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Characterization of Squid-Processing Byproduct Hydrolysate and Its Potential as Aquaculture Feed Ingredient

P. Z. LIAN,[†] C. M. LEE,*[,†] AND E. PARK[§]

Department of Nutrition and Food Sciences, University of Rhode Island, Kingston, Rhode Island 02881

The squid (*Loligo pealei*) byproduct composed of heads, viscera, skin, fins, and small tubes was subjected to hydrolysis at 55 °C and natural pH (6.8) using endogenous proteases. Squid hydrolysate was characterized during the course of hydrolysis for changes in the degree of hydrolysis, viscosity, electrophoretic pattern of proteins and peptides, and amino acid and fatty acid profiles. The change in viscosity can be used to monitor the progress of protein hydrolysis up to the molecular mass of 26.63 kDa. The 2 h hydrolysis resulted in a 2-fold increase in the total free amino acids and yielded hydrolysate with protein molecular mass of ≤45 kDa having feed attractability and good amino acid and fatty acid profiles with high contents of essential amino acids and fatty acids. Such hydrolysis-induced changes can make squid byproduct hydrolysate a good source of aquaculture feed ingredient, especially for a starter diet for larval fish.

KEYWORDS: Squid hydrolysate; SDS-PAGE; viscosity; degree of hydrolysis; amino acids; fatty acids

INTRODUCTION

In the northeastern United States, ∼19100 MT of squid (*Loligo pealei*) was landed annually from 1988 to 1997 (*1*) and processed into various squid products. It is estimated that ∼40% of the total body weight ends up as processing byproduct that is not utilized, causing a serious disposal problem. The major component in squid-processing byproduct (SPB) is protein in the range of 72−77% of the total solid mass. The squid protein fraction has shown growth-promoting and attractant properties in shrimp culture at levels as low as 1.5% due to the presence of unknown low molecular weight peptides (*2*) and chemo-attractant free amino acids and betaine (*3*). The level of protein in squid-processing byproduct is high enough for proteolytic hydrolysis for the generation of peptides and free amino acids. It also possesses most of the amino acids essential to the growth and survival of fish (*4*). Thus, the conversion of the processing byproduct into high-value aquaculture feed ingredients can be a viable approach to solving the waste disposal problems while simultaneously gaining economic returns.

One of the approaches for SPB conversion is to hydrolyze the proteins, the primary component, into smaller and more bioavailable materials, namely, peptides and free amino acids. This makes the product more digestible and could be conveniently formulated into a microdiet to be used as a starter feed. Furthermore, the released peptides and free amino acids could be potential chemoattractants as well as feeding stimulants to carnivorous species according to Carr (*5*) and Goddard (*6*). Hydrolysis is achieved with the aid of acid or enzymes. Acid hydrolysis causes the destruction of tryptophan and the formation of NaCl following the neutralization, which can make the product unpalatable, whereas enzymatic hydrolysis produces less undesirable byproducts (*7*). Goldhor and others (*8*) reported that acidified cod hydrolysates were less palatable than the control when semimoist diets were tested in Atlantic salmon. Fish silage is produced from fish-processing wastes by the autolysis through the addition of acids (*9*) or lactic acid fermentation to inhibit the growth of spoilage microorganisms (*10*). Although the product has good storability, nutritional components might be decomposed in the acid environment.

