

Differential Scanning Calorimetry of Beef Muscle: Influence of Postmortem Conditioning

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ABSTRACT

Differential scanning calorimetry (DSC) was used to follow the changes in the endothermic transitions of beef muscle during conditioning. Sternomandibularis muscle held at 5°C from 2-8 days postmortem resulted in a significant ($P < 0.05$) drop in the total heat of transition (ΔH) from 3.8 to 3.0 J/g. The myosin transition decreased from 57.8° to 55.2°C while the actin transition increased from 81.8° to 83.2°C ($P < 0.05$). Storage time and temperature were varied to generate a response surface of thermal data for psoas major and semimembraneosus muscle. The decrease in ΔH of psoas major was optimal between 10° and 13°C. Total ΔH of semimembraneosus (3.9 J/g) was significantly greater ($P < 0.05$) than that of psoas major (3.4 J/g).

INTRODUCTION

THE PRACTICE OF HANGING BEEF carcasses to improve tenderness was established long before the enzymology of conditioning was scientifically investigated. The enzymes responsible for the degradation of myofibrillar proteins during conditioning have been studied extensively and the literature was reviewed comprehensively by Penny (1980). Calcium activated neutral protease (CANP) has been implicated in the breakdown of the Z-disc and the fragmentation of myofibrils. Cathepsins B and D, which have pH optima below 4, have also been shown to degrade myofibrillar proteins (Yates et al., 1983). The rate and nature of proteolysis varies between muscles depending on their available enzyme concentration and holding temperature. It was recently reported that at 4°C and pH 7 catheptic activity is minimized, while at 37°C and pH 5.4 CANP activity is low (Yates et al., 1983). These researchers also noted the degradation of myosin and related this to a reduction in the rigor crossbridges effecting a mechanical weakening of the myofibril. Smith et al. (1978) concluded that maximum tenderizing was achieved for most beef muscles after 11 days storage at 4°C, but that some muscles reached their minimum shear values in 5 days while others required 28 days. Differential scanning calorimetry (DSC) has been used to relate the denaturation of muscle proteins to the textural

changes caused by cooking (Findlay and Stanley, 1984a; Martens et al., 1982). The transition temperature and ΔH of muscle proteins have been shown to be sensitive to changes in pH and ionic strength (Wright et al., 1977; Wright and Wilding, 1984). Because DSC has been shown to be useful in accurately measuring thermal properties of muscle, this study was undertaken to determine the effect of conditioning of beef on DSC thermal curves.

MATERIALS & METHODS

BEEF NECK MUSCLE (sternomandibularis) was obtained from four 18 month-old Charolais crossbreed heifers immediately postmortem and restrained to prevent shortening. The samples were wrapped in damp paper towel and held at 20°C for 8 hr before holding isothermally at 5°C for 2, 4, 6 and 8 days. Sarcomere length, as measured by laser diffraction, averaged 1.95 μm (sd 0.12 μm).

Beef psoas major (sarcomere length 3.12, sd 0.09 μm) and semimembraneosus (sarcomere length 2.33, sd 0.06 μm) muscles were taken from another group of four 18 month-old Charolais crossbreed heifers 8 hr postmortem, comminuted, blended, divided into 50g samples and sealed in plastic bags. A two variable rotatable design was used to optimize the experiment and yield response surface data on the effect of varying time and temperature of conditioning (Mullen and Ennis, 1979). Temperature levels of 3°, 3°, 15.5°, 23° and 28°C were used for storage times of 8, 22, 56, 90 and 104 hr.

Differential scanning calorimetry was performed using the methods previously described by Findlay and Stanley (1984b). Thermal curves were analyzed for temperatures of maximum transition, T_{max} (T_1 , T_2 , T_3 and T_4) and corresponding heats of transition (ΔH). The data were analyzed by multiple regression and response surface plots were generated using the Statistical Analysis System (Helwig and Council, 1979).

