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Use of trout by-products (*Oncorhynchus mykiss*) for the production of an extruded fish feed

Uso de subproductos de trucha (*Oncorhynchus mykiss*) para la producción de alimento extruído para peces

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Abstract

Extrusion cooking is a continuous mixing, cooking, and forming processing method. The main objective of the study was to determine the effect of the inclusion of chemical silage residues from the benefit of trout (*Oncorhynchus mykiss*) in the extrusion process of a food diet for fish. The evaluation of the inclusion of hydrolyzed protein flour (HPF) was carried out in a simple screw extruder, using a 2k factorial design, with four factors: extrusