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COMPARATIVE MICROSCOPY AND MORPHOMETRY OF SKELETAL MUSCLE FIBERS IN POULTRY

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Abstract

Two experiments comparing microscopic analyses were performed using two cooking methods and two different muscles from both chickens and turkeys. In the first experiment, breast (pectoralis) and thigh (quadriceps) muscle taken from male and female Rhode Island Red chickens at three ages (10 weeks, 25 weeks and 52 weeks) were cooked in a microwave oven. Samples were collected for observation with brightfield, phase contrast, interference contrast (Nomarski) and transmission electron microscopy. Samples from the same muscle areas were provided for taste panel evaluation. In the second experiment, breast and thigh samples were collected from 10-week old male and female turkeys with one control sample uncooked while duplicate samples were cooked via a dry heat convection oven. Samples from the same muscle areas were taken for microscopic analyses, taste panel evaluation and shear press analysis. A decrease in sarcomere length and an increase in Z-disc fragmentation were noted in breast and thigh muscles with both cooking methods. Results indicated that type of microscopy (and the ancillary tissue preparative techniques used) significantly affected the morphometrical data. Using similar muscle samples, brightfield microscopy of paraffin sections resulted in significantly shorter ($P<0.05$) sarcomere measurements than other types of microscopy, regardless of age, sex or muscle type. Samples displaying longer sarcomere distances were judged more "tender" by the taste panel and recorded smaller forces with shear press analysis.

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Introduction

Muscle tenderness is probably one of the most important aspects of palatability and is a major contributing factor to consumer acceptance of a meat product. Locker (1960) observed that longer sarcomeres were found in more "tender" meat while shorter sarcomeres were noted in less tender meat. Since that time evaluation of muscle with light microscopy and transmission electron microscopy has shown that postmortem tenderization may be associated with structural changes in the myofibrils (Stanley, 1983; Cassens et al., 1984 and Locker, 1984). Using turkey breast muscle, Johnson and Bowers (1976) showed that, during postmortem aging, loss of lateral attachments between fibrils and deterioration of the Z-line were most likely responsible for decreases in shear values and increased tenderness. The relationships between muscle structure and composition and the resulting quality of meat have been extensively discussed and reviewed by Asghar and Pearson (1980).

Various mechanical methods of assessing meat tenderness quantitatively, such as the widely used Warner-Bratzler shear device, have been in existence for many years and have been reviewed in the literature (Voisey, 1976). As the degree of muscular contraction increased, tenderness, as measured by the Warner-Bratzler shear apparatus, decreased and fiber diameter increased (Kastner et al., 1976). Mechanical shear force values may correlate poorly with other methods of assessment of tenderness, especially where there are large differences in connective tissue strength (Bouton et al., 1973). Using bovine samples Bouton et al. (1975) concluded that force applied in Warner-Bratzler shear analysis would be borne initially by myofibrillar structure, which had been coagulated and stiffened by cooking, rather than by the denatured connective tissue with its rubberlike properties while additional force reflects increasing strain on the connective tissue.

Changes that occur during heating or cooking are complex and are related primarily to two structural components of the muscle tissue - muscle fibers and connective tissue fibers (Hearne et al., 1978a). Paul (1963) described a clumping of perimysial collagen in heated muscle

samples but found that the endomysial reticulum remained virtually intact. Heat-induced differences in shear press force and ease of fragmentation have been associated with microscopic changes in muscle structure including decreased sarcomere length, cracks in muscle fibers, and irregular sarcomere patterns (Hearne et al., 1978b; Cheng and Parrish, 1976). Rate of heating, time of cooking and final temperature affect the quality of meat by inducing changes in structure (Hegarty and Allen, 1975; Hearne et al., 1978a; Bouton et al., 1981). Chambers et al. (1982) examined samples of raw or heat treated (conventional or microwave oven) bovine or porcine muscle and found that no histological characteristic except sarcomere length of porcine muscle fibers was affected significantly by type of heat or type of oven. Since correlation coefficients for both beef and pork data indicated that relationships between histological measurements and sensory data varied greatly, they concluded that these measurements should not be used exclusively to study relationships between muscle structural components and sensory evaluation of muscle tenderness. Khan et al. (1981) pointed out differences in morphometric analyses based on methods of measuring and sample selection. Bello et al. (1981), using fish muscle, compared several different methods of histological (light microscope) tissue preparation and described artifacts related to several types of fixation protocol.

The purpose of this paper is to compare several tissue preparative techniques and types of microscopy using sarcomere measurements from breast and thigh muscle samples in poultry of different ages and sexes. Brightfield, phase contrast, interference contrast (Nomarski) and transmission electron microscopy (TEM), are compared with respect to consistency of observation and applicability as an assay technique in the meat industry.

