EFFECT OF ENDOGENOUS PROTEINASES ON POSTMORTEM PROTEOLYSIS IN RABBIT LONGISSIMUS MUSCLE

A.S. BABIKER, A.L. SCHROEDER, M. KOOHMARAIE, P.H. BERNTHAL, T.R. DUTSON AND R.A. MERKEL

Depts. of Animal Science and Food Science and Human Nutrition
Michigan State University
East Lansing, MI 48824 USA

INTRODUCTION

Meat tenderness increases substantially during postmortem aging (Goll et al., 1964; Etherington, 1981). The changes in tenderness have been attributed to modification of the myofibrils by proteolysis (Bechtel and Parrish, 1983; Ouali et al., 1983; Yates et al., 1983). The changes include the loss of the Z-disks (Penny, 1980; Etherington, 1981) and fragmentation of myofibrils (Olson et al., 1976). Gradual disappearance of troponin-T and desmin with the appearance of bands in the 27 to 32 kilodalton (kd) region of electrophoretograms of myofibrillar proteins also have been well documented (Dabrowska et al., 1973; Olson and Parrish, 1977).

High temperature (22 - 37°C) conditioning (HT) and electrical stimulation (ES) have been widely used to avoid cold-shortening and enhance meat tenderness (Carse, 1973; Dutson et al., 1977; Lochner et al., 1980). HT is believed to improve tenderness through enhancement of proteolytic enzyme activities, either lysosomal (Dutson et al., 1977) or the calcium-dependent proteinases (CDP's) (Marsh, 1983). The mechanism whereby ES produces its effect is unresolved. The combination of low pH and high muscle temperature, produced by HT conditioning and ES, are believed to cause rupture of lysosomes and release of catheptic enzymes which degrade myofibrillar proteins and lead to tenderization (Dutson et al., 1980b; Wu et al., 1985). ES improvement of meat tenderness via fiber fracture also has been reported (Marsh et al., 1981).

The use of enzyme inhibitors in vivo to assess the contribution of proteolysis to postmortem tenderization has not been explored to date. Leupeptin, a peptide-aldehyde, readily diffuses across cell membranes. Leupeptin inhibits degradation of proteins in isolated muscle tissue (Libby and Goldberg, 1978) and in vivo (Stracher et al., 1978; Sher et al., 1981). It inhibits cathepsin B (Kirschke et al., 1980), cathepsin L (Kirschke et al., 1980) and the CDP's (Sher et al., 1981). Thus, leupeptin would appear to be useful to assess the role of these enzymes in the degradation of myofibrillar proteins and enhanced postmortem tenderization.

This study has been undertaken to: 1) explore the in vivo use of enzyme inhibitors to assess the role of proteolysis in postmortem tenderization; 2) determine the in vivo inhibitory activity of a cysteine proteinase inhibitor (leupeptin) against some lysosomal and sarcoplasmic cysteine proteinases and 3) determine whether proteolysis by these selected enzymes is responsible for the tenderizing effect of HT conditioning and ES of rabbit carcasses.

MATERIALS AND METHODS

Thirty-two rabbits (3 kg live weight) were allotted to a symmetrical two-level factorial design in incomplete blocks. The factorial design includes: 1) electrically stimulated (ES) vs non-stimulated (NES) carcasses; 2) carcass conditioning temperature of 2° (LT) or 22° C (HT); and 3) with (L) or without (NL) leupeptin injection. Leupeptin, 100 mg/kg body weight, in 0.9% saline was injected intraperitoneally. One h after injection, the animals were slaughtered and electrical stimulated using a Grass S5 stimulater delivering square waves with 14.3 pules/s and duration of 5 ms at 80 v for 3 min. The rabbits were dressed, swabbed with 1 mM NaN_3 and held at their respective conditioning temperature. Longissimus muscle samples were removed for myofibrillar frag-

mentation index (MFI), calcium-dependent proteinases (CDP's), cathepsins B, H and L activities and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of myofibrillar proteins. Four h postmortem another sample was taken to assess effects of ES and HT on distribution of lysosomal enzymes (β-glucuronidase, cathepsins B, H and L). Determination of MFI and SDS-PAGE of myofibrillar proteins also was conducted at 24, 72 and 168 h post-mortem.

