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Acceleration of Postmortem Tenderization in Ovine Carcasses Through Activation of Ca^{2+}-Dependent Proteases

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ABSTRACT

Infusion of lamb carcasses with 0.3M $CaCl_2$ resulted in acceleration of postmortem tenderization process. Control and treated animals had similar cathepsin B, H, and L activities. Various control groups had similar CDP-I, -II and inhibitor activities, whereas these were all decreased in $CaCl_2$ infused animals. Hence, it was concluded that the activation of Ca^{2+}-dependent proteases was responsible for the observed postmortem proteolysis and tenderization and, it seems unlikely that activity of these cathepsins is related to postmortem tenderization under conditions used in this experiment.

INTRODUCTION

TENDERNESS is the prominent quality determinant and probably the most important sensory characteristic of meat. Currently, postmortem aging appears to be one of the best methods for producing tender meat. Although the increase in meat tenderness is measurable both subjectively and objectively, the exact mechanism of postmortem tenderization still remains an unresolved issue.

Many studies have been carried out on the changes of myofibrillar proteins during postmortem storage, the causes of these changes, and the relationship between these changes and meat tenderization (Penny, 1980; Asghar and Bhatti, 1987). Proteolysis of muscle appear to be a major contributor to the tenderization process during postmortem aging. Proteolytic changes in collagen during postmortem storage comparable to those of myofibrillar proteins (Tarrant, 1987) have not been observed. Therefore, the principal mechanism of meat tenderization during postmortem storage may be the limited hydrolysis of myofibrillar proteins by endogenous muscle proteases (Goll et al., 1983a).

Of the proteases indigenous to skeletal muscle, two likely proteolytic systems include, (1) the Ca^{2+}-dependent proteases (Goll et al., 1974a; Olson et al., 1977; Goll et al., 1983a; Koohmaraie et al., 1986; Zeece et al., 1986; Koohmaraie et al., 1987; Koohmaraie et al., 1988a,b) and (2) the cathepsins (Moeller et al., 1977; Dutson, 1983; Ouali et al., 1987). Some have suggested that cooperative action of these two protease systems also may occur (Penny and Ferguson-Pryce, 1979; Dutson, 1983; Asghar and Bhatti, 1987).

Incubation of bovine longissimus muscle slices obtained at 12 hrs postmortem (Koohmaraie et al., 1988b) in a buffer solution containing $CaCl_2$ demonstrated that most of the postmortem changes occurred within the first 24 hrs of incubation. On the other hand, when muscle slices were incubated in the same buffer containing 10 mM EGTA or 10 mM EDTA, these changes were completely blocked (Koohmaraie et al., 1988b). Because none of the treatments affected lysosomal enzyme activities (cathepsin B, H and L) and because there was a decrease in calcium-dependent protease activity, Koohmaraie et al. (1988b) concluded that: (1) the postmortem tenderization events are Ca^{2+} mediated, and that (2) calcium-dependent proteases are responsible for postmortem tenderization. The present experiment was conducted to determine whether the tenderization process could be accelerated by infusion of carcasses with $CaCl_2$ immediately after death.

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MATERIAL & METHODS

A TOTAL of 24 lambs (8–12 month old) were slaughtered in groups of four; one lamb for each of the four treatments: (1) control (animals slaughtered according to normal procedures); (2) electrically stimulated (6 cycles, each cycle consists of 20 sec on, 20 sec off, 21 volts, rectangular waves, 14 cycle/sec); (3) electrically stimulated and then infused (10% of the live weight) with water (distilled, deionized, glass distilled), and (4) electrically stimulated and then infused (10% of the live weight) with 0.3M $CaCl_2$. The above solutions were infused through the carotid artery (following electrical stimulation and prior to evisceration) with a ham pumping device (Presto Precision Product, Inc. Farmingdale, NY). After completion of the infusion process, the carcasses were transferred to a holding cooler (1–2°C). Twenty-four hr after slaughter, the entire loin and rib were removed from each carcass and divided into three sections. Each section was then randomly assigned to day 1, 3, or 6 for the following determinations. **Day 1:** shear force, Ca^{2+}-dependent protease-I, II, and their inhibitor activities, cathepsin B, H and L activities, sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of isolated myofibrils, and Ca^{2+} content. **Day 3:** SDS-PAGE of isolated myofibrils. **Day 6:** shear force, SDS-PAGE of isolated myofibrils.

