INTRAMUSCULAR COLLAGEN CONTENT AND SOLUBILITY: THEIR RELATIONSHIP TO TENDERNESS AND ALTERATION BY POSTMORTEM AGING

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INTRODUCTION

Numerous workers have reported the tenderness of beef to be significantly related to the amount of the connective tissue (stromal) component of the muscle (Jeremiah, 1978). Relative to this Cross et al. (1973a, b) reported that the content and solubility of collagen and elastin within a beef muscle were the main determinants of the stromal component of tenderness; and Reagan et al. (1976) concluded that the animal's chronological age and the total collagen content of the muscle accounted for the largest proportion of the variation observed in the tenderness of beef longissimus dorsi muscles. Alexander and Fox (1975) observed that the amount of residual connective tissue after cooking was significantly and inversely related to both raw and cooked beef tenderness. However, Pfeiffer et al. (1972) reported that tenderness was more closely related to the amount of cross-linking within and between collagen molecules than to the total collagen content or the proportion of soluble collagen present. Numerous other workers have also emphasized the importance of the proportion of thermally stable to labile bonds in the collagen molecule to tenderness (Jeremiah, 1978). However, various other reports have indicated that neither collagen content nor collagen solubility were significantly related to the tenderness of beef muscle (Jeremiah, 1978).

Many other investigators have documented that postmortem storage (aging) at refrigeration temperature significantly improved the tenderness of beef (Jeremiah, 1978). In addition, Lin et al. (1976) indicated that the deleterious effects of the cross-linking capacity of collagen could be completely offset by proteolytic enzyme activity; and Laakkonen et al. (1969) established the existence of a natural collagenolytic enzymatic activity within the water-soluble fraction of beef muscle. Stanley and Brown (1973) concluded that 13 days of aging increased the solubility of intramuscular collagen by 29%, and various other workers have reported a reduction in the amount of adhesion between muscle fibers (McIntosh, 1967) and alterations of collagen solubility (Dutson et al., 1974; McClain et al., 1965, 1970; McIntosh, 1967; Pfeiffer et al., 1972) with postmortem aging. However, other reports have indicated that the tenderization associated with postmortem aging was not due to changes within the stromal proteins (Wierbicki et al., 1954; Davey and Gilbert, 1966), or that the solubility of collagen was not affected by either the temperature or time of aging (Pierson and Fox, 1976; Herring et al., 1967; Pfeiffer et al., 1972; Wierbicki et al., 1954).

In view of the contentious nature of these findings the present study was designed to determine the relationship of intramuscular collagen content and solubility to bovine muscle tenderness, the interrelationships of collagen contents and solubilities among muscles, and the effect of postmortem aging upon the intramuscular contents and solubilities of beef muscles.

MATERIALS AND METHODS

The longissimus dorsi (LD) muscles from 108 crossbred bovine carcasses (36 bulls, 36 heifers, and 36 steers) predominantly 1/2 or 3/4 blood Charolais, Simmental, or Chianina, ranging in chronological age from 11 to 15 months, and with carcass weights of approximately 270 kg were sampled at various postmortem intervals (1, 24, 144, 312, and 480 hr). The semitendinosus (ST) muscles of the steer carcasses were also sampled after 144, 312, and 480 hr of postmortem aging. Prerigor LD samples (1 hr) were removed as a 10 cm thick muscle section from the region of the 12th thoracic vertebra of the right side of each of the 108 carcasses. The shortloins from the left sides of each of the 108 carcasses and the ST muscles from the left sides of each of the 36 steer carcasses were removed after chilling for 24 and 144 hr, respectively, at 0°C, and transferred to a holding cooler (2°C) for the remainder of the aging period. Although a tenderness gradient had been shown to exist along the longitudinal axis of the beef LD muscle (Martin et al., 1970) and the possibility that such a gradient also existed for collagen content and/or solubility, the shortloins were aged intact and samples were removed anterior to posterior starting at the 12th thoracic vertebra, in order to simulate commercial aging of wholesale cuts as closely as possible.

