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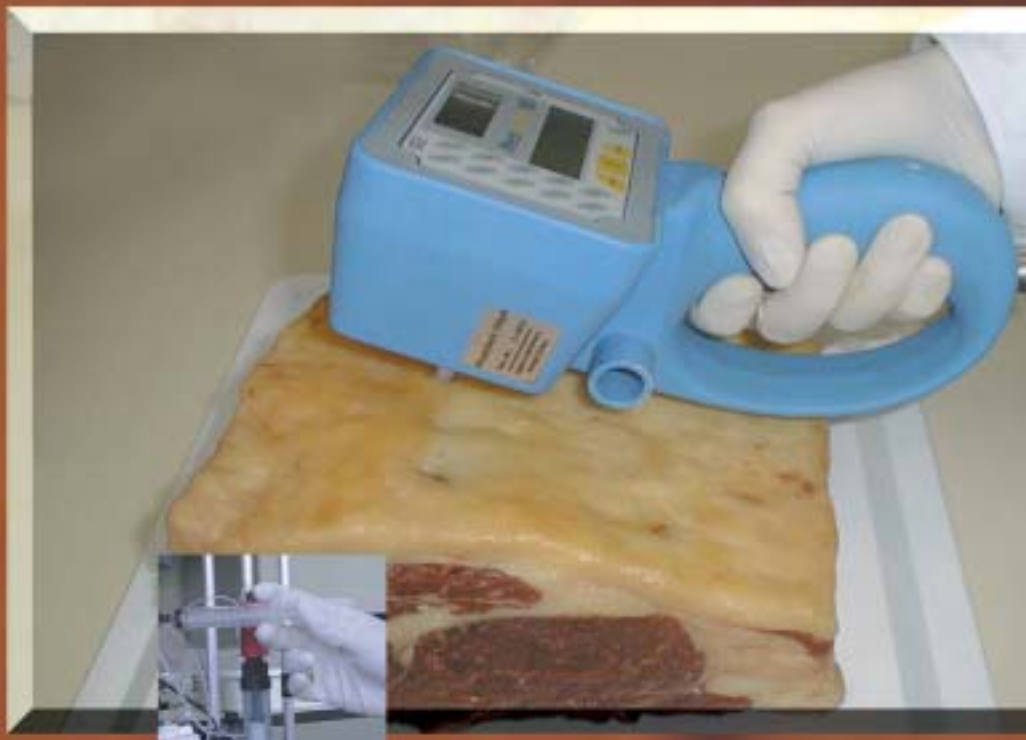
Biochemical and Physical Indicators of Beef Quality



The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 13

Contents

BIOCHEMICAL AND PHYSICAL INDICATORS OF BEEF QUALITY

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SUMMARY

pH measurements taken at 3 and 7 hours post-mortem were significantly related to both sensory tenderness and shear force. Furthermore, pH at these times was also correlated to water-holding capacity. Further refinement of these measurements could prove useful in indicting some quality attributes of beef. There was a large variation between carcasses in the rate of pH decline post-mortem resulting in large differences in pH values at 3 and 7 hours post-mortem. This may explain the inherent variation in tenderness between carcasses.

Evidence was found that the variation in pH decline exerts its effect on meat tenderness through the level of activity of the proteolytic enzymes. Relationships between calpains, calpastatin and cathepsins to meat quality attributes were sought. Of these the calpain inhibitor, calpastatin, measured at 6 hours post-mortem, was significantly related to tenderness.

The action of calpains and cathepsins on myofibrillar proteins during post-mortem storage results in the formation of a number of fragments of the substrate protein. Detailed examination of these fragments and subsequent correlations with quality attributes were undertaken.

The appearance of three fragments (30 kDa, 32 kDa and 34 kDa) was related to tenderness. These fragments appeared more strongly in tender meat and less strongly in tough meat. As mentioned above, these fragments seem to have the ability to indicate tough or tender meat but their measurement is complex and cumbersome. A more rapid detection of these fragments could be of great benefit to the meat industry.

Physical aspects were also studied. Results showed that electrical properties alter concomitantly with ageing. Shear force values and sensory tenderness scores were significantly correlated to conductivity and impedance. Also, some colour properties were significantly related to these electrical properties. The properties were measured using robust on-line probes and are industrially well suited. Refinement of optimum depth, location and direction should be made to strengthen the correlations.

NIR spectral data were found to be highly correlated with meat quality attributes particularly shear force and sensory tenderness measurements. Although the precise chemical and physical basis for the relationship remains unclear, it is evident that NIR has potential to act as a meat quality indicator particularly as a predictor of tenderness.

Supplementation of cattle feed with dietary vitamin E improved the oxidation and colour stability of meat packaged under various conditions. It also reduced cholesterol oxidation in cooked beef and reduced warmed-over flavours. Predictive modelling showed that vitamin E could reduce drip loss in fresh beef cuts.

Very fast chilling of hot deboned beef produced meat as tender as conventionally chilled meat. It appears that a physical restraint through crust freezing prevents cold-shortening and toughening.

Introduction to meat quality

Beef of a consistent quality is required by the meat industry in order to maintain and expand markets. Measurement of beef quality is difficult at factory level. There are many tests for quality attributes (tenderness, colour, flavour and water-holding capacity) which can be applied to a piece of meat only after it leaves the beef plant. These methods are destructive and time consuming and are therefore poorly suited for on-line use in industry. Measurements to indicate the final eating quality are not well developed yet.

This project examined novel approaches to this problem using biochemical and physical methods.

BIOCHEMICAL INDICATORS OF BEEF QUALITY

pH as an indicator of beef quality

It is well documented that pH can influence many quality traits of beef such as tenderness, colour, flavour and water-holding capacity. There are two aspects of pH which must be considered. Ultimate pH (pH_u ; taken at 24 or 48 hours post-mortem) gives very reliable data as to whether the beef is dark-cutting (above 5.9) or not. Dark-cutting beef is dark, firm and dry (DFD) and has a short shelf-life due to its high pH. It is generally unacceptable and can be almost completely avoided by good stress-free handling of cattle. It accounts for no more than 5 to 10% of beef produced at any given time.

