THE RELATIONSHIP OF TRACE MINERAL CONTENT AND ULTRASTRUCTURE TO POULTRY MUSCLE TENDERNESS

by

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INTRODUCTION

Tenderness is one of the most important palatability attributes of poultry meat. The amount of inter- and intra-molecular bonding of collagen, amount of elastin and collagen in connective tissue, state of contraction and protein coagulation of myofibrils, conditions of processing and storage, water holding capacity, method of cooking, muscle pH, sex, age, and breed of the bird are interrelated factors that play a role in tenderness of poultry meat. Research has been conducted on the effect of those factors on poultry muscle tenderness; however, the results are not definitive.

Poultry meat is a good source of calcium, potassium, phosphorus, and iron. Calcium and potassium play an important role in muscle contraction and may contribute to the ultimate contracture state in postmortem muscle, thus, affecting tenderness of meat. The relationship between muscle toughness and trace element content has not been evaluated in poultry muscle.

The most direct evidence for the Z line undergoing substantial disruption and degradation during postmortem storage comes from ultrastructural investigations. Postmortem weakening of the actin-myosin interaction is also seen ultrastructurally as a lengthening or relaxation of rigor shortened sarcomeres in the absence of ATP. The degradation of the Z line and changes in actin and myosin interactions are related to increase in muscle tenderness (Goll et al., 1970).

Many studies on tenderness have been conducted on beef. However, in some aspects, poultry meat is different from beef meat. In chicken dark meat, for example, the Z line of chicken dark meat is little affected by aging (Hay et al., 1973).

The objectives of this study were to investigate (1) the trace mineral content of poultry muscle by x-ray fluorescence, (2) the ultrastructure of the

poultry myofibril as determined by scanning electron microscopy, and (3) the relationship of those myofibrillar characteristics to shear value of raw and cooked poultry muscle.

REVIEW OF LITERATURE

Effect of heating on tenderness of muscle.

Hegarty and Allen (1976) indicated that an increase in internal temperature between 50 and 100°C. increased the shear value of aged and unaged turkey (adult tom turkey) pectoralis muscle when cooked by dry heat. However, work by Goodwin et al. (1962) indicated that tom turkey muscle cooked by moist heat to 55°C. had significantly higher shear values than that cooked to 77°C. or above; but no significant differences were found in shear values of turkey cooked to 77, 82, 88, and 94°C. There was a gradual decline for each shear value of the breast meat as the end-point temperature increased. Those authors also showed that the outer 0-3 mm layer of the pectoralis major gave significantly higher shear values than did the inner 3-6 mm and 6-9 mm layers.

Schmidt and Parrish (1971) studied the effect of heat on beef muscle connective tissue. Muscle connective tissue shrinkage began at approximately 50°C. and continued until fragmentation eventually occurred at higher temperatures, but perimysial connective tissue shrinkage required an internal temperature of 70°C. and higher before any significant fiber changes were observed. Machlik and Draudt (1963) claimed that collagen shrank at about 58°C., and Hamm (1966) 63°C. Goll et al. (1964) found that 55°C. was required to solubilize collagen from veal muscle but 60°C. and above was needed to solubilize collagen from more mature bovine muscle.

Heating brings about changes in the ultrastructure of muscle tissue. The length of sarcomeres decrease when muscle tissue was heated (Aronson, 1966;

Hostetler and Landmann, 1968; Schmidt and Parrish, 1971; Dube et al., 1972; Hegarty and Allen, 1972). Lowe (1948) and Marsh et al. (1974) suggested an association between shortened muscles and toughness of meat. However, no significant correlation between tenderness and sarcomere length in turkey muscles was reported (Varadarajulu and Cunningham, 1971; Hegarty and Allen, 1972). The greatest effect on fiber length occurs below 70°C. (Hostetler and Landmann, 1968; Hegarty and Allen, 1972). Using phase contrast microscopy, Aronson (1966) showed that heat shortened both I band and A band of rabbit myofibrils.

Scanning electron microscope used to study ultrastructural change of poultry muscle.

Scanning electron microscopy (SEM) is used to examine solid specimens and to produce images that have a high visual impact, especially when viewed in stereo. It can show the consequences of heating, cooling, bending, ion etching, electromigration and other dynamic processes (Wells,1974). Few workers have utilized the techniques of SEM to evaluate structural changes in poultry muscle tissue. Schaller and Powrie (1971) used a cryofracture technique coupled with air drying to prepare muscle samples for SEM evaluation. In pre-rigor turkey pectoralis muscle they noted an extensive sarcoplasmic reticulum which was not differentiated clearly from the transverse element of fibers. After aging 3 days, the size of transverse elements had not decreased; however, their surface had become rough as if disruption of elements was beginning. After 6 days, transverse elements had collapsed and breaks in fibrils and perforations in the sarcolemma were noted.

Johnson and Bowers (1976) followed the method of Schaller and Powrie (1971) to work on turkey breast muscle. They found pronounced transverse elements at the Z line and a less prominent element in the center of the A band in pre-rigor muscle. Rigor muscle showed curved fibers. Transverse elements at the Z line

were slightly flatter than in pre-rigor muscle and those in the M line region still were apparent. Fibrils still were packed densely but more intercellular space was evident in "rigor" than in pre-rigor muscle. Muscle aged 24-48 hr exhibited transverse elements that were flattened but still visible. They were less pronounced than those reported by Schaller and Powrie (1971) in turkey breast muscle aged 3 days. Myofibrils tended to fracture readily at the Z line. In addition, fiber surfaces were broken and perforated, and intermyofibrillar spaces increased in size with post-rigor aging.

Using scanning electron microscopy, Schaller and Powrie (1972) indicated that the structural integrity of myofibrils was lost near or at the transverse elements when chicken muscle was heated to 97°C. Granular matter was observed beneath the sarcolemma of fibers. Endomysial fibers were not detected in chicken muscle heated to 97°C but in tissue heated to 60°C. swollen fibers were apparent.

Effects of aging on ultrastructure of myofibrils.

The sliding filament theory for muscle structure originally proposed by Huxley and Hanson (1954) has been verified by microscopic examination of skeletal muscle. The myofibrillar proteins are assembled into thick and thin filaments making up the myofibril. Thick filaments are made up of myosin and component C while thin filaments contain actin, tropomyosin, troponin, and β-actinin. In addition, there is a tranverse element, the Z line composed of α-actinin which also may contain tropomyosin and actin depending on the definition of its boundary. The M line is composed of M protein which functions to maintain the thick filaments in proper register longitudinally and laterally. With light microscopy, muscle fibers appear as a series of thin, parallel, cross-straited fibrils resulting in a longitudinally striated muscle (Bloom and Fawcett, 1968).

During aging of beef muscle, a loss of adhesion between adjacent myofibrils

occurs. Alterations also appear at the region of the Z lines, leading to a weakening of intermyofibrillar linkages (Davey and Gilbert, 1969). Davey and Dickson (1970) also noted that a weakening of the myofibrillar structures at the junction of the I filaments with the Z discs of the sarcomeres during beef muscle aging was the cause of loss of tensil strength of the myofibrils. In addition, the Z discs underwent progressive changes and lost ground substance as aging proceeded.

Takahashi et al. (1967) worked on chicken pectoral muscle and showed that (1) fragmentation of the myofibrils and (2) reversible or irreversible contraction of the sarcomeres were two morphological changes which occur during postmortem aging at 5°C. The tendency for myofibrils, from pectoral muscles, to break into small fragments consisting of 1-4 sarcomeres (upon blending in a Waring Blendor) increased progressively with time of postmortem. Based on an electron microscopic study, Fukazawa and Yasui (1967) suggested that the fragmentation of the chicken pectoral myofibril might be due to the destruction of the specific configuration of the Z line in the myofibrils. The degradation and/or disappearance of the Z line, and the breakdown of the junction of the Z line and the I filament of chicken pectoral muscle during postmortem storage at 5°C. were two types of destruction in the Z line of sarcomeres and myofibrillar fragments, according to Fukazawa et al. (1969). They confirmed that a change in the state of the Z line and the junction of the Z line and I filaments appeared to be indispensable for the fragmentation of the myofibrils. The tendency of myofibrils to fragment might however have a direct relationship to tenderness. Hay et al. (1973) using electron microscopy, compared the effect of postmortem aging on chicken breast and leg muscle and claimed that the breast muscle sarcomeres were shorter and that breast muscle was deficient in glycogen granules.

Very few studies have been conducted on the ultrastructure of chicken dark muscle as affected by aging. That the effects of aging on longus adductor were quite different from the effects on pectoralis superficialis has been indicated

by Hay et al. (1973), who also found that the Z line of leg muscle was less affected by aging than that of breast muscle.

