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Precipitation of protein, proximate analysis and amino acid composition of waste effluent of prawn processing

(Pemendakan protein, analisis proksimat dan komposisi asid amino air buangan pemprosesan udang)

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Key words: prawn processing effluent, precipitation, proximate analysis, amino acids

Abstract

The precipitation of protein from waste water of shrimp processing was done by changing the pH from 9.2 to pH 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 with 4 N HCl. The precipitation of protein at pH 4.0 gave the highest amount of precipitate as well as the highest crude protein content. Proximate analysis of the precipitate indicated the presence of 77.7% crude protein, 16.9% crude fat, 2.73% ash and 1.42% moisture on dry weight basis. The HPLC analysis indicated the presence of 18 amino acids (Asp, Glu, Ser, Thr, Arg, Gly, Ala, Pro, Val, Met, Leu, Ile, Phe, Cys, Lys, His, Trp and Tyr) in the precipitate. The total amount of free amino acids in the precipitate was 80.04 g ± 0.97/100 g samples with glutamic acid being the most abundant. Amino acid analysis also indicated that the precipitate contained high amount of essential amino acids (43.72%).

Introduction

The worldwide production and processing of shrimp represents an industry valued at several hundred million dollars (Mandeville et al. 1992). However the crustacean waste generated a pollution problem. The waste generated from the fishing industry represents approximately 70% of the total landings (Mandeville et al. 1991). This abundant waste has a detrimental effect on the environment due to the deterioration of fish tissue in the landfill sites. A better economic use of the waste would minimise the pollution problem and at the same time maximise the profits of the processor. Alternatively, the waste can be utilised by extracting useful components such as pigments and flavour active components and incorporating them into desirable marketable products.

Numerous researchers (Hayashi et al. 1981; Kato et al. 1989; Voight et al. 1990) have identified the primary flavour active components of shellfish as being umami (free amino acids and sugars, nucleotides, inorganic salts and betaine). Simpson and Haard (1985) extracted carotenoproteins and pigments from shrimp waste using trypsin and EDTA. Jaswal (1989) investigated the production of amino acids from protein hydrolysates of the shrimp and found that the shrimp offal contained approximately 40–42% essential amino acids. This paper describes the precipitation of protein from the waste water of prawn processing at different pH, proximate analysis and the

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composition of amino acids of the precipitate.

Materials and methods

Precipitation of protein

The waste water of prawn processing was collected from Eastern Global (M) Pte. Ltd., Parit Buntar, Perak. Protein was precipitated from the waste water of prawn processing using 4 N HCl (Del Valle and Aguilera 1990). Six samples of 200 ml of prawn processing waste water were treated with 4 N HCl and the pH was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 respectively. The initial pH of the waste water was 9.2. The solution was allowed to stand at room temperature for 2 h to precipitate the protein. The experiment was done in triplicate to confirm the reliability of the results. The precipitate was then freeze-dried and stored until further use.

Proximate composition

Analyses for proximate composition were carried out by AOAC (1990) standard methods. A triplicate of 5 g samples (treated with 4 N HCl at pH 4.0) was dried for 24 h at 105 °C. The loss in weight after drying was recorded as moisture. The ash content of the sample was determined by incinerating a known quantity of the homogenate, previously dried to a constant weight in a silica crucible. The ashing was done in a muffle furnace at 550 °C for 24 h until whitish or greyish ash was obtained. The crude fat content was determined by extracting the dried, homogenised sample with petroleum ether using Soxhlet apparatus for about 16 h on an electrothermal extraction unit.

The semi-micro kjeldhal method, which essentially determines the total nitrogen content, was used to determine the crude protein content. A triplicate of 0.5 g of the homogenised sample was digested with 20 ml nitrogen free concentrated sulphuric acid until the solution became clear. The solution was made alkaline by adding 40% sodium hydroxide solution, steam distilled with 2% boric acid and titrated with 0.02 N sulphuric acid. A factor of 6.25 was used to calculate the protein content.

