S3P04.WP

EFFECT OF STRESS-INDUCED HIGH POSTMORTEM ULTIMATE pH ON PROTEASE ACTIVITY AND SENSORY PROPERTIES OF BEEF.

J.A. BELTRAN [1], I. JAIME [1], P. SANTOLARIA [2], C. SAÑUDO [1]
and P. ALBERTI [2] and P. RONCALES [1]

[1] Dep. Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad de Zaragoza. 50013 Zaragoza, Spain
[2] Dep. Producción Animal. Servicio de Investigación Agraria. Diputación General de Aragón. Apartado 727. 50080 Zaragoza, Spain

INTRODUCTION

It is well known that meat tenderness improves during aging. Among the mechanisms involved, proteolytic degradation of myofibrillar proteins plays the most important role. Calpains and cathepsins have been shown to be able to degrade myofibrillar proteins; the activity of both enzymatic systems is influenced by several factors, one of the most determinant being pH (Ouali, 1992).

Ultimate pH in post-mortem bovine muscle may vary between 5.4 and 7.2. High pH is associated with an increased tenderness (Penny *et al.*, 1963; Bouton *et al.*, 1973; Yu and Lee, 1986). One explanation of this could be that high pH favours the action of calpains.

Ante-mortem stress determines some important modifications in post-mortem biochemistry of muscle. The high pH of muscles from stressed animals is one of them (Mohan Raj *et al.*, 1992).

The aim of this study was to asses the effect of high pH obtained by stress on protease activity (calpains and cathepsins B and L) and selected sensory characteristics (tenderness and juiciness) in beef meat, with a view to relate post-mortem enzyme activity to sensory properties.

MATERIALS AND METHODS

Material

Longissimus thoracis (6th rib) of 44 stressed and unstressed Swiss Brown steer carcasses were analyzed for post-mortem pH, protease activities and selected sensory characteristics.

pH of tissue

About 3g of muscle were homogenised in 20ml distilled water for 15s. The measurement was carried out immediately using a Crison pH-meter with a combined glass electrode.

Preparation of muscle extracts

A portion of *Longissimus thoracis* was removed from each animal as soon as possible after slaughter (30 to 60 minutes) and processed immediately. The other portion of muscle was excised from the carcasses on the 7th day post-mortem.

Muscle was trimmed and minced.

Preparation of calpains

Partial purification of calpains and calpastatin from muscle tissue was performed according to the method described by Ducastaing *et al.* (1985) and adapted to HPLC. Briefly, muscle tissue (20 g) was homogenised with Ultraturrax in three volumes of 10mM Tris-HCl buffer pH7.5 containing 0.05M NaCl, 4mM EDTA, 2mM 2-mercaptoethanol and 1mM NaN_3. After one hour extraction under magnetic stirring, the homogenate was centrifuged at 30,000g for 30 minutes, the supernatant filtered through cheese cloth and then adjusted to pH7.5. Precipitated material was eliminated by centrifugation at 50,000g for 50 minutes. All operations were performed at 0-4°C with pre-cooled solutions. Aliquots of 50.000g supernatant were filtered through 0.22μm millipore membrane and loaded on a mono Q HR 10/10 column (Pharmacia) equilibrated in 5mM Tris-HCl buffer pH7.5, 0.1mM EDTA, 0.05M NaCl and 2mM 2-mercaptoethanol. Protein elution by a non-linear NaCl gradient (0.05-0.5M) was performed at a flow rate of 1ml/min and fractions of 1ml were collected.

Assay for calpain activity

Calcium-dependent proteolytic activity was assayed, according to the procedure described by Koohmaraie *et al.* in 1986, using casein (Hammerstein) as substrate at 25°C in 10mM KCl, 50mM tris-acetate, pH7.5, 10mM MCE, 2.5mM Ca^{2+} and 5mg/ml casein. Total reaction volume was 2ml. Control for enzyme as substrate accompanied each assay. The reaction was initiated by addition of calpain and stopped by addition of 2ml of 5% trichloroacetic acid (TCA).

The assay tubes were then centrifuged at 1000xg for 15 minutes and the absorbency of the supernatant was measured at 278nm.

Assay for inhibitor activity

The activity of the inhibitor was determined by pre-incubating appropriate amounts of inhibitor and enzyme at 25°C for 60 minutes in 1.5ml reaction mixture (Koohmaraie *et al.*, 1986).

Preparation of B+L cathepsins

A portion of muscle tissue was used for the preparation of muscle lysosomes by a modification of the method of Obled *et al.* (1984). Briefly, muscle tissue was homogenized into seven volumes of ice-cold 0.25M sucrose, pH7.2, containing 0.15M KCl and mMEDTA. Tissue debris was recovered by centrifugation at 1000g for 10 minutes, re-suspended in a further seven volumes of sucrose solution and again homogenized. The homogenate was centrifuged as before and combined supernatants centrifuged at 3000g for 10 minutes. The lysosomal pellet was obtained by centrifuging at 25000g for 20 minutes and then extracted overnight at 4°C in 50mM sodium acetate buffer, pH5.0 containing 1mM EDTA and 0.2% (v/v) Triton X -100. The lysosomal extract was clarified by centrifugating at 25000g for 20 minutes.