Shear force determination

Shear force was determined on unfrozen lamb chops according to standard procedures. Briefly, chops were broiled on a Farberware "open hearth" broiler and internal temperature was monitored with iron constant thremocouple wires attached to a Honeywell 112 multipoint recorder. Chops were turned when internal temperature reached 40°C and removed from broiler when they reached 70°C. Chops were cooled for 24 hr at 2–4°C and six cores (1.27 cm diameter) were removed from each chop. Each core was sheared three times (total of 18 readings per chop) with a Warner-Bratzler device.

Preparation and assay of cathepsins B, H and L

Extraction and assay of catheptic enzymes were done according to the procedure described by Koohmaraie et al. (1988a).

Preparation and assay of Ca^{2+}-dependent protease-I, -II, and their inhibitor

Ca^{2+}-dependent protease-I (CDP-I), -II (CDP-II) and their inhibitor were prepared from 200g longissimus muscle. The muscles were trimmed of fat and connective tissue, cut into 2 × 2 cm pieces and stored at −70°C (not more than 14 days). The samples were removed from the freezer, allowed to stand at 2°C for 90 min and then homogenized in 2.5 vol 50 mM Tris-HCl, pH 8.3 containing 5 mM ethylene glycol-bis (β-aminoethyl ether) N, N, N', N'-tetraacetic acid (EGTA) and 10 mM 2-meracapetoethanol (MCE), in a Waring Blendor. To prevent Ca^{2+}-stimulated autolysis of CDP-I and CDP-II during extraction of muscle (Hathaway et al., 1982; Mellgren et al., 1982; Goll et al., 1983b), from lambs infused with $CaCl_2$, the samples were homogenized in 2.5 vol 150 mM Tris-HCl, pH 8.3 containing 50 mM EGTA, and 10 mM MCE. Homogenization was done twice for 30 sec at the low speed setting and twice for 30 sec at the high

speed setting, with a 30 sec cooling period between bursts. The homogenate was centrifuged at 30,000 x g_{max} for 60 min. The supernate was filtered through two layers of cheese cloth and pH was adjusted to 7.5. The pH-adjusted supernate was then passed through glass wool and dialyzed against 20 mM Tris-HCl, pH 7.5 containing 5 mM EGTA and 10 mM MCE, two changes for 18–24 hr. After dialysis, the extracts were centrifuged at 30,000 × g_{max} for 60 min, chromatographed on DEAE-Sephacel column that had been equilibrated with 20 mM Tris-HCl, pH 7.40 containing 10 mM NaCl, 0.1 mM EGTA and 10 mM MCE. Columns were then washed with the same buffer to remove unbound protein, until absorbance of the outflow at 278 nm was between 0.1 to 0.4. The bound proteins were then eluted with a continuous gradient (total volume of 700 mL) of 10–500 mM NaCl in the elution buffer.

Activities of CDP-I and CDP-II were determined by using casein as substrate. The reaction mixture contained 5 mg/mL casein in 100 mM Tris-acetate, pH 7.5 containing 10 mM MCE, 1 mM NaN_3 and 5 mM $CaCl_2$ in a total volume of 2.0 mL. To determine Ca^{2+}-independent activity for each fraction assayed, 5 mM $CaCl_2$ was replaced with 10 mM EDTA. After 50 min incubation at 25°C, the reaction was stopped by adding 2 mL 5% trichloroacetic acid. After centrifugation at 3000 × g_{max} for 30 min, the A_{278} of the supernatant was measured. The inhibitor activities were determined as described by Koohmaraie et al. (1988a).