RESULTS

SEVERAL THERMAL TRANSITIONS changed significantly ($P < 0.05$) during the time course of 5°C isothermal storage of beef sternomandibularis muscle (Table 1). The first transition (T_1), attributed to myosin (Wright et al., 1977; Stabursvik and Martens, 1980), shifted from 57.8° to 55.2°C, while the ΔH decreased from ca. 0.72 to 0.55 J/g. The second transition (T_2) showed a major decrease in ΔH from 1.97 to 1.47 J/g, but remained at ca. 66°C. The actin transition (T_3) increased from 81.8° to 83.2°C and dropped

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Table 1—Effect of isothermal conditioning on the T_{max} and ΔH values beef sternomandibularis muscle ($n = 15$)

Aging (days) +	Transition temperature (T_{max} °C)			Heat of transition (J/g)			
	T_1	T_2	T_3	ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
2	57.82 a*	66.33 a	81.84 a	0.725 a	1.969 a	1.127 a	3.821 a
(Std dev)	(1.77)	(1.16)	(0.37)	(0.115)	(0.329)	(0.179)	(0.477)
4	56.04 b,c	65.69 b	82.59 b	0.655 a,b	1.875 a,b	1.098 a	3.629 a
	(2.34)	(0.99)	(0.70)	(0.102)	(0.387)	(0.195)	(0.499)
6	56.60 b	66.15 b	82.96 c	0.599 b,c	1.601 b,c	1.027 a,b	3.227 b
	(2.35)	(1.13)	(0.72)	(0.126)	(0.394)	(0.207)	(0.578)
8	55.16 c	66.97 a	83.20 c	0.551 c	1.465 c	0.967 b	2.983 b
	(2.91)	(1.23)	(0.73)	(0.118)	(0.393)	(0.176)	(0.505)

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

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from 1.13 to 0.97 J/g. The total ΔH over the range 45-92°C dropped from ca. 3.8 to 3.0 J/g. Thermal curves of 2, 4, 6 and 8 days storage, normalized to 10 mg wet muscle, appear in Fig. 1.

During conditioning at 5°C, the ΔH values for all transitions of beef sternomandibularis muscle declined steadily. The decrease in the ΔH of myosin (T₁) was accompanied by a drop in T_{max} while actin (T₃) increased in transition temperature. It is clear that these three major endothermic transitions of beef muscle are not the discrete events attributed to myosin, collagen and actin by Stabursvik and Martens (1980) but a net response of the muscle proteins reflecting their association and environment as discussed below.

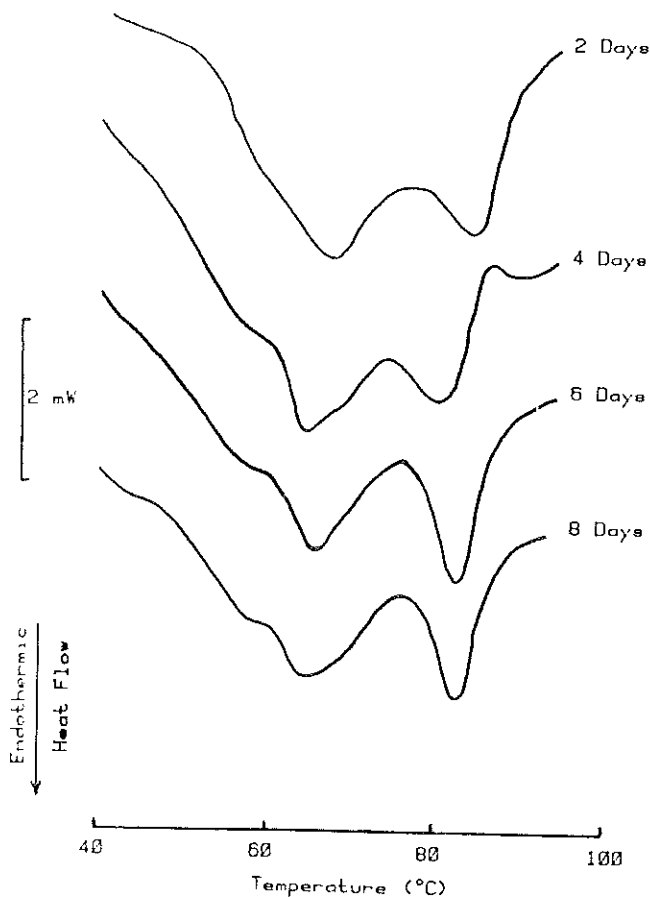


Fig. 1—DSC thermal curves of beef sternomandibularis muscle stored at 5°C for 2, 4, 6 and 8 days postmortem. Each curve is the sum of 10 samples normalized to 10 mg wet muscle.