Davey and Dickson (1970) summarized changes probably related to aging within the fibrillar structures of beef muscle. The first change was a weakening of lateral attachments which maintain myofibrils in precise register within the muscle febers, probably at the level of the Z discs and involving elements of the sarcoplasmic reticulum. The second change was a weakening of the myofibrils themselves, resulting in a breaking at the jumction of the I filament and the Z disc, and occasionally at the edge of the A band due to the loss of association of actin and tropomyosin within the I filaments of the sarcomeres. The third change was a loss of material from the Z discs leading in some cases to a complete dissolution of those structures.

X-ray fluorescence as a method of trace mineral analysis.

When an element is irradiated with x-ray of sufficiently high energy, secondary, or fluorescence, x-rays are emitted that are characteristic of the element. To induce characteristic x-rays of the atom, the photoelectric absorption is the desired interaction. X-ray fluorescence exhibits a characteristic dependence on energy, as well as on the atomic number of the atom in which the interaction take place. Measurement of the intensity and wavelength of fluorescence radiation is now a well established method of analysis and has been applied to the determination of the elements, from sodium to uranium in powder, liquid or metal samples (Brown, 1959). Sensitivity range is less than 1 p.p.m. (Woldseth, 1973a).

The basic components of a system for x-ray fluorescence consists of three units, the first is the excitation source, the second detects the fluorescent spectrum emitted by the sample, and the third extracts the information for qualitative (x-ray energy or wavelength) and quantitative (x-ray intensity) analysis

(Woldseth, 1973b).

The method is rapid (1-4 min), independent of the chemical combination of the element, and nondestructive (in the sense that the specimen examined is not destroyed), though some specimen preparation may be required (Pomeranz & Meloan, 1971). The samples are excited which in turn produces the characteristic x-rays of the elements in the sample. This photon flux coming from the sample is detected by a lithium drifted silicon [Si(Li)] solid state detector which yields a pulses amplitude proportional to the energy of the detected x-ray. The detector pulses are amplified, processed, and sorted according to amplitude with an amplifier and pulse height analyzer. A pulse height spectrum is the resulting histogram of the number of pulses versus pulse height (Cooper, 1973).

Effect of trace mineral content on the muscle.

Greaser et al. (1967) suggested that Ca^{++} might play a role in the change of myofibrillar proteins during postmortem storage because within several hours after death, sarcoplasmic reticulum in porcine muscle began to lose its ability to sequester Ca^{++}. Goll et al. (1970) also stated that onset of tension development or shortening in postmortem muscle strips was closely correlated to the time at which sarcoplasmic reticulum began to lose its Ca^{++} sequestering ability, and that postmortem Z line degradation was caused by Ca^{++} which was released when sarcoplasmic reticular membranes in postmortem muscle lose the ability to accumulate Ca^{++} against a concentration gradient. Work by Weiner & Pearson (1969) showed that intravenous antemortem injections of ethylenediaminetetraacetic acid (EDTA), or ethyleneglycol-bis-N,N'-tetraacetic acid (EGTA), or 1, 2-cyclohexanediaminetetraacetic acid (CDTA) significantly inhibited rabbit and pig muscle shortening during development of rigor mortis. On the other hand, increased levels of calcium resulted in a greater amount of fiber shortening.

However $MgCl_2$ had no effect on muscle shortening.

Polyvalent metals such as iron, zinc, copper, magnesium, manganese, and cobalt have been indicated as potential crosslinkers (Bjorksten,1968). Crosslinking has been proven for collagen (Bakerman, 1969). In human skin, the transition of extractable collagen to the insoluble form is due to the formation of intermolecular crosslinks (Bakerman, 1962). High correlations between element concentration (Fe, Zn, Ca, and Co) and Warner-Bratzler shear value was shown by Vavak et al. (1976) in bovine heart and muscle. Webb et al. (1967), working on beef muscle, showed that as tenderness improved during aging, extractable sodium, magnesium, and calcium were released from the muscle proteins, while potassium, phosphate, and nitrogen were retained. Tenderness decreased as the quantity of magnesium decreased in the exudate from water-holding capacity determinations.

Almost all polyvalent metals accumulate on aging, particularly in the aorta, but also in other organs in close contact with blood circulation (Vavak et al., 1976). Pennington and Calloway (1973) claimed that age seemed to be inversely related to copper concentration. Younger animals have a higher copper concentration in organs and muscle meats than do older animals of the same species. Helander (1966) believed that the concentrations of K, Mg, and P decreased with age and that the concentrations of Na, Cl, and Ca increased.

Shear force as related to meat tenderness.

The force required to shear animal muscle has been extensively used as a measurement of tenderness. Presently, the Warner-Bratzler and L.E.E.-Kramer shear presses are the most widely used physical method of measuring the shear force of muscle. According to Sharrah et al. (1965), sensory scores for tenderness correlated better with the Warner-Bratzler than with the L.E.E.-Kramer instrument.

Correlation of objective methods, such as the Warner-Bratzler shear, with

subjective assessments have been reported as highly variable (Szczesniak and Torgeson, 1965). The reasons are: 1. both the instrumental and taste panel measurements being subject to sampling variation, 2. the stress and strain patterns developed in the mouth, during the chewing and mastication of meat not being adquately represented by the instrumental techniques employed, 3. the taste panel scoring system being oversimplified by assuming a linear tenderness scale (Bouton et al., 1975). Khan and Voisey (1973) claimed that within muscle, variability was smaller in muscle having lower shear force values than in muscle having higher shear force values. However, Webb et al. (1964), and Khan et al. (1973) showed that panel tenderness values were significantly correlated with Warner-Bratzler shear values in beef muscle. In addition, a correlation coefficient of 0.85 ($P<0.001$) between panel rating and Warner-Bratzler shear values on chicken breast muscle was reported by de Fremery and Streeter (1969).

Comparison of results of shear force from different laboratories is impractical because of lack of standardization of procedures and texture measuring devices (Khan et al., 1973).

MATERIALS AND METHODS

Frozen, dressed young turkeys and chicken hens were purchased from a local supermarket. After thawing at 4°C. for 48 hr (Cunningham and Lee, 1975), the birds were cooked in boiling water for 3 hr, and muscles were removed from the breast and thigh for;(1) trace mineral analysis, (2) shear force measurement, and (3) ultrastructure observation. For the examination of raw muscle, samples were taken immediately after thawing.

Trace mineral analysis:

The excised poultry muscles were ground in an Osterizer food blendor (25-60 cycle). Ground samples were held frozen until analyzed. Duplicate 4.9-5.0 g samples, in a porcelain crucible, were dried in an electric oven (103°C.) over-

night and then weighed. Ash of poultry muscles was determined from the dried samples using a Muffle furance for 12 hr at 520-530°C. The resulting ash was weighed into lucite sample holders (5cm across with a 0.3cm deep circular indentation, 1.5cm in diameter) and exposed to x-rays using a Cd^{109} source with an activity of 0.5 mC (milli-Curies). Elements with atomic weights of 39.1 to 95.94 (K to Mo) emitted characteristic x-rays which were detected with a $\langle Si(Li) \rangle$ solid state detector (Ovtec, Model 7416-10195), analyzed and stored in a pulse height analyzer (Nuclear Data, 100). The resulting spectra were printed on paper tape, and later transferred to magnetic tape. A computer analysis of the fluorescence spectrum was used to determine the area under each peak. The area was compared with previously determined standards to calculate p.p.m. of each element per sample. Standards for each element were made by adding 1% of the element to ashed turkey muscle.

Tenderness measurements:

Ten-gram portions of meat (5.08x5.08x0,5 cm) were cut parallel to the muscle fibers for shear force measurement on a Kramer shear press with an electronic recorder. The press was set in the 100 range with a 227 Kg ring and a 15-second down stroke. Shear values were made on duplicate samples (Cunningham et al., 1972).

Ultrastructure observations:

The differences in ultrastructure among meat samples were observed from scanning electron micrographs. Samples were prepared by a method modified from that of Jones et al. (1977). Glutaraldehyde-fixed samples were frozen in liquid nitrogen, shattered between plexiglass plates and dehydrated in ethanol. Dried samples (critical point drying) were glued to aluminum stubs with silver paste, coated with gold heated on tungsten wire and examined with an ETEC Autoscan scanning electron microscope operating at 20 Kv accelerating voltage. The following formula was used for measuring the length of sarcomeres from the

micrographs:

$$\text{Actual size } (\mu m) = \frac{\text{Size from photomicrograph (cm)}}{\text{Magnification}}$$

Statistical analysis:

The analysis of variance was applied to the data and means were separated by the LSD(least significant differences) method.

