

EXPERIMENTAL CONDITIONS FOR OPTIMAL TENDERISATION OF SQUID WITH EXOGENOUS PROTEASES.

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INTRODUCTION

In the companion paper "Tenderisation of squid rings with bromelain and a crude spleen extract" a general introduction is made which applies also to this paper.

The objective of this work was to set the experimental conditions for obtaining the presumable tenderising effect on squid mantle of two exogenous proteases. One was commercial bromelain, whose beneficial action on dry sausage has been already reported (Melendo et al., 1996). The second, a crude lysosomal extract from bovine spleen, whose properties are currently being investigated in our laboratory.

MATERIAL AND METHODS

Forty kg of fresh squids of the species *Loligo vulgaris* from the same catch were purchased at the Zaragoza fish market, and transported to the laboratory on ice. All squids were cleaned (skin, head, tentacles and viscera removed); 20-25 cm long squid mantles were packed in individual plastic bags and stored at -20°C until use, with the exception of those used for the first experiment as fresh. Prior to treatment, frozen mantles were thawed at 0-2°C overnight, they were cut either perpendicular to the longitudinal axis of the mantle (rings), or parallel to it (strips) according to the experiment.

Bromelain was obtained from Sigma Chemical Co. Ltd. Bovine spleen extracts 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.

Squid samples were subjected to different conditions and treatments according to the following outline: either rings or strips obtained from mantles were marinated for either 0.5, 2 or 8 hr, at 4, 20 or 37°C, pH 5.0 or 7.0, with a 3:1 ratio of water to squid (w:w), containing 2% salt, in the presence or absence of enzyme (80 U/100 g). Cooking was brought about by heating this marinade at a rate of 10°C/min up to 100°C, and boiled for 30 min. (Stanley and Hultin, 1982; Sikorski et al., 1986). After cooking, they were allowed to cool to 25°C at room temperature.

Moisture, total protein nitrogen and non-protein nitrogen were determined by official methods (Ministerio de Sanidad y Consumo, 1985). Squid rings or strips were weighed before and after cooking to assess weight loss.

For instrumental texture measurement, a Warner-Bratzler shear cell installed on an Instron universal testing machine was used. Maximum stress and 'toughness' were recorded.

RESULTS AND DISCUSSION

Table 1 shows the results for fresh squid rings after 0.5, 2 and 8 hr of marinating. Two facts were highly noticeable; first, marinating for 8 hr resulted in very large weight losses and, most likely as a direct consequence, in tougher texture (Otwell and Hamann, 1979a). Second, both bromelain and the spleen extract exerted a tenderising effect, the former with a higher significance. It was concluded that marinating time for next experiments should be close to 30 min. The same experiment was performed with frozen squid (Table 2). Freezing is the usual way of storage of squids, and it is known to exert a tenderising effect (Kolodziejska, et al., 1987). Results confirmed the tenderising effect of freezing; they also allowed us to draw the same conclusions as those of Table 1.

The effect of pH is presented in Table 3. A pH decrease from 7.0 to 5.0 seems to have an almost negligible effect; a very small toughening, as reported by Sikorski and Kolodziejska, 1986 and Kolodziejska, et al., 1987, was observed. Neutral pH appeared to be therefore preferable.

Table 4 shows the results of varying the temperature of marinating. When marinating at 37°C was compared to lower processing temperatures, it became evident that the tenderising effect of both enzymes was significantly higher at that temperature.

Finally, squid samples prepared as rings or strips were compared. Results shown in Table 5 clearly demonstrated that mantle strips were significantly more tender than mantle rings. This agreed with structural differences reported by Otwell and Hamann, (1979ab) and Otwell and Giddins, (1980). An effective and significant tenderisation due to both proteases, however, was achieved only when squid was presented in the form of rings.

CONCLUSIONS

In order to obtain an optimal tenderising effect of exogenous proteases on squid, the following conditions should be met: use of frozen squid mantles, transversally cut as rings and marinating with the enzyme at pH 7.0 and 37°C for 30 min.

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Table 1: Effect of time of marinating on maximum stress (kg/mm²), toughness (kg/mm²), weight loss (%) and non protein nitrogen (NPN; mg/g D.M.) for fresh squid rings treated with enzymes at pH 7.0 and 20°C.*

Treatment	30 min			2 hr			8 hr		
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	46,7	43,5	45,5	49,8	46,22	48,47	54,15	53,66	54,17
No. texture assays	14	14	12	14	15	17	10	11	14
Maximum Stress	0,0175± 0,0025ae	0,0163± 0,0028ad	0,0145± 0,0028bd	0,0183± 0,0038a	0,0142± 0,0038bd	0,0136± 0,0033b	0,0259± 0,0019c	0,0269± 0,0025c	0,0196± 0,0045e
Toughness	0,0110± 0,0024a	0,0095± 0,0021ab	0,0106± 0,0027a	0,0121± 0,0024a	0,0088± 0,0025b	0,0079± 0,0029b	0,0167± 0,0021cd	0,0184± 0,0022c	0,0149± 0,0034d
NPN	0,881± 0,031a	1,144± 0,067b	1,064± 0,075bf	0,837± 0,015ae	0,992± 0,021f	0,983± 0,022f	0,638± 0,004c	0,937± 0,036da	0,784± 0,011e

* Different letters in the same row denote significant difference (p<0.5).