MFI was determined according to the procedure of Culler et al. (1978). Lysosomal enzymes activities was performed by a modification of the method of Moeller et al. (1976). The two supernatants after centrifugation in the MFI procedure were combined and centrifuged at 105,000 x g for 2 h at 2°C to yield an unsedimentable fraction (supernatant) and a microsomal fraction (pellet). The pellet was homogenized in 10 ml of MFI isolating medium containing 0.1% Triton X-100. The myofibril suspension, after MFI determination, was diluted with an equal volume of isolating medium containing 0.2% Triton X-100 and used as the nuclear fraction. Protein concentration in the three fractions (unsedimentable, US; sedimentable, S; and nuclear, NF, respectively) was determined by Biuret (Gornall et al., 1949), aliquots diluted with 0.1% Brij-35 to contain 1 mg protein/ml and used for determination of lysosomal enzyme activities.

β-glucuronidase activity (lysosomal enzyme marker) was assessed by the fluometric procedure of Wu et al. (1985). Cathepsins B, H and L activities were determined fluorometrically (Barrett, 1980) as described by Kirschke et al. (1983).

Separation of CDP's and their inhibitor on DEAE-Sephacel was as described by Koohmaraie et al. (1987). The inhibitor was eluted with 0.145 M NaCl and the CDP's with 0.5 M NaCl. Activity of the CDP's was determined by caseinolytic assay (Dayton et al., 1976).

Myofibrils for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were purified according to Goll et al. (1974). SDS-PAGE was conducted on slab gels (12.5% acrylamide, 37.5:1.0 acrylamide : bis-acrylamide) according to Laemmli (1970).

Data for the enzyme activities, percent release of lysosomal enzymes and MFI were analyzed using a 2^3 factorial in 4 replicates and 2 blocks per replicate. Each interaction term was confounded in one replicate (Gill, 1978).

RESULTS AND DISCUSSION

Table 1 shows the activities of CDP's, cathepsins B, L and H as affected by leupeptin and the percent inhibition of each enzyme. Leupeptin decreased ($P<0.001$) the activity of the CDP's and the free activity of cathepsins B and L. Leupeptin did not affect ($P>0.05$) the free activity of cathepsin H or the bound activities of any of the lysosomal enzymes. These observations are consistent with the data of others who reported that leupeptin inhibited cathepsin B activity in vitro (Libby and Goldberg, 1978) and in vivo (Sutherland and Greenbaum, 1983). Injection of leupeptin in dystrophic mice inhibited CDP activity (Sher et al., 1981). Leupeptin did not inhibit activity of cathepsin H which agrees with results of others (Kirschke et al., 1980). The lack of leupeptin inhibition of bound activities of cathepsins B and L may indicate that leupeptin did not enter lysosomes.

Leupeptin did not completely inhibit activities of the CDP's, cathepsins B or L. This may be due to low levels of leupeptin reaching the muscles. Tanoka (1983) reported that leupeptin was rapidly metabolized in animals. Leupeptin inactivating enzyme in tissues of mice have also been observed (Place et al., 1985). Thus, leupeptin in this study may have been partially degraded and the amount that reached the muscle was too low to completely inhibit these enzymes.

TABLE 1. THE EFFECT OF IN VIVO INJECTION OF LEUPEPTIN ON THE ACTIVIT CDP'S, CATHEPSINS B, L AND H AT ZERO HOUR POSTMORTEM

Enzyme	No Leupeptin	Leupeptin Injected	SE[a]	Percent Inhibition
CDP's[b]	37.31	19.58***	2.62	47.5
cathepsin B (free)[c,d]	4.42	1.99***	0.34	55.0
(bound)[c,e]	3.07	3.52	0.23	----
cathepsin L (free)[c,d]	5.31	1.26***	0.29	76.2
(bound)[c,e]	3.50	3.07	0.21	----
cathepsin H (free)[c,d]	20.76	20.33	0.98	2.1
(bound)[c,e]	5.92	6.94	0.36	----

[a] - standard error of mean

[b] - CDP's total activity in OD units/ 50g muscle.

[c] - catheptic enzymes total activities in mol of product released $.min^{-1}$. 4.0 g $muscle^{-1}$.

[d] - Free activity represent the activity in unsedimentable fraction (105,000 xg supernate).