Some studies have revealed the presence of highly active proteases in squid muscle and viscera (*11*−*13*). The enzyme activity in squid muscle covered a wide pH range of 2.6−7.4, indicating the presence of both acid and alkaline proteases (*14*). According to the study of Pappas (*15*), the optimal pH and temperature of proteolytic activity from squid waste were found to be 5.0 and 55 °C, respectively. In our preliminary study, well-chopped squid-processing byproduct underwent a complete autolysis, leading to liquefaction with its own endogenous enzymes under the proper hydrolysis condition. This avoids the use of the costly commercial enzymes, making the digestion process economical. The enzymatic conversion of fish protein has been extensively studied according to a paper by Kristinsson and Rasco (*7*). However, no studies have been done on the enzymatic hydrolysis of SPB. The objective of this study was to characterize SPB for the changes in degree of hydrolysis (DH), viscosity, protein and peptide profiles, and free amino acid and fatty acid profiles during hydrolysis and their inter-relationships. The results would provide necessary information

* Author to whom correspondence should be addressed (e-mail uricml@uriacc.uri.edu).
[†] Department of Nutrition and Food Sciences.
[§] Department of Chemical Engineering.

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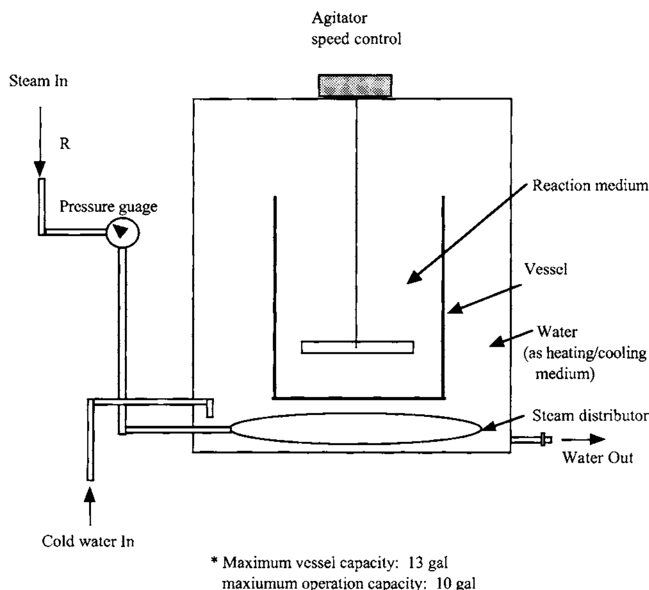

Figure 1. Setup of enzyme hydrolysis system with temperature and agitation speed controls.

for the commercial production of squid byproduct hydrolysate that can be used by the aquaculture feed industry.

MATERIALS AND METHODS

Materials. Fresh squid (*L. pealei*) processing byproduct consisting of heads, viscera, skin, fins, and small tubes were obtained from the local seafood-processing plants (Narragansett, RI). Standard protein and polypeptide markers were obtained from Bio-Rad Laboratories (Hercules, CA). The amino acid reference and C-21 internal standard were obtained from Sigma-Aldrich (St. Louis, MO), and the standard fatty acid methyl ester (FAME) mix was from AccuStandard (New Haven, CT).

Sample Preparation. The weight distribution of each part of squid byproduct was determined. For the preparation of squid hydrolysate, 15 kg of byproduct (collected in February 2002) was chopped in a Hobart meat chopper (model VCM 40) at a high speed for 2 min and put into a 13 gal stainless steel vessel (40 cm height and 40 cm diameter). The vessel was then placed in a 55 °C water bath and hydrolyzed under constant mixing at natural pH for various intervals. The water bath was set up in a laboratory retort (Dixie Canner Equipment Co., Athens, GA) by filling the chamber to an appropriate level with water. The temperature of the reaction medium (squid homogenate) was regulated by the water, the temperature of which was controlled by steam injection (**Figure 1**). Mixing was carried out using a Stir-Pak mixer (Cole-Parmer Instrument, Vernon Hills, IL) fitted with a dual cross blade propeller (18.5 cm × 4 cm). The temperature was chosen based on the study of Pappas (*15*). Approximately 1 kg of samples was taken after 0, 30, 60, 90, 120, 180, 240, and 300 min of hydrolysis and analyzed for the DH, viscosity, electrophoretic patterns of proteins and peptides, and amino acid and fatty acid profiles.

