USE OF A BOVINE SPLEEN LYSOSOMAL-ENRICHED EXTRACT FOR TENDERISATION OF BEEF

Jesús A. Melendo, José Antonio Beltrán and Pedro Roncalés

Dept. of Animal Production and Food Science. Laboratory of Food Technology. Faculty of Veterinary Science. University of Zaragoza. C. Miguel Servet 177. 50013 ZARAGOZA. SPAIN.

INTRODUCTION AND OBJECTIVES

The role of cathepsins in post-mortem tenderisation of meat is subjected to controversy, although it does not seem to be of primary importance (Roncalés et al., 1995). However, since Robbins and Cohen (1976) observed structural changes in the region of the Z bands of bovine myofibrils treated with an extract of bovine spleen, their potential as an exogenous muscle tenderiser has been strongly suggested (Cohen et al., 1979; Robbins et al., 1979). Furthermore, Kopp and Valin (1981) concluded that enzymes of muscle lysosomal fraction reduced the strength of muscle collagen at pH 5.5. Collagenolytic activity of cathepsins was also described by Beltrán et al. (1993).

Recently, we applied a lysosomal-enriched extract from bovine spleen (BSLE) for the tenderisation of squid (Melendo et al., 1997). Tenderisation was achieved without modifying extensively the characteristic texture of squid.

The aim of our research was to investigate the use of this extract for meat tenderisation.

MATERIAL AND METHODS.

Experimental setup. Bovine spleen lysosomal-enriched extracts (BSLE) were obtained as described by Lardeux et al. (1983). One unit was defined as the increase of 0.001 absorbance units at 278 nm at 30°C in acetate buffer, pH 5, containing 10 mg/ml of miofibrillar protein, 0.5 mM DTT and 0.1 mM EDTA.

In a first experiment, M. *Semimembranosus* was minced, mixed and maintained for 1 hour at 37°C with solutions: 0, 0.0037, 0.037, 0.19, 0.57 y 1.5 U/g of BSLE. For the second experiment, *Longissimus dorsi et thoracis.* was used. Steaks (of about 150 g), were treated by injection and superficially with 10 U/100g of liophylized BSLE or a commercial tenderiser, and stored either 48 hours at 0-4ºC or 42 hours at 0-4°C and 6 hours at 20°C (the latter only the injected sample). In the third experiment, loin steaks were injected with 10 U/100g of BSLE and stored 7 days at either 0 or 10°C.

Analyses. Total protein nitrogen (PN) and non-protein nitrogen (NPN) were determined by official methods. Protein solubility in TCA 5% and protein extractability were assayed according to Melendo et al. (1997). For instrumental texture measurement, a Warner-Bratzler shear cell installed on a TA-XT-2 texture analyzer was used. Maximum shear force and 'toughness' were recorded. For sensory analysis, meat was placed in a preheated grill at 160°C and removed when internal temperature reached 70°C; tenderness of samples presented at random was evaluated by a trained panel of 4 members using a 9-point scale. Electrophoresis was performed in a 7.5% polyacrylamide gel with SDS using a Phast System, Pharmacia Biotech AB. One μmole sample was run at 10 mA during 10 Vh and the separation was carried out at 1 mA for 100 Vh, at 15¡C. The gel was stained with Coomassie blue. Statistical analysis was performed.

RESULTS

Figure 1 shows results for NPN, protein solubility and protein extractability of minced meat as a function of the concentration of BSLE added. All three parameters increased with increasing enzyme concentration, although its rate was slowered after reaching 0.1-0.2 U/g. Therefore, proteolysis of meat proteins by effect of relatively low concentrations of BSLE was demonstrated. Besides this, proteolysis was also evidentiated by electrophoresis (Figure 2); in fact, both MHC hydrolysis and 30kDa peptide appearance were clearly recorded.

Results of experiment 2, displayed in Tables 1 and 2, showed that beef was significantly ($p<0.05$) tenderised by either injection or surface application of the extract (10U/100g) after two days of treatment. This was demonstrated by shear force, shear energy and sensory measurements. The effect was very similar to that of a commercial bromelain tenderiser.

Experiment 3, in which the BSL extract was injected at the same concentration to beef steaks and stored for 7 days, demonstrated that it was most efficient in tenderising meat. Both shear force and sensory scores (Figure 3) resulted in a very significant tenderisation ($p<0.01$). This effect was enhanced by temperature, although meat with BSLE stored at 4°C was already scored as very tender.

All these results confirmed those reported by Cohen et al. (1979) and Robbins et al. (1979) for meat tenderisation by using a spleen extract, as well as those of Melendo et al. (1998) regarding tenderisation of squid, both in the form of rings and strips, by BSLE.

CONCLUSION

Injection of beef steaks with a lysosomal-enriched extract from bovine spleen (BSLE; 10U/100g), containing mainly cathepsins, resulted in significant tenderisation of meat. Therefore, BSLE may be envisaged as a novel and unexpensive meat tenderiser.