[e] - Bound activity represent the sum of the activities in the microsomal (105,000 xg pellet) and nuclear (1,000 xg pellet) fractions.

*** P<0.001.

TABLE 2. EFFECTS OF ES AND HT ON THE RELEASE OF LYSOSOMAL ENZYMES AT 4 HOUR POSTMORTEM

Enzyme	LT	HT	NS	ES
β-Glucuronidase	52.9	56.1	53.2	55.8
Cathepsin B	71.9***	78.2	74.7	75.4
Cathepsin L	74.8	79.3	76.7	77.5
Cathepsin H	88.8*	90.5	88.9*	90.4

Values are % free activity calculated as [activity in US fraction/ total activity] X 100.

Total activity = activity in unsedimentable + sedimentable fractions.

*, *** Indicates a significant difference between adjacent means on a line (P<0.05 and P<0.001, respectively).

Effects of ES and HT conditioning on the release of lysosomal enzymes at 4 h postmortem are presented in Tables 2 and 3. Moeller et al. (1976) reported that HT and Dutson et al. (1980b) found that ES increased the percent of activity of β-glucuronidase and cathepsin C released. Also, the increased percent of activity released of β-glucuronidase, cathepsins B and H in the ES treatment group was largely due to the decreased activity of the microsomal fraction (Wu et al., 1985). However, the data in Tables 2 and 3 do not agree with those of Wu et al. (1985). It is evident that when the activity in the nuclear fraction was omitted from the calculation of total activity HT increased ($P<0.05$) the percent of released activity of cathepsins B and H but had no effect on the activity of β-glucuronidase and cathepsin L. On the other hand, ES increased ($P<0.05$) the percent of released activity of cathepsin H with no effect on the other enzymes. When the nuclear fraction was included in the calculation of total activity, HT did not affect ($P>0.05$) the percent of released activity of any of the enzymes. ES increased ($P<0.05$) percent of released activity of β-glucuronidase and cathepsin L but did not affect those of cathepsins B and H. Thus, the effect of HT and ES on percent of activity released of lysosomal enzymes was not consistent and depended on how total activity was calculated.

The effects of conditioning temperature, ES and leupeptin on MFI are presented in Table 4. Leupeptin decreased ($P<0.01$) MFI at 24, 72 and 168 h postmortem. This suggests that myofibril fragmentation is, at least in part, due to the activities of CDP, cathepsins B or L. Since complete inhibition of these enzymes was not achieved, it is not known whether the increase in MFI of the leupeptin injected animals was due to the uninhibited activities of these enzymes or to other enzymes not inhibited by leupeptin. However, Ouali et al. (1987) reported that cathepsin H degraded barely detectable levels of the myofibrillar proteins when incubated with purified myofibrils. In addition, Matsukura et al. (1984) concluded that despite the partial destruction of Z-disks, myofibril fragmentation was not caused by cathepsin D. Koohmaraie et al. (1986) showed that 24-28% of CDP-I activity was found to cause most of the changes in MFI and SDS-PAGE of myofibrillar proteins. We recently showed that MFI was completely inhibited when bovine longissimus muscle slices were incubated with Ca^{2+} chelators (EDTA and EGTA). These data suggest that myofibril fragmentation and hence tenderization was caused by CDP activity rather than catheptic enzymes (Koohmaraie et al., 1988).

Although the effect of temperature on MFI was not significant, leupeptin reduced ($P<0.05$) the effect of HT on MFI at 24 h postmortem. This observation suggests that the effect of HT on myofibril fragmentation is mediated through proteolytic enzymes that are inhibited by leupeptin. ES increased ($P<0.001$) myofibril fragmentation at 24 h postmortem regardless of leupeptin injection. This indicates that the increase in myofibril fragmentation due to ES may not be mediated through proteolysis. This suggestion agrees with Sonaiya et al. (1982) who reported that although ES increased MFI it did not affect the time of appearance of the 30 kd band. Others also have concluded that ES enhances tenderness via fiber fracture (Marsh et al., 1981). Fiber fracture was not measured in our study.

