Differential Scanning Calorimetry of Beef Muscle: Influence of Sarcomere Length

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ABSTRACT

Contraction state of beef muscle at onset of rigor influences tenderness of cooked meat. Loss in tenderness during cooking has been related, through use of differential scanning calorimetry (DSC), to thermal denaturation of myofibrillar proteins. Contraction of beef sternomandibularis muscle was controlled at sarcomere lengths of 2.4, 2.1, 1.9, 1.7, and 1.4 μm. Samples were scanned from 25-105°C at 10°C/min; ΔH (change in heat of transition) between 45° and 92°C dropped from ca. 4 J/g muscle at 2.4 μm to ca. 3 J/g at 1.4 μm. This difference ($P < 0.05$) amounts to less than 1% of the total energy required to heat meat from 45° to 92°C. The decrease is attributed to a greater actomyosin contribution to the overall thermal curve resulting from increased overlap of the filaments.

INTRODUCTION

THE STATE OF muscle contraction at the onset of rigor has been associated with meat tenderness since Ramsbottom and Strandine (1949) concluded that shortened beef muscle was tougher than similar muscle that had been stretched before rigor. Subsequent studies have related the loss of tenderness to the increased overlap of thick and thin filaments and the concurrent decrease in sarcomere length (Locker, 1958). Cold shortened beef was found to require four times the shear force of rest length muscle (Marsh and Leet, 1966). The contribution of myofibrillar proteins to overall meat tenderness has received a great deal of attention since commercial conditioning reduces myofibrillar toughness while the background toughness due to connective tissue is largely unaffected (Penny, 1980).

Regardless of the improvement in tenderness caused by conditioning, final assessment of meat quality can only be made after cooking. The effect of cooking on tenderness hass been explained on the basis of denaturation of muscle proteins and dehydration (Hamm, 1966). Recently, differential scanning calorimetry (DSC) has been applied to the muscle system to investigate the fundamental thermodynamic changes associated with the heating of meat. The endothermic transitions observed in muscle have been attributed to the principal protein constituents (Martens and Vold, 1976; Wright et al., 1977; Stabursvik and Martens, 1980). These transitions in normal (pH 5.4) post-rigor beef muscle have been related to sensory panel response (Martens et al., 1982), but in none of these studies has contraction state been considered.

The objective of this study is to examine the effect of the contraction state of beef muscle on DSC thermal curves.

MATERIALS & METHODS

Samples

Beef neck muscle (sternomandibularis) was obtained immediately postmortem from four 18 month-old Charolais crossbreed heifers at the University abattoir. Each muscle was divided laterally into four sections and wrapped in damp paper towel to prevent surface dehydration. A cold shortened sample was prepared by refrigerating at 5°C. The remaining samples were stretched to different tensions, restrained with cord, wrapped in damp paper towel and held at 20°C for 8 hr before refrigerating at 5°C for 2, 4, 6 and 8 days.

Laser diffraction

Individual muscle fibers were teased from the beef samples and diffraction measurements were made using the method of Varcoe and Jones (1983). Ten measurements were taken to provide an average sarcomere length. Five groups of samples were selected at the following sarcomere length; 1.4 μm, sd = 0.09; 1.7 μm, sd = 0.07; 1.9 μm, sd = 0.11; 2.1 μm, sd = 0.09 and 2.4 μm, sd = 0.13.

