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Abstract

To convert the waste material into valued marketable products, prawn heads were selected for the production of microbiological peptone by autolytic and added proteolytic enzyme digestion. Acceptable conditions for autolysis were phosphate-citrate buffer pH 7.5, temperature 52.5°C, time 3 h and prawn head concentration 200 g/L with an average protein yield of about 71%. Crude protein yield increased to 92% and 82% when prawn heads were digested by pepsin (3 h, pH 2.0, 37 °C) and papain (3 h, pH 6.2, 25°C), respectively at 0.5% (w/w). The chemical composition and ability to support microbial growth of the products from enzymic digestion were comparable to or slightly better than for peptones produced by autolytic digestion.

Introduction

All prawn processing plants generate about 40-80 % of solid wastes depending upon the prawn species, the size of the plant, the type of products being processed and whether prawns are beheaded at sea. These wastes tend to be degraded very rapidly due to enzymic and bacteriological processes, causing noxious odours and environmental pollution. Thus, processors must continually search for cost-effective disposal methods or viable method for utilization of processing wastes. Conversion of waste material into valued marketable products not only increases

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Therefore, the aim of this study was to examine the optimum conditions for the digestion of prawn heads by autolysis and addition of proteolytic enzymes and to evaluate the suitability of prawn head peptones as microbiological growth media compared to commercially available peptones.

Material and Methods

Autolytic digestion Frozen prawn heads, a mixture of Banana, Tiger, Eastern King and Endeavour species, were macerated for 2 min with phosphate-citrate buffer. The pH of the slurry was adjusted to 6.5, 7.0, 7.5 and 8.0 with 0.1N HCl and 0.1N NaOH and sufficient water was added to make the final volume to the desired concentration of 100, 200, 300, 400, 500 and 600 g prawn head/L. After dilution, the slurry was shaken in a reciprocating shaking water bath at a temperature of 30°, 37.5° 45 °, 52.5° or 60°C. The crude protein content of aliquots after filtration and boiling was determined after 0, 1, 2, 3, 4, 6 and 8 h using Lowry's method (Lowry et al. 1951).

Digestion by adding proteolytic enzymes Proteolytic enzymes used in this study were papain (Type 1 ex papaya latex - activity 1 - 2 units/g solid) and pepsin (1:10000 ex porcine stomach-mucosa - activity 1200 - 2000 units/mg protein). Prawn head slurry was prepared as for autolytic digestion but the pH was adjusted as follows: papain 4.2, 5.2, 6.2, 7.2 and 8.2; pepsin 1.0, 1.5, 2.0, 2.5 and 3.0. The enzyme solutions were added to a concentration of 0.1, 0.3, 0.5, 0.7, and 1.0% (w/w of frozen prawn head) and the contents were shaken in a reciprocating shaking water bath at a temperature of 25°, 30°, 37 , 45 ° or 60°C. Aliquots of slurry were taken after 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 h digestion, and the crude protein content of the filtrates was determined after boiling to inactivate all enzymes.

For the production of prawn head peptone, two different types of drier were used: a spouted-bed drier under the conditions of inlet air temperature 90°-100°C, air flow rate 70 mL/s, outlet air temperature 60°- 65°C, and the freeze-drier at a temperature of -35°C to -40°C and vacuum of 0.2 mm Hg.

Chemical evaluation of prawn head peptones

Prepared prawn head peptones and two commercial peptones (Oxoid Bacteriological peptone and Difco Bactopeptone) were analysed for proximate composition (AOAC 1984), chitin (Winzler 1955), amino nitrogen (Cobb *et al*. 1973) and non-protein nitrogen (Menzincescu and Szabo 1936).

Evaluation of peptones as microbiological media. Six prawn head peptones were compared with commercial Oxoid and Difco peptone for their ability to support microbial growth which was assessed by measuring the amount of biomass produced by four pure cultures of bacteria (*Escherichia coli*, *Bacillus subtilis*, *Lactobacillus plantarum*, and *Staphylococcus aureus*), yeast (*Saccharomyces cerevisiae*) and fungus (*Rhizopus oligosporus*).

