Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

GONZÁLEZ-FÉLIX, Mayra Lizett; PEREZ-VELAZQUEZ, Martin; EZQUERRA-BRAUER, Josafat Marina; Bringas-ALVARADO, Lorena; SÁNCHEZ-SÁNCHEZ, Anabel; TORRES-ARREOLA, Wilfrido

Evaluation of jumbo squid (Dosidicus gigas) byproduct hydrolysates obtained by acid-enzymatic hydrolysis and by autohydrolysis in practical diets for Pacific white shrimp (Litopenaeus vannamei)

Ciência e Tecnologia de Alimentos, vol. 34, núm. 3, julio-septiembre, 2014, pp. 552-558
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

DOI: http://dx.doi.org/10.1590/1678-457X.6414

Evaluation of jumbo squid (*Dosidicus gigas*) byproduct hydrolysates obtained by acid-enzymatic hydrolysis and by autohydrolysis in practical diets for Pacific white shrimp (*Litopenaeus vannamei*)

Mayra Lizett GONZÁLEZ-FÉLIX[1]*, Martin PEREZ-VELAZQUEZ[1], Josafat Marina EZQUERRA-BRAUER[2], Lorena Bringas-ALVARADO[1], Anabel SÁNCHEZ-SÁNCHEZ[2], Wilfrido TORRES-ARREOLA[2]

Abstract

The marine bioprocessing industry offers great potential to utilize byproducts for fish meal replacement in aquafeeds. Jumbo squid is an important fishery commodity in Mexico, but only the mantle is marketed. Head, fins, guts and tentacles are discarded in spite of being protein-rich byproducts. This study evaluated the use of two jumbo squid byproduct hydrolysates obtained by acid-enzymatic hydrolysis (AEH) and by autohydrolysis (AH) as ingredients in practical diets for shrimp. The hydrolysates were included at levels of 2.5 and 5.0% of the diet dry weight in four practical diets, including a control diet without hydrolysate. Shrimp growth and survival were not significantly affected by the dietary treatments. Postharvest quality of abdominal muscle was evaluated in terms of proximate composition and sensory evaluation. Significantly higher crude protein was observed in the muscle of shrimp fed the highest hydrolysate levels, AH 5% (204.8 g kg^{-1}) or AEH 5% (201.3 g kg^{-1}). Sensory analysis of cooked muscle showed significant differences for all variables evaluated: color, odor, flavor, and firmness. It was concluded that Jumbo squid byproducts can be successfully processed by autohydrolysis or acid-enzymatic hydrolysis, and that up to 5.0% of the hydrolysates can be incorporated into shrimp diets without affecting growth or survival.

Keywords: *Dosidicus gigas*; jumbo squid; byproduct hydrolysates; *Litopenaeus vannamei*; shrimp aquafeeds; fishmeal replacement.

1 Introduction

The replacement of fish meal in aquafeeds has been one of the most emphasized research goals at the turn of the century. The marine bioprocessing industry offers great potential to convert and utilize byproducts for such purpose. Humboldt squid, *Dosidicus gigas*, also known as jumbo squid, was among the five most important fishery commodities produced in Mexico by volume from 2006 to 2011(Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2011); however, only the mantle is marketed, whereas the head, fins, guts, and tentacles are discarded, in spite of being protein-rich byproducts (Lin & Ba-Fang, 2006).

Protein hydrolysates, consisting of small peptides and amino acids, may be obtained through thermal, chemical, or enzymatic modification of proteins or combinations of these processes. Enzymatic hydrolysis of byproducts is a widely used process (Liaset et al., 2000; Jae-Young et al., 2007; Nielsang et al., 2005; Baek & Cadwallader, 1995) that can modify or improve the properties of proteins, for instance, their digestibility (Panyam & Kilara, 1996). Nevertheless, endogenous enzymes already present in byproducts may induce autohydrolysis. In squid muscle and gut, a high endogenous proteolytic activity has been reported (Leblanc & Gill, 1982; Rodger et al., 1984; Sugiyama et al., 1989), and when chopped, the tissues may be hydrolyzed rendering a hydrolysate rich in free essential amino acids that could potentially be used as a feed ingredient for aquafeeds (Lian et al., 2005).

Shrimp aquaculture in Mexico is an established economic activity that can profit from reduced feed costs. Replacing fish meal in aquafeeds with inexpensive protein sources, such as unexploited byproducts that are locally or regionally available, may be a great opportunity for a sustainable utilization of resources. Tacon & Metian (2008) estimated that the global fish meal use and demand will decline from 1,008.6 thousand tonnes of fish meal used in compound aquafeeds for shrimp (including *Litopenaeus vannamei*, *L. stylirostris, Penaeus monodon*, *Marsopenaeus japonicus*, *Fenneropenaeus chinensis*, *F. indicus*, *F. merguiensis*, *Metapenaeus ensis*, etc.) in 2007 to 790.0 thousand tonnes in 2015 and 616.7 thousand tonnes in 2020. The reason why fish meal use is expected to decline is due to the decreasing market availability of fish from capture fisheries, which would in turn increase market cost for this limited commodity. Evidently, this would increase the global use of cheaper plant and animal alternative protein sources; thus, squid hydrolysates may be a convenient and acceptable alternative for fish meal replacement in shrimp aquafeeds, provided that they produce adequate growth performance. In such an event, replacement levels would need to be determined.