Differential scanning calorimetry

A portion of muscle (ca. 5 mg) from the same muscle fiber used for laser diffraction measurement was transferred to an aluminum hermetic pan and weighed to within 10 mcg using a Model G Cahn electrobalance. To adjust for small natural variations in pH, ensure an excess of moisture and promote good thermal contact, 5 μL of 0.07M Sorenson's phosphate buffer (pH 5.4) were included in the pan before sealing. The quantity of buffer was selected to give approximately 1:1 proportions. Stabursvik and Martens (1980) showed that pH of the muscle affected the resultant thermal curve; they concluded that buffers could be used to adjust muscle samples of different natural pH values to a common pH. Wright and Wilding (1984) concluded that unless samples are studied under equivalent conditions, the results obtained are not necessarily comparable. Sorenson's phosphate buffer was used for the preparation of beef muscle for electron microscopy. Consequently it was selected for use in DSC at a pH of 5.4 to assure that the muscle was in the lower range of pH of normal post-rigor muscle. The characteristic exotherm between 45° and 55°C found in pre-rigor muscle and disappearing with rigor (Wright et al., 1977) was absent in all samples used in this study. A Dupont Model 1090 Differential Scanning Calorimeter (DSC) with a pressure DSC cell was used to scan the samples from 25-105°C at 10°C/min under ambient pressure with a nitrogen flush of 25 mL/min. The differential heat flow was recorded at 0.2 sec intervals on a magnetic disk for subsequent computerized analysis. Each sample was cooled and rescanned to provide a measure of the reversible transitions and a baseline for analysis. Plots of heat flow versus temperature were obtained for each specimen. Data analysis programs were applied to the thermal curves to provide heat of transitions (ΔH) and temperatures of maximum transition (T_{max}). Rescanned thermal curves were subtracted from initial runs to compensate for the change in baseline. All thermal curves were normalized to 10 mg of whole muscle (80% moisture) to facilitate plotting of thermal curves and permit comparison. Analysis of variance and multiple linear regression were performed using the Statistical Analysis System (Helwig and Council, 1979).

RESULTS

STRETCHING AND RESTRAINING MUSCLE does not produce uniform sarcomere length, but, by taking samples for DSC from the same fiber used for measurement of sarcomere length it was possible to classify samples into sarcomere length groups. Three samples were taken from within each group at 2, 4, 6 and 8 days postmortem for each animal. The thermal curves were analyzed in two different ways. Discrete data for ΔH values and T_{max} were obtained over a range of 45-92°C. Partial area analysis provided the T_{max} and ΔH values for individual transitions

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that fell within integration limits of 45-60°C (T_1), 60-76°C (T_2) and 76-92°C (T_3). The means and standard deviations of the data for sarcomere length are shown in Table 1. Analysis of variance results are given in Table 2. A linear model was applied to determine the contribution of each source of variation to the regression model (Table 3).

Individual thermal curves for each sarcomere length group were combined and normalized to 10 mg of wet muscle for the baseline corrected scans (Fig. 1). Since muscle thermal curves are not comprised of discrete events, but are a net response resulting from the overlapping transitions of the protein constituents, the second derivative was plotted to help locate subtle changes in heat flow hidden in the shoulders of larger transitions (Fig. 2). To examine the difference between the extremes of sarcomere length the thermal curve of the 2.4 μm sample was subtracted from that of the 1.4 μm sample to yield a thermal curve of the difference (Fig. 3).

The first transition, (T_1), which has been attributed to myosin (Wright et al., 1977; Stabursvik and Martens, 1980), decreased significantly ($P < 0.05$) with increased sarcomere length. The heat of transition (ΔH_1) increased significantly as sarcomere length increased from 2.1 to 2.4 μm. The T_2 results showed no significant difference due to sarcomere length; however, the contribution of sarcoplasmic proteins, actomyosin and connective tissue to this transition makes interpretation difficult. The heat of transition, ΔH_2, increased significantly with sarcomere length. The actin transition, T_3, increased significantly with increased sarcomere length, indicating greater stabilization of this protein. ΔH_3 also increased significantly with sarcomere length. The total heat flow over the range 45-92°C increased significantly from 2.94 J/g at 1.4 μm to 4.04 J/g at 2.4 μm ($P < 0.05$). These enthalpies are in the range of those found by Wright et al. (1977) for muscle and constituent proteins. No significant difference was found between animals for any of the variables tested (Table 2).

The relative contribution of the independent variables sarcomere length, aging and animal to a linear regression model (Table 3) showed that aging had the greatest effect on transition temperatures while sarcomere length influenced the heat of transition, particularly ΔH_3. The contribution of interation to R^2 was relatively small with the exception of T_3.

