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Predicting Variability of Ageing and Toughness in Beef *M. Longissimus lumborum et thoracis*

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ABSTRACT

The object of this study was to determine muscle characteristics which might predict meat toughness. Eleven Charolais cattle were slaughtered at approximately 26 months of age and the Longissimus lumborum et thoracis *muscle was taken 1 hr post mortem and stored at 12°C for 24 hr and then at 4°C.*

The average half-life for ageing in these raw muscles was 4.6 days but the toughness varied widely between the animals. Toughness varied 3-fold and the rate of ageing varied 20-fold between animals.

Correlations were done to determine which characteristics might explain this variability. Toughness was correlated positively with increase in oxidative status of muscle and the initial levels of calpastatin. Toughness was correlated negatively with the initial levels of μ- and m-calpains and cysteine and serine proteinase inhibitors, the initial pH values and the rates of their decline. The rates of ageing were highly correlated positively with the initial levels of proteinase inhibitors and the rates of decline of calpastatin and negatively with the ultimate amounts of expressible juice.

There was a wide variability in tenderness in M. Longissimus lumborum et thoracis *from similar animals. Variations in metabolism and enzyme activity controlled by inhibitors and calpains appear to be largely responsible for this variability. Copyright © 1996 Elsevier Science Ltd*

INTRODUCTION

Since the early 1980s, the consumption of meat, especially beef, has decreased in France. One reason for this reduction is the variability in organoleptic quality, particularly tenderness, which is the major palatability trait affecting consumer acceptance of beef.

It has been known for many years that, after death, muscles undergo physical and biochemical changes which are responsible for their conversion to meat (Bendall, 1978). After slaughter, there is a decrease in temperature and pH (Marsh *et al.*, 1988) and an increase in osmotic pressure (Bonnet *et al.*, 1992) and expressible juice (Offer & Knight, 1989). Consequently, there is a weakening of the myofibrillar structure and an improvement in tenderness of the cooked meat.

The increase in tenderness during post-rigor ageing may be due to the action of endogenous proteolytic enzymes (Koohmaraie, 1994). Two proteolytic systems have been implicated in the tenderisation of meat: the calpain proteinases (Dransfield, 1994*a*) and the lysosomal proteinases (Ouali & Valin, 1980). Equally, enzyme inhibitors may play a role in controlling enzyme activity and meat ageing (Geesink *et al.*, 1992).

The ageing of meat during storage of carcasses at refrigerated temperatures has been known since the turn of the century, but the basis for the variations among similar animals (same species, breed, sex and age) is not well understood. Our research efforts have been directed toward understanding the causes of variation in meat tenderness from similar beef animals.

MATERIALS AND METHODS

Animals and sampling

Eleven Charolais cattle from 24 to 30 months (average 26 months) were brought from a commercial market to the laboratory. After captive-bolt stunning, exsanguination and dressing, carcass weights ranged from 334 to 511 kg, from which the *M. longissimus lumborum et thoracis* (Ld) were excised within 30 min of stunning. Each muscle was divided into 20 parts, each 5 cm thick, 10 for rheological measurements and 10 for biochemical and biophysical measurements, vacuum packaged and kept at 12 °C for the first 24 hr *post mortem*. Subsequently, the packs were stored at 4 °C for up to 14 days.

All the measurements were made from 1 to 14 days (352 hr) *post mortem*, except for lactate dehydrogenase (LDH), citrate synthase (CS), myofibrillar Mg/Ca-dependent ATPase (ATPase) activities and cysteine and serine proteinase inhibitory levels, which were measured at 1 hr *post mortem*.

Temperature, pH and expressible juice determination

The temperature at the centre of the slice was determined using a digital thermometer (model KM 455 XP, Kane-May, England).

To determine pH, 2 g of ground Ld were homogenized using a Polytron for 10–15 s in 20 ml of 5 mM sodium iodoacetate (Bendall, 1978). The pH of the homogenate was determined with an electronic pH meter (type WTW 537, Amilabo, Lyon, France).

The expressible juice (g/g of wet muscle) was determined from 5 g of ground muscle centrifuged at 100 000 ***g*** for 30 min by weighing the released liquid.

Osmotic pressure determination

The osmotic pressure was assessed by Differential Scanning Calorimetry (Setaram, model DSC121b), according to the method of Bonnet *et al.* (1992).