Fish hydrolysates derived from byproducts of the Alaskan fishing industry have been evaluated as replacement for Menhaden fish meal in *L. vannamei* diets (Forster et al., 2011). The hydrolysates replaced 50% of Menhaden fish meal included at 13% in the control diet on an isonitrogenous basis and were fed to shrimp cultured in an outdoor zero-water exchange

Received 18 June, 2014
Accepted 18 July, 2014 (006414)
[1]*Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora – UNISON, Hermosillo, Sonora, México, e-mail: mgonzale@dictus.uson.mx*
[2]*Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora – UNISON, Hermosillo, Sonora 83000, México*
**Corresponding author*

system for 8 weeks. The growth performance of shrimp fed two different hydrolysates (a liquid and acidified to pH 3.8 and the other acidified, neutralized to pH 6.5, and drum-dried) was not different from that of shrimp fed the control diet. The authors concluded that these two hydrolysates may replace 50% of the Menhaden fish meal. Other two hydrolysates evaluated (one acidified to pH 3.8 and drum-dried and the other hydrolyzed, but not acidified, and then drum-dried) induced lower final weight and growth of shrimp compared to animals fed the control diet. The authors suggested that the growth differences were mostly the result of differences in feed intake rather than the nutritional quality of the diets. In addition, a tuna byproduct protein hydrolysate (TBPH) was evaluated at 2.5, 5, 7.5, and 10% of a diet containing porcine meat meal as the main protein source to improve the quality and digestibility for *L. vannamei* (Hernández et al., 2011). Shrimp fed diets containing 5% TBPH showed significantly greater weight gain, better feed conversion ratio (FCR), and higher specific growth rate than that of shrimp fed greater levels of TBPH or the basal diet. The authors suggested that the hydrolysate at that level provided additional attractants and improved overall protein digestibility and amino acid profile of the diets resulting in improved growth performance of shrimp. An additional study on fish hydrolysate (Córdova-Murueta & García-Carreño, 2002) demonstrated that high dietary inclusions produced lower growth performance of *L. vannamei*, most likely due to the presence of large amounts of free amino acids and lower trypsin, chymotrypsin, and total proteolytic activity in shrimp fed those diets. At the same time, this study demonstrated that the use of squid meal prepared from dried jumbo squid mantle produced good growth performance of shrimp at a 3% inclusion level on a total crude protein basis although other studies have reported improvement of growth at slightly higher inclusion levels of squid meal (Sánchez et al., 2012; Cruz-Suárez et al., 1992). The objective of this study was to evaluate two jumbo squid byproduct hydrolysates obtained by acid-enzymatic hydrolysis and by autohydrolysis in practical diets for Pacific white shrimp, *L. vannamei*.

2 Materials and methods

2.1 Preparation of hydrolysates

Within 8 hours of capture, jumbo squids were transported on ice to the Seafood Laboratory of the Department of Food Research (DIPA) of the University of Sonora, Hermosillo, Sonora, Mexico. The head, tentacles, and skin were cut into small pieces and stored at –80°C until preparation of the hydrolysates.

Autohydrolysis. The head, tentacle, and skin pieces were homogenized for two minutes. The homogenate was then placed in a shaking water bath at 55°C and kept at a pH of 5.0 (Lian et al., 2005) for 2 h to allow the autohydrolysis process to take place, indicated by a pH drop to 4.1 with no further drop.

Acid-enzymatic hydrolysis. For this homogenate, a mixture of acid (HCl) and enzyme, specifically pepsin, was used according to the methods described by Lin & Ba-fang (2006). Firstly, 500 mL of a 50 mM phosphate buffer (pH 7.5) were used to homogenize the squid tissues, followed by a pH adjustment to 2.5 with HCl 3M. The homogenate was then placed in a shaking water bath at 45°C, and 5 mL of pepsin (Faga Lab, Mocorito, Sin., Mexico) solution (8 mg/mL) were added. The products were obtained after allowing the reaction to take place for 20 min at 45°C, indicated by a rise in pH from 2.5 to 2.9.

For both types of hydrolysis, pH drop or rise was monitored continuously. The reaction was stopped, after confirming that the pH values were no longer dropping or increasing, by placing the sample at 100°C for 10 min. Both hydrolysates were lyophilized (LabConco FreezeDryer 3, Kansas, MO, USA) at –50°C and 10 mBar of pressure for 72 h until dry, followed by characterization. The degree of hydrolysis (DH, %) of each hydrolysate was determined using the OPA (o-phthaldialdehyde) method (Nielsen et al., 2001) with serine as a standard; the soluble protein content (SPC, mg mL^{-1}) was determined using the Bradford method (Bradford, 1976) with bovine serum albumin (1 mg mL^{-1}) as a standard; crude protein, crude fat, moisture, and ash (all expressed as g kg^{-1}) were determined using AOAC standard methods (Association of Official Analytical Chemists, 2005), and the amino acid profile (% of protein) was determined by reverse-phase HPLC (Hewlett-Packard 1100, Waldbronn, Germany) using the method of Vázquez-Ortiz et al. (1995), and they were identified and quantified using an external standard of amino acids (Table 1).

Table 1. Degree of hydrolysis (DH: %), soluble protein content (SPC: mg mL^{-1}), proximate composition (g kg^{-1}), and amino acid (AA) profile (% of protein) of jumbo squid byproduct hydrolysates by autohydrolysis (AH) and acid-enzymatic hydrolysis (AEH)[1].