The pH_u of the majority of beef is around 5.5 - 5.8. Despite this the variability in tenderness and other attributes in this beef is not explained on the basis of its pH_u. Previous work has shown that the rate of pH decline (pH 7.0 to 5.5) immediately after slaughter is not only highly variable between animals but also is in some way related to the subsequent tenderness of beef (Troy, 1995).

This study was undertaken to investigate the use of early post-mortem pH measurements in predicting the quality attributes of beef.

A total of 50 Hereford cross heifers were randomly selected in the lairage of a commercial beef plant. The animals were slaughtered by captive bolt and chilled for 24 hours. The right striploin (M. *longissimus dorsi*) was used for all analyses.

pH measurements were taken on the hour from 1 to 8 hours post-mortem and then again at 1 and 2 days post-mortem. The striploin (M. *longissimus dorsi*) was excised at 48 hours post-mortem and allowed to age up to 14 days at
2 - 4^{0}C.

Figure 1 shows mean pH values and standard deviations for 50 beef striploins. The pH decline between the animals was highly variable. For instance, by 3 hours post-mortem the pH_3 range was 6.1 to 6.6, by seven hours the pH ranged from 5.7 to 6.2. The pH_u ranged from 5.6 to 5.4.

When all the pH data was related to Warner Bratzler shear force (WBSF) i.e. the amount of force required to shear a standard piece of meat, significant correlations were obtained (Table 1).

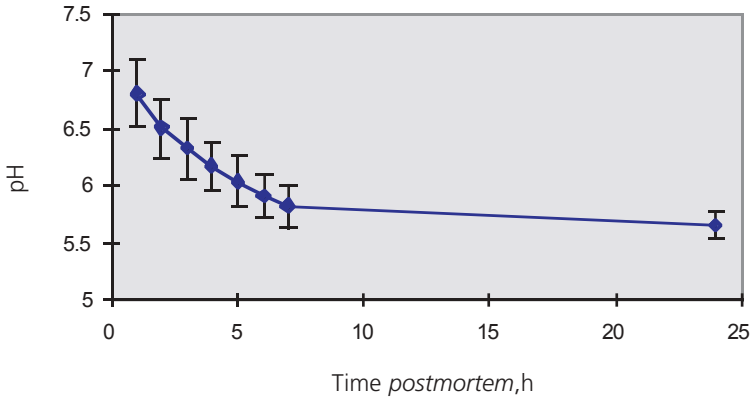

Figure1: pH of beef striploin during the first 24 hours after slaughter

Table1: Statistical correlations between early post-mortem pH values, shear force and tenderness scores of beef striploins

	Ageing time, d	pH_3	pH_7
WBSF	2	+0.63***	+0.50***
	7	+0.54**	+0.49***
	14	+0.46***	+0.46***
Tenderness[1]	2	-0.38**	-0.41**
	7	-0.35*	-0.35*
	14	-0.44**	-0.52***

Significance level *** p<0.001 ** p<0.01 * p<0.05

[1] Tenderness: 1 = extremely tough, 8 = extremely tender

n = 50

This demonstrates that the decline in pH is related to the shear force of aged beef. Generally higher correlations were obtained during the early period of ageing (2 days post-mortem). pH measured at 3 hours post-mortem was most closely related to the shear force at 2 days (Table 1).

When these pH values were related to sensory tenderness scores (Table 1), similar correlations were obtained but to a slightly less significant degree. However, strong relationships were maintained up to 14 days ageing. The results agree with previous work (Troy, 1995) which showed that the lower the pH early post-mortem, the lower the WBSF, and the higher the tenderness ratings of panellists for aged beef.

With regard to other quality parameters, the early post-mortem pH measurements were not related to flavour or meat juiciness as rated by trained panellists. However, there was a significant negative correlation between pH_3 and drip loss. No significant correlations were found between pH measurements early post-mortem and colour parameters as measured by the Hunter L, a and b system. It should be noted that pH_{24} (ultimate pH) was strongly related to colour parameters and drip loss.

Results show that early post-mortem pH measurements can indicate the tenderness of striploin steaks and furthermore that the rate of pH decline is highly variable between carcasses.

Protease activity as a potential indicator of meat tenderness

Introduction: It is widely accepted that two structural components contribute to toughness: the connective tissue component and the myofibrillar component. Since the ageing of meat appears to have no major effect on the structure of collagen, the principal component of connective tissue (Lawrie, 1985), it is generally accepted that changes in the myofibrillar proteins are responsible for tenderisation.

Two proteolytic systems are believed to have a role in the degradation of myofibrillar proteins. The first is composed of the calcium-dependent neutral proteinases, calpain I and II, and their inhibitor, calpastatin. The calpain system, which was discovered in the 1970s, is considered by many to be primarily responsible for post-mortem tenderisation. It has been proposed that when the post-mortem pH of muscle falls to about 6.1 calpain I is activated and proteolysis and tenderisation are initiated (Koohmaraie, 1988). Proteolysis is believed to continue at a rate governed by the concentration of calpain I, until calpain I becomes depleted and tenderisation stops (Koohmaraie, 1988). A model for the role of calpains and calpastatin in meat tenderisation has been proposed by Dransfield (1993, 1994). According to this model, calpain I contributes to early post-mortem proteolysis, while calpain II is partially activated and contributes to post-mortem tenderisation during prolonged ageing. Although evidence for the involvement of the calpain system in meat tenderisation seems convincing, it

is unlikely that the action of this system is solely responsible for post-mortem tenderisation. The ultrastructural changes in myofibrils induced by calpains in vitro are different from those normally seen during ageing. In addition, proteolytic changes occur meat even at low pHs when calpain activity is not favoured.