Materials and Methods

Birds

Two types of poultry common to the consumer were used to investigate, using several types of microscopy, the comparative aspects of muscle structure after cooking. Rhode Island Red breed chickens were selected from a single hatch provided by the Tuskegee University Poultry Farm. Four males and four females were taken at three different ages (10 weeks, 25 weeks and 52 weeks). In addition, three male and three female turkeys aged 10 weeks from a single hatch were provided by Auburn University School of Veterinary Medicine.

Sacrifice and post-mortem treatment

All birds were exsanguinated via the jugular vein. Following scalding, their feathers were removed and carcasses eviscerated. After washing, birds were sealed in plastic bags and placed in an ice bath for 24 hours. This time period permitted a complete transition through rigor mortis (Lyon and Wilson, 1986).

Cooking

Following removal from the ice bath, birds were brought to room temperature and rinsed. Chickens were placed in a shallow glass cooking dish, covered with plastic paper wrap and cooked to an internal temperature of 88°C using a 625-watt microwave oven. A temperature probe (thermometer) was inserted into the pectoralis major muscle using care not to touch any bone. Birds were rotated during the cooking process to assure even heating. Turkeys were split longitudinally through the vertebral column and breast bone. One half of each bird was used for "raw" samples; the other half was prepared by placing the half-carcass, skin side up, in a shallow baking pan and cooking by a dry heat convection oven at 350°F (177°C) to an internal temperature of 88°C. A meat thermometer inserted into the pectoralis major muscle was used to monitor the temperature. Turkeys were prepared in this manner rather than by microwave since this is a common consumer cooking method (AHEA, 1980).

Sample selection

Five (5) samples each of pectoralis and quadriceps muscles from each of the birds were taken for processing for each type of microscopy. All samples were taken from subsurface parts of the muscle. Thus the same muscle from each bird was compared using each type of microscopy.

Phase contrast and interference contrast microscopy

Unfixed, unstained muscle samples (0.5 cm^3) were placed in Sorenson's buffer (pH 6.8) made 60% in glycerol (60 ml buffer, 40 ml glycerol), dissected, macerated and spread on a glass slide. Observations utilizing either phase contrast or interference contrast optics were made with a Leitz Orthoplan microscope using a planapochromat 100x oil immersion objective. Sarcomere measurements were made manually on a Hitachi video monitor which had been calibrated using a stage micrometer.

Brightfield and transmission electron microscopy

Small samples of muscle (1 mm^3) were fixed in Karnovsky's (1965) glutaraldehyde/paraformaldehyde solution in 0.1M sodium phosphate (pH 7) for 1 to 2 h at 4°C. After a number of rinses with 0.1 M sodium phosphate buffer, the tissue was post-fixed with 2% osmium tetroxide buffered in sodium phosphate (0.1M, pH 7) for 1 h at 4°C. A second series of rinses with 0.1 M phosphate buffer followed by distilled water preceded en bloc staining for 2 h in 0.5% uranyl acetate (Terzakis, 1968). Following dehydration with ethanol, the specimens were cleared in propylene oxide, infiltrated and embedded with an Epon/Araldite epoxy resin (Luft, 1961). Blocks were placed in a 60°C oven for 24-36 h or until desired hardness was obtained. For brightfield observation, 1 micrometer sections were cut using an LKB ultramicrotome equipped with a glass knife. Sections were placed on gelatinized glass slides and stained with 0.1% Toluidine Blue in 1% sodium borate. Coverslips were mounted using Permount and slides were observed with a Zeiss Standard microscope using

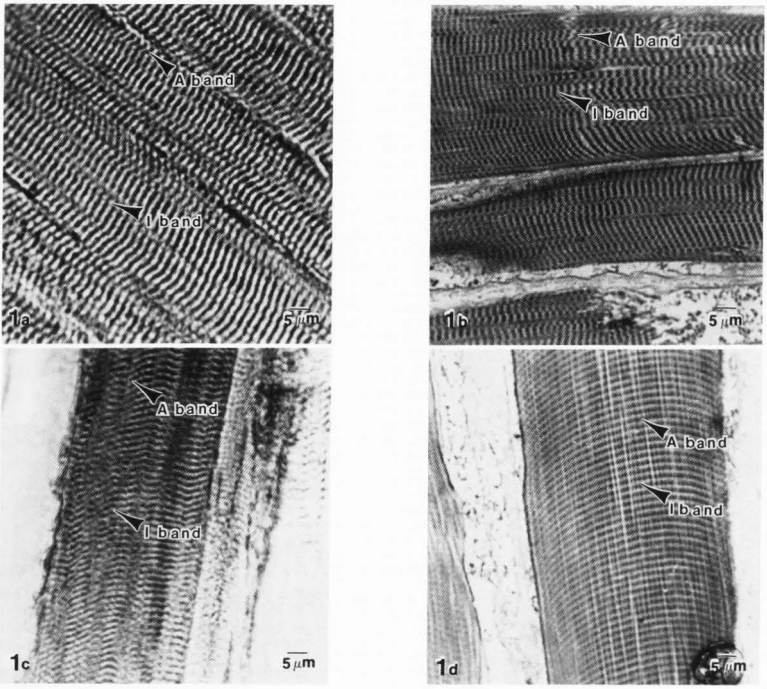


FIGURE 1. Brightfield microscopy. Pectoralis muscle from 10 week old male chicken cooked by microwave oven (a) paraffin procedure (b) epoxy resin procedure and from 52 week old male chicken cooked by microwave oven (c) paraffin procedure (d) epoxy resin procedure. Bar = 5 μm.