The effect of leupeptin, conditioning temperature and ES on the SDS-PAGE pattern of myofibrillar proteins of the rabbit longissimus muscles were compared at 0, 24, 72 and 168 h postmortem. For comparison, beef longissimus samples were taken at the same periods during postmortem storage. The desmin and troponin-T (TN-T) bands were lost by 168 h in beef muscle with the concomitant appearance of a major band at 30 kd.

TABLE 3. EFFECTS OF ES AND HT ON THE RELEASE OF LYSOSOMAL ENZYMES AT 4 HOUR POSTMORTEM

Enzyme	LT	HT	NS	ES
β-Glucuronidase	42.1	45.0	41.6*	45.5
Cathepsin B	47.1	50.0	46.8	50.2
Cathepsin L	43.0	47.4	40.4*	50.0
Cathepsin H	73.0	77.1	73.1	77.1

Total activity = Activity in unsedimentable + sedimentable + nuclear fractions.

* Indicates a significant difference between adjacent means on a line ($P<0.05$).

TABLE 4. EFFECTS OF CONDITIONING TEMPERATURE, ES AND LEUPEPTIN ON MYOFIBRILLAR FRAGMENTATION INDEX[a]

Time Postmortem	Treatment		SE[b]
	NL	L	
0	44.45	43.10	1.15
24	69.96	64.42***	1.28
72	73.20	66.90**	2.15
168	76.00	68.86**	2.50
	LT	HT	
0	44.20	43.35	1.15
24	67.77	66.80	1.28
72	70.43	69.68	2.15
168	72.85	72.00	2.50
	NS	ES	
0	43.05	44.50	1.15
24	63.83	70.55***	1.28
72	68.15	71.95	2.15
168	72.30	72.55	2.50

[a] - values of MFI reported as (A_{540} X 200).
[b] - standard error of means.
[c] - significant interaction (temperature X leupeptin)

LT,NL	LT,L	HT,NL	HT,L
69.4	66.1	70.4	62.8

** $P<.01$.
*** $P<.001$.

A shift in the troponin-I (TN-I) band to a lower molecular weight also occurred. These changes which occurred in beef muscle did not occur in rabbit samples. A loss in intensity of the TN-T band and appearance of two protein bands at 32 kd and 27kd were evident in the ES, LT, NL samples. Additionally, two new bands appeared just below the TN-I band in the 24 and 72 h postmortem samples. These changes were completely inhibited by leupeptin since they did not appear in the ES, LT, L samples. Other investigators have observed that, unlike that observed in beef muscle, aging of rabbit muscle or treatment of rabbit myofibrils with proteinases produced new protein bands at 32 and 27 kd with no band at 30 kd (Ouali et al., 1987).

Rabbit longissimus muscle from the NES, HT, NL treatments and those of NES, HT, L showed that in NL samples, the bands at 32 and 27 kd appeared after 24 h but decreased in intensity by 168 h postmortem. In the corresponding L samples, the two bands also appeared at 24 h but had completely disappeared at 72 and 168 h. No explanation is apparent for this observation. The NL samples clearly showed the bands at 32 and 27 kd at 24, 72 and 168 h postmortem. The L samples showed many faint bands in the 32 to 27 kd region. Thus, the effects of leupeptin, aging temperature and ES on the SDS-PAGE banding pattern of myofibrillar proteins of rabbit muscles did not appear to be consistent when compared to beef.

CONCLUSIONS

This experiment has demonstrated the potential of using enzyme inhibitors in vivo for postmortem aging studies to resolve the involvement of proteolysis in postmortem tenderization. The mechanism whereby ES produces its effects on meat tenderness was also determined. Leupeptin decreased ($P<0.001$) the activities of the CDP's, cathepsins B and L with concomitant reduction ($P<0.01$) of myofibril fragmentation. In some animals, leupeptin inhibited the appearance of myofibrillar protein degradation products visualized by SDS-PAGE banding pattern but the effects were not consistent among rabbits. Effects of ES and HT (4 h) on the release of lysosomal enzymes was not consistent among animals. The data also suggest that the release of the lysosomal enzymes may not be a major contributor to the mechanism whereby ES and HT improve tenderness. ES increased ($P<0.001$) MFI values at 24 h postmortem regardless of leupeptin injection suggesting that ES may have caused fiber fracture rather than releasing lysosomal enzymes.

