ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645
Indian Journal of Science and Technology, *Vol 11(16), DOI: 10.17485/ijst/2018/v11i16/118634, April 2018*

Chemical Characterization of Hydrolyzed Protein Meal Obtained from Trout (*Oncorynchus Mykiss*) By-Products Silage

José Luis Hoyos Concha[1*], Héctor Samuel Villada Castillo[1], Alejandro Fernández Quintero[2] and Jeimmy Rocío Bonilla Méndez[1]

[1]Agroindustry Department, Agrarian Sciences School, University of Cauca, Popayán, Cauca, Colombia; jlhoyos@unicauca.edu.co, villada@unicauca.edu.co, jrbonilla@unicauca.edu.co
[2]Food Engineering School, University of Valle, Santiago de Cali, Valle del Cauca, Colombia; alejandro.fernandez@correounivalle.edu.co

Abstract

Objective: To assess conditions of yielding and chemical characterization of hydrolyzed protein meal obtained from chemical silage of trout by-products. **Methods:** Trout by-products chemical silage was obtained by adding formic acid. Hydrolysis, pH and acidity were tracked to define the extraction day of the hydrolyzed protein meal. This hydrolyzed meal was characterized by determining soluble and volatile nitrogen, peroxide value, molecular mass profile, amino acids profile, lactic and formic acid concentration and digestibility with pepsin; and it was compared with a commercial fish meal. **Findings:** Resulting by-products of rainbow trout showed a lipids and protein level close to 90% in dry matter, which makes them a viable exploitation alternative. Degree of hydrolysis obtained was close to 62%, obtaining molecular masses of protein fractions between 0.2 and 0.6 KDA, condition that made easier the extraction of the lipid fraction and obtaining a fat-free hydrolyzed protein meal by endogenous enzymatic hydrolysis, with a chemical compositions that allows its use as raw material or additive in formulation for animal diet, complying with the requirements of fish meal legislation. Pepsin digestibility test showed a superior digestibility compared with commercial fish meal, which creates and advantage in relation to absorption and nutritional exploitation. **Novelty/Improvement:** A product with technical-functional features in relation to its nutritional advantages and chemical composition, was obtained, which can represent differentiation to advance in innovation in animal food and nutrition.

Keywords: Digestibility, Fish Meal, Hydrolyzed Protein, Silage, Trout

1. Introduction

Nowadays, fish agro industry depends in great manner on high-cost raw materials, like fish oil and meal, protein carriers and diet lipids, respectively. Such dependence has an effect in production costs, given that availability of these raw materials is unstable, which generates fluctuation in price and limits profitability of this productive sector[1]. Fish silage is an interesting alternative to fish meal, since it is a safe, simple, environmentally friendly and low-cost technology compared to meal obtaining. Besides, it reduces effluents and bad smell problems[2–11]. For its obtaining, a whole fish or pieces of it are added with acids, enzymes or lactic acid bacteria, which causes protein hydrolysis and creates microbiological and physical-chemical changes that can prevent undesired processes such as fat oxidation, putrefaction processes and allow recovery of essential fatty acids and other functional ingredients such as hydrolyzed protein or hydrolyzed protein meal, collagen, oil, among others[12,13].

*Author for correspondence

With the addition of acids, the mix reaches a pH lower than 4.0, at which proteases are usually inactive, while pepsin and cathepsin are highly active. Pepsin content can be very high in fish entrails, which is considered as the main responsible of silage production, and, its reaction rate depends on conditions such as room temperature and the type and quality of the organs in place[14]. Endogenous enzymatic hydrolysis that occurs during silage process by acid addition (chemical silage) affects mainly the protein, hydrolyzing it in peptide fragments between 2 and 20 aminoacids[15] and the physical-chemical changes that happen can generate effects in the process conditions and the final quality of the product when this hydrolyzed protein meal is used as substitute raw material or additive for obtaining extruded diets for animals[16] optimized the Pallone ditchela by-product autolysis using a mixture of sulfuric acid and formic acid and obtained hydrolysates that can serve as a nutritional source for industrial applications for both humans and animals. Similarly, author in[17] studied the use of by-products of fish and sludge obtained from the coagulation of water treatment waste from fish processing industries, applying the silage process with sulfuric acid and phosphoric acid for obtaining hydrolysates of fish. They found higher degrees of hydrolysis when applying phosphoric acid. On the other hand, report the positive effect of free peptides and amino acids resulting from the hydrolysis of proteins obtained by chemical silage with formic acid and their action as stimulators of nonspecific immunity in fish, in addition to being a source of proteins and essential amino acids in tilapia diets.

Other investigations have studied fish protein hydrolysates by autolysis[18–20] or through the use of endogenous enzymes isolated from fish in order to obtain hydrolysates with different properties[21,22]. These studies have further demonstrated the potential of such products as agents with various functional properties such as antioxidant, antimicrobial activity, emulsifying ability and foaming.

Changes in the size of the protein present in the chemical silage influence the type of properties in the final hydrolyzate[23]. Therefore, the objective of this study was to evaluate the changes occurred during the chemical silage process of trout (*Oncorhynchus mykiss*) by-products and chemical characteristics of hydrolyzed protein meal obtained, to analyze its effects on nutritional exploitation and potential use on diets for animal feeding.

2. Methods and Materials

2.1 Materials

2,4,6 trinitrobencene-sulphonic acid (5%, Sigma Aldrich), sodium sulfite (>98%, Emsure), L-Leucine (>98.5, Sigma Aldrich), sodium hydroxide (>99%, Emsure), Sulphuric acid (98%, Emsure), hydrochloric acid (37%, Merck), potassium hydroxide (85%, Emsure), perchloric acid (70%, Emsure), acetic acid (>99.8%, Honeywell), chloroform (99%, Emsure), potassium iodide (99%, Carlo Erba), sodium thiosulfate (99.5%, Merck), diethyl ether (99.7%, Panreac) and other analytic-degree reactive were used. Water (HPLC degree, Sigma Aldrich), acetonitrile (HPLC degree 99.9% Sharlout), formic acid (HPLC degree 99.9%, Panreac), monobasic potassium phosphate (>99%, Emsure), dibasic potassium phosphate (>98%, Emsure), pepsin (activity 3.200–4.500 units/mg of protein, Sigma Aldrich), Plate Count Agar (Oxoid), Agar Yeast Glucose Chloramphenicol (Oxoid), Agar Man, Rogosa and Sharpe (Oxoid), Lauril sulphate triptose culture (Oxoid), bile bright green culture (Oxoid).

