paul (1972) related that generally, tenderness should increase (thus the WB shear value should decrease) with a decrease in fibrous collagenous tissue.

The quantity of elastic tissue correlated moderately with the initial tenderness score for both dry heat treatments and with the final tenderness score for MD. For CM, the quantity of elastic tissue correlated moderately with final tenderness. Cross et al. (173) reported that elastic tissue is highly variable in its relationship to tenderness and, therefore, is a poor meausrement to use in estimating tenderness.

The score for total collagenous tissue plus elastic tissue correlated positively with the initital tenderness score, the final tenderness score and the WB shear value for both dry heat treatments, and correlated negatively with those variates for both moist heat treatments. The results were unexpected because tenderness usually decreases when the WB shear value increases. For this study, both tenderness scores and the WB shear values increased as total connective tissue increased with moist heat treatments.

The score for fibrous collagenous tissue plus elastic tissue increased as tenderness increased in CD, MD and CM. For MM, initial tenderness decreased as the score for fibrous collagenous tissue plus elastic tissue increased. For all cooking methods, the WB shear value decreased as the fibrous collagenous tissue plus elastic tissue score increased. The relationships of the fibrous collagenous tissue plus elastic tissue score to initial tenderness or the WB shear value were unexpected, because generally tenderness is thought to decrease with an increase in connective tissue. Similar results were noted when the relationship of fibrous collagenous tissue score vs the initial

tenderness or vs the WB shear value without adding the elastic tissue score.

Generally, for MD and MM steaks, correlation coefficients were moderate for fat quantity vs juiciness score, flavor score, ether extract or the WB shear value (Table 5). Also, with CM, the fat quantity score was related moderately to the juiciness score and to the WB shear value. With CD, correlations for the fat quantity score vs juiciness and flavor scores, ether extract or WB shear values were low. The lower the quantity of fat the higher the juiciness score for MD, CM and MM steaks. Paul (1972) reported that data concerning the importance of fat to the sensation of juiciness are variable. It seems probable that the juiciness of the steaks used in this study was dependent on some factor other than fat quantity.

As the fat quantity score increased, ether extract also increased for microwave-cooked beef. For MM, that relationship was high and for MD it was moderate. Because ether extract is a chemical determinant of fat quantity the positive relationship was expected. A significant ($P<0.05$) moderate negative correlation was found for the fat quantity score and the flavor score for MM steaks (Table 5). On the other hand, for MD steaks fat quantity and flavor scores correlated positively. Brennand and Lindsay (1978) reported that fat did not appear to influence the intensity of beef flavor. Carpenter et al. (1963) reported a highly significant, positive, but rather low correlation ($r = 0.38^{**}$) between flavor intensity and histologically observed fat quantity.

For MD, CM and MM, a negative and moderate correlation coefficient occured for the fat distribution score vs. the juiciness score, or vs. the WB shear value (Table 5). Juiciness increased as fat was observed

in fewer areas in the muscle tissue. When fat is present in a few areas it may be more agglomerated than when it is evenly distributed throughout the muscle tissue, which could cause the mouth to detect more fat at one time and give the impression of greater juiciness. Lewis (1955) stated that there was "an inverse relationship between the juiciness scores and distribution of fat" in turkey muscle.

As fat distribution decreased, the WB shear value increased for moist heat cooked beef. Ramsbottom et al. (1945) reported that fat has a low shear value. In this study, it appears that as fat became less evenly distributed in CM and MM it did not provide a tenderizing effect throughout the muscle tissue.

SUMMARY

Selected histological characteristics of thirty-two beef top round steaks cooked in a microwave or in a conventional oven by moist (oven-film bag) or dry (modified roasting) heat were studied. Samples for microscopic examination were taken from center and edge positions of the cooked steaks. Data for sensory, chemical and physical characteristics of the same steaks were available to study relationships between those characteristics and the histological characteristics. Data were analyzed by analysis of variance for a split,split plot design to study influences of type of oven, type of heat, steak position or sample position and interactions of those variables on the histological properties of the muscle. Correlation coefficients were calculated for selected paired variates on the basis of oven/heat treatment.

Differences in histological characteristics attributable to type of oven, type of heat, steak position or sample position were not

significant. Calculation of LSDs for significant ($P<0.05$) interactions indicated that the only significant difference observed for mean scores was between the fat distribution scores for center and edge samples of conventionally-cooked steaks from the inside of the top round. Differences in beef top round steaks cooked in a conventional or microwave oven by dry or moist heat (Moore, 1978) cannot be explained by differences in mean values for selected histological characteristics of those steaks.

Moderate to high negative correlation coefficients were found for muscle fiber width vs initial and final tenderness in microwave-cooked steaks, which indicates that decreased muscle fiber width was an indicator of increased tenderness in microwave-cooked beef top round.