Proximate Composition Analysis. AOAC methods (938.08 and 950.46B) (*16*) were followed to analyze ash and moisture contents. Lipid was determined by the solvent extraction method using chloroform/methanol (1:1) instead of (2:1) (*17*) in consideration of squid lipid being high in phospholipids (*18*). The protein content was determined by subtracting ash and lipid from solids because the subtraction values came out close to those by the Kjeldahl nitrogen analysis, where the protein was computed as N × 6.25. The Kjeldahl analysis was done using the Kjeltec System 1002 (Tecator, Höganäs, Sweden). The degree of closeness was determined from a set of 20 samples, where the percent mean difference [= Kjeldahl − subtraction values)(100)/Kjeldahl values] came out to be in the range of 0−0.09%. This indicates the negligible amount of carbohydrate present in the SPB.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). Protein and peptide profiles of squid hydrolysate were determined by employing SDS-PAGE. Squid hydrolysate (1 g) was homogenized for 1.5 min with 19 mL of extraction solution (2.5% SDS−5 mM Na_2EDTA−1% *β*-mercaptoethanol−100 mM Tris/glycine buffer at pH 8.8) in an Omni-mixer (Type DM, Ivan Sorvall, Inc., Norwalk, CT). The mixtures were heated at 95 °C for 10 min to inactivate proteolytic enzymes and centrifuged for 20 min at 18000*g* (type SS-4 centrifuge with SS-34 rotor; Ivan Sorvall Inc.). The supernatant (0.5 mL) was taken and mixed with 0.5 mL of sample diluent in a 2 mL vial and used for the SDS-PAGE analysis. Protein molecules were separated using 12% polyacrylamide gels and a 4% stacking gel overlay according to the procedure of Laemmli (*19*) in a Mini-Protean II system (Bio-Rad). A 200 V constant voltage setting was used until the bromophenol blue reached the front of the gel (∼45 min). The molecular masses of the main proteins in the sample were estimated by comparison with standard protein markers including trypsin inhibitor (21.25 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), bovine albumin (66.2 kDa), phosphorylase *b* (97.4 kDa), and *β*-galactosidase (116.25 kDa).

Table 1. Weight Distribution of Squid Byproducts from Different Processing Plants

plant	heads + arms	fins	funnels	tubes	viscera	pens
A	42.80	25.27	12.87	8.00	6.10	1.60
B	52.40		29.50		10.80	0.30

Viscosity. Viscosity was measured at 25 °C using a Bohlin CVO rheometer (Bohlin Instruments Inc., East Brunswick, NJ) equipped with a C25 concentric cylinder measuring system. After preshearing of the hydrolysate (13.50 g) for 10 s at a shear rate of 60 s^{-1}, viscosity was measured at the same shear rate.

Degree of Hydrolysis. The DH was determined following the method of Nielsen et al. (*20*). The *o*-phthaldialdehyde (OPA) reagent was prepared as follows: 9.550 g of disodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) and 250 mg SDS were dissolved in 187.5 mL of deionized water and then mixed with 200 mg of OPA (97% OPA predissolved in 5 mL of ethanol) and 220 mg of 99% dithiothreitol (DTT)). The final solution was made up to 250 mL with deionized water. Sample (1 g of hydrolysate) was mixed with 19 mL of 4% SDS solution and heated immediately at 95 °C for 10 min to inactivate enzymes. Five milliliters of the resulting solution was centrifuged at 920*g* for 10 min using a Dynac centrifuge (Becton, Dickison and Co., Parsippany, NJ), and 4 mL of supernatant was diluted to 50 mL with deionized water. To measure the absorbance, 3 mL of OPA reagents was added to 15 mL tubes and then 400 *μ*L of serine standard (50 mg of serine diluted in 500 mL of deionized water) or sample solution was added, using four tubes for each sample, standard and blank. The mixture was mixed for 5 s and held for exactly 2 min before being read at 340 nm (Lambda 4B UV/VIS spectrophotometer, Perkin-Elmer Instruments, Norwalk, CT). Blanks were prepared with 400 *μ*L of deionized water and tested as described above. The means of the standards and blanks were used for calculation. The DH was calculated as