Results and Discussion

Autolytic digestion pH and autolytic digestion time of frozen prawn head incubated at various temperatures produced a highly significant ($P<0.001$) difference in crude protein yield (Figure 1). The maximum crude protein yield, approximately 78 % of the original protein content of the prawn head, was obtained at pH 7.5 which is near the normal pH of frozen prawn head (7.8 - 8.0). Therefore, subsequent digestion was performed at a slightly basic pH, not only to obtain the maximum protein yield but also to reduce the cost of acid for pH adjustment.

The autolytic digestion of prawn heads is highly dependent ($P < 0.001$) on temperature (Figure 2). The amount of protein in the digest increased as the temperature increased up to 52.5° C, but above this temperature the protein yield decreased, probably caused by denaturation of the protein at the elevated temperature, in agreement with the finding of Suzuki and Matsumoto (1978) on krill native protein. The effect of digestion time on autolytic digestion is shown in Figure 3 ; the digestion occurred rapidly within the first hour and slowly increased up to 3 h digestion, after which the protein yield decreased. Protein yield at varying prawn head concentration is shown in Figure 4 ; the yield increased from 56 to 74% of the original protein content of the prawn heads when the prawn head concentration was increased from 100 to 200 g/L. Thus, the optimum condition for protein extraction by autolytic digestion from frozen prawn heads on a laboratory scale are phosphate-citrate buffer pH 7.5 , temperature 52.5°C, time 3 h and prawn head concentration 200 g/L.

Digestion by added proteolytic enzymes The crude protein yield obtained from pepsin digestion was significantly higher at pH 2.0 than at other pHs for temperatures in the range 30°to 60°C (Figure 5). The maximum protein yield was obtained at 37° C for each pH studied except for pH 1.0 at which the optimum temperature was 45°C.

For papain digestion , the crude protein yield was significantly affected by both pH and temperature. Papain activity was shown to be optimum at pH 6.2 for protein hydrolysis of prawn heads (Figure 6). As temperature increased from 25° to 60° C the protein yield decreased by approximately 12 % for each increase in pH. The maximum yield was obtained at 25°C.

The digestion of prawn heads by five levels of pepsin and papain showed similar results for both enzymes with the higher protein yield from pepsin (Figure 7). The minimum concentration of enzyme for digestion was judged to be 0.5 % w/w of original prawn heads.

Chemical composition of prawn head peptones The chemical composition of prepared prawn head peptones (Table 1) showed higher fat content than the two commercial peptones. This is responsible for the cloudy appearance of a 0.5% (w/v) peptone solution. Total protein content of derived prawn head peptones was both considerably lower than that of the commercial peptones. The level of non-protein nitrogen indicated that the protein quality was contributed by chitin and other nitrogen compounds such as ammonia; the higher the non-protein nitrogen, the lower the protein quality obtained. The results of non-protein nitrogen content of all samples were not significantly different, although the levels in the prawn head peptones were slightly higher than those for the commercial products.

Evaluation of peptones as microbiological media The ability of peptones to support microbial growth in terms of biomass production (Table 2) showed that the prawn head peptone solution prepared by papain digestion and freeze-drying supported equivalent or better growth for all bacteria tested compared to the commercial Difco and Oxoid peptone solutions. Biomass production in peptone solutions prepared by papain digestion ranked first, followed by that in the peptone solutions prepared by autolysis and pepsin digestion. There was no significant difference between the prawn head peptones and the commercial peptones in supporting growth of yeast except for the peptones prepared by pepsin digestion which were significantly inferior. Biomass production of the fungus was higher on media prepared from various types of prawn head peptones than it was on the commercial peptones. The amount of biomass was closely related to the fat content of the peptone; a higher fat content peptone solution produced a significantly higher amount of biomass.

Conclusions

The microbiological peptones can be derived from prawn processing wastes such as heads by autolytic digestion or by digestion with added proteolytic enzymes. Although the amount of protein digested from prawn heads is significantly increased when proteolytic enzymes are added to the digest, the chemical composition and ability to support microbial growth of the final products from enzymic digestion are comparable to or only slightly better than for the peptones produced by autolytic digestion.

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Table 1. Chemical composition of commercial and prawn head peptones.