The second enzyme system of interest is the acidic lysosomal proteolytic system (Moeller et al., 1977). The lysosomal proteinases, cathepsins B and L, have been shown to exhibit proteolytic action on myofibrillar proteins (Schwartz & Bird, 1977). Cathepsin B has an optimum pH for most substrates of about pH 6.0. Cathepsin L is probably the least stable of the lysosomal enzymes at neutral or alkaline pH. It has been shown that its stability can be significantly increased by the presence of substrates and/or protein inhibitors (Turk et al., 1995). However, cathepsin L is a powerful cysteine endopeptidase, degrading proteins ten times faster than other cellular cysteine endopeptidases. Cathepsin L has activity towards many substrates including collagen and myofibrillar proteins such as myosin and actin (Matsukura et al., 1981).

The objective of this study was to investigate the potential use of measurement of proteolytic enzyme activity in the early post-mortem period as a predictor of ultimate meat tenderness.

Thirty - six heifers (predominantly Hereford cross) of similar grade (R, 4L), weight (390 - 420 kg) and age (18-24 months) were randomly selected in the lairage and slaughtered at a commercial facility (Meadow meats, Rathdowney, Co. Laois). Two individual trials were carried out. The first trial involving 18 animals (the calpain trial) was carried out to determine the usefulness of measuring calpain I, II and calpastatin activity as potential indicators of meat tenderness. The second trial involving a further 18 animals (the cathepsin trial) was carried out to determine the usefulness of measuring cathepsin B and L activity as potential indicators of meat tenderness.

▲ *Measuring enzyme activity in striploins*

CALPAIN I, II AND CALPASTATIN ACTIVITY

In general, activities of these components decreased during the early post-mortem period (Table 2). Calpain I measured at 6 h post-mortem was not highly correlated to meat tenderness as judged by sensory analysis or WBSF. There was a significant correlation found however, between calpain I at 6 h post-mortem and sensory tenderness at 7 days of ageing ($P<0.05$). It is thought that tenderisation begins when calpain I is activated by the progressive release of calcium ions (at about 6 h post-mortem). However, lack of other strong relationships with this measurement does not allow us believe that this measurement is of significance. Even lower relationships were found between calpain I measured at 2 h post-mortem and sensory attributes (results not shown).

Table 2: Calpain I, calpain II and calpastatin (mean ± standard deviation) activity in bovine striploin (n=18)

Enzyme/inhibitor	Hours post-mortem	
	6	24
calpain I	490 ± 156	430 ± 173
calpain II	880 ± 312	866 ± 358
calpastatin	4356 ± 2236	3510 ± 2662

Activity expressed in units/kg meat.

No significant differences were observed between activities at 6 h and 24 h post-mortem.Calpain II measured at 6 h and 24 h post-mortem showed no significant relationship between tenderness and WBSF. Vidalenc et al., (1983) reported that (as in this work) the activity of calpain II does not seem to be linked directly to the tenderisation process. Calpastatin on the other hand measured at 6 h and 24 h post-mortem showed some significant relationships to tenderness. These measurements were not strong against both tenderness (14 days) and WBSF (at 2 days). In fact of all the factors involved in the calpain system, calpastatin seems to be the most closely linked to tenderness. Development of a rapid method for the detection of calpastatin could prove useful.

Table 3: Statistical correlations (r) between calpain I, II and calpastatin activity in bovine striploin and shear force

	Days post-mortem	
	2	7
Calpain I	-0.627	0.333
Calpain II	0.324	0.295
Calpastatin	0.613*	0.607

Level of significance *** = p<0.001 ** = p<0.01 * = p<0.05

n = 18

Table 4: Statistical correlations (r) between calpain I, II and calpastatin activity in bovine striploin and sensory tenderness score

Enzyme	Tenderness score		
	Days post-mortem		
	2	7	14
Calpain I	+0.131	+0.575*	+0.117
Calpain II	-0.232	-0.442	-0.201
Calpastatin	-0.255	-0.147	-0.466*

Level of significance = * = p<0.05

n=18

Cathepsin B and cathepsin B&L activities in relation to beef ageing

The level of activity of cathepsin B and cathepsin B&L in the soluble fraction, as a percentage of the total activity, increased with increasing time post-mortem (Table 5). The most noticeable increase in the level of cathepsin B and B&L activity occurred between 6 h post-mortem and 24 h post-mortem. Ertbjerg (1996) suggested that the increase in activity was the result of a time-dependent degradation of endogenous inhibitors resulting in increased measured activities of cathepsins.

Table 5: Activity of cathepsin B and B&L in bovine striploin at 6 h, 24 h and 7 d post-mortem

Enzyme	Time post-mortem		
	6 h	24 h	7 d
Cathepsin B	+43.15 ± 5.10	59.07 ± 10.30	60.65 ± 4.20
Cathepsin B&L	51.36 ± 9.20	63.36 ± 8.75	65.21 ± 3.96

+ mean ± standard deviation of 18 samples. Cathepsin activity is expressed as the activity in soluble fraction expressed as a percentage of total activity.

Relationship between cathepsin B and cathepsin B&L activity and WBSF values

The level of cathepsin B activity measured at 6 h, 24 h and 7 d was negatively but not significantly correlated with WBSF values measured at 2 and 7 d post-mortem (Table 6).

The level of cathepsin B was positively but not significantly correlated with WBSF values measured at 14 d post-mortem (Table 6). Results from both the present study and that carried out by Whipple *et al.* (1990) would suggest that cathepsin B is not useful as an early post-mortem indicator of meat tenderness.

There was a significant negative correlation between the level of cathepsin B&L activity at 6 h and WBSF values at 14 d post-mortem (Table 6). This result indicates that the higher the level of cathepsin B&L activity at 6 h post-mortem the more tender the meat at 14 d and this measurement, if developed as a more rapid method, could be a useful indicator of beef tenderness.