RESULTS AND DISCUSSION

Effect of heating on tenderness of poultry muscles

Tenderness of poultry muscles was measured with a L.E.E.-Kramer shear press and expressed as Kg force needed to shear 1 g of muscle as shown in Tables 1, 2 & 3 and Figs. 1 & 2.

Turkey muscle was tenderer than chicken hen muscle (Table 1). The cooked poultry dark muscles were significantly more tender ($P<0.01$) than cooked light muscles (Table 2), which agrees with Cunningham and Lee (1975) for chicken broiler muscles, and Varadarajulu and Cunningham (1971) for turkey muscles. However, raw dark muscles were tougher ($P<0.01$, Table 2) than raw light muscles because dark muscles contained more connective tissue per unit volume of muscle than light muscles did. The finding that dark muscles were more tender than light muscles after cooking agreed with the statement of Bratzler (1971) that during cooking, the muscle fibers become tougher and the connective tissues become tenderer. Thus, for muscles containing relatively large amounts of connective tissue, such as poultry dark muscle, the toughing of the fibers was less important than the softening of connective tissue; and tenderness of dark muscles were improved very much by the combination of a long heating period and a moist-heat cooking. However, long period and moist-heat cooking induced tough poultry light muscles, which contained only small amounts of connective tissue,

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Table 1 Sarcomere lengths and shear values of poultry muscles.

Muscle	Sarcomere length (μm)	Shear value[a]
Turkey		
Raw dark	2.40	6.54
Cooked dark	1.83	3.33
Raw light	1.73	5.43
Cooked light	1.19	4.55
Chicken hen		
Raw dark	1.94	6.66
Cooked dark	1.37	3.88
Raw light	1.77	5.54
Cooked light	1.21	4.94

2.12(m) 2.12(m) * 1.46(m) 1.46(m) * 1.66(m) 1.66(m) * 1.49(m) 1.49(m)

4.93(m) 4.93(m) * 4.99(m) 4.99(m) 4.96(m) 4.96(m) ** 5.25(m) 5.25(m)

[a] Kg force/g muscle
**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line

Table 2 A comparison of sarcomere lengths and shear values of raw and cooked poultry muscles.

Muscle	Sarcomere length (μm)	Shear value[a]
Raw		
Turkey dark	2.40	6.54
Chicken dark	1.94	6.66
	1.96(m)	6.60(m)
Turkey light	1.73	5.43
Chicken light	1.77	5.54
	1.47(m)**1.88(m) **	6.04(m) ** **
		5.48(m) **
Cooked		
Turkey dark	1.83	3.33
Chicken dark	1.37	3.88
	1.40(m)	3.60(m)
Turkey light	1.19	4.55
Chicken light	1.21	4.94
		4.17(m) **
		4.75(m) **

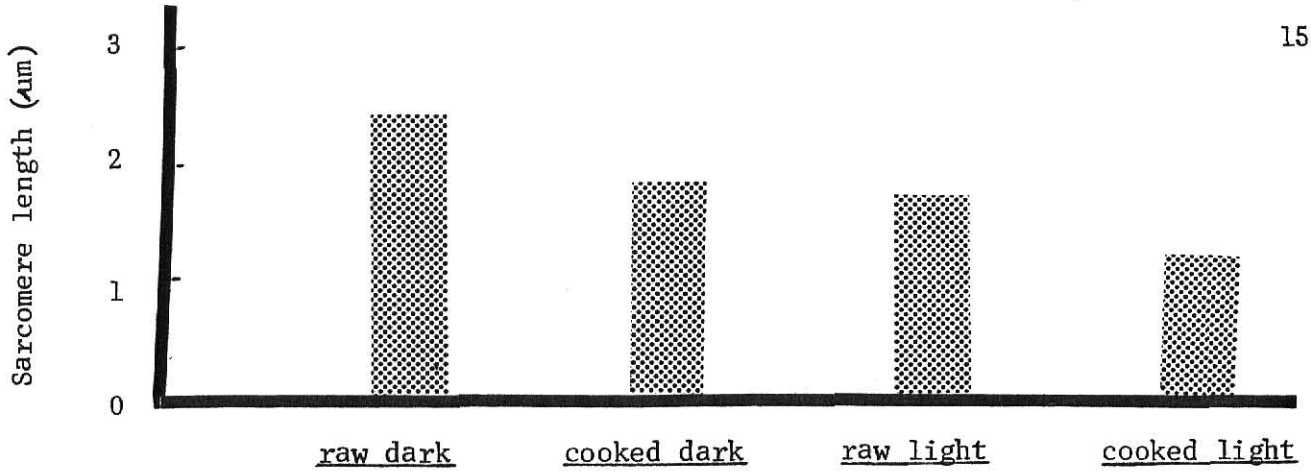
[a]Kg force/g muscle
**Significant at the 1% level
(m) mean of figures connected by line

Table 3 Sarcomere lengths and shear values of poultry muscles.

Muscle	Sarcomere length (μm)	Shear value[a]
Turkey		
Raw dark	2.40	6.54
		5.98(m)
Raw light	1.73	5.43
	1.79(m)	
Cooked dark	1.83	3.33
		3.94(m)
Cooked light	1.19	4.55
	*	
Chicken hen		
Raw dark	1.94	6.66
		6.10(m)
Raw light	1.77	5.54
	1.57(m)	
Cooked dark	1.37	3.88
		4.41(m)
Cooked light	1.21	4.94

5.98(m) 5.98(m)	**
4.41(m) 4.41(m)	**
6.10(m)	**

[a]Kg force/g muscle
**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line



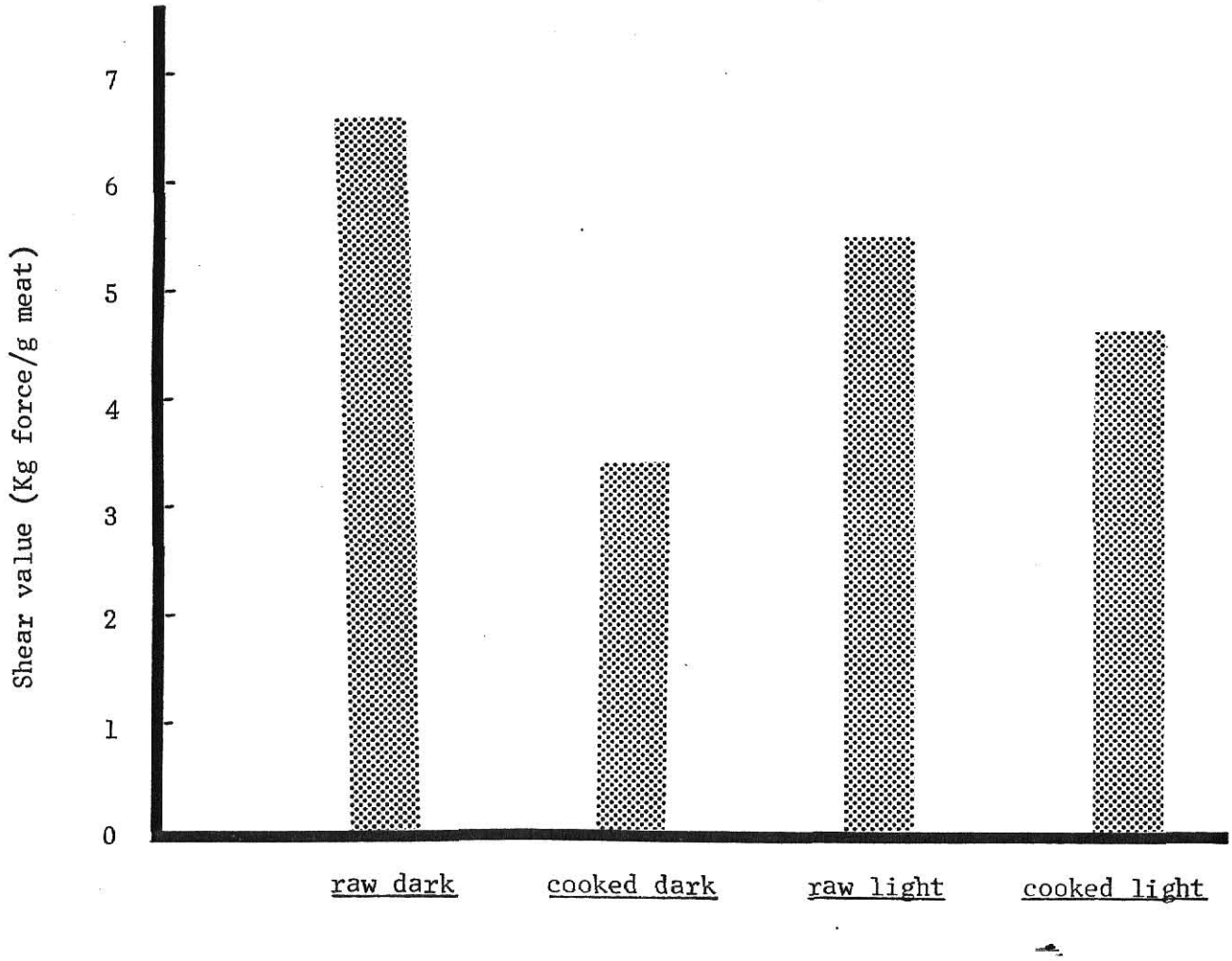
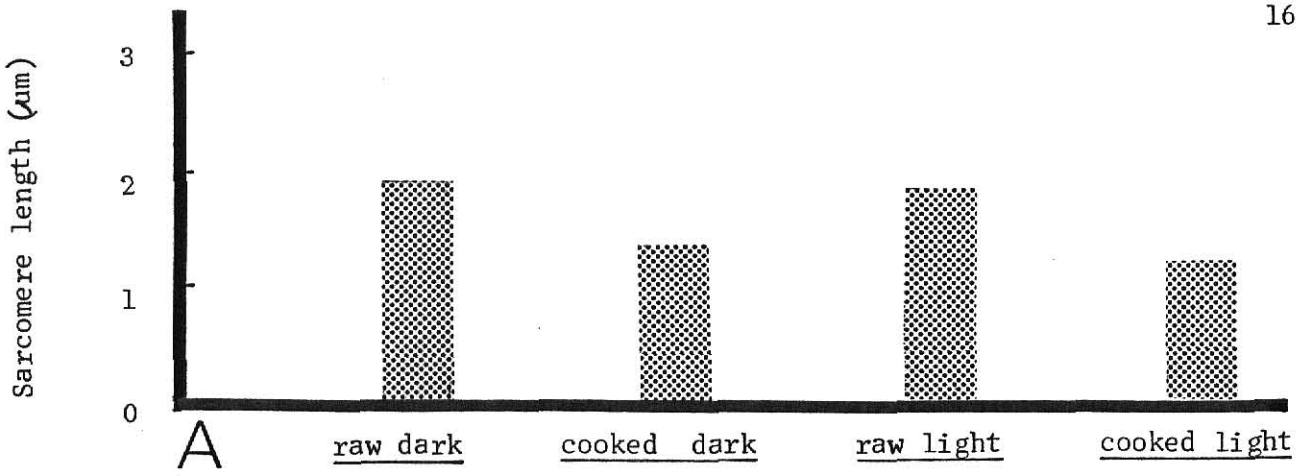


