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Thermomechanical Properties of Beef Muscle

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SUMMARY

Differential scanning calorimetry (DSC) was used to follow the three major endothermic transitions (T_1, T_2 and T_3) of beef muscle during heating. Borchardt and Daniels reaction kinetics were used to predict the three time and temperature treatments required to sequentially eliminate each transition. Longissimus dorsi *and* semimembranosus *muscles were removed from beef carcasses suspended by Achilles tendon or pelvis. Samples prepared by heating for 5 min at 57°C (I), 70°C (II) and 81°C (III) were assessed by sensory panel for tenderness, juiciness and residual connective tissue. Weight loss, Warner–Bratzler (W–B) shear and microstructure using transmission electron microscopy (TEM) were also determined. The I treatment showed a significant difference in tenderness and residual connective tissue between muscles, but not between contraction states. The II treatment produced collagen shrinkage and a significant drop in W–B shear and residual connective tissue, coupled with increased tenderness in* semimembranosus *muscle. An increased W–B value, decreased juiciness, increased weight loss and a reduction in sarcomere and A-band length accompanied the III transition. Muscles from carcasses that had been suspended by the pelvis were found to be significantly more tender than the same muscles from Achilles hung carcasses. It is concluded that DSC is capable of determining amount of protein denaturation and, hence, degree of cooking.*

INTRODUCTION

Differential scanning calorimetry (DSC) is a powerful technique that is starting to find wide application in the study of the structure and thermal

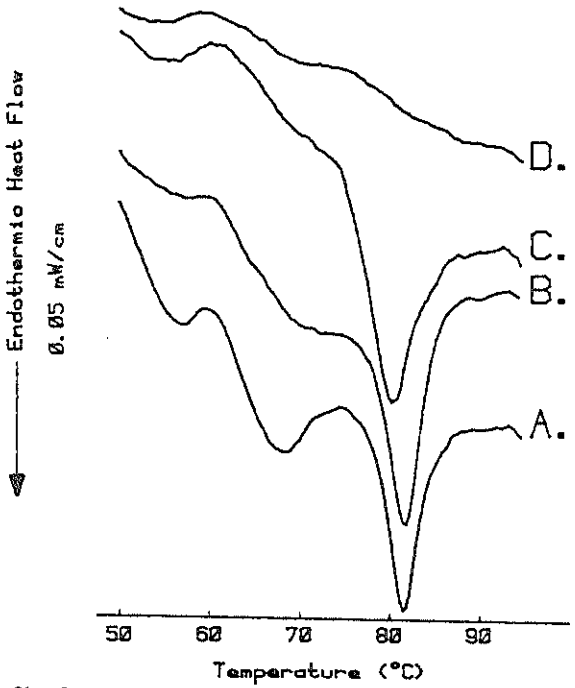

Fig. 1. DSC of beef *semimembranosus* muscle showing the sequential elimination of the three major endothermic transitions through controlled heat treatment. A, Control. B, 57 °C for 5 min (I). C, 70 °C for 5 min (II). D, 81 °C for 5 min (III).

properties of natural polymers (Donovan, 1984). DSC has been used to relate the denaturation of individual muscle proteins to the textural changes in meat caused by cooking (Martens *et al.*, 1982, Findlay & Stanley, 1984*a*). The three major endothermic transitions of beef muscle, attributed to myosin, collagen and actin, have been associated with specific changes in sensory texture (Martens *et al.*, 1982). Transition temperature and heat of transition (ΔH) of muscle proteins have been shown to be sensitive to changes in pH and ionic strength (Wright *et al.*, 1977, Wright & Wilding, 1984). Contraction state and conditioning of beef muscle have also been shown to influence the endothermic profile (Findlay & Stanley, 1984*b*,*c*). Application of Borchardt and Daniels reaction kinetics to the endothermic transitions of beef muscle (Findlay *et al.*, 1985) has permitted the calculation of time and temperature conditions that sequentially eliminate each endothermic transition, allowing the production of cooked meat of controlled denaturation of muscle proteins, as described in Fig. 1. The effect of three thermal

treatments on meat texture was determined by sensory panel. Their responses were related to cooking loss, Warner–Bratzler shear and microstructure of the sarcomere as observed using transmission electron microscopy (TEM).

