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RESEARCH ARTICLE

A STUDY ON RECOVERY OF PROTEIN HYDROLYSATE FROM INDUSTRIAL SHRIMP WASTE AND ITS NUTRITIONAL STATUS

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ABSTRACT

Shrimp waste, extraction of protein hydrolysate active compounds from the waste and using them as useful marketable products is a smart solution here which would minimize the pollution problem and at the same time maximize the profits of the processors. Although part of the waste which mainly consists of exoskeleton and cephalothorax is traditionally used for chitin/chitosan preparation, feed manufacture and manure, and a major portion remains unused or underutilized. Only 65% of the shrimp is edible. The remainder is discarded as inedible waste (Cephalothorax and exoskeleton) over the years; techniques have been developed for the exploitation and recovery of these byproducts in valuable biopolymers. About 35-45% by weight of shrimp raw material is discarded. This can generate an unpalatable high salt content product. Enzymatic hydrolysis of proteins is an attractive means of producing better functional and nutritional properties in food proteins generated from by-product The current trend of changing food preference / habit is giving importance to the food industry highlighting the need like protein designing, food engineering and tailor made proteins. In fish processing sector, highly prized fish proteins include mainly the shrimp waste proteins.

INTRODUCTION

Shrimp processing bio wastes include the head, shell and tail portions (non- edible parts) which account for about 50-70 % of the total volume of raw material. The global implications of this scenario are that enormous shrimp bio wastes are being generated by the sea food industry due to the escalating demand for shrimp products. In addition, continuous disposal of these voluminous bio wastes in to costal and near shore environments has contributed to intense environmental pollution and consequent deterioration in affected ecosystems. On other hand, by disposing the valuable by products without recycling and proper utilization, the sea food industries are missing prime opportunities for deriving several value added products such as chitin and other bioactive substances. In India a major producer of shrimp, more than 1, 00,000 tons shrimp bio-wastes generated annually and only an insignificant amount of that bio-waste is utilized for the extraction of protein hydrolysate. Enzymatic hydrolysis, however, produces less undesirable by-product and improves the functional and nutritional properties of food proteins. There is an increasing interest in the development of fast and gentle enzymatic production methods as an alternative to mechanical or chemical treatments that frequently damage the products and reduce product recovery and functionality. Enzymatic hydrolysis of proteins is an attractive means of producing better functional and nutritional properties in food proteins generated from by-product. The present study on "Recovery of Protein hydrolysate from shrimp shell waste and its Storage study" was taken up with following objectives-

- Recovery methods of protein hydrolysate from shrimp waste
- To study physical, biochemical and nutritional quality analysis of protein hydrolysate.

MATERIALS AND METHODS

Raw material

The shrimp waste was collected from Nizham Rekha Seafood Pvt. LTD processing plant located near Kolkata, India and transported to our laboratory in iced condition in insulated container. As the sea food processing plant mainly deals with *Penaeus monodon, Fenneropenaeus indicus*, and *Macrobrachium rosenbergii* the waste disposed mainly comprises of head, exoskeleton and tail of these species.

Chemicals and glassware used

Most of the chemicals used in the analysis were either of 'Analytical' or 'Guaranteed' reagent grades. Chemicals were

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prepared according to their specification and precaution measures were followed in storing them. The glassware used were all of 'Borosil' make. Total Plate Count (TPC) was estimated by using Nutrient Agar procured from 'Hi-media'.

- Alkali used-NaOH, KOH
- Enzymes used-Pancreatin3X (Himedia)
- Microbial strain of –*Lactobacillus* (Strain name-*Lactobacillus plantarum* abs. A =1.7 measured at 535nm)
- Chemicals for Amino acid analysis (Hydrochloric acid, Borate buffer, FMOC reagent)

Packaging Material

Borosil glass bottles were used for packing of shrimp waste protein hydrolysates during storage period.

METHODS

Study of raw material

Certain physical, chemical, and sensory characteristics of shrimp waste were analyzed.

Physical characteristics

All physical characteristics of shrimp waste are given in Table-I the total length and round weight of shrimp waste were 13 count and 10 kg shrimp waste taken for experimental study. Out of which, 9.4 kg shrimp waste was obtained after washing. From washed shrimp waste, 4.6 kg of dried shrimp waste was obtained from washed shrimp waste. From dried shrimp waste, 3.8 kg of shrimp waste powder, 38% of yield was obtained from fresh shrimp waste.