REFERENCES

Barrett, A.J. (1980): Flurometric assays for cathepsin B and cathepsin H with methylcoumaryl-amide substrates. Biochem. J. 187:909.

Bechtel, P.J. & Parrish, F.C., Jr. (1983): Effects of postmortem storage and temperature on muscle protein degra-dation: Analysis by SDS gel elec-trophoresis. J. Food Sci. 48:294.

Bohley, P., Riemann, S., Koelsch, R. & Lasch, J. (1977): Protein degradation in rat liver cells. Acta Biol. Med. Germ. 36:1821.

Carse, W.A. (1973): Meat quality and the acceleration of postmortem glycolysis by electrical stimulation. J. Food Technol. 8:163.

Culler, R.D., Parrish, F.C., Jr., Smith, G.C. & Cross, H.R. (1978): Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177.

Dabrowska, R., Barylko, B., Nowak, E. & Drabikowski, W. (1973): The origin of 30,000 dalton protein in troponin preparations. FEBS Letters 29:239.

Dayton, W.R., Reville, W.J., Goll, D.E. & Stromer, M.H. (1976): A Ca^{2+}-activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochemistry 15:2159.

Dutson, T.R. & Lawrie, R>A. (1974): Release of lysosomal enzymes during postmortem conditioning and their relationship to tenderness. J. Food Technol. 9:43.

Dutson, T.R., Smith, G.C. & Carpenter, Z.L. (1980a): Lysosomal enzyme distribution in electrically stimulated ovine muscle. J. Food Sci. 45:1097.

Dutson, T.R., Smith, G.C., Savell, J.W. & Carpenter, Z.L. (1980b): Possible mechanisms by which electrical stimulation improves meat tenderness. Proc. 26th Meet. Eur. Meat Res. Workers, Vol. 2:84. Colorado Springs, CO.

Dutson, T.R., Yates, L.D., Smith, G.C., Carpenter, Z.L. & Hostetler, R.L. (1977): Rigor onset before chilling. Proc. Recip. Meat Conf. 30:79.

Etherington, D.J. (1981): Enzyme actions in the conditioning of meat. In "Proteinases and Their Inhibitors: Structure, Function and Applied Aspects" (V. Turk and L.J. Vitale, eds.) pp. 279-290. Pergamon Press, Oxford.

Gill, J.L. (1978): Design and analysis of experiments in the animal and medical sciences. Vol. 2. The Iowa State University Press, Ames, IA.

Goll, D.E., Henderson, D.W. & Kline, E.A. (1964): Postmortem changes in physical and chemical properties of bovine muscle. J. Food Sci. 29:590.

Goll, D.E., Otsuka, Y., Nagainis, P.A., Shannon, J.D., Sathe, S.K., Muguruma, M. (1983): Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137.

Goll, D.E., Young, R.B. & Stromer, M.H. (1974): Separation of subcellular organelles by differential and density gradient centrifugation. Proc. Recip. Meat Conf. 27:250.

Kirschke, H., Langner, J., Riemann, S., Wiederanders, B., Ansorge, S. & Bohley, P. (1980): Lysosomal cysteine proteinases. In "Protein Degradation in Health and Disease". (D. Evered and J. Welan, eds.) pp 15-35. Excerpita Medica, New York, NY.

Kirschke, H., Wood, L., Roisen, F.J. & Bird, J.W.C. (1983): Activity of lysosomal cysteine proteinase during differentiation of rat skeletal muscle. Biochem. J. 214:871.

Koohmaraie, M., Babiker, A.S., Merkel, R.A. & Dutson, T.R. (1988): Role of Ca^{2+}-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 83:1253.

Koohmaraie, M., Schollmeyer, J.E. & Dutson, T.R. (1986): Effect of low calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. J. Food Sci. 51:28.

Koohmaraie, M., Seideman, S.C., Schollmeyer, J.E., Dutson, T.R. & Crouse, J.D. (1987): Effect of postmortem storage on Ca^{++}-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19:187.

Libby, P. & Goldberg, A.L. (1978): Leupeptin, a protease inhibitor, decreases protein degradation in normal and diseased muscle. Science 199:534.

Libby, P. & Goldberg, A.L. (1980): Effects of chymostatin and other proteinase inhibitors on protein breakdown and proteolytic activities in muscle. Biochem. J. 188:213.