MATERIALS AND METHODS

Animals

Four homozygous 18-months-old Charolais crossbred heifers were obtained from the University abbatoir. Their carcasses were split and one side was suspended by the Achilles tendon, the other by the pelvis. After 3 days at 10 °C the *longissimus dorsi* and *semimembranosus* muscles were excised, vacuum bagged and stored at −30 °C. Sarcomere length measurements were taken using laser diffraction (Varcoe & Jones, 1983). A-band lengths were measured directly from TEM photographs.

Sample preparation

Cubes of 1·4 cm, free of visible fat and connective tissue, were prepared after cutting frozen meat with a band saw. These were sealed in plastic bags, weighed and cooked by immersion in a constant temperature water bath and the time and temperature determined using DSC kinetics. Immediately following this the bags were immersed in cold running tap water to effect rapid chilling to 25 °C in preparation for sensory evaluation. After the bags were opened the cubes were removed and wiped to remove free liquid. The cubes were weighed and the cooked weight subtracted from the initial weight to obtain cooking loss which was expressed as per cent of initial weight.

Sensory analysis

Sensory analysis was designed to test muscle type, suspension and thermal treatment using the four homozygous animals as replicates. Eight panelists, selected from an initial pool of twelve, were trained to differentiate tenderness, juiciness and residual connective tissue content of cooked beef muscle samples defined as follows; tenderness perceived after three chews, juiciness perceived as moistness in the mouth after five chews and residual connective tissue perceived to be in the sample after

Evaluate the three meat samples from left to right in the order of presentation. Be sure to turn the cube so that you bite across the fiber grain and not parallel to the fibers. Mark your response with a vertical line at the point on the line that best describes your reaction to the attribute. Use the sample number to identify each mark.

Tenderness:

Very tough — *Very tender*

Juiciness:

Very dry — *Very juicy*

Residual connective tissue:

Very much — *Very little*

Fig. 2. Sample ballot for sensory texture panel.

continued chewing just before swallowing (Gullett *et al.*, 1983). Sets of three coded samples (one cube of each treatment) were presented to the panelists randomly, at 25°C under red lighting to mask small colour differences. Panelists were instructed to orient the cube so that their first bite was across the fibers. Responses were recorded on an unstructured 15 cm scale (Fig. 2) using extreme descriptors to anchor the line (Stanley *et al.*, 1972). The results were analysed using statistical programs from the SAS library (Helwig & Council, 1979).

Differential scanning calorimetry

Differential scanning calorimetry was performed using the methods previously described by Findlay & Stanley (1984*b*). Thermal curves of uncooked muscle were analysed using the Borchardt and Daniels kinetics program (Dupont Instruments, Wilmington, Delaware) to determine the time and temperature treatment that denatured 90% of the protein involved in each transition. Thermal curves to confirm the effect of heat treatment were obtained from each sample.

Physical testing

Equivalent samples were subjected to the Warner–Bratzler (W–B) shear test using an instrument equipped with a single blade moving at 0·065 m/s and the average of ten replicates was expressed in Newtons per centimeter (N/cm) (Stanley & Swatland, 1976). Transmission electron microscope (TEM) images were obtained of sections of heated muscle from two of the four animals. Samples were held in Sorenson's phosphate buffer (0·07 M, pH 5·4) prior to dehydration; fixation and examination were as described by Findlay & Stanley (1984*a*).

RESULTS

The Achilles hung *longissimus dorsi* yielded sarcomeres of 1·87 μm (standard deviation = 0·08) while the pelvic hung muscle measured 2·42 μm (standard deviation = 0·10). The length of sarcomeres from *semimembranosus* was found to be 2·01 μm (standard deviation = 0·07) for the Achilles hung and 2·52 μm (standard deviation = 0·09) for the pelvic suspension. The mean transition temperatures and corresponding heats of transition (ΔH) for both muscles are shown in Table 1.