Chemical characteristics

Methods used to assess proximate composition and the freshness parameters of the raw material and protein hydrolysate are described in sections.

Sensory characteristics

The shrimp shell waste was assessed organoleptically based on the general appearance and colour, flavour, odour using 10 point hedonic scale (CIFE, 2001) given in Appendix I,II and III. On the basis of sensory technique, the quality of shrimp shell waste was assessed (CIFE, 2001). Occurrence of melanosis was also observed for whole shrimp shell waste based on melanosis rating scale. The overall acceptance of the shrimp waste was then assessed based on the mean score of the panelist. Nine- point hedonic scale was used to assess the sensory quality of the extracted shrimp waste protein hydrolysate.

Sample preparation

Industrial shrimp waste from a processing plant situated at Kolkata, India was collected which comprises cephalothorax, shell and tail. The waste of (*M. rosenbergii*) was washed under running water. Portions (100 gm) were packed into plastic bags and kept frozen at - 20°C until used. About 1000 gm of the raw waste was ground and passed through a 60 mesh sieve and freezed. The freeze dried sample was subjected to three types of hydrolysis methods to recovery of twelve types of samples. Choose the best three samples (protein hydrolysates) kept in glass bottles stored at 4°c (refrigerated) during 120 days storage period. Each 15 days storage period to check with biochemical, microbiological characteristics and estimate the shelf life of product.

Extraction of protein hydrolysate from shrimp waste

Extraction of protein in the form of protein hydrolysate from shrimp shell waste is the criteria of the present study. Extraction methods mainly three different hydrolysis process.

Protein recovery by alkaline hydrolysis method

Two bases used were NaOH and KOH, at a concentration of 1%, 3% and 5% (w/v). The raw waste was diluted with the solution of alkali at a ratio 1:10 (w/v) and heated at three different temperatures (50°C 70°Cand 90°C) with stirring for 1, 2 and 3 hrs. After extraction, solution was diluted and centrifuged at 12,000 rpm for 15 mins at 4°C. The supernatants were processed into a dry powder using freeze-drying method.

Protein recovery by enzymatic hydrolysis

Shrimp waste protein hydrolysate was prepared according to method of Holanda and Netto (2002) with slight modification. Shrimp waste was suspended (1:1, w/v) in distilled water. The mixture was heated at a temperature of 90°C for 30 min to inactivate the endogenous hydrolyzing enzyme. The mixture was then homogenized and adjusted to pH 8.5 with 1 N NaOH at 60°C to optimize protease activity. Alkalase 2, 4L, (Sigma Pvt. Ltd., India) which is a serine endopeptidase enzyme obtained from *Bacillus licheniformis*, was mixed at a enzyme/substrate ratio (E/S) of 3%. The hydrolysis reaction was carried out in jacketed glass reactor with controlled temperature and pH monitored by pH stat method using an automatic Mettler DL 25 titration unit. Hydrolysis was continued until degree of hydrolysis of 6% and 12% was reached, when reaction was stopped by heating at 90°C for 5 minutes. The insoluble fraction was separated by centrifugation at 16000 rpm for 15 minutes at 4°C. The supernatant obtained was made into a powder, by freeze-drying process.

Protein hydrolysate recovery by fermentation method

Protein hydrolysates were prepared from shrimp waste through lactic acid fermentation following the method of Bueno-Solano et al. (2008). Shrimp waste (500gm) was placed into 1000 mL glass flasks and mixed with 10% (by mass) cane sugar and 5% (by volume per mass) commercial innoculum (abs. A= 1.7, measured at 535nm), stirred and incubated in a water bath at 30°C for 36 hours. At the start of fermentation, the pH was adjusted to 6 with 2M citric acid or 5M Acetic acid or 5M Lactic acid. The fermentation was carried out with constant stirring. The silage obtained was centrifuged at 6,440 rpm for 15 mins at 5°C to obtain a chitin rich fraction (sediment) and the protein rich liquor and lipid fraction. The protein rich liquor (aqueous phase) was finally processed into a dry powder using a freeze drying method. The shrimp waste

protein hydrolysates derived in liquid form by alkaline, enzymatic and fermentative methods was referred as sample 1, 2 and 3, respectively. To produce protein hydrolysate in dry powdered form for better shelf life and storage stability, the liquid hydrolysate, rich in protein, was dehydrated using in animal nutrition.