Component (%)[a]	Oxoid	Difco	Prawn head peptone A1[b]	A2	A3	B1	B2	B3
Moisture	6.0	1.5	5.5	6.0	5.6	6.1	5.9	6.0
Fat	0.6	0.5	7.0	7.6	7.2	7.3	7.9	7.1
Ash	6.4	5.9	19.7	22.0	16.8	17.4	21.4	14.4
Crude fibre	0.5	0.4	1.8	2.8	1.9	2.1	2.8	1.8
Crude protein	96.2	97.8	73.5	68.3	75.2	74.9	69.0	79.1
Chitin	0.3	0.2	1.1	2.4	1.8	1.6	2.4	1.1
Corrected protein[c]	96.1	97.8	73.1	67.3	74.5	74.2	67.9	74.9
Amino nitrogen	3.1	3.0	2.0	1.3	2.2	2.1	1.3	2.3
Non-protein nitrogen	1.1	1.2	1.4	1.4	1.6	1.6	1.4	1.3

[a] Calculated on dry weight basis

[b] A1 = Autolytic digestion + spouted-bed drying

A2 = Pepsin digestion + spouted-bed drying

A3 = Papain digestion + spouted-bed drying

B1 = Autolytic digestion + freeze drying

B2 = Pepsin digestion + freeze drying

B3 = Papain digestion + freeze drying

[c] Corrected for chitin

Table 2. Biomass production[a] of test microorganism growth on commercial and prawn head peptone solutions.

Test microorganism	Oxoid	Difco	Prawn head peptone A1[b]	A2	A3	B1	B2	B3
Bacteria								
E. coli	70	66	64	54	68	66	54	72
B. subtilis	104	103	94	77	96	90	88	100
S. aureus	95	100	89	73	90	92	75	92
L. plantarum	60	68	56	36	58	56	38	64
Yeast								
S. cerevisiae	106	110	104	78	104	100	80	104
Fungus								
R. oligosporus	81	60	165	296	218	257	320	140

[a] Expressed as mg dry weight biomass / 100 mL medium.