A thin (approximately 3 mm) slices and a 3.5 cm thick steak were removed from the anterior end of each prerigor muscle section and shortloin and from the center of each ST muscle upon removal from the carcass. After each subsequent aging interval the dehydrated surface was removed from the anterior end and center of each shortloin and ST muscle, respectively, and a thin cross-sectional slice (approximately 3 mm) and a 3.5 cm thick steak were removed.

Upon removal all steaks were cooked in a microwave oven to an internal temperature of approximately 75°C (±3°C), and refrigerated overnight at 2°C, prior to removing 3 cores 2 cm in diameter. Each core was then sheared using the Ottawa Texture Measuring System (L'Hirondelle and Martin, 1975) and the mean shear force value from the 3 cores was recorded.

All of the subcutaneous fat and epimysium was removed from the LD muscle of each 3 mm slice and a 15 gm subsample was removed from the central portion of the muscle cross-section and freeze dried, using a Virtis 14-port manifold freeze dryer attached to a Duo-Seal model R-1405 vacuum pump, for approximately 48 hr or until completely dry (Jeremiah et al., 1980). Each subsample was weighed following the freeze drying procedure to determine moisture loss, fragmented with a Virtis model "45" homogenizer (5 to 10 sec at high speed, i.e. approximately 40,000 rpm), and separated into heat-soluble and insoluble fractions following the procedure

of Hill (1966). The individual muscle fractions were then hydrolyzed for 15 hr in 6N hydrochloric acid in sealed pyrex culture tubes; and the hydroxyproline content of the individual hydrolyzates were determined following the procedure of Woessner (1961). The collagen contents of the individual fractions were determined by multiplying the hydroxyproline contents by 7.25 as described by Goll et al. (1964); and the total collagen content for each muscle sample was obtained by combining the collagen contents of the heat-soluble and insoluble fractions. The percent soluble collagen in each muscle sample was determined using the technique of Hill (1966).

Simple correlation coefficients were obtained through linear regression analyses and numerical differences between means were tested for significance using the Student "t" test (Steele and Torrie, 1960).

RESULTS AND DISCUSSION

Shear force values from the LD decreased 39.3% ($P < 0.05$) from 1 to 144 hr postmortem, 13% ($P < 0.05$) from 144 to 312 hr, and 8.5% ($P < 0.05$) from 312 to 480 hr, and from the ST they decreased 19.9% ($P < 0.05$) from 144 to 480 hr (Figure 1). Such findings lend support to the reports of numerous other workers that postmortem aging significantly improved tenderness (Jeremiah, 1978). In addition, it is possible that the tenderization produced in the LD by aging was in fact greater than has been shown in the present study, due to the method of sampling, since Martin et al. (1970) demonstrated that the tenderness of the LD decreased anteriorly to posteriorly along the longitudinal axis of the portion of the muscle sampled.

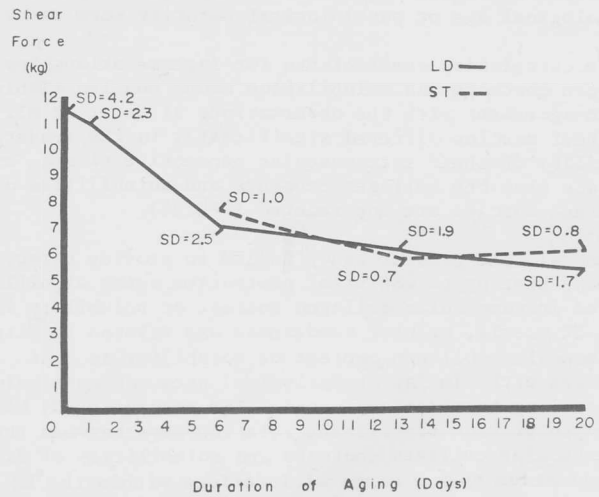

Fig. 1 Effect of storage at 2°C on mechanical shear force values.