Nutritional analysis

Amino acid composition

The amino acid composition of the hydrolysates obtained after each 30 minutes was estimated by using a amino acid analyzer (Waters India Pvt. Ltd., USA), (I.I.C.B-Kolkata) according to PICO.TAG system. Hydrolysate protein 20mg was extensively dialyzed overnight against deionised distilled water, dried and was hydrolysed with 6 N HCl containing 1% phenol for 22 h at 105º C in the PICO.TAG work station. Hydrolysis was carried out at vapour phase. Hydrolysed sample and standard amino acid mixture, standard H 0.005 ml were taken in respective tubes, introduced into the reaction vial and were dried completely. These were then derivatized by phenyl isothyocyanate (PITC) solution (ethanol: triethylamine: water: PITC, 7:1:1:1, by volume) for 20 min at 25º C. Then the vials were dried and samples were reconstituted in diluents solution (Na2 HPO4 0.071%, w/w, pH 7.4 containing acetonitrile 5%, v/v). The samples were analyzed by HPLC at 38ºC as per PICO.TAG manual. Detector setting, chart speed and run time were at 254 nm, 2 cm and 12 min respectively. An amino acid present in unknown sample was determined quantitatively by comparing the peak areas (745B data modules print out) of amino acids present in standard H. The numbers of residues were determined on the basis of molecular weight of 56240 and carbohydrate content 7.75% (w/w). For determination of tryptophan, the sample (weight equivalent to about 2 mg tryptophan) was treated with 4.2 M NaOH (100 ml) and 0.3 ml triglycerine and placed in the oven at 110 º C for 24 h. Seven ml of 6 M HCl were then added to the mixture and the pH adjusted to 4.5 using pH 4.2 citric acid buffer solution and the mixture was made to a certain volume. Tryptophan content was determined by colorimetric analysis (UV-1700, Shimadzu Co., Kyoto, Japan) at 400 nm under the condition of pH 5.0–5.5, column oven temperature 55 º C, reactor temperature 100 ºC, and reaction time 10–15 min.

Statistical analyses

Statistical analyses were performed as per Snedecor and Cochran (1968). Correlation coefficient (r) was calculated for the chemical quality parameters for the raw material to observe their acceptance level. One way analysis of variance (ANOVA) and three way ANOVA followed by least significant test in the form of critical difference was performed to test the significant difference between samples and storage days in the case of dried fish product.

RESULTS AND DISCUSSION

Characteristics of raw material

Physical characteristics

All physical characteristics of shrimp waste are given in Table 1. The total length and round weight of shrimp waste were 13 count and 10 kg shrimp waste taken for experimental study. Out of which 9.4 kg shrimp waste was obtained after washing. From washed shrimp waste 4.6 kg 0f dried shrimp waste was obtained from washed shrimp waste.

Table 1. Physical characteristics of shrimp waste

S. no.	Physical characteristics	Results
1.	Total weight of shrimp waste	10 kg
2.	After washing	9.4 kg
3.	After drying	4.6 kg
4.	Weight of shrimp waste powder	3.8 kg
5.	Yield percentage of shrimp waste powder	38 %

From dried shrimp waste 3.8 kg of shrimp waste powder, 38 % of yield was obtained from fresh shrimp waste. The fairly high yield obtained in present study may be due to comparatively M. rosenbergii shrimp waste used. The yield% obtained in present study fall well within the ranges obtained by Synowiecki *et al.* (2000). The proximate composition of shrimp waste is presented in Table 2. The result of moisture, protein, fat, and ash percentage of the shrimp waste was 76.31±0.2%%, 13.5±0.272%, 8.5±0.140%, and 1.54±0.140% respectively.