Myofibril isolation

Myofibrils were isolated according to the procedure described by Goll et al. (1974b).

Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted according to Laemmli (1970) on 0% polyacrylamide slab gels.

Protein concentration

Protein concentration were determined by the biuret method (Gornall et al., 1949).

Calcium determination

The total calcium contents of the longissimus muscle was measured using standard techniques, with an atomic absorption spectrophotometer (model D 951, Instrument Lab. Inc., Lexington, MA). Briefly, 5–7g muscle (at 24 hr postmortem) were ashed at 450°C followed by HNO_3-$HC10_4$ digestion. Upon completion of the digestion process, the amount of Ca^{2+} was measured by atomic absorption spectrometry.

Statistical analysis

Animals were randomly assigned to treatments. Data were analyzed by one way analysis of variance. When significant differences among treatments were detected, treatment means were compared using the least significant difference method. The comparisonwise error rate was 0.05 (SAS, 1982).

RESULTS

THE CALCIUM CONTENT (μg/g wet tissue) of the carcasses infused with $CaCl_2$ was increased about 40-fold as compared to the other treatments (Table 1). Infusion of carcasses with $CaCl_2$ significantly ($P<0.05$) lowered longissimus muscle shear force values by 24 hr and these values did not change with further postmortem storage time. To avoid the Ca^{2+}-induced muscle shortening, low frequency electrical stimulation (LFES) was used to diminish the ATP supply in the prerigor muscle. LFES improved tenderness (shear value) by about 17%. To ensure that the observed effects were not due to infused water lamb carcasses were infused with water only after LFES. There was no affect on shear value. Catheptic enzyme activities were not affected by treatment. CDP-I and CDP-II and their inhibitor had similar activities for the CON, LFES, and LFES + H_2O treatments while they were significantly lowered in carcasses infused with $CaCl_2$.

The effect of these treatments on myofibrillar proteins were studied on myofibrils purified from the longissimus muscle after 1, 3, and 6 days of postmortem storage and subjected to SDS-PAGE (Fig. 1). The 30,000 dalton component was already present in longissimus muscle of $CaCl_2$ infused lamb 24 hrs postmortem and its intensity increased only slightly with further postmortem storage. In contrast, the 30,000 dalton component appeared gradually with postmortem storage time for muscle from CON lambs but it was readily apparent after 6 days of storage. It appears that LFES accelerated the appearance of the 30,000 dalton component, because this proteolytic fragment was present in LFES and in LFES + H_2O groups after 3 days of postmortem storage. Appearance of the 30,000 dalton component has been reported to be the most consistent and noticeable change that occurs in myofibrillar proteins during postmortem storage (MacBride and Parrish, 1977; Olson and Parrish, 1977; Olson et al., 1977; Parrish et al., 1981; Penny, 1980; Koohmaraie et al., 1984a,b,c).

DISCUSSION

INFUSION of carcasses with $CaCl_2$, eliminated the requirement for extended postmortem storage as indicated by shear force measurements at 24 hrs postmortem (Table 1). SDS-PAGE results indicate that changes in the myofibrillar proteins were completed within 24 hr as opposed to 3–7 days required for the same changes to occur in the other treatment groups. Because these treatments had no effect on the lysosomal enzymes and a clear and definable decrease in activities (autolysis) of CDP-I, CDP-II and their inhibitor, we conclude that: (1) postmortem tenderization is a Ca^{2+} mediated process and that (2) there is sufficient evidence to conclude that Ca^{2+} functions through activation of the CDPs.

