TENDERISATION OF SQUID RINGS WITH BROMELAIN AND A CRUDE SPLEEN EXTRACT.

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INTRODUCTION

Economic significance of squid fisheries is increasing largely over the last decades. World catch is estimated at about 2.5 million tones. The number of new consumers increases accordingly, both in countries in which cephalopods have been consumed traditionally and in those in which they have been not. Concern exists however on the negative opinion of many new consumers about squid eating quality, due to its rather tough texture (Otwell and Hamann, 1979a). Squid mantle texture is known to be related to its particular structure. This has been well described by Otwell and Hamann (1979ab) and Otwell and Giddins (1980). Essentially, muscular fibres show both radial and circular arrangement, and they are supported by connective tissue with longitudinal, radial and circular orientations.

With the aim of reducing squid toughness, a number of methods have been proposed based on different systems. Sikorski and Kolodziejska (1986) and Kolodziejska, et al. (1987) studied the effect of cooking time and treatment with polyphosphates; Kolodziejska, et al. (1992 and 1994) demonstrated the tenderising effect of an hepatopancreas extract of the own squid. Very recently, Montet, et al. (1996) have reported the autolytic tenderisation of the squid mantle by activating the expression of endogenous proteases.

The objective of this research was to assess the effect of two exogenous proteases on the sensory properties of two Atlantic species of squid: *Loligo vulgaris* and *Illex coindetti*. Proteases used were commercial bromelain, whose beneficial action on dry sausage was already reported (Melendo et al., 1996), and a crude lysosomal extract from bovine spleen, whose properties are currently being investigated in our laboratory. Optimum experimental conditions for the tenderising effect on squid mantle of the two proteases have been established in the companion paper "Experimental conditions for optimal tenderisation of squid with exogenous proteases".

MATERIAL AND METHODS

Twenty kg of fresh squids of each species *Loligo vulgaris* and *Illex coindetti* were purchased and handled as described in the companion paper. After thawing, they were cut perpendicular to the longitudinal axis of the mantle as squid rings. Squid samples were marinated in the presence or absence of enzyme (80 U/100 g) at 37°C, pH 7.0, for 30 min. Cooking was brought about as in the previous paper. Bromelain and bovine spleen extracts were obtained and defined as described. Non protein nitrogen, weight loss, maximum stress and 'toughness' were determined as described, too.

Sensory analysis: cooked squid rings were evaluated for a profile of textural sensory attributes by a jury of 20 trained members using a 9-point intensity scale; significance between sample means was tested by ANOVA. Triangle and duo-trio tests were made according to the International Standard ISO CTC 34/SC 12 Regulation.

Electrophoretic study: myofibrillar proteins were obtained as described by Olson et al. (1976). 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. It was stained with Coomassie blue.

RESULTS AND DISCUSSION

Table 1 shows the results obtained for both *Loligo vulgaris* (Lv) and *Illex coindetti* (Ic) by effect of proteases under optimal conditions. First to point out is the fact that weight loss was significantly lower in Lv than in Ic. This, according to Otwell and Hamann (1979a), should result in toughening of cooked squid. If shear force parameters are compared for controls, it may be observed that in fact 'toughness' was significantly higher for Ic. Instrumental texture parameters also show that the two studied proteases exerted a significant tenderising effect on both squid species. This effect appeared to be more intense for bromelain, since maximum stress failed to reveal the tenderisation of Ic by the spleen extract. Non protein nitrogen (NPN), as a proteolysis index, did not present any significant difference; this was already reported by Kolodziejska et al. (1987). A triangle test carried out by a consumer taste panel confirmed the tenderness differences revealed by shear force parameters, although statistical significance was very low ($p<0.1$) for the extract and low ($p<0.05$) for bromelain.

Figure 1 depicts the sensory textural profile of Lv squid control and marinated with either of the two proteases, evaluated by a trained taste panel. It is worth to mention the significant effect of both proteases on the squid texture profile. However, the profiles revealed differences in enzyme action. The spleen extract significantly decreased initial bite force and springiness, giving rise to a more tender texture without modifying the particular characteristics of squid texture. Bromelain exerted a more dramatic effect, since all initial force, chewiness and springiness were greatly reduced, while the usually low adhesiveness was significantly increased. Therefore, bromelain, at the concentration used, modified largely squid texture besides tenderising.

The electrophoretic patterns showed in Figure 2 did not reveal any difference due to the presence of any of the proteases. Thus, proteolytic events seemed not to be as intense as to explain the textural changes found by instrumental and sensory analysis.