	AH	AEH
DH	21.1[a] ± 2.7	14.3[b] ± 1.4
SPC	0.93 ± 0.001	0.91 ± 0.005
Crude protein	876.1[a]	808.0[b]
Crude fat	2.1	2.0
Crude fiber	-	-
Ash	17.0[b]	110.2[a]
Moisture	104.8[a]	79.8[b]
NFE[2]	-	-
Histidine	2.2[a] ± 0.3	1.6[b] ± 0.4
Leucine	6.1[a] ± 1.1	3.8[b] ± 1.3
Isoleucine	3.0[a] ± 0.6	2.0[b] ± 0.5
Lysine	8.7[a] ± 0.5	5.2[b] ± 1.3
Methionine	2.7[a] ± 0.4	1.9[b] ± 0.7
Phenylalanine	2.8 ± 0.8	1.1 ± 0.4
Threonine	3.7 ± 1.4	3.0 ± 0.2
Valine	4.1[a] ± 0.8	2.7[b] ± 0.7
Alanine	1.2 ± 1.6	0.9 ± 0.4
Arginine	12.9[a] ± 1.8	6.8[b] ± 0.7
Serine	3.8 ± 0.7	2.7 ± 0.7
Tyrosine	7.7[a] ± 1.1	4.6[b] ± 1.3
Glycine	21.2[a] ± 1.9	12.9[b] ± 1.2
Aspartic acid	8.8 ± 1.8	9.7 ± 1.7
Glutamic acid	17.9 ± 1.8	19.6 ± 2.2
Taurine	2.5[b] ± 1.7	9.9[a] ± 1.6
Essential AA/Non-essential AA	0.7	0.4

[1]Values are means of 3 replicates ± S.D; Means within rows with different letters are significantly different (Duncan's alpha = 0.05); [2]NFE (Nitrogen Free Extract) = 100 – (% Crude protein+ % Crude fat + % Crude fiber + % Ash + % Moisture).

2.2 Diet preparation

Five practical diets containing 35% crude protein were tested in this trial. Sardine fish meal (FM) was included in the control diet at 10% on a dry weight basis, and it was replaced at two levels, 25 and 50% of FM with the autohydrolysis hydrolysate (AH) and the acid-enzymatic hydrolysate (AEH), corresponding to the inclusion levels of 2.5 and 5% of dry weight (AH 2.5%, AH 5%, AEH 2.5%, and AEH 5%) were evaluated. The ingredient composition and proximate composition of the experimental diets are shown in Table 2. Diets were prepared at the Nutrition Laboratory, Department of Scientific and Technological Research (DICTUS) of the University of Sonora by cold extrusion using a Hobart A-200 extruder (Hobart, Troy, OH) and dried at 45°C until reaching a moisture content of 5 to 10%. Prior to use, each diet was crumbled and sieved to an appropriate size and stored frozen (–20°C) until required. Proximate composition of the hydrolysates and diets was determined by a commercial laboratory (Analitica del Noroeste S.A. de C.V.) in Hermosillo, Sonora, Mexico.

2.3 Shrimp feeding experiment

A 35-day growth trial was conducted in an indoor recirculating system at the Wet Aquaculture Nutrition Laboratory of Kino Bay Experiment Station, University of Sonora. The experimental system consists of 100 circular polyethylene tanks (0.07 m^2 bottom area, 19 L total volume capacity) filled with 15 L of filtered seawater, a 1,100-L sump tank, a biofilter, a sand filter (Jacuzzi, Model L-190-7, Little Rock, AR, USA), a 1.5-HP pump (Jacuzzi, Model 150MF-T, Little Rock, AR, USA), a 20-µL pore size cartridge filter (Pentek, Model Big Blue PL5010BB, Sheboygan, WI, USA), a 120-W ultraviolet light chamber (Rainbow Lifeguard, Model UV97, El Monte, CA, USA), and a 1/2 HP in-line chiller (Aquatic Ecosystems, Model AE62B, Apopka, FL, USA). Aeration was provided by a 1.0-HP blower (Fuji, Model VFC40, Saddle Brook, NJ, USA). Seawater recirculation rate in each tank was 1.9 L min^{-1}, and 5–10% of the water volume was replaced daily with newly filtered seawater. Juvenile *L. vannamei* were obtained from the laboratory Maricultura del Pacifico (Kino Bay, Sonora, Mexico). Four shrimp with an average individual weight (± SD) of 1.17 ± 0.11 g were stocked into each tank at a stocking density of 56 shrimp per m^2. Each dietary treatment was randomly assigned to twenty replicate tanks, and the shrimp were manually fed the experimental diets twice a day (09:00 and 19:00 h). The feeding rate was adjusted to provide 4% of the body weight during the course of the study by weekly weighing the animals in five designated tanks per treatment. Uneaten feed and fecal wastes were removed daily before the next feeding. Daily measurements of temperature, salinity, and dissolved oxygen were performed using a multi-function oxygen meter (model Y85, YSI, Yellow Springs, Ohio, USA); the average values were 30.1 ± 1.2°C, 35.0 ± 1.7 g L^{-1} and 5.62 ± 0.29 mg L^{-1}, respectively. Ammonia, nitrite, nitrate, and pH were monitored weekly and

Table 2. Ingredient (g kg^{-1}) and proximate composition (g kg^{-1}) of experimental diets.

