Enzymatic Protein Hydrolysate from Tuna Canning Wastes - Standardisation of Hydrolysis Parameters

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Tuna canning wastes, consisting of cooked tuna red meat was hydrolysed using pineapple juice as a source of crude Bromelain. Optimum conditions for hydrolysis as monitored by solids and nitrogen solubilization were: 0.008 units enzyme/mg substrate protein, 5-6 pH and 60-70°C temperature. Substrate concentration did not appear to have much influence on solubilization of solids. Material balance under optimized conditions of hydrolysis showed that soluble solids and soluble nitrogen increased by 62.6 and 92% respectively over initial levels and could be recovered with minimal losses.

The annual world catch of tuna in 1989 was 2.5 million tonnes making it one of the major fisheries of the world (Anon, 1991). India too, has a major tuna fishery of nearly 62,000 tonnes (Anon, 1990), which is expected to go up with the expansion of the deep sea fishing fleet. A major portion of the world catch amounting to 0.4 million tonnes is canned (Anon, 1991). Since only the white meat of tuna is used in canning, a sizable amount of red meat is available as canning waste, which is mostly used as an ingredient by the pet food canning industry or locally disposed off as animal feed. Tuna red meat is nutritionally comparable to the white meat (Mukundan *et al.*, 1979) and can be a good source of protein, iron and vitamins A & D for humans (Mukundan, 1986). This study was undertaken to find out the possibility of using Bromelain mediated hydrolysis of tuna canning wastes, which principally consist of red meat to produce a protein hydrolysate. The use of protein hydrolysates in food is well known and widely practised on account of their good solubility and functional characteristics (Adler-Nissen, 1986). Pineapple, the source of crude bromelain is abundantly available in Kerala and the enzyme's broad pH optimum avoids the necessity of acid/alkali addition during hydrolysis.

Materials and Methods

Yellowfin tuna (*Thunnus albacares*) canning waste was obtained from the processing plant, Integrated Fisheries Project, Cochin, which mainly consisted of cooked tuna red meat (TRM) with some small bone pieces. The meat was thoroughly blended in a silent cutter and stored at -20°C in 0.5 kg lots.

Crude Bromelain for hydrolysis was obtained by comminuting pineapple pieces in a blender and pressing through cheese cloth or by using a commercial juicer. The juice obtained was centrifuged at 21000 x G for 15 min at 20°C. The clear supernatant was stored at -20°C till required. Enzyme activity at pH 5.5 was assayed by the Caseinolytic procedure of Murachi (1970). A unit of enzyme activity is defined as 1 μ mol of tyrosine released/ml enzyme/min under the assay conditions.

For hydrolysis of the tuna red meat (TRM), known quantities of TRM, water, and pineapple juice (all at 0-5°C) were blended in a varing blender for 3 min. 2-mercaptoethanol or cysteine hydrochloride at 0.005 M was added during the last min of blending. Zero time samples were drawn immediately unless otherwise mentioned, and the enzyme-substrate mixture placed in a thermostatic water bath. Following temperature equilibration (10 min) samples were drawn at intervals and the enzyme inactivated by keeping in a boiling water bath for 20 min. Solid content was determined by drying at 105°C overnight and nitrogen by the semi- micro kjeldahl procedure (AACC, 1976). Soluble solids and nitrogen were determined similarly in the filtrate of the sample extracted 4-5 times with water. Further extracts of the sample contained negligible amounts of solids and nitrogen. Deionised water and AR grade chemicals were used throughout the experiments.

Results and Discussion

Enzyme and substrate concentrations, pH and temperature being the 4 major variables in any protein hydrolysis experiment (Adler-Nissen, 1986), the same were optimized for this system.

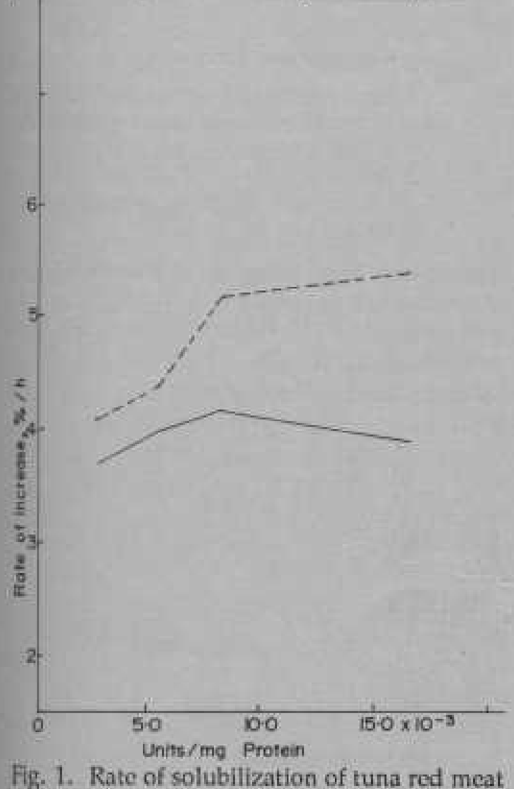

Fig. 1. Rate of solubilization of tuna red meat at different enzyme concentrations for the first 4 hours; — soluble solids as % of total solids; — soluble nitrogen as % of total nitrogen. Experimental conditions as in Table 1.