Table 6: Statistical correlations between cathepsin B and B&L activity in bovine striploin samples and shear force values following ageing

Enzyme		WBSF		
	Analysis time post-mortem	2 d	7 d	14 d
Cathepsin B	6 h	-0.256	-0.221	+0.483
	24 h	-0.382	+0.534	+0.067
	7 d		+0.145	+0.040
Cathepsin B&L	6 h	-0.337	-0.452	-0.750*
	24 h	+0.199	-0.203	-0.009
	7 d		-0.397	-0.474

Level of significance * = $p < 0.05$ n = 18 striploins

CONCLUSION

It is concluded that early post-mortem protease activity is not strongly related to meat quality attributes. It should be stressed that these measurements are highly complex and are performed on extracts and not *in situ*. This may explain some of the weak correlations found. Despite this, there is merit in further examination of the early post-mortem level of calpastatin as an indication of beef tenderness.

Protein fragments as an indication of beef tenderness

Introduction: The increase in beef tenderness following storage at refrigeration temperatures is widely believed to be due to enzymatic breakdown of muscle proteins (Koohmaraie, 1994). Muscle proteins can be classified as sarcoplasmic, myofibrillar or connective tissue proteins. Of the three groups of proteins only minor changes occur in connective tissue proteins during post-mortem ageing and it is generally accepted that meat tenderness increases during ageing as a result of degradation of myofibrillar or sarcoplasmic proteins (Lawrie, 1985).

In contrast to myofibrillar proteins, proteolytic changes in sarcoplasmic proteins have received little attention in the study of meat tenderisation.

In recent years, high performance capillary gel electrophoresis (CGE) has been developed for the separation and molecular weight determination of peptides and proteins (Cohen & Karger, 1987). CGE is directly comparable to traditional slab gel SDS-PAGE since the separation mechanisms are identical. The CGE format offers a number of advantages over SDS-PAGE. The speed of analysis is the major advantage, with results being available in hours rather than days. Another important advantage is the on-line detection system.

The aim of this study was to determine if sarcoplasmic and myofibrillar protein degradation, monitored by SDS-PAGE or CGE, could be used as an indicator of meat tenderness. In the case of myofibrillar proteins CGE was investigated as an approach to detecting previously undetected myofibrillar protein degradation products during post-mortem ageing.

Animals and samples: The muscle samples from the animals described above were also used in this study. The results of SDS-PAGE and CGE carried out on the same striploin samples are reported. SDS-PAGE and CGE analysis was performed on all fifty animals but the results obtained from only 2 animals, one “tender” and one “tough”, are reported here. These animals were chosen for presentation because they differed in pH_3 and pH_7 values, tenderness scores and WBSF (Table 7).

Table 7: Tenderness, shear force and pH values for "tender" and "tough" striploins

Sample	pH_3	pH_7	Tenderness score[1]	WBSF[2]
Tender	6.41	5.66	7.63	3.52
Tough	6.90	6.25	4.13	9.72

[1]Scale 1-8; 1 = extremely tough, 8 = extremely tender

[2]Warner-Bratzler shear force measured in kg.

GEL ELECTROPHORESIS OF SARCOPLASMIC PROTEINS

The 15% SDS-PAGE gel profiles of sarcoplasmic proteins from "tender" and "tough" striploins were similar to profiles reported previously (Borchert et al., 1969). Based on the calculation of molecular weights from the standards, the molecular weight of sample proteins ranged from 74 kDa to 12 kDa.

No visible changes in the SDS-PAGE profiles were observed during ageing from 3 h to 14 d post-mortem. Similar results were obtained when the polyacrylamide concentration was varied. Furthermore, there were no apparent differences between "tender" and "tough" in the SDS-PAGE profiles of sarcoplasmic proteins.

CAPILLARY GEL ELECTROPHORESIS OF SARCOPLASMIC PROTEINS

The electropherograms of sarcoplasmic proteins extracted at 3 h and 14 d post-mortem from "tender" meat showed peaks which corresponded to proteins of different molecular weights. Using an internal standard (benzoic acid) and a CGE standard, the molecular weights of unknown proteins or fragments were calculated. The molecular weight range of the sarcoplasmic proteins separated by CGE, 7 kDa to 78 kDa, was similar to that obtained on the 15% SDS-PAGE gel. The first obvious differences between the 3 h

and 14 d samples were the decreases in the peaks corresponding to a molecular weights of 8 kDa, 14 kDa, 25 kDa, 48kDa and 73 kDa. The peak corresponding to 48 kDa could possibly be creatine kinase. On the other hand the series of peaks in the 9 kDa to 12 kDa region increased. These increases in peak height could be due to the degradation of larger sarcoplasmic or myofibrillar proteins to give protein fragments. The electropherograms of all animals studied were similar.

Based on these preliminary results, and in comparison to SDS-PAGE, CGE seemed to be more sensitive to changes in sarcoplasmic proteins post-mortem. However, protein sequencing would be necessary to identify the peaks which have increased with ageing and to determine whether or not they were proteolytic fragments of sarcoplasmic or myofibrillar proteins.

Myofibrillar proteins

GEL ELECTROPHORESIS OF MYOFIBRILLAR PROTEINS

The SDS-PAGE profiles of myofibrillar proteins were analysed and the results generalised in Table 8. The molecular weights of the proteins or protein fragments on the gel lay between approximately 500 kDa and 12 kDa. The two most prominent bands on this gel can be attributed to myosin (210 kDa) and actin (45 kDa). Major alterations in the intensity of these bands have not been detected previously (Bechtel & Parrish, 1983) and actin or myosin degradation is not generally considered as a potential indicator of meat tenderness.

A number of proteolytic fragments in the 28 to 34 kDa region of the gel appeared and increased with post-mortem ageing of the muscle samples (Table 8). The 30 kDa band has been used previously as an indicator of tenderness (Olson *et al.*, 1977)

The 30 and the 32 kDa bands were more visible in “tender” than “tough” after 48 h storage (Table 8). It has been confirmed that the 30 and 32 kDa bands are degradation products of troponin T. However, the structural role, if any, which troponin T plays in the tenderisation process is unclear.