Table 2. Proximate composition of shrimp sp waste (*M. rosenbergii*)

Species	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
M.rosenbergii	76.31 ±0.2%	13.5 ±0.272%	8.5 ±0.140%	1.54 ±0.140%

The moisture content of shrimp waste ranges between 73.1 to76.9% and protein content ranges between10.5% to 13.9%, which fairly tallies with the result of the present study.

Chemical characteristics

Generally chemical tests are estimated to shrimp shell waste mainly to determine the quality and freshness of shrimp waste. A present study result of shrimp waste TVB-N value was 14.5±0.167 (mg %) presented in Table 3. The TVB-N value of shrimp waste was 14.5mg% within the acceptable limit. These results fairly coincide with the present study and shrimp waste was fairly fresh condition.

Table 3. Bio-chemical characteristics

Parameter	Range
TMA	6.16±O.07 (mg %)
TVB-N	14.5±0.167 (mg %)
FFA	3.44±0.072 (%of oleic acid)
PV	12.44±0.076 (mill equivalent of O2/kg of fat)
Chitin	5.62 ±o.130

The PV is a good index to judge quality of fat. The PV is a measure of the first stage of oxidative rancidity value above 10-20 mill equivalent of O2/kg of fat, the shrimp in all probability will smell and taste rancid. The PV recorded in the present investigation was12.44±0.076 (mill equivalent of O2/kg of fat), which was within the acceptable limit. The recommended microbiological limit for the shrimp waste is 3.5×105±0.20/gm this is well within the limit.

Sensory and Microbial characteristics

The sensory characteristics of shrimp waste are presented in Table 4. The results show that waste having bright and shining

appearance with less that 5% discoloration. There was no evidence of black spot on shell. Considering these sensory characteristics the quality of raw material was regarded as very good.

Table 4. Sensory characteristics of shrimp shell waste

Appearance	Bright and shining
Discoloration of shell	Below 5%
Black spot on shell	Nil
Objectionable foreign matter	Nil
Texture	Moderate tough and elastic
Overall quality	Very good

Protein recovery (%) from shrimp waste by different methods

Protein recovery from the raw waste depends on the method and degree of hydrolysis and time (Chakraborty, 2002). In case of enzymatic hydrolysis, the curve depicts high initial reaction rate followed by a decrease up to the stationary phase, where apparently hydrolysis no longer occurred. This profile could be associated with product inhibition by compounds found during the hydrolysis and the action of insoluble peptides, which act as an effective substrate competitor for the non hydrolysed protein (Rebecca *et al.,* 1991). Whereas in case of LAB fermentation, the curve showed a continuous process of reaction and time taken was more than enzymatic method.

Table 5. Protein Recovery (%) from. shrimp shell waste

Samples	Degree of hydrolysis (%)	Time (Minutes/hours)	Protein recovery (%)
Enzymatic hydrolysis method	11.4%	30 (minutes)	45.626±0.725%
	19.3%	60 (minutes)	57.283±0.345%
	28.5%	90 (minutes)	61.126±0.205% (Max)
Fermentative method	12.3%	24 hrs	33.483±0.261%
	18.6%	30 hrs	36.826±0.556%
	29.2%	36 hrs	37.33±0.574 % (Max)
Alkaline method	NaOH	3 hrs	84.23±2.120%
		4 hrs	87.43±1.005%
		5 hrs	88.75±0.231%
	KOH	3 hrs	83.89±1.034%
		4 hrs	86.45±0.884%
		5 hrs	88.39±0.562% (Max)