It was noted by Wright et al. (1977) and Wright and Wilding (1984) that although myosin appeared to have a single transition in fresh muscle, isolated myosin had three transitions that have been related to the three structural subunits, light meromyosin and heavy meromyosins S₁ and S₂ and was sensitive to changes in pH and ionic environment. Proteolysis of the thick filament may be responsible for cleavage of the myosin and the liberation of more labile subunits. Conversely, the dissociation of actin from the actomyosin complex may lead to greater thermal stability and the higher actin transition temperature observed with conditioning.

The interaction of temperature and storage time was investigated using a rotatable design experiment. Psoas major was selected for its tenderness and low connective tissue content while semimembraneosus is known to possess more connective tissue and is a tougher cut of meat (Swatland, 1984). Multiple regression was used to provide an equation to generate a response surface. Table 2 contains the mean, standard deviation, probability and R² values for T_{max} and ΔH values for both muscles. The total ΔH for semimembraneosus was 3.9 J/g while that for psoas major was only 3.4 J/g (P < 0.05), probably reflecting the lower connective tissue content of psoas major.

The response surface models generated by regression analysis of psoas major thermal curves revealed that the effect of storage temperature and time on ΔH₁ accounted for 68% of the variation at P = 0.0913 (Fig. 2). The model for total ΔH (Fig. 3) accounted for 70% of the variation (P = 0.0714). The T₄ transition gave a significant (P = 0.0145) temperature response (Fig. 4). Analysis of the ther-

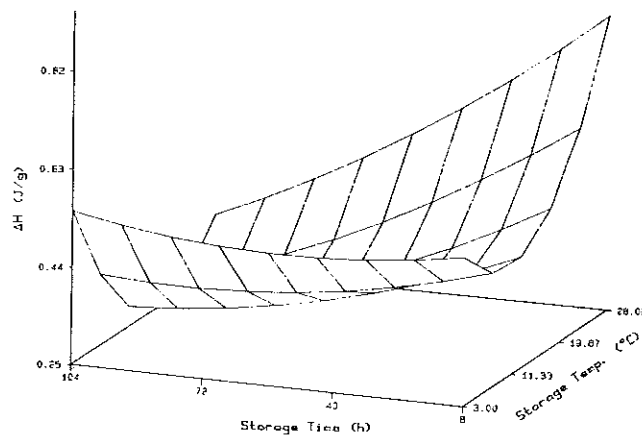


Fig. 2—ΔH₁ (Z) of psoas major as a function of storage time (X) and temperature (Y). (Z = 0.615 - 0.00107 X + 0.02528 Y - 0.000016 X² + 0.00123 Y² - 0.000198 X*Y; R² = 0.683; P = 0.0913).

Table 2—Regression analysis of the rotatable design thermal curves of beef muscle (n = 13)

Muscle	Temperature of Transition (T _{max} °C)				Heat of Transition (J/g)				
	T ₁	T ₂	T ₃	T ₄	ΔH ₁	ΔH ₂	ΔH ₃	ΔH ₄	ΔH _{Total}
Psoas major									
Mean	54.49	66.77	80.79	89.97	0.398	0.516	0.686	0.046	3.413
Std dev	0.91	1.78	0.42	0.43	0.084	0.096	0.082	0.024	0.420
Probability =	0.3867	0.8779	0.3902	0.0145	0.0913	0.2878	0.2295	0.3884	0.0714
R ²	0.4677	0.1921	0.4658	0.8229	0.6829	0.5257	0.5639	0.4668	0.7076
Semimembraneosus									
Mean	55.12	66.62	80.77	89.95	0.481	0.519	0.624	0.038	3.928
Std dev	1.15	1.41	0.36	0.57	0.124	0.150	0.024	0.012	0.581
Probability =	0.1442	0.3900	0.0067	0.2531	0.3541	0.3054	0.0001	0.8321	0.8847
R ²	0.6300	0.4659	0.8597	0.5480	0.4861	0.5148	0.9670	0.2240	0.1870

Regression model: d.f. = 5; Z = aX + bX² + cY + dY² + eXY + f; X = storage time (h); Y = storage temperature (°C).

mal curves of semimembraneosus showed a significant response for temperature, $P = 0.0067$, (Fig. 5) and ΔH , $P = 0.0001$ (Fig. 6) for the actin transition (T_3).