Amino acid composition

Hydrolysis Individual amino acids were determined after digestion of the samples in 6 N HCl at 110 °C as described by Blackburn (1968). The sample was hydrolysed in triplicates by using the sealed-tube hydrolysis method (Davies and Thomas 1973). About 0.05–0.1 g of the homogenised sample was weighed into a medium wall borosilicate test tube. It was then added with 10 ml of 6 N HCl and the tube was flushed with nitrogen before being hydrolysed in the oven at 110 °C for 24 h. After hydrolysis, the sealed end of the tube was opened and the tube was allowed to cool. The hydrolysate was transferred to a 100 ml volumetric flask and 400 µl of 50 µmole/ml of AABA was then added before being made up to volume with deionised water. The hydolysate was then filtered through filter paper (Whatman No. 541) and re-filtered with syringe filter.

Cysteine and methionine were determined by performic acid oxidation prior to their digestion in 6 N HCl and were measured as cysteic acid and methionine sulphone, respectively (Blackburn 1968). About 0.05–0.1 g of homogenised sample was weighed into a pear shaped flask. It was then added with 4.0 ml of cold performic acid and the solution was kept in the refrigerator at 4 °C for 16 h. Cold bromic acid (5 ml) was then added and the flask with its contents were placed again in the refrigerator for 30 min before being evaporated in the rotary evaporator at 80 °C. Subsequently, the residue was hydrolysed with 6 N HCl.

Tryptophan was determined by alkaline hydrolysis with 4.3 N $LiOH.H_2O$ (AOAC 2000). About 0.05–0.1 g of the homogenised sample was weighed into a medium wall borosilicate test tube. A volume of 15 ml of 4.3 N $LiOH.H_2O$ was added to the sample and the solution was

flushed with nitrogen to remove soluble oxygen before being hydrolysed in the oven at 120 °C for 16 h. After hydrolysis, the sample was transferred into a beaker and 9 ml of 6 N HCl was added. The pH of the solution was adjusted to 4.5 with 2 N HCl or 2 N NaOH. The solution was then transferred into 100 ml volumetric flask and made up to volume with deionised water. The solution was then filtered with filter paper (Whatman No. 2) and then re-filtered with syringe filter and 20 µl of the solution was then injected into HPLC column.

Derivatisation Derivatisation was done according to Cohen and De Antonis (1994) using borate buffer and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (ACCQ-tag reagent). Borate buffer (70 µl) and 10 µl of sample were mixed and vortex to increase the pH. Then, 20 µl of ACCQ-tag reagent was added and vortex immediately. The sample was kept at room temperature for 1 min for the derivatisation to complete.

Amino acid analysis The amino acid content of the samples was analysed and quantitatively determined using Waters 510 HPLC system (Waters, USA) and detected by Waters 470 fluorescence detector at E_λ and E_m of 250 nm and 395 nm respectively for 6 N HCl and performic acid hydrolysate. Meanwhile, for tryptophan analysis the E_λ and E_m was 285 nm and 345 nm respectively. A volume of 10 µl of derivatised sample was injected in gradient mode into the column and the amino acids were eluted automatically. The column used was Waters AccQ-Tag Amino Acid Analysis Column (3.9 mm id x 150 mm) at 36 °C. For tryptophan analysis Pico Tag C 18 column (3.9 id x 300 mm) was used at 31 °C. The quantity of each amino acid was determined from the chromatogram.

The calculation of each amino acid is as follows:
Calculation of response factor, Rf:

$$Rf = \frac{W_{is} \times A_{std}}{W_{std} \times A_{is}}$$

Where: Rf = Standard response factor
W_{is} = Wt. of internal standard, AABA
A_{is} = Peak area of internal standard
W_{std} = Wt. of standard
A_{std} = Peak area of standard

Amino acids content (g/100 g sample)

$$= \frac{A_s \times W_{is} \times 100}{A_{is} \times W_s \times Rf}$$

where: A_s = Peak area of sample
A_{is} = Peak area of internal standard
W_s = Wt. of sample (g)
W_{is} = Wt. of internal standard
Rf = Response factor

Mobile phase preparation
Mobile phase A was prepared by diluting 200 ml of AccQ-Tag Eluent A (concentrate) with 2,000 ml of distilled water. Mobile phase B was 60% acetonitrile.