	Control	AH 2.5%	AH 5%	AEH 2.5%	AEH 5%
Sardine fish meal[a]	100.0	75.0	50.0	75.0	50.0
Soybean meal solvent extracted[b]	401.0	410.8	420.8	412.6	424.3
Corn gluten meal[c]	60.0	60.0	60.0	60.0	60.0
Squid Hidrolysate	-	12.5	25.0	12.5	25.0
Sardine fish oil[d]	30.0	31.0	32.0	31.0	31.9
Soybean oil[e]	10.5	11.8	13.2	11.8	13.3
Whole wheat[f]	332.0	332.4	332.5	330.6	329.0
Mineral premix[g]	5.0	5.0	5.0	5.0	5.0
Vitamin premix[h] without choline	18.0	18.0	18.0	18.0	18.0
Choline chloride[i]	2.0	2.0	2.0	2.0	2.0
Vitamin Stay C[j] 25%	1.0	1.0	1.0	1.0	1.0
CaP-dibasic[i]	30.0	30.0	30.0	30.0	30.0
Soy lecithin[k]	10.0	10.0	10.0	10.0	10.0
Cholesterol[i]	0.5	0.5	0.5	0.5	0.5
Crude protein	353.6	353.1	352.5	350.9	350.2
Crude fat	65.1	65.7	66.1	65.3	65.8
Crude fiber	13.6	11.6	11.4	13.3	14.2
Ash	81.6	77.9	79.2	85.1	87.7
Moisture	55.7	56.7	54.6	55.9	55.0
NFE[l]	430.4	435.0	436.2	429.5	427.1

[a]Conservera San Carlos S.A. de C.V., Puerto San Carlos, B.C.S., Mexico; [b]Consorcio Super, S.A. de C.V., Guadalajara, Jalisco, Mexico; [c]CPIngredientes, S.A. de C.V., Guadalajara, Jalisco, Mexico; [d]Productos Pesqueros de Guaymas, S.A de C.V., Guaymas, Sonora, Mexico; [e]Ragasa Industrias S.A. de C.V., Monterrey, Nuevo Leon, Mexico; [f]Molino La Fama, S.A de C.V. Hermosillo, Sonora, Mexico; [g]ICN, Aurora, Ohio, USA. g/100 g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428; [h]Fisher Scientific, Pittsburgh, Pennsylvania, USA. g/kg premix: thiamin HCl 0.5, riboflavin 3.0, pyrodoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D3 (400,000 IU/g) 0.002, dl-alpha-tocopheryl acetate (250 IU/g) 8.0, Alpha-cellulose 865.266; [i]Sigma-Aldrich Co., St. Louis, MO, USA; [j]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA; [k]Impulsora Golden, S.A. de C.V., D.F., Mexico; [l]NFE (Nitrogen Free Extract) = 100 – (% Crude protein+ % Crude fat + % Crude fiber + % Ash + % Moisture).

maintained within the ranges recommended for commercial production of shrimp (Samocha et al., 1993), with average values of 0.32 ± 0.09 mg NH_4-N L^{-1}, 0.46 ± 0.14 mg NO_2-N L^{-1}, 2.17 ± 0.63 mg NO_3-N L^{-1}, and 8.06 ± 0.13, respectively.

2.4 Postharvest evaluation of shrimp

At the end of the feeding trial, an evaluation of postharvest quality of shrimp was performed. The shrimp were transported on ice to the Seafood Laboratory of DIPA, University of Sonora. Ten shrimp from each treatment were sampled to determine the proximate composition, following AOAC standard methods (Association of Official Analytical Chemists, 2005). The heads were removed from the remaining harvested shrimp and stored at −80°C until used for sensory evaluation. Next, they were thawed in a refrigerator (4°C) and then cooked in boiling water for 5 min. An untrained group of 30 panelists assessed the color, odor, flavor, and firmness of the shrimp using a hedonic scale (Pedrero & Pongborn, 1996). The sensorial firmness of the cooked shrimp was evaluated on a scale of 1 to 7: (1) extremely soft, (2) very soft, (3) soft, (4) neither soft nor firm, (5) lesser firmness, (6) good firmness, and (7) excellent firmness. The color, odor, and flavor of the cooked shrimp were also evaluated on a scale of 1 to 7: (1) dislike very much, (2) dislike much, (3) dislike, (4) neither like nor dislike, (5) like slightly, (6) like much, and (7) like very much. In addition, the instrumental firmness was measured by recording the force required to penetrate the material using a texturometer (Model Chatillon 2-3b, Empire Scale Co., Santa Fe Springs, CA, USA) with a cylindrical plunger of 0.6 cm in diameter. The shearing direction was set perpendicular to the orientation of the muscle cells; the results were expressed in Newtons.

2.5 Statistical analyses

Final weight (g), instantaneous growth rate (IGR, % day^{-1}), survival (%), and feed conversion ratio (FCR) were the indices used to evaluate shrimp performance. Instrumental firmness (Newtons) and proximate composition (g kg^{-1}) of shrimp muscle were the indices used to evaluate postharvest shrimp quality. Survival was transformed by arcsine square root before statistical analysis. One-way ANOVA was used to evaluate the effect of dietary fish meal replacement with two jumbo squid byproduct hydrolysates on shrimp performance and postharvest shrimp quality variables. Duncan's multiple range test was used as the mean separation procedure ($P < 0.05$). Sensory evaluation data were analyzed by non-parametric Kruskal–Wallis test. Statistical analyses were performed using the SAS software package (Statistical Analisys System Institute, 1999-2000).

3 Results and discussion

The characterization of the hydrolysates (Table 1) demonstrated the AH hydrolysate had significantly higher DH (21.1%), as well as significantly higher crude protein (876.1 g kg^{-1}) and moisture (104.8 g kg^{-1}), as compared to those of the AEH hydrolysate (14.3%, 808.0 g kg^{-1} and 79.8 g kg^{-1}, respectively) although it showed significantly lower ash content (17.0 vs 110.2 g kg^{-1}). Thus, these data showed that a successful hydrolysis of jumbo squid byproduct obtained from mixed head, tentacles, and skin can be achieved using both methods. The differences in protein content may be partly explained by differences in the processes; the AH was left for 2 h at 55°C to allow for the autohydrolysis process to take place, whereas the AEH was left for 20 min at 45°C to allow for the hydrolyzation, in the presence of pepsin, to take place. The longer time period and higher temperature resulted in a more efficient hydrolyzation of the squid byproduct in the AH process. Furthermore, pepsin is an endopeptidase that breaks peptide bonds in the interior of a polypeptide chain, whereas in the autohydrolysis process, several proteolytic peptidases with different specificities work simultaneously (Stanley & Hultin, 1984), which could possibly explain not only the differences in the DH, but also the differences in the amino acid profile of the two hydrolysates.