Assay for B+L cathepsins activity

Cathepsins B and L together were assayed fluorimetrically with the common substrate, N-CBZ-L-phenylalanyl-l-arginine 7-amido- 4-methylcoumarin substrate (Z-Phe-Arg-NHMec) according to Barret (1980).

Sensory evaluation

The 7th day post-mortem, after aging at 4°C under vacuum, ribs were cut into 2.5cm steaks and frozen for subsequent taste panel evaluation. The steaks were thawed at 4°C for 24 hours prior to cooking and serving. Steaks were placed in a preheated grill at 160°C and removed when internal temperature had reached 70°C. Muscle strips were served on pre-heated plates to be evaluated by a trained panel consisting of eleven members.

Tenderness, juiciness, flavour intensity and overall acceptability were scored by placing a mark on an unstructured 100mm line scale anchored at the ends with the terms "extremely tender", "extremely juicy", "intense beef flavour", "high quality" and "extremely tough", "extremely dry", 'tasteless", "low quality".

RESULTS AND DISCUSSION

As a consequence of stress, ultimate pH of muscles varied between 5.4 and 6.9. Three muscle groups were established: >6.3; 6.3-5.8; <5.8 according to their ultimate pH.

Table 1 shows the activity of μ-calpain, m-calpain and calpastatin at pre-rigor and 7th day post-mortem for the three groups of pH. The only highly significant difference related to pH was found in the m-calpain activity at 7th day, this was higher for the group of pH>6.3. It was noticeable that calpastatin activity was not modified by pH. Results shown in Table 1 indicate that cathepsins (B+L) activity was not affected by pH. There was no difference among the three groups above mentioned.

Post-mortem pH also exerted a highly significant effect upon sensory parameters (Table 2) such as overall tenderness, juiciness or acceptability. Quality parameters were in all cases directly related to pH, i.e., the higher the pH the more tender and juicy the meat. These results agreed with Dransfield (1980) for the increase in juiciness and Penny *et al.* (1963), Bouton *et al.*, (1973), Yu and Lee, (1986) for the increase in tenderness.

Our results (Table 3) indicated a high, statistically significant correlation (r=0.776) between tenderness and the levels of m-calpain activity at 7th day post-mortem , while they were not correlated to μ-calpain (there was not activity at 7th day post-mortem), calpastatin and cathepsins (B+L). Calpains are claimed as the main proteolytic system responsible for post-mortem proteolysis (Koohmaraie, 1992). Most researchers (Koohmaraie *et al.*, 1987; Geesink *et al.*, 1992; Dransfield, 1993) attributed to μ-calpain a major contribution to proteolysis and thus to meat tenderization. It has been also demonstrated that μ-calpain activity decreases very rapidly in post-mortem muscle, as well as that high ultimate pH may accelerate this process (Geesink *et al.*, 1992). On the contrary, m-calpain has been shown to maintain high activity levels throughout meat aging (Melloni *et al.*, 1992). The fact that m-calpain is able to autolyse and decrease its calcium requirements (Suzuki, 1987) allows its involvement in meat proteolysis.

High ultimate pH may lead to an improvement in tenderness due to increased proteolytic activity, as the calcium-dependent proteases are known to have a pH optimum close to 7 (Asghar and Bhatti, 1987; Ceña *et al.*, 1992).

According to the results of our investigation we conclude that high post-mortem ultimate pH, as a consequence of stress, significantly increases m-calpain activity measured at 7th day post-mortem, which results in a greatly enhanced tenderization of beef meat.

ACKNOWLEDGEMENTS

The authors wish to thank the University of Zaragoza (grant 92/229-40) and the Interministerial Commission of Food Science and Technology (CICYT) (grant ALI 92/0644) for partial support of this research.

REFERENCES

ASGHAR, A., and BHATTI, A.R. 1987. Endogenous enzymes in skeletal muscle: their significance in muscle physiology an during post-mortem aging events in carcasses. *Adv. Food Res.* 31:343-445.

BARRET, A.J. 1980. Fluorimetric assays for cathepsin B and H with methylcoumarylamide substrates. *Biochem. J.* 187:909-912.

BOUTON, P.E., CARROLL, F.D., HARRIS, P.V., and SHORTHOUSE, W.R. 1973. Influence of pH and fibre state upon factors affecting the tenderness of bovine muscle. *J. Food Sci.* 38:404-407.

CEÑA, P., JAIME, I., BELTRÀN, J.A., and RONCALÉS, P. 1992. Proteolytic activity of isolated lamb calpains on myofibrils under the conditions of pH, Ca^{2+} concentration and temperature existing in postmortem muscle. *Z. Lebensm. Unters. Forsch.* 194:248-251.