Fig. 1. Effect of cooking on sarcomere length (A) and shear value (B) of dark and light turkey muscles



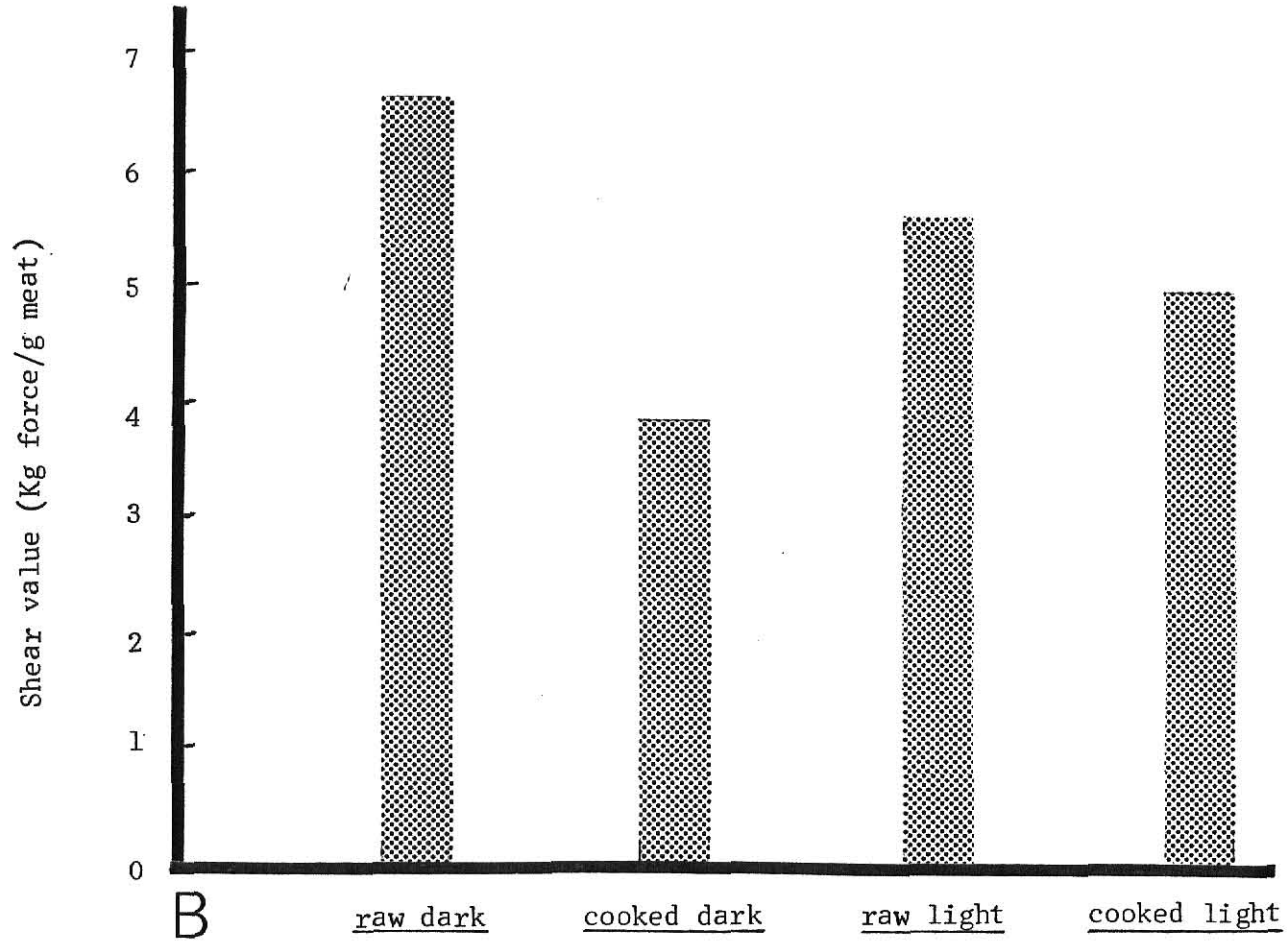


Fig. 2. Effect of cooking on sarcomere length (A) and shear value (B) of dark and light chicken hen muscles

because the toughness of muscle fibers overwhelmed the minor influence of connective tissue. Kramer shear values also showed that raw poultry muscles were tougher than cooked poultry muscles ($P<0.01$, Table 2). It was also true when comparison was made between raw and cooked muscles within turkey or chicken hen (Table 3).

Shear force values observed by Varadarajulu and Cunningham (1971) from cooked turkey muscles were higher than the values of cooked turkey muscles reported in this study. The former study used dry-heat cooking; but, instead of oven roast, I cooked turkey muscles in boiling water. Heating muscles above $60^{o}C$. with dry-heat caused drying, harding, and coagulating of the myofibrillar proteins. Toughness, due to drying and coagulating of myofibrillar proteins, might originate from the loss of water of hydration surrounding thick and thin filaments (Schmidt et al., 1970).

There was a significant difference ($P<0.05$) between turkey light muscles and dark muscles (Table 1); but no difference ($P<0.05$) was observed between chicken light and dark muscles. Chicken raw muscles and cooked muscles were significantly tougher ($P<0.01$) than turkey raw muscles, and cooked muscles, respectively (Table 3). The statement of Marsh (1977) that the collagen contribution to toughness was due to the presence of intermolecular crosslinks which, with increasing animal age, become more thermally resistant and thus less readily broken during cooking could be an explanation of tougher chicken hen muscles in comparison with less mature turkey muscles.

Effect of temperature on ultrastructure.

The micrographs of raw and cooked poultry muscles obtained from the scanning electron microscope are shown in Figs. 3 to 10. Each muscle sample will be discussed separately for its unique structural changes. Sarcomere lengths of turkey muscles were significantly different ($P<0.05$) from those of chicken hen

muscles (Table 3). Dark and raw poultry muscles had significantly longer ($P<0.01$) sarcomere length than those of light and cooked poultry muscles, respectively (Table 2). With the comparison within turkey or chicken hen muscles, sarcomere length of dark muscle was significantly ($P<0.05$) longer than that of light muscle (Table 1).