About 100 mg of muscle were sealed in a hermetic stainless steel pot, an empty sealed identical pot being used as reference. Samples were frozen from +20 °C to −20 °C at 5 °C/min. After 5 min, the sample was heated at 2 °C/min to 30 °C. Melting endotherms were recorded and analysed by a computer (IBM, PC 486); a baseline was interpolated between −12 °C and 21 °C, temperatures which surrounded the endothermic peak. The melting onset temperature (T) was read as the intersection of a tangent to the first part of endotherm extrapolated to the baseline.

The osmolarity (osmol/g) of muscle was calculated from the equation:

$$\mathrm{Os} = 0.086 - 0.229 \times T$$

determined similarly for NaCl solutions with osmolalities ranging from 0 to 1 osmol/litre (Wolf *et al.*, 1973).

Preparation of myofibrils

One g of minced muscle was homogenized in 10 volumes of a modified Ringer solution (0.15 M NaCl, 0.025 M KCl, 3 mM $MgCl_2$, 4 mM EGTA, pH 6.5). The homogenate was filtered through cheese cloth and the filtrate, after 30 min on ice, was centrifuged at 10 000 ***g*** for 15 min. The pellet (myofibrils) was washed twice with a solution of 0.05 M KCl, 5 mM β-mercaptoethanol, 1 mM EDTA at pH 6.5 and once with a similar solution without EDTA. The pellet was then suspended in this solution. The myofibrillar protein content was determined by the Biuret method (Gornall *et al.*, 1949) and expressed in mg of protein/ml of suspension.

Myofibrillar ATPase activity

The ATPase activity was measured with a Radiometer TTA31 titrator, in a total volume of 3.25 ml at 30 °C, pH 7.4 by titrating the prepared myofibrils with 10 mM KOH (Ouali & Valin, 1980). The ATPase activity was measured in the presence of 4 mM ATP, 4 mM $MgCl_2$, 170 mM KCl, 0.2 mM $CaCl_2$ and 1–2 mg of myofibrils/ml. Three replicate measurements were made and the ATPase activity was expressed as μ-equivalents of KOH/min/mg of myofibrillar proteins.

LDH and CS activities

One g of minced muscle was homogenized using a Polytron for 15 s in 20 ml of buffer containing 0.063 mM glycylglycine, 0.5 mM saccharose, 6.2 mM EDTA, 125 mM NaF, 5 mM DTT at pH 7.6. After a one hr extraction at 20 °C, the homogenate was centrifuged at 5000 ***g*** for 15 min. LDH and CS activities were determined in the supernatant.

LDH activity was measured according to Ansay (1974) using 2.5 mM pyruvate and CS activity was measured according to Srere (1969). LDH and CS activities were expressed in μmole of substrate liberated/sec/g of muscle.

Cysteine and serine proteinase inhibitors

Cysteine and serine proteinase inhibitors were extracted according to Bige *et al.* (1985) by salting-out between 40 and 70% ammonium sulfate saturation.

Activities of cysteine and serine proteinase inhibitors were determined by Barrett and Salvasen (1986) with papain (EC 3.4.22.2; Sigma, P4762) and trypsin (EC 3.4.21.4; Sigma, T1763), respectively.

Titration of trypsin was carried out as described by Chase and Shaw (1967) using p-nitrophenyl-p'-guanidinobenzoate-HCl (Sigma) as substrate and measuring the absorbance at 410 nm (Uvikon 860).

Titration of papain was carried out as described by Barrett and Salvasen (1986) using a stock solution of 0.1 mM E64 and N-Phe-Arg-NHMec (Sigma) as substrate. The fluorescence was measured with a Perkin Elmer, LS-50 B (Excitation $\lambda = 360$ nm, Emission $\lambda = 460$ nm). The concentration of active papain was determined by extrapolating the initial slope of the curve relating fluorescence and concentration of E64 to zero fluorescence. Cysteine and serine proteinase inhibitor activities were expressed in nmole of enzyme inhibited/g of muscle.