$$DH = h/h_{total} \times 100\%;\ h = (\text{serine-NH}_2 - \beta)/\alpha \text{ mequiv/g of protein}$$

where h is the number of hydrolyzed bonds and h_{total} is the total number of peptide bonds per protein equivalent; for fish protein $\alpha = 1.00$, $\beta = 0.40$, and $h_{total} = 8.6$ (*21*).

$$\text{serine-NH}_2 = (OD_{sample} - OD_{blank})/(OD_{standard} - OD_{blank}) \times 0.9516 \text{ mequiv/L} \times 0.05/(W \times V_2/V_1 \times P)$$

where serine-NH_2 = mequiv of serine NH_2/g of protein, W = g of sample weight, P = protein (%) in sample, V_1 = extraction volume, V_2 = volume of extraction solution for dilution, and 0.05 is the sample volume/L.

Total Amino Acid and Free Amino Acid Analyses. Total amino acid analysis followed the standard protocol established by Waters

Table 2. Proximate Compositions of Squid-Processing Byproduct and Hydrolysate[a]

sample	lipid (%)	moisture (%)	ash (%)	protein[b] (%)	protein (%) (solid wt basis)
plant A					
Dec 2001, squid byproduct	2.30 ± 0.04	85.30 ± 0.07	1.80 ± 0.11	10.60	72.11
Feb 2002, squid byproduct	1.82 ± 0.08	86.70 ± 0.11	1.21 ± 0.06	10.27	77.22
April 2002, squid byproduct	2.23 ± 0.07	86.51 ± 0.04	1.26 ± 0.08	10.00	74.13
April 2002, whole squid	2.17 ± 0.03	81.49 ± 0.07	1.90 ± 0.05	14.44	78.01
plant B					
squid byproduct	2.87 ± 0.15	81.20 ± 0.05	1.76 ± 0.05	14.10	75.00
plant A					
Feb 2002, hydrolysate	1.78 ± 0.15	87.05 ± 0.03	1.03 ± 0.01	10.76	83.09

[a] Values are means and standard deviations ($n = 2$). [b] Protein content (%) = 100 − % moisture − % lipid − % ash.

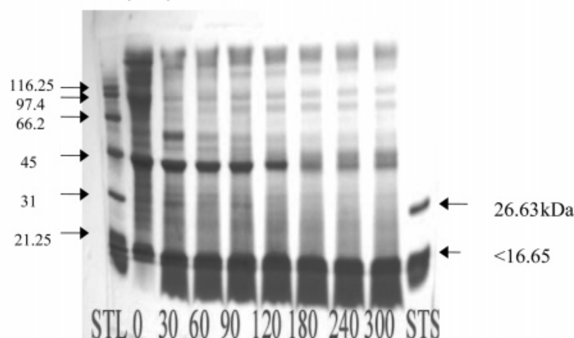

Figure 2. SDS-PAGE profiles of squid byproduct during autolysis. STL, larger molecular weight marker; STS, small molecular weight marker (Sigma Chemical Co. and Bio-Rad Laboratories); 0, 30, 60, 90, 120, 180, 240, and 300, hydrolysis time (min); gel, 12% T with 3% C running gel, 4% T stacking gel, at 200 constant volts for ~45 min; stain, 30 min with 0.1% Coomassie blue R-250 in fixative (40% MeOH–10% HAc); destain, 40% MeOH–10% HAc.