40x and 100x planachromat objectives. For transmission electron microscopy, 60nm - 90nm sections were cut using a DuPont Sorvall MT2B ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963), and observed with a Philips 201 electron microscope at 60kV. Sarcomere measurements were taken from light and transmission electron micrographs.

Paraffin embedding

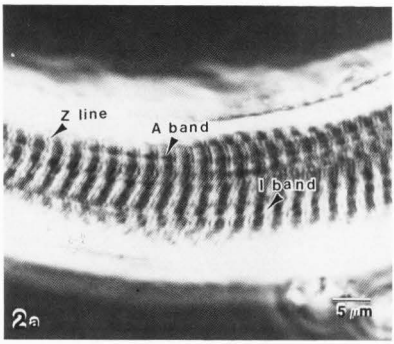
Samples (1 cm^3) were fixed for 12 h in 10% neutral buffered formalin, dehydrated in ethylene glycol monoethyl ether (3 changes at 2 h each), rinsed in methyl benzoate (3 changes at 30 minutes each) and cleared in benzene (3 changes at 10 minutes each) (Benson, 1964). Tissue was embedded in paraffin, sectioned (6 micrometers) with a rotary microtome, and stained routinely with hematoxylin and eosin.

Measurements

In all cases, measurements were taken from five randomly selected areas of each muscle sample. To facilitate measurements made using light microscopy where it was difficult to measure single sarcomeres, lengths were determined by calculating an average length based on 5 or 10 sarcomeres measured as a unit. This mean was considered as a single measurement. Twenty measurements were collected from each of the five sample areas, resulting in a total of 100 measurements per muscle.

Taste panel

A six member taste panel was selected and instructed. Duplicate samples (1 cm^3) were cut from muscle areas near sites selected for microscopic analysis and presented to the panel. Evaluation of tenderness was based on a scale of 1 (tender) to 10 (tough).



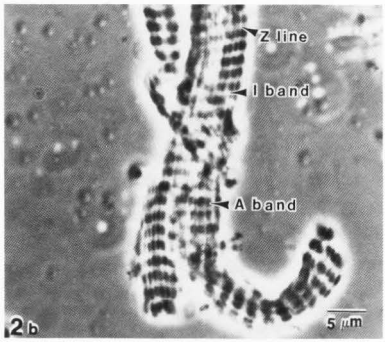
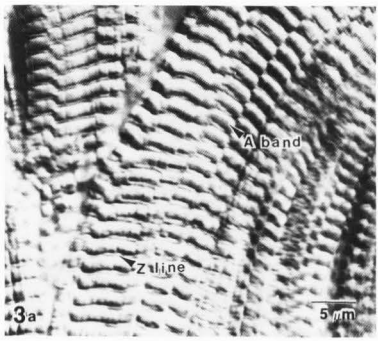
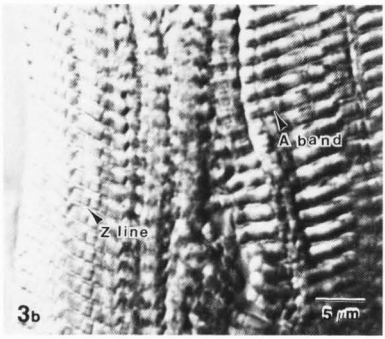
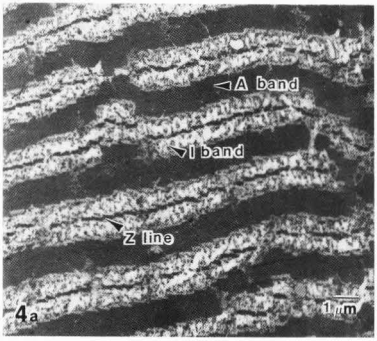
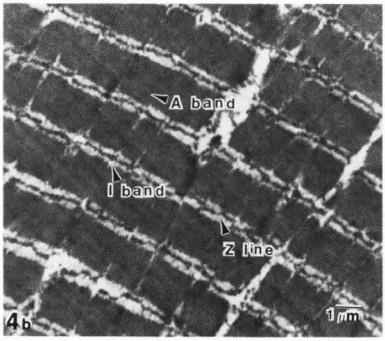


FIGURE 2. Phase contrast microscopy. Quadriceps muscle from 10 week old (a) and 52 week old (b) male chickens cooked my microwave oven. Bar = 5 µm.