Calpains and calpastatin levels

Muscle samples (20 g) were homogenized in 100 ml of buffer containing 50 mM Tris-HCl, 3 mM EDTA, 10 mM β-mercaptoethanol, 150 nM pepstatin A at pH 7.5 and the calpains were separated from calpastatin using hydrophobic chromatography (Geesink *et al.*, 1992) by injecting 30 ml on a phenylsepharose CL-4B column (1.5×8 cm, Pharmacia) eluted at 3.5 ml/min. The eluted calpains were separated by ion exchange chromatography (Geesink *et al.*, 1992) on a DEAE Sephacel column (1.2×11 cm, Pharmacia) eluted at 1 ml/min using a linear gradient of 0–500 mM NaCl. The μ-calpain and m-calpain eluted at about 150 mM and 300 mM NaCl, respectively. After pooling the active fractions and dialysing for 1 hr to remove the salt, μ- and m-calpain activities were determined according to Crawford *et al.* (1987) using Suc-Leu-Tyr-4-methyl-7-coumarylamide (125 μM) as substrate. One unit of calpain activity was defined as liberating one picomole NH_2Mec per min. Activities were expressed in units per gram of muscle. Calpastatin activity in the extract was determined under similar conditions with μ-calpain and one unit of calpastatin activity was defined as the amount which inhibited one unit of calpain activity.

Rheological measurements

The resistance of the myofibrillar structure was measured according to Lepetit and Buffière (1995). A rectangular (L = 1.5, W = 1 and H = 1 cm) raw meat sample was compressed by a 1 cm^2 flat-ended probe in a cell which had lateral walls. The probe had a constant speed of 50 mm/min. The compression was perpendicular to the muscle fibers and, during compression, the sample could deform only in the direction of muscle fibers. The stress (force/probe area) obtained at a sample deformation of 20% was recorded and expressed in N/cm^2. The term 'resistance' (and more generally, toughness) refers to that stress measured at 20% deformation.

Statistical analysis

Analysis of variance and correlations were obtained with STATITCF software (ITCF, 1991).

The rate of decline of pH was determined as $(pH_{24\ hr} - pH_{1\ hr})/23$. An exponential equation with time *post mortem* was used for modelling the evolution of the other characteristics after 24 hr, that is, μ-calpain and calpastatin activities and resistance.

$$C = a - b \times \exp(-ct)$$

where C represents the characteristic,

a, its ultimate value,
b, the extent of its decline,
c, the rate of its decline,
and t, the time (hr) *post mortem*.

The parameters a, b and c were calculated by minimizing the sum of squared residuals (Dransfield, 1994*a*) using a non-linear method (Newton Raphson algorithm).

Correlations were used to study the relationships between the ultimate resistance and the rate of decrease in resistance with storage (ageing) of the muscles and their biochemical and physical traits. The ultimate value of the characteristics was determined by extrapolation of the exponential curves relating the different characteristics and time *post mortem*.

The correlations were performed on data from 8 of the 11 animals because one animal had missing biochemical values, another missing rheological values and the third had outlying values for cysteine proteinase inhibitor and ATPase activity.

RESULTS

The mean values characteristic of muscle type (Table 1) were 35 μmole of substrate liberated/sec/g for LDH activity, 0.15 μmole of substrate liberated/sec/g for CS activity and 0.47 μeq KOH/min/mg of protein for the ATPase activity. Cysteine and serine proteinase inhibitory activities were approximately the same, around 1.8 nmole of enzyme inhibited/g of muscle (Table 1). The 'animal' effect (Table 1) was highly significant for enzymatic and contractile activities.

Animals differed very significantly in pH, expressible juice, m-calpain, calpastatin and resistance, significantly in the level of μ-calpain and not significantly in temperature and osmotic pressure (Table 2). The 'time *post mortem*' effect (Table 2) was highly significant

TABLE 1

Bovine *M. Longissimus lumborum et thoracis*, Inhibitor Activities and their Variations Among Animals. Values are the Mean ± Standard Error (SEM) of 11 Animals; Each Value of Which was from 6 Replicate Determinations, and the Mean Square (MS), *F* Ratio and Significance were from Analysis of Variance. LDH and CS Activities were Expressed in μmol of Substrate Liberated/sec/g of Muscle, ATPase Activity in μeq of KOH/min/mg of Protein and Cysteine and Serine Proteinase Inhibitors Activities in nmol of Enzyme Inhibited/g of Muscle