TABLE 1
Mean Transition Temperatures and Heats from DSC Thermal Curves of Beef Muscle ($n = 5$). Means in the Same Column Bearing the Same Letter are NSD at $P < 0·05$ using Duncan's Test

Muscle	*Suspension*	*Transition temperature (°C)*		
		T_1	T_2	T_3
Longissimus	Achilles	55·5[a]	66·8[a]	80·9[a]
	Pelvic	55·6[a]	66·8[a]	82·1[b]
Semimembranosus	Achilles	55·8[a]	66·6[a]	82·5[b]
	Pelvic	55·4[a]	66·6[a]	81·9[ab]

Muscle	*Suspension*	*Heat of transition (J/g)*			
		ΔH_1	ΔH_2	ΔH_3	*Total*
Longissimus	Achilles	0·66[a]	1·62[a]	1·32[a]	3·60[a]
	Pelvic	0·71[a]	1·74[a]	1·35[a]	3·80[ab]
Semimembranosus	Achilles	0·64[a]	1·68[a]	1·06[b]	3·38[c]
	Pelvic	0·75[a]	1·87[b]	1·41[c]	4·03[b]

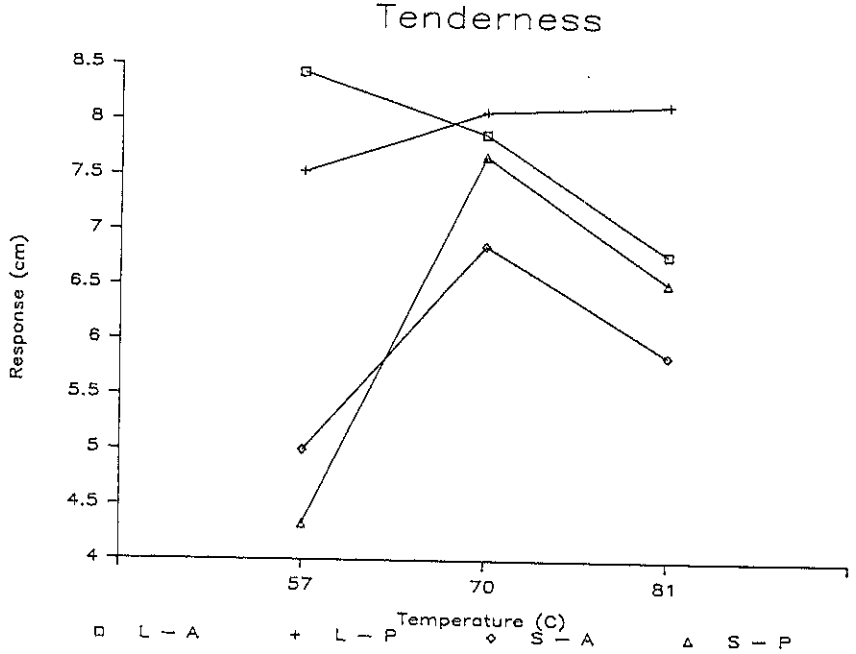

Fig. 3. The effect of three heat treatments (I, II and III) on sensory tenderness of beef *longissimus dorsi* (L) and *semimembranosus* (S) muscle from Achilles (A) and pelvic (P) carcass suspension.

TABLE 2

Mean Values for Sensory Attributes of Beef Muscle by Muscle Type and Carcass Suspension (Eight Panelists, Four Replicates).

Means in the Same Row Bearing the Same Letter are NSD at $P < 0{\cdot}05$ using Duncan's Test.