These results clearly demonstrate that infusion of lamb carcasses with $CaCl_2$ resulted in loss of CDP-I and inhibitor activities and a significant decrease in the activity of CDP-II within the first 24 hr (Table 1). Without further investigations, it would be impossible to explain the effect of $CaCl_2$ infusion treatment on this proteolytic system. However, it has been clearly demonstrated that calcium-dependent proteases autolyze in the presence of calcium (Hathaway et al., 1982; Mellgren et al., 1982; Goll et al., 1983b; Parkes et al., 1985; Crawford et al., 1987) and continued incubation in the presence of calcium results in loss of enzymatic activity (Mellgren et al., 1982; Parkes et al., 1985; Crawford et al., 1987). Therefore, the loss of CDP-I activity and significant decrease in CDP-II activity could be due to autolysis of these proteases in the presence of calcium (Table 1). Studies conducted to examine the effect of postmortem storage on CDPs in bovine skeletal muscle (Ducasting et al., 1985; Koohmaraie et al., 1987) have demonstrated that conventional postmortem storage had no effect on CDP-II activity. Based on these observations (Ducasting et al., 1985; Koohmaraie et al., 1986) and the results of present study (Table 1) we have concluded that autolysis in the presence of calcium is the reason for loss of CDPs activities. Whether the process of autolysis and loss of CDP activity occurred before myofibrillar proteins degradations (Fig. 1) and tenderization (Table 1) cannot be determined without further studies. However, Crawford et al. (1987) reported that the loss of enzymatic activity due to autolysis in the presence of calcium is a slow process. For chicken CDP-II the large subunit is degraded, over 2 hr at 22°C, to active-site-containing fragments of 54,000 and 37,000 daltons, after which no further significant autolysis occurred (Parkes et al. 1985). However, the loss of enzyme activity due to autolysis in CDP-II from chicken muscles proceeds much more slowly such that even after 3 hr of exposure to 5 mM $CaCl_2$ at 22°C, it retained over 50% of its original activity (before autolysis). Therefore, it seems reasonable to suggest that myofibrillar protein degra-

Table 1—Effect of treatments on calcium content, shear forces and enzyme activities in ovine longissimus muscle

Traits	Treatments			
	Control	Electrically stimulated	Electrically stimulated, then infused with H_2O	Electrically stimulated, then infused with $CaCl_2$
Ca (μg/g tissue)[d]	53.0 ± 4.1[a]	57.0 ± 4.8[a]	56.9 ± 4.8[a]	1577.0 ± 186.4[b]
Shear force at day 1 (kg)[e]	7.60 ± 0.48[a]	6.32 ± 0.27[b]	6.23 ± 0.30[b]	3.56 ± 0.16[c]
Shear force at day 6 (kg)[e]	4.61 ± 0.85[a]	3.60 ± 0.31[ab]	3.03 ± 0.28[b]	2.77 ± 0.29[b]
Cathepsin B[f]				
Unsedimentable	25.6 ± 3.7[a]	31.4 ± 2.4[a]	41.1 ± 6.6[ab]	51.1 ± 7.7[b]
Sedimentable	16.3 ± 2.2	20.6 ± 1.9	27.2 ± 7.5	17.6 ± 3.7
Total	42.9 ± 5.8	52.1 ± 3.7	68.3 ± 13.9	68.7 ± 7.5
Cathepsin H[f]				
Unsedimentable	153.2 ± 21.8	163.5 ± 19.5	185.7 ± 20.5	204.7 ± 21.2
Sedimentable	20.1 ± 1.5	21.0 ± 2.2	28.4 ± 3.2	25.1 ± 2.95
Total	173.3 ± 22.5	184.5 ± 21.2	214.1 ± 21.6	229.9 ± 33.0
Cathepsin L[f]				
Unsedimentable	25.1 ± 2.2[a]	29.5 ± 3.4[a]	37.3 ± 6.9[a]	56.5 ± 9.2[b]
Sedimentable	183.6 ± 34.4	264.8 ± 77.9	246.1 ± 67.4	246.8 ± 65.5
Total	208.7 ± 34.7	294.3 ± 81.2	283.8 ± 73.5	303.3 ± 71.6
CDP-I[g]	81.7 ± 7.4[a]	77.3 ± 6.7[a]	84.3 ± 3.1[a]	0.0 ± 0.0[b]
CDP-II[h]	136.9 ± 5.7[a]	124.8 ± 5.5[a]	126.5 ± 1.2[a]	57.6 ± 5.7[b]
CDP-I + CDP-II	218.6 ± 8.9[a]	202.2 ± 9.0[a]	210.8 ± 2.7[a]	57.6 ± 5.7[b]
Inhibitor[i]	78.3 ± 7.0[a]	68.3 ± 4.5[a]	69.2 ± 7.9[a]	0.0 ± 0.0[b]