The recovery of protein hydrolysate from shrimp waste was mainly done by three methods which are Alkaline, Enzymatic, and Fermentative methods. The protein recovery (%) was mainly dependent on time and degree of hydrolysis (%). Always protein recovery (%) was directly proportional to time and degree of hydrolysis (%). In Alkaline method using NaOH, time of 5 hrs protein recovery (%) was 88.75±0.231% (max) and using KOH, time of 5 hrs protein recovery (%) was 88.39±0.562% .In enzymatic method Degree of hydrolysis 11.4% protein recovery (%) was 45.626±0.725% and Degree of hydrolysis 19.3% protein recovery % was 57.283±0.345%. Protein recovery (%) was lower in fermentative hydrolysis method Compare to other two methods (Alkaline, enzymatic). Although there is a relationship between PR% and DH% (Baek and Cadwallader, 1995), previous work has shown that PR% didn't improve significantly at DH% value less than 12% (Holanda and Netto, 2002). So protein recovery at a DH of 12.6%, 18.5% and 29.2% adopting two different methods has been depicted in Table-6. PR% with Pancreatin 3X was about 33.483±0.261%, 36.826±0.556% and 37.33±0.574 % higher than that of fermentative method at a DH% of 12.3%, 18.6% and 29.2% respectively. Pancreatin 3X results in higher protein recovery, in addition to providing hydrolysates with good functional properties with a mild bitter taste as compared to other proteolytic enzymes (Shahidi and others, 1995, Miazani et al. 2005). With the increase in DH from 11.43 to 19.3 %, PR increased by 57.283±0.345% and 36.826±0.556% similarly with increase in DH from 20 to 30% PR increased by 61.126±0.205% and 37.33±0.574 % respectively for enzymatic and fermentative method of hydrolysis. Synowiecki and Alkhatteb (2000) obtained a PR of 64.6% from previously demineralized *Cragon cragon* shrimp waste cephalothorax at a DH of 19% while Gildberg and Stenberg (2001) obtained 68.5% from Pandalus borealis waste after 2 hrs of hydrolysis with alkalase.

Table 5. Showed the values for alkaline deproteinization using KOH and NaOH. Deproteinization varied from 36.49 to 88.39% and was generally favoured by increase in alkali concentration, temperature and at a lower concentration, by the reaction time. Chang and Tsai (1997) obtained 68% deproteinization of pink Shrimp waste using 10% NaOH at 75° C for 6 hrs. The main factor involved in the efficiency of protein removal is the solution concentration and the temperature in addition to the crustacean species (Synowiecki and Alkhateeb, 2000). Though alkaline hydrolysis was more efficient in protein recovery giving a maximum value of 88.39±0.562% as against 57.283±0.345% and 36.826±0.556% for enzymatic and fermentative method, the protein recovered lose amino acid to a large extent, resulting in decreased nutritional and biotechnological quality. Also protein hydrolysis at extreme temperature and pH generally yields products with reduced nutritional quality, poor functionality and restricted use as flavor enhancers (Loffler, 1986).

Study of Protein Hydrolysate

According to the protein recovery (%) from the raw shrimp waste, the best sample was chosen from three different hydrolysis methods (alkaline, enzymatic, and fermentative) designated as Sample-1 (S1), Sample-2 (S2), Sample-3 (S3) and with those further studies was undertaken. Taken further studies choose three samples. In alkaline hydrolysis method using NaOH and time of 5hrs protein recovery (%) was 88.75±0.231% designated as S1, similarly in enzymatic hydrolysis method degree of hydrolysis 28.5 (%) and time of 90 minutes protein recovery (%) was 61.126±0.205% designated as S2. In fermentative hydrolysis method degree of hydrolysis 29.2 (%) and time of 36 hrs protein recovery (%) was 37.33±0.574 % designated as S3.

Proximate composition of protein hydrolysates

Proximate composition of products mainly depends on the preparation method, raw material quality and environment. The S1 protein hydrolysate proximate composition were moisture 5.546±0.08%, Protein 5.546±0.08% Lipids 1.873±0.100 % and Ash 17.283±0.070%. In S2 protein hydrolysate proximate composition were moisture 8.56±0.05 % Protein 75.05±0.135% Lipids 2.65±0.050% and Ash14.17±0.052%. In S3 protein hydrolysate proximate

composition were moisture 7.15±0.050% Protein 49.57±0.056% Lipids 1.66±0.051% and Ash 7.56±0.055%.The moisture content of three hydrolysates extracted in terms of percentage was 5.546±0.08%, 8.56±0.05% and 7.15±0.050% for alkaline, enzymatic and fermentative method respectively of protein recovery. The moisture content is related to kind of sample and temperature employed during the process of evaporation. Table 6. Presents the proximate analysis of the hydrolysate produced following three recovery process.