Turkey dark muscle:

In raw turkey dark muscle (Fig. 3), the transverse elements were not prominent; but still distinguishable. The A band was pronounced and occupied most area of the sarcomere. H zone located at the middle of the A band could not be detected. At either side of the Z line, the I band was indented. Aging effect was indicated by the indentation of the I band and the degeneration of the Z line. When compared with raw turkey light muscle (Fig. 5), (1) Z line of light muscle was thinner than in dark muscle, (2) A band of light muscle became less pronounced than the A band of dark muscle, and (3) I band perforation occurred in raw light muscle but not in raw dark muscle. The observations implied that dark muscle was more resistant to aging. Hay et al. (1973) reported that, at 0 hr postmortem, the Z line width of broiler breast and leg muscles were almost the same. At 48 hr postmortem, the Z line of breast muscle appeared to be ruptured; but in leg muscles, the Z line was very evident. Those authors believed that the Z line of leg muscles was more refractory to factors which resulted in the disruption of the Z line in breast muscle and that the effects of aging on leg muscles were quite different from the effects on breast muscle.

Heat shrank the sarcomere of turkey dark muscle, from approximately 2.40 μm to 1.83 μm (Table 1). Sarcomeres of turkey dark muscle were significantly longer ($P<0.05$) than those of chicken hen dark muscles (Table 1). Besides the change of sarcomere length, micrographical features also changed considerably after cooking (Fig. 4) as compared to Fig. 3. A bands became shortened and flattened. Transverse elements that overlay the Z lines of myofibrils became

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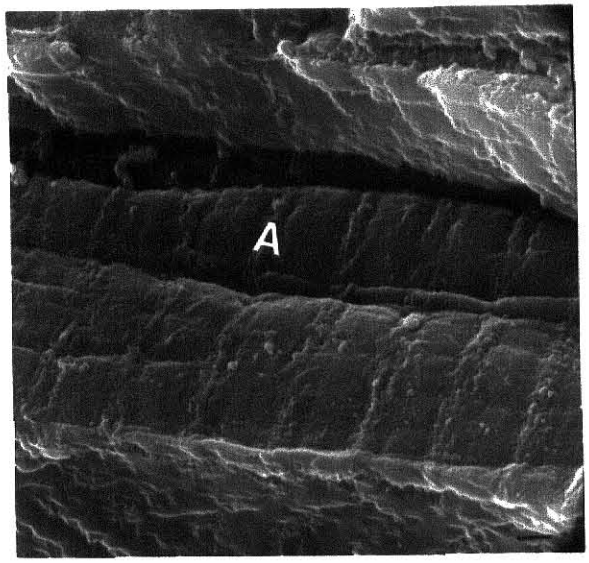


Fig. 3 Ultrastructure of myofibrils of raw turkey dark muscle showing flattened transverse elements (A) (X 5,000; marker=1 μm).

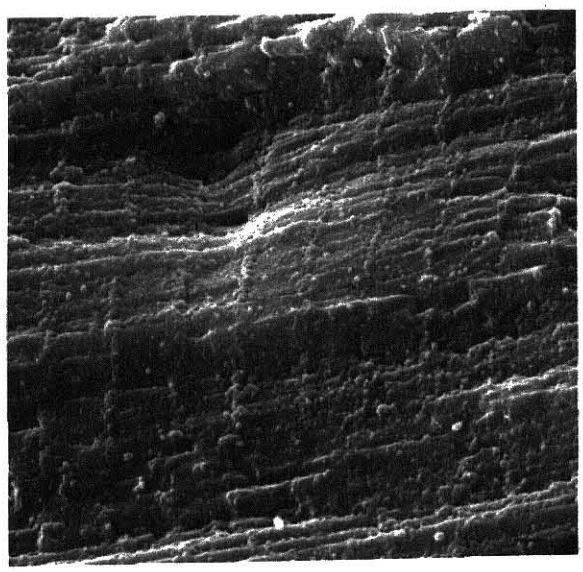


Fig. 5 Longitudinal fractures of myofibrils in raw post-rigor turkey pectoralis major muscle (X 5,000; marker= 1 μm).

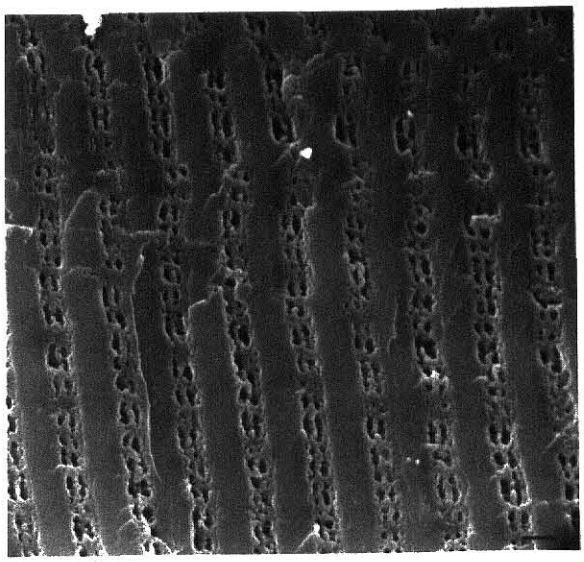


Fig. 4 Myofibrils of cooked turkey dark muscle showing significantly changed I band and transverse elements (X 5,000; marker= 1 μm).

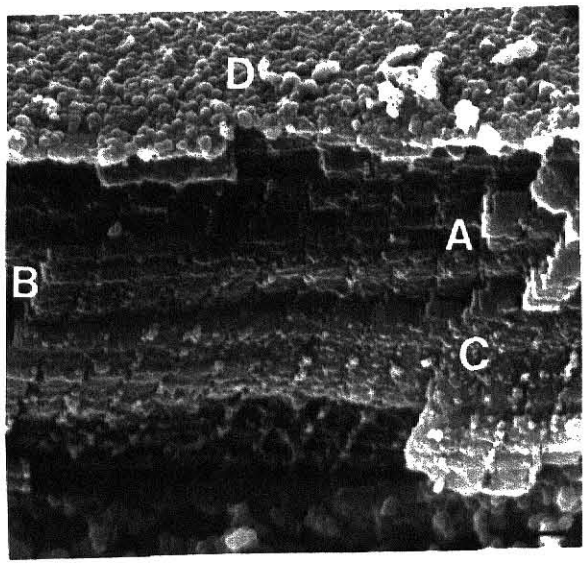


Fig. 6 View of myofibrillar structure in cooked turkey light muscle showing missing sections of sarcomeres (A), transverse fracture planes (B), granular matter on sarcomeres (C), and granulation of connective tissue (D) (X 5,000; marker= 1 μm).

less apparent, some part even disappeared. Transverse elements would be difficult to identify without the degradation of the I band. Fracture planes, caused by cryofracture (Jones, 1977), were distributed over all the surface of cooked dark muscle, but not over raw muscle. The appearance of fracture planes in cooked muscle indicated that heat had weakened myofibrillar structure. This inference concurred with the finding of Jones et al. (1977), that fracture planes occurred at weak point of muscle structure.

The most important change caused by heating, in comparison with raw turkey muscle, was the degradation of the I bands and Z lines (Fig. 4 & 3). The degree of I band breakage in cooked turkey muscle was higher than that of I band breakage in cooked light muscle (Fig. 6). I band degradation might contribute to the tenderness of cooked dark muscle since it was the most tender muscle of all turkey muscle samples.

Turkey light muscles:

Transverse elements of raw turkey light pectoralis muscle were flattened but still visible (Fig. 5). Those elements were less pronounced than those observed by Johnson and Bowers (1976) in turkey pectoralis major muscle aged 24-48 hr. However, the samples used in this study were aged more than 48 hr. The degradation and/or disappearance of the Z lines and the breakdown of the junction of the Z lines and I filaments were two important types of change in chicken pectoralis major muscle during aging (Fukazawa et al., 1969).

Sarcomeres of raw light muscle (Fig. 5), approximately 1.7 μm long, were demarcated by transverse elements located along the Z lines and continued through the space between the myofibrils. Those structures were either T-tubule or Z band substances (Sybers and Ashraf, 1973). Sarcomeres were shorter than those (2.64 μm) reported by Varadarajulu and Cunningham (1971), who measured sarcomeres on pre-rigor muscle; however in this study, sarcomere measurements were taken on postrigor muscle. According to Hay et al. (1973) and Smith et al.

(1969), sarcomeres of avian muscle shortened upon cold aging. Lying on each side of the Z line areas were short, faint I bands. The A bands, located in the middle of the sarcomeres, were not clearly defined. Tissue perforation, caused by aging, was shown on the junction between the Z lines and I filaments.