Correlation coefficients for total quantity of collagenous tissue did not indicate a consistent relationship to tenderness. The initial tenderness score was explained, partially, by the fibrous collagenous tissue score only for MM steaks. The correlation coefficient for the initial tenderness score vs the fibrous collagenous tissue score for CD, MD and CM steaks, and the coefficients for the WB shear value vs the fibrous collagenous tissue score for all oven/heat treatments did not indicate the expected relationship.

Tenderness could be explained, partially, by the observed quantity of elastic tissue only for dry heat treatments. The two combined scores for collagenous and elastic tissue did not help to explain differences in tenderness of beef top rounds any better than did scores for the individual connective tissue components.

For microwave cooking, the fat quantity score agreed with the percentage ether extract, and for all cooking methods, the WB shear

value was explained, partially, by the fat quantity score. Fat distribution appeared to account, partially, for the juiciness score given to MD, CM and MM steaks.

Generally, correlation coefficients indicated that the histological study did not measure the same attributes of top round steaks that were measured by a sensory panel or by objective measurements related to sensory attributes.

CONCLUSIONS

1. Top round steaks cooked by dry or moist heat in a conventional or microwave oven to an end point temperature of 65°C, do not exhibit differences in muscle fiber width, total collagenous tissue, fibrous collagenous tissue, total elastic tissue, fat quantity or fat distribution observed histologically.
2. Generally, histological characteristics of microwave-cooked steaks tend to help explain more about the sensory, physical or chemical properties of those steaks than the histological characteristics of conventionally-cooked steaks explain about the properties of steaks cooked conventionally.
3. The variability of correlation coefficients for histological characteristics vs sensory properties mandates that histological characteristics not be used alone to predict the sensory qualities of beef top round steaks.

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APPENDIX

Meat used - handling and sampling

Four U.S. Choice fresh, unfrozen beef top rounds, approximately 9 kg, were obtained from a local wholesale meat company. They were vacuum packed in Cryovac B-620 "Barrier bag" using a Cryovac 8200 vacuum chamber 1 to 4 days after slaughter. The rounds were purchased 16 to 30 days after vacuum packing. The external fat covering was removed, the semimembranosus and adductor muscles were quared off and divided into eight steaks, each 3.8 cm thick. Steaks were assigned to treatments according to the position of the steak within the round (Figure 2). Weights of the four inside steaks (B,C,F,G) ranged from 467 to 752 g; the four outside steaks (A,D,E,H) ranged in weight from 468 to 633 g.

Individual steaks (except steaks for the fist cooking period from each round) were wrapped in aluminum foil (guage 0.0015) and frozen in an upright freezer at an average temperature of $-23.5^{\circ}C \pm 2.5^{\circ}C$ until used (3-10 days).

Experimental design for cooking

Treatment combinations studied were: CD, conventional oven, dry heat; CM, conventional oven, moist heat; MD, microwave oven, dry heat, MM, microwave oven, moist heat. The experimental design for cooking was a split plot with eight replications with the steak positions in the round as the main plots and the treatment combinations as the subplots. There were 16 evaluation periods with two steaks cooked at each period. Each top round provided steaks for two replications of each oven/heat treatment combination (Table 6).

Figure 2 - Sampling plan for beef top round

A-H - Steaks for cooked sample analyses

J - Strip for raw sample analyses

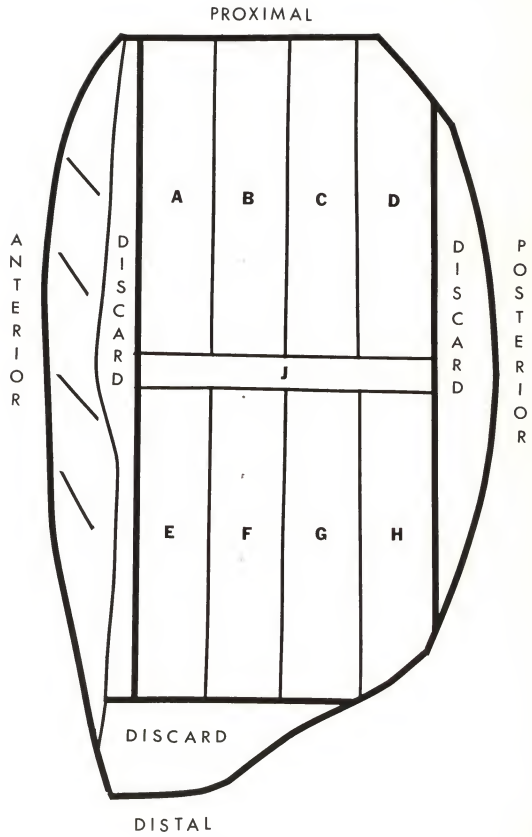
PROXIMAL
ANTERIOR
DISCARD
A
B
C
D
J
E
F
G
H
DISCARD
DISCARD
POSTERIOR
DISTAL

Table 6-Experimental design for cooking

Cooking period	Round	Replication	Steak position[a]	Treatment[b]
1	I	1	C	3
			F	4
2			B	1
			G	2
3		2	A	3
			H	4
4			D	1
			E	2
5	II	3	G	1
			F	4
6			E	2
			C	3
7		4	D	1
			B	2
8			H	3
			A	4
9	III	5	D	3
			A	4
10			F	1
			E	2
11		6	B	2
			G	3
12			H	1
			C	4
13	IV	7	A	2
			C	3
14			H	1
			E	4
15		8	G	1
			F	4
16			B	2
			D	3