Attribute	*Treatment*	*Muscle*				*Combined mean*
		Longissimus		*Semimembranosus*		
		Achilles	*Pelvic*	*Achilles*	*Pelvic*	
Tenderness	I	8·43^a	7·54^a	5·00^b	4·33^b	6·33
	II	7·88^a	8·08^a	6·87^a	7·68^a	7·63
	III	6·79^a	8·15^b	5·87^c	6·53^a	6·84
Juiciness	I	11·23^a	9·25^a	10·32^a	10·87^a	10·42
	II	7·64^a	8·27^a	5·98^b	6·71^b	7·15
	III	5·35^a	7·53^b	4·13^c	4·11^c	5·28
Residual connective tissue	I	8·10^a	7·63^a	5·53^b	4·98^b	6·56
	II	8·47^a	8·18^a	8·12^a	8·64^a	8·35
	III	7·87^a	8·73^a	7·72^a	8·23^a	8·14

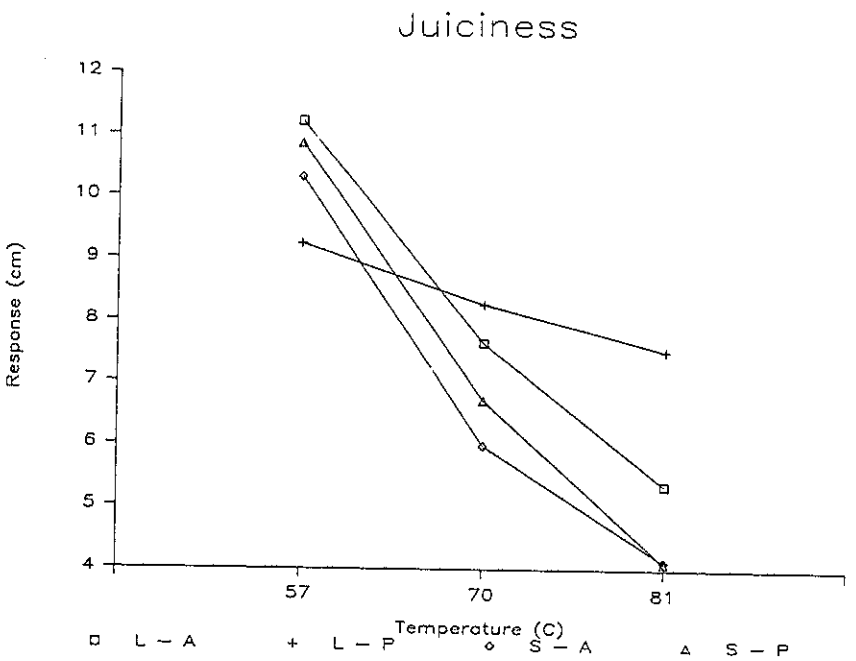

Fig. 4. The effect of three heat treatments (I, II and III) on sensory juiciness of beef *longissimus dorsi* (L) and *semimembranosus* (S) muscle from Achilles (A) and pelvic (P) carcass suspension.

The response of the sensory panel to beef cubes prepared using the three heat treatments, I, II and III, are shown in Figs 3, 4 and 5. *Semimembranosus* from both methods of suspension showed a significant ($P < 0{\cdot}05$) increase in tenderness between the 57 °C (I) and 70 °C (II) treatments (Fig. 3). This is most likely due to the collagen transition (around 67 °C) resulting in a softening of the connective tissue. All muscle samples showed a significant reduction in juiciness with increased heat treatment (Fig. 4). Both the Achilles and pelvic hung *semimembranosus* showed a significant drop ($P < 0{\cdot}05$) in residual connective tissue between I and II treatments (Fig. 5), paralleling the increased tenderness and also attributable to collagen denaturation (Fig. 3). Differences found between muscles and carcass suspension (Table 2) were in keeping with the current understanding of the effect of connective tissue contribution and filament overlap to muscle texture (Bendall & Restall, 1983). *Semimembranosus* was found to be significantly ($P < 0{\cdot}05$) less tender than *longissimus* after I treatment. No significant difference was found between muscle samples