Shear press

After meat was cooled to room temperature, uniform cores 1 cm thick were removed from two areas of each sample and evaluated in duplicate (each core was sheared twice) for tenderness using an Instron Food Testing System equipped with a Warner-Bratzler meat shear. The cores were oriented such that they were cut across the fibers. The peak height on the curve was taken as the shear force (Newton/cm^2).

Statistics

A completely randomized selection using a factorial design of five factors (5 microscopy techniques, 3 ages of birds (first experiment) or 2 cooking methods (second experiment), 2 sexes, 2 muscle types, 5 sample cores from each muscle type, with 20 observations from each muscle core as replications) was used. Analysis of Variance and Duncan's New Multiple Range Test were performed to identify significant differences between treatment means (Steel and Torrie, 1980). The Tuskegee University's Time Share VAX 11-780 mini-computer system was utilized.

Results and Discussion

Figures 1 through 4 are representative micrographs of cooked muscle taken from chickens of different ages. Figure 1 shows muscle as seen with brightfield microscopy using either paraffin embedded material (Figs. 1a, 1c) or "thick" (1 µm) sections of epoxy embedded material (Figs. 1b, 1d). Breast (pectoralis) muscle samples from young (10 week) male birds are shown in Figures 1a and 1b while pectoralis samples from older male (52 week) birds are shown in Figures 1c and 1d. The striated pattern was quite evident although in many instances structure had been altered to such an extent by cooking that the Z-lines were not visualized and sarcomere lengths difficult to measure. Figures 2a,b and 3a,b are representative of quadriceps (thigh) muscle samples as visualized with phase contrast and differential interference contrast (Nomarski) microscopy. Figures 2a and 3a show samples from 10 week male chickens while Figures 2b and 3b are of 52 week male chicken quadriceps. Z-lines were usually visible with both of these microscopic techniques. Ruddick and Richards (1975) have shown that conventional phase contrast microscopy of chicken pectoralis muscle was consistent in terms of sarcomere measurements and correlated well with an alternative measurement method, laser diffraction. Figures 4a,b, transmission electron micrographs of 10 week (Fig. 4a) and 52 week (Fig. 4b) old chicken pectoralis muscle, show all structural areas of skeletal muscle sarcomeres. With the resolving power achievable using the electron microscope, Z-lines were easily distinguished.

When cooked and raw turkey pectoralis muscle samples (Figs. 5a-d) were compared using transmission electron microscopy, changes induced by cooking became evident. Sarcomere lengths were shorter and myofibrillar proteins were coagulated in cooked samples (Figs. 5a,b). Fragmentation of myofibers in the area of the Z-disc was also observed and myofilaments in the I-area virtually disappeared but a banding pattern was still evident (Fig. 5b). Giles (1969) found that after 20 minutes at 60°C myosin could still be seen but not actin, and at longer heating times, Z-lines lost their structural detail and became disorganized. Schmidt and Parrish (1971) observed progressive myofibrillar shrinkage and degradation, thin filament disintegration and thick filament coagulation in heated bovine longissimus dorsi muscle. Similar thermal-induced changes were described by Voyle (1981) using scanning electron microscopy. Leander et al. (1980), investigating thermal effects on bovine muscle, suggested that the actin filaments of the I-band were disintegrated by thermal treatment, whereas the thicker filaments of the A-band were less affected. Raw samples of similar muscle (Figs. 5c,d) displayed intact myofilaments and obvious bands. Thin actin filaments were present in the I area and exhibited no evidence of degradation.

Table 1 presents the mean sarcomere lengths of chicken muscle from different sexes as seen with the different types of microscopy used. Measurements of the same samples using Nomarski optics and phase contrast optics resulted in sarcomere lengths which were similar. They are presented as a single value. Paraffin preparation resulted in significantly ($P<0.01$) shorter sarcomeres than those observed in similar muscle prepared using other methods. The reason for this artifactual shrinkage is not clear although Bello et al. (1981) described similar problems in fish muscle samples fixed with 10% formalin and embedded in paraffin and recommended a pre-embedding infiltration with nitrocellulose to minimize distortion. Quadriceps muscle had significantly ($P<0.01$) longer sarcomeres than pectoralis muscle when observed with Nomarski, phase contrast, brightfield ("thick" epoxy sections) and transmission electron microscopy. Transmission electron microscopy resulted in shorter sarcomere lengths than those seen in Nomarski/phase preparations in pectoralis muscle from male and female birds. Similar results were described by Hegarty et al. (1973) who proposed that the hyperosmolarity of the fixative used in tissue preparation for transmission electron microscopy (Karnovsky's fixative) would be expected to produce shrinkage.