Rainbow trout (*Oncorhynchus mykiss*) by-products were obtained from slaughter of animals in fattening stage with average size and weight of 18.5±1.0 cm and 400±10.0 g respectively, put on a 24 hour fast and supplied by Aquaculture and Agricultural Products Marketer and Producer Association from Silvia, Cauca (Asociación Productora y Comercializadora de Productos Acuícolas y Agrícolas de Silvia, Cauca)–APROPESCA. Immediately after entrails removal, these were stored in portable coolers and moved in dry ice to the Biotechnology Laboratory of the University of Cauca for analysis.

2.2 Proximal Analysis of By-products

Proximal composition of by-products was determined according to AOAC, 2005. Parameters measured were moisture[24–28]. Nitrogen Free Extract (NFE), was calculated by difference between 100 and crude fiber, crude lipids, crude protein and ash.

2.3 Endogenous Enzymatic Hydrolysis

For preparation of endogenous enzymatic hydrolyzed (chemical silage), formic acid at 85% was added in a relation of 25g per kg of entrails, according to methodology proposed. This procedure ensures to obtain a pH

between 3.0 and 3.2. Butylhydroxytoluene was used as antioxidant in a concentration of 200 mg per liter of lipids present in the by-products. The hydrolysis was carried out for a period of 10 days at a temperature of 15 °C ± 0.5. Analyses such as degree of hydrolysis (DH) and pH were performed daily in triplicate. Total Titratable Acidity (TTA) was performed every two days and microbiological assays were carried out on days 0, 5 and 10, in triplicate.

2.3.1 Degree of Hydrolysis (DH) Determination

DH was measured by 2,4,6-trinitrobencene-sulphonic acid method (TNBS)[29]. Degree of hydrolysis was calculated according to Equation (1).

$$\%DH = \frac{(NH_2)_{tx*100}}{(NH_2)_T} \quad (1)$$

Where, $(NH_2)tx$ = α groups concentration – amino terminal, expressed as L-leucine mMoles at *tx* time, $(NH_2)_T$ = α groups concentration – total amino terminal, expressed as L-leucine mMoles in sample after a total acid hydrolysis.

2.3.2 pH and Total Titratable Acidity (TTA) Determination

pH measurement was performed directly over the silage matrix. TTA was estimated by titrating with NaOH 0.1 N of 2.0 g of sample were taken and mixed with 25ML of distilled water, until reaching pH 8.3[30].

2.4 Hydrolyzed Protein Meal Characterization

Extraction of chemical silage was performed by filtrating through a No 30 Tyler series sieve to remove solid residuals. Then, filtering was fed by centrifuge separator Cinc industries model V02, at a separation speed of 70 MHz to obtain fat-free hydrolyzed protein meal. Hydrolyzed protein was dried at a temperature of 70°C for 15 hours. The resulting paste was ground and sieved through a No 40 Tylerseries sieve to be stored. Ash content of hydrolyzed protein meal was adjusted by adding bone meal. Hydrolyzed protein meal features were compared with those of a South-American commercial fish meal from Siquality S.A. Company (Guayaquil-Ecuador). Parameters assessed were as follows: proximate analysis, Total Volatile Nitrogen (TVN), soluble nitrogen, degree of hydrolysis, peroxide value, pepsin digestibility, molecular masses profile analysis, aminoacid profile, formic and lactic acid content.

2.4.1 Soluble and Volatile Nitrogen Determination

Soluble protein fraction was estimated following AOAC 968.06 official method. TVN was determined according to method described in the European Union official journal[31].

2.4.2 Peroxide Value Determination

Peroxide value was determined according AOAC 965.33 official method[32].

2.4.3 Molecular Mass Profile Determination

The analysis was carried out by Ultra-High Resolution Liquid Chromatography (UHPLC) in an Ultimate 3000 equipment coupled to a mass detector Exactive™ Plus Orbitrap, Thermo Fisher Scientific. As stationary phase, a C18 Titancolumn was used in reverse phase (2.09 mm diameter, 100 mm length and 1.9 µm particle size). The mobile phase corresponded to a mixture water: acetonitrile (20:80) adjusted to a pH 4.0 with formic acid 0.1%, with a gradient change up to 100% of acetonitrile and constant flow of 0.300mL/min. Column compartment temperature was 40°C. Masses range evaluated was 100 to 1200 m/z. Mass analysis parameters were carried out by adjusting of ionization source to 6 kW (*spray voltage*) and with nitrogen as auxiliary gas at 190°C. The information was processed through Thermo Xcalibur software, Version 3.1.

2.4.4 Amino Acids Profile

The analysis was carried out by UHPLC in a Prominence set with UV Visible detector brand Shimadzu, column ACEC18-AR.

2.4.5 Lactic and Formic Acid Concentration

The analysis was carried out by UHPLC in an LC-2010 set with UV Visible detector brand Shimadzu, column Aminex HPX-87C Bio-Rad.

2.4.6 Pepsin Digestibility (DivPepsin)

The determination of *DivPepsin* was performed according to AOAC 971.09 official method[33]. The digested protein was determined by the methodology described by AOAC 968.06 official method. This parameter was calculated by the Equation (2).

$$\%Div\,Pep\,sin = \frac{Digested\ \ Protein * 100}{Total\ \ Protein} \qquad (2)$$

2.5 Statistical Analysis

Changes in degree of hydrolysis, TTA and pH were tested using a simple measure-repeated design, in which intra-subject factor corresponded to time, as observed in Table 1. Statistical analysis of data obtained was carried out by IBM SPSS Statistics 20 software, which verified normality of the data and performed the variance analysis (ANOVA) for intra-subject factors, as well as pair comparison to determine differences in time and between treatments. All effects are reported with a significance $p<0.05$.

Table 1. Experimental design for selection of extraction time through silage

Design	Intra-subject Factor (FW)	FW levels	Response Variables
Simple repeated Measures	Time	Days	Degree of hydrolysis, pH, TTA

To determine the differences between hydrolyzed protein meal and the comparison pattern (fish meal), a T Student test was used. All effects are reported with a significance $p<0.05$.

3. Results

3.1 By-Product Chemical Characterization

Table 2 shows the results of proximate composition of rainbow trout entrails. The lipids were the predominant component with a 69.3%, followed by crude protein with 27.2%, macromolecules that represent nearly 90% in dry matter. Authors report similar values for the same species[34,35] with fat content around 70% and protein of 23%. In contrast, the muscle shows an inverse relation with a higher protein value (65%) concerning fat content (20 %)[36]. The lesser fraction of fiber and carbohydrates is related to glycogen accumulated in the liver and the striate muscles and pentoses present in nucleic acids, released as a consequence of post-mortem autolytic changes[37,38].