[a,b,c] Means within the same row with different superscripts differ ($P<0.05$).
[d] on wet basis.
[e] kg/1.27 cm core
[f] μunits/mg protein/min.
[g] Low Ca^{2+}-requiring Ca^{2+}-dependent protease, total activity/200 g muscle (caseinolytic activity)
[h] High Ca^{2+}-requiring Ca^{2+}-dependent protease, total activity/200 g muscle (caseinolytic activity)
[i] Inhibitor of CDP-I and CDP-II, A_{278}/200g muscle (inhibition of casein hydrolysis by CDP-II).

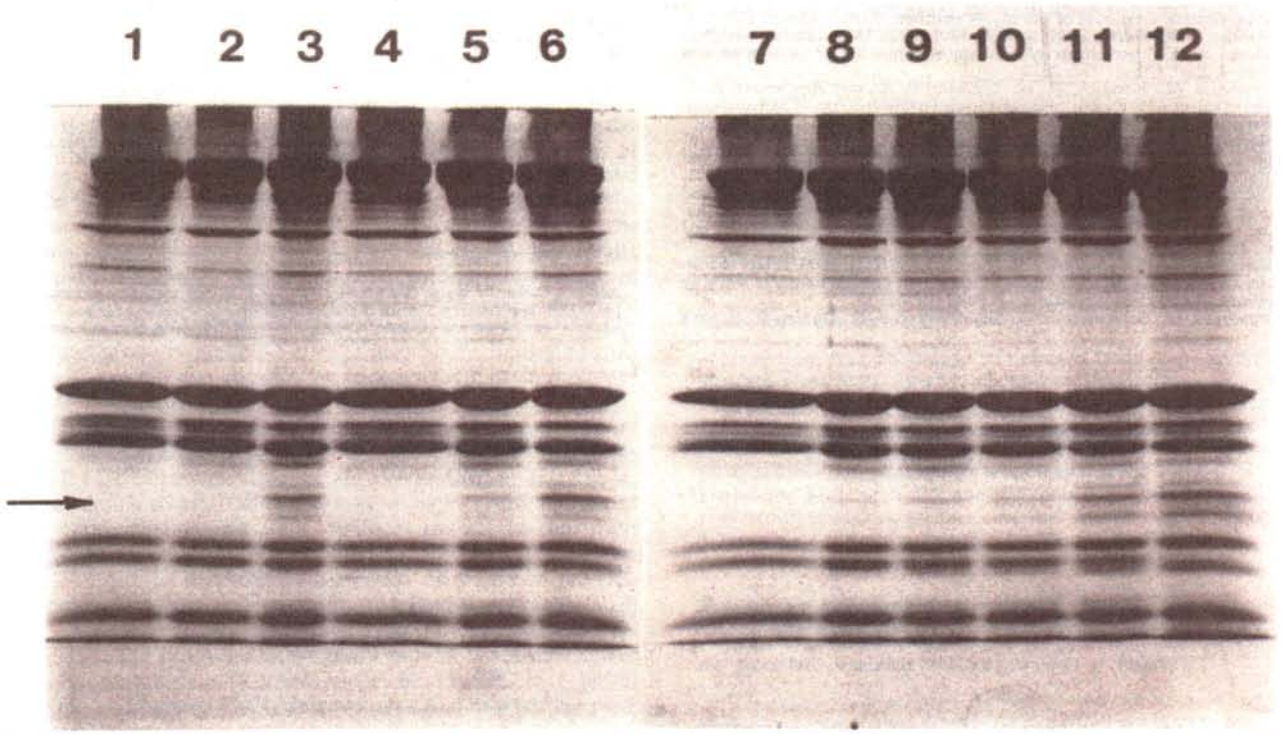

Fig. 1—SDS-PAGE of myofibrils isolated from longissimus muscle of treatments at different days postmortem. Gels 1, 2, and 3 represent Control; 4, 5, and 6 represent electrically stimulated; gels 7, 8 and 9 represent electrically stimulated then infused with water, and gels 10, 11 and 12 represent electrically stimulated then infused with $CaCl_2$ at 1, 3 and 6 days postmortem. Arrow indicates the position of 30,000 dalton fragment.

dation and tenderization took place prior to total loss of enzymatic activity.

Reasons for the loss of inhibitor activity with $CaCl_2$ infusion of carcasses are not readily apparent. It has been demonstrated that both CDP-I (Goll, personal communications) and CDP-II (Mellgren et al., 1986) are capable of hydrolyzing their specific inhibitor. However, Mellgren et al. (1986) reported that even after extensive cleavage of the inhibitor by calcium-dependent protease it remained a potent inhibitor of the protease. Mellgren et al. (1986) suggested that calcium-dependent proteolysis of inhibitor may represent an initial step in the turnover of the inhibitor and further degradation of the inhibitor may occur by other proteases.

In summary, we report a procedure that would eliminate the requirement for postmortem storage beyond 24 hr to ensure meat tenderness. The procedure involves low frequency elec-