Table 2 summarizes mean sarcomere lengths of muscles from chickens whose ages are different. Again it can be noted that paraffin preparation resulted in significantly shorter sarcomeres. With the exception of samples from 52-week old chickens as seen with brightfield microscopy of

FIGURE 3. Differential interference contrast microscopy (Nomarski). Quadriceps muscle from 10 week old (a) and 52 week old (b) male chickens cooked by microwave oven. Bar = 5 µm.

FIGURE 4. Transmission electron microscopy. Pectoralis muscle from 10 week old (a) and 52 week old (b) male chickens cooked by microwave oven. Bar = 1 µm.

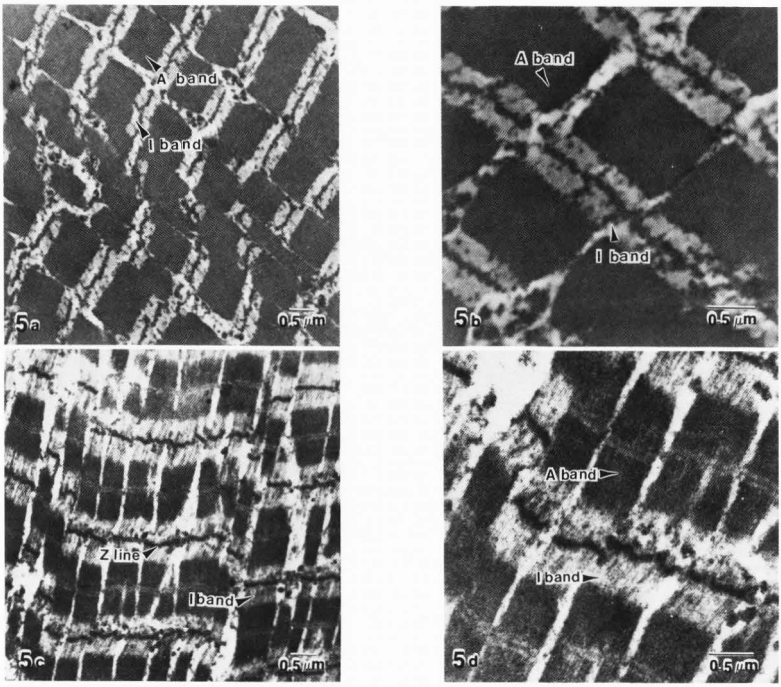


FIGURE 5. Transmission electron microscopy. Pectoralis muscle from 10 week old turkey cooked by dry heat convection oven (a and b) and raw (c and d). Bar = 0.5 µm.

"thick" epoxy sections, quadriceps muscle had significantly longer sarcomeres than pectoralis muscle. Among all age groups, sarcomeres as seen in teased preparations with Nomarski and phase contrast optics and brightfield of "thick" sections were not significantly different. Within the 52 week group sarcomeres were not significantly different with different types of microscopy when similar muscle types were compared, except in the paraffin preparation.

Table 3 presents a comparison of mean sarcomere lengths from cooked and raw turkey pectoralis and quadriceps muscles. It is apparent that data are influenced significantly by type of microscopy used for observation. In comparing brightfield microscopy, which requires fixation, dehydration, embedding and sectioning of tissue samples, with interference contrast (Nomarski) or phase contrast microscopy, which requires only gentle "squashing" in buffer for sample observation, significant ($P<0.05$) differences were observed in sarcomere lengths from similar samples (Table 3). These differences were present in both raw and cooked samples, both breast and thigh samples and in samples from both sexes. Quadriceps muscle from either sex had significantly ($P< 0.05$) longer sarcomeres than pectoralis as measured using all types of microscopy. Raw pectoralis and quadriceps muscle fibers from male and female birds had significantly ($P<0.05$) longer sarcomeres as measured using Nomarski optics when compared to other types of microscopy. Using Nomarski optics also resulted in longer sarcomere measurements in cooked muscle samples although differences between raw and cooked sarcomere lengths were not as great. With the exception of female pectoralis muscle, cooking significantly ($P<0.05$) shortened sarcomeres in both types of muscle from both sexes regardless of type of microscopic measurement technique. Giles (1969) observed a slight amount of heat related shrinkage at 60°C, more at 70°C, with maximal shrinkage of 20% occurring after 100 minutes at 70°C. Hegarty and Allen (1972) reported no decrease in sarcomere length in turkey

Table 1

MEAN SARCOMERE LENGTHS (μm) OF PECTORALIS AND QUADRICEPS MUSCLES FROM CHICKENS OF DIFFERENT SEXES USING VARIOUS TYPES OF MICROSCOPY*

	Method of Microscopy			
Sex	Nomarski/ Phase	BF-Epon	BF-Paraffin	TEM
Male				
Pectoralis	1.7^{a}	1.7^{a}	1.1^{b}	1.4^{c}
Quadriceps	2.0^{d}	1.9^{d}	1.2^{b}	2.3^{e}
Female				
Pectoralis	1.6^{a}	1.6^{a}	1.1^{b}	1.4^{c}
Quadriceps	2.3^{e}	2.0^{d}	1.2^{b}	1.9^{d}

*Means calculated based on 12 birds; 4 from each of 3 age groups.