Table 8: Fragments of myofibrillar proteins extracted from "tender" and "tough" striploins at 24h, 48 h, 7 d and 14 d post-mortem

Protein (kDa)	Tender	Tough
28 to 34	+ + + + +	+ +
Troponin-T	+ +	+ + + + +
55	+	+ + +
110	+ + +	+
Nebulin	+	+ + +

+ = level of appearance

It has been suggested by Ertbjerg (1996) that the degradation of troponin-T is probably a marker for some other changes rather than a primary event in the weakening of the myofibril structure. The 34 kDa band was also evident in "tender" after 48 h but not in "tough".

Detecting protein fragments during the conditioning period

The intensity of the 55 kDa band, believed to be the cytoskeletal protein desmin (Troy, 1987), decreased with post-mortem ageing and was absent in all animals studied by 14 d post-mortem. Degradation of desmin during meat ageing has been noted by several researchers (Young *et al.*, 1980; Penny *et al.*, 1984; Weber, 1984). The susceptibility of desmin to the action of cathepsin B and L is uncertain. It has been shown that desmin is resistant to cathepsin D. Desmin has also been shown to be a suitable substrate for calpains (Lazarides, 1980). Hwan & Bandman (1989) concluded, after incubation of muscle homogenates at different pHs with a variety of protease inhibitors, that it was unlikely that both neutral calpains and acidic cathepsins were responsible for the degradation of desmin during post-mortem ageing at 4^0C. While the intensity of the 55 kDa band decreased with time, the rate of decrease was not obviously different from sample to sample suggesting that it would not be suitable as an indicator of tenderness (Fig.2).

In 10% SDS-PAGE gels of myofibrillar proteins (results not shown) the degradation of two other important structural proteins, titin and nebulin, was observed. Titin degradation began at 48 h post-mortem and appeared to be complete in all samples at 14 d post-mortem. Penny *et al.* (1984) reported that titin is degraded by lysosomal cathepsins during post-mortem storage. The action of the neutral calpains on titin is still unclear (O'Halloran, 1996). Like desmin, the degradation of titin, as observed using SDS-PAGE, could not be used as an indicator of meat tenderness as its degradation was observed to be identical in both "tender" and "tough".

Nebulin was observed to be completely degraded after 48 h of post-mortem ageing in all muscles used in this study. It has been reported that nebulin can be degraded by cathepsin L (Penny et al., 1984) and calpains (Huff-Lonergan et al., 1995). Nebulin is believed to link the thin filament to the Z-line structure. Therefore, degradation of nebulin would result in the weakening of this link and consequently lead to an increase in tenderness. However in this study there was no difference in the rate of nebulin degradation with post-mortem ageing in different animals.

Conclusion

Regarding myofibrillar proteins, a number of protein fragments appeared on the SDS-PAGE profiles with increasing time post-mortem. In agreement with previous studies the most important of these were the 30 kDa, 32 kDa and the 34 kDa fragments and the rate of their appearance could be related subjectively to tenderness. No additional information relating to the potential use of myofibrillar protein profiles to predict tenderness was obtained in this study. A more quantitative approach is presently being undertaken.

There is no evidence from this study to suggest that SDS-PAGE profiles of sarcoplasmic proteins could be used in the detection of muscles with different post-mortem pH values or ultimate tenderness. SDS-PAGE may not be a sensitive enough technique to identify proteolytic changes in sarcoplasmic proteins. However, some changes were observed in the electropherograms obtained following CGE separation of sarcoplasmic proteins from LD muscle aged for different times. As yet the proteins or protein fragments responsible for these changes have not been identified, although their molecular weights are known. The CGE electropherograms were similar for all animals although the data for only two animals is shown. It would appear that sarcoplasmic proteins are of little use as predictors of meat tenderness.

PHYSICAL INDICATORS OF BEEF QUALITY

Electrical properties of muscle tissues

Two electrical properties of meat were studied i.e. electrical conductivity and impedance. Both these properties are altered during the ageing of meat when damage occurs in the membrane systems of muscle, particularly at high temperatures. Conductivity will rise with an increase in free fluids within a muscle; conversely, impedance (a combinant of resistance and capacitance) will decrease as muscle ages.

The extent of damage to the muscle membranes is thought to be caused by the activity of proteolytic enzymes. Therefore the extent of the increase in conductivity or decrease in impedance is thought to be related to meat tenderness, water-holding capacity and colour.

Post-mortem changes in muscle electrical properties and their relationship to meat quality attributes

The electrical properties of bovine muscle tissue was investigated to determine their relationship to meat quality attributes. The experiment used 47 carcasses from heifers which were randomly selected in an industrial meat plant.

All heifers were chilled at 12-13^{0}C for 10-11h, followed by storage at 0^{0}C until 24h post-mortem at which time the right hand side striploins were excised. The rate of temperature and pH fall were monitored up to 24h. Electrical impedance, conductivity and capacitance measurements were taken at hourly intervals up to 8 hours post-mortem and again at 1, 2, 7 and 14 days post-mortem. Samples were taken at various times post-mortem between 2 and 14d for sensory analysis, Warner Bratzler shear force (WBSF), sarcomere length, drip loss, cook loss and Hunter Lab colour measurements. Results are presented for 47 carcasses (mean values ± standard deviation).

Measuring the conductivity of beef striploins

ELECTRICAL IMPEDANCE (PY) AND CONDUCTIVITY (EC)

Py decreased on average from 75 at 7 hours post-mortem to 6 at 14 days post-mortem (Fig. 3). However there was a large variation in Py between striploins at a given time post-mortem particularly at 1 and 2 days.