Table 6-concluded

[a]Steak positions are illustrated in Figure 2

Steak	Position
A	Proximal-anterior
B	Proximal-center
C	Proximal-center
D	Proximal-posterior
E	Distal-anterior
F	Distal-center
G	Distal-center
H	Distal-posterior

[b]Treatments randomly assigned to the steaks

1	Dry heat, conventional oven, CD
2	Dry heat, microwave oven, MD
3	Moist heat, conventional oven, CM
4	Moist heat, microwave oven, MM

Before each cooking period, except for the first cooking period for each round, steaks designated by the experimental design were defrosted in the foil wrap four hours at approximately 25°C and 20 hours at approximately 4°C, then unwrapped and weighed. Steaks for the first cooking period were stored at 4°C for 12 hours, then unwrapped and weighed.

Thermometers (-20° to 105°C, 15 cm long) were inserted with the bulb (approximately 1.3 cm long) in the geometric center, and at positions 4.0 cm from the proximal and distal edges of each steak. Temperatures at the three positions were recorded initially, upon removal from the oven and after a post-oven temperature rise. Glass thermometers with a nonpolar liquid in the column were used for microwave cooked steaks.

In preliminary work the weight and the cooking time required for steaks were plotted on a graph and a line that best fit the points on the graph was drawn for each oven/heat treatment. From that line, cooking time for steaks assigned to each oven/heat treatment in the main study was estimated based on the weight of the steaks. The CM, MD and MM steaks were removed from the oven at a center temperature of 58°, 59° and 55°C, respectively, to achieve a final temperature of 65°C at the center of the steak. CD steaks were cooked to 65°C; preliminary work showed no post-oven temperature rise for that treatment.

For conventional modified roasting (CD) each steak was placed on a wire rack 12.7 cm high set in a shallow pan. Steaks were cooked in an electric rotary hearth oven at 177°C. For microwave roasting (MD), each steak was placed on a Pyrex casserole lid (diameter, 15.5 cm) in a 22.8 cm Pyrex pie plate, placed in the center of the rotary

hearth in the microwave oven (Sharp R-8200) and cooked at the roast setting (approximately 455 watts).

For cooking in oven film bags, each steak was placed in an oven film bag and closed with a twister tie or masking tape for microwave cooked steaks. Six slits (approximately 1.5 cm long) were made in each bag to allow steam to escape and prevent the bag from breaking. The thermometers were inserted through the oven film bag in the same positions described for dry heat treatments. For CM, the entire system was placed on a low rack in a shallow roasting pan and cooked in a electric rotary hearth oven at 177°C. For MM, the entire system was placed on a Pyrex casserole lid (diameter 15.5 cm) in a 22.8 cm Pyrex pie plate in the center of the rotary hearth in the microwave oven and cooked at the roast setting.

Sensory evaluation

Flavor, juiciness, texture and tenderness of 1.3 x 2 cm cores of cooked meat were evaluated by an 8-member panel using a 5 to 1 intensity scale (Form IV, Appendix, p.55). Instructions for evaluation (Form V, Appendix, p.56) were given to panel members during preliminary work.

Cores were presented to panel members in the top of half-pint double boilers set over warm water (approximately 65°C) and the entire system was placed on an electric hot tray set at low heat (approximately 35°C). All sensory evaluation took place within 15 minutes after preparation of samples.

Shear value

Tenderness was measured on cooked samples cooled to room temperature

by shearing 1.3 cm cores with a Warner-Bratzler shearing apparatus with an 11.25 kg dynamometer. Four cores were taken from the proximal (c), center (b) and distal (a) positions in each steak (Figure 1). Duplicate measurements were made on each core and the over-all shear value was the average for the four shear cores.

Ether extract

Percentage of ether extract in samples of both raw and cooked meat were measured in triplicate by the analytical laboratory of the Department of Animal Sciences and Industry using a modified AOAC method.

MUSCLE FIBERS AND FAT STAINING AND MOUNTING PROCEDURE[a]

1. Dip tissue in tap water
2. Stain in Alum Hematoxylin[b] - 2 minutes
3. Rinse in tap water - 1 minute
4. Rinse in tap water - 1 minute
5. Stain in Sudan IV solution[c] -

   Raw tissue - 2 minutes

   Cooked tissue - 3 minutes
6. Dip in ethyl alcohol, 50%
7. Dip in ethyl alcohol, 70%
8. Dip in ethyl alcohol, 95%
9. Rinse in tap water

Muscle fibers stain blue and fat stains red.