$^{a-e}$Means with the same superscript letter (regardless of column or row) are not significantly different at the P<0.01 level, Analysis of Variance and Duncan's Multiple Range Test.

semitendinosus and sartorius muscles after application of heat if the unheated sarcomere values were 2.4 micrometers to 2.1 micrometers. However, longer sarcomeres decreased with the application of heat.

Table 4 summarizes taste panel and shear press data from turkey pectoralis and quadriceps muscles while Table 5 includes taste panel evaluation of muscle from chickens of different ages. Data were used primarily to confirm the relationship of sarcomere length to shear measurements and consumer satisfaction of meat in cooked samples. Brady and Hunecke (1985) demonstrated significant correlations between taste panel evaluation and instrumental tests of shear, penetration and compressive force. It has been shown that changes occur in meat texture and taste in older chickens (Fry et al., 1958). In our work younger chickens were graded as more tender than older chickens (Table 5) but no significant differences in sarcomere lengths were demonstrated in chickens due to age (Table 2). Thigh muscle regardless of age, sex, or species was graded as more tender by the taste panel than breast meat (Tables 4 and 5). This may be attributable, in part, to higher fat content of thigh muscle compared to breast muscle (Ang et al., 1984). Thigh muscle samples, regardless of age or sex, displayed longer sarcomere lengths than breast samples in both chickens (Tables 1,2) and turkeys (Table 3). Shear press analysis was utilized to provide a comparison of mechanical assessment of "tenderness" of turkey samples with taste panel data and microscopy. Larger force values were recorded for cooked pectoralis muscle samples than for cooked quadriceps samples (Table 4). The relationship of sarcomere length and shear force values was investigated by Dutson et al. (1976) who demonstrated that shear force decreases at a faster rate in sternomandibularis muscle due to increasing sarcomere length, than in psoas major muscle. Differences in connective tissue content also influence the relative shear force values.

Pectoralis samples from female turkeys displayed short sarcomeres (Table 3) and high shear forces (Table 4) with little change due to cooking. Taste panel evaluation of these samples confirmed this "toughness" (Table 4). The authors can offer no explanation as to why turkey hens had such tough breast muscle but this peculiarity in the data provided us with a unique opportunity to evaluate the validity of our premise that sarcomere length is related to, and in fact can be used to predict, final tenderness of the cooked product. These hens exhibited statistically shorter (P<0.05) sarcomere lengths for their pectoralis muscle, regardless of type of microscopy, for both cooked and raw samples. There was no statistical difference in length of sarcomeres due to cooking, data which correlate well with shear force observations. Lyon and Wilson (1986) noted that female samples of broiler breast muscle required approximately 1 kg more shear force than male samples regardless of cooking method or rigor condition. Shear values for male broilers were significantly lower than those for females cooked in an autoclave for 20 minutes and held under refrigeration for 2 to 4 h (Simpson and Goodwin, 1975). The same effect due to sex of the bird was noted when meat was cooked in a microwave oven (Farr et al., 1983).

There are many morphological parameters in addition to sarcomere length which play a role in the perception of meat quality, particularly

Table 2

MEAN SARCOMERE LENGTHS (μm) OF PECTORALIS AND QUADRICEPS MUSCLES FROM CHICKENS OF DIFFERENT AGES USING VARIOUS TYPES OF MICROSCOPY*

	Method of Microscopy			
	Nomarski/ Phase	BF-Epon	BF-Paraffin	TEM
10 week				
Pectoralis	$1.7^{a,b,c}$	$1.6^{b,c}$	1.1^{d}	1.2^{d}
Quadriceps	$2.2^{e,f,g}$	$2.1^{e,f}$	1.2^{d}	$1.9^{a,b,g,h}$
25 week				
Pectoralis	1.5^{c}	$1.6^{b,c}$	1.1^{d}	1.3^{d}
Quadriceps	$2.2^{e,g}$	$1.9^{a,g,h}$	$1.2^{d,f}$	$2.4^{e,f,g}$
52 week				
Pectoralis	$1.6^{b,c}_{f,g,h}$	$1.7^{b,c}$	1.1^{d}	$1.7^{b,c}$
Quadriceps	$2.1^{f,g,h}$	$1.8^{a,b,c,h}$	1.1^{d}	$2.0^{f,g,h}$

*Means calculated based on 8 birds; 4 males and 4 females.