Although total collagen content of the LD increased by 13.9% ($P < 0.05$) on a fresh-weight basis and by 11.2% ($P < 0.05$) on a moisture-free basis between 144 and 312 hr, the total collagen content of the ST did not differ significantly ($P > 0.05$) among postmortem sampling periods on either a fresh-weight or moisture-free basis (Figures 2 and 3). It is probable that the increase observed in the collagen content of the LD was attributable to an anatomical effect, since sampling proceeded posteriorly from the 12th thoracic vertebra, rather than from any change in composition within the muscle due to postmortem aging. Thus it would appear that these differences in total collagen content masked any alteration in collagen content that may have been produced by postmortem aging. The substantially higher collagen content of the ST muscle lends support to the reports of Cover et al. (1962) and Cover and Smith (1956) that muscles differed significantly in their collagen contents.

The percentage of soluble collagen increased in the LD by 12.1% ($P < 0.05$) between 24 and 480 hr but decreased by 16.1% ($P < 0.05$) in the ST between 312 and 480 hr (Figure 4). The increase in the proportion of heat-soluble collagen in the LD with aging, observed in the present study, provides some support for the findings of Stanley and Brown (1973) that 13 days of postmortem aging substantially increased the solubility of intramuscular collagen. However, the inconsistency of response observed between the two muscles evaluated lends greater support to the reports of various other workers that postmortem aging produced little effect on collagen solubility (Herring et al., 1967; Pfeiffer et al., 1972; Pierson and Fox, 1976; Wierbicki et al., 1954; etc.). The significant differences in the proportion of soluble collagen between the LD and ST are in accordance with the previous report of Field and Pearson (1969) that collagen solubility differed significantly between muscles.

The simple correlation coefficients for relationships of intramuscular collagen content and solubility with shear force values presented in Table 1 indicate that neither collagen content nor solubility of the LD and ST

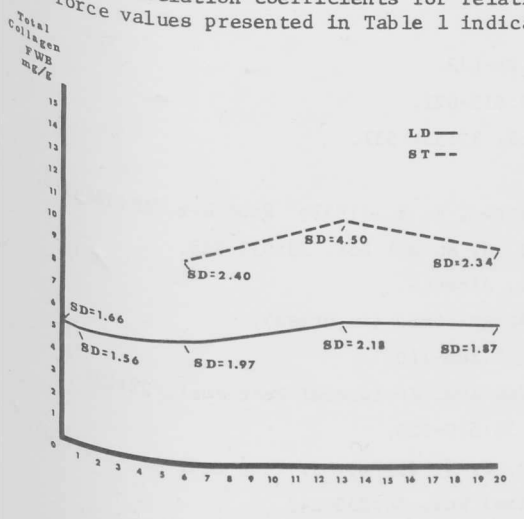

Fig. 2 Effect of storage at 2°C on total collagen content on a fresh-weight basis.

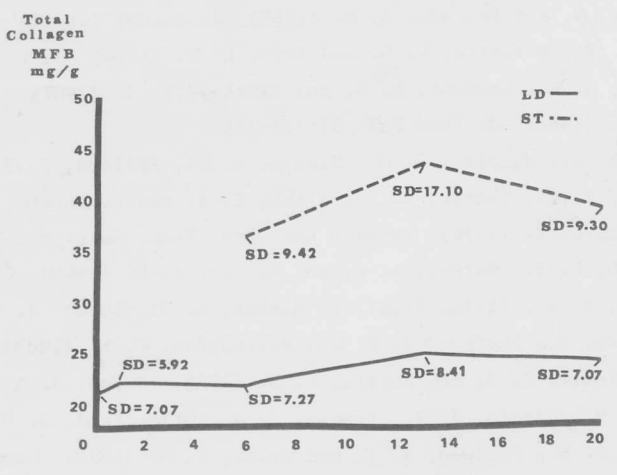

Fig. 3 Effect of storage at 2°C on total collagen content on a moisture free basis.