Most of the amino acids analyzed were significantly higher in the AH hydrolysate compared to those of the AEH hydrolysate, some amino acids by between 40-50%, while others not by that much. Thus, the AH hydrolysate, in general, had more essential amino acids than the AEH hydrolysate. However, taurine was significantly higher in the AEH hydrolysate (9.9%) than in the AH hydrolysate (2.5%), an important attribute to be exploited in aquafeeds for marine carnivorous fish and shrimp in which fish meal is replaced by vegetable protein since taurine has proven to be an essential non-protein amino acid absent in terrestrial plants (Watson et al., 2013). In spite of this, the differences were minimized through the formulation by the inclusion of the other ingredients. The formulated amino acid profile of the experimental diets met the essential amino acid requirements of the National Research Council (National Research Council, 2011) for marine shrimp, *e.g.*, *Marsupenaeus japonicus* and *Penaeus monodon*, since for *L. vannamei* the only recommended amino acid inclusion is Lysine (1.6% on a dry-matter basis. The remaining essential amino acid requirements have not been tested or determined so far. The analysis of the amino acid profile of the experimental diets was not performed, but the lack of significant differences in shrimp biological performance supports the premise, albeit theoretically, that amino acid requirements of shrimp were met by all experimental diets.

It is important to draw attention to the fact that endogenous enzyme activity in squid byproduct proved to be sufficient to obtain a good quality hydrolysate, and that pepsin utilization under these conditions was less efficient than the other proteases already present in squid byproduct. The proximate characterization of the hydrolysates helped achieve accurate formulations of the experimental diets, which may be corroborated by their proximate composition (Table 2). The isoproteic and isolipidic experimental diets showed average crude protein and crude fat values of 352.1 and 65.5 g kg^{-1}, respectively. Crude fiber was slightly lower in the AH hydrolysate diets, whereas ash content was higher in the AEH hydrolysate diets.

With respect to the biological performance of shrimp, Forster et al. (2011) suggested that up to 50% of Menhaden fish meal included at 13% of *L. vannamei* diets could be replaced with hydrolysates (a liquid and acidified to pH 3.8 and the other

acidified, neutralized to pH 6.5, and drum-dried) derived from byproducts of the Alaskan fishing industry without affecting growth performance, which represents a dietary inclusion level of 6.5% of the hydrolysates in the diet. Hernández et al. (2011) demonstrated that shrimp fed diets containing 5% of TBPH had better growth performance than that of shrimp fed greater levels or the basal diet. In the present study, two levels of fish meal replacement, 2.5% and 5%, using two jumbo squid byproduct hydrolysates obtained by acid-enzymatic hydrolysis and by autohydrolysis in diets for *L. vannamei* were evaluated. No significant differences ($P < 0.05$) in the weight of shrimp were observed at the beginning of the trial (Table 3). At the end, the final weight ranged from 4.11 to 4.87 g, IGR from 3.01 to 3.63% day^{-1} and survival from 67.5 to 76.3%; these variables were not significantly affected by the dietary treatments (Table 3). Similarly to observations by Forster et al. (2011), no statistically significant adverse effects on growth performance were evident with the dietary inclusion levels of either hydrolysate. However, the trend in shrimp growth suggests that a higher replacement level of fish meal would probably result in decreased shrimp growth (Table 3), particularly in the case of the AH hydrolysate, as demonstrated by Hernández et al. (2011) in shrimp fed 7.5 or 10% of TBPH, or by Espe et al. (2012), who analyzed feeding increasing levels of hydrolyzed fish protein concentrate (3.75, 7.5, 11.25, and 15%) to replace fish meal in *Salmo salar* fed plant-protein-based diets.

FCR did not show significant differences between the treatments and ranged from 1.43 to 1.73; this last FCR value corresponded to shrimp fed the AH 5%, which resulted in the smallest shrimp (4.11 g) recorded at the end of the experiment. In their study, Forster et al. (2011) suggested that the growth differences in shrimp fed different hydrolysates were mostly the result of differences in feed intake, rather than the nutritional quality of the diets they were fed. The same consideration may apply here. Even though the characterization of the AH and the AEH jumbo squid hydrolysates exhibited differences (Table 1), they were compensated for or minimized through the formulation of the experimental diets and verified by their proximate composition, which showed general nutritional similarities and overall good quality. Consequently, detailed characterization of the hydrolysates helped improve and optimize the use of these products in the experimental diets, and it is valuable information for the aquafeeds industry although feed intake still needs to be enhanced.

On the other hand, among the postharvest variables evaluated, the proximate composition of shrimp muscle fed the experimental diets showed statistical differences (Table 4). Muscle of shrimp fed the AH 5% and AEH 5% diets had significantly higher content of crude protein (204.8 and 201.3 g kg^{-1}, respectively) and significantly lower crude fat (8.4 and 6.0 g kg^{-1}, respectively) as compared to those of the other treatments and the control treatment. Ash was significantly lower (12 g kg^{-1}) and moisture was significantly higher (813.7 g kg^{-1}) in shrimp muscle of the AH 2.5% treatment, but the rest of the treatments showed no statistical differences between each other for these variables (Table 4). Nevertheless, shrimp in the AH 5% and AEH 5% dietary treatments did not successfully outgrow animals in the other groups. Thus, including protein hydrolysates as a source of very digestible small peptides and amino acids may not necessarily guarantee a beneficial effect on shrimp growth.