DRANSFIELD, E. 1993. Modelling post-mortem tenderisation-IV: role of calpains and calpastatin in conditioning. *Meat Sci.* 34:217-234.

DUCASTAING, A., VALIN, C., SCHOLLMEYER, J.E. and CROSS, R. 1985. Effects of electrical stimulation on post-mortem changes in the activities of two Ca dependent neutral proteinases and their inhibitor in beef muscle. *Meat Sci.* 15:193-202.

GEESINK, G.H., OUALI, A., SMULDERS, F.J.M., TALMANT, A., TASSY, C., GUIGNOT, F., and van LAACK, H.L.J.M. 1992. The role of ultimate pH in proteolysis and calpain/calpastatin activity in bovine muscle. *Biochimie.* 74:283-289.

GREASER, M.L. 1986. In: BECHTEL, P.J. (ed). *Muscle as Food.* Academic Press, Orlando, pp.37-102.

KOOHMARAIE, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in post-mortem proteolysis and meat tenderness. *Biochimie.* 74:239-245.

KOOHMARAIE, M., SCHOLLMEYER, J.E., and DUTSON, T.R. 1986. Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *J. Food Sci.* 51:28-32.

KOOHMARAIE, M., SEIDEMAN, S.C., SCHOLLMEYER, J.E., DUTSON, T.R., and CROUSE, J.D. 1987. Effect of post-mortem storage on Ca^{2+}-dependent proteases, their inhibitor and myofibril fragmentation. *Meat Sci.* 19:187-196.

MOHAN RAJ, A.B., RICE, D.A., KILPATRICK, D.J., McCAUGHEY, W.J., and McLAUCHLAN, W. 1992. Effect of mixing male sex types of cattle on their meat quality and stress-related parameters. *Meat Sci.* 32:367-386.

OBLED, A., OUALI, A., and VALIN, C. 1984. Cysteine proteinase content of rat muscle lysosomes. *Biochimie.* 66:609.

OUALI, A. 1992. Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie.* 74:251-265.

PENNY, I.F., VOYLE, C.A., and LAWRIE, R.A. 1963. A comparison of freeze-dried beef muscles of high or low ultimate pH. *J. Sci. Food Agric.* 14:535-543.

SUZUKI, K. 1987. Calcium-activated neutral protease: domain structure and activity regulation. *TIBS.* 12:103-105.

YU, L.P., and LEE, B. 1986. Effects of post-mortem pH and temperature on bovine muscle structure and meat tenderness. *J. Food Sci.* 51:774-780.

Table 1. Mean values (±SD) calpains (U/g)[1], calpastatin (inhibtion percentage) and B+L cathepsin[2] at two hours post-mortem and 7th day of aging in bovine *longissimus thoracis* muscle classified into three groups according to their ultimate pH values.

	<5.8	5.8-6.3	>6.3
μ-calpain 2h pm	34.8±15.2[a]	41.6±22.0[a]	39.2±19.2[a]
m-calpain 2h pm	50.8±18.8[a]	63.2±21.6[a]	59.2±26.8[a]
calpastatin 2h pm	60.2±24.7[a]	69.0±34.9[a]	69.9±22.4[a]
B+L 2h pm	0.100±0.05[a]	0.104±0.022[a]	0.108±0.053[a]
m-calpain 7d pm	16.8± 8.8[a]	35.4±17.6[a]	70.8±23.2[b]
calpastatin 7d pm	64.7±29.6[a]	73.4±43.5[a]	58.0±25.6[a]
B+L 7d pm	0.082±0.046[a]	0.125±0.076[a]	0.121±0.062[a]

[a,b] Different superscripts within a row indicate significant differences ($P<0.01$) between the three pH groups.
[1] 1 unit of activity is defined as an increase of OD (278nm) of 0.001/min/g muscle.
[2] Activity values are given in μmol substrate hydrolysed/min/g muscle.

Table 2. Mean values (±SD) of Warner-Bratzler shear force and sensory parameters at 7th day of aging in bovine *longissimus thoracis* muscle classified into three groups according to their ultimate pH values.

	<5.8	5.8-6.3	>6.3
WB	34.06^{a}±19.90	41.09^{a}± 6.73	23.37^{b}± 5.8
Tenderness	47.67^{a}± 9.19	51.48^{a}± 4.60	66.00^{b}± 7.66
Juiciness	49.24^{a}± 5.35	51.60^{a}± 7.91	62.74^{b}± 4.79
Flavour	63.28^{a}± 4.19	62.56^{a}± 4.12	65.34^{a}± 2.90
Overall acceptability	49.21^{a}± 8.41	49.42^{a}± 7.47	60.66^{b}± 4.36

^{a,b} Different superscripts within a column indicate significant differences ($P<0.01$) between the three pH groups.