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[a]procedure modified from Wu (1977)

[b]Manufactured by Paragon C. and C. Co., Inc. 190 Willow Avenue, Bronx, N.Y. 10454

[c]Formula for stain is in Appendix, p.50.

Mounting the cover glass

Glycerine jelly was used as the mounting medium. After staining, the slides were dried with disposable paper wipers, care being taken to avoid damage to the muscle tissue section. Two drops of warm glycerine jelly (stored at 37°C in a paraffin warming oven, and heated in a hot-water bath to approximately 80°C during mounting periods) was dropped onto the section. A cover slip was placed on the glycerine jelly covered section.

COLLAGENOUS AND ELASTIC CONNECTIVE TISSUE STAINING PROCEDURE[a]

1. Dip tissue in xylene
2. Dip in ethyl alcohol, absolute
3. Dip in ethyl alcohol, 95%
4. Dip in distilled water
5. Stain in Elastic tissue stain[b] - 15 minutes
6. Differentiate in 2% Ferric Chloride solution - 3-8 minutes
7. Dip in distilled water
8. Dip in ethyl alcohol, 95%
9. Rinse in running tap water - 5 minutes
10. Stain in Picro-ponceau solution[b] - 1 minute
11. Dip in ethyl alcohol, 95%
12. Dip in ethyl alcohol, 95%
13. Dip in ethyl alcohol, absolute
14. Dip in ethyl alcohol, absolute
15. Dip in acidified xylene[b]

Collagenous tissue stains red, degraded collagenous tissue does not stain, elastic tissue stains black, and other components stain yellow.

---

[a]procedure modified from Thompson (1966) and Reid (1971).

[b]Formula is in Appendix, p.50.

Mounting the cover glass

Permount was used as the mounting medium. After staining, the slides were dried with disposable paper wipers, care being taken to avoid damage to the muscle tissue section. Two drops of Permount were dropped onto the section. A cover slip was placed on the Permount covered section.

FORMULAS FOR HISTOLOGICAL STAINS

Sudan IV solution:

1.0 g Sudan IV
50 ml ethyl alcohol, 70%
50 ml acetone

Mix thoroughly. Keep the saturated solution in a tightly stoppered bottle and filter before using. Make fresh after 250-300 sections have been stained.

Elastic tissue stain

20 ml alcoholic hematoxylin, 5%
8 ml aqueous ferric chloride, 10%
8 ml Lugol's iodine

Mix thoroughly. Make fresh daily.

Alcoholic hematoxylin, 5%

5 g hematoxylin
110 ml ethyl alcohol, absolute

Mix thoroughly, then dissolve with the aid of heat.

Lugol's iodine

2 g potassium iodide (KI)
1 g iodine crystals
100 ml distilled water

Dissolve the KI in a few ml of water, then dissolve the iodine crystals in this solution. Add the remainder of the water and mix thoroughly.

Picro-ponceau solution

10 ml aqueous Ponceau S, 1%
86 ml aqueous picric acid, saturated
4 ml acetic acid, 1%

Mix thoroughly. Make fresh after 300 sections have been stained.

Acidified xylene

100 ml xylene
2 drops acetic acid, glacial

Mix thoroughly. Make as needed.

Form I. Score card for histological evaluation of fiber width and fat in beef top round steaks.

Panel Member_____ Code_____ Date_____

Measurement	Section Number 1	2	3	4	5	Average
Muscle fiber width, mm						
Fiber 1						
Fiber 2						
Fiber 3						
Average	____	____	____	____	____	_____
Fat						
Relative quantity[a]						_____
distribution[b]						_____

Quantity[a]	Distribution[b]
7 - large	7 - Present in all areas
5 - medium	5 - Present in many areas
3 - small	3 - Present in moderate number of areas
1 - None*	1 - Present in few areas

*If quantity is none leave distribution blank

Form II. Score card for histological evaluation of connective tissue in beef top round steaks.

Panel Member_____ Code_____ Date_____

Measurement	Section Number 1	2	3	4	5	Average
Collagenous tissue						
Quantity[a]						_____
% Fibrous						_____
% Granular						_____
Elastic tissue						
Quantity[b]						_____

Quantity[a]	Quantity[b]
7 - large	7 - large
5 - medium	5 - medium
3 - small	3 - small
1 - none	2 - trace
	1 - none

Form III. Instructions for microscopic measurement of fiber width

The virtual image of a tiny scale is engraved on a clear glass disc, the ocular micrometer. Insert this disc into the eyepiece by unscrewing the top lens and inserting the disc into the shelf within the eyepiece. This disc is marked in equal units with the center further divided into five smaller units.

To determine the width of each ocular unit, compare the ocular disc to a stage micrometer. The stage micrometer is a slide with a measurement line divided into 0.01 mm units. The slide is inserted onto the stage of the microscope under high power (43X objective and 10X eyepiece). The dynazoom knob should be set on 1 to give a magnification of 430X. Match a line of the scale on the stage micrometer with a line on the squared scale of the ocular (eyepiece) micrometer. Count the number of ocular and stage units until another line on the ocular micrometer matches another line on the stage micrometer. Determine the distance covered by the ocular units. Each unit on the stage micrometer equals 0.01 mm. In this experiment, 1 large ocular unit equals 0.03 mm (30) and one small ocular unit equals 0.006 mm (6).