Table 1. *Increase in solubilization parameters during hydrolysis* with different enzyme concentrations*

A. *Increase in soluble solids as % of total solids*

Enzyme: protein ratio+	Hours of hydrolysis 0	4	8	ml Bromelain/g meat
0.0027	23.01	37.82	40.92	0.22
0.0055	26.85	42.72	47.19	0.43
0.0083	29.88	46.62	50.12	0.65
0.0165	38.89	54.45	56.76	1.30

B. *Increase in soluble nitrogen as % of total nitrogen*

Enzyme: protein ratio+	Hours of hydrolysis 0	4	8
0.0027	20.00	36.36	39.27
0.0055	21.48	39.16	43.70
0.0083	22.92	43.10	46.04
0.0165	29.64	50.99	51.19

*Hydrolysis carried out in a 1:3 suspension at 40°C and 0.005 M 2-mercapto ethanol. No agitation.

+ units enzyme/mg protein

Optimum enzyme concentration: The maximum amount of solubilization of solids and total nitrogen from TRM was obtained at the highest enzyme concentration used (0.0165 units/mg protein) as seen in Table 1. However, the rate of solubilization of solids was marginally greater at a lower enzyme concentration (Fig.1) of 0.008 units/mg protein, which gave a slightly decreased rate of nitrogen solubilization than that obtained with the maximum enzyme concentration. Thankamma *et al.* (1979) and Nair *et al.* (1985) have similarly reported obtaining maximum yields of

Table 2. *Increase in solubilization parameters during hydrolysis* at different meat: liquid sespension ratios*

A. *Increase in soluble solids as % of total solids*

Meat:liquid ratio	Hours of hydrolysis 0	4	8
1:100	35.70	49.42	50.42
1:64	32.45	48.16	53.06
1:32	31.50	48.39	53.76
1:16	32.79	48.01	53.31
1:8	31.88	49.10	53.00
1:4	30.60	47.38	52.62

B. *Increase in soluble nitrogen as % of total nitrogen*

Meat:liquid ratio	Hours of hydrolysis 0	4	8
1:100	23.53	32.35	44.12
1:64	20.57	41.80	41.50
1:32	22.81	40.17	41.60
1:16	24.53	48.03	55.16
1:8	32.60	50.34	55.00
1:4	42.86	48.34	63.80

* Hydrolysis carried out at 0.0083 units enzyme/mg protein, 0.005M 2-mercapto ethanol and 40°C. No agitation

hydrolysate from different fish at the maximum enzyme: substrate ratios used in their experiments. But as substantial quantities of pineapple juice were required at the maximal enzyme concentration (Table 1), it may be desirable to carry out the hydrolysis at the lower enzyme concentration with longer periods of incubation or with agitation.

Optimum substrate concentration: At the end of 8 h of hydrolysis, substantial differences between different substrate con-

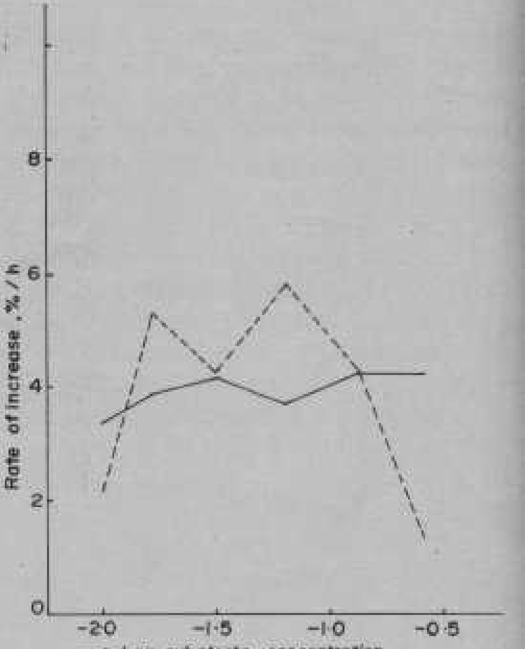

Fig. 2. Rate of solubilization of tuna red meat at different substrate concentrations for the first 4 hours; — soluble solids as % of total solids; --- soluble nitrogen as % of total nitrogen. Experimental conditions as in Table 2

centrations in the extent of solubilization of solids were not observed (Table 2), which was reflected in the relative rates of solids solubilization (Fig.2). But the extent of solubilization of total nitrogen was observed to be slightly higher at the higher substrate concentrations (Table 2). This difference was however not borne out in the rate of nitrogen solubilization which was observed to fluctuate widely with no apparent trend (Fig.2). This wide variation could be traced to the variations in the total nitrogen values in the same substrate concentrations with time. This was due to the difficulty of obtaining a small but representative sample for analysis from higher substrate concentrations which were highly viscous and particulate.