[b] A1 = Autolytic digestion + spouted-bed drying

A2 = Pepsin digestion + spouted-bed drying

A3 = Papain digestion + spouted-bed drying

B1 = Autolytic digestion + freeze drying

B2 = Pepsin digestion + freeze drying

B3 = Papain digestion + freeze drying

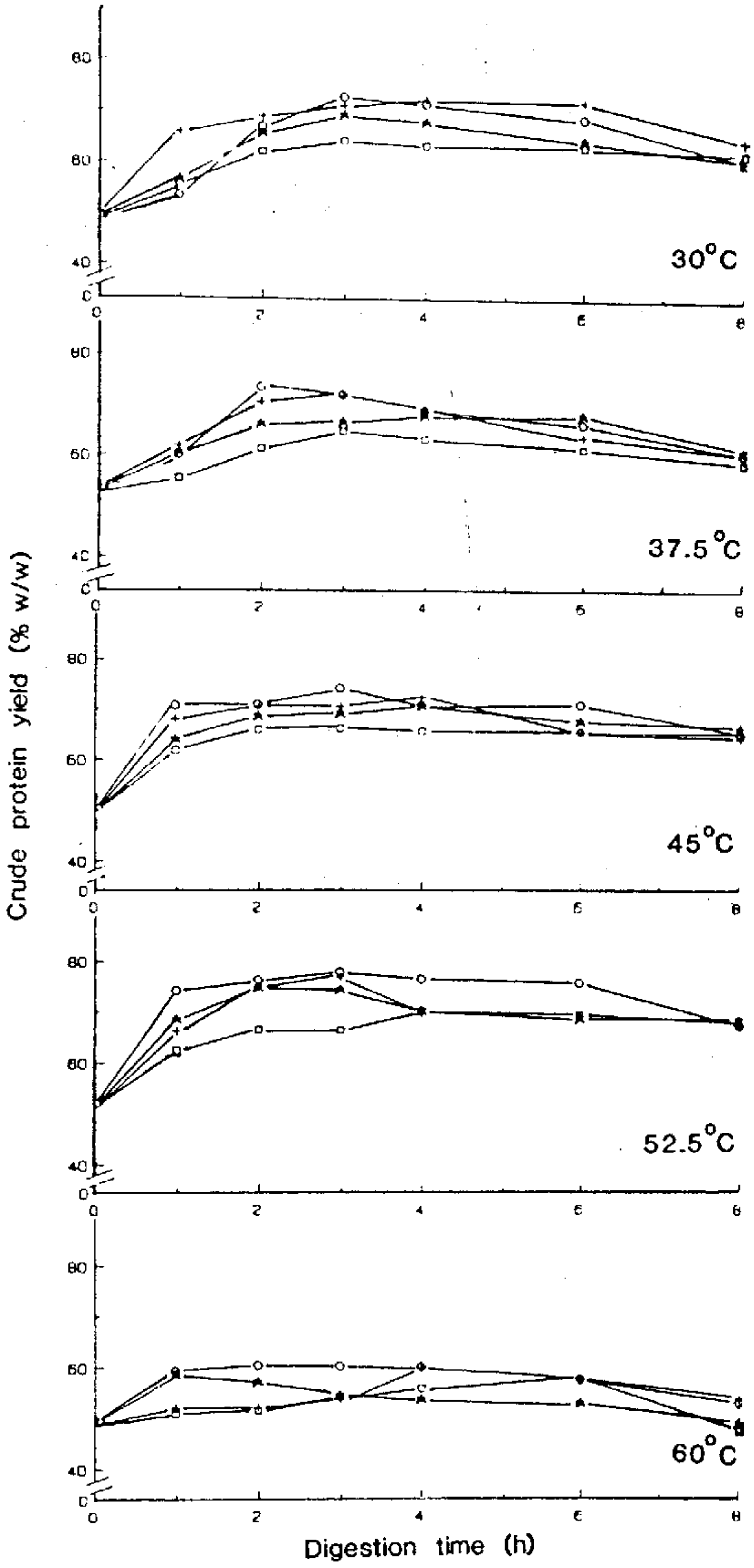

Figure 1. Effect of pH, temperature and time on autolytic digestion of prawn heads