The instrumental firmness of shrimp abdominal muscle in the animals fed diets with the two squid byproduct hydrolysates did not show statistical differences between each other or the control group (Table 4). It is known that firmness of meat depends on its myofibrillar proteins and collagen content, and that firmness is lost in muscle protein by the action of proteases such as collagenases during postmortem degradation (Jiang, 2000), particularly when postmortem handling and storage are not done properly (Sriket, 2014); moreover, reduced instrumental firmness of shrimp muscle has been reported when

Table 3. Individual, initial, and final weight; IGR; survival; and FCR of *L. vannamei* fed diets with two jumbo squid byproduct hydrolysates obtained by autohydrolysis (AH) and by acid-enzymatic hydrolysis (AEH)[1].

	Control	AH 2.5%	AH 5%	AEH 2.5%	AEH 5%	Pr >F
Initial weight (g)	1.19 ± 0.13	1.16 ± 0.10	1.15 ± 0.11	1.19 ± 0.13	1.19 ± 0.11	0.6920
Final weight (g)	4.87 ± 1.03	4.64 ± 0.88	4.11 ± 0.89	4.82 ± 1.08	4.69 ± 1.10	0.1325
IGR (% day^{-1})	3.63 ± 0.73	3.48 ± 0.68	3.01 ± 0.76	3.58 ± 0.78	3.48 ± 0.73	0.0666
Survival (%)	76.3 ± 17.2	68.8 ± 19.7	75.0 ± 21.5	67.5 ± 21.6	67.5 ± 21.6	0.5356
FCR	1.43 ± 0.30	1.49 ± 0.39	1.73 ± 0.45	1.47± 0.41	1.51 ± 0.34	0.1226

[1]Values are means of 20 replicates ± S.D; Means within rows with different letters are significantly different (Duncan's alpha = 0.05).

Table 4. Instrumental firmness (Newtons) and proximate composition (g kg^{-1}) of *L. vannamei* muscle fed diets with two jumbo squid byproduct hydrolysates obtained by autohydrolysis (AH) and by acid-enzymatic hydrolysis (AEH)[1].

	Control	AH 2.5%	AH 5%	AEH 2.5%	AEH 5%	Pr >F
Instrumental firmness	2.2 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	0.870
Crude protein	189.5^{b} ± 1.0	154.5^{c} ± 5.1	204.8^{a} ± 2.1	185.2^{b} ± 3.8	201.3^{a} ± 12.7	<0.0001
Crude fat	20.2^{b} ± 0.9	19.9^{b} ± 4.7	8.4^{c} ± 2.9	32.3^{a} ± 2.9	6.0^{c} ± 0.9	<0.0001
Ash	16.8^{a} ± 0.2	12.0^{b} ± 0.2	16.7^{a} ± 0.2	17.1^{a} ± 0.4	16.8^{a} ± 1.0	<0.0001
Moisture	773.4^{b} ± 0.6	813.7^{a} ± 0.8	770.1^{b} ± 1.5	765.4^{b} ± 2.0	776.0^{b} ± 14.2	<0.0001

[1]Values are means of 10 replicates (for instrumental firmness) or 3 replicates (for proximate composition) ± S.D; Means within rows with different letters are significantly different (Duncan's alpha = 0.05).

Table 5. Sensory analysis of *L. vannamei* cooked abdominal muscle from animals fed diets with two jumbo squid byproduct hydrolysates obtained by autohydrolysis (AH) and by acid-enzymatic hydrolysis (AEH)[1].

Experimental diet	Control	AH 2.5%	AH 5%	AEH 2.5%	AEH 5%	Prob>ChiSq
Color	$4.8^{a} \pm 1.9$	$5.0^{a} \pm 1.7$	$4.6^{b} \pm 1.7$	$4.7^{ab} \pm 1.6$	$5.0^{a} \pm 1.9$	0.0029
Odor	$5.3^{b} \pm 1.6$	$5.4^{b} \pm 1.6$	$5.8^{a} \pm 1.4$	$4.7^{c} \pm 1.8$	$5.8^{a} \pm 1.5$	<0.0001
Flavor	$5.3^{c} \pm 1.9$	$4.9^{d} \pm 1.9$	$5.8^{b} \pm 1.4$	$4.4^{e} \pm 1.9$	$6.1^{a} \pm 1.3$	<0.0001
Sensorial firmness	$6.0^{b} \pm 1.6$	$5.9^{b} \pm 1.8$	$6.3^{a} \pm 1.5$	$5.6^{c} \pm 1.6$	$6.4^{a} \pm 1.2$	<0.0001

[1]Values are means of 30 replicates ± S.D. Means within rows with different letters are significantly different (P>0.05); Color, odor, and flavor scored from 1 (dislike very much) to 7 (like very much). Sensorial firmness scored from 1 (extremely soft) to 7 (excellent firmness).