trical stimulation of carcasses after death followed by infusion of the carcasses with a $CaCl_2$-containing solution prior to evisceration. However, further studies will have to be conducted to ascertain the effect of this procedure on other important meat characteristics (e.g. flavor and protein functionality) before any recommendation of its commercial application can be made.

REFERENCES

Ashgar, A. and Bhatti, R. A. 1987. Endogenous proteolytic enzyme in skeletal muscle: Their significance in muscle physiology and during postmortem aging events in carcasses. Adv. Food Res. 31: 343.

Crawford, C., Willis, A. C., and Gagnon, J. 1987. The effects of autolysis on the structure of chicken calpain II. Biochem J. 248: 579.

Ducasting, A., Valin, C., Schollmeyer, J., and Cross, R. 1985. Effect of electrical stimulation on postmortem changes in the activities of two Ca dependent neutral proteinases and their inhibitor in beef muscle. Meat Sci. 15: 193.

Dutson, T. R. 1983. Relationship of pH and temperature to distribution of specific muscle proteins and activity of lysosomal proteinases. J. Food Biochem. 7: 223.

Goll, D. E., Stromer, M. H., Olson, D. G., Dayton, W. R, Suzuki, A., and Robson, R. M. 1974a. The role of myofibrillar proteins in meat tenderness. In "Proceedings of the Meat Industry Research Conference," p. 75, issued by the American Meat Institute Foundation, Arlington, VA.

Goll, D. E., Young, R. B., and Stromer, M. H. 1974b. Separation of subcellular organelles by differential and density centrifugation. 27th Ann. Recp. Meat Conf., p. 250.

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

Goll, D. E., Shannon, J. D., Edmunds, T., Sathe, S. K., Kleese, W. C., and Nagainis, P. A. 1983b. Properties and regulation of the Ca^{2+}-dependent proteinase. In "Calcium-Binding Proteins," B. deBernard et al., (Ed.), p. 19. Elsevier Science Publishers, London.

Gornall, A. G., Bardawill, C. J., and David. M. M. 1949. Determination of serum-protein by means of the biuret reaction. J. Biol. Chem. 177: 751.