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 corresponding to the width of a fiber and multiplying that by the size of the unit of measure.

Example: muscle fiber width = 1 large ocular unit and three small ocular units.

(1 X 0.03 mm) + (3 X 0.006mm) = 0.048 mm (48)

Note:

The eyepiece can be turned in the tube, thus turning the ocular

scale. In this way, fibers can be measured even though they do not lie in a perfectly vertical or horizontal direction. For each section, select 3 fibers at random, measure, calculate width in mm and record on score sheet.

Once the ocular micrometer has been set up, it should not be removed from the eyepiece of the microscope. If the disc is removed from the eyepiece, the calibrations for unit determinations need to be repeated for each magnification used, because turning the disc over changes the calibration readings.

Form IV. Score Card for Sensory Evaluation of Beef Top Round Steaks (Semimembranosus and Adductor Muscles)

Panel Member _____ Code_____ Date_____

Sample	Flavor	Juiciness	Texture	Tenderness			Comments
				Initial	# chews	score	
A							
B							

Flavor

5 Intense beef flavor
4 Moderately intense beef flavor
3 Slightly Intense beef flavor
2 Perceptible beef flavor
1 No beef flavor

Juiciness

5 Juicy
4 Moderately juicy
3 Neither juicy nor dry
2 Slightly dry
1 Dry

Tenderness

5 Tender
4 Moderately tender
3 Neiter tender nor tough
2 Slightly tough
1 Tough

Texture

5 Mealy (fine, friable)
4 Moderately mealy
3 Neither mealy nor chewy
2 Slightly chewy
1 Chewy

Form V. Instructions to judges for sensory evaluation of beef top round

For scoring sensory characteristics, each judge is to select two cores of meat at random from each double boiler. Use one core for assessing flavor, juiciness and texture, and one core for counting chews and evaluating tenderness.

Scoring for flavor and juiciness

Record a score for flavor and another for juiciness within a range of 5 to 1 that describes your impression of the sample. Refer to the score card for descriptive terms for specific scores within the range of 5 to 1. Record the score describing your impression of flavor and juiciness at the beginning of the chewing process.

Scoring for texture

Mealiness is fragmentation of the meat resulting in tiny, dry pieces of meat that cling to the cheek, gum and tongue. Record a score for mealiness within the range of 5 to 1 that describes your impression of the sample. Refer to the score card for descriptive terms for specific scores within the range of 5 to 1.

Scoring for tenderness

Record a score describing your initial impression of tenderness at the beginning of the chewing process within a range of 5 to 1. Refer to the score card for descriptive terms for specific scores within the range of 5 to 1.

Count the number of times you chew the core of meat before swallowing. Chew until the core is masticated completely, then swallow. Record the number of chews required to masticate the core. Record a score from 5 to 1 that describes your impression of the tenderness of the core. Refer to the score card for descriptive terms for specific scores within the range of 5 to 1.

Use the number of chews to help you standardize your tenderness scores from day to day. Set up for yourself a range of the number of chews for each score from 5 to 1. For example, if you chew 10-24 times, a score of 5; 25-34 times, a score of 4; 35-44 times, a score of 3; continuing to reduce the score by a given number of increased chews. Each judge sets his own range of chews for a given score.

Comments

Comments about the samples and/or explaining your reason for giving a particular score are helpful.

Take your time to score each sample. Water is provided for rinsing your mouth between samples.

Table 7- Mean squares and F-values for selected histological characteristics

Source of variation	D/F	Muscle fiber width		Fat quantity		Fat distribution	
		Mean square	F-value	Mean square	F-value	Mean square	F-value
Round (R)	3	17.29	3.45	2.851	3.18	5.101	5.67
Steak Position (S)	1	2.031	0.39	0.7876	0.88	0.01265	0.01
Error (a)	3	5.017		0.8956		0.8993	
Type of Oven (O)	1	3.802	0.51	8.338	2.69	13.60	2.72
Type of Heat (H)	1	18.71	2.50	0.4064	0.13	0.001406	0.00
S X O	1	18.06	2.41	0.3752	0.12	0.6215	0.12
S X H	1	0.2756	0.04	0.01890	0.01	0.04515	0.01
O X H	1	0.3025	0.04	0.7877	0.25	0.01891	0.00
S X O X H	1	0.1600	0.02	6.825	2.20	13.78	2.76
Error (b)	18	7.492		3.096		4.991	
Sample Position (L)	1	3.610	0.56	0.5077	0.45	1.658	2.35
S X L	1	6.002	0.94	0.08265	0.07	1.238	1.76
O X L	1	4.101	0.64	3.106	2.76	1.856	2.64
H X L	1	8.122	1.27	0.9752	0.87	0.007656	0.01
S X O X L	1	5.641	0.88	0.2889	0.26	3.019	4.29*
S X H X L	1	0.3025	0.05	5.348	4.75*	0.8789	1.25
O X H X L	1	15.41	2.40	0.1501	0.13	0.9264	1.32
S X O X H X L	1	0.7656	0.12	1.410	1.25	0.1702	0.24
Error (c)	24	6.414		1.127		0.7042	