CONCLUSIONS

Both squids *Loligo vulgaris* and *Illex coindetti* were significantly tenderised by effect of bromelain and a bovine spleen extract. The latter did not noticeably modify the characteristic texture profile of squid, while bromelain did introduce extensive changes.

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Table 1: Effect of specie on maximum stress (kg/mm^2), toughness (kg/mm^2), weight loss (%) and non protein nitrogen (NPN; mg/g D.M.) for frozen squid rings treated with enzymes at pH 7.0 at 37°C and 20°C during 30 min.

Species		*Illex coindetti*			*Loligo vulgaris*	
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	56,4	57,3	59,2	38,6	40,1	38,9
No. texture assays	18	17	18	15	11	12
Maximum Stress	0,0131± 0,0026a	0,0119± 0,0027a	0,0094± 0,0019b	0,0120± 0,0021a	0,0082± 0,0017b	0,0093± 0,0009b
Toughness	0,0124± 0,0028a	0,0104± 0,0009b	0,0096± 0,0013b	0,0077± 0,0017c	0,0055± 0,0012d	0,0051± 0,0009d
NPN	0,733± 0,017a	0,724± 0,028a	0,771± 0,005b	1,263± 0,035c	1,190± 0,094c	1,295± 0,045c
Triangle test+	-	-	-	Co.	p<0,1	p<0,05

+ Difference with the control samples by a 20-consumer taste panel.

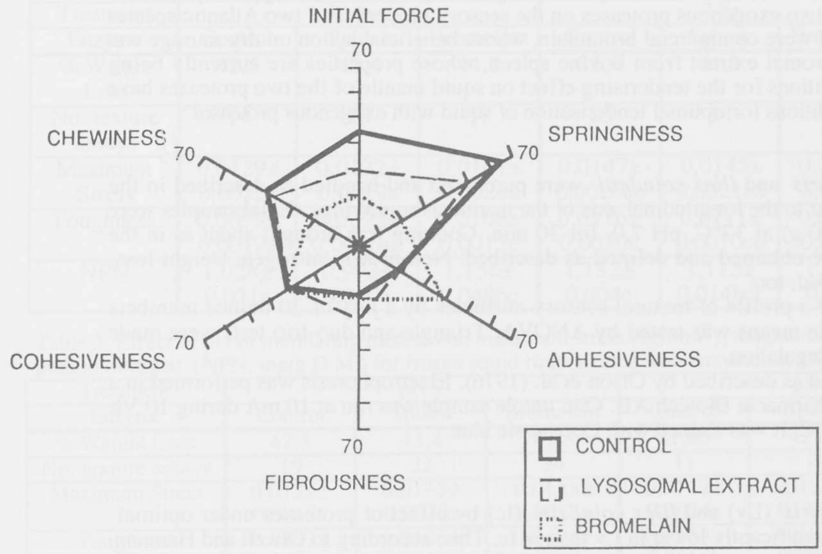

Figure 1. Texture profile of squid rings marinated with proteases (pH 7.0, 37°C, 30 min), evaluated by a 6-member trained taste panel using a non-structured 100 mm scale.

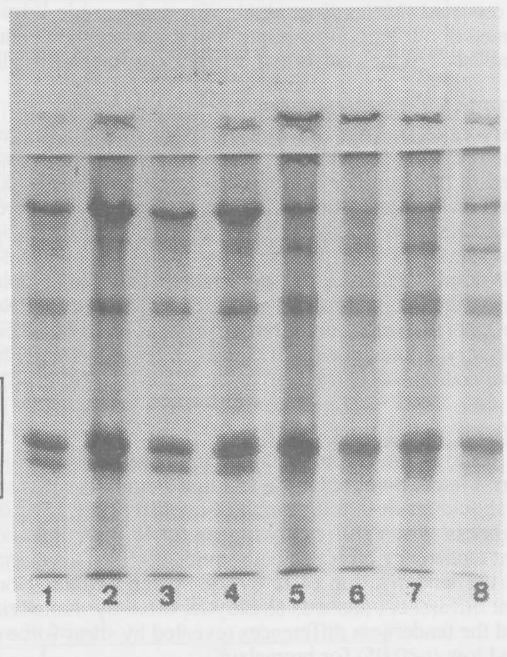

Figure 2. SDS-PAGE using 7.5% acrylamide of myofibrillar proteins from squid rings marinated with proteases: 1,2) *Loligo vulgaris* (Lv), with bromelain, 3) Lv, with spleen extract, 4) Lv, control, 5) *Illex coindetti* (Ic), with bromelain, 6) Ic, with spleen extract, 7,8) Ic, control.