Statistical analysis
A triplicate analysis was performed in a completely randomised design. Statistical significance of observed differences among means of experimental results was evaluated by analysis of variance (ANOVA) followed pairwise comparison of means by least significant differences (LSD). The computerised program of SAS system version 6.12 for Elementary Statistical Analysis was used.

Results and discussion

Precipitation of protein

Precipitation of protein from waste water of prawn processing was done at different pH. The precipitate of solids content *(Figure 1)* was significantly higher at pH 4.0 than at the other pH. The amount of precipitate was 0.44 g/200 ml. Thus, the solids were extracted best at pH 4.0. The amounts of precipitate at pH 3.0 and 5.0 were not significantly different. As the pH increased, the amounts of precipitate decreased. The results were similar to those of Rodriguez et al. (1994) where the precipitate of solid content obtained was significantly higher at pH 4.5. Adjusting the pH to 4.5 was effective in increasing precipitate solids content and this was consistent with isoelectric point (pl) of fish proteins (Chetfel et al. 1985; Del Valle and Aguilera 1990). Precipitation of protein has usually been by adjusting pH to (pl) range (3.8–4.9) for fish protein (Maeda and Ozawa 1975; Emelyanova et al. 1977; Vega and Brennan 1987; Del Valle and Aguilera 1990).

The crude protein content was significantly higher (77.73%) at pH 4.0 compared to other pH *(Figure 2)*. The crude protein content of the precipitate at precipitation less or more than pH 4.0 was lower compared to at pH 4.0. As the pH increased, the protein content decreased.

Figure 3 shows the content of crude protein in the supernatant after the precipitation and centrifugation. The crude protein content at pH 4.0 was the lowest while the highest crude protein content was obtained at pH 8.0.

Proximate analysis

The chemical composition of the precipitate of prawn processing waste water is summarised in *Table 1*. The crude protein content was determined by using kjeldhal system and was found to be 77.73%. It was higher than the crude protein content of the shrimp waste effluent (58.98%) reported by Toma and Meyers (1975), and lower than the crude protein content of commercial shrimp waste (94.6%) as reported by Mandeville et al. (1992). The high protein content could represent a good source of protein for food industry. The difference of crude protein content between those findings was due to the differences of other compounds in the precipitate.

The crude fat content was determined by soxhlet fat extraction method and was

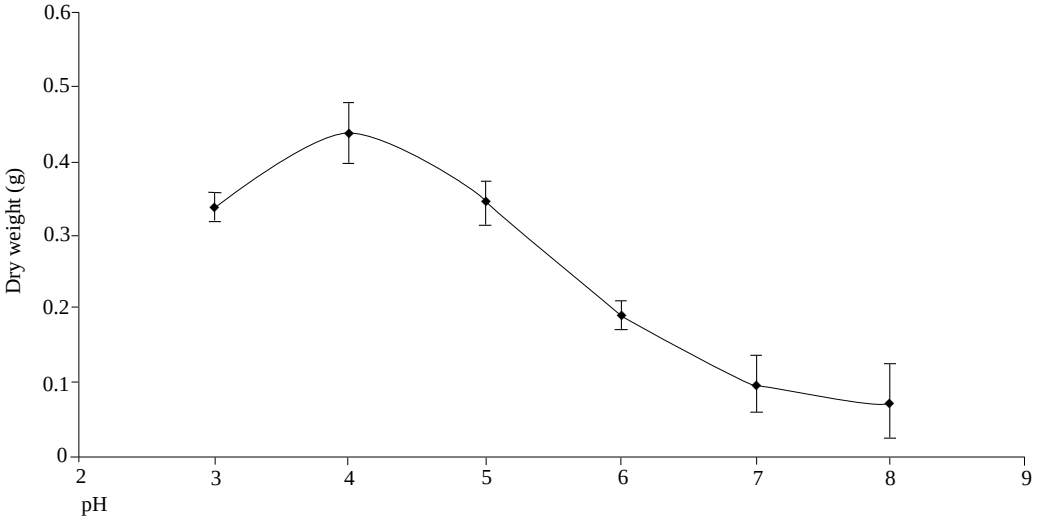

Figure 1. Weight (dry wt.) of the precipitate after the precipitation of protein by 4 N HCl at different pH (n = 3)

