THE USE OF DIFFERENTIAL SCANNING CALORIMETRY TO PREDICT SARCOMERE LENGTH

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Story in Brief

The thermal profiles of 5 different muscles (Infraspinatus, Triceps brachii, Supraspinatus, Longissimus dorsi, and Psoas major) as obtained by differential scanning calorimetry were analyzed for their relationship to sarcomere length. Regression equations to predict sarcomere length were developed using measurements associated with the transition temperatures, heat flows and energies. Analyses were performed on 6 cores from each muscle. No differences were observed between muscles for the first 2 transition temperatures. The Longissimus dorsi showed the lowest temperature of transition at the third peak of the profile. The greatest amount of heat flow was observed at the second transition temperature when the Infraspinatus was analyzed. Multivariate regression indicated that differential scanning calorimetry thermograms can account for a large amount of the variability in sarcomere length but the variables are not the same for all muscles. These data suggest that the differential scanning calorimeter may be useful to determine factors responsible for tenderness in beef muscles.

(Key Words: Differential Scanning Calorimetry, Sarcomere Length, Tenderness, Beef.)

Introduction

The meat industry has historically been concerned with factors that influence tenderness of retail cuts of beef, such as postmortem processing conditions and state of muscle fiber contraction (Locker, 1958). Many investigators feel that much information is still unclear and attempts to determine factors related to tenderness of muscle tissue is still in need of refinement. The use of differential scanning calorimetry (DSC) may provide the meat industry with more a reproducible and quantitative method for characterizing properties of muscle systems. Some research has provided

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information about the relationship between beef tenderness and sarcomere length. Studies have related an increase in tenderness to an increase in sarcomere length (Herring et al., 1965). The influence of sarcomere length, or state of muscle contraction, was investigated using differential scanning calorimetry (Findlay et al., 1984) and it was noted that a possible relationship exists. This research provides insight into the possible relationships between sarcomere length and heat denaturation with associated thermal transition states. The purpose of this work was to study the effect of muscle type on sarcomere length and differential scanning calorimetric thermograms.

Materials and Methods

Five beef muscles (Infraspinatus, Triceps brachii, Supraspinatus, Longissimus dorsi, and Psoas major) were obtained from beef carcasses (3-7 days postmortem) processed at the University abattoir. After excision, the individual muscles were cut immediately into uniform 1" steaks. Adjoining steaks were assigned for DSC analysis, vacuum packaged and stored overnight at 40 +/- 3 ^{o}F.

Six cores were taken from each steak for DSC analysis. A Black and Decker 1/4" Standing Utility Drill equipped with a 1/4" coring bit was used to excise the cores from each steak. These cores were placed in sterile Whirl-Pak bags and frozen. Care was taken to uniformly sample each muscle in order to accurately reflect the characteristics of the entire muscle. Major fat and connective tissue deposits were avoided. Once cores were removed the remaining steak was trimmed of all excess visible fat and connective tissue and frozen for further analysis.

Differential Scanning Calorimetry

Sample cores were removed from the freezer, carefully positioned such that precise removal of only lean tissue was accomplished using a dissecting scalpel and forceps thereby reducing the incorporation of excessive connective tissue or intermuscular fat. Lean tissue samples were then placed in preweighed Perkin-Elmer volatile aluminum sample pans and crimped using a Perkin-Elmer volatile sealer assembly. Care was taken to pack the tissue in the sample pans such that the tissue was in direct contact with the entire bottom in order to eliminate the possible variation in heat transfer which might occur if not all of the surface area of the pan is utilized. Samples were then

placed in the platinum-iridium sample furnace of a Perkin-Elmer DSC7 differential scanning calorimeter with the reference furnace containing an empty aluminum volatile sample pan. The system was calibrated using Indium (m.p.=313.88^{O}F, J/g=28.45) and Zinc (m.p.=786.20^{O}F, J/g=108.37) at a scanning rate of 18^{O}F /min. Nitrogen gas (20cc/min) was used for purging the exhaust away from the DSC sample holder. An ice water bath was used to maintain temperature control. Samples were weighed to the nearest 0.1 mg and scanned from 68^{O}F to 212^{O}F at a scanning rate of 18^{O}F/min. Data collection and analysis were performed using an IBM Personal System/2 Model 55 SX Computer equipped with Perkin-Elmer Thermal Analysis Software. After samples were scanned they were removed from the DSC, punctured and placed in a Fisher Scientific Isotemp Oven (Model 655F) overnight at 212 ^{O}F and reweighed for dry weight determinations.