Table 2. Proximal composition on dry base of products

Component	Percentage (m/m)
Ash	3.2 ± 0.1
Crude protein	27.277 ± 0.008
Crude lipids	69.2 ± 0.3
Crudefiber	0.013 ± 0.001
NFE	0.323

3.2 Endogenous Enzymatic Hydrolysis

Changes in the degree of hydrolysisare observed in Figure 1. These are related to the endogenous enzymatic hydrolysis, in which pepsin is the most important aspartate-protease in fish entrails that can act. Vannabun[39], report high protease activity in fish by-products at a pH 3.0 with a stability higher than 95% under these conditions.

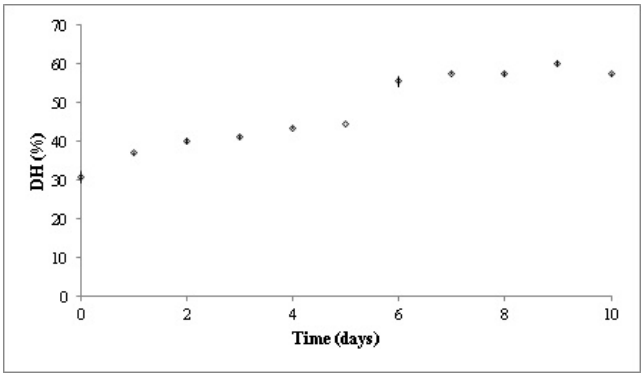

Figure 1. Degree of hydrolysis on tested silages.

There are two main stages observed during the hydrolysis. The first one with incremental values until day 6, where the increase of the degree of hydrolysis is directly related to the endogenous content of gastric enzymes, mainly pepsins, in the entrails of rainbow trout. Pepsins, classified as aspartic endo-peptidases (EC

3.4.23), which show affinity for aromatic aminoacids, are responsible of protein digestion in animal stomach in pH ranges between 2.0 and 3.0[40,41] reported isolation of 3 pepsinogens in rainbow trout, with a superior enzymatic activity in pH ranges between 1.5 and 4.5 and a temperature between 30 and 40°C. During the elaboration of chemical silage, adding formic acid generates a pH of 3.0, a condition in which the pepsinogen precursor synthetized in gastric mucus secreted in lumen, auto-catalytically turns into pepsins by removal of a N-terminal segment[42] showing its highest activity and beginning protein hydrolysis process. The second stage, posterior to day 6, shows stability in the degree of hydrolysis, statistically corroborated by not reporting significant difference in pair comparison ($p > 0.05$). This behavior possibly relates to the depletion of residual amino acids, reducing the available substrate as degree of hydrolysis increases[43], as well as enzymatic inhibition by increase of low-molecular-weight peptides[44,45].

According to Figure 2, TTA and pH did not show significant differences in time ($p> 0,05$) according to the expected behavior, since, unlike processes such as biological silage, there is not continual production and lactic acid accumulation by lactic bacteria[46] in this one. Just the same, proteolytic action produces aminoacids that, depending on ionization degree, may generate absorbing capacity[47]. Acidity and pH stability shown in values around 3,0, avoid proliferation of microorganisms causing deterioration that may lead to nitrogenous compounds such as amines, ammonium and other low-molecular-weight that can affect the absorbing capacity and physical-chemical characteristics of the product[48,49].

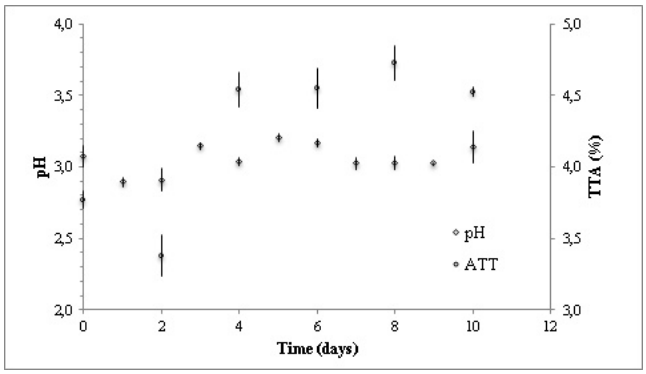

Figure 2. PH and TTA on silage.

3.3 Characterization of Hydrolyzed Protein Meal

Table 3 presents the results obtained from the chemical characterization of the hydrolyzed protein meal, compared to a conventional fish meal.

The results do not show significant differences in proximate analysis between hydrolyzed protein meal and South-American type fish meal, and their results adjust to NTC 646[50]. The nutritional value of fish meal depends on protein content, which is the macromolecule that fixes the conditions of cost and commercialization. The high protein content (higher than 60%) in tested meals is directly related to the removal of non-soluble particles by mechanic separation[51,52] reports average values of protein content up to 65% in white fish meals and South-American type, and up to 72% in herring-type fish, where percentage varies mainly due to fish type and processing form[53,54]. Several authors have reported protein content in hydrolyzed meal in a range between 60 to 90% concerning total composition[55–60] Compositional and nutritional quality of protein meals can be affected by high temperature and long drying periods, conditions that decrease to amino acids availability because of formation of Maillard products[61]. Likewise, factors such as product freshness, temperature and storage conditions may accelerate deterioration due to bacterial, enzymatic or rancidity as a consequence of peroxides, TVN and toxic biogenic amines content[62].

Lipid content in raw materials tested was around 6%, a value similar to the one obtained by other authors[63], a behavior related to the removal of the non-soluble fraction by centrifugation. Ash content depends on the origin of raw materials, becoming a quality factor due to its relation as evidence of adulteration with other meals of mineral nature such as plaster or talc. Fish meal contains ash values usually under 20%, which matches the results of this study, which showed values around 18%, percentage related to bone content and calcium and phosphorous levels[64]. As to percentage of ashes in hydrolyzed protein meal, values lower than 10% are reported in hydrolyzed protein meals, however, for this study and with the goal of obtaining similar conditions of the process concerning conventional fish meal, ash content was adjusted by adding bone meal, given the low content of ashes in entrails, with values ranging between 3.37% and 3.91%.