Characteristic	*Mean ± SEM*	*MS*	*F Ratio*
LDH activity	35.3 ± 1.5	64	36***
CS activity	0.15 ± 0.01	0.01	86***
ATPase activity	0.47 ± 0.03	0.07	50***
Cysteine proteinase inhibitors	1.76 ± 0.60		
Serine proteinase inhibitors	1.73 ± 0.39		

***, $p < 0.001$.

TABLE 2

Variation of Post-mortem Characteristics and their Changes with Storage. The Values are the Results of Analysis of Variance from 10 Muscles, Stored under Controlled Temperature Conditions and Measured at 10 times *Post mortem*

	Animals			*Times post mortem*		
Characteristic	*MS*	*F ratio*		*MS*	*F ratio*	
Temperature	2.27	1.59	NS	856	601	***
pH	0.06	5.50	***	2.0	180	***
Osmotic pressure	0.00	1.95	NS	0.0	31	***
Expressible juice	0.00	4.13	***	0.1	248	***
μ-Calpain	39.2	5.60	*	18.6	2.7	***
m-Calpain	41.4	2.13	***	1124	58	**
Calpastatin	9588	7.74	***	2579	34	***
Resistance	144.5	5.90	***	812	33	***

***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$.
MS, Mean square.

for all the characteristics (temperature, pH, osmotic pressure, expressible juice, μ-calpain, calpastatin and resistance) except for m-calpain which was very significant.

The pH decreased linearly from 6.76 at 1 hr to an ultimate value of 5.6 at about 24 hr (Fig. 1). The expressible juice increased from 0.004 g/g of muscle at 1 hr to approximately 0.3 g/g of muscle at 24 hr and then remained approximately constant. The osmotic pressure increased from 0.47 osmol/g to 0.67 osmol/g in 47 hr. The osmotic pressure continued to increase after the ultimate pH had been reached (Fig. 1).

The level of μ-calpain (about 28 units/g of muscle) did not change between 1 and 9 hr *post mortem* (Fig. 2). At 24 hr *post mortem*, μ-calpain activity in the extract (12 units/g of muscle) was 43% of the initial activity. From 24 hr *post mortem*, μ-calpain activity decreased asymptotically to 14 days *post mortem* with a final value close to zero. The m-calpain activity (6 units/g of muscle) in the extract (Fig. 2) remained constant up to 7 days and then decreased to 3 units/g of muscle at 14 days *post mortem*. Calpastatin level (44 units/g of muscle) did not change significantly over the first 9 hr *post mortem* (Fig. 2). From 24 hr, calpastatin activity in the extract decreased asymptotically by 83% by the end of the storage period (final value approximately equal to 8 units/g of muscle).

Resistance (Fig. 2) of pre-rigor muscle between 1 and 9 hr *post mortem* was approximately 4 N/cm^2. At 24 hr *post mortem*, the resistance was at the maximum value recorded of 26 N/cm^2. In this study, toughness varied 3-fold between the animals. After 24 hr, the resistance decreased asymptotically until it reached approximately 8 N/cm^2 at 7 days *post mortem* and decreased little thereafter. Among animals, the rate of ageing varied 20-fold between the maximum and the minimum.

The rate of pH decline during the first 24 hr varied from 0.035 to 0.066 units/hr and averaged 0.053 units/hr (Table 3). The rate of the decline of resistance, μ-calpain and calpastatin activities were significantly different among the animals (Table 3). After 24 hr, the extractable μ-calpain activity decline (0.03 hr^{-1}) was similar to that of resistance (0.03 hr^{-1}) and faster (0.02 hr^{-1}) than calpastatin (Table 3).