DSC thermal curves are difficult to analyze in the form of discrete data due to the net heat flow being measured and the probability of interactions between proteins. However, to utilize the power of three dimensional plotting a discrete response is required. The thermal data that proved to be significant demonstrated changes that are consistent with the current understanding of the enzymology of conditioning. The ΔH_1 of beef psoas major (Fig. 2) showed its greatest reduction in ΔH_1 around 12–13°C, the temperature used commercially for carcass conditioning. Almost no change occurred at 3°C, while the initially higher ΔH at higher storage temperature declined rapidly as proteolysis progressed. The total ΔH for psoas major (Fig. 3) had its highest value at 3°C and at the initial time. With an increase in storage temperature there was a rapid decrease in ΔH commensurate with proteolysis. Again, it appears that temperatures of 10–15°C resulted in a more rapid reduction in ΔH to the 3 J/g value that is typical of aged meat.

The currently unidentified (F-actin, desmin?) T_4 transition of psoas major accounted for less than 2% of the total ΔH . This transition temperature increased gradually (Fig. 4) at low storage temperatures but rose more rapidly

at higher initial storage temperature. At higher storage temperatures there was a significant decrease in T_{max} paralleling the catheptic activity noted by Yates et al. (1983).

Semimembraneosus gave highly significant responses for both temperature and heat of the actin transition. The response surfaces (Fig. 5 and 6) displayed the same general shape with maxima at 28°C initially but at 3°C after 104 hr. At high storage temperature there was a steady decline in T_{max} and ΔH of the actin transition resulting from proteolytic activity. At low temperature there was a gradual increase in ΔH and in T_{max} similar to that found in the beef sternomandibularis stored isothermally at 5°C.

SUMMARY & CONCLUSIONS

THE INTERACTIONS of muscle proteins during heating must be understood before the changes in endothermic profiles of meat can be quantitatively related to the degradation observed during conditioning. In general, the total ΔH value for muscle declined during conditioning. The increase in the transition temperature of actin as a result of conditioning in both sternomandibularis and semimembraneosus paralleled the actin shift observed when sarcomere length was increased (Findlay and Stanley, 1984b).

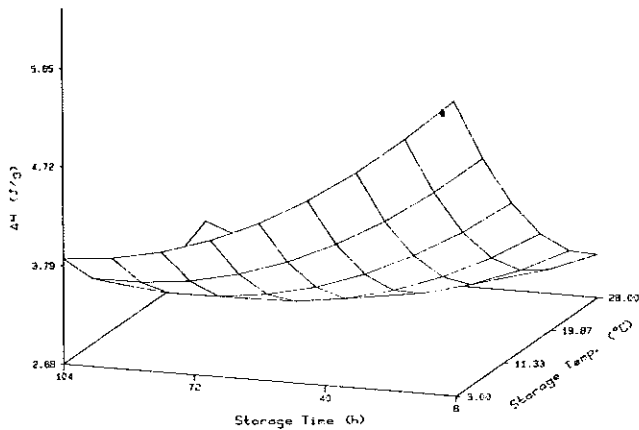


Fig. 3—Total ΔH (Z) of psoas major as a function of storage time (X) and temperature (Y). ($Z = 6.46 - 0.04087 X - 0.1796 Y + 0.000178 X^2 + 0.00253 Y^2 + 0.00075 X*Y$; $R^2 = 0.708$; $P = 0.0714$).

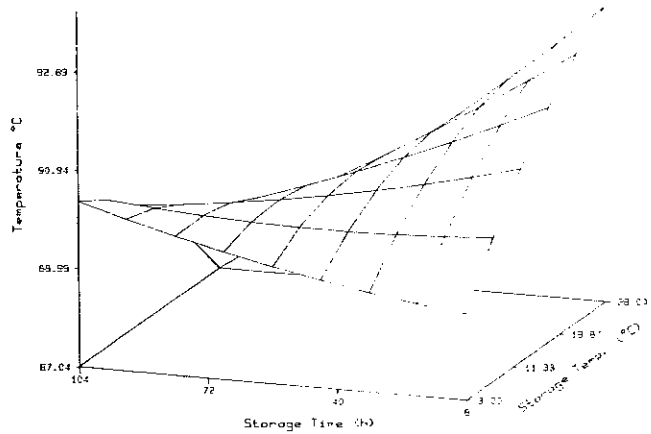


Fig. 4— T_4 transition temperature (Z) of psoas major as a function of storage time (X) and temperature (Y). ($Z = 87.9 + 0.01714 X + 0.2841 Y + 0.000075 X^2 - 0.00302 Y^2 - 0.00308 X*Y$; $R^2 = 0.823$; $P = 0.0145$).