Value of total volatile nitrogen did not show significant differences between the tested samples. Values

Table 3. Characterization of hydrolyzed protein meal

Analysis	Fish meal*	Hydrolyzed protein meal	NTC 646/98 Fish Flour	
			Minimum	Maximum
Crude protein (%, m/m)	63.20±0.06	62.92±0.05	56	-
Crude lipid(%, m/m)	6.81±0.02	6.45±0.02	-	-
Ash (%, m/m)	18.7±0.1	17.9±0.1	-	20
Total Volatile Nitrogen (mg/100 g sample)	109.70±0.09	109.4±0.2	-	120
Peroxidevalue (meq of oxygen/kg)	0.753±0.001	0.88±0.05	-	2
Pepsin digestibility (%, m/m)	80.17±0.006	91.2±0.2	80	
Crude fiber (%, m/m)	1.0±0.2	0.6±0.2	-	
NFE(%, m/m)	10.3	12.2	-	
Degree of hydrolysis(%, m/m)	5.61±0.05	62.95±0.01	-	
Soluble Protein (%, m/m)	17.33±0.06	68.94±0.09	-	
Formic Acid(%, m/m)	0.19	0.41	-	
Lactic acid(%, m/m)	0.18	0.18	-	

* Fish meal South-American type. Siquality S.A

obtained are below the maximum permitted for fishmeal, which is 120mg/100 g according to NTC 646. This behavior is probably related to action of formic acid in silage acidification, which inhibits microbial action responsible for ammonification, resulting from oxidative domination of free amino acids from bacteria that causes their reduction by generating ammonium, which affects negatively nutritional value and protein percentage at the same time. Ammonia nitrogenous level in silage is one of the key indicators in protein quality and how much true protein has been preserved and not as non-protein nitrogen; at higher ammonia nitrogen, the poorer the quality of the protein and less the palatability of silage[65,66].

Pepsin digestibility of the protein is an important chemical property of proteins concerning their nutritional value[67]. Hydrolyzed protein meal with 91.24% digestibility showed significant differences in contrast with conventional fish meal with 80.17%. Low-molecular-weight protein fractions have been reported as digestible protein supplement in young fish feeding[68] and *in vitro* as well as *in vivo* experiments with fish indicate that low-molecular-weight peptidic fractions (between 0.5–3.0 KDA) stimulate positively nutritional aspects in fish[69]. Tested meals presented values of protein pepsin digestibility higher than 80%, a behavior related to manufacture and obtaining conditions of themselves, which could be related to use of moderate temperature that prevent the hydroperoxide formation from lipids, which may react with the protein and reduce its availability.

Concerning the value of peroxide value, this showed a value of 0.88 and 0.75 meq of oxygen/kg in hydrolyzed protein meal and conventional fish meal, respectively; values that comply with NTC 646. Presence of unstable lipid substrates such as phospholipids and highly unsaturated fatty acids in the membrane[70], and pro-oxidants such as hemoglobin for its iron content[71], increase oil oxidation process, contributing to the appearance of undesirable smells[72]. To reduce oxidation processes in hydrolyzed protein meal, BHT (Butyl-hydroxytoluene), a lipid antioxidant of synthetic origin, was added to reach product stabilization, increasing its shelf life[73], preserving organoleptic properties and keeping nutritional quality.

Degree of hydrolysis data reported in Table 3, show significant differences, presenting a value of 5.61% for fish meal and 62.95 % for hydrolyzed protein meal. The result of fish meal is related to its production process, which normally comes from pelagic fish capture that are stored to be put under thermal treatment for oil and meal production. This process inactivates hydrolytic enzymes responsible of the reduction of molecular weight of protein and increase in degree of hydrolysis. Samuelsen[74] report degree of hydrolysis values for fish meal between 6.4 and 8.9, value that reflects the protein breakage degree. Endogenous gastric enzyme content is activated at a pH close to 3.0 as it happens in chemical silage by adding formic acid, generating proteolytic action and therefore, the elevated value of degree of hydrolysis for hydrolyzed protein meal.

In Table 4 are described molecular masses in a range from 100 to 1200 (m/z). As observed, hydrolyzed protein meal shows fractions of molecular mass with values under 566.4, while conventional fish meal show fractions up to 1048.7 m/z. This difference infers the effect of endogenous hydrolytic action, which generates a higher number of low-molecular-weight fractions.

Table 4. Molecular mass distribution in a 100 to 1200 range (m/z)

Hydrolyzed protein meal		Fish Meal	
Retaining Time (min)	Molecular mass (m/z)	Retaining Time (min)	Molecular mass (m/z)
0.656	255.944	0.624	391.929
0.724	226.951	2.286	608.384
0.724	362.926	2.625	654.107
0.724	498.900	2.977	696.436
3.072	283.717	3.298	740.463
3.072	566.427	3.611	784.489
3.873	340.260	3.911	812.519
4.483	396.802	4.193	856.546
4.990	453.344	4.441	900.572
7.052	246.242	4.684	899.541
9.424	274.274	4.921	960.594
9.811	290.269	5.340	1048.651
12.117	302.305	10.581	391.284
12.399	318.300	11.010	290.269
15.788	356.352	12.317	467.114
18.499	391.284		
21.716	391.284		

In Table 5, it is observed that 82.4% of protein fractions show molecular masses between 0.2 and 0.6 kDa, while for the same range, conventional fish meal shows only a 26.7% of fractions with those characteristics. Considering that the average weight of an amino acid is approximately 110 Da[75], it is possible to assume that in the range of masses tested, in hydrolyzed protein meal there is predominance of fractions between two and five amino acids report for fish meal from Norwegian herring (Clupea harengus), percentages between 21.5 and 66.7% of peptides of molecular mass under 0.2 KDA, with the majortarian percentage in the water-soluble protein fraction, values associated to the amounts of soluble added to the cake to prepare whole fish meals and the way of processing form of the same.

Table 5. Mass frequency in a 0.2 to 1.2 kDa range

Mass ranges (kDa)	Hydrolyzed protein meal	Fish meal
0.2–0.4	82.4%	20.0%
0.4–0.6	17.6%	6.7%
0.6–0.8		33.3%
0.8–1.0		33.3%
1.0–1.2		6.7%

In[76], mention that low-molecular-weight peptides and free amino acids affect processes like extrusion due to the plasticizer effect. It increase molecular mobility and reduce vitreous fusion temperature[77]. Peptides and amino acids show amphipathic characteristics desirable in extrusion processes, since these contribute to the stability of the final product, through the formation of networks of intermolecular links such as hydrogen and ionic bondings, that lead to different types of protein-protein interactions or proteins with other components.

The value found for water-soluble protein was 17.33% for fish meal and 62.95% for hydrolyzed protein meal. Water-soluble protein level of protein meals is an important commercial specification and depends on the kind of fish, endogenous proteolysis activity variation, storage temperature, time and temperature of the meal obtainance process[78]. The normal range of water-soluble protein is between 20 and 30% of the total protein content[79], range that fits within the values obtained in the current study for fish meal and that indirectly relates protein molecular weight, since protein fraction with lower molecular weight will tend to be more water-soluble. Samuelsen, reported values between 9 and 27.5% of soluble protein for 15 fish meals from Norwegian herring (Clupea harengus). Concerning hydrolyzed protein meal, solubility increase to 62.95% is the most evident answer to the endogenous enzymatic hydrolysis process. Red salmon protein hydrolysates reached a solubility of 95%, after being hydrolyzed with exogenous enzymes, while raw red salmon protein solubility was only 20%[80], observing the same tendency in hydrolysates of shark protein[81]. An increase in enzymatic hydrolysis corresponds to a considerable increase of soluble nitrogen, in pH close to neutrality[82]. This increase of solubility concerning

protein in original state is related to the reduction of its secondary structure and the release of low-molecular-weight peptides[83], which causes an increase of the polar hydrophilic groups, which leads to an increase of their water solubility. Improved solubility allows hydrolysates to be applied easily to feeding systems[84], especially emulsions, foams and gels, because protein provides a homogeneous dispersion of molecules in colloidal systems and improves interphase properties.