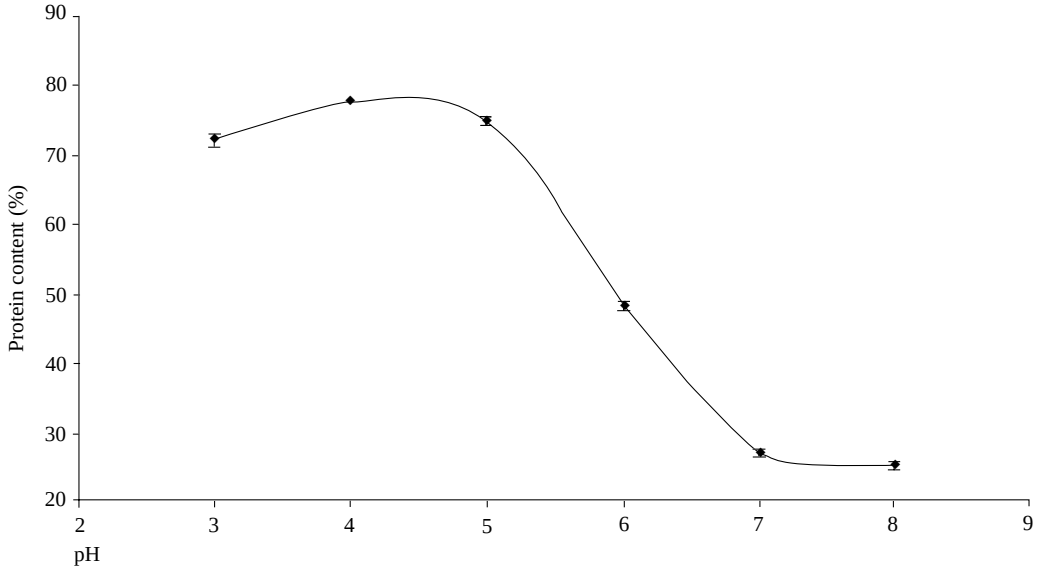

Figure 2. Protein content of the precipitate at different pH (n = 3)

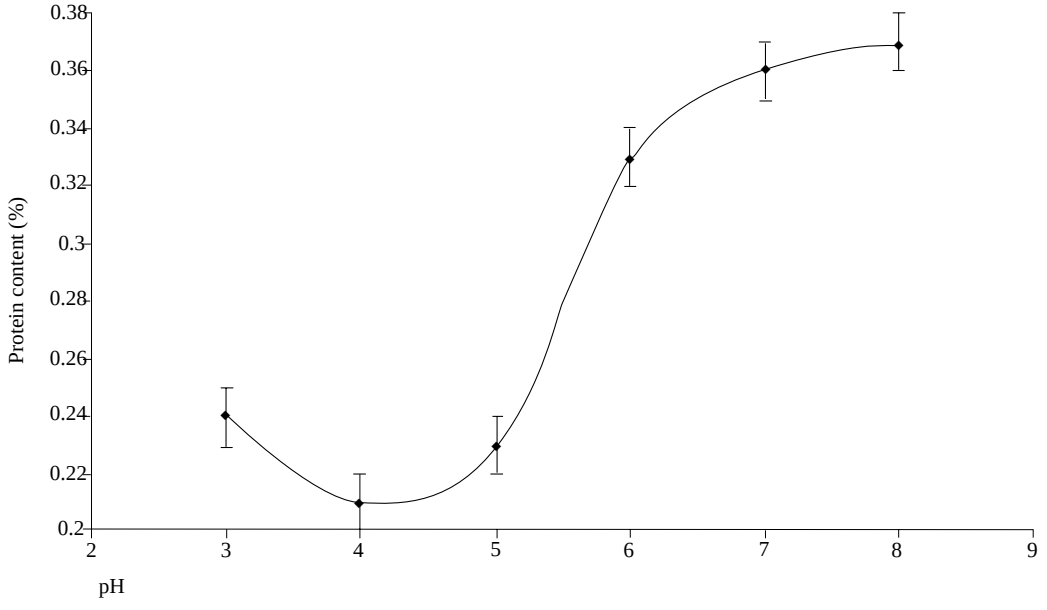

Figure 3. Protein content of the supernatant at different pH (n = 3)