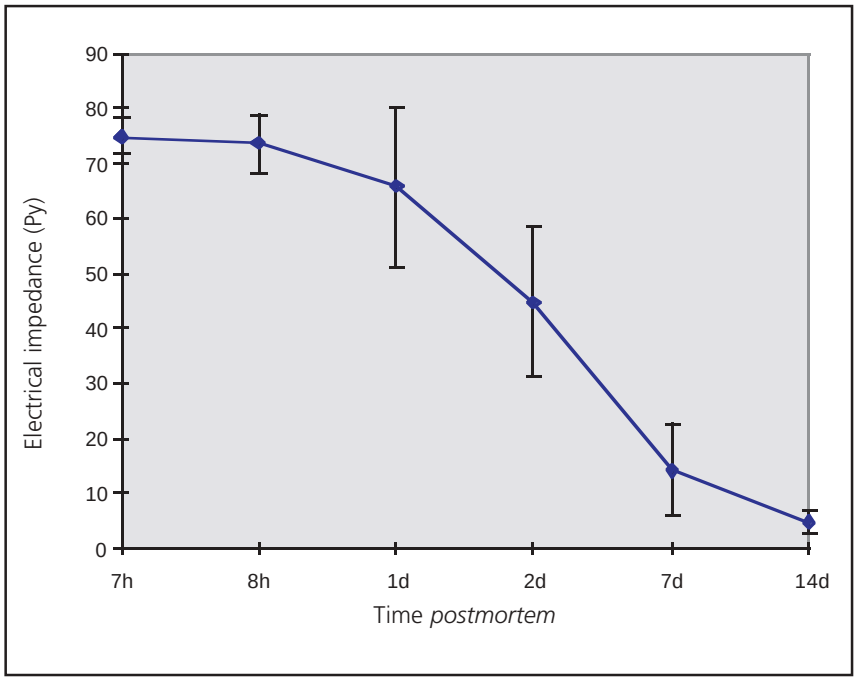

Figure 2: Decrease of electrical impedance (Py) in beef striploin during ageing for 14 days

EC increased throughout the storage period (Fig 3). Again, a large variation between striploins was observed especially at 1 and 2 days post-mortem.

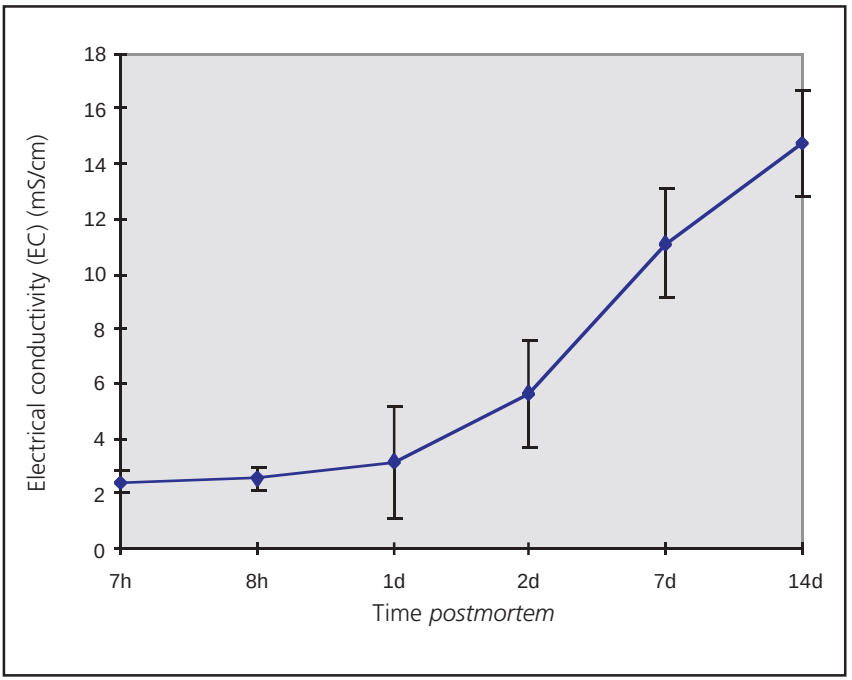

Figure 3: Increase of electrical conductivity (EC) in beef striploin during ageing for 14 days

CORRELATIONS WITH SENSORY ATTRIBUTES

Relationships between actual electrical measurements and quality parameters at that point of ageing, yield highly significant correlation coefficients. Tenderness, colour and shear force were all significantly correlated to both impedance and conductivity values (Table 9). This demonstrates that measurement of these electrical properties are useful in segregating tough and tender meat and gives an indication as to whether the meat was aged or not. It could prove useful to the retail and catering trade with further refinement.

Data were analysed to assess the use of early post-mortem electrical measurements (up to 2 days) to predict meat quality (up to 14 days). Results showed that no significant relationships were found and so the ability of electrical measurements to predict meat quality was not demonstrated. However the ability of electrical measurements to indicate the sensory attributes of meat at a given time post-mortem was shown. This is probably due to the strong relationship between the electrical properties and the extent of muscle ageing.

Table 9: Simple correlation coefficients obtained between electrical properties and selected meat quality attributes evaluated in beef striploins aged for 2, 7 and 14 days

	Py	EC
Warner Bratzler shear force	-0.65***	0.68***
Hunter a value	0.65***	-0.66***
Hunter b value	0.56***	-0.57***
Tenderness	0.56***	-0.60***
Overall acceptability	0.55***	-0.58***

Significance level : *** $p<0.001$, NS = not significant

Near infrared (NIR)

NIR has been used in the meat industry to analyse the fat, moisture and protein composition. Few studies of the relationships between sensory properties and NIR measurements of beef during ageing have been identified in the literature. The ability of NIR to detect changes in the state of water and hydrogen bond interactions in foods has been observed. Since such changes evidently occur in meat during tenderisation and ageing, a

relationship may exist between NIR measurements and meat quality attributes. This was initially investigated by Mitsumoto et al. (1991), who obtained high correlation coefficients between NIR spectral data and Warner Bratzler Shear Force values. Since then various workers have demonstrated high correlations between NIR spectra and various quality attributes of sausages and beef cuts, showing promise for NIR as a meat quality indicator. NIR is a rapid, easy to use, non-destructive, safe and automated system.