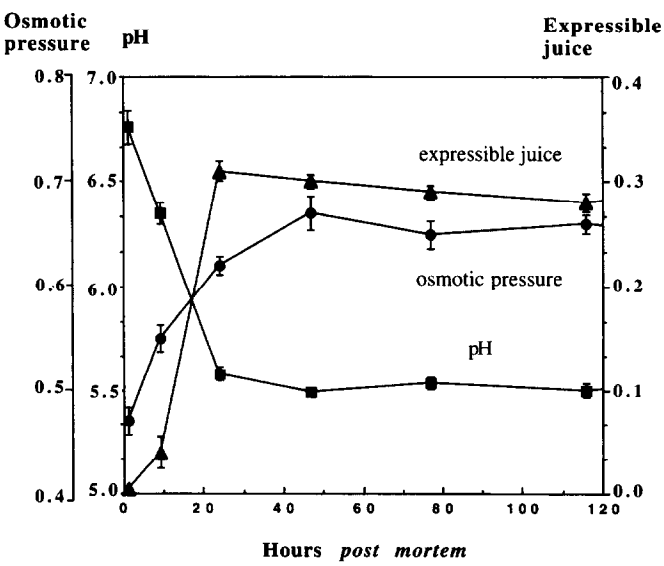

Fig. 1. Variations in pH, osmotic pressure and expressible juice during storage. Values are the means, osmol/g of muscle for osmotic pressure and g/g of muscle for expressible juice, with bars equal to 2 standard errors of 11 muscles stored at 12 °C for 24 hr and then at 4 °C.

Correlations between the ultimate resistance of the different muscles, the rate of ageing and the different muscle characteristics measured at 10 times *post mortem* are shown in Table 4.

The ultimate resistance of the muscles was correlated positively ($+0.65$; $p < 0.1$) with the CS activity of the muscles (Table 4): the more 'red' the muscle, the tougher it was after ageing. The ultimate resistance of the muscles was negatively correlated (-0.50; $p < 0.1$) with the initial levels of μ- and m-calpains (Table 4): the richer the muscle was in enzymes, the less resistant it became. Futhermore, the ultimate resistance of the muscles was correlated positively ($+0.65$; $p < 0.1$) with calpastatin at 1 hr: the richer the muscle was in this inhibitor, the more resistant was the meat. Ultimate resistance was also negatively correlated (-0.60; $p < 0.1$) with serine and cysteine proteinase inhibitor activities: the more there was of these inhibitors, the more tender the meat. For the biophysical characteristics (Table 4), ultimate resistance was negatively correlated (-0.59; $p < 0.1$) with initial pH and the rate of its decline (-0.62; $p < 0.1$). The faster the rate of pH decline, the less resistant was the muscle.

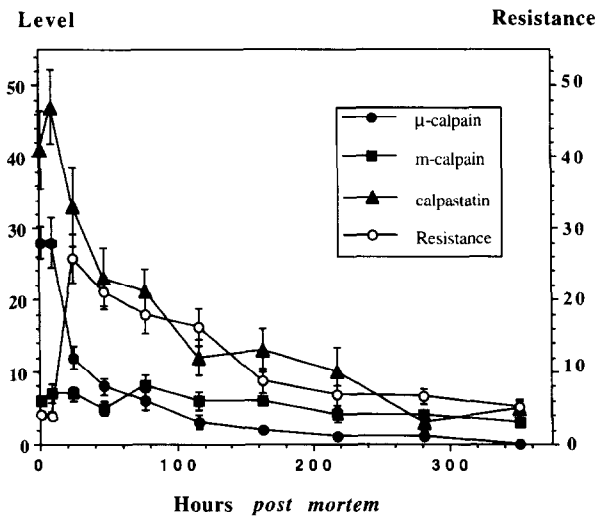

Fig. 2. Variations in activities of extracted μ-calpain, m-calpain and calpastatin, and resistance during storage. Values are the means, units/g of muscle extract for calpains and calpastatin and N/cm^2 for resistance, with bars equal to 2 standard errors of 11 muscles stored at 12 °C for 24 hr and then at 4 °C.

TABLE 3

Rate of Decrease of Principal Characteristics Intervening in the Ageing or Ultimate Resistance Among Animals. Values were Obtained from the Exponential Curves Relating Each Characteristic and Time *Post mortem* for μ-calpain Activity, Calpastatin Activity and Resistance, and were Determined for pH as $(pH_{24\ hr} - pH_{1\ hr})/23$ from 8 animals. The rate of decline of μ-calpain activity, calpastatin activity in the extract and resistance are expressed in hr^{-1} and pH in units/h

Characteristic	*Mean*	*Standard Deviation*
pH	0.053	0.008
μ-Calpain	0.031	0.020
Calpastatin	0.016	0.012
Resistance	0.026	0.039

The rate of ageing was positively correlated (+0.90; $p < 0.001$) with the rate of decline of calpastatin activity; the higher the rate of the decline, the more ageing. The rate of ageing (Table 4) increased with the activities of serine and cysteine proteinase inhibitors (+0.88; $p < 0.05$): the richer the muscle was in these inhibitors, the greater its rate of ageing. The rate of ageing was also related to the ultimate value of the expressible juice of the muscles (−0.73; $p < 0.05$).