$^{a-h}$Means with the same superscript letter (regardless of column or row) are not significantly different at the P<0.01 level, Analysis of Variance and Duncan's Multiple Range Test.

Table 3

MEAN SARCOMERE LENGTHS (μm) OF PECTORALIS AND QUADRICEPS MUSCLES FROM TURKEYS USING VARIOUS TYPES OF MICROSCOPY*

	Type of Microscopy		
	Nomarski/ Phase	Bright-field	TEM
Male Pectoralis			
Raw	1.94[a,f]	1.54[b]	1.78[c]
Cooked	1.67[d]	1.32[e]	1.60[b,d]
% Change	-14%	-14%	-10%
Male Quadriceps			
Raw	1.98[a]	1.84[f]	1.78[c]
Cooked	1.87[f]	1.71[d]	1.37[e]
% Change	-5%	-7%	-23%
Female Pectoralis			
Raw	1.58[b,d]	1.19[g]	1.41[e]
Cooked	1.48[b,e]	1.12[g]	1.41[e]
% Change	-6%	-6%	-0-
Female Quadriceps			
Raw	1.89[f]	1.54[b]	1.58[b,d]
Cooked	1.70[d]	1.37[e]	1.47[e]
% Change	-10%	-11%	-7%

*Means calculated based on 4 birds of each sex.

$$\%\text{Change} = \frac{\text{length of raw} - \text{length of cooked}}{\text{length of raw}} \times 100$$

[a-g]Means with the same superscript (regardless of column or row) are not significantly different at the P<0.05 level, Analysis of Variance and Duncan's Multiple Range Test.

Table 4

"TENDERNESS" ASSESSMENT OF PECTORALIS AND QUADRICEPS MUSCLE SAMPLES FROM TURKEYS

	Shear Press[a] (kg/1.0cm core)	Taste Panel[b]
Male Pectoralis		
Raw	24.0 ± 2.8	----
Cooked	16.8 ± 1.9*	3.7 ± 0.7
% Change**	-30%	
Male Quadriceps		
Raw	27.5 ± 3.0	----
Cooked	12.5 ± 1.7*	3.3 ± 0.5
% Change	-55%	
Female Pectoralis		
Raw	29.2 ± 3.1	----
Cooked	25.0 ± 2.6	6.7 ± 1.3
% Change	-14%	
Female Quadriceps		
Raw	23.0 ± 2.9	----
Cooked	11.7 ± 1.4*	2.7 ± 0.4
% Change	-49%	

[a]Instron Model 1132 Food Testing System equipped with a Warner Bratzler Meat Shear Fixture. Mean of 5 determinations for each of 4 birds; total of 20 determinations for each muscle type.

[b]Six member laboratory panel using a 10-point scoring scale (1 = tender; 10 = tough). Each muscle sample was assessed in duplicate.

*Significantly (P<0.05) different from raw samples; Student's t-test.

$$**\%\text{change} = \frac{\text{force for raw} - \text{force for cooked}}{\text{force for raw}} \times 100$$

tenderness, which have not been addressed by the present study. Wu et al. (1985) using scanning electron microscopy noted heat-induced changes in collagen, particularly in the perimysium and the endomysium and suggested that if a correlation among collagen crosslinking, connective tissues solubilization and meat toughness can be established, then scanning electron microscopy could provide a direct means by which the contribution of collagen to meat toughness can be determined. The type and quantity of connective tissue present, particularly perimysial collagen and its reaction to thermal stress have been investigated by Carroll et al. (1978). More recently the role of cytoskeletal elements, especially the fibrous proteins desmin and connectin, in the perception of tenderness has been investigated and reviewed (Locker, 1984).

Cooking temperature and method affect morphology and hence consumer acceptability. Moody et al. (1978) found that microwave cooked meat had the greatest cooking losses but Lyon and Wilson (1986) reported smaller shear values in male and female broiler breast with microwave cooking compared to water heating. Thermal effects could account for the uniformity in sarcomere lengths of similar muscle types from chickens in various age groups (Table 2). Since all samples had been prepared using similar cooking procedures (microwave), moisture loss may have caused a "shrinkage" of tissue. Several researchers have confirmed that meats (broiler, turkey, beef and pork) heated with microwave energy had a higher total moisture loss and lower percentage moisture than meat heated in water or air (Kylen et al., 1963; Culotta and Chen, 1973; Bowers et al., 1974). Likewise, the observation of shorter sarcomere lengths in cooked turkey muscle as compared to raw turkey muscle (Table 3) can be attributed to the thermal effects of cooking. Chambers et al. (1982) demonstrated decreased sarcomere lengths with dry heat and higher end point temperatures.