Near infrared reflectance spectra as indicators of beef quality

This study was carried out to investigate the relationship between NIR spectral data and meat quality attributes. It was designed to mimic industrial requirements and to this end spectra were collected using a fibre optic surface interactance probe.

Carcasses (n=24) were chilled at 12-13^{0}C for 10-11h, followed by storage in a 0^{0}C chill until 24h post-mortem at which time the sirloin muscles were excised and stored at 4^{0}C for 14 days. Freshly cut samples (2.5cm thick) were taken at 1, 2, 7 and 14d post-mortem for NIR analysis, sensory analysis, Hunter Lab colour measurements, cook loss, and sarcomere length determination. Reflectance spectra between 750 and 1098nm were recorded. NIR measurements and sensory analysis were carried out.

Models were generated for the prediction of meat quality attributes by principle component regression (PCR) using the optimal number of components. PCR proved to be accurate in predicting meat quality attributes, having high correlation coefficients for all attributes listed (Table 10).

Table 10: Prediction of Warner Bratzler shear force, sensory tenderness, flavour, texture and acceptability of sirloin using near infrared spectral data by principle component regression

Quality attribute	r	SEP	RPD	n	Optimal PCs
WBSF	0.82	1.72	1.7	84	7
Tenderness	0.67	0.71	1.3	64	7
Flavour	0.51	0.35	1.2	64	7
Texture	0.53	0.41	1.2	64	7
Acceptability	0.46	0.52	1.1	64	7

r = Correlation coefficient

SEP = Standard error of prediction

RPD = Ratio of the standard error of prediction to the standard deviation

n = Number of samples used

Optimal PCs = Optimal number of principal components used in model generation.

Figure 4 shows a representative sample of the spectra collected. The major features are two broad peaks and a large offset between the individual spectra. Seven components were identified as the optimal number to describe the variance in the spectral data and used to generate predictive models for WBSF, tenderness, flavour, texture and acceptability (Table 10). Correlation coefficients calculated in the case of WBSF and tenderness values are high, being capable of explaining approximately 67% and 45%, respectively, of the variation in these two quality attributes. This suggests that NIR spectroscopy in the range studied may have potential for the prediction of these two parameters. The sample with high WBSF values has a higher overall absorbance at all wavelengths measured than that with low

WBSF value, with the mean lying somewhere between the two except at lower wavelengths (<830nm). In the case of the other sensory properties, correlation coefficients are low, with the derived models only explaining approximately 26, 28 and 21% of the variation in flavour, texture and overall acceptability respectively (Table 10), suggesting that this model may not be as useful for the prediction of these attributes.

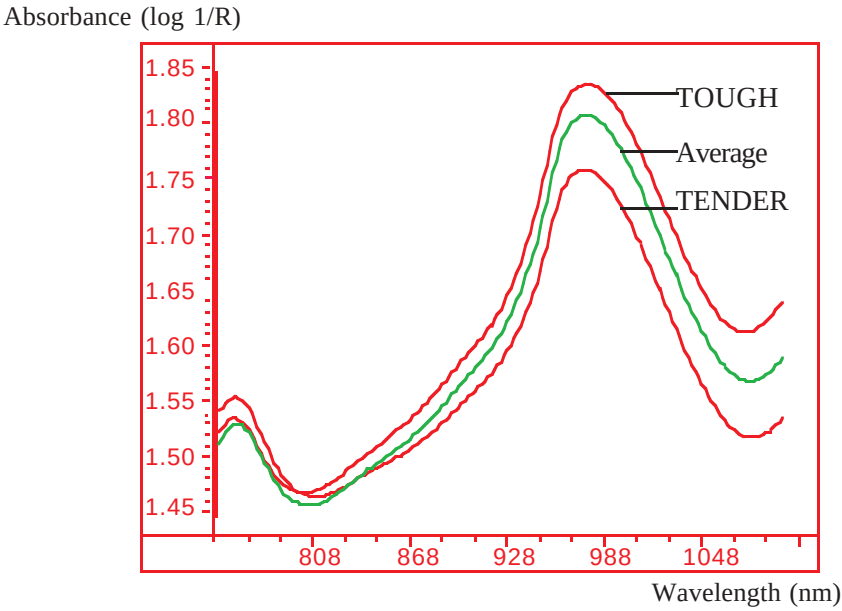

Figure 4: Mean near infrared spectra from bovine striploins (n=24), together with spectra of the toughest and most tender samples in the set according to sensory analysis

Hunter 'L', 'a' and 'b' values demonstrated high correlation coefficients of 0.56, 0.82 and 0.84 respectively with NIR spectral data. Sarcomere length and cook loss were also moderately correlated (0.53 and 0.44, respectively) with NIR data.

The results described so far relate to the ability of combined NIR spectra recorded at 1, 2, 7 and 14d post-mortem to predict meat sensory parameters measured over the ageing period. Another specific area of interest lies in determining the ability of spectra recorded at various times post-slaughter to predict the sensory properties of meat after 14d storage. To investigate this issue, spectra recorded at 1 (Table 11), 2, 7 and 14d post-mortem were used to predict sensory data recorded after 14d storage.

Table 11: Prediction of Warner Bratzler Shear Force, sensory tenderness, flavour, texture and acceptability of sirloin stored for 14 days at 4°C by principal component regression using spectra recorded on day 1

Time of spectral collection	Quality attribute	r	SEP	RPD	n	Optimal PCs
1d	WBSF	0.89	0.67	2.3	20	6
	Tenderness	0.80	0.46	1.7	20	6
	Flavour	0.72	0.38	1.5	20	6
	Texture	0.73	0.30	1.5	20	6
	Acceptability	0.78	0.41	1.6	20	6

r = Correlation coefficient

SEP = Standard error of prediction

RPD = Ratio of the standard error of prediction to the standard deviation

n = Number of samples used

Optimal PCs = Optimal number of principal components used in model generation.