Sarcomere Length

Remaining DSC sample cores were individually analyzed using the laser diffraction method described by Cross et al. (1981). Twenty-five measurements were taken from each prepared sample and mean sarcomere length was calculated to the nearest 0.01 um.

Statistical Analysis

Data were analyzed using analysis of variance least squares, fixed model procedure. Prediction equations were generated using multiple linear forward stepwise regression to maximize R^2 by the Statistical Analysis System (SAS, 1986).

Results

Variables T1, T2, and T3 represent the three observed transition temperatures of the meat sample thermograms (Figure 1). These temperatures represent the peak maxima based on the calculated peak onset and identify the temperature at which protein denaturation occurs. Table 1 indicates the means of transition temperatures observed when samples of the various muscles were subjected to DSC analysis. Transition 1 and 2 (T1 and T2) were not different between muscles. The third transition (T3) was lowest ($P<0.01$) for the Longissimus dorsi when compared to other muscles. The Psoas major was higher at T3 than the Longissimus dorsi. Previous research found that thermal analysis of post-rigor M. sternomandibularis provided three thermal transitions

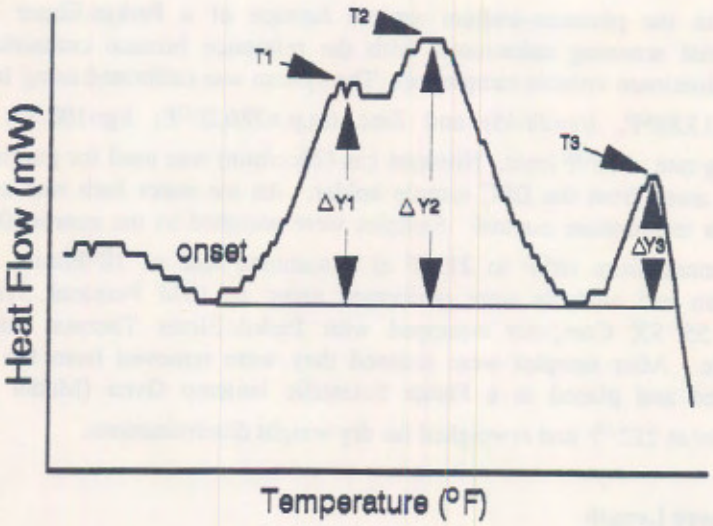

Figure 1. DSC thermogram indicating transition temperatures (T1,T2, T3) and transition heat flow (ΔY1,ΔY2,ΔY3).

Table 1. Transition temperatures (°F) obtained from dsc thermograms of five fresh beef muscles.

	Transition Temperatures (°F)		
Muscle	T1	T2	T3
Infraspinatus	135.18[a]	146.43[a]	175.89[a]
Triceps brachii	133.79[a]	148.28[a]	175.06[a]
Supraspinatus	134.17[a]	147.07[a]	175.60[a]
Longissimus dorsi	133.36[a]	148.60[a]	173.28[c]
Psoas major	134.19[a]	147.52[a]	174.60[b]
SE	0.52	0.67	0.40

[a,b,c] Means within the same column with different superscripts differ ($P<0.01$).

of 134.6 °F, 149.0 °F and 176.0 °F (Wright et al, 1977). The authors further suggested that myosin, sarcoplasmic proteins and actin were responsible for T1, T2, and T3, respectively . The temperature at which these proteins denature are be consistent among muscles.

Transition energies, or enthalpy (J/g) are noted as ΔH1 and ΔH2 and calculated by using the onset temperature and the peak maxima. These values are representative of the area under the thermal transition curves (Figure 2). ΔH1 represents the area under peaks 1 and 2 since separation of the area under these two peaks was difficult due to the complexity of the reactions taking place. ΔH2 is the area under the third peak. The enthalpy associated with the first two peaks (ΔH1) was significantly lower ($P<0.01$) for the Psoas major than for the other muscles, generating 0.89 J/g. These data suggest that less energy was associated with the endothermic reactions involving myosin and sarcoplasmic proteins for the Psoas than for other muscles. The actin transition energy (ΔH2) for the Psoas major and the Infraspinatus were also lower ($P<0.01$) than the Supraspinatus and the Longissimus dorsi. The Triceps brachii averaged 0.50 J/g which was not different ($P>0.05$) from the other muscles. The summation of ΔH1 and ΔH2 corresponds to the total energy (ΔHt) associated with the thermal denaturation of muscle proteins. ΔHt for the Psoas major was significantly lower ($P<0.01$) at 1.32 J/g than for the other muscles. The Longissimus dorsi and the Triceps brachii tended to require the