found to be 16.69%. A similar result was also obtained by Toma and Meyers (1975). However, Mandeville et al. (1992) reported that the crude fat content of the precipitate is 4.1%. Ash and moisture percentages are 2.73 and 1.42% respectively. These amounts were lower than that (6.33% and 10.0%) reported by Toma and Meyers (1975) but higher than that (1.3% and 0%) reported by Mandeville et al. (1992).

Amino acid composition

The HPLC analysis showed that the precipitate contained 18 amino acids namely

Table 1. Proximate analysis of the precipitate of prawn waste water

	% Average (n = 3)*	Std. deviation
Protein	77.73	0.17
Fat	16.69	0.23
Ash	2.73	0.07
Moisture	1.42	0.15

*Mean of three replicates

aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleusine, leusine, phenylalanine, tryptophan and lysine *(Figures 4, 5* and *6)*. The results obtained were similar to those reported by Mandeville et al. (1992). The free amino acid in the precipitate was calculated to be 80.04 g ± 0.97 per 100 g sample. The most abundant amino acid was glutamic acid followed by methionine *(Table 2)*. The amino acid content of glutamic acid, aspartic acid, methionine, leucine and arginine accounted for 48.46% of the total free amino acids in the precipitate.

The adequacy of a protein for human depends upon its content of essential amino acids (leusine, isoleusine, lysine, methionine, cysteine, phenylalanine, threonine, valine, tryptophan and histidine). The essential amino acids in the protein under study represented 43.72% of the total amino acids. This is an attractive feature in terms of nutritive value. The result was slightly higher than that reported by Jaswal (1989) and Mandeville et al. (1992). Jaswal investigated the production of amino acids from protein hydrolysates of shrimp and found that the shrimp offal contains approximately 40–42% essential amino acids. The contents of essential amino acids in the raw and cooked commercial shrimp waste are 34.6% and 42.8%, respectively (Mandeville et al. 1992). The high content of these amino acids is generally associated with crustacean species (Konosu and Yamaguchi 1982).

Table 2. Amino acid composition in the precipitate of waste water of prawn processing (n = 3)

Amino acid	Amount (g/100 g sampel)
Alanine	4.15 ± 0.14
Arginine	5.18 ± 0.28
Aspartic acid	9.97 ± 0.33
Cysteine	0.73 ± 0.05
Glutamic acid	12.75 ± 1.03
Glysine	2.99 ± 0.09
Histidine	1.40 ± 0.26
Isoleusine	3.08 ± 0.09
Leusine	5.18 ± 0.15
Lysine	5.04 ± 0.06
Methionine	5.71 ± 0.15
Phenylalanine	4.62 ± 0.05
Proline	2.72 ± 0.20
Serine	3.37 ± 0.23
Threonine	3.46 ± 0.22
Tryptophan	3.35 ± 0.07
Tyrosine	3.22 ± 0.07
Valine	3.15 ± 0.04

Mean are average of three determinations

Furthermore, the concentration of arginine is an indicator of the quality of the waste, since arginine usually decomposes to ornithine and urea by arginase enzyme as a result of long storage condition (Cobb et al. 1974; Miyagawa et al. 1990). The sweetness of the shrimp usually parallels the amount of His, Pro, Ala, Ser and Glu present (Konosu 1979). These amino acids represent 30.47% of the total amino acids. Glutamic acid and glycine were demonstrated to be the main contributors to the taste of an extract of dried shrimp (Konosu 1979). Results from this study showed that these amino acids represented 19.67% of the total amino acids and was higher than that reported by Mandeville et al. (1992).