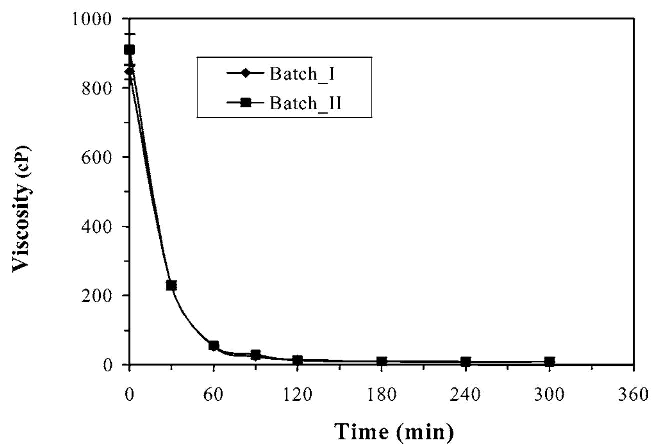

Figure 3. Changes in the viscosity of squid hydrolysate with hydrolysis time.

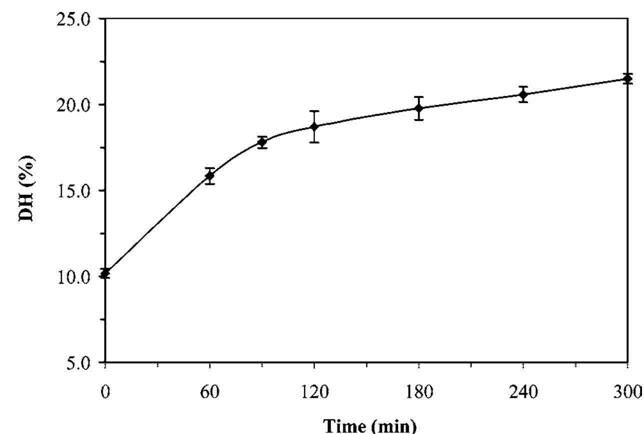

Figure 4. Changes in the degree of hydrolysis of squid-processing byproduct with time.

(Milford, MA) (*21*). Ten microliters of 100× diluted squid hydrolysate was vacuum-dried in a 6 mm × 50 mm tube, hydrolyzed with 6 N HCl vapor in a sealed vacuum vial at 112 °C for 24 h in a PICO.TAG workstation (Millipore Corp., Milford, MA), vacuum-dried again, mixed with 15 μL of methanol/water/ trimethylamine (2:2:1 by volume), and redried. This was followed by derivatization with 20 μL of methanol/ triethylamine/water/phenylisothiocyanate solution (7:1:1:1 by volume) and dilution with 200 μL of PICO.TAG sample diluent solution. After filtration through a 0.2 μm pore size PVDF syringe filter (Whatman Inc., Clifton, NJ), amino acids were separated on a reversed phase PICO.TAG column (3.9 mm × 15 cm) using a HPLC (series 200, Perkin-Elmer, Shelton, CT) fitted with an UV-absorbance detector (model 228, ISCO Inc., Lincoln, NE).

For the free amino acid analysis, squid hydrolysate (5 g) was homogenized with 10 mL of 7.5% TCA for 1 min and then centrifuged at 10000*g* (type SS-4 centrifuge with SS-34 rotor; Ivan Sorvall Inc.) for 10 min. The supernatant (10 μL) was subjected to the drying, derivatization, dilution, and separation in the same manner as described for total amino acid analysis.