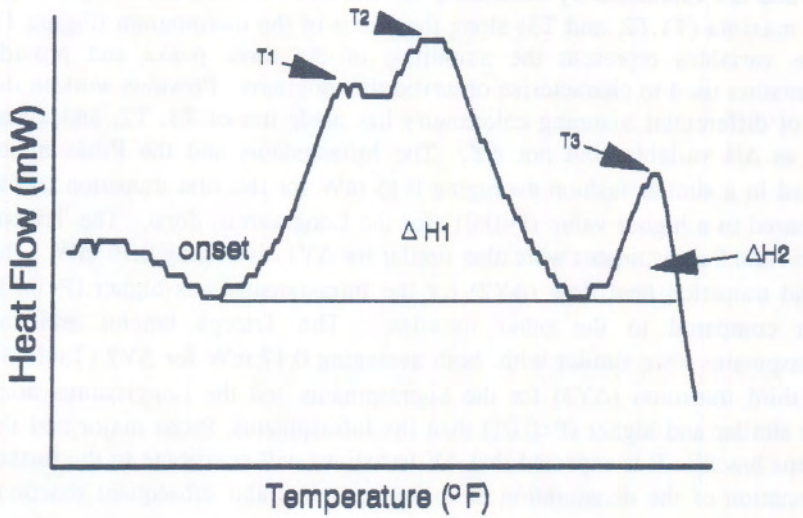

Figure 2. DSC thermogram indicting transition energies (H1, ΔH2).

Table 2. Enthalpy (J/g)values obtained from dsc thermograms of five fresh beef muscles.

	Enthalpy (J/g)	
Muscle	ΔH1	ΔH2
Infraspinatus	1.35[a]	0.47[bc]
Triceps brachii	1.30[a]	0.50[ab]
Supraspinatus	1.30[a]	0.54[a]
Longissimus dorsi	1.20[a]	0.56[a]
Psoas major	0.89[b]	0.43[c]
SE	0.07	0.02

[a,b,c] Means within the same column with different superscripts differ (P<0.01).

next lowest total energy (1.76 and 1.80 J/g, respectively) followed by the Infraspinatus (1.82 J/g) and the Supraspinatus (1.84 J/g).

Transition heat flow (mW) is represented by variables ΔY1, ΔY2, and ΔY3 and are calculated by measuring the distance between the onset and the peak maxima (T1,T2, and T3) along the y-axis of the thermogram (Figure 1). These variables represent the amplitude of the three peaks and provide information used to characterize observed thermograms. Previous work in the area of differential scanning calorimetry has made use of T1, T2, and T3 as well as ΔH variables but not ΔY. The Infraspinatus and the Psoas major reacted in a similar fashion averaging 0.15 mW for the first transition (ΔY1) compared to a higher value (P<0.01) for the Longissimus dorsi. The Triceps brachii and Supraspinatus were also similar for ΔY1, averaging 0.16 mW. The second transition heat flow (ΔY2) for the Infraspinatus was higher (P<0.01) when compared to the other muscles. The Triceps brachii and the Supraspinatus were similar with both averaging 0.17 mW for ΔY2 (Table 3). The third transition (ΔY3) for the Supraspinatus and the Longissimus dorsi were similar and higher (P<0.01) than the Infraspinatus, Psoas major and the Triceps brachii. It is expected that ΔY variations will contribute to the further explanation of the denaturation of muscle proteins and subsequent reactions taking place.

As expected, sarcomere length for the Psoas major was the longest (P<0.01) at 3.27 um followed by the Triceps brachii and the Infraspinatus. The

Table 3. Transition heat flow (mW) obtained from dsc thermograms of five fresh beef muscles.

	Transition heat flow (mW)		
Muscle	ΔY1	ΔY2	ΔY3
Infraspinatus	0.15[b]	0.24[a]	0.19[b]
Triceps brachii	0.16[a]	0.17[b]	0.21[b]
Supraspinatus	0.16[a]	0.17[b]	0.23[a]
Longissimus dorsi	0.18[a]	0.13[b]	0.24[a]
Psoas major	0.15[b]	0.15[b]	0.20[b]
SE	0.01	0.02	0.01

[a,b] Means within the same column with different superscripts differ ($P<0.01$).

Longissimus dorsi and the Supraspinatus had the shortest ($P<0.01$) sarcomere lengths when compared to other muscles (Table 4).