Disintegration of I bands and deformation of Z lines of cooked light muscle are shown in Fig. 6. After cooking, sarcomeres became shortened, from about 1.73 μm to 1.19 μm (Table 1). Cryofracture caused the missing sections of sarcomeres (A). Most myofibrils observed in this picture were fused or nondistinct (Schaller and Powrie, 1972). Evidence of the effect of heating on myofibrillar structure was seen at transverse fracture planes (B) which were caused by heating and then by cryofracture. Granules derived from the heated sarcoplasm are revealed in the lower part of the figure. Schaller and Powrie (1972) reported that rapid heat induced small granules; and slow heat produced large granules. Other coagulated material is shown deposited on sarcomeres (C). At the upper part of the picture, the granular feature was believed to be the connective tissue (D) which was changed by heating. According to Schaller and Powrie (1972), granular matter found in heated beef and chicken muscles were from sarcoplasm. However, work by Paul (1965) on rabbit muscle, concluded that heating appeared to produce some granulation from the muscle fibers themselves, from the fine collagen strands, and from the endomysial reticulum, rather than primarily from the muscle fiber as in beef.

Chicken hen dark muscles:

The finding of Hay et al. (1973) (that during aging, structural changes were more rigid in chicken broiler leg muscle than in breast muscle) was also found to apply to chicken hen muscles in this study, since the results show that transverse elements of raw dark muscle (Fig. 7) have collapsed; but are still more pronounced than that of raw light muscle (Fig. 9). In the micrograph of raw chicken hen dark muscle, the A bands were the same as that of tyrkey raw dark muscle, occupying most area of the sarcomeres; however, the H zone (A)

that was not shown in turkey muscle could be seen in the chicken muscle, although it was not very clear. The short, indented I bands appear at either side of the Z lines. Strong effects of cryofracture are shown on several areas of this figure. It was inferred that muscle from which this picture was obtained might have received much more force than other samples during cryofracturing.

Schaller and Powrie (1972) reported that transverse elements that overlay the Z lines of myofibrils were still evident in chicken broiler semitendinosus muscle heated to 97°C., and I bands were severely disrupted. However, cooked chicken hen dark muscle (Fig. 8) in this study showed less structural change, even when boiled for 3 hr. Chicken hen muscle was more resistant to heat than chicken broiler muscle.

The ultrastructural changes in cooked chicken hen dark muscle (Fig. 8) were very comparable to changes of beef semitendinosus muscle heated to 90°C. for 45 min, reported by Jones et al. (1977). Sarcomeres shortened to about 1.37 µm (Table 3). Heat shrank muscle fibers and brought the sarcomeres closer together (Table 3, and Fig. 8). Cooked hen dark muscle showed more severe breakage of myofibrils along the Z lines compared with cooked chicken hen light muscle. Transverse elements of cooked hen dark muscle were still visible between the sarcomeres. It was difficult to distinguish the ultrastructure of the A and I bands.

Chicken hen light muscles:

The tissue perforation observed in raw turkey light muscle caused by aging was not observed in raw chicken hen light muscle (Fig. 9). A possible reason was that myofibrillar structure of chicken hen light muscle were mature; whereas those of turkey light muscle were immature. Transverse elements of raw hen light muscle were flattened. That area of sarcomeres between the transverse elements was undistinguishable A band and I band. H zones could not be seen. There were fewer transverse fracture planes in raw hen light muscle than in raw hen dark muscle. Transverse elements of chicken hen light muscle became narrow

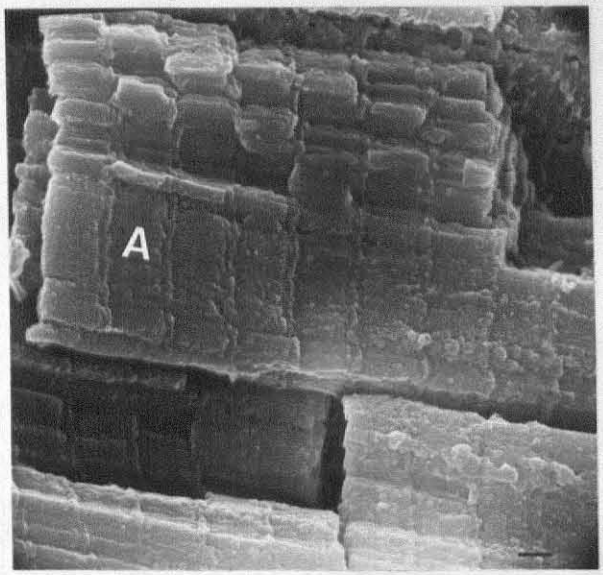


Fig. 7 Strong effect of cryofracture was shown in raw chicken hen dark muscle. Faint H zone was indicated by the letter A (X 5,000; marker= 1 μm).

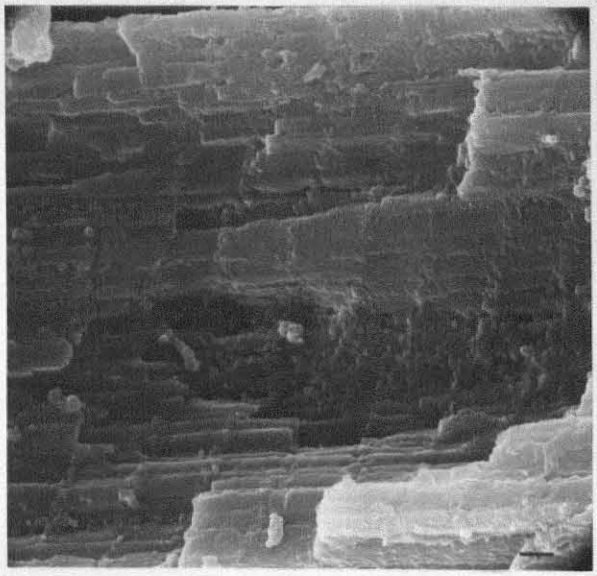


Fig. 9 Transverse elements of raw hen light muscle were less prominent after aging; but they were still visible (X 5,000; marker= 1 μm).

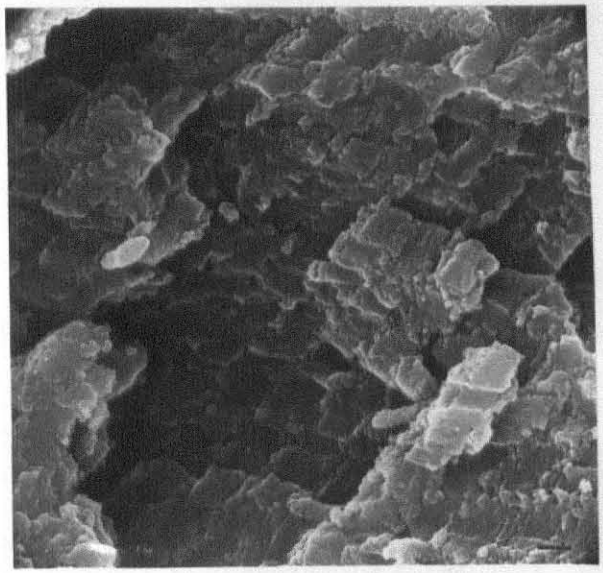


Fig. 8 In cooked, dark muscle of chicken hen, proteins were coagulated along z-lines. There was no evidence of I band disintegration (X 5,000; marker= 1 μm).

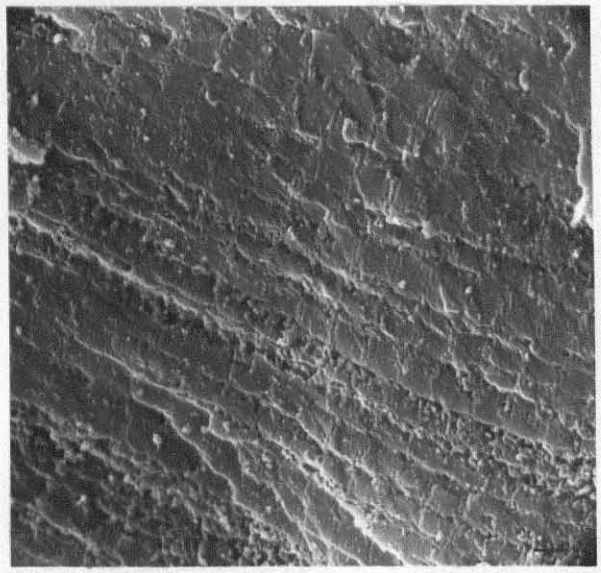


Fig. 10 Heat induced degradation of the junction between I band and z-line was shown in cooked chicken hen light muscle (X 5,000; marker= 1 μm).

and less apparent after cooking (Fig. 10). The I bands were degenerated but the degree of degradation was less than in cooked turkey light muscle (Fig. 6). Sarcomeres were shortened after cooking and fracture planes were distributed over the surface of muscle. Coagulated material, myofibrillar and/or collagenous protein, was deposited on myofibrils as granular matter. Ultrastructural changes in cooked chicken hen light muscle was less in comparison to cooked chicken broiler light muscle which was reported by Schaooer and Powrie (1972).