Table 7- continued

Source of variation	D/F	Quantity of collagenous connective tissue		% Fibrous collagenous connective tissue		% Fibrous collagenous connective tissue score	
		Mean square	F-value	Mean square	F-value	Mean square	F-value
Round (R)	3	1.575	1.70	2714	14.32*	2.565	5.43
Steak Position (S)	1	0.003906	0.00	110.2	0.58	0.8556	1.81
Error (a)	3	0.9256		189.5		0.4716	
Type of Oven (O)	1	1.025	1.14	75.25	0.25	0.2756	0.29
Type of Heat (H)	1	0.1314	0.15	5.062	0.02	0.1225	0.13
S X O	1	1.995	2.21	105.1	0.36	0.1600	0.17
S X H	1	1.410	1.56	132.2	0.46	0.07562	0.08
O X H	1	0.5814	1.56	225.0	0.78	0.05062	0.05
S X O X H	1	2.066	2.29	495.1	1.71	0.09000	0.10
Error (b)	18	0.9024		289.6		0.9443	
Sample Position (L)	1	0.001406	0.00	49.00	0.22	0.03062	0.05
S X L	1	0.4389	0.50	27.56	0.12	0.3600	0.62
O X L	1	1.183	1.34	637.6	2.82	3.610	6.18*
H X L	1	0.1502	0.17	100.0	0.44	0.01562	0.03
S X O X L	1	0.3139	0.24	196.0	0.87	0.5256	0.90
S X H X L	1	0.0001562	0.00	150.1	0.66	0.3600	0.62
O X H X L	1	2.066	2.34	52.56	0.23	0.09000	0.15
S X O X H X L	1	1.925	2.18	20.25	0.09	0.5256	0.90
Error (c)	24	0.8848		225.8		0.5843	

Table 7- concluded

Source of Variation	D/F	Quantity of elastic connective tissue		Collagenous + elastic connective tissue score		Fibrous collagenous + elastic connective tissue score	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Round (R)	3	0.4152	0.94	0.5839	0.46	4.425	13.03*
Steak Position (S)	1	0.04000	0.09	0.06891	0.05	0.1600	0.47
Error (a)	3	0.4396		1.269		0.3395	
Type of Oven (O)	1	0.03062	0.10	0.7014	0.45	0.06250	0.04
Type of Heat (H)	1	0.7225	2.26	0.2377	0.15	0.3025	0.21
S X O	1	0.3600	1.13	4.050	2.58	0.9506	0.66
S X H	1	0.3906	1.22	3.285	2.09	0.8556	0.60
O X H	1	0.5625	1.76	2.288	1.46	0.5256	0.37
S X O X H	1	0.1056	0.33	1.238	0.79	0.06250	0.04
Error (b)	18	0.3197		1.571		1.431	
Sample Position (L)	1	0.1056	0.41	0.08266	0.07	0.05062	0.05
S X L	1	0.01000	0.04	0.3164	0.26	0.3025	0.29
O X L	1	0.005625	0.02	1.351	1.11	4.840	4.70*
H X L	1	0.4225	1.63	1.076	0.88	0.8100	0.79
S X O X L	1	0.1225	0.47	0.6602	0.54	0.8556	0.83
S X H X L	1	0.005625	0.02	0.003906	0.00	0.1056	0.10
O X H X L	1	0.04000	0.15	2.681	2.20	0.2756	0.27
S X O X H X L	1	0.4556	1.76	4.254	3.49+	1.690	1.64
Error (c)	24	0.2589		1.219		1.030	

*, $P < 0.05$

Table 8- Histological evaluation scores

Measurement	Replication	Heat Treatments							
		Conventional				Microwave			
		Dry		Moist		Dry		Moist	
		Center	Edge	Center	Edge	Center	Edge	Center	Edge
Muscle fiber width, μ	1	48.5	45.1	48.3	49.6	46.9	53.2	49.6	49.3
	2	49.1	42.4	54.1	48.1	47.3	48.0	52.1	51.3
	3	47.9	45.9	48.1	51.2	48.0	49.7	51.9	50.3
	4	46.1	50.1	45.9	54.3	47.9	50.8	48.5	51.2
	5	49.5	45.1	43.6	48.4	48.3	47.9	43.7	48.1
	6	49.7	47.7	44.5	47.5	46.7	45.2	49.2	45.3
	7	43.7	44.9	50.5	50.8	47.7	51.1	48.9	49.5
	8	52.7	52.2	48.4	46.8	47.5	44.4	46.1	50.7
Fat quantity score	1	3.9	4.2	5.3	5.1	6.7	5.8	6.6	6.5
	2	4.1	4.7	3.0	5.7	6.3	4.1	3.8	5.5
	3	4.9	6.1	3.0	3.8	7.0	5.0	5.4	6.5
	4	1.9	2.9	3.8	7.0	1.9	4.5	6.9	3.3
	5	3.3	2.5	3.7	4.3	6.6	5.1	2.7	2.9
	6	6.2	3.8	6.1	5.7	3.1	4.2	2.7	3.9
	7	3.0	4.6	3.8	2.6	5.4	5.7	6.3	6.1
	8	5.0	5.7	3.9	6.1	5.6	4.9	6.5	5.3