Lochner, J.N. Kauffman, R.G. & Marsh, B.B. (1980): Early postmortem cooling rate and beef tenderness. Meat Sci. 4:227.

Marsh, B.B., Lochner, J.V., Takahashi, G. & Kragness, D.D. (1981): Effects of early postmortem pH and temperature on beef tenderness. A research note. Meat Sci. 5:479.

Matsukura, W., Matsumoto, T., Tashiro, Y., Okitani, A. & Kato, H. (1984): Morphological changes in myofibrils and glycerinated muscle fibers on treatment with cathepsins D and L. Int. J. Biochem. 16:957.

Moeller, P.W., Fields, P.A., Dutson, T.R., Landmann, W.A. & Carpenter, Z.L. (1976): Effects of high temperature conditioning on subcellular distribution and levels of lysosomal enzymes. J. Food Sci. 41:216.

Olson, D.G., Parrish, F.C., Jr. & Stromer, M.H. (1976): Myofibril fragmentation and shear resistance of three bovine muscles during postmortem aging. J. Food Sci. 41:1036.

Ouali, A., Garrel, N., Obled, A., Devol, C., Valin, C. & Penny, I.F. (1987): Comparative action of cathepsins D, B, H, L and of a new lysosomal cysteine proteinase on rabbit myofibrils. Meat Sci. 19:83.

Ouali, A., Obled, A., Cottin, P., Merdaci, N., Ducastaing, A. & Valin, C. (1983): Comparative effects of postmortem storage and low-calcium-requiring neutral proteinase on bovine and rabbit myofibrillar proteins. J. Sci. Food Agr. 34:466.

Penny, I.F. (1980): The enzymology of conditioning. In: "Developments in Meat Science", Vol. 1 (R.A. Lawrie, ed.) pp. 115-143. Applied Science Publishers, Barking, Essex.

Place, G.A., Ning, M.C.Y.J. & Beynon, R.J. (1985): A leupeptin inactivating enzyme from rat liver. In: "Intracellular Protein Catabolism" (Khairalla, E.A., J.S. Bond and J.W.C. Bird, eds.) pp 269-271. Alan R. Liss, Inc., New York, NY.

Sher, J.H., Stracher, A., Shafiq, S.A. & Hardy-Stashin, J. (1981): Successful treatment of murine muscular dystrophy with the proteinase inhibitor leupeptin. Proc. Natl. Acad. Sci. USA 78:7742.

Sonaiya, E.B., Stouffer, J.R. & Beermann, D.H. (1982): Electrical stimulation of mature cow carcasses and its effect on tenderness, myofibril protein degradation and fragmentation. J. Food Sci. 47:889.

Stracher, A., McGowan, E.B. & Shafiq, S.A. (1978): Muscular dystrophy: Inhibition of degeneration in vivo with protease inhibitors. Science 200:50.

Sutherland, J.H.R. & Greenbaum, L.M. (1983): Paradoxical effect of leupeptin in vivo on cathepsin B activity. Biochem. Biophys. Res. Comm. 110:332.

Takahashi, K., Kim, O.H. & Yano, K. (1987a): Calcium-induced weakening of Z-disks in postmortem skeletal muscle. J. Biochem. 101:767.

Takahashi, K., Yamanoue, M., Murakami, T., Nishimura, T. & Yoshikawa, R. (1987b): Paratropomyosin, a new myofibrillar protein, weakens rigor linkages formed between actin and myosin. J. Biochem. 102:1187.

Tanaka, W. (1983):
Absorption, distribution, metabolism and excretion of leupeptin. In: "Proteinase Inhibitors: Medical and Biological Aspects". (Katunuma, N., H. Umezawa and H. Holzer, eds.) pp 17-24. Springer-Verlag, New York, NY.

Wu, F.Y., Dutson, T.R., Valin, C., Cross, H.R. & Smith, S.B. (1985):
Aging index, lysosomal enzyme activities and meat tenderness in muscles from electrically stimulated bull and steer carcasses. J. Food Sci. 50:1025.

Yates, L.D., Dutson, T.R., Caldwell, J. & Carpenter, Z.L. (1983):
Effect of temperature and pH on the postmortem degradation of myofibrillar proteins. Meat Sci. 9:157.