Trace mineral content of poultry muscles.

Trace mineral content of turkey and chicken muscles is presented in Tables 4 to 8. Except Cu and Zn, all trace minerals tested here showed significant difference ($P<0.05$, and also $P<0.01$ for Fe and Rb) between raw and cooked muscles (Table 6 and 8). Turkey muscles contained significantly higher ($P<0.05$) amounts of K-Ca and Rb, and lower Fe (wet tissue) concentration than did chicken hen muscles (Table 7 and 5). Iron concentration (wet tissue) of chicken dark muscle was significantly higher ($P<0.05$) than that of turkey dark muscle (Table 5); but turkey light muscle (dry tissue) had higher ($P<0.05$) rubidium concentration than did chicken light muscle (Table 7). Raw dark and raw light poultry muscles showed higher concentrations of Fe ($P<0.05$, Table 6) and Rb ($P<0.01$, and $P<0.05$ only for wet light tissue, Table 8) than did cooked dark and cooked light poultry muscles, respectively.

The results of this study agreed with the report of Pennington and Calloway (1973) that dark meat had a higher mineral content than light meat. Results of my study also showed significant differences ($P<0.01$) in Fe (wet tissue, Table 5) and Rb (Table 7) between dark and light poultry muscles. It was also true when comparison was made between dark and light muscles in both raw and cooked poultry muscles for Fe ($P<0.05$, Table 6) and Rb ($P<0.01$, Table 8). Methods of cooking affected the result of trace mineral content (Pennington and Calloway,

1973). Dry heat cooking reduced the moisture of muscles thereby increasing the percent of trace mineral, on a wet weight basis. Poultry muscles cooked in moist heat showed less trace mineral content because minerals leached out into the cooking water. With dry heat cooking, Murphy et al. (1975) believed that cooked poultry meat contained more zinc than did raw meat. However, the opposite was revealed in this study with moist heat cooking (Table 4). My results concurred with the report of Fox et al. (1960) that cooked (moist heat) chicken hen muscle contained less mineral than did raw hen muscle.

Values of K and Ca were reported together because the detector could not separate K and Ca. The amount of K-Ca in either raw dark or raw light muscle in this study was higher than that (3430 µg/g meat) reported by Bowes and Church (1975) who did not specify dark or light meat. Table 7 also showed that dark poultry muscle contained more K-Ca than did light poultry muscle. But Jacob and Nair (1975) reported that chicken biceps femoris muscle contained less Ca than did the pectoralis muscle. In cooked turkey light muscle, the amounts of iron, zinc and rubidium in this study were all less than the values reported by Zenoble et al. (1977) who used x-ray fluorescence to detect trace minerals of turkey light muscle. One possible reason for the difference was that Zenoble used a dry heat cooking method. Those authors also could not detect copper; but copper was found in every sample of this study. Pennington and Calloway (1973) showed a higher copper content in raw turkey dark and light muscles than was found in this study (wet tissue). However, they did not indicate their method of cooking or analysis.

Chicken muscle contains less minerals than turkey muscle, except for iron in dark muscles, as shown in Tables 4 to 8. Age could be a reason for the higher amount of iron in hen dark muscles. This study showed higher iron but lower copper in raw chicken hen dark and light muscles compared with the values reported by Jacob and Nair (1975). However, the chickens used by Jacob and Nair were younger than those used in this study. According to Pennington and Calloway

Table 4 Copper, and zinc content of poultry muscles (µg/g muscle).

Muscle	Cu		Zn	
	Dry tissue	Wet tissue	Dry tissue	Wet tissue
Turkey				
Raw dark	3.11	0.80	71.35	24.48
Cooked dark	1.37	0.50	60.51	18.25
Raw light	2.00	0.53	49.75	17.71
Cooked light	1.14	0.39	36.41	9.71
			(m)39.72—*—61.48(m)	
Chicken hen				
Raw dark	2.40	0.62	61.20	23.82
Cooked dark	1.33	0.35	52.88	15.86
Raw light	1.42	0.35	39.74	12.01
Cooked light	0.86	0.30	32.99	8.66

*Significant at the 5% level
(m) mean of figures connected by line

Table 5 Iron content of poultry muscles (μg/g muscle).

Muscle	Fe		Fe	
	Dry tissue	Wet tissue	Dry tissue	Wet tissue
Turkey				
Raw dark	42.52	15.73	42.52	15.73
		13.84(m)		
Cooked dark	32.32	11.95	32.32	11.95
		12.05(m) *		
Raw light	33.24	11.64	33.24	11.64
		10.25(m)		
Cooked light	26.37	8.87	26.37	8.87
		* *		10.28(m)-**-14.26(m)
Chicken hen				
Raw dark	49.71	16.75	49.71	16.75
		14.69(m)		
Cooked dark	32.48	12.63	32.48	12.63
		12.50(m) *		
Raw light	32.54	11.88	32.54	11.88
		10.30(m)		
Cooked light	26.08	8.73	26.08	8.73

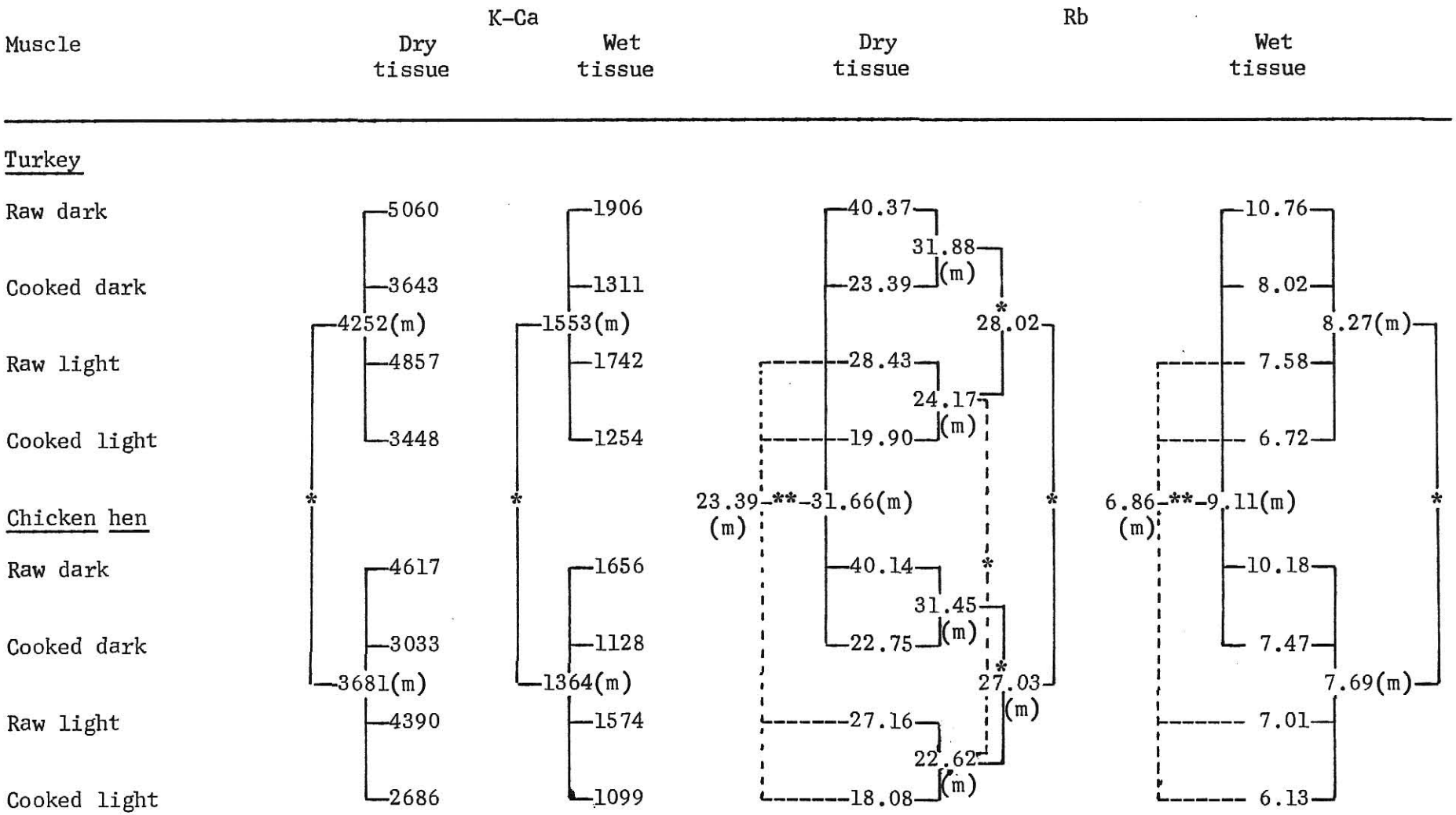
**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line

Table 6 A comparison of iron content in raw and cooked poultry muscles (ug/g muscle).