The solubilization of insoluble proteolytic substrates has been observed to proceed with the adsorption of major part of the proteinase on to substrate particles

Table 3. *Increase in solubility parameters during hydrolysis* at different pH*

A. *Increase in soluble solids as % of total solids*

pH of hydrolysis	Hours of hydrolysis	
	0**	2
4.0	69.15	78.86
4.5	72.56	79.60
5.0	72.84	82.30
5.5	62.79	78.65
6.0	64.30	77.41

B. *Increase in soluble nitrogen as % of total nitrogen*

pH of hydrolysis	Hours of hydrolysis	
	0**	2
4.0	20.28	34.79
4.5	22.23	37.69
5.0	22.44	41.08
5.5	19.06	46.22
6.0	19.80	48.26

*Hydrolysis carried out at 0.05 units enzyme/mg protein, 0.005M 2-mercaptoethanol, 40°C.

** Zero time analyses carried out separately on meat suspension and bromelain

(Archer *et al.*, 1973). This mode of proteolytic attack would neutralize the dilution effect of the substrate as observed in the solubilization of total solids. Thus, the substrate concentration while not of much influence on the extent and rate of solids solubilization, is of practical importance in the amount of water that has to be finally evaporated to concentrate the hydorlysate.

Optimum pH for hydrolysis: The solubilization of TRM solids and nitrogen at various pH of hydrolysis by Bromelain are shown in Table 3. The higher SS/TS values seen in comparison to the earlier values are as a result of using much higher

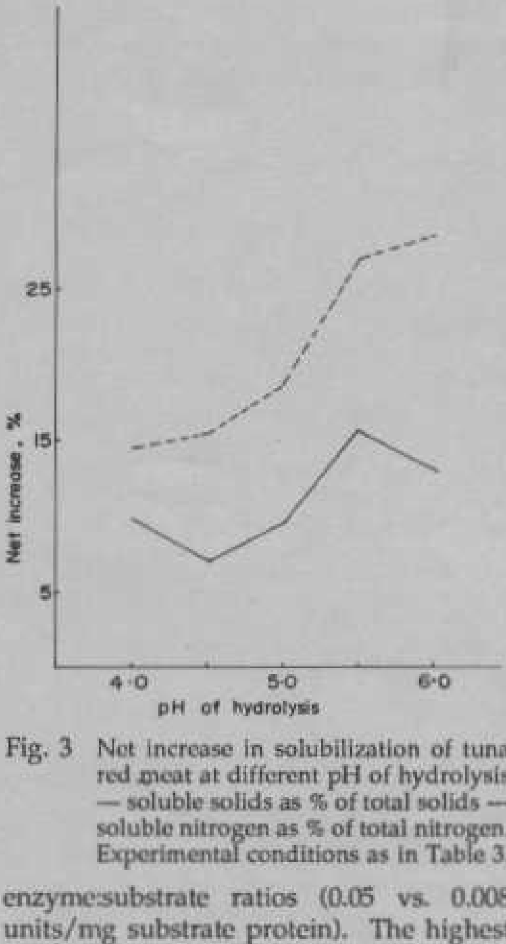

Fig. 3 Net increase in solubilization of tuna red meat at different pH of hydrolysis — soluble solids as % of total solids --- soluble nitrogen as % of total nitrogen. Experimental conditions as in Table 3.

enzyme:substrate ratios (0.05 vs. 0.008 units/mg substrate protein). The highest net increase in soluble solids was at pH 5.5 (Fig.3), but the maximum increase in soluble nitrogen was recorded at pH 6.0. However, the difference between the two pH for increase in net soluble nitrogen was very little. Thus, the pH range of 5.5 - 6.0 appeared to be most suitable for TRM hydrolysis with bromelain, which agrees well with the earlier reports of a broad optimum pH range for this enzyme (pH 5-8, Adler-Nissen, 1986). Higher pH of hydrolysis were not tried as they involved the addition of excessive amounts of alkali.