DISCUSSION

Post-mortem physicochemical changes

Immediately after slaughter, the pH varied from 6.29 to 7.24 between the animals and declined gradually to about 5.6 over 24 hr (5.38–5.53).

The osmolarity increased to an ultimate value between 0.62 and 0.74 osmol/kg of muscle at 47 hr *post mortem*, which is slightly higher than the 0.48 and 0.54 osmol/kg of muscle for beef *Sternomandibularis* and *Psoas major* muscles, respectively, reported by Winger and Pope (1980) and the 0.5–0.6 osmol/kg for fast-twitch beef muscles (Geesink *et al.*, 1995). The pH decline is generally associated with an increase in osmotic pressure (Ouali, 1991) but, in this study, the osmotic pressure continued to increase slightly after the ultimate pH value was attained. Factors other than pH decline could therefore be responsible for some of the increase in the osmotic pressure in muscle.

Expressible juice increased during *rigor* to an ultimate value between 0.26 and 0.31 g/g of muscle at 47 hr *post mortem*, slightly more than the 0.25 g/g of muscle reported by Ouali *et al.* (1991). The amount of expressible juice increased throughout *rigor* reaching an ultimate value at approximately 24 hr *post mortem* as did pH confirming that these two characteristics are related (Ouali *et al.*, 1991).

Post-mortem biochemical changes

The decrease in temperature from about 37 °C to about 4 °C, the decline in pH and the increase in osmolarity and expressible juice will undoubtedly affect enzyme activities. As the pH declined and the osmolarity increased, μ- and m-calpain remained constant up to 9 hr

TABLE 4

Characteristics Important in Determining the Rate of Ageing and the Ultimate Resistance. Values are the Correlation Coefficients Between the Characteristics and the Rate of Ageing or the Ultimate Resistance. Only Those Characteristics Having Correlation Probabilities < 0.1 are Reported

Characteristic	*Rate of ageing*	*Ultimate resistance*
Expressible juice	−0.73	
Serine proteinase inhibitors	0.87	−0.61
Cysteine proteinase inhibitors	0.85	−0.53
Initial calpastatin activity		0.65
Rate of decline of calpastatin	0.90	
Initial μ-calpain		−0.53
Initial m-calpain		−0.46
Citrate synthase activity		0.65
Initial pH		−0.59
Rate of decline of pH		−0.62

post mortem. Thereafter, m-calpain decreased erratically as described *in situ* by Kendall *et al.* (1993) and *in vitro* by Wang and Jiang (1991). After 5 days of post-mortem storage, m-calpain activity extracted was 97% of the original activity similar to the values found by Vidalenc *et al.* (1983) for rabbit and Ducastaing *et al.* (1985) for beef. After 14 days, only 50% of the original activity remained, a much lower value than the 80% found by Koohmaraie *et al.* (1987).

The level of extracted μ-calpain decreased asymptotically during storage for all the animals as previously described by Vidalenc *et al.* (1983). From 0 to 24 hr, μ-calpain activity decreased by 55% which is slightly less than the 60% reported by Alarcon-Rojo and Dransfield (1995). After 3 days, 80% of the initially extracted μ-calpain activity was lost, which is in agreement with Koohmaraie *et al.* (1986) and slightly less than the 100% reported by Ducastaing *et al.* (1985). All the activity extracted was lost at 14 days *post mortem* which is in agreement with other published findings.

The level of extracted calpastatin activity also decreased as is generally found. After 24 hr, 77% of the original activity was still present which is slightly less than the 90% reported by Ducastaing *et al.* (1985). 50% of its activity was lost by 72 hr, lower than the 70% reported by Vidalenc *et al.* (1983) but much more than the 10% reported by Ducastaing *et al.* (1985). Vidalenc *et al.* (1983) and Koohmaraie *et al.* (1987) reported that the inhibitor activity was not detectable after 6 days *post mortem* while in this study, even at 14 days, 17% of the extractable activity remained. It is clear that further understanding is needed of the post-mortem changes in calpains and calpastatin to reconcile these wide differences.