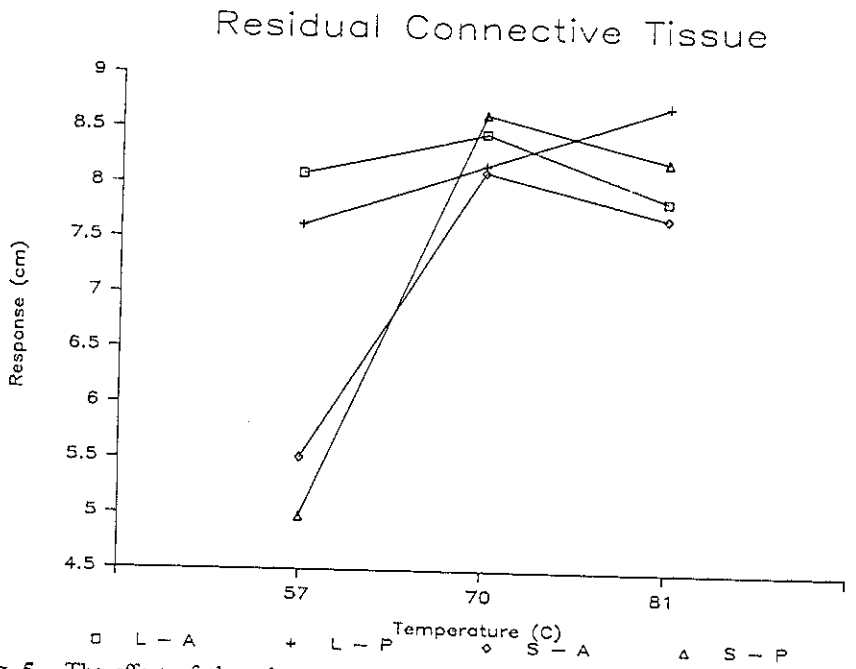

Fig. 5. The effect of three heat treatments (I, II and III) on sensory assessment of residual connective tissue of beef *longissimus dorsi* (L) and *semimembranosus* (S) muscle from Achilles (A) and pelvic (P) carcass suspension.

at II. At III, Achilles suspended *semimembranosus* was judged toughest, with pelvic hung *semimembranosus* and Achilles hung *longissimus* being equally more tender and pelvic stretched *longissimus* the most tender. A difference in juiciness was noted at II where *longissimus* was found juicier than *semimembranosus*. Pelvic suspended *longissimus* was notably juicier than Achilles suspension for all other muscle at III.

W–B shear values (Table 3) followed the same general trend noted for sensory tenderness ($R^2 = 0{\cdot}71$, $P < 0{\cdot}001$) and residual connective tissue ($R^2 = 0{\cdot}84$, $P < 0{\cdot}001$). High shear values for both samples of *semimembranosus* at I dropped significantly at II and increased slightly at III. *Longissimus dorsi* demonstrated a steady increase in shear for the Achilles hung sample, while the pelvic hung muscle showed a small decrease from I to II followed by an increase at III.

Weight loss in all four muscle treatments was virtually linear over the range of temperature treatments (Table 4). Initial weight loss to I was approximately 26 %. At II the loss was around 32 %, while III samples had

TABLE 3

The Effect of Three Heat Treatments (I, II and III) on Warner–Bratzler Shear Values of Beef *longissimus dorsi* and *semimembranosus* Muscle from Achilles and Pelvic Carcass Suspension ($n = 40$).

Means in the Same Row Bearing the Same Letter are NSD at $P < 0{\cdot}05$ using Duncan's Test

Muscle	*Suspension*	*I* (N/cm^2)	*II* (N/cm^2)	*III* (N/cm^2)
Longissimus				
	Achilles	$36{\cdot}7^{a}$	$43{\cdot}5^{ab}$	$48{\cdot}4^{b}$
	Pelvic	$45{\cdot}5^{a}$	$36{\cdot}5^{b}$	$42{\cdot}6^{a,b}$
Semimembranosus				
	Achilles	$63{\cdot}5^{a}$	$38{\cdot}4^{b}$	$41{\cdot}3^{b}$
	Pelvic	$66{\cdot}7^{a}$	$39{\cdot}6^{b}$	$44{\cdot}5^{b}$
Combined	Mean	$53{\cdot}1^{a}$	$39{\cdot}5^{b}$	$44{\cdot}2^{b}$

lost 40 % of their original raw weight. This loss follows the sensory panel's perception of reduced juiciness, with the notable exception of *longissimus dorsi* pelvic suspension which was significantly ($P < 0{\cdot}05$) juicier.