Table 2: Effect of time of marinating on maximum stress (kg/mm²), toughness (kg/mm²), weight loss (%) and non protein nitrogen (NPN; mg/g D.M.) for frozen squid rings treated with enzymes at pH 7.0 and 20°C.*

Treatment	30 min			2 hr			8 hr		
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	42,5	43,4	44,8	35,9	41,1	44,9	48,4	48,1	57,4
No. texture assays	14	13	17	15	14	18	17	16	12
Maximum Stress	0,0129± 0,0022a	0,0132± 0,0019a	0,0141± 0,0026a	0,0147± 0,0020ac	0,0145± 0,0021ad	0,0128± 0,0022a	0,0167± 0,0021b	0,0154± 0,0023bcd	0,0157± 0,0025bd
Toughness	0,0099± 0,0019a	0,0095± 0,0016ab	0,0108± 0,0012c	0,0116± 0,0020c	0,0111± 0,0010cb	0,0095± 0,0014a	0,0130± 0,0021d	0,0111± 0,0014c	0,0106± 0,0019bc
NPN	1,028± 0,021a	0,993± 0,050a	1,156± 0,048bc	1,132± 0,004c	1,115± 0,014bcf	1,171± 0,011c	0,841± 0,030d	0,776± 0,045e	1,109± 0,002f

Table 3: Effect of pH of marinating medium on maximum stress (kg/mm²), toughness (kg/mm²), weight loss (%) and non protein nitrogen (NPN; mg/g D.M.) for frozen squid rings treated with enzymes at 20°C for 30 min.*

Treatment	pH 7			pH 5		
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	42,5	43,4	44,8	41,05	42,86	36,6
No. texture assays	19	22	34	11	11	12
Maximum Stress	0,0127± 0,0017a	0,0135± 0,0020a	0,0133± 0,0024a	0,0142± 0,0024a	0,0126± 0,0018a	0,0123± 0,0028a
Toughness	0,0107± 0,0020a	0,0106± 0,0018a	0,0104± 0,0017a	0,0124± 0,0016b	0,0103± 0,0018a	0,0106± 0,0021a
NPN	1,080± 0,061ab	1,004± 0,015bc	1,163± 0,030d	0,999± 0,058c	1,107± 0,067ad	1,117± 0,006ad

Table 4: Effect of temperature of marinating on maximum stress (kg/mm²), toughness (kg/mm²), weight loss (%) and non protein nitrogen (NPN; mg/g D.M.) for frozen squid rings treated with enzymes at pH 7.0 for 30 min.*

Treatment	4°C			20°C			37°C		
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	32,9	37,6	35,8	42,5	43,4	44,8	38,6	40,1	38,9
No. texture assays	11	12	13	43	40	47	15	11	12
Maximum Stress	0,0128± 0,0020bd	0,0111± 0,0014ae	0,0101± 0,0017a	0,0133± 0,0026b	0,0137± 0,0024b	0,0133± 0,0026bd	0,0120± 0,0021de	0,0082± 0,0017c	0,0093± 0,0009ac
Toughness	0,0118± 0,0012bf	0,0112± 0,0011be	0,0094± 0,0017a	0,0111± 0,0020ef	0,0105± 0,0018e	0,0105± 0,0018e	0,0077± 0,0017d	0,0055± 0,0012c	0,0051± 0,0009c
NPN	1,193± 0,131a	1,109± 0,111a	1,313± 0,055b	1,027± 0,109a	1,025± 0,075a	1,145± 0,037a	1,130± 0,150a	1,006± 0,150a	1,093± 0,124a

Table 5: Effect of mantle cuts on maximum stress (kg/mm²), toughness (kg/mm²), 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.*

Treatment	strips			rings		
Enzyme	Control	Spleen	Bromelain	Control	Spleen	Bromelain
% Weight Loss	41,6	42,5	45,3	38,6	40,1	38,9
No. texture assays	15	14	15	22	16	18
Maximum Stress	0,0052± 0,0011a	0,0050± 0,0009a	0,0052± 0,0009a	0,0130± 0,0027b	0,0102± 0,0044c	0,0108± 0,0025c
Toughness	0,0070± 0,0010a	0,0065± 0,0009a	0,0071± 0,0013a	0,0090± 0,0031b	0,0082± 0,0032ab	0,0078± 0,0026ab