Post-mortem ageing

The myofibrillar component of resistance measured on raw muscle decreased exponentially from 24 hr *post mortem* to the end of storage. The decline in resistance followed the decrease of extracted μ-calpain and calpastatin as previously described by Dransfield (1994*a*,*b*). The maximum resistance obtained was at 24 hr, but varied considerably among animals, the minimum was 60% of the maximum, which is more than the 40% reported by Lepetit *et al.* (1986) using similar methods. At 24 hr *post mortem*, resistance was approximately 25 N/cm^2 and, after 3 days of storage at 4 °C, it had decreased by 31% which is in agreement with Geesink *et al.* (1992). For all the animals, the ultimate resistance was approached after 7 days when it varied from 17% to 57% (average 34%) of the maximum resistances at 24 h. Similar variability (44%) was reported by Geesink *et al.* (1992). The rate of ageing is highly temperature-dependent (Alarcon-Rojo and Dransfield, 1995) but the overall reduction in resistance at 4 °C is the same as at 10 °C. At 14 days, resistance between animals varied 30% from the maximum to the minimum showing that its variability between animals had halved with time *post mortem*. The average half-life for ageing in these raw muscles was 4.6 days which is very similar to the 5 days in beef muscle measured after cooking (Dransfield *et al.*, 1980). The weakening in the raw muscle appears, therefore, to be related to that in cooked meat.

Metabolic characteristics of the muscles

Skeletal muscles consist of a mixture of fibre types: slow-twitch oxidative or type I; fast-twitch oxidative-glycolytic or type IIA; fast-twitch glycolytic or type IIB; and intermediate or type IIC (Hunt & Hedricks, 1977). These fibre types typify the contractile and metabolic properties and the amounts of oxidative and glycolytic enzymes.

Many biochemical studies of muscle have been made in pig (Karlsson *et al.*, 1993), rabbit (Briand & Briand, 1986), sheep (Briand *et al.*, 1981) and lamb (Valin *et al.*, 1982),

but most studies on bovine muscle metabolism have been done using histological or histochemical methods (Hunt & Hedricks, 1977; Totland *et al.*, 1988). In this study, the metabolism of the beef Ld muscle was estimated by its lactate dehydrogenase (LDH) activity, which represents the glycolytic capacity of the muscle, and citrate synthase (CS) activity, which represents oxidative capacity of the muscle. Ansay (1974) showed that the Ld muscle contains a high percentage of type II fibers and a low oxidative capacity. From the data shown in Table 1, Ld muscle could be classified as type IIA which is in agreement with the histological and histochemical data of Hunt and Hedricks (1977). Talmant *et al.* (1986) using 18 muscles from bulls, steers and cows from 2 to 10 year-old Charolais, Friesian and crossbreeds found a value equal to 85 μmol of liberated substrate/sec/g of proteins for the LDH activity of Ld. In this study, with a homogenous group of 11 Charolais bulls, approximately 26 months old, values varied from 27.3 to 45.5 μmol. This 2-fold discrepancy may be related to an increase in glycolytic capacity with age since LDH activity in *Semitendinosus* muscle from Limousin cattle increased from 12 months to 16 months (Jurie *et al.*, 1995).

Oxidative capacity increases with age from 12 to 16 months (Talmant *et al.*, 1986; Jurie *et al.*, 1995) along with an increase in pigment content in Afrikaner or Friesland cattle (Boccard *et al.*, 1979). In this study, values varied from 0.11 to 0.19 μmol of substrate liberated/sec/g of muscle for the CS activity of all the animals.

The contractile activity of the Ld muscle classifies it as a 'fast-twitch' muscle (Ansay, 1974). In this study, we determined the contractile activity as ATPase activity which is directly linked to the speed of contraction (Barany *et al.*, 1965). Values varied from 0.28 to 0.68 μeq KOH/min/mg of myofibrillar protein, in close agreement with those found by Ouali *et al.* (1983), but twice the value obtained using older cattle (Talmant *et al.*, 1986).

In this study, using similar Ld muscles, we found no significant correlation between the level of serine proteinase inhibitors and muscle type but a positive correlation ($+7.0$; $p < 0.05$) between the level of cysteine proteinase inhibitors and ATPase activity. The activities of both these inhibitors and their enzymes are higher in slow-twitch than in fast-twitch muscles (Spanier & Bird, 1982), but cystatin levels are similar in lamb *Supraspinatus*, *Longissimus* and *Gluteus medius* muscles (Whipple & Koohmaraie, 1992).