Acid lactic contain of tested samples did not show differences, obtaining a value for samples of 0.18%. This value is related to the production of lactic acid from glycogen present in the liver and muscle of animals that suffers a metabolic conversion due to lack of oxygen accumulating as lactic acid after capture. pH of live fish muscle tissue is near the neutrality and due to post-mortem anaerobic formation of lactic acid, pH diminishes and varies from species to species, fishing area, time of the year and the fish energy reserve. The presence of lactic bacteria in the digestive tract of fish can also affect this value, these bacteria generate acidity from soluble carbohydrates present in the digestive tract. Formic acid content showed differences between the samples tested, and showed a formic acid residue of 0.41% in hydrolyzed protein meal. Formic acid is a low-molecular-weight acid, highly hydrophilic and with a boiling temperature of 105°C[85]. During hydrolyzed protein meal production, entrails are added with formic acid, degreased and dried in a drying process at 70°C, this temperature makes it possible to reduce formic acid by evaporation, going from a value of 2.5% to 0.41%.

On Table 6, amino acid composition of two hydrolyzed protein meals from trout is observed. The first one corresponds to the composition of meal studied in this paper and the second to the one obtained by Wald, as well as a conventional fish meal. It is observed that the most abundant amino acids are leucine (6.78), lysine (9.3), arginine (9.2), serine (15.7), glutamic acid (14.5) and glycine (7.4). Wald et al., reported a similar composition and predominant amino acids in hydrolysates from trout entrails to those ones found in this current research. During acid hydrolysis, asparagine is converted in aspartic acid and glutamine in glutamic acid; therefore, the reported amount represents the total quantity of glutamic acid and glutamine, and of aspartic acid and asparagine[86] report high contents of glutamic acid in tilapia hydrolysates; just the same[87] and Wald report high contents of glutamic acid in trout hydrolysates, according to the results obtained in this study. Percentage of residuals with hydrophilic character (32.4%) was superior to that one of the ones of hydrophobic character (27.8%) reported values of 35.6% and 35.3% for polar character residuals and non-polar, respectively; similar to the ones obtained on this study. High percentages of non-polar amino acids, is a desirable characteristic in hydrolysates with bioactive applications, because they lead to the increase of permeability in the membrane, which improves its antimicrobial capacity[88], besides from interaction between antibacterial peptides and the bacteria membrane, they could be associated to the formation of hydrophobes[89]. As observed on Table 6, there is no tryptophan value reported, whose stability, increases in a free state or bonded to proteins during the manufacture of foods and depends on temperature, oxygen presence and per oxidation agents. In absence of oxidative agents, tryptophan is an amino acid stable before strongly acid conditions, basic and sterilization temperatures as extruded cooking. Lipid hydroperoxides react with cytokine and tryptophan, and fish is one of the products most prone to suffer this kind of reactions due to its high content of poly-unsaturated fatty acids, that reduces its availability in the first moments of lipid oxidation[90], which possibly explains the absence of this amino acid in tested samples.

Concerning microbiological analysis of protein meal, results show absence of all tested microorganisms. Hydrolyzed protein meal obtained in the research presented on this paper reports a formic acid value of 0.41% and lactic acid of 0.18% and a pH value of 4.7; conditions that may have influenced directly in the absence of microorganisms in the sample[91], report the use of formic and propionic acid in proportion (70:30) in fish meal in percentages of 0.4 to 1.2%, and observed a proportional effect in the reduction of *Escherichia coli* at higher inclusion of the acid. Lückstädts[92], relates the use of acidifiers in animal feeding, which have an effect in the food, intestinal tract and metabolism of the animal. For the current study, adding formic acid has an effect in the reduction of pH of trout entrails, condition that allows pepsin action and reduces pH, preventing microorganism growth.

Table 6. Amino acids profile

Amino acid (%)	Hydrolyzed protein meal	Fish meal	Trout protein meal[22]
Hydrophobic Amino acids			
Isoleucine (Ile; I)	3.622	3.832	4.169
Leucine (Leu; L)	6.795	6.717	7.442
Methionine (Met; M)	2.378	2.469	3.105
Phenylalanine (Phe; F)	3.249	3.440	3.777
Valine (Val; V)	4.298	4.514	5.242
Alanine (Ala; A)	3.642	5.240	7.002
Proline (Pro; P)	3.799	4.157	6.106
Total	27.782	30.370	36.844
Basic Amino acids			
Lysine (Lys; K)	9.310	10.343	8.227
Arginine (Arg; R)	9.229	8.303	7.730
Histidine (His; H)	2.932	3.114	2.985
Total	21.471	21.760	18.942
Polar Amino acids			
Tyrosine (Tyr; W)	3.583	3.100	3.609
Threonine (Thr; T)	5.705	4.566	4.545
Glycine (Gly; G)	7.400	6.998	8.595
Serine (Ser; S)	15.680	14.439	3.793
Cistein (Cys; C)	-	-	0.256
Total	32.368	29.102	20.799
Aspartic Acid (Asp; D)	3.850	3.117	9.403
Glutamic acid (Glu; E)	14.529	15.651	14.012

4. Conclusions

Resulting by-products of eviscerations of rainbow trout present a content of around 90% of protein and fat in dry matter, macromolecules of great importance in the food industry for animals and especially considering the importance of finding substitutes for conventional fish meal, entrails become a feasible alternative for exploitation. The treatment performed by chemical silage, generated an acidic environment that avoids microbial decomposition, due to endogenous enzymaticaction performed by pepsins, facilitating oil release from tissues and boosting protein hydrolysis, presenting protein fractions with molecular masses between 0.2 and 0.6 KDA. The degree of hydrolysis obtained, around 62%, allows the obtaining of a product denominated hydrolyzed protein meal, which complies with the regulations for fish meal. The test of pepsin digestibility showed a differentiation in favor of hydrolyzed protein meal in contrast with conventional fish meal, by obtaining a superior digestibility percentage, granting it an advantage in relation to absorption and nutritional exploitation.