Second derivative analysis of the thermal curves (Fig. 2) indicated the emergence of a transition below 60°C in the 1.4 μm curve which was not apparent at 2.4 μm. The pattern of the 1.9 μm curve shows a doublet in the vicinity of 54°C which warrants further investigation. A direct subtraction of the 2.4 μm thermal curve from the 1.4 μm thermal curve gave a thermal difference curve that might explain some of the differences due to sarcomere length. The T_{max} values for the thermal curve are 54°, 65°, 80°, and 85°C; the temperatures cited by Wright et al. (1977) for actomyosin are 54°, 65°, and 80°C. Greater overlap of thick and thin filaments will increase the probability of interaction between actin and myosin. Wright et al. (1977) found actomyosin to require 20% less heat than whole muscle and 30% less than myofibrils. On this basis it may be hypothesized that greater overlap associated with shorter

Table 1—Effect of sarcomere length on T_{max} and ΔH values in beef sternomandibularis muscle

Sarcomere length (n = 12)		Transition Temperature °C			Heat of transition J/g			
		T_1	T_2	T_3	ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
1.40		57.03 a*	66.07 a	82.38 a	0.551 a	1.502 a	0.891 a	2.943 a
	(Std. Dev.)	(2.75)	(0.91)	(0.29)	(0.073)	(0.302)	(0.114)	(0.347)
1.70		57.38 a	66.18 a	82.21 a	0.615 a	1.598 a	0.959 a	3.173 a,b
		(2.38)	(1.06)	(0.36)	(0.135)	(0.381)	(0.133)	(0.543)
1.90		56.63 a,b	66.48 a	82.83 b	0.633 a	1.700 a,b	0.990 a	3.323 b,c
		(1.70)	(1.31)	(1.06)	(0.123)	(0.404)	(0.148)	(0.450)
2.10		55.49 b	66.09 a	82.93 b	0.622 a	1.810 a,b	1.156 b	3.588 c
		(3.26)	(1.35)	(1.10)	(0.135)	(0.379)	(0.153)	(0.469)
2.40		55.59 b	66.62 a	82.88 b	0.742 b	2.028 b	1.277 c	4.047 d
		(1.99)	(1.37)	(0.72)	(0.116)	(0.473)	(0.140)	(0.571)

* Means in the same column with the same letter are not significantly different $P < 0.05$ using Duncan's Multiple Range Test.

Table 2—Analysis of variance of beef sternomandibularis DSC data

Source	d.f.	T_1	T_2	T_3	ΔH_1 Probability	ΔH_2	ΔH_3	ΔH_{Total}
Sarcomere length	4	0.1765	0.6882	0.0032	0.0008	0.0050	0.0001	0.0001
Aging	3	0.0199	0.0303	0.0001	0.0001	0.0005	0.0056	0.0001
Animal	3	0.1541	0.3288	0.1560	0.4602	0.6197	0.8487	0.9215
Error	23							
Total	59							

Table 3—Contributions to R^2 for a linear model composed of sarcomere length, age and animal

Source*	T_1	T_2	T_3	R^2 (%) ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
Sarcomere length	6.70	1.64	8.39	19.42	18.53	49.59	38.92
Aging	11.10	5.13	37.86	25.10	22.99	10.13	29.80
Animal	0.98	0.96	2.36	0.04	8.73	1.33	6.14
Total**	20.82***	7.02	56.17***	48.17***	42.68***	60.18***	68.73***

* d.f. for linear model = 10; ** Includes interactions; *** $P < 0.01$.

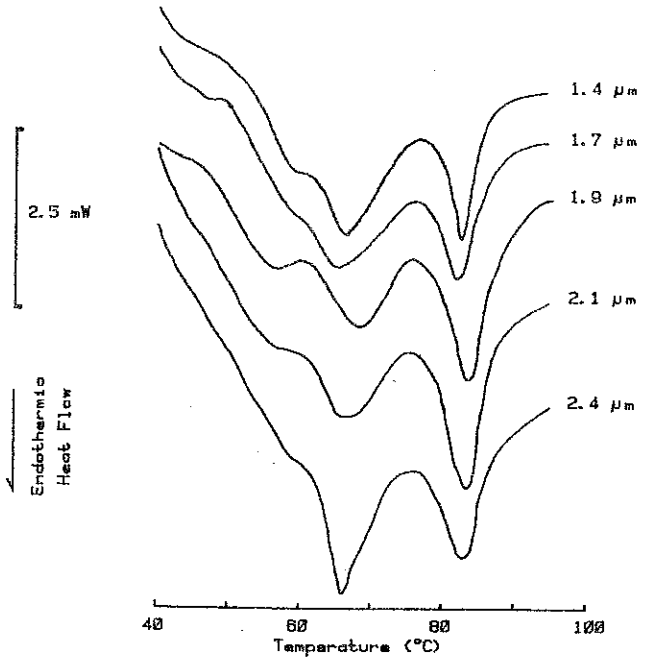

Fig. 1—DSC thermal curves of beef sternomandibularis muscle as a function of sarcomere length. Each curve represents the addition of 12 samples normalized to 10 mg of wet muscle.