Conclusion

The precipitation of protein from waste water of prawn processing at pH 4.0 with 4 N HCl gave the highest yield as well as the highest crude protein content. The precipitate provided protein of high nutritive quality on the basis of its essential amino

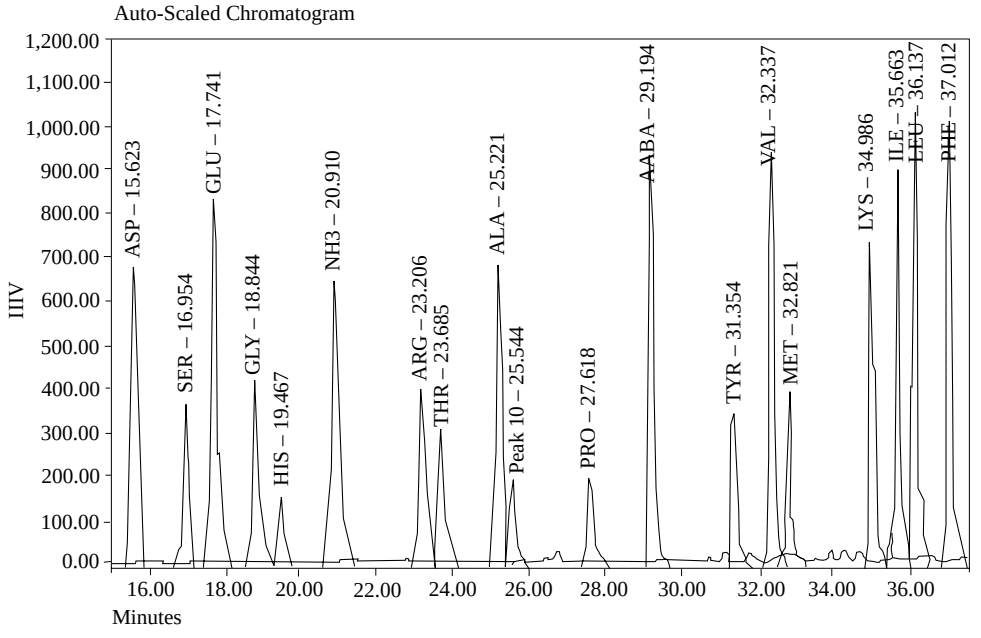

Figure 4. Chromatogram of HPLC separation of amino acids in the precipitate of waste water of prawn processing

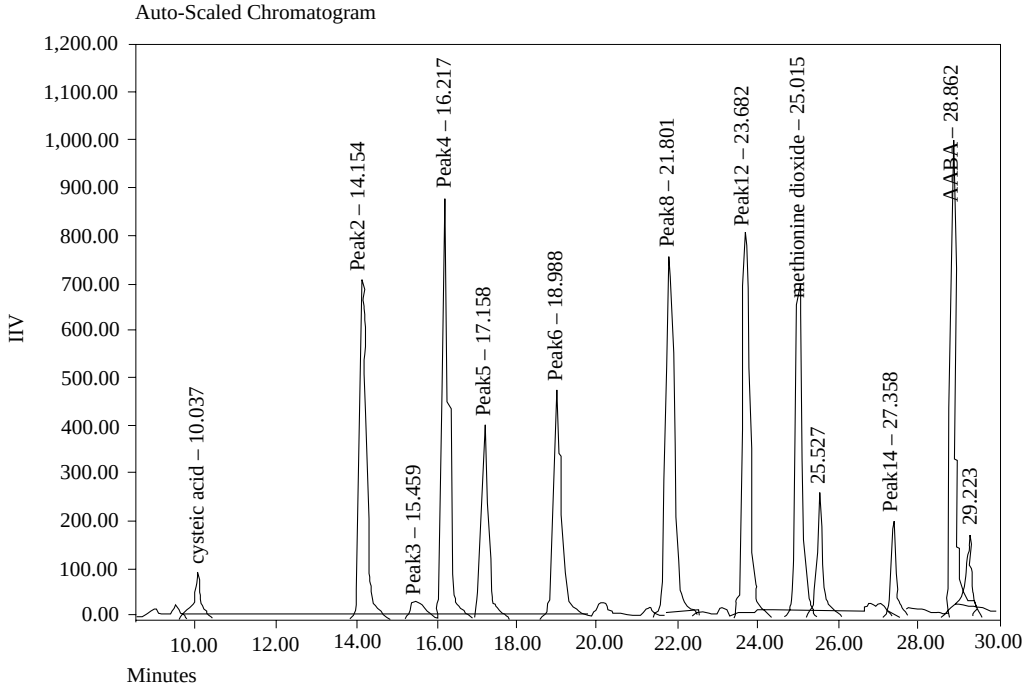

Figure 5. Chromatogram of HPLC separation of methionine (represented as methionine dioxide) and cysteine (represented as cysteic acid) in the precipitate of waste water of prawn processing