were significantly related to the tenderness of these muscles. Such findings are in agreement with the reports of other workers that neither collagen content (Field, 1968; Herring et al., 1967; Hunsley et al., 1971; Kruggel et al., 1970; etc.) nor collagen solubility (Rea et al., 1970; Stewart et al., 1974; etc.) were related to the tenderness of beef muscle. In addition, they concur with the conclusions of Wierbicki et al. (1954) and Davey and Gilbert (1966) that the tenderization produced by postmortem aging was not due to changes within the stromal proteins. However, such results are in general disagreement with numerous other reports of significant relationships between the tenderness of beef and collagen content and solubility (Jeremiah, 1978). Similarly the conclusions of Hiner et al. (1953) and Reagan et al. (1976) that the amount and character of the intramuscular connective tissue were closely related to beef tenderness, and that the animal's chronological age and the total collagen content of the muscle were the most important determinants of the variability associated with the tenderness of bovine LD muscle, respectively, are not supported by the results of the present study.

However, it should be stressed that the range in chronological age for cattle used in the present study was relatively narrow, which may explain why significant relationships were not obtained between tenderness and intramuscular collagen content or solubility. Such a conclusion is in accord with the report of Herring et al. (1967) that the influence of collagen solubility upon tenderness lost significance when the effect of chronological age or physiological maturity were removed.

Simple correlation coefficients for interrelationships of collagen contents and solubilities among muscles (Table 2) are in agreement with the observations of Cover et al. (1962) that beef muscles differed significantly in the tenderness and solubility of their intramuscular connective tissue, and indicate that the collagen contents and solubilities of different muscles are not related ($P>0.05$).

In general, the present study failed to provide direct evidence that up to 480 hr of postmortem aging significantly altered intramuscular collagen content or solubility in the LD or ST muscle, or that tenderness was related to either intramuscular collagen content or solubility in beef carcasses with similar chronological ages, carcass weights, and breeding to those evaluated. The present study has also failed to provide significant relationships between the intramuscular collagen contents and solubilities of different muscles. The present study did however provide an indication that anatomical locations within the LD differ in their total collagen contents and that further research is required to substantiate and document such differences.

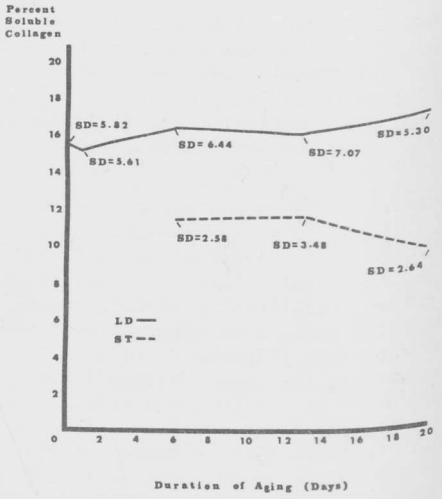

Fig. 4 Effect of storage at 2 °C on the percent soluble collagen

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Table 1. Simple Correlation Coefficients for Relationships of Intramuscular Collagen Content and Solubility with Shear Force Values after Various Intervals of Postmortem Aging

	Muscle							
	Longissimus dorsi				Semitendinosus			
	Aging Interval[1]							
Relationship	1	24	144	312	480	144	312	480
Soluble Collagen/Shear Force	-.03	0.05	-.06	0.04	-.10	-.01	-.05	-.15
Insoluble Collagen/Shear Force	-.17	-.10	-.14	-.01	-.01	0.11	-.02	0.06
Total Collagen/Shear Force	-.14	-.06	-.13	0.00	-.03	0.10	-.02	0.04
% Soluble Collagen/Shear Force	-.01	0.09	0.08	0.06	-.13	-.12	-.08	-.16

[1] Hours

Table 2. Simple Correlation Coefficients for the Relationships of Intramuscular Collagen Content and Solubility among Muscles at Various Aging Intervals

	Aging Interval[1]		
Relationship	144	312	480
LD Soluble Collagen/ST Soluble Collagen	0.20	-.19	0.09
LD Insoluble Collagen/ST Insoluble Collagen	-.23	0.04	-.08
LD Total Collagen/ST Total Collagen	-.20	-.01	-.09
LD % Soluble Collagen/ST % Soluble Collagen	0.21	-.03	0.02

[1] Hours