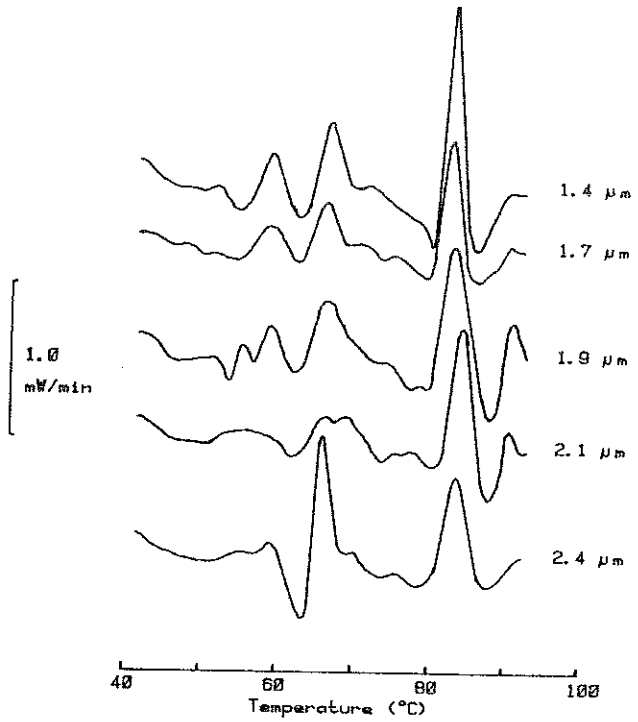

Fig. 2—Second derivative curves of beef sternomandibularis muscle DSC thermal curves as a function of sarcomere length.

sarcomere length leads to a greater interaction between actin and myosin. Thereby resulting in a greater actomyosin contribution to the overall muscle thermal curve reducing the total heat of transition. The heat contribution of the actin transition is affected the most since F-actin has a higher T_{max} (Wright et al., 1977) and thus a concomitant higher stability.

The contribution of aging to the shift in transition temperature and decrease in ΔH is demonstrated by the data in Table 3. The magnitude of the effect on R^2 suggests that the tenderization due to proteolysis also causes a reduction in the ΔH values in meat.

SUMMARY & CONCLUSIONS

THE MAJOR THERMAL EFFECT of the contraction state of beef sternomandibularis muscle, measured as a decrease in sarcomere length from 2.4 to 1.4 µm, is a reduction in ΔH from 4 J/g to 3 J/g. This change appears to be due to the increase in overlap of thin and thick filaments. The major contribution to this decrease in ΔH is found in the actin transition (from 1.3 J/g to 0.9 J/g, $P < 0.05$) indicating a reduction in the stability of actin when it interacts with myosin as actomyosin, instead of aggregating with itself.

The effect of aging on the thermal properties of muscle is in keeping with the current understanding of the enzymology of meat conditioning (Penny, 1980). Proteolysis breaks down the structure of the sarcomere making it more heat labile. However, a more extensive examination of the effect of conditioning on the thermal properties of beef is warranted.

Meat is usually cooked at a fixed external temperature for a predetermined time or by monitoring the internal temperature. It is apparent that contraction state will have an influence on the temperature at which the proteins undergo denaturation and the resultant loss in tenderness. The difference in ΔH value between contracted and rest length muscle is approximately 1 J/g. The heat capacity of beef is related to its moisture content and is in the vicinity of 3.3 J/g °C (Mohsenin, 1980). Over the temperature range used

