SELECTED CHARACTERISTICS OF CONNECTIVE TISSUES INFLUENCING MUSCLE QUALITY *

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Basic characterization of factors affecting eating qualities of muscle is of utmost importance. If improvements are to be made in these factors from genetic or environmental interactions or from processing innovations, more definitive measures are paramout.. With the advent of more sensitive and exacting procedures for defining and comparison of the various structural, biochemical and physiological parameters in tissues, hopefully, compositional differences and postmortem changes can be elucidated. It is recognized that all physiological and biochemical changes in muscle must involve connective tissues intimately associated with each muscle cell. Certainly, a more complete understanding is needed of the structural and compositional differences occurring in these connective tissues during aging, stress associated with slaughter, and during postmortem holding and preparation of muscle as a food.

Earlier work in this laboratory identified differences in reticular tissues between longissimus dorsi (LD) muscles of extreme shear value differences. Further work by McClain et al. (1965) demonstrated that tenderness variations in LD muscles were not attributable to alkali-insoluble collagen contained in these muscles. This study was initiated to further characterize reticular tissues differences before and after cooking and to measure the acid- and salt-soluble fractions of collagen in selected muscles.

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Materials and Methods

One hundred twenty carcasses from yearling steers were surveyed for tenderness utilizing shear values (Warner-Bratzler) of the LD muscle. The 12th rib was removed from each carcass after 72 hrs. in a chill room (approx. 3° C) and allowed to remain at 2-3° C for an additional 96 hrs. before cooking and shearing for tenderness values. From this group, 30 samples were selected. Half of the samples were taken from muscles classified as low shear value (less than 10.6 kg on a 2.54 cm core), and the remaining samples represented high shear value muscles (more than 17.5 kg on a 2.54 cm core).

Shear value determinations.

Each 12th rib sample was standardized to a thickness of 3.2 cm. Steaks were cooked in deep fat (135° C) to an internal temperature of 70° C. Three 2.5 cm cores were removed from each steak and sheared on the Warner-Bratzler shear machine. Samples, approximately 4 x 4 x 3 mm, were removed for argyrophilic fiber staining procedures from the median core of each steak after shearing. Similar samples were removed from the median area of an adjacent uncooked portion for comparisons of argyrophilic tissues with the cooked samples.

Reticular fiber staining procedures.

Tissue sections were stained using a modification of the procedure of Soule (1962). Samples were placed immediately into 10 percent buffered (pH 7.0-7.2) neutral formalin, and fixed for a minimum of 72 hrs. All samples were then dehydrated in graded alcohol baths, cleared in xylene and embedded for cross-sectioning in Paraplast (M.P. 56-58° C). After sectioning at 6 microns, tissue ribbons were floated on a water bath (50-52° C) separated and picked up on acid clean slides coated with Mayers-Albumin fixative, and allowed to dry for 12 hrs. Slide-mounted sections were cleared in xylene and taken to water in graded alcohols. Staining was accomplished in one percent gold chloride for 30 minutes at 35° C, followed by dipping into distilled deionized water and transferring to a five percent sodium carbonate, 0.5 percent potassium hydroxide bath for 10 minutes. Slides were then transferred directly to a five percent potassium iodide solution for 3

minutes, followed by rinsing twice in distilled deionized water. Counter-staining was done in a 0.25 percent methylene blue chloride solution for 30 seconds followed by rinsing two times in distilled deionized water. Sections were dehydrated in 95 percent ethyl alcohol, cleared in xylene and mounted in Permount.

Reticular fiber intactness determinations.

Five fields were surveyed per section at a magnification of 400X utilizing an American-Optic binocular model scope. A subjective score was assigned based upon the relative intactness of the reticular fiber net surrounding the individual muscle cells. This score ranged from one through five with one corresponding to 0 percent intactness and five corresponding to 100 percent intactness. Values for each section viewed were expressed as the mean of the five observations.

Acid- and salt-soluble collagen determinations.