Fatty Acid Analysis. Lipids were extracted in the same manner as described previously for the proximate analysis except for an ice water jacketed blending jar in place of an Eberbach jar. The chloroform layer with 1 mg of lipid was accurately transferred into a test tube, dried under a nitrogen stream in a 40 °C water bath, and then mixed with 1 mL of methylene chloride ($MeCl_2$) with 50 μg of C_{21} as internal standard. Transmethylation was conducted by adding 3 mL of 5% HCl/ MeOH, sealing under nitrogen, and placing in aa 70 °C oven for 120 min. After cooling, 4 mL of 6% K_2CO_3 was added and vortex-stirred. The $MeCl_2$ layer was dried under N_2 in a 40 °C water bath, redissolved in 1 mL of $MeCl_2$, and filtered through a 2.5 μm filter into an amber GC vial. The vial was capped under N_2 for the analysis of FAMEs, which was performed with a Perkin-Elmer AutoSystem XL GC equipped with a flame ionization detector. The methyl esters were separated in a DB-Wax column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (J&W Scientific, Folsom, CA) under the following operation conditions: injection, 2.0 μL; injector temperature, 250 °C; detector temperature, 300 °C; flow rate of carrier gas He, 20 mL/min; oven temperature, 50 °C, held for 2 min following injection; ramp, 40 °C/ min to 200 °C, held for 16 min, 210 °C, held for 11 min, and 220 °C, held for 10 min. The relative content of each fatty acid methyl ester is reported as percent peak area of total fatty acid methyl esters using the FAME quantitative standard mix and C_{21} internal standard (Accu-Standard, New Haven, CT).

RESULTS AND DISCUSSION

Proximate Composition of Squid-Processing Byproduct and Hydrolysate. The weight distribution and composition of SPBs varied with the seafood-processing plants. As for the

Table 3. Total Amino Acids and Changes in Free Amino Acids of Squid Byproduct during Hydrolysis

amino acid	hydrolysate wt (%)	per protein wt (%)	free amino acids (mg/g of hydrolysate[e]) at hydrolysis time of 0 min	30 min	60 min	90 min	120 min	150 min	180 min	% change
Asp	1.03	9.46	1.17	2.77	2.75	3.55	3.83	4.02	4.71	301.95
Glu	1.38	12.63	1.79	4.57	4.86	5.68	6.14	6.48	7.07	296.02
Ser	0.34	4.01	0.54	1.01	1.03	1.14	1.29	1.35	1.49	175.41
Gly	0.59	4.01	0.75	1.67	2.06	2.22	2.52	2.61	2.82	275.44
His*	0.16	1.45	0.28	0.51	0.53	0.55	0.61	0.63	0.67	137.74
Arg*	1.1	10.02	6.26	7.21	7.07	7.39	7.84	8.48	8.63	37.89
Thr*	0.33	2.97	0.64	1.36	1.79	1.92	2.19	2.33	2.54	297.73
Ala	0.54	4.96	0.88	1.86	1.92	2.16	2.40	2.48	2.73	210.01
Pro	0.47	4.29	1.40	2.21	2.57	2.60	2.88	3.03	3.01	114.66
Tyr	0.34	3.11	0.68	0.81	0.99	1.11	1.27	1.31	1.47	114.86
Val*	0.45	4.08	0.45	1.08	1.13	1.29	1.48	1.50	1.73	284.84
Met*	0.36	3.32	0.04	0.13	0.17	0.21	0.22	0.21	0.23	456.51
Cys2	0.05	0.49		0.01	0.02	0.00	0.01	0.01	0.00	
Ile*	0.42	3.87	0.34	0.91	1.01	1.04	1.23	1.21	1.45	326.03
Leu*	0.82	7.47	0.47	1.80	1.93	2.14	2.43	2.41	3.04	541.15
Phe*	0.41	3.73	0.41	0.80	1.44	1.45	1.79	1.79	2.11	420.43
Lys*	0.66	6.07	0.88	1.64	1.94	1.66	1.95	2.41	2.84	222.94
total FAA[a] (mg/g)			16.99	30.36	33.19	36.11	40.08	42.26	46.55	
fold[b]			1.00	1.79	1.95	2.13	2.36	2.49	2.74	
EAA[c] (mg/g)			9.77	15.45	17.00	17.65	19.74	20.97	23.25	
fold[d]			1.00	1.58	1.74	1.81	2.02	2.15	2.38	