□ pH 6.5, ★ pH 7.0, o pH 7.5, + pH 8.0

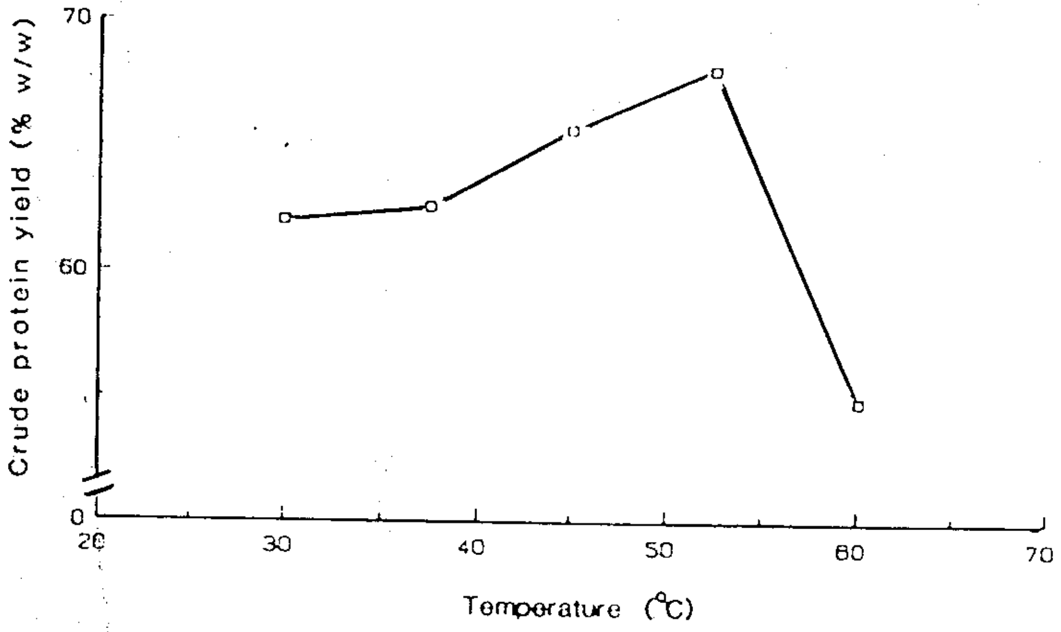

Figure 2. Effect of temperature on autolytic digestion of prawn heads

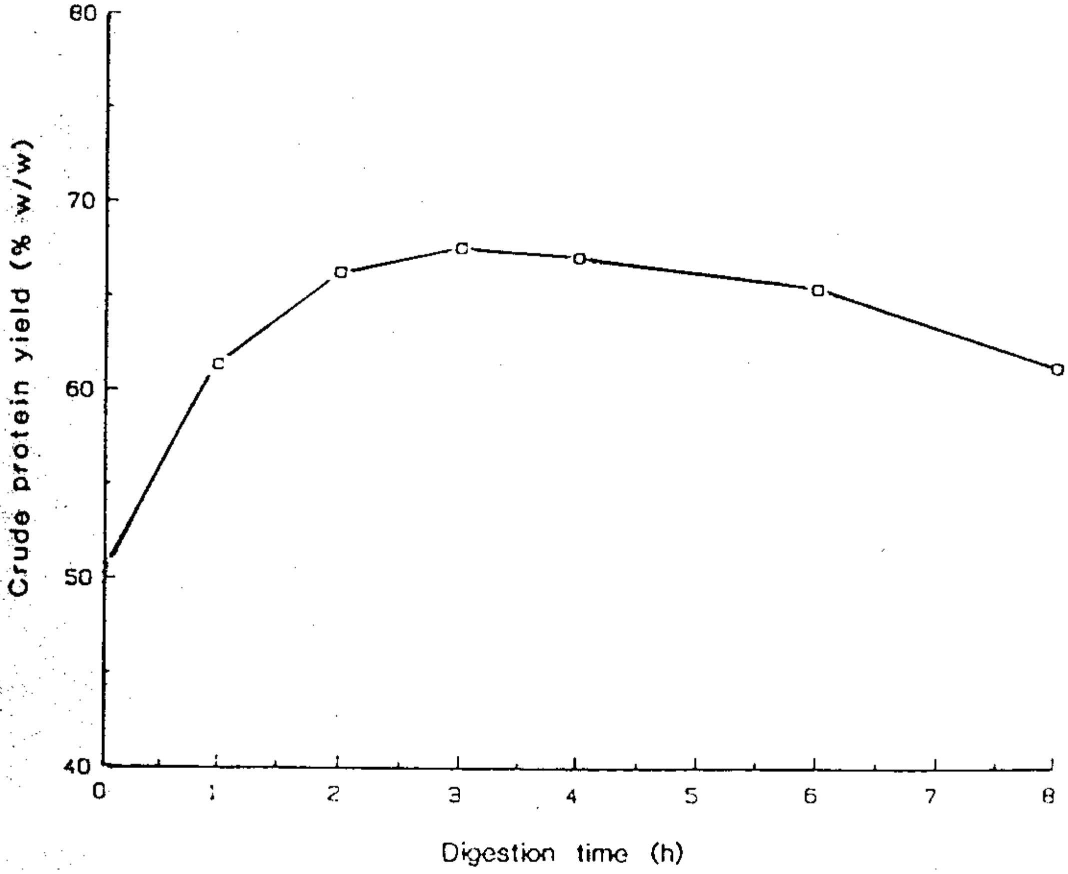