Hathaway, D. R., Werth, D. K., and Haeberle, J. R. 1982. Limited autolysis reduces the Ca^{2+} requirement of a smooth muscle Ca^{2+}-activated protease. J. Biol. Chem. 257: 9072.

Koohmaraie, M., Kennick, W. H., Elgasim, E. A., and Anglemier, A. F. 1984a. Effect of pre-rigor pressurization on the activity of calcium-activated factor. J. Food Sci. 49: 680.

Koohmaraie, M., Kennick, W. H., Elgasim, E. A., and Anglemier, A. F. 1984b. Effect of postmortem storage on muscle protein degradation: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49: 292.

Koohmaraie, M., Kennick, W. H., Anglemier, A. F., Elgasim, E. A., and Jones, T. K. 1984c. Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49: 290.

Koohmaraie, M., Schollmeyer, J. E., and Duston, 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., and Crouse, J. D. 1987. Effect of postmortem storage on Ca^{2+}-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19: 187.

Koohmaraie, M., Seideman, S. C., Schollmeyer, J. E., Dutson, T. R., and Babiker, A.S. 1988a. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53: 407.

Koohmaraie, M., Babiker, A. S., Merkel, R. A., and Dutson, T. R. 1988b. The role of Ca^{2+}-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. In press.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680.

Locker, R. H. and Hagyard, C. J. 1963. A cold-shortening effect in beef muscles. J. Sci. Fd. Agric. 14: 787.

MacBride, M. A. and Parrish, F. C. Jr. 1977. The 30,000-dalton component of tender bovine longissimus muscle. J. Food Sci. 42: 1627.

Mellgren, R. L., Repetti, A., Muck, T. C., and Easly, J. 1982. Rabbit skeletal muscle calcium-dependent protease requiring Millimolar Ca^{2+}. J. Biol. Chem. 257: 7203.

Mellgren, R. L., Mericle, M. T., and Lane, R. D. 1986. Proteolysis of the calcium-dependent protease inhibitor by myocardial calcium-dependent protease. Arch. Bioch. Biophys. 246: 233.

Moeller, P. W., Fields, P. A., Dutson, T. R., Landmann, W. A., and Carpenter, Z. L. 1977. High temperature effects on lysosomal enzyme distribution and fragmentation of bovine muscle. J. Food Sci. 42: 510.

Olson, D. G. and Parish, F. C. Jr. 1977. Relationship of myofibril fragmentation index to measures of beefsteak tenderness. J. Food Sci. 42: 506.

Olson, D. G, Parrish, F. C. Jr., Dayton, W. R., and Goll, D. E. 1977. Effect of postmortem storage and calcium-activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42: 117.

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

Parkes, C., Kembhavi, A. A. and Barratt, A. J. 1985. Calpain inhibition by peptide epoxides. Biochem. J. 230: 509.

Parrish, F. C. Jr., Selvig, C. J., Culler, R. D., and Zeece, M. G. 1981. CAF activity calcium concentration, and the 30,000-dalton component of tough and tender bovine longissimus muscle. J. Food Sci. 46: 308.

Penny, I. F. 1980. The enzymology of conditioning. In "Developments in Meat Science—1," R. Lawrie (Ed.). Applied Science Publishers Ltd., London.

Penny, I. F. and Ferguson-Pryce, R. 1979. Measurement of autolysis in beef muscle homogenate. Meat Sci. 3: 121.

SAS. 1982. "SAS User's Guide: Statistics." Statistical Analysis System Institute, Inc., Cary, NC.

Tarrant, P. V. 1987. Review: Muscle biology and biochemistry. Proc. Eur. Meeting of Meat Research Workers, p. 1.

Zeece, M. G., Robson, R. M., Lusby, M. L., and Parrish, F. C. Jr. 1986. Effect of calcium activated protease (CAF) on bovine myofibrils under different conditions of pH and temperature. J. Food Sci. 51: 797.

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