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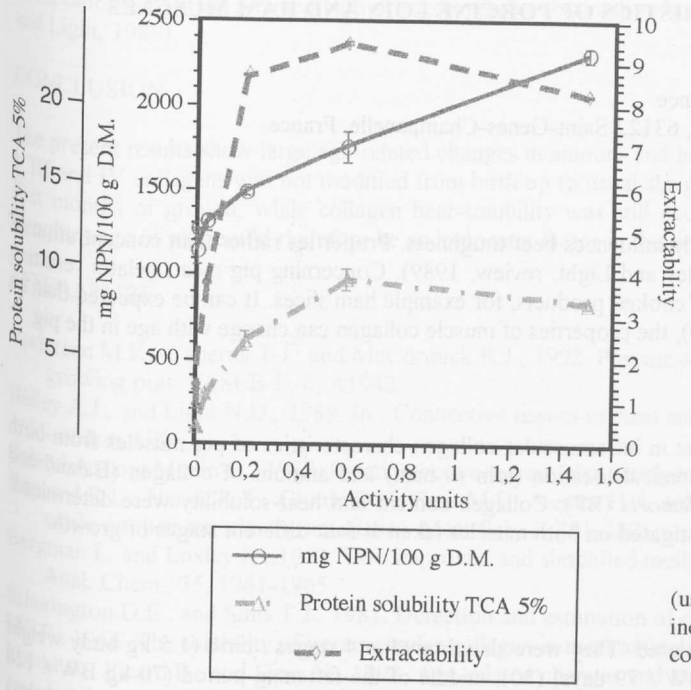

Figure 1. Effect of the addition to fresh minced meat with BSLE at different concentrations. Measurement of differents fractions of nitrogen: non-proteic nitrogen (NPN), protein solubility or soluble nitrogen in TCA 5%, and extractability.

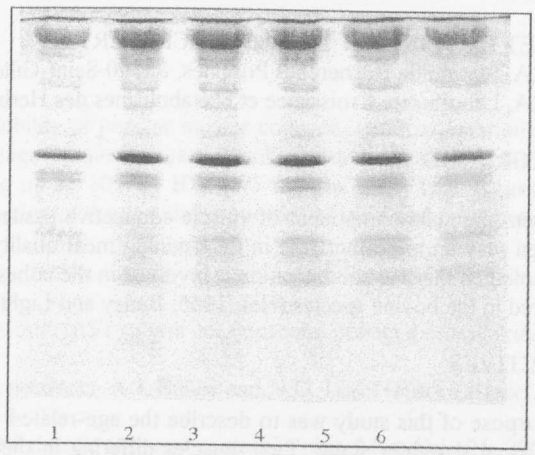

Figure2. Sodiumdodecylsulphate-polyacrylamidegelelectrophoresis (using 20% acrylamide) of myofibrillar protein from fresh minced meat incubated during 1 hour at 37°C with different concentrations of BSLE: 1) control; 2) 1.5 U/g; 3) 0.57 U/g; 4) 0.19 U/g; 5) 0.037 U/g; 6) 0.0037 U/g.

Table 1: Effect on meat texture of the injection to fresh meat of BSLE (10 U/100 g) or a commercial enzyme, and ageing for 48 hours at 0-4°C, or 42 hours at 0-4°C and 6 h at 20°C.

		Ageing at 4°C/48 hours		Ageing at 4°C/48 hours and 20°C/6 hours	
	Control	Extract	Commer. Enzyme	Extract	Commer. Enzyme
Shear force (kg)	6.34±0.75a	4.80±0.42b	4.55±0.58b	5.20±0.54b	4.97±0.43b
Shear energy (J)	0.53±0.05a	0.48±0.06b	0.45±0.04b	0.46±0.05b	0.49±0.03b
NPN (mg/g D.M.)	216.8±6.1a	200.4±8.0a	170.7±10.8b	234.6±21.9a	150.6±10.1b
Sensory analysis*	3.6	5.2	4.0	4.5	4.5

*Evaluated using a structured 1-9 scale; 1 was extremely tough and 9 extremely tender.
a,b Different letters in the same row denote significant difference ($P<0.05$).

Table 2: Effect on meat texture of the superficial addition to fresh meat of BSLE (10 U/100 g) or a commercial enzyme, and ageing for 48 hours at 0-4°C.

	Ageing at 4°C/48 hours		
	Control	Extract	Commer. Enzyme
Shear force (kg)	6.00±0.69a	4.77±0.51b	5.06±0.69b
Shear energy (J)	0.53±0.06a	0.47±0.05b	0.47±0.06b
NPN (mg/g D.M.)	224.1±25.9a	213.2±26.6a	158.9±1.5b
Sensory analysis*	3.6	4.5	4.7

*Evaluated using a structured 1-9 scale; 1 was extremely tough and 9 extremely tender.
a,b Different letters in the same row denote significant difference ($P<0.05$).

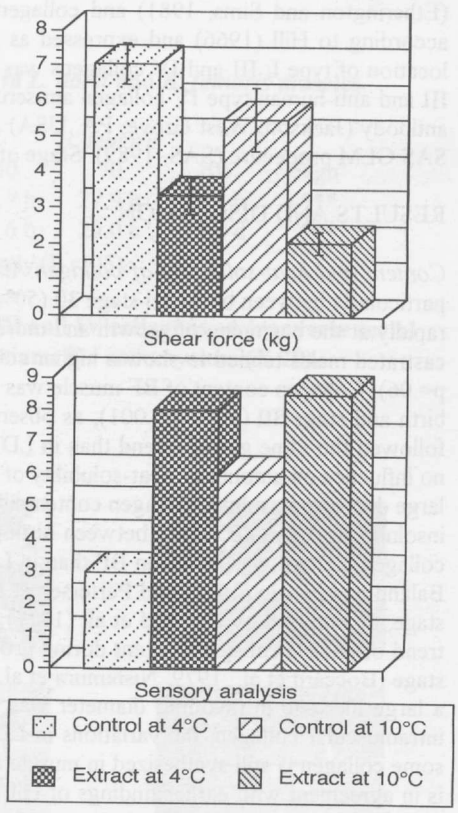

Figure 3: Shear force and sensory evaluation of beef steaks injected with BSLE (10U/100g) and aged for 7 days at 4 or 10°C.