Microstructural examination of the tissue by TEM revealed that heating caused a degradation of myofilaments, 'thermal induced contraction' (Leander, 1977) and, at II, gelatinization of collagen. Changes in sarcomere length and the A-band are given in Table 5. Both Achilles suspended muscles showed no significant change in sarcomere

TABLE 4

The Effect of Three Heat Treatments (I, II and III) on the Weight of Beef *longissimus dorsi* and *semimembranosus* Muscle from Achilles and Pelvic Carcass Suspension ($n = 40$).

Means in the Same Row Bearing the Same Letter are NSD at $P < 0{\cdot}05$ using Duncan's Test

Muscle	*Suspension*	*I*	*II*	*III*
		(*Per cent of initial weight*)		
Longissimus				
	Achilles	$74{\cdot}1^{a}$	$67{\cdot}1^{b}$	$60{\cdot}2^{c}$
	Pelvic	$74{\cdot}6^{a}$	$68{\cdot}3^{b}$	$59{\cdot}9^{c}$
Semimembranosus				
	Achilles	$73{\cdot}2^{a}$	$66{\cdot}4^{b}$	$58{\cdot}4^{c}$
	Pelvic	$74{\cdot}5^{a}$	$66{\cdot}5^{b}$	$57{\cdot}6^{c}$
Combined	Mean	$74{\cdot}1^{a}$	$67{\cdot}1^{b}$	$59{\cdot}0^{c}$

TABLE 5
The Effect of Three Heat Treatments (I, II and III) on Sarcomere and A-band Lengths of Beef *longissimus dorsi* and *semimembranosus* Muscle from Achilles and Pelvic Carcass Suspension ($n = 20$).
Means in the Same Row Bearing the Same Letter are NSD at $P < 0{\cdot}05$ using Duncan's Test

Muscle	*Suspension*	*Length* (μm)			
		Initial	*I*	*II*	*III*
Longissimus					
Sarcomere	Achilles	$2{\cdot}13^{a}$	$2{\cdot}13^{a}$	$2{\cdot}05^{a}$	$1{\cdot}81^{b}$
A-band			$1{\cdot}52^{a}$	$1{\cdot}40^{ab}$	$1{\cdot}31^{b}$
Sarcomere	Pelvic	$2{\cdot}42^{a}$	$2{\cdot}38^{a}$	$2{\cdot}23^{b}$	$1{\cdot}94^{c}$
A-band			$1{\cdot}44^{a}$	$1{\cdot}31^{b}$	$1{\cdot}27^{b}$
Semimembranosus					
Sarcomere	Achilles	$2{\cdot}10^{a}$	$1{\cdot}94^{a}$	$1{\cdot}91^{a}$	$1{\cdot}69^{b}$
A-band			$1{\cdot}30^{a}$	$1{\cdot}30^{a}$	$1{\cdot}08^{b}$
Sarcomere	Pelvic	$2{\cdot}52^{a}$	$2{\cdot}41^{a}$	$2{\cdot}19^{b}$	$1{\cdot}87^{c}$
A-band			$1{\cdot}36^{a}$	$1{\cdot}28^{a}$	$1{\cdot}10^{b}$

length between the unheated muscle and the I and II treatments. The longer sarcomere length pelvic suspended muscles showed a significant reduction ($P < 0{\cdot}05$) in length from I to II. All muscles had significantly ($P < 0{\cdot}05$) shorter sarcomeres at III. The A-band length of both muscles declined significantly ($P < 0{\cdot}05$) from I to III with no significant difference between carcass suspension. These microstructural changes paralleled the sensory response for juiciness. The sarcomere and A-band length of pelvic hung *longissimus* showed less change than *semimembranosus*, possibly allowing the retention of more intracellular moisture and thus contributing to juiciness.

Changes in ultrastructure may be seen in the TEM micrographs of Fig. 6. A view of *longissimus* from the pelvic suspension heated to I appears in Fig. 6A. At this temperature the myosin transition has been completed. It is evident that the A-band has coagulated and does not exhibit the distinct thick filament arrangement typical of unheated muscle. However, a non-myosin filament structure is still visible in the A-band, particularly in the M-line region. The actin thin filaments are clearly intact and connected to the Z-disc. Inspection of the Achilles hung *semimembranosus* (Fig. 6C) reveals the same features, but with greater filament overlap.

At III the A-band of *longissimus dorsi* (Fig. 6B) filament structure is still

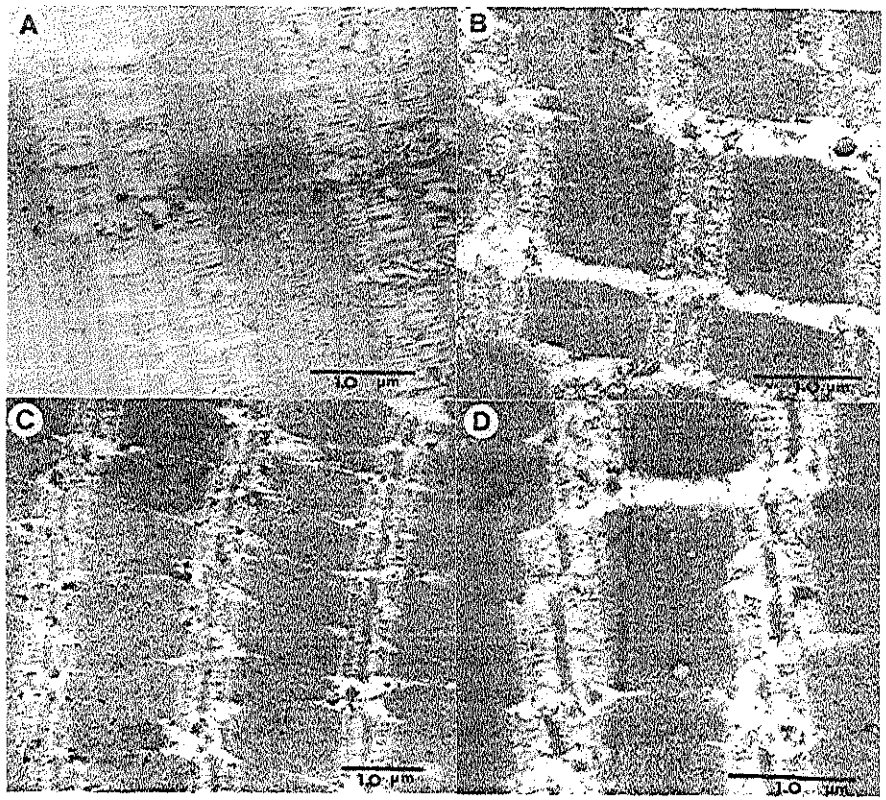

Fig. 6. Transmission electron microscope photographs of longitudinal sections of sarcomeres from heated beef muscle; A, *longissimus dorsi*, pelvic suspension, I (57 °C for 5 min). B, *longissimus dorsi*, pelvic suspension, III (81 °C for 5 min). C, *semimembranosus*, Achilles suspension, I (57 °C for 5 min). D, *semimembranosus*, Achilles suspension, III (81 °C for 5 min).

visible passing through the I-band to the Z-disc. It would seem that this cannot be actin or myosin from the thin and thick filaments since they have both undergone irreversible transitions and have formed a random coil gel matrix. The filaments observed here may be the ultra-thin gap filaments described in cooked meat by Locker & Wild (1982). Although the thermal degradation of connectin has been noted at temperatures of 60 °C, complete destruction is not achieved until 40 min at 80 °C (Locker, 1984). In this work, heating was limited to 5 min at 81 °C, a treatment that would be kinetically insufficient to result in complete connectin denaturation.

