E30

# EFFECT OF SODIUM CHLORIDE TREATMENTS ON THE MICROSTRUCTURE OF MUSCLE FIBRES

M.L. PÉREZ CHABELA, M.E. AGUILAR ALVAREZ, A. PÉREZ TORRES*, P. LARA CALDERÓN, I. GUERRERO LEGARRETA.
Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa. Apartado Postal 55-535, C.P. 09340 México D.F., México. *Departamento de Biología Celular y Tisular, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F.

KEYWORD

Microstructure, meat tenderization, calpaines. myofibrils

## BACKGROUND

Meat tenderization mechanisms have mainly been studied in beef however histological studies show no significant differences in muscle structures between species. Disruption of I-band and position of Z-line are related to aged meat, with no species-specificity (Etherington *et al.*, 1987).

The mechanical properties of meat are related to final pH, postmortem temperature, sarcomere length and enzymatic proteolysis of myofibrillar proteins, particularly troponine and desmine (Yu and Lee, 1986). One group of enzymes significantly involved in meat tenderization are the calpaines, sarcoplasmic cystein-proteases, of two types: calpain I, activated in the presence of 50 to 70 mM Ca, and calpain II activated with 1 to 5 mM Ca (Koohmaraie, *et al.*, 1988). Optimal conditions for calpain activation are 25°C and pH=7.5 (Cottin, *et al.*, 1991). When meat is marinated with sodium chloride, myofibrillar proteolysis takes place at an accelerated rate, reducing the time necessary for postmortem conditioning. It has been demonstrated that calpaines produce breakdown of Z-line, T-troponine, I-troponine, tropomyosine, a-actinine, titine and nebuline (Koohmaraie *et al.*, 1984).

However, reports in the literature regarding identification of T-tubules, transverse bridges, Z-disks and inter-myofibrillar connection during tenderisation is scattered. Studies on structure identification by electron microscopy are not consistent (Silva, *et al.* 1992).

## OBJECTIVES

To study the mechanisms of any breakdown of myofibrillar proteins, caused by marinating with calcium chloride during meat conditioning.

## METHODS

Samples from beef *Longissimus dorsi* were obtained just after slaughter and were divided into 1500 g portions. Half of the samples were marinated with calcium chloride (0.150 mM for 48 h, 4°C) with continuous stirring. Control samples were stored under parallel conditions. After marinating, each portion was divided into 5 sub-portions and kept at 4°C. Analyses were carried out on day 1 and every other day thereafter, up to a total of 14 days. Sensory analysis was carried out at the same sampling times in order to evaluate tenderness, juiciness and flavor with an untrained panel.

Sections of 1 $cm^3$ were taken and fixed with Bouin reagent (6 h). They were then included in O.C.T. and frozen at -70°C. Sections of 5 x 1 mm were fixed with 2.5% glutaraldehyde in a 0.1 M sodium cocodilate buffer at pH 7.4 (3 h, 4°C). The samples were sectioned, stained and placed under a transmission electron microscope.

## RESULTS AND DISCUSSION

Marinated meat showed considerable tenderization at day 1 of study, however there was limited subsequent tenderisation. This could be due to a self degradation of calpaines

when exposed to calcium, hence meat does not undergo excessive tenderization when calpaines are applied, in contrast to the action of plant proteases.

Sensory analysis show that there were no significant differences ($P>0.05$) with respect to juiciness and flavor, although tenderness was considerably different between marinated meat and control.

Prolonged exposure to calcium resulted in calpain inactivation, in agreement with Koohmaraie, *et al.*, (1992) and Zimmerman and Schalaepfer (1991) who demonstrated that autolysis of calpains is a common and irreversible process.
Structural differences were observed under the TEM between marinated and control samples, mainly as small holes and disruptions at the line Z region. A 30,000 Dalton component appeared in marinated samples after the first 24 hours of storage, in agreement with Koohmaraie, *et al.*, (1988)

REFERENCES

Cottin, P., Poussard, J.P., Desmazes, D., Georgescauld and Ducastaing, A. 1991. Free calcium and Calpain I activity. Biochimica et Biophysica Acta 1079:139.

Etherington, D.J., Taylor, M.A.J. and Dransfield, E. 1987. Conditioning of meat from different species. Relationship between tenderizing and the levels of Cathepsin B, Cathepsin L, Calpain I, Calpain II and Beta-glucoronidase. Meat Sci. 20:1.

Koohmaraie, M. 1992. Effect of pH, temperature and inhibitors on autolysis and catalytic activity of bovine skeletal muscle μ-calpain. J. Anim. Sci. 70:3071.

Koohmaraie, M., Babiker, A.S., Merkel, R.A. and Dutson, T.R. 1988. Role of calcium-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 53:1253.

Koohmaraie, M., Kennick, W.H., Anglemeir., Elgasin, E.A. and Jones, T.K. 1984. Effects of postmortem storage on muscle protein degradation: analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:229.

Silva, T.J.P., Bracker, C.E., Orcutt, M.W., Forrest, J.C. and Judge, M.D. 1992. Preparation of muscle samples for comparative electron microscopy. J. Anim. Sci. 70:2452.

Yu, L.P. and Lee, Y.B. 1986. Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. J. Food Sci. 51:774.

Zimmerman, U.P. and Schlaepfer, W.M. 1991. Two-stage autolysis of the catalytic subunit that initiates activation of calpain I. Biochim. Biophys. Acta 1078:192.