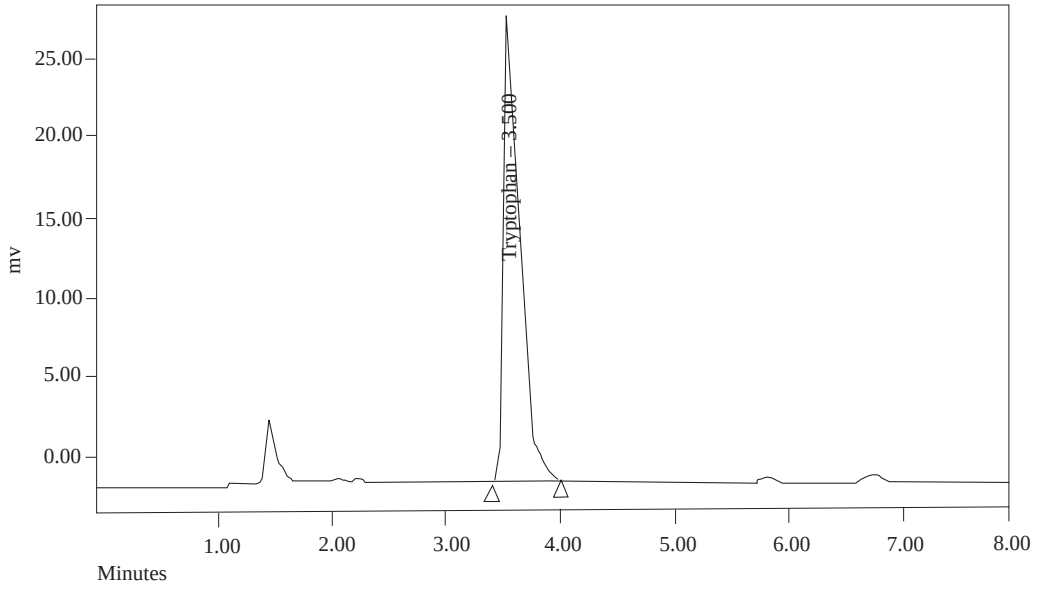

Figure 6. Chromatogram of tryptophan in the precipitate of waste water of prawn processing

acids content. Glutamic acid, which is the main contributor to the taste and the palatability of the sample, was the most abundant amino acid in the precipitate. This fact makes the waste water of prawn processing an attractive raw material for extracting useful components and utilising them in seafood products.

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Abstrak

Pemendakan protein daripada air buangan pemprosesan udang telah dilakukan dengan mengubah pH larutan daripada 9.2 kepada pH 3.0, 4.0, 5.0, 6.0, 7.0 dan 8.0 dengan menggunakan 4 N HCl. Pemendakan pada pH 4.0 memberikan hasil mendakan dan kandungan protein kasar tertinggi berbanding dengan pH yang lain. Analisis proksimat menunjukkan mendakan air buangan udang pada pH 4.0 mengandungi 77.7% protein kasar, 16.9% lemak kasar, 2.73% abu dan 1.42% air (berat kering). Analisis menggunakan HPLC menunjukkan terdapat 18 jenis asid amino iaitu Asp, Glu, Ser, Thr, Arg, Gly, Ala, Pro, Val, Met, Leu, Ile, Phe, Cys, Lys, His, Trp dan Tyr. Jumlah asid amino di dalam mendakan ialah 80.04 g ± 0.97/100 g sampel dan kandungan asid glutamik ialah yang tertinggi berbanding dengan asid amino lain. Mendakan air buangan udang mengandungi asid amino perlu yang tinggi iaitu 43.72%.

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