Table 8- continued

Measurement	Replication	Heat Treatments							
		Conventional				Microwave			
		Dry		Moist		Dry		Moist	
		Center	Edge	Center	Edge	Center	Edge	Center	Edge
Fat distribution score	1	3.5	3.8	2.1	5.0	6.1	5.9	5.8	5.1
	2	1.7	5.1	2.6	2.2	5.4	4.3	3.5	2.7
	3	3.9	5.1	1.8	1.8	6.3	3.9	4.3	5.7
	4	1.0	1.6	3.4	6.1	2.2	2.9	6.7	5.8
	5	1.4	1.7	2.5	2.3	4.2	4.3	1.0	1.0
	6	5.0	4.7	5.0	4.6	1.9	2.5	1.4	3.1
	7	1.5	3.0	2.9	1.1	3.7	5.0	5.3	5.4
	8	4.5	4.9	4.5	4.9	5.0	4.1	4.7	5.5
Total collagenous connective tissue score	1	3.6	4.5	4.1	3.5	4.9	3.4	3.3	4.5
	2	3.7	3.3	4.6	5.3	4.3	3.5	5.1	3.8
	3	3.5	4.9	4.6	3.9	5.5	4.9	5.4	5.8
	4	3.5	4.1	6.9	4.5	5.8	4.9	5.7	3.1
	5	5.0	4.5	3.4	3.1	3.9	5.5	4.1	6.1
	6	4.1	6.1	4.7	5.7	4.3	5.0	3.0	3.9
	7	3.5	5.0	3.9	4.1	6.3	2.9	3.7	5.8
	8	5.4	4.1	3.0	5.1	3.5	4.9	4.7	3.4

Table 8- continued

Measurement	Replication	Heat Treatments: Conventional, Dry, Center	Conventional, Dry, Edge	Conventional, Moist, Center	Conventional, Moist, Edge	Microwave, Dry, Center	Microwave, Dry, Edge	Microwave, Moist, Center	Microwave, Moist, Edge
Fibrous collagenous connective tissue, %	1	75	78	84	82	76	75	82	72
	2	73	69	81	92	88	84	40	78
	3	55	64	27	23	60	52	68	42
	4	45	64	25	59	42	55	66	51
	5	32	58	44	26	23	37	75	46
	6	60	45	47	72	58	39	92	51
	7	49	78	49	58	70	80	62	41
	8	84	59	87	63	79	44	71	76
% Fibrous collagenous connective tissue score	1	2.7	3.5	3.4	2.9	3.5	2.6	2.7	3.0
	2	2.7	2.2	3.9	4.8	3.8	2.9	2.0	2.9
	3	2.0	3.1	1.2	0.9	3.2	2.4	3.6	2.3
	4	1.6	2.6	1.7	2.5	2.5	2.7	3.7	1.6
	5	1.5	2.5	1.5	0.8	1.4	2.0	3.0	2.8
	6	2.3	2.7	2.2	4.1	2.2	2.0	2.8	1.9
	7	1.7	3.9	1.8	2.3	2.5	2.3	2.2	2.6
	8	4.6	2.4	2.6	1.8	5.0	2.2	3.0	2.6

Table 8- Continued

Measurement	Replication	Heat Treatments: Conventional, Dry, Center	Conventional, Dry, Edge	Conventional, Moist, Center	Conventional, Moist, Edge	Microwave, Dry, Center	Microwave, Dry, Edge	Microwave, Moist, Center	Microwave, Moist, Edge
Quantity of elastic connective tissue score	1	2.1	1.6	4.1	2.6	1.5	1.3	1.8	1.9
	2	1.8	2.7	2.2	2.8	2.0	1.7	1.5	2.0
	3	1.7	2.0	1.8	2.2	2.1	2.0	2.1	1.4
	4	1.1	1.1	1.4	2.2	1.3	2.1	2.2	1.6
	5	2.2	1.7	1.5	1.9	2.5	1.6	2.0	2.7
	6	1.8	1.2	1.2	1.9	1.4	2.1	1.3	1.3
	7	1.3	1.1	1.9	2.1	1.5	1.7	1.3	2.9
	8	1.5	2.0	1.7	1.8	2.9	1.5	1.5	2.1
Collagenous + elastic connective tissue score	1	5.7	6.1	8.2	6.1	6.4	4.7	5.1	6.4
	2	5.5	6.0	6.8	8.1	6.3	5.2	6.6	5.8
	3	5.2	6.9	6.4	6.1	7.6	6.9	7.5	7.2
	4	4.6	5.2	8.3	6.7	7.1	7.0	7.9	4.7
	5	7.2	6.2	4.9	5.0	8.4	7.1	6.1	8.8
	6	5.9	7.3	5.9	7.6	5.3	7.1	4.3	5.2
	7	4.8	6.1	5.8	6.2	5.8	4.6	5.0	8.7
	8	6.9	6.1	4.7	6.9	9.2	6.4	6.2	5.5