exposed to fumonisin B_1-containing feed (García-Morales et al., 2013). None of the above conditions were present in this study, which could explain the absence of differences in shrimp muscle as measured by instrumental firmness. However, the sensory analysis of cooked muscle showed significant differences for all variables evaluated (Table 5). In this case, sensorial firmness differed from instrumental firmness; the panelists perceived significantly more firmness in cooked muscle of shrimp fed either one of the hydrolysates at the highest inclusion level, AH 5%, and AEH 5%, as well as more odor and flavor. According to Albrektsen (2009), by adding the amino acid hydroxyproline to fish feed, a major constituent of collagen that promotes its stability, it is possible to achieve a higher level of muscle firmness in salmon by stimulated collagen production and increased strength in the connective tissue, rendering better meat quality and product properties that could increase profitability of the aquaculture industry. Since AH 5% and AEH 5% diets had higher inclusion levels of the hydrolysates, potentially, more free amino acids, including proline, the precursor of hydroxyproline, could have been available to incorporate into the muscle of growing shrimp, resulting in significantly higher firmness (6.3 and 6.4, respectively) of cooked shrimp according to the panelists, as well as better flavor (5.8 and 6.1, respectively), and odor (5.8 for both) of animals fed these experimental diets. Nevertheless, proline or hydroxyproline were not quantified in the hydrolysates since they are not essential amino acids and they were of no particular interest at that moment. Both total collagen content and soluble collagen content (based on hydroxyproline content) have been used to explain stability of cooked salmon fillet and chicken breast (Kong et al., 2008). Higher collagen content and collagen cross-linkages are usually correlated with tougher meat (Tornberg, 1996). Therefore, hydroxyproline and collagen quantification is something that needs to be pursued in future research when sensory analysis of cooked shrimp muscle is analyzed to establish if firmness can be correlated to these variables.

4 Conclusions

Under the conditions prevailing in this study, it can be concluded that hydrolysates from byproduct of jumbo squid (head, tentacles, and skin) can be successfully produced by either autohydrolysis or acid-enzymatic hydrolysis, and they can potentially be utilized to partially replace sardine fishmeal in the aquafeeds industry. In *L. vannamei* diets, a 5% level of inclusion of either hydrolysate is recommended. Although including the squid hydrolysates as a source of very digestible small peptides and amino acids may not necessarily guarantee a beneficial effect on growth of shrimp, it may increase the content of crude protein in shrimp muscle and improve muscle firmness, flavor, and odor, as perceived by consumers.

Acknowledgements

The authors would like to express their appreciation for the assistance provided by the staff at Kino Bay Experiment Station in Kino Bay, Sonora, Mexico. The soybean meal and vitamin Stay C 25% were kindly donated by Consorcio Super, S.A. de C.V., Guadalajara, Jalisco, Mexico. Mention of trademarks or proprietary products does not constitute an endorsement of the products by the University of Sonora and does not imply its approval to the exclusion of other products that may also be suitable.

References

Albrektsen, S. (2014). *Discovery increases muscle firmness*. Tromsø: Nofima, Food Research Institute. Retrieved from http://www.nofima.no/en/nyhet/2009/08/discovery-increases-muscle-firmness

Association of Official Analytical Chemists - AOAC. (2005). *International Official Methods of Analysis* (17th ed). Washington.

Baek, H. H., & Cadwallader, K. R. (1995). Enzymatic hydrolysis of crayfish processing by-products. *Journal of Food Science*, *60*(5), 929-935. http://dx.doi.org/10.1111/j.1365-2621.1995.tb06264.x

Bradford, M. M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Córdova-Murueta, J. H., & García-Carreño, F. L. (2002). Nutritive value of squid and hydrolyzed protein supplement in shrimp feed. *Aquaculture*, *210*(1-4), 371-384. http://dx.doi.org/10.1016/S0044-8486(02)00011-X

Cruz-Suárez, L. E., Ricque, D., & AQUACOP. (1992). Effect of squid meal on growth of *Penaues monodon* juveniles reared in pond pens and tanks. *Aquaculture*, *106*(3-4), 293-299. http://dx.doi.org/10.1016/0044-8486(92)90261-I

Espe, M., Ruohonen, K., & El-Mowafi, A. (2012). Hydrolysed fish protein concentrate (FPC) reduces viscera mass in Atlantic salmon (*Salmo salar*) fed plant-protein-based diets. *Aquaculture Nutrition*, *18*(6), 599-609. http://dx.doi.org/10.1111/j.1365-2095.2012.00944.x

Forster, I., Bechtel, P., Dominy, W. G., Lane, S., Avena, R., Ju, Z. Y., & Conquest, L. (2011). Use of fish hydrolysates and fish meal byproducts of the Alaskan fishing industry in diets for Pacific white shrimp *Litopennaeus vannamei*. *North American Journal of*

Aquaculture, *73*(3), 288-295. http://dx.doi.org/10.1080/15222055.2011.598371

García-Morales, M. H., Perez-Velazquez, M., González-Félix, M. L., Burgos-Hernández, A., Cortez-Rocha, M. O., Bringas-Alvarado, L., & Ezquerra-Brauer, J. M. (2013). Effects of fumonisin B1-containing feed on the muscle proteins and ice-storage life of white shrimp (*Litopenaeus vannamei*). *Journal of Aquatic Food Product Technology*. http://dx.doi.org/10.1080/10498850.2013.775621

Hernández, C., Olvera-Novoa, M. A., Smith, D. M., Hardy, R. W., & González-Rodríguez, B. (2011). Enhancement of shrimp *Litopennaeus vannamei* diets based on terrestrial protein sources via the inclusion of tuna by-product protein hydrolysates. *Aquaculture*, *317*(1-4), 117-123. http://dx.doi.org/10.1016/j.aquaculture.2011.03.041

Jae-Young, J., Zhong-JI, Q., Hee-Guk, B., & Se-Kwon, K. (2007). Purification and characterization of an antioxidant peptide from tuna backbone protein by enzymatic hydrolysis. *Process Biochemistry*, *42*(5), 840-846. http://dx.doi.org/10.1016/j.procbio.2007.02.006

Jiang, S. T. (2000). Effect of proteinases on the meat texture and seafood quality. *Food Science and Agricultural Chemistry*, *2*(2), 55-74.