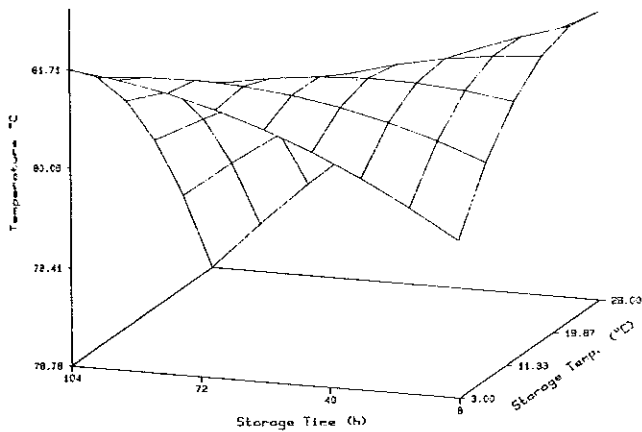


Fig. 5— T_3 transition temperature (Z) of semimembraneosus as a function of storage time (X) and temperature (Y). ($Z = 0.0382 + 0.00825 X + 0.0598 Y - 0.000005 X^2 - 0.00113 Y^2 - 0.00054 X*Y$; $R^2 = 0.967$; $P = 0.0001$).

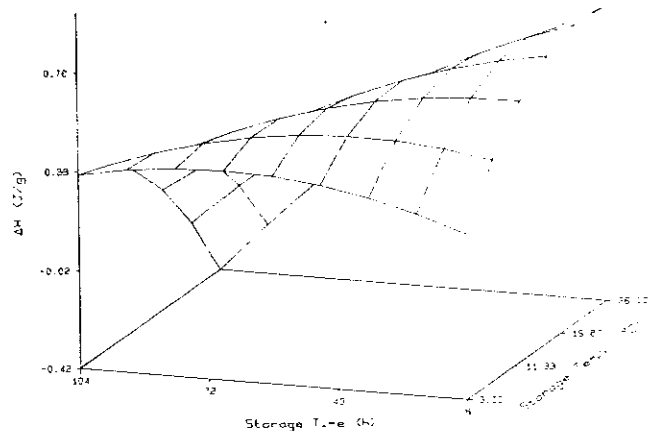


Fig. 6— ΔH_3 (Z) of semimembraneosus as a function of storage time (X) and temperature (Y). ($Z = 78.3 + 0.0529 X + 0.2728 Y - 0.000179 X^2 - 0.00526 Y^2 - 0.00296 X*Y$; $R^2 = 0.860$; $P = 0.0067$).

The minor reduction in ΔH of protein transitions during conditioning, less than 1% of the energy required to increase the temperature of meat during cooking, emphasizes the importance of the temperature of transition. A shift towards higher transition temperatures in meat cooked to a specific internal temperature will result in a greater proportion of undenatured myofibrillar proteins and, it would be supposed, concomitantly less myofibrillar toughening. The tenderizing effect of conditioning on beef muscle has been well established; however, the relative importance of CANP or cathepsins B and D in meat tenderness is still not clear. DSC may provide a way to follow the degradation of myofibrillar proteins during conditioning. The contribution and interaction of individual muscle proteins to the endothermic profile of muscle requires further research before its effect on cooked meat tenderness is understood.

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the proportion of nontoxic and very toxic samples received during a season. Over a 5-month period, an autoanalyzer system would have eliminated the bioassays on 70-80% of the approximately 1500 samples routinely analyzed. Another advantage of autoanalyzer screening is the availability of an estimate of toxin level for all samples, thus reducing the number of trials necessary to establish the proper dilution for those samples which are retested by the bioassay.

The results of this study show that the autoanalyzer has great potential as a routine monitoring system for PSP. Additional work is currently being conducted to refine the technique and to determine the exact correlation between the mouse bioassay and the autoanalyzer in this region. Further comparison testing will help to establish an exact relationship between the results of the autoanalyzer and the bioassay and may lead to a predictive mathematical factor, the use of which would allow a closer correspondence between the two techniques. As described earlier, laboratories in other regions of the country will need to do extensive comparison testing to determine the level of correlation between the two techniques in their particular region.

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