Table 8 - concluded

Measurement	Replication	Heat Treatments							
		Conventional				Microwave			
		Dry		Moist		Dry		Moist	
		Center	Edge	Center	Edge	Center	Edge	Center	Edge
Fibrous collagenous + elastic connective tissue score	1	4.8	5.1	7.5	5.5	5.0	3.7	4.5	4.9
	2	4.5	5.0	6.1	7.6	5.8	4.6	3.5	4.9
	3	3.6	5.1	3.0	3.1	5.3	4.4	5.7	3.7
	4	2.7	3.7	3.2	4.7	3.8	4.8	5.9	3.2
	5	3.7	4.3	3.0	3.7	3.9	3.6	5.0	5.5
	6	4.1	3.9	3.4	5.9	3.6	4.1	4.0	3.2
	7	3.0	5.0	3.7	4.4	4.6	4.1	3.5	5.5
	8	6.0	4.4	4.3	4.9	7.8	3.7	4.5	4.6

[a]Scores are an average of three panelist's scores which are each an average score for histological characteristics of five randomly selected muscle sections

HISTOLOGICAL CHARACTERISTICS OF BEEF TOP ROUND STEAKS COOKED BY DRY OR MOIST HEAT IN A CONVENTIONAL OR MICROWAVE OVEN

by

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Since the introduction of microwave ovens for consumer use there has been a dramatic rise in their popularity. Cooking time for meat in the microwave oven is four to five times faster than cooking by conventional methods and consumers are using this appliance increasingly. The histological characteristics of skeletal muscle explain, in part, some sensory characteristics of meat. Histological characteristics of meat cooked by dry heat in conventional ovens have been studied extensively. Information is needed on the histological characteristics of meat cooked by moist heat in a conventional or microwave oven and by dry heat in a microwave oven.

Selected histological characteristics of thirty-two beef top round steaks cooked in a microwave or in a conventional oven by moist (oven-film bag) or dry (modified roasting) heat were studied. Samples for microscopic examination were taken from center and edge positions of the cooked steaks. Data for sensory, chemical and physical characteristics of the same steaks were available to study relationships between those characteristics and the histological characteristics. Data were analyzed by analysis of vairance for a split, split plot design to study influences of type of oven, type of heat, steak position or sample position and interactions of those variables on the histological properties of the muscle. Correlation coefficients were calculated for selected paired variates on the basis of oven/heat treatment.

Differences in histological characteristics attributable to type of oven, type of heat, steak position or sample position were not significant. Calculation of LSDs for significant ($P<0.05$) interactions indicated that the only significant difference observed for mean scores was between the fat distribution scores for center and edge samples of

conventionally-cooked steaks from the inside of the top round. Differences in beef top round steaks cooked in a conventional or microwave oven by dry or moist heat cannot be explained by differences in mean values for selected histological characteristics of those steaks.

Moderate to high negative correlation coefficients were found for muscle fiber width vs initial tenderness in microwave-cooked steaks, which indicates that decreased muscle fiber width was an indicator of tenderness in microwave-cooked beef top round steaks.

Correlation coefficients for total quantity of collagenous tissue did not indicate a consistent relationship to tenderness. The initial tenderness score was explained, partially, by the fibrous collagneous tissue score only for microwave/moist (MM) heated steaks. The correlation coefficient for the initial tenderness score vs the fibrous collagenous tissue score for conventional/dry (CD), microwave/dry (MD) and conventional/moist (CM) heated steaks, and the coefficients for the WB shear value vs the fibrous collagenous tissue score for all oven/heat treatments did not indicate the expected relationship.

Tenderness could be explained, partially, by the observed quantity of elastic tissue only for dry heat treatments. The two combined scores for collagneous and elastic tissue did not help to explain differences in tenderness of beef top round any better than did scores for the individual connective tissue components.

For microwave cooking, the fat quantity score agreed with the percentage ether extract, and for all cooking methods, the WB shear value was explained, partially, by the fat quantity score. Fat distribution appeared to account, partially, for the juiciness score given to MD, CM and MM steaks.

Generally, correlation coefficients indicated that the histological study did not measure the same attributes of top round steaks that were measured by a sensory panel or by objective measurements related to sensory properties.