Table 6. proximate composition of shrimp protein hydrolysates

Samples	Moisture (%)	Protein (%)	Total lipids (%)	Ash (%)
S1	5.546 ±0.08%	51.05 ±0.371%	1.873 ±0.100%	17.283 ±0.070%
S2	8.56 ±0.05 %	75.05 ±0.135%	2.65 ±0.050%	14.17 ±0.052%
S3	7.15 ±0.050%	49.57 ±0.056%	1.66 ±0.051%	7.56 ±0.055%

S1 = SWPH extracted by alkaline hydrolysis method
S2 = SWPH extracted by enzymatic hydrolysis method
S3 = SWPH extracted by fermentative hydrolysis method

On dry weight basis, protein content was maximum in case of enzymatic recovery method and it was greater by 51.05±0.371% and 49.57±0.056% from alkaline and LAB fermentation method respectively, which may be due to intactness of disulphide bonds and retention of amino acids. Hayashi and Kameda (1980) hypothesized that the protein recovered by enzymatic hydrolysis method preserves its nutritional quality and results in improvements in some characteristics such as solubility and better absorption by the organisms. Hydrolysates with DH of 20% obtained with Pancreatin contained more lipids than hydrolysates obtained by LAB fermentation method. This could be due to formation of peptides with accessible hydrophobic regions, which are more lipid binding than those with hydrophobic regions embedded in the interior (Dauksas *et al.,* 2005). Low lipid content is an important factor for hydrolysate storage stability. The ash content was highest for alkaline method than the other two methods.

Physical characteristics

Yield percentage

The yield of protein hydrolysate depended on the Degree of Hydrolysis (DH) of the shell proteins. The degree of hydrolysis 10%, yields (%) of protein hydrolysates 48.66±3.00%, 57.5±2.786% and 39.6±0.987% Alkaline, Enzymatic and Fermentation methods respectively. Similar results are produced Synowiecki *et al.* (1999) the best nitrogen recovery was 69.2% of that present in the protein fraction of C. crangon processing discards at DH about 30%. This yield was achieved after 4 h reaction at 55°C using an enzyme concentration 20 AU/kg of protein. The degree of hydrolysis 20%, yields (%) of protein hydrolysates 57.283±0.345% 36.826±0.556% enzymatic and fermentation methods respectively. The observed relationship between the protein hydrolysates yield and DH values is in a good agreement with the for protein hydrolysates from bovine blood. The final yield of the hydro- lysate was reduced by partial extraction of the proteins during preliminary demineralization of the shells. The degree of hydrolysis 30%, yields (%) of protein hydrolysates 61.126±0.205%, 37.33±0.574% Enzymatic and Fermentation methods respectively. Above comments on concluded to yield (%) is mainly dependent on degree of hydrolysis. Show the results in Table 7.

Table 7. Physical characteristics of shrimp waste protein hydrolysates

Methods	Yieldg/100gm of raw shrimp waste (dry weight basis)	pH	Water activity (A_W)
S1	48.66±3.00%	8.333±0.577	0.72±0.015
S2	57.5±2.786%	8.426±0.345	0.78±0.045
S3	39.6±0.987%	8.21±0.12	0.81±0.032

Amino acid profile present in the hydrolysate

Generally shrimp waste hydrolysate has a high content of essential amino acids indicating a high nutritional value for use as feed or as a nitrogen source in growth media for microorganisms (Gildberg and Stenberg, 2001). Table 15 shows the total amino acid profile of three different hydrolysates. The total content of amino acid in the hydrolysates recovered by alkaline, enzymatic and LAB fermentation method was 24.21, 51.35 and 35.93 gm per gm of dry weight of hydrolysate respectively. In both alkaline and fermentation method, tyrosine was the amino acid present in hydrolysate in superior quantity at 3.55 ±2.01 and 6.64 ±2.01 % respectively. As for the enzymatic method, glutamic acid was the most abundant amino acid measuring 5.25 ±3.21%. The total essential amino acids present in hydrolysate derived by alkaline, enzymatic and fermentative method were 11.52, 25.74 and 19.0 % respectively. The higher concentration of histidine was1.28 ±2.02 % (alkaline method), arginine 1.56 ±3.12 % (alkaline method), threonine 2.42 ±1.88 % (enzymatic method), tyrosine 6.64 ±2.01% (fermentative method), valine 2.95 ±1.88% (enzymatic method), methionine 1.12 ±2.12% (alkaline method), isoleucine 1.32 ±1.23% (alkaline method), leucine 4.25 ±7.56% (enzymatic method) and phenylalanine 2.37 ±4.07% (enzymatic method). The limiting essential amino acids in case of hydrolysate extracted by alkaline, enzymatic and fermentative method were leucine, methionine and phenyl alanin respectively. The essential amino acid of higher concentration in the three samples was tyrosine. Due to the high temperatures subjected to the hydrolysate, both in the process of spray drying and in the