Figure 3. Effect of digestion time on autolytic digestion of prawn heads

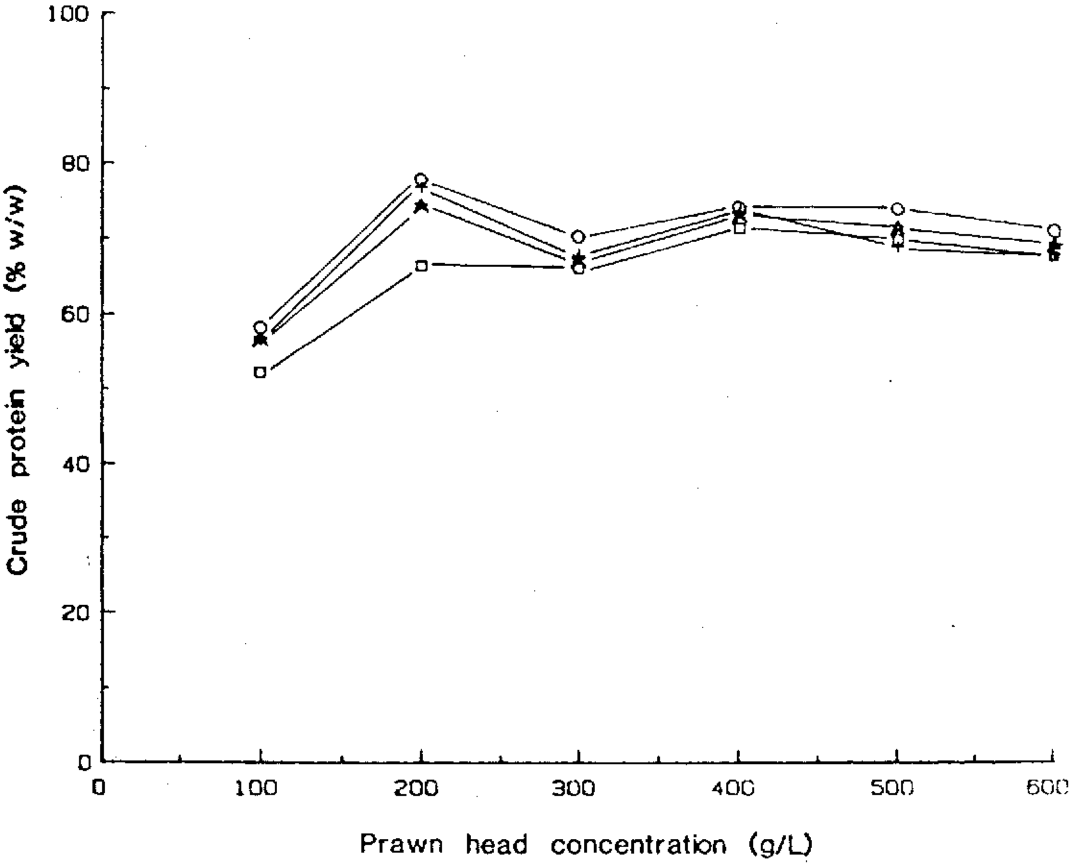

Figure 4. Effect of prawn head concentration on autolytic digestion of prawn heads for 3 h at 52.5°C