5. Acknowledgements

Thanks to the Colombian Royalty General System for funding this research through the Project "Alternatives for usage of by-products from fish-growth activity" (Alternativas para el uso de subproductos derivados de la actividad piscícola), carried out by University of Cauca (Universidad del Cauca).

6. References

1. Ministerio de Agriculturay Desarrollo Rural MADR. Available from: http://www.ceniacua.org/archivos/Diagnostico_para_revision_Dic_5_2011_v1.pdf
2. Lanes J, Borquez A, Alcaino J, Toledo J. Physicochemical composition and digestibility of silages from fishery residues in the Atlantic salmon Salmo salar. Cuban Journal of Agricultural Science. 2011; 45(4):417–22.
3. Toledo J, Llanes J. Estudio comparativo de los residuos de pescado ensilados por víias bioquimica y biologica. Revista Aquatic. 2006; 25:28–33.
4. Gutierrez O, Savon L, Toledo J, Llanes J. Utilizacion de silos pesqueros en la formulacion de dietas semi-humedas para tilapias rojas Oreochromis niloticus mossambicus. Revista Cubana de Ciencia Agricola. 2012; 46(1):67–72.
5. Garces Y, Perea C, Valencia NF, Hoyos JL, Gomez JA. Efecto nutricional del ensilado quimico de subproductos piscicolas en la alimentacion de pollos de engorde (Gallus domesticuset al). Cuban Journal of Agricultural Science. 2015; 49(4):503–8.
6. Pantoja J, Sanchez S, Hoyos J. Obtaining extruded nourishment for red tilapia (Oreochromis ssp) using fish biological silage. Biotecnol en el Sect Agropecu y Agroindustrial. 2011; 9(2):178–87.
7. Munoz A, Bucheli J, Bonilla R, Hoyos JL. Evaluacion de la tecnología de ensilaje como metodo de extraccion de aceite

de subproductos de trucha arcoíris (*Oncorhynchus mykiss*). Vitae. 2016; 23(1):278–82.

8. Goosen NJ, Wet LF, Gorgens JF, Jacobs K, De Bruyn A. Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia Oreochromis mossambicus. Animal Feed Science Technology. 2014; 188:74–84. Crossref.
9. Ramasubburayan R, Iyapparaj P, Subhashini K, Chandran M, Palavesam A, Immanuel G. Characterization and nutritional quality of formic acid silage developed from marine fishery waste and their potential utilization as feed stuff for common carp Cyprinus carpio fingerlings. Turkish Journal of Fisheries and Aquatic Sciences. 2013; 13:281–9. Crossref.
10. Ferraz DAL, Borghesi R, Oetterer M. Use of fish waste as silage- A review. Brazilian Archives Biology Technology. 2007; 50(5):879–86. Crossref.
11. Goosen NJ, Wet LF, Gorgens JF. Rainbow trout silage as immune stimulant and feed ingredient in diets for Mozambique tilapia (Oreochromis mossambicus). Aquaculture Research. 2016 Jan; 3(47):329–40. Crossref.
12. Rubio N, Beltran S, Jaime I, Diego SM De, Sanz MT, Carballido JR. Production of omega-3 polyunsaturated fatty acid concentrates : A review. Innovative Food Science and emerging Technologies. 2010; 11:1–12. Crossref.
13. Rai AK, Swapna HC, Bhaskar N, Halami PM, Sachindra NM. Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. Enzyme and Microbial Technology. 2010; 46(1):9–13. Crossref.
14. Kristinsson HG, Rasco BA. Fish protein hydrolysates: Production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition. 2000; 40(1):43–81. Crossref. PMid:10674201
15. Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chemical. 2012; 135(4):3020–38. Crossref. PMid:22980905.
16. Prabha J, Narikimelli A, Sajini MI, Vincent S. Optimization for autolysis assisted production of fish protein hydrolysate from underutilized fish Pellona ditchela. International Journal of Scientific and Engineering Research. 2013; 4(12):1863–9.
17. Tomczak-Wandzel R, Medrzycka K. Preparation, composition and properties of fish silage produced with post-coagulation sludge. Environment Protection Engineering. 2013; 39(4):39–49.
18. Ovissipour M, Rasco B, Shiroodi SG, Modanlow M, Gholami S, Nemati M. Antioxidant activity of protein hydrolysates from whole anchovy sprat (Clupeonella engrauliformis) prepared using endogenous enzymes and commercial proteases. Journal Science Food Agriculture. 2013; 93(7):1718–26. Crossref. PMid:23184898.
19. Mazorra MA, Pacheco R, Ramírez JC, Garcia G, Lugo ME. Endogenous proteases in Pacific whiting (Merluccius productus) muscle as a processing aid in functional fish protein hydrolysate production. Food bioprocess technology. 2012; 5(1):130–7. Crossref.
20. Samaranayaka AGP, Li-Chan ECY. Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (Merluccius productus). Food Chemical. 2008; 107(2):768–76. Crossref.
21. Silva JFX, Ribeiro K, Silva JF, Cahu TB, Bezerra RS. Utilization of tilapia processing waste for the production of fish protein hydrolysate. Animal feed science and technology. 2014; 196:96–106. Crossref.
22. Wald M, Schwarz K, Rehbein H, Bußmann B, Beermann C. Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout by-products with trout pepsin. Food Chemical. 2016 Aug; 205:221–8. Crossref. PMid:27006234.
23. Wald M. Manufacture, isolation and characterization of antibacterial peptides from rainbow trout (*Oncorhynchus mykiss*). Tesis Doctoral; 2016. p. 1. PMid:27224642
24. AOAC. 934.01. Moisture in animal feed. Official Methods of Analysis of Association Analytical Chemist's. (17th) Gaithersburg; 2005. p. 1–5.
25. AOAC 942.05. Determination of ash in animal feed. Official Methods of Analysis of Association Analytical Chemist's. 17th Gaithersburg, USA; 2005 Oct. p. 1392–7.
26. AOAC 991.36. Fat (Crude) in meat and meat products. Official Methods of Analysis of Association Analytical Chemist's. 17th Gaithersburg; 2005.
27. AOAC 968.06. Protein (crude) in animal feed: Dumas method. Official Methods of Analysis of Association Analytical Chemist's. 17th Gaithersburg; 2005. p. 1–2.
28. AOAC 962.09. Fiber (crude) in animal feed and pet food. Official Methods of Analysis of Association Analytical Chemist's 17th Gaithersburg; 2005. p. 1–4.
29. Adler-Nissen J. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. Journal Agriculture Food Chemical. 1979; 27(6):1256–62. Crossref.
30. Zakaria Z, Hall GM, Shama G. Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. Process Biological Chemical. 1998; 33(1):1–6. Crossref.
31. UE Reglamento (CE) n° 2074/2005 de la comision. Diario Oficial de la union europea; 2005. p. 1–33.
32. AOAC 965.33. Peroxide value of oils and fats. Official methods of analysis of Association Analytical Chemist's. 17th Gaithersburg; 2005. p. 1.