Samples were removed from the longissimus dorsi, semimembranosus and triceps brachii muscles from six cattle at 1 hr. and 168 hrs. postmortem. Acid- and salt-soluble collagen fractions were determined by a modified procedure of Woessner (1961) reported by McClain et al. (1965). Cooking and shearing of samples were as described above.

Results and Discussion

Reticular fiber intactness score and shear values.

Mean shear values and reticular fiber intactness scores are shown in table 1.

Table 1. Shear values and Reticular Fiber Intactness Scores for LD Muscles

Shear Value Groups	N	Shear Values	Reticular Fiber Intactness Scores Cooked tissue	Uncooked tissue
High S.V.[a]	15	20.12[b]	4.11[c]	4.12
Sta. Dev.		2.41	0.65	0.68
Low S.V.	15	9.40[b]	3.57[c]	4.15[c]
Sta. Dev.		0.74	0.97	0.50

a. S.V. Shear value expressed as kg/2.54 cm core
b. Shear value differed significantly ($P > .01$)
c. Intactness scores between shear value groups differed significantly ($P > .05$)

Shear values differed significantly ($P > .01$) when tested by the t-test (Snedecor, 1956). There was also a significant difference ($P < .05$) between argyrophilic intactness scores between shear value groups and between cooked and uncooked tissue of the low shear value group. However, no significant difference was observed between intactness scores between cooked and uncooked tissues within the high shear value group.

Correlation coefficients between shear values and reticular fiber intactness scores as shown in table 2 revealed a significant relationship ($r = .49$) in the low shear value group.

Table 2. Correlation Coefficients Between Shear Values and Reticular Fiber Intactness Scores

	High Shear Value Group	Low Shear Value Group
Reticular Fiber Intactness		
Uncooked	0.28	0.49 *
Cooked	0.29	0.15

N = 14

* Significant at 0.05 level

Reticular fibers of cooked and uncooked tissue did not appear to differ in their affinity for gold chloride. However, it was observed on the high and low shear value cooked tissue sections that an unidentified substance was present in and around the reticular net which had a strong affinity for methylene blue.

Acid- and salt-soluble collagens.

The primary assumption throughout the literature regarding methods of determining the content of connective tissue in striated muscle is that intracellular constituents of muscle are solubilized by alkali solution, but connective tissue collagen is not. However, more recently the solubilization of collagen has been observed in dilute acetic acid.

It has been postulated (Orekhovitch and Shpikiter, 1957) that citrate-soluble collagens are biological precursors of insoluble mature collagen. Cold salt-soluble fractions of collagens have been demonstrated and shown to change into a mature-like collagen substance by warming to body temperature (Gross, 1961). It has been demonstrated that soluble collagens may be altered by growth rate, nutrition, temperature and various endocrine compounds. Therefore, it is apparent that the long-held theory that collagen is a metabolically inert substance with the primary function of passive support of the cellular constituents of the body is now untenable.

Samples were selected from the LD, SM and TB muscles from six mature bovine animals (2-5 years of age) for determinations of acid- and salt-soluble collagens at 1 hr. and 168 hrs. post-mortem. Results are shown in table 3.

Table 3. Means for Acid- and Salt-soluble Collagens in Selected Bovine Muscles at 1 hr. and 168 hrs. Post-mortem

An. No.	Muscle[1]	Shear Value[2]	Salt-soluble		Acid-Soluble	
			1 hr.	168 hrs.	1 hr.[3]	168 hrs.
			μg Hp/g - muscle			
1	LD	11.13	16.47	3.43	1.02	1.44
	SM	16.41	6.26	6.47	1.08	1.53
	TB	15.50	6.11	5.82	1.67	0.36
2	LD	10.90	9.92	12.40	1.06	2.19
	SM	13.23	7.43	12.20	0.72	0.74
	TB	18.23	7.47	7.96	0.61	0.21
3	LD	9.92	6.92	4.88	1.44	1.08
	SM	10.24	5.94	5.33	1.25	1.54
	TB	11.55	7.49	4.23	1.56	0.08
4	LD	10.24	10.47	11.93	0.89	0.03
	SM	9.30	8.68	11.39	0.90	1.04
	TB	12.20	9.85	9.71	1.50	0.95
5	LD	11.34	10.54	12.08	0.51	0.27
	SM	11.29	15.52	13.28	0.53	--
	TB	9.13	12.48	9.74	0.55	0.43
6	LD	9.49	17.06	10.27	--	0.49
	SM	7.18	10.18	10.74	--	0.70
	TB	8.82	11.58	13.51	0.92	0.23