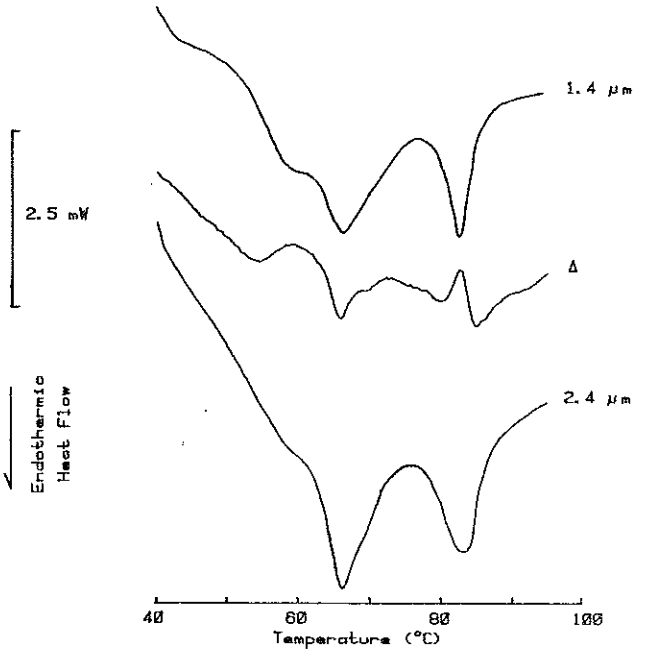

Fig. 3 — The difference in net heat flow between beef sternomandibularis muscle of sarcomere length 2.4 and 1.4 µm.

for integration of ΔH, 45-92°C, the total heat required to increase the temperature would be 155 J/g. It is clear from this that the variation in heat required for denaturation of protein will contribute less than 1% to the total heat required in cooking. However, the stabilizing shift of the actin transition towards a higher denaturation temperature that was observed with increased sarcomere lengths may have a noticeable effect on the tenderness of cooked meat. This relationship shall be the subject of further investigation.

—Continued on page 1534

cantly at the 5% level by time and treatments with mean values for the steam treated kernels being significantly greater than values for the other treatments (Table 1).

The b values (Fig. 1) indicate that all heat treatments caused an initial decrease in yellowness of the kernels in relation to the control. Mean b values for all treatments over time were significantly less than the control, but b values for D-1, D-2, and steam treated kernels were not significantly different.

The hue angle ($\theta = \tan^{-1}$ b/a) and saturation index [SI = $(a^2 + b^2)^{1/2}$] are more effective for predicting visual color appearance than either the L, a, or b values alone (Little, 1975). Values of θ for pecan kernels fall within the first quadrant of the Hunter a, b diagram; therefore, kernels with higher values of θ appear more golden while kernels with lower values of θ appear more reddish brown (Forbus et al., 1983).

Initial values of θ for dielectrically heated kernels were considerably higher than for the steam treated kernels (Fig. 1), again indicating the darkening and reddening effects of the steam heat treatment. Hue of the kernels changed significantly with time and treatment, with mean values for the D-3 and steam treated kernels being significantly lower (or more reddish brown) than kernels subjected to the other treatments. Values for θ at T_{16} decreased progressively with increasing kernel temperature of the dielectric heating treatments, resulting in final values of 48, 46.5 and 45°, respectively (Fig. 1). Values for all treatments and the control at T_{16} fell within the "medium brown" color classification as related to values established previously (Forbus et al., 1983).

The differences in kernel color saturation are indicated in Fig. 1 by values of SI at T_0 and T_{16} for the various treatments. SI values for the steam treated kernels and the control kernels were significantly higher initially and indicated a more saturated reddish brown and golden color, respectively, for these treatments than was apparent in the dielectrically heated kernels. However, the control and steam treated kernels underwent a greater loss in color saturation with time than did the dielectrically heated kernels at T_{16}. Mean values of SI for all treatments over time (Table 1) indicate nonsignificant differences in color saturation for kernels receiving the dielectric heating treatments.

CONCLUSION

OUR RESULTS show that the heating of pecan kernels in a high moisture atmosphere causes greater changes in kernel color initially and during storage than equivalent treatment in a low moisture atmosphere. Differences are apparently due to enhancement of the oxidative transformation of color precursors in the testa of the kernels (Senter et al., 1978) by heat in the presence of high moisture since leaching of tannins from the shells of the steam treated pecans was not apparent in this study.

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DSC OF BEEF MUSCLE . . . From page 1531

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