Table 8. Amino acid composition of protein hydrolysates (gm per gm dry weight)

Amino acids	S1	S2	S3
Asp	2.34± 4.12	4.33 ±1.75	3.46 ±2.36
Glu	2.19 ±3.45	5.25 ±3.21	4.01 ±7.18
Ser	0.68 ±7.23	2.67 ±6.09	0.83 ±4.05
His	1.28 ±2.02	1.11 ±2.05	1.02 ±2.94
Gly	2.12 ±2.14	4.83 ±1.53	2.42 ±7.89
Thr	1.09 ±0.42	2.42 ±1.88	1.33 ±5.64
Ala	2.53 ±1.12	5.11 ±2.08	3.74 ±1.23
Pro	2.33 ±5.33	3.42 ±1.03	2.47 ±4.01
Tyr	3.55 ±2.01	3.22 ±4.56	6.64 ±2.01
Arg	1.56 ±3.12	3.25 ±3.52	2.35 ±1.03
Val	0.85 ±6.23	2.95 ±1.88	1.69 ±4.15
Met	1.12 ±2.12	2.12 ±2.56	1.64 ±1.98
Ile	1.32 ±1.23	2.45 ±8.26	1.83 ±6.22
Leu	0.11 ±1.45	4.25 ±7.56	1.18 ±4.28
Phe	0.94 ±1.34	2.37 ±4.07	1.02 ±3.14
∑ Amino acid total	24.21	51.35	35.93
∑ Essential amino acid	11.52	25.74	19.00

formation of a paste, there may be a loss of a portion of the amino acids in the samples (Abdul-Hamid et al., 2002).). In non-essential quantified amino acids, the highest concentration in the dry powder was the glutamic acid (5.25 ±3.21 &4.01 ±7.18 gm/g dry weight) and alanin (5.11 ±2.08 gm/g dry weight) for alkaline, enzymatic fermentative and alkaline method respectively. The amino acid in lower concentrations in the three hydrolysates was serine, with concentrations of 0.68 ±7.23, 2.67 ±6.09 and 0.83 ±4.05 g/g dry weight in sample 1, 2 and 3 respectively. The concentrations obtained were slightly lower than those reported earlier, where the shrimp by-products were treated with an enzyme hydrolysis. Ruttanapornvareesakul et al. (2006) have reported that the amino acid in higher concentration was glutamic acid and the limiting amino acid was methionine. Gildberg and Stenberg (2001) and Shahidi and Synowiecki (1991) have reported histidine as a limiting essential amino acid and leucine as the biggest concentration and in both the cases, the proteins were removed from shrimp by-products. Mizani et al. (2005) pointed histidine as the limiting amino acid and threonine as the more concentrated. Hydrolysate obtained by alkaline method showed overall result inferior than that of other two methods which may be due to hydrogen abstraction including racemization of L amino acid. Also disulphide bonds may get splitted with a loss of cystiene, serine and threonine due to β elimination reactions (Krause and Freimouth. 1985). Enzymatic method produced hydrolysate of superior quality in terms of amino acid, which can be attributed to low temperature accelerated hydrolysis minimizing undesirable reactions.

Statistical Analysis

All the parameters analyzed are in triplicate and the data are tabulated in a form of mean ± standard deviation using Descriptive statistics method through Excel (MS-Office) software. The changes in mean sensory scores of the Organoleptic parameters for the DWM, surimi prepared by Cryoprotectant I and II, between treatment and between storage days, during frozen storage were also statistically analyzed using two factor ANOVA (Analysis of variance) without replication technique through Excel (MS-Office) software.The results of all bio-chemical and physico-chemical parameters studied during frozen storage were statistically analyzed using two factor ANOVA without replication technique to find the significant difference or changes in a same parameter between storage period and between treatments.

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