DISCUSSION

DSC has established the stepwise process of denaturation of muscle proteins that occurs during the cooking of meat. The initial transition, attributed to myosin, is completed after a thermal treatment of 57 °C for 5 min. This is accompanied by moisture loss and coagulation of the myosin of the A-band without significant changes in either sarcomere or A-band length.

The second transition, completed after 5 min at 70 °C, encompasses the denaturation of sarcoplasmic proteins, myosin fragments and tropomyosin and the shrinkage of various types of collagen fibrils. The impact of these events on the texture of cooked meat is complex. Both carcass suspensions of *semimembranosus*, higher in connective tissue than *longissimus*, showed a significant ($P < 0{\cdot}05$) drop in W–B shear concomitant with an increase in sensory tenderness, a drop in juiciness and perception of residual connective tissue. *Longissimus* did not change significantly between I and II, probably due to the reduced effect of the lower quantity of connective tissue on the texture of this muscle. It should be noted that, over the temperature range of 57 °C to 70 °C, Bailey (1984) noticed an increase in toughness rather than the decrease experienced in this study. This can be explained in terms of the kinetics of collagen denaturation. The II treatment is controlled to prevent the denaturation of actin, limiting its contribution to hardening of the cooked meat. The loss of elasticity when collagen undergoes shrinkage around 65 °C reduces the chewiness of the meat, resulting in the increased perception of tenderness.

The III transition accounts for the denaturation of actin, both in the actomyosin complex and as F and G actin, as well as a possible contribution of the troponins and actinins. The W–B shear of all muscle samples increased significantly with decreased juiciness, increased weight loss and reduction in sarcomere and A-band length. A significant difference in sensory tenderness due to greater sarcomere length was noted for both pelvic hung muscles at III. The significantly higher juiciness of the *longissimus* pelvic suspended meat may be due to the orientation of the reticular fibers. It was noted by Stanley & Swatland (1976) that the reticular fibers of stretched muscle were pulled almost parallel to the long axis of the muscle fibers. As muscles shortened the orientation of the reticular fibers became more perpendicular to the muscle fiber. Bendall & Restall (1983) showed that heating caused

shrinkage of collagen and a reduction in fiber cross-section, rather than length. It appears that the tension of reticular fibers oriented around the fiber may be responsible for the extrusion of free water from the muscle fiber. Since the orientation of the reticular fibers of stretched muscle is lengthwise, the tension may not be sufficient to cause extrusion of free water, resulting in a 'juicier' cooked meat. *Semimembranosus* possesses more connective tissue than *longissimus* which may account for the apparent absence of this effect in the pelvic suspended muscle. There is very good agreement between the sensory perception of both tenderness and residual connective tissue and W–B shear values.

DSC allows the reproducible determination of the temperature and ΔH for the denaturation of muscle protein groups within muscle type and suspension. Controlled heating, designed to denature the muscle protein groups, virtually eliminates the effect of contraction and muscle type upon the sensory perception of tenderness, juiciness and residual connective tissue.

This study shows the importance of the thermal behaviour of individual muscle proteins and its interaction on the sensory perception of cooked meat quality. DSC has demonstrated its usefulness in predicting the time and temperature treatments required for controlled denaturation of muscle proteins. Researchers investigating quality aspects of cooked meat will benefit from the fundamental information provided by DSC. It is now possible to eliminate cooking as a source of variation by examining meat prepared to the same level of denaturation of constituent proteins rather than preparation under fixed conditions. For those studies that require fixed time and temperature treatments DSC allows the assessment of the effect of that treatment on muscle proteins.

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