[1] LD - *longissimus dorsi*; SM - *semimembranosus*; TB - *triceps brachii*

[2] Shear value expressed as kg/2.54 cm core cooked 7 days post-mortem

[3] Expressed as μg hydroxyproline/g muscle tissue

Previous work in this laboratory by McClain *et al.* (1965), studying acid- and salt-soluble collagens in shank muscle from bovine animals, revealed that salt-soluble collagen decreased with postmortem aging while acid-soluble collagen increased. The above data did not reveal this relationship; however, the quantities of extractable collagens differed tremendously between shank muscles and those muscles studied here. As reported earlier, the amount of salt- and acid-soluble collagen in shank muscles did not differ significantly between tenderness groups. These data failed to reveal consistent relationships with regard to changes upon aging or with relation to shear value of each respective muscle.

Summary

The relationship between reticular fiber intactness scores of cooked and uncooked tissues, acid- and salt-soluble collagen fractions and shear values (Warner-Bratzler) of selected bovine muscles was examined. Staining procedures used for reticular fibers were modifications of the method of Soule, 1962. Acid- and salt-soluble collagen fractions were determined at 1 hr. and 168 hrs. postmortem by modified procedures of Woessner *et al.*, 1961. *Longissimus dorsi* muscles were selected on the basis of shear values 7 days postmortem and classified as low shear value (less than 10.6 kg on a 2.54 cm core) and high shear value (more than 17.5 kg on a 2.54 core). Reticular fiber intactness scores ranged from one through five with one representing 0 percent intactness and five representing 100 percent intactness.

Reticular fiber intactness scores were significantly different ($P < .05$) between shear value groups and between cooked and uncooked tissues of the low shear value group. However, reticular fiber intactness scores did not differ significantly between cooked and uncooked tissues within the high shear value group. The correlation coefficient ($r = .49$) between reticular fiber intactness score and shear value was significant ($P < .05$).

No significant relationship was observed between amounts of acid- and/or salt-soluble collagen fractions and shear values of LD, SM or TB muscles. Quantities of acid- and salt-soluble collagens were extremely small in these muscles and these limited data revealed no consistent change in these fractions with postmortem aging.

Literature Cited

Gross, J. 1961 Collagen. Scient. Amer. 204(5):88.

McClain, P.E., A.M. Mullins, S.L. Hansard, J.D. Fox and R.F. Boulware. 1965. Relationship of Alkali-Insoluble Collagen to Tenderness of Three Bovine Muscles. J. Animal Sci. 24:4, 1107.

McClain, P.E., A.M. Mullins, S.L. Hansard, J.D. Fox and R.F. Boulware. 1965. Acid- and Salt-soluble Collagen in Bovine Muscle. Soc. Exp. Biol. and Med. 119:492.

Orekhovitch, V.N. and V.O. Shpikiter. 1957. Procollagens as Biological Precursors of Collagen and the Physicochemical Nature of These Proteins. Coun. Tis. Sym. Backwell Publications, Oxford.

Soule, J.D. 1962. Direct Staining of Reticular Fibers with Gold Chloride. Stain Tech. 37:31.

Woessner, J.F., Jr. 1961. The Determination of Hydroxyproline in Tissue and Protein Samples Containing Small Protein of This Amino Acid. Arch. Biochem. Bioph. 93:440.

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