33. AOAC 971.09. Pepsin digestibility of animal protein feeds. Filtration method final action. Official Methods of Analysis of Association Analytical Chemist's. 17th Gaithersburg; 2005.
34. Dumas A, de Lange CFM, France J, Bureau DP. Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2007 Nov; 273(1):165–81. Crossref.
35. Yamamoto T, Konishi K, Shima T, Furuita H, Suzuki N, Tabata M. Influence of dietary fat and carbohydrate levels on growth and body composition of rainbow trout *oncorhynchus mykiss* under self-feeding conditions. Fish Science. 2001; 67:221–7. Crossref.
36. Perea A, Gomez E, Mayorga Y, Triana CY. Caracterizacion nutricional de pescados de produccion y consumo regional en Bucaramanga. Archivos latinoamericanos de nutricion; 2008; 58:91–7. PMid:18589578
38. Beltran DE. Aprovechamiento de los productos pesqueros. Krogdahl. Mexicali, Baja California, Mexico: Editorial Universidad Autonoma de Baja California; 2006 Jan. p. 1–231.
39. Vannabun A, Ketnawa S, Phongthai S. Characterization of acid and alkaline proteases from viscera of farmed giant catfish. Food Biology Science. 2014; 6:9–16. Crossref.
40. Bougatef A, Hajji M, Balti R, Lassoued I, Triki-Ellouz Y, Nasri M. Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. Food Chemical. 2009; 114:1198–205. Crossref.
41. Wald M, Rehbein H, Beermann C, Bußmann B, Schwarz K. Purification and characterization of pepsinogen and pepsin from the stomach of rainbow trout (*Oncorhynchus mykiss*). European Food Research and Technology. 2016; 242(11):1925–35. Crossref.
42. Kageyama T. Pepsinogens, progastricsins, and prochymosins: structure, function, evolution, and development. Cellular and Molecular Life Sciences. 2002 Feb; 59(2):288–306. Crossref. PMid:11915945.
43. Klomklao S, Kishimura H, Benjakul S. Use of viscera extract from hybrid catfish (Clarias macrocephalus x Clarias gariepinus) for the production of protein hydrolysate from toothed ponyfish (Gazza minuta) muscle. Food Chemical. 2013; 136(2):1006–12. Crossref. PMid:23122156.
44. Nguyen HTM, Sylla KSB, Randriamahatody Z, Donnay-Moreno C, Moreau J, Tran LT. Enzymatic hydrolysis of yellowfin tuna (Thunnus albacares) by-products using protamex protease. Food Technology Biotechnology. 2011; 49(1):48–55.
45. Guerard F, Guimas L, Binet A. Production of tuna waste hydrolysates by a commercial neutral protease preparation. Journal of Molecular Catalysis B: Enzymatic. 2002; 19(20):489–98. Crossref.
46. Zahar M, Benkerroum N, Guerouali A, Laraki Y, El Yakoubi K. Effect of temperature, anaerobiosis, stirring and salt addition on natural fermentation silage of sardine and sardine wastes in sugarcane molasses. Bioresource Technology. 2002; 82(2):171–6. Crossref.
47. García E. Ensilado de pescado adicionado con pasta de Jatropha curcas no toxica como alternativa para la alimentación de tilapia (Oreochromis niloticus). Sinaloa, Mexico: Instituto Politecnico Nacional; 2012. p. 1–115.
48. Llanes J, Toledo J, Fernandez I, Lazo J. Estudio del ensilado biologico de pescado como inoculo de bacterias lacticas en la conservacion de desechos pesqueros. Revista Electronica de Veterinaria. 2007; 8(9):1–6.
49. Castillo M, Rojas A, WinChing R. Valor nutricional del ensilaje de maiz cultivado. Agron Costarric. 2009; 33(1):133–46.
50. NTC 646. Norma Tecnica Colombiana. NTC 646. Alimentos para animales. harinas de pescado; 1998.
51. Chalamaiah M, Rao GN, Rao DG, Jyothirmayi T. Protein hydrolysates from meriga (Cirrhinus mrigala) egg and evaluation of their functional properties. Food Chemical. 2010; 120(3):652–7. Crossref.
52. Sandbol P. Nueva tecnologia en la produccion de harina de pescado para piensos: Implicaciones sobre la evaluacion de la calidad. IX Curso de Especializacion FEDNA; Barcelona, Espana. 1993.
53. Opstvedt J, Nygard E, Samuelsen TA, Venturini G, Luzzana U, Mundheim H. Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. Journal Science Food Agriculture. 2003; 83:775–82. Crossref.
54. Samuelsen TA, Mjos SA, Oterhals A. Impact of variability in fishmeal physicochemical properties on the extrusion process, starch gelatinization and pellet durability and hardness. Animal Feed Science Technology. 2013; 179(1–4): 77–84. Crossref.
55. Choi YJ, Hur S, Choi BD, Konno K, Park JW. Enzymatic hydrolysis of recovered protein from frozen small croaker and functional properties of its hydrolysates. Journal Science Food. 2009; 74(1):17–24. Crossref. PMid:19200081
56. Dong YL, Sheng GY, Fu JM, Wen KW. Chemical characterization and anti-anaemia activity of fish protein hydrolysate from Saurida elongata. Journal Science Food Agriculture. 2005; 85:2033–9. Crossref.
57. Sathivel S, Smiley S, Prinyawiwatkul W, Bechtel PJ. Functional and nutritional properties of red salmon (Oncorhynchus nerka) enzymatic hydrolysates. Journal Science Food. 2005; 70(6):401–6. Crossref.
58. He S, Franco C, Zhang W. Functions, applications and production of protein hydrolysates from Fish Processing Co-Products (FPCP). Food Research International. 2013; 50:289–97. Crossref.