□ pH 6.5, ☆ pH 7.0, ○ pH 7.5, + pH 8.0

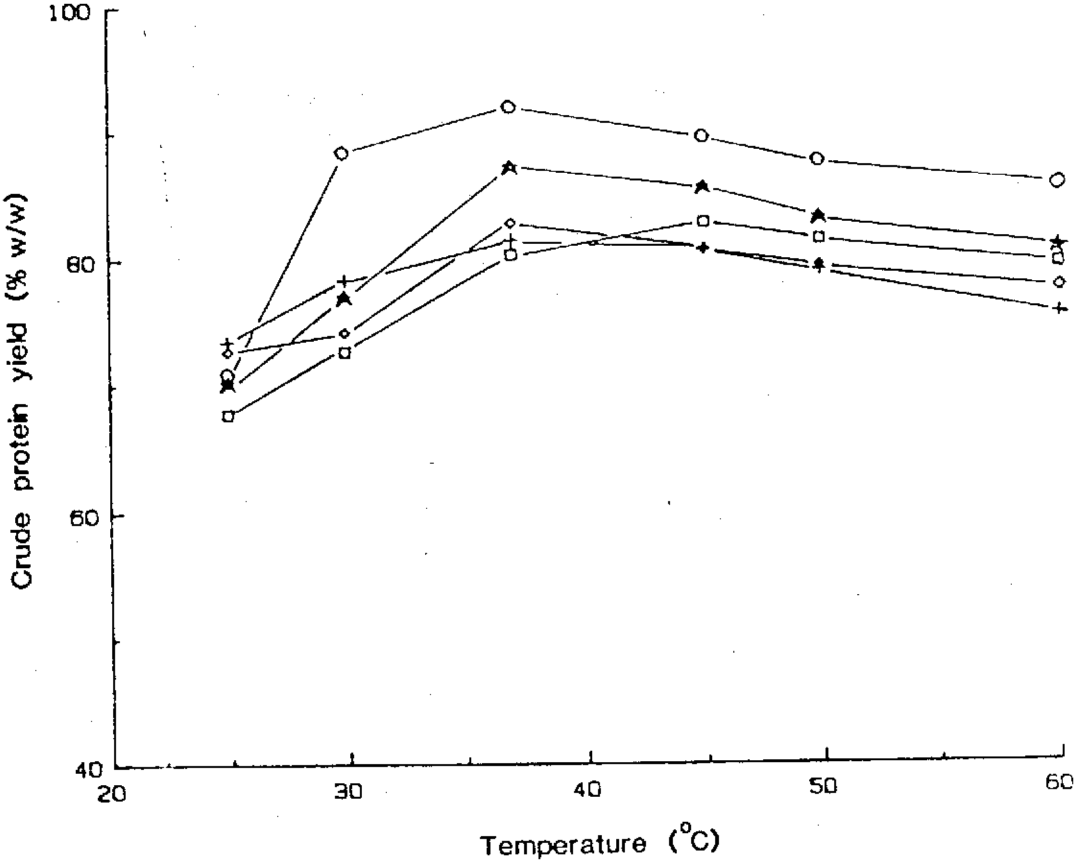

Figure 5. Effect of pH and temperature on crude protein yield from pepsin digestion of prawn heads