Optimum temperature for hydrolysis: The solubilization of TRM by Bromelain at different temperatures showed that the maximum increase in both soluble solids and

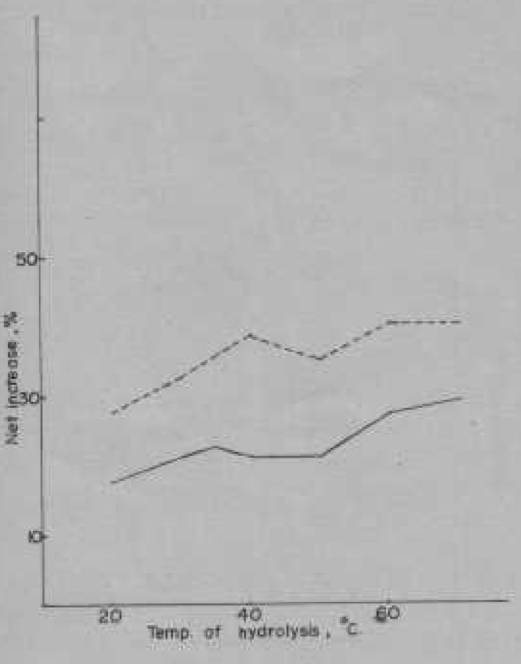

Fig. 4. Net increase in solubilization of tuna red meat at different temperatures of hydrolysis — soluble solids as % of total solids; --- soluble nitrogen as % of total nitrogen. Hydrolysis conditions: 1:10 suspension, 0.05 units enzyme/mg protein, 0.005 M 2-mercaptoethanol. No agitation.

soluble nitrogen occurred between 60-70°C (Fig.4). This agrees well with the temperature optimum reported earlier for this enzyme (Hale, 1969). Nair *et al*. (1985) used a temperature of 30°C for hydrolysis of jew fish with bromelain, but reported a gross yield of only 11.2% as compared to 42-45% (net) yields in this experiment, albeit at higher enzyme: protein ratios.

Material balance of TRM hydrolysis with Bromelain in a bulk batch: In order to obtain data on yield of hydrolysis and losses under optimized conditions of hydrolysis, a bulk batch (5 kg) was hydrolysed, the results of which are shown in Fig.5. As a result of hydrolysis, a net increase of 47.6 g soluble

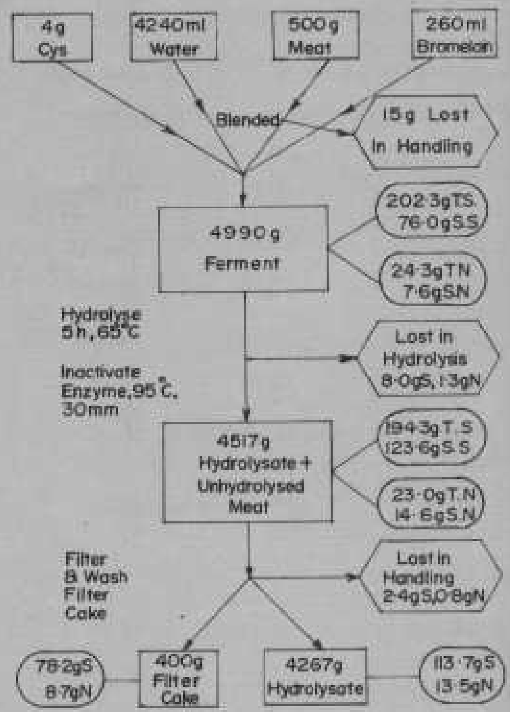

Fig. 5 Material Balance of tuna red meat hydrolysed with crude uncentrifuged pineapple juice. Hydrolysis conditions: 1:10 suspension, 0.008 units enzyme/mg protein, natural pH (5.6) 0.005 M Cysteine HCl, temperature 65°C, agitated with slow speed stirrer. S - Solids, N - nitrogen, TS - total solids, TN - total nitrogen, SS - soluble solids, SN - soluble nitrogen.

solids and 7 g soluble nitrogen was obtained in 5 h, which represented an increase of 62.6 and 92% respectively over initial levels. During hydrolysis and recovery of hydrolysate, about 10 g of solids and 2 g of nitrogen amounting to 5 and 8% of total solids and total nitrogen respectively were lost. Thus, recovery of both solids and nitrogen were good. However, a substantial fraction of TRM, amounting to 38.7% of total solids and 35.7% of total nitrogen was left unhydrolysed. Resistance of heated meat proteins (O'Meara & Munro, 1985) and denatured fish proteins

(Devadas, 1980: Langmyhr, 1981) to enzymatic hydrolysis, especially when subjected to severe heat treatments have been reported earlier. This could be the reason for the limited hydrolysis of TRM which had been cooked under pressure prior to canning. Raw or mild heat treated TRM may perhaps be more completely solubilized upon enzymatic digestion which needs further investigation. The final hydrolysate recovered after filtration had the characteristic tuna flavour and very little bitterness.

Tuna red meat, a waste product of the tuna canning industry could be hydrolysed with good hydrolysate yields using pineapple juice as a source of crude bromelain. However, the solubilization of TRM was not complete and the residual sugars of the pineapple juice may lead to non-enzymatic browning when the hydrolysate is dried at high temperature.

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