Although all muscles in this study are physiologically similar and their physicochemical and biochemical changes follow a similar pattern, ultimate resistance and rate of ageing varied significantly.

Determination of the characteristics which might explain the different resistances of the muscles

Correlations were used to estimate which characteristics of the animals were related to the ultimate resistance and rate of ageing.

The ultimate resistance (final toughness) was higher in muscles which had higher CS activity, that is, as the oxidative capacity increased. As far as we know, no other publication relates resistance to a biochemical index of oxidative metabolism in beef although several publications describe histological measures of fiber type. The correlation between muscle resistance and muscle fiber type is far from clear. In agreement with our results, Whipple *et al.* (1990) reported that the percentage of red fibers was positively correlated with shear force in Ld. Also, Totland *et al.* (1988) related the increase in shear force value with the proportion of type I fibers. Other histological studies (Seideman *et al.*, 1987) found a negative relationship between tenderness of bovine *M. longissimus* and fibre area. Moreover, Geesink *et al.* (1995) reported that the extent of ageing between 1 and 14 days *post mortem* could be explained by the average surface area of type I fibres. Clearly further work is required to clarify the role, if any, of muscle type to tenderness.

The ultimate resistance was lower in those muscles with higher initial levels of μ- and m-calpains. Olson *et al.* (1976) observed a low level of calpains and little ageing in *M. Psoas major*, and Koohmaraie *et al.* (1987) demonstrated that the Ld muscle, which had the highest degree of ageing, also had the highest μ-calpain activity. In this study, m-calpain was also correlated to the ultimate resistance which suggests that m-calpain might also be used to indicate tenderness, as suggested by Jiang *et al.* (1995).

The ultimate resistance was higher in muscles with higher calpastatin levels at 1 hr *post mortem*. There are no reports of a relationship between ultimate resistance and the level of calpastatin at 1 hr *post mortem*, but several authors have reported a positive correlation between calpastatin activity at 24 hr and shear force (Whipple *et al.*, 1990; Shackelford *et al.*, 1991).

The levels of the other inhibitors in muscle, that is, cysteine and serine proteinase inhibitors were negatively correlated with the ultimate resistance. This correlation is surprising because few publications report a role for these inhibitors in meat ageing. One specific cysteine inhibitor of cathepsins, cystatin, was positively correlated with shear force up to 14 days *post mortem* and 31% of its variation at 1 day was accounted for by cystatin activities at 24 hr *post mortem* (Shackelford *et al.*, 1991). Cysteine and serine proteinase inhibitors may therefore play a role in tenderisation, but their role may be indirect.

The ultimate resistance was higher in muscles which had lower pH at 1 hr and, as previously suggested by Purchas (1990), the rate of its decline. Muscle pH at 3 hr *post mortem* may sometimes be a predictor of tenderness (Marsh *et al.*, 1988), but not always (Shackelford *et al.*, 1994). The rate of pH and temperature decline appeared to be related to the shear force at 1 day *post mortem*, the tenderisation starting when muscles reach a pH of approximately 6.1, emphasizing the importance of *rigor mortis* development to tenderness (Dransfield, 1994*a*).

Several workers (Wu & Smith, 1987; Geesink *et al.*, 1992) have reported that osmolarity, through an effect on the degree of shrinking of the myofibrillar lattice and/or facilitation of the action of proteases, relates to tenderness but we found no significant relationship, largely due to the consistency of osmolarity among muscles.

The rate of the ageing was higher in muscles with higher cysteine and serine proteinase inhibitor activities and was negatively correlated with the ultimate expressible juice of the muscle. Expressible juice represents the lack of hydration of proteins. It is possible that less hydrated substrates and enzymes are less effective and account for the slower ageing.

Eighty percent of the rate of ageing among animals was accounted for by the rate of calpastatin decline. However, calpastatin is inactive *in vitro* at pH 5.5 which is approximately the muscle pH at 24 hr *post mortem*. Therefore, calpastatin, rather than playing a direct role, could indicate general proteolysis by being a substrate for the μ-calpain as suggested by Dransfield (1994*b*). We believe the average rate of decline of μ-calpain best reflects the proteolysis of ageing although its precise mode of operation is unknown.

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