Kong, F., Tang, J., Lin, M., & Rasco, B. (2008). Thermal effects on chicken and salmon muscles: Tenderness, cook loss, area shrinkage, collagen solubility and microstructure. *LWT-Food Science and Technology*, *41*(7), 1210-1222. http://dx.doi.org/10.1016/j.lwt.2007.07.020

Leblanc, E. L., & Gill, T. A. (1982). Comparative study of proteolysis in short-finned (*Illex illecebrosus*) and long-finned (*Loligo pealeileseur*) squid. *Comparative Biochemistry and Physiology Part B*, *73*(2), 201-210. http://dx.doi.org/10.1016/0305-0491(82)90272-3

Lian, P. Z., Lee, C. M., & Park, E. (2005). Characterization of squid-processing byproduct hydrolysates and its potential as aquaculture feed ingredient. *Journal of Agricultural and Food Chemistry*, *53*(14), 5587-5592. PMid:15998119. http://dx.doi.org/10.1021/jf050402w

Liaset, B., Lied, E., & Espe, M. (2000). Enzymatic hydrolysis of by-products from the fish-filleting industry; chemical characterization and nutritional evaluation. *Journal of the Science of Food and Agriculture*, *80*(5), 581-589. http://dx.doi.org/10.1002/(SICI)1097-0010(200004)80:5<581::AID-JSFA578>3.0.CO;2-I

Lin, L., & Ba-Fang, L. (2006). Radical scavenging properties of protein hydrolysates from jumbo flying squid (*Dosidicus eschrichitii* Steenstrup) skin gelatin. *Journal of the Science of Food and Agriculture*, *86*(14), 2290-2295. http://dx.doi.org/10.1002/jsfa.2600

National Research Council - NRC. (2011). *Nutrient requirements of fish and shrimp*. Washington: National Academies Press. 376 p.

Nielsang, S., Lertsiri, S., Suphantharika, M., & Assavanig, A. (2005). Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *Journal of Food Engineering*, *70*(4), 571-578. http://dx.doi.org/10.1016/j.jfoodeng.2004.10.011

Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, *66*(5), 642-646. http://dx.doi.org/10.1111/j.1365-2621.2001.tb04614.x

Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology*, *7*(4), 120-125. http://dx.doi.org/10.1016/0924-2244(96)10012-1

Pedrero, F. D. L., & Pongborn, R. M. (1996). *Evaluación Sensorial de los Alimentos: métodos Analíticos*. México: Alhambra Mexicana. 251 p.

Rodger, G., Weddle, R. B., Carig, P., & Hastings, R. (1984). Effect of alkaline protease activity on some properties of comminuted squid. *Journal of Food Science*, *49*(1), 117-123. http://dx.doi.org/10.1111/j.1365-2621.1984.tb13685.x

Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación - SAGARPA. (2011). *Anuario Estadístico de Acuacultura y Pesca 2011*. México: Comisión Nacional de Acuacultura y Pesca. 305 p.

Samocha, T. M., Lawrence, A. L., & Bray, W. A. (1993). Design and operation of an intensive nursery raceway system for peneid shrimp. In J. P. McVey. *CRC Handbook of mariculture*. (2nd ed., pp. 173-210). Boca Raton: CRC Press. Crustacean Aquaculture 1.

Sánchez, D. R., Fox, J. M., Gatlin, III, D. M., & Lawrence, A. L. (2012). Dietary effect of squid and fish meals an growth and survival of Pacific white shrimp *Litopenaeus vannamei* in the presence or absence of phytoplankton in an indoor tank system. *Aquaculture Research*, *43*(12), 1880-1890. http://dx.doi.org/10.1111/j.1365-2109.2011.02997.x

Statistical Analisys System Institute - SAS. (1999-2000). *The SAS system for Windows, Software Release 8.1*. Cary.

Sriket, C. (2014). Proteases in fish and shellfish: role on muscle softening and prevention. *International Food Research Journal*, *21*(1), 433-445.

Stanley, D. W., & Hultin, H. O. (1984). Protoelytic activity in North America squid and its relation to quality. *Canadian Institute of Food Science and Technology Journal*, *17*(3), 163-167. http://dx.doi.org/10.1016/S0315-5463(84)72507-7

Sugiyama, M., Kousu, S., Hanabe, M., & Okuda, Y. (1989). Chemical properties. In Sugiyama M., Kousu S, Hanabe M., & Okuda Y. (Eds.), *Utilization of squid*. New Dheli: Oxonian Press. 251 p.

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, *285*(1-4), 146-158. http://dx.doi.org/10.1016/j.aquaculture.2008.08.015

Tornberg, E. (1996). Biophysical aspects of meat tenderness. *Meat Science*, *43*(Supl. 1), 175-191. http://dx.doi.org/10.1016/0309-1740(96)00064-2

Vázquez-Ortiz, F. A., Caire, G., Higuera-Ciapara, I., & Hernandez, G. (1995). High-performance liquid-chromatographic determination of free amino-acids in shrimp. *Journal of Liquid Chromatography*, *18*(10), 2059-2068. http://dx.doi.org/10.1080/10826079508013960

Watson, A. M., Barrows, F. T., & Place, A. R. (2013). Taurine supplementation of plant derived protein and n-3 fatty acids are critical for optimal growth and development of cobia, *Rachycentron canadum*. *Lipids*, *48*(9), 899-913. PMid:23884630. http://dx.doi.org/10.1007/s11745-013-3814-2