□ pH 1.0, ☆ pH 1.5, ○ pH 2.0, ◊ pH 2.5, + pH 3.0

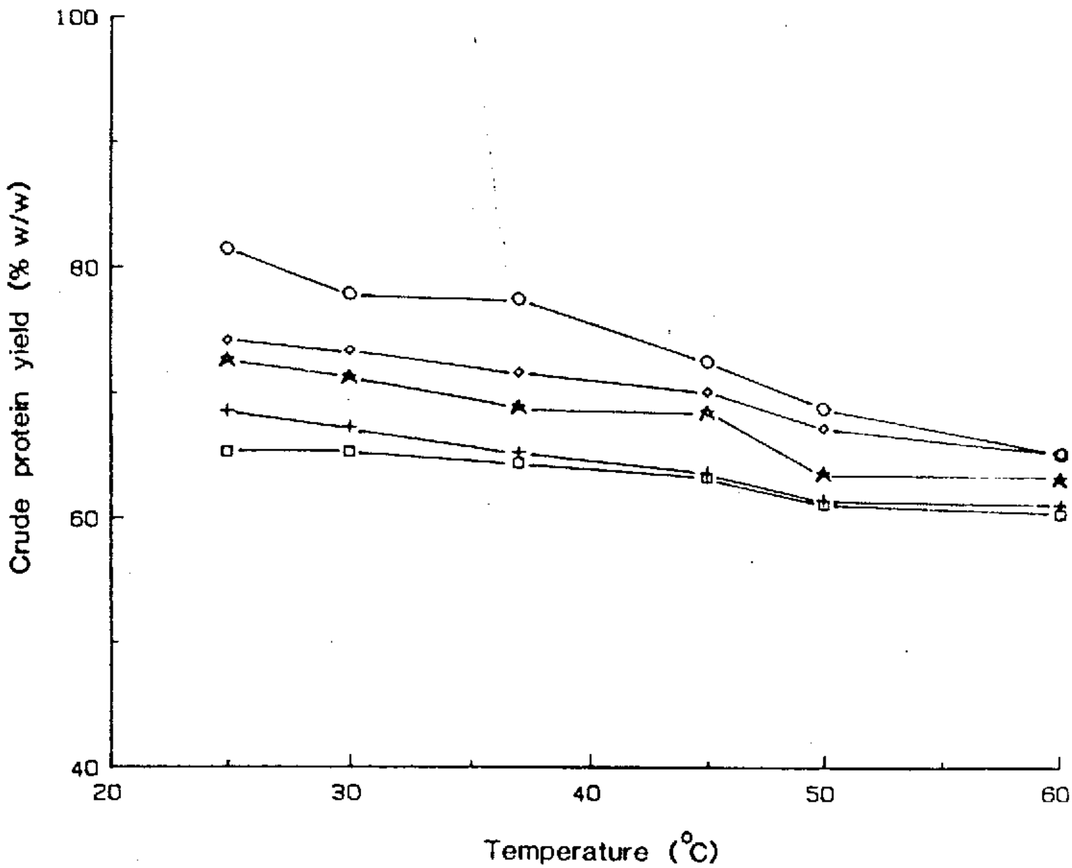

Figure 6. Effect of pH and temperature on crude protein yield from papain digestion of prawn heads
□ pH 4.2, ★ pH 5.2, ○ pH 6.2, ◊ pH 7.2, + pH 8.2

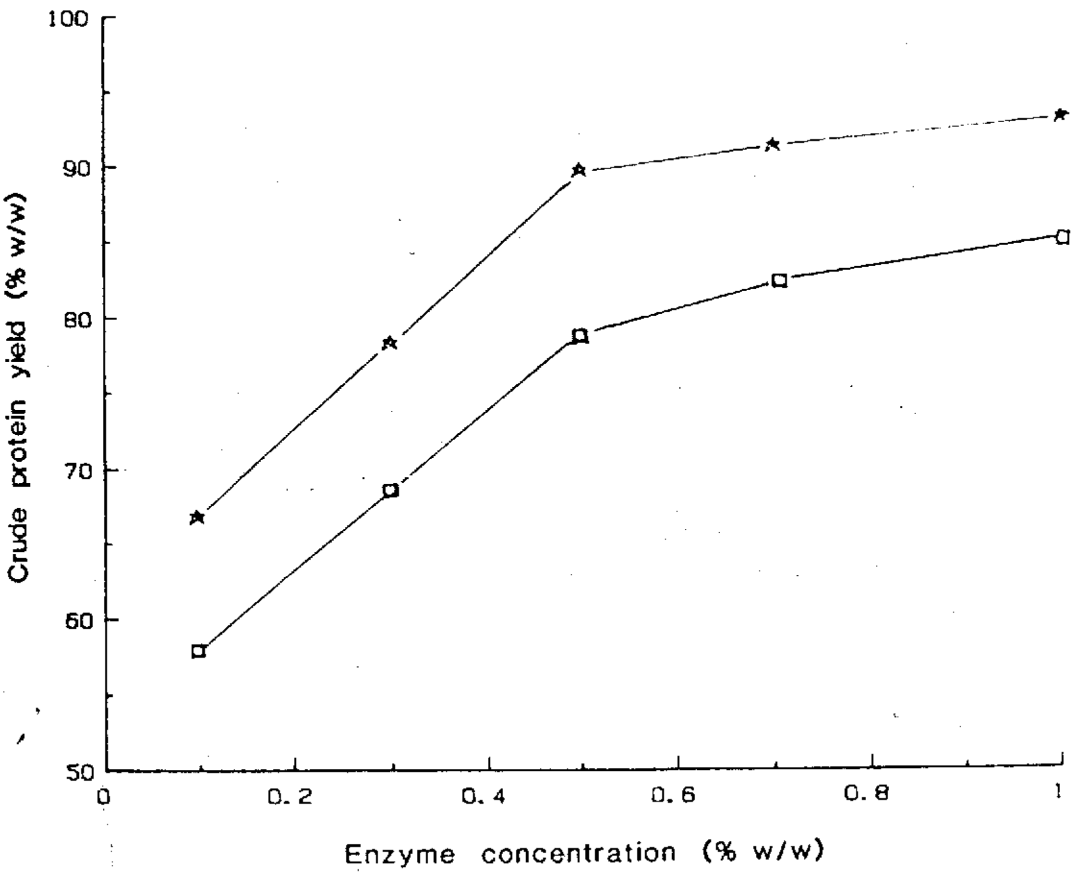

Figure 7. Effect of enzyme concentration on crude protein yield from pepsin (★) and papain (□) digestion of prawn heads