[a] Total free amino acid contents. [b] Increase of total free amino acids at certain time of hydrolysate divided by the free amino acid contents at 0 min. [c] Essential amino acids for fish feed. [d] Increase of essential amino acids at certain time of hydrolysis divided by the essential amino acid content at 0 min. [e] Hydrolysate: 86.7% moisture.

weight distribution (**Table 1**), the byproducts from plant A consisted of largely heads and arms and fins, followed by funnels, tubes, and viscera. However, in the byproduct from plant B, the major fraction was heads and arms, followed by funnels and viscera; fins and tubes were not present. As for proximate compositions, the sample from plant A had a higher moisture content (85.30−87.05%) than that from plant B (81.49%) (**Table 2**). The reason is believed to be due to the difference in the processing technique used. The former uses a water-jet machine to remove the skin, resulting in the extra moisture inclusion and disruption of viscera. In the latter, the head and viscera are manually removed, and the skin is removed by a rotating brush machine. No marked differences in lipid, protein, ash, and moisture contents in the same plant were observed with season. The viscera consisted of liver, kidney, gonads, gill, stomach, and intestine, having strong enzyme activities. The extremely active proteases were found in squid muscle, which was thought to be responsible for the rapid deterioration of quality in post-mortem tissue (*14*). LeBlanc and Gill (*11*) reported that cathepsins D and E were the major proteases recovered from *L. pealei*. These findings support the hydrolysis of squid byproduct through autolysis without the addition of exogenous enzymes.

The proximate composition of hydrolysate basically reflected that of byproduct (**Table 2**), although unhydrolyzed membranes and chitogenous pens were removed during filtration.

SDS-PAGE. Molecular mass distribution of squid hydrolysate during hydrolysis was determined by SDS-PAGE. Before hydrolysis, various molecular mass bands were observed in the SDS-soluble fraction using 12% gel (**Figure 2**). Molecular masses >116.25 kDa completely disappeared during 30 min of hydrolysis concomitantly with the appearance of a new band with a molecular mass between 66.2 and 45 kDa. There was a slight decrease in actin fraction (45 kDa) observed before 2 h of hydrolysis. After this point, however, the actin band gradually disappeared. No obvious new bands appeared between 45 and 21.25 kDa. The remaining main peptides of squid hydrolysate have molecular masses <21.25 kDa, indicating the formation of larger amounts of low molecular weight peptides and free amino acids.

Viscosity. The intermolecular protein interaction is believed to cause the change in viscosity. The initially viscous squid byproduct paste rapidly degraded into a free-flowing liquid upon 60 min of hydrolysis when the disappearance of molecular mass >45 kDa occurred as shown in **Figure 2**. The viscosity of hydrolysate decreased at an exponential rate up to 120 min of hydrolysis (**Figure 3**). This clearly reflects the electrophoretic pattern, where the fraction having a molecular mass >26.63 was further hydrolyzed to completion at 120 min with minimum viscosity (**Figure 2**). This suggests that proteolytic degradation of proteins can be monitored by measuring changes in viscosity. However, further hydrolysis did not cause a marked change in viscosity due to the formation of low molecular weight peptides.

Degree of Hydrolysis. The DH of squid byproduct during autolysis is shown in **Figure 4**. As expected, the DH increased with hydrolysis time. The rate of hydrolysis increased rapidly during the initial 60 min of hydrolysis and decreased thereafter. The shape of the hydrolysis curve was similar to those of shrimp (*22*) and fish protein (*23*) hydrolyses. The change in DH was inversely related to that in viscosity during the first 60 min (**Figure 4**), after which the DH showed a slight but steady increase with no changes in the viscosity observed.