Muscle	Fe Dry tissue	Fe Wet tissue	Fe Dry tissue	Fe Wet tissue
Raw				
Turkey dark	42.52	15.73	42.52	15.73
		16.24(m)		13.68 (m)
Chicken dark	49.71	16.75	49.71	16.75
		*		14.00(m) *
Turkey light	33.24	11.64	33.24	11.64
		11.76(m) 11.76(m)		14.32(m)
Chicken light	32.54	* 11.88	32.54	11.88
				**
Cooked				
Turkey dark	32.32	11.95	32.32	11.95
		12.28(m) *		10.41 (m)
Chicken dark	32.48	12.63	32.48	12.63
		*		10.54(m) *
Turkey light	26.37	8.87	26.37	8.87
		8.80(m) 8.80(m)		10.68(m)
Chicken light	26.08	8.73	26.08	8.73

**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line

Table 7 Potassium-calcium, and rubidium content of poultry muscles (µg/g muscle).



**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line

Table 8 A comparasion of potassium-calcium, and rubidium content of raw and cooked poultry muscles (ug/g muscle).

Muscle	K-Ca		Rb	
	Dry tissue	Wet tissue	Dry tissue	Wet tissue
Raw				
Turkey dark	5060	1906	40.37	10.76
Chicken dark	4617	1656	40.14	10.18
	4731(m)	1719(m)	40.25(m)	10.47(m)
Turkey light	4857	1742	28.43	7.58
Chicken light	4390	1574	27.16	7.01
			27.79(m)	7.29(m)
			34.02(m)	8.88(m)
Cooked				
Turkey dark	3643	1311	23.39	8.02
Chicken dark	3033	1128	22.75	7.47
	3202(m)	1198(m)	23.07(m)	7.74(m)
Turkey light	3448	1254	19.90	6.72
Chicken light	2686	1099	18.08	6.13
			18.99(m)	6.42(m)
			21.03(m)	7.08(m)

**Significant at the 1% level
*Significant at the 5% level
(m) mean of figures connected by line

(1973), younger animal muscles have a higher copper concentration than do older animals of the same species. As far as iron was concerned, again, age could be a factor affecting the iron content between old and young poultry of the same species because almost all polyvalent metals accumulate on aging (Vavak et al., 1976). Zinc values reported in this study were higher than the values reported by Murphy et al. (1975) and Osis et al. (1972). Those two reports showed similar values (28 μg/g) for cooked chicken dark meat and (9-11 μg/g) for cooked chicken light meat, using the same method, atomic absorption spectrophotometry, to measure zinc content of chicken muscle.

When comparison was made within individual species, poultry dark muscle contained significantly ($P<0.05$) higher amount of Fe (wet tissue, Table 5) and Rb (dry tissue, Table 7) than did light muscle. As far as iron was concerned, the greater abundance of myoglobin and cytochromes in the dark meat probably account for the higher iron contents of dark muscle (Jacob and Nair, 1975).

Almost all the values of trace minerals detected by x-ray fluorescence in this study were higher than those reported in studies where other methods were used. It implies that sensitivity of x-ray fluorescence is higher than that of other methods.

Relationship between ultrastructural changes and tenderness.

The more the change in ultrastructure (Figs 3 to 10), the tenderer the poultry muscle (Table 1, 2 & 3, Fig. 1 & 2). In turkey muscles, the tenderest muscle was that having the most significant change in ultrastructure. That was also true for chicken hen muscles. Shear values of raw poultry muscle were inversely proportional to the degree of degradation of the transverse elements. There was no correlation between sarcomere length and shear value of raw poultry muscles but a positive correlation existed between sarcomere length and tenderness in cooked poultry muscles. Heat caused sarcomeres to shrink and increased

the tenderness of poultry muscles simultaneously. The conclusion of previous studies (Locker, 1960; Marsh & Leet, 1966; Marsh et al., 1974) that shortening muscle decreased the tenderness of meat was not substantiated in raw poultry muscle in this study; however, my results on raw poultry muscle agreed with the findings of Varadarajulu and Cunningham (1971) that no significant correlation exists between tenderness and sarcomere length in turkey muscles. Those authors stated that that might be due to the fact that fiber measurements were made on pre-rigor meat, whereas sensory evaluation was done on postrigor samples. An interesting report on turkey thigh muscle was made by Hegarty and Allen (1972) that no significant correlation existed for shear force values between the cooked folded and stretched muscles. Another agreement between the report of Varadarajulu and Cunningham (1971) and my results was that turkey dark meat had longer sarcomere length than light meat. I found that is also the case in chicken hen muscles. Regarding the sarcomere length of raw turkey dark and light muscles, the values reported by Varadarajulu and Cunningham (1971), 2.64 μm for light and 3.10 μm for dark meat, were all longer than the sarcomere lengths of raw turkey dark and light muscles reported here. That could have been due to the samples measured at different stages postmortem—the former authors measured sarcomere length at pre-rigor; whereas my measurements were postrigor. Another reason might be the samples coming from different positions of the muscle (Hegarty and Allen, 1972).

Relationship between trace mineral content and tenderness.

Calcium has been reported to play an important role in muscle tenderness: (1) to weaken the strength of muscle fibers by disrupting the Z lines and thus increasing tenderness (Nakamura, 1973), and (2) to weaken or disrupt interfibrillar attachments (Goll, 1970). The calcium content of muscle could therefore be

a major factor affecting the tenderness of muscle. Unfortunately, my results could not show the exact amount of Ca; therefore, the relationship between Ca and muscle tenderness can not be determined. A substantial relationship between the Z line degradation and muscle tenderness was found; but that does not imply the more the degree of Z line degradation, the higher the content of Ca. Dark muscles are generally the most active and thus have a larger blood supply. Consequently, more iron is present in dark muscle because iron is an important component of blood. The relationship between iron and muscle tenderness was still uncertain since exercise is another factor known to induce toughness of muscles. Zinc content of beef juice extract has been reported to be positively associated with tenderness (El-Badawai et al., 1964). However, although my study showed a positive relationship between zinc content and tenderness of cooked poultry muscles; a negative association in raw muscle was observed.

This study showed that poultry raw dark muscles had a higher concentration of trace minerals and higher shear values than raw light muscles; but that cooked dark muscles were higher in trace minerals and had lower shear values than cooked light muscles. Therefore, it is difficult to establish a relationship between trace mineral content and tenderness for both raw and cooked poultry muscles, although Vavak et al. (1976) concluded that a multiple correlation of 0.97 was found between trace mineral content (Fe, Co, Zn, and Ca) and shear value (Warner-Bratzler) of cooked beef semimembranosus muscle.

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THE RELATIONSHIP OF TRACE MINERAL CONTENT AND ULTRASTRUCTURE TO POULTRY MUSCLE TENDERNESS

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ABSTRACT

Relationship between tenderness, trace mineral content, and ultrastructure of poultry muscles were investigated. Trace mineral concentration was determined with x-ray fluorescence, ultrastructural change was observed with a scanning electron microscope, and tenderness was measured with a L.E.E.-Kramer shear press.

Dark muscles had greater amounts of trace minerals than light muscles in both turkeys and chicken hens. Turkey muscles contained more trace minerals than did chicken hen muscles, except for iron in dark muscles. Sensitive x-ray flourescence was capable of detecting greater amounts of trace minerals than had been reported in the literature. Ultrastructural changes were more pronounced in cooked dark muscles than in cooked light muscles. The most obvious changes in raw poultry muscle due to heat were the degradation of the transverse elements and the junctions between the I bands and Z lines. Ultrastructurally, the transverse elements of raw dark muscles were more stable to cold aging than those of raw light muscles. L.E.E.-Kramer shear values showed cooked dark muscle was tenderer than cooked light muscle; but raw dark muscle was tougher than raw light muscle.

Increased tenderness was associated with degree of ultrastructural change. A high positive correlation was found between sarcomere lengths and tenderness for cooked poultry muscle, but not for raw muscle. The relationship between trace mineral content and tenderness of poultry muscle was not established because high trace mineral concentrations occurred together with high shear values in raw poultry muscle; but the opposite conditions were found in cooked poultry muscle.