Prediction of sarcomere length using the variables generated from the differential scanning calorimetry thermoprofiles of the five beef muscles was successful for all muscles except the Longissimus dorsi (Table 5). Transition variables T2, ΔY1, ΔY2 could account for 90.8% of the variation in sarcomere length in the Psoas major. T2, ΔY1, ΔY3 and ΔH2 accounted for 79.9 and 76.6 % of the variation in sarcomere length for the Infraspinatus and Triceps brachii, respectively. The third transition temperature (T3) alone accounted for more than 47 percent in variation for sarcomere length in the Supraspinatus. Of the regression models that contributed significantly ($P<0.05$) to the prediction of sarcomere length it can be noted the ΔY1 contributed most to the prediction of sarcomere length in both the Infraspinatus and Psoas major, while it was ΔY3 in the Triceps brachii and T3 for the Supraspinatus.

Discussion

Isolation and purification of myosin, actomyosin, and actin with associated thermograms have made it possible to assign thermal transition T1

Table 4. Sarcomere lengths (um) of five different beef muscles.

Muscle	Sarcomere length (um)
Infraspinatus	2.33[b]
Triceps brachii	2.44[b]
Supraspinatus	1.94[c]
Longissimus dorsi	1.86[c]
Psoas major	3.27[a]
SE	0.04

[a,b,c] Means within the same column with different superscripts differ ($P<0.01$)

to myosin and T3 to actin (Wright et al., 1977). It was also suggested that T2 resulted from the thermal denaturation of sarcoplasmic proteins and collagen. $\Delta H1$ and $\Delta H2$ are the result of the energy associated with the endothermic reactions taking place during the denaturation of muscle proteins. This allows for the assessment of how much energy was required for the unfolding and breakdown of myosin, actin, and sarcoplasmic proteins and thereby qualifying the various proteins within muscle tissue. ΔHt expressed the total amount of energy attributed to muscle protein denaturation. Of the five muscles analyzed, the Psoas major, which exhibited the longest sarcomere length, yielded the lowest total energy, suggesting less energy was required to denature these muscle proteins than those of the other four muscles. Based on sarcomere length, the amount of energy required to cause a transition may be related to actin-myosin interactions.

Variations in transition heat flow may suggest that there are variations in the denaturation process of different muscles. Denaturation involves endothermic heat flow resulting in the unfolding of proteins and the alteration of internal bond energies. Differences in bond energies are measured by differences in transition heat flow. This is important since the transition heat flow may be dependent upon the extent of overlap between thick and thin filaments within a muscle. Myosin can be found in the thick filaments and actin is located in the thin filaments. These proteins are the major components of the myofibrillar fraction of muscle proteins and are responsible for contraction of muscle fibers (Pearson et al., 1989). Sarcomere length provides

Table 5. Transition temperatures, heat flow and enthalpy associated with sarcomere length of different muscles.

Y	Transition temperatures (°F)			Transition heat flow (mW)			Transition energies (J/g)		Bo	R^2*100
	T1	T2	T3	ΔY1	ΔY2	ΔY3	ΔH1	ΔH2		
Infraspinatus										
Sarcomere Length	-	0.130	-	4.370	-	-2.718	-	-	-8.218	79.96
Triceps brachii										
Sarcomere Length	-	-	-	2.594	-	-4.757	-	-1.577	3.806	76.60
Supraspinatus										
Sarcomere Length	-	-	-0.235	-	-	-	-	-	20.716	47.68
Longissimus dorsi										
Sarcomere Length	-	-	-	-	-	-	-	-	-	-
Psoas major										
Sarcomere Length	-	-0.131	-	6.758	-10.172	-	-	-	12.332	90.82

an estimate of the extent that muscle fibers are contracted and thus the extent of overlap between thick and thin filaments. The greater the extent of overlap between thick and thin filaments the shorter the sarcomere length. The five muscles used in this experiment were selected based on their degree of tenderness. The Psoas major is a more tender muscle followed by the Longissimus dorsi, Infraspinatus, Triceps brachii and the Supraspinatus (Johnson et al., 1988). As stated, $\Delta Y1$ contributed the most to the prediction of sarcomere length for the Infraspinatus and the Psoas major. Since $\Delta Y1$ reflects myosin's response to heat denaturation, the myosin fraction may be more readily exposed to the added energy when sarcomere length is extended. With the lack of overlap of thick and thin filaments (longer sarcomere length) the ability for heat to penetrate and alter internal bonds of myosin during the first transition is greater than that of a muscle which might have filaments that are more closely inter-linked.

Variation in sarcomere length of the Triceps brachii and the Supraspinatus are best accounted for by either T3 or $\Delta Y3$. Since these muscles had shorter sarcomeres, the thermal transition of actin is more closely associated with sarcomere length. As an increased number of crosslinks are formed, less myosin is exposed. Research is continuing to assess the relationship of DSC measurements to objective tenderness ratings. As the DSC thermograms continue to be analyzed, more information may be gained to assess the relationship of components to tenderness.

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