Total Amino Acid and Free Amino Acid Profiles. The total amino acid profile and changes in free amino acid contents of the hydrolysate during the course of 3 h of hydrolysis are shown in **Table 3**. It has been well established that all teleost fish require 10 essential amino acids (EAA), namely, arginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine, and valine, which should account for at least 50% of the total amount of available amino acids (*24*). The total EAA in squid hydrolysate was found to be 50.71% of the total amino acids when 1.22% of the tryptophan value in the water-extracted squid muscle protein was used in computation (*25*). The amount of total EAA present in water-

Table 4. Fatty Acid Profile of Lipid in Squid Hydrolysate[a]

fatty acid	%	fatty acid	%
14	1.35	20	0.05
15	0.29	20:1	2.44
15:1	0.00	20:3*n*3	1.49
16	17.73	20:4*n*6	0.14
16:1	0.59	20:5*n*3	11.16
17	0.60	22	0.05
17:1	0.20	22:1*n*9	0.24
18	2.75	22:6*n*3	24.45
18:1*n*9	1.88		
18:2*n*6	0.19		
18:3*n*3	0.08		
identified fatty acids (%)	65.69		
EPA + DHA	35.61		

[a] Contained 1.78% lipid and 87% moisture.

extracted squid muscle protein was 53.41%. This indicates that squid byproduct hydrolysate can be a good source of high-quality protein. With the progression of hydrolysis, the large molecules of proteins degraded gradually into small peptides and free amino acids. This resulted in a 2-fold increase in free amino acids during the initial 60 min of hydrolysis. All individual free amino acids also increased at different levels during hydrolysis. Obviously, this is attributed to the work of proteolytic enzymes.

In general, carnivorous species show a positive response to alkaline and neutral substances, such as glycine, proline, valine, taurine, and betaine (*6*). The squid muscle is rich in taurine and betaine, 97−481 and 671−1010 mg/100 g, respectively (*13*). According to studies by Pawson (*26*) and Ellingsen and Døving (*27*), the glycine and alanine are the most potent attractant amino acids for cod. Takaoka et al. (*3*) reported that the mixture of serine, aspartic acid, glycine, and alanine plus betaine showed a markedly high feeding stimulant activity for tiger puffer (*Takifugu rubripes*). Attractant amino acids, such as glycine and alanine, increased by 236.07 and 172.89%, respectively, upon 2 h of hydrolysis. The chemoattractant properties of squid hydrolysate were confirmed in our previous study using trout fingerlings (*28*). The study showed that the 2 h hydrolysis sample exhibited stronger attraction than that without hydrolysis or 3 h of hydrolysis. This indicates that over-hydrolysis may have weakened the attractability by either reduction of attracting compounds or formation of counterattracting ones.

Fatty Acid Profile. The fatty acid profile (**Table 4**) shows that the oil fraction (1.78% of hydrolysate) contained 11.16% EPA and 24.45% DHA, indicating that there is twice as much DHA as EPA. The concentrations of EPA and DHA appear to be higher than those found in salmon oil (8.65% EPA and 10.67% DHA, our unpublished data). For larval fish nutrition, it is desirable to have a higher level of DHA delivered in the form of phospholipids rather than triacylgycerols (*29*). The majority of lipid class present in squid is reported to be phospholipids (*18*).

Conclusions. Fish attractants can be produced by proper hydrolysis of squid processing byproduct without the addition of commercial enzymes. There were clear relationships among DH, viscosity, electrophoretic pattern of proteins and peptides, and amino acid profiles of squid hydrolysate. Changes in viscosity can be used to monitor the progress of hydrolysis up to the point when molecules >26.63 kDa are no longer present. During the course of hydrolysis, all free amino acids increased to varying extents due to the wide presence of proteolytic enzymes. Over-hydrolysis may reduce the attractant properties of hydrolysate by reduction of attracting compounds or the formation of counterattracting ones. In view of the good amino acid and fatty acid profiles with high contents of essential amino acids and fatty acids (DHA and EPA), the squid byproduct hydrolysate can be used as an aquaculture feed ingredient, especially for a starter diet for larval fish, which require good protein and lipid nutrition, digestibility, and feeding stimulant.

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