Technical aspects of tissue preparation also "alter" morphology as viewed with various types of microscopy. Most of these alterations are artifactual and should be minimized or at least standardized to be of value in interpretation. Varriano-Marston et al. (1978) showed that the average sarcomere of at-death,

Table 5

TASTE PANEL EVALUATION* OF MUSCLE SAMPLES FROM CHICKENS OF DIFFERENT AGES

Age	Meat Type: Quadriceps	Pectoralis
10 week	4.06 ± 1.70	5.56 ± 2.20
25 week	5.47 ± 1.93	6.74 ± 2.09
52 week	6.25 ± 2.19	7.35 ± 2.44

*Six member laboratory panel using a 10-point scoring scale (1 = tender; 10 = tough). Each muscle sample was assessed in duplicate.

frozen hydrated samples is longer than that observed in fixed, dehydrated embedded samples. Bello et al. (1981) devised an improved technique for histological preparation of muscle which minimizes damage and distortion in the arrangement and organization of muscle. In addition, Khan et al. (1981) caution researchers with respect to tissue sampling techniques in histological and morphometric studies and the effect of these techniques on data collection.

Birds in this study had similar histories. They were from the same hatch, were raised under similar conditions, fed the same feed, etc. Samples from similar muscle areas were selected for each type of microscopy as well as for taste panel and shear press assessment. In effect, the same muscle from the same bird was assayed using all techniques, permitting a valid comparison of methods with minimal sample variation. What was seen as differences in sarcomere length from similar muscle samples (type of muscle, age, sex, etc.) as observed with different types of microscopy was primarily attributable to differences in tissue preparation and instrumentation.

Microscopy has proven to be a valuable tool in the study of texture. It is obvious that a knowledge of microstructure is necessary if one is to manipulate or regulate the texture of a food product. However, the limitations of microscopy, both in terms of the capabilities of the instrumentation and in the possible technical induction artifact, must be recognized and taken into consideration in data interpretation.

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Discussion With Reviewers

Reviewer I: Why didn't the authors use the procedures of Bello et al. (1981)?
Authors: Bello et al. tested several different fixatives (including formalin) and embedding procedures (including paraffin) for use with light microscopy procedures and sectioned muscle tissue. It is the purpose of our work to compare several different types of microscopy (of which brightfield of paraffin is one) with respect to consistency and ease of morphometric observation. We selected what we considered to be the most common type of routine histological procedure for our example of brightfield light microscopy.

C.A. Voyle: Did the authors notice whether the initial sarcomere length influenced the degree of damage due to heat?
Authors: The authors presume that degree of "damage" means degree of shrinkage of sarcomere lengths. Our results indicate that longer initial (raw) sarcomere lengths from male pectoralis, female pectoralis and female quadriceps in turkeys are subject to a greater percentage of shrinkage when heated as observed with Nomarski or phase contrast optics. Hegarty and Allen (1972) noted similar results in turkey with respect to sarcomere shrinkage.

C.A. Voyle: Hsieh et al. (Meat Sci. 4, 299-311, 1980) reported that bovine muscles reached cooking temperature more rapidly when cooked in a microwave oven than in a conventional oven. What is the effect of rate of heating on poultry muscle structure?
Authors: In this study, poultry muscles reached cooking temperature more rapidly when cooked in a microwave oven than in a conventional oven. We made no attempt to quantify cooking changes in sarcomere lengths in chicken (cooked by microwave) as we did with turkey (cooked by conventional heat) so the question cannot be addressed directly. However, Hearne et al. (1978a) showed in bovine semitendinosus that muscle fibers disintegrated as endpoint temperature increased and that fiber disintegration was greater at a faster rate of heating. Lyon and Wilson (1986) noted overall physical shrinkage of poultry muscle heated by microwave but presented no sarcomere data.

C.A. Voyle: Can the authors explain the lack of statistical difference between raw male turkey pectoralis and quadriceps muscles?
Authors: We have no explanation for this.

H.J. Swatland: Can the authors comment on the aging effect of connective tissue in poultry?
Authors: We made no quantitative measurements of connective tissue either as birds grew older or as the meat aged. deFremery and Streeter (J. Food Sci. 34, 176-180, 1969) saw no decrease in alkali-insoluble hydroxyproline during post mortem tenderization periods in broilers. They concluded that the changes in tenderness that occur during post mortem aging are not caused by the breakdown or dissolution of connective tissue proteins.

R.H. Locker: Are longer sarcomere lengths in quadriceps muscle due to skeletal restraint during chilling?
Authors: This is certainly a contributing factor. Hegarty et al. (1973) described significant differences in sarcomere lengths of rigor stretched and unstretched muscles from turkey legs but no significant difference in pectoralis muscle.

R.H. Locker: Can the authors explain the sarcomere length data from female turkey pectoralis muscle?
Authors: We can offer no explanation for the data but can cite several other investigations in which similar findings were reported (see text of paper).