59. Bhaskar N, Benila T, Radha C, Lalitha RG. Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (Catla catla) for preparing protein hydrolysate using a commercial protease. Bioresource Technology. 2008; 99:335–43. Crossref.
60. Nilsang S, Lertsiri S, Suphantharika M, Assavanig A. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. Journal of Food Engineering. 2005; 70:571–8. Crossref.
61. Nakayama T, Hayase F, Kato H. Formation of e-(2-formyl-5-hydroxymethyl-pyrrol-1-yl)-L-norleucine in the Maillard reaction between D-glucose and L-lysine. Agriculture Biology Chemical. 1980; 4(5):1201–2. Crossref.
62. Samuelsen TA. Fishmeal physichochemical properties. Impact on the fish feed extrusion process, phase transitions and physical pellet quality. Doctoral Thesis. University of Bergen; 2015 Jun.
63. Souissi N, Bougatef A, Triki-ellouz Y, Nasri M. Biochemical and functional properties of Sardinella (Sardinella aurita) by-product hydrolysates. Food Technology Biotecnology. 2007; 45(2):187–94.
64. Anderson JS, Higgs DA, Beames RM, Rowshandeli M. Fish meal quality assessment for Atlantic salmon (Salmo salar L.) reared in sea water. Aquaculture Nutrition. 1997; 3:25–38. Crossref.
65. Toledo J, Botello A, Llanes JE. Evaluacion del ensilado quimico de pescado en la alimentacion de Clarias gariepinus. Revista Electronica de Veterinaria. 2007; 8(9):1–6.
66. Oliveira IS de, Lourenco L de FH, Sousa CL, Peixoto Joele MRS, Ribeiro S da C do A. Composition of MSM from Brazilian catfish and technological properties of fish flour. Food Control. 2015; 50:38–44. Crossref.
67. Sarker AK, Saha D, Begum H, Zaman A, Rahman MM. Comparison of cake compositions, pepsin digestibility and amino acids concentration of proteins isolated from black mustard and yellow mustard cakes. AMB Express. 2015; 5(22):1–6. Crossref.
68. Raghunath MR, Gopakumar K. Trends in production and utilization of fish silage. Journal of Food Science and Technology. Association of Food Scientists and Technologists. 2002; 39:103–10.
69. Gildberg A. Enzymes and bioactive peptides from fish waste related to fish silage, fish feed and fish sauce production. Journal of Aquatic Food Product Technology. 2004; 13(2):1–10. Crossref.
70. Liang Y, Hultin HO. Separation of membranes from acid-solubilized fish muscle proteins with the aid of calcium ions and organic acids. Journal Agriculture Food Chemical. 2005; 53:3008–16. Crossref. PMid:16366688
71. Raghavan S, Kristinsson HG. Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: A comparative study of five enzymes. Journal Agriculture Food Chemical. 2008; 56:1434–41. Crossref. PMid:18247531
72. Maqsood S, Benjakul S. Comparative studies on molecular changes and pro-oxidative activity of haemoglobin from different fish species as influenced. Food Chemical. 2011; 124:875–83. Crossref.
73. Domingos AK, Saad EB, Vechiatto WWD, Wilhelm HM, Ramos LP. The influence of BHA, BHT and TBHQ on the oxidation stability of soybean oil ethyl esters (biodiesel). Journal of the Brazilian Chemical Society. 2007 Apr; 18(2):416–23. Crossref.
74. Samuelsen TA, Mjos SA, Oterhals A. Influence of type of raw material on fishmeal physicochemical properties, the extrusion process, starch gelatinization and physical quality of fish feed. Aquaculture Nutrition. 2014; 20:410–20. Crossref.
75. Beaulieu L, Thibodeau J, Bonnet C, Bryl P, Carbonneau M-E. Detection of antibacterial activity in an enzymatic hydrolysate fraction obtained from processing of Atlantic rock crab (Cancer irroratus) by-products. Pharma Nutrition. 2013 Oct; 1(4):149–57. Crossref.
76. Samuelsen T, Oterhals A. A powdered protein composition having binding properties adapted for use as protein source and binder in a formulated feed; 1999.
77. Di Gioia L, Guilbert S. Corn protein-based thermoplastic resins: Effect of some polar and amphiphilic plasticizers. Journal Agriculture Food Chemical. 1999; 47(3):1254–61. Crossref.
78. Oterhals A, Samuelsen TA. Plasticization effect of solubles in fishmeal. Food Resources. 2015; 69:313–21. Crossref,
79. Samuelsen TA, Oterhals A. Water-soluble protein level in fishmeal affects extrusion behavior, phase transitions and physical quality of feed. Aquaculture Nutrition. 2015; 12–4.
80. Gbogouri GA, Linder M, Fanni J, Parmentier M. Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. Food Chemical Toxicol. 2004; 69(8):615–22. Crossref.
81. Diniz FM, Martin AM. Effects of the extent of enzymatic hydrolysis on functional properties of shark protein hydrolysate. LWT - Food Science and Technology. 1997 May; 30(3):266–72. Crossref.
82. Foh MBK, Amadou I, Kamara MT, Foh BM, Xia W. Effect of enzymatic hydrolysis on the nutritional and functional properties of Nile Tilapia (Oreochromis niloticus) proteins. American Journal of Biochemistry and Molecular Biology. 2011 Jan; 1(1):54–67. Crossref.
83. Adler-Nissen J. Enzymic hydrolysis of proteins for increased solubility. Journal Agriculture Food Chemical. 1976 Nov; 24(6):1090–3. Crossref. PMid:794085
84. Thiansilakul Y, Benjakul S, Shahidi F. Compositions functional properties and antioxidative activity of pro-

tein hydrolysates prepared from round scad (Decapterus maruadsi). Food Chemical. 2007; 103:1385–94. Crossref.
85. Acido formico Fichas Internacionales de Seguridad Quimica; 2010. p. 1–2.
86. Robert M, Zatylny-Gaudin C, Fournier V, Corre E, Le Corguille G, Bernay B. Molecular characterization of peptide fractions of a Tilapia (Oreochromis niloticus) by-product hydrolysate and in vitro evaluation of antibacterial activity. Process Bbiochemical. 2015 Mar; 50(3):487–92. Crossref.
87. Taheri A, Anvar SAA, Ahari H, Fogliano V. Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout (Onchorhynchus mykiss) viscera. Iranian Journal of Fisheries Sciences. 2013; 12(1):154–69.
88. Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. Pharmacological Reviews. 2003; 55(1):27–55. Crossref. PMid:12615953
89. Cheng X, Tang X, Wang Q, Mao XY. Antibacterial effect and hydrophobicity of yak κ-casein hydrolysate and its fractions. International Dairy Journal. 2013 Aug; 31(2):111–6. Crossref.
90. Gil A. Tratado de nutricion. Madrid, Espana: Medica Panamericana; 2010. p. 1–765.
91. Malicki A, Zawadzki W, Bruzewicz S, Graczyk S, Czerski A. Effect of formic and propionic acid mixture on Escherichia coli in fish meal stored at 12°C. Pakistan Journal of Nutrition. 2004; 3(6):353–6. Crossref.
92. Luckstadts C. The use of acidifiers in fisheries and aquaculture. Reviwe Perspect Agriculture Science Nutrition Resources. 2008; 3(44):1–9.