Spectra recorded at 1d after slaughter revealed high correlations (Table 11) with all of the sensory variables suggesting that spectra recorded soon after slaughter may have the potential to predict the eventual magnitude of these parameters several days later. In general, there is little difference between the correlations achieved with either 1d or 2d spectra although WBSF was better predicted using spectra recorded at the earlier time.

Shear force as an indicator of tenderness

Analysis of Warner-Bratzler shear force measurements throughout the course of this study revealed that shear force taken at 2 days post-mortem had a strong relationship to sensory tenderness scores at 7 and 14 days (mean correlation of 0.53, $P<0.001$). This suggests that a shear force taken at 2 days reveals some information on the tenderness of muscle during the ageing process. If strict sampling procedures in terms of location, fibre direction, cooling etc., were specified it is possible that this measurement (shear force at 2 days post-mortem) could be a useful indicator and indeed a predictor of tenderness.

Measuring the shear force of beef striploins

CONCLUSIONS

Correlations between the beef quality attributes and near infrared data suggest that this non-destructive technique has the potential to produce useful predictive models, especially in the case of Warner Bratzler shear force and sensory tenderness. These models possess significant accuracy to determine the selected quality variables in beef during ageing but more importantly NIR spectroscopy appears to have the potential to predict the 14d quality of beef through measurements taken as early as 1 or 2d post-mortem. A trial is required to report the predictive ability of NIR spectroscopy in an industrial environment.

RESULTS FROM OTHER RESEARCH ACTIVITIES FROM THIS PROJECT

University College Cork examined the influence of dietary vitamin E content and fatty acid composition, post-mortem ageing and storage on the colour, flavour and texture of beef and other meats. In addition, the use of predictive modelling to assess simple meat quality measurements from samples taken shortly after slaughter was investigated.

A consistently high level of quality is essential for Irish meats to compete on the supermarket shelves of Europe. Increasing the stability of muscle lipids and myoglobin by vitamin E dietary supplementation and modified atmosphere packaging will prolong shelf life.

Dietary vitamin E in cattle feeds was deposited throughout the muscles. This increased oxidative and colour stability of meat held under different packaging (aerobic, MAP, vacuum) and temperature (4^0C, -20^0C) conditions. At a molecular level, vitamin E interacted with the cytochrome b system to improve colour stability. Vitamin E supplementation reduced cholesterol oxidation in cooked beef and reduced warmed-over flavour. Predictive modelling of meat quality showed that vitamin E had a positive effect on reducing drip loss in fresh beef cuts.

University College Dublin assessed the impact of rapid post-slaughter chilling systems on beef texture and eating quality in hot deboned beef cuts.

It was demonstrated that accelerated chilling could produce meat as tender as conventionally chilled meat and that crust freezing is essential (in addition to proteolysis) if cold-shortening is to be avoided. The exact mechanism is unclear. Electrical stimulation, in addition to fast chilling, was found to produce tender beef. However, the relative effects of the restraint provided by crust freezing and stimulation, though positive, remains unclear. A higher degree of proteolysis was observed in the stimulated samples due to the rapid fall in muscle pH. In general, the main effect of rapid chilling is in the provision of a rigid outer surface that physically prevents shortening rather than in providing an increase in proteolytic activity.

OVERALL CONCLUSIONS

The following section outlines the main conclusions of this study.

- pH values taken at 3 and 7 h post-mortem (pH3 and pH7, respectively) were significantly correlated with tenderness assessed by WBSF measurement and sensory analysis.
- In terms of early post-mortem predictors of tenderness it appears that the activity of calpastatin offers the most potential. A significant correlation was found between the level of calpastatin at 6 h post-mortem and tenderness scores at 14 d post-mortem. The level of calpastatin activity at 24 h post-mortem was also shown to be a potential indicator of meat tenderness with significant correlations between its activity and WBSF at 2 d post-mortem. More research is required to develop a more rapid and non-destructive test for this marker.
- A number of protein fragments appeared on the SDS-PAGE profiles with increasing time post-mortem. The most important of these were the 30 kDa, 32 kDa and the 34 kDa fragments and the rate of their appearance could be related subjectively to tenderness. However, a more rapid and quantitative method is required for use in the industry such as an ELISA test kit.

- Electrical measurements can segregate tough and tender beef in so far as it indicates whether meat has undergone the ageing process or not. This would be useful for the retail trade.
- Near infra-red spectra related strongly with texture measurements and offers an exciting potential as a reliable indicator of tenderness. However, it presently is an off-line measurement and requires further refinement.

Future research needs

There is still a great deal of research to be carried out in order to confidently identify meat with desirable quality attributes such as tenderness.

In the area of biochemical markers, much more rapid, easy-to-use and non-destructive tests are required. The measurement of a sensitive biochemical marker to meat tenderness remains elusive. Perhaps protein fragments and protease activities will provide these markers but in both cases the measurement is highly technical and cumbersome. The development of ELISA test kits which could be used on soluble extracts (e.g. the drip or exudate of meat) would be a reasonable answer. Transferring technologies from other disciplines such as medical research (e.g. biosensors) could help in delivering a solution more quickly.

Physical indicators probably provide a more realistic solution. Many are rapid, non-destructive and easy to use. However the interpretation of the signal in terms of quality attributes is more complex and will require more detailed studies. Near infra-red technology is emerging as an exciting tool for meat quality measurements. More effort should be made to develop on-line NIR probes for use in the meat industry. In the field of engineering, much more mechanically sensitive devices are being developed. There is a need to import state of the art engineering technologies into the area of meat science.

Developing meat quality predictors involves more than one scientific discipline and perhaps that is the reason none are readily available. Future research in this area demands a multi-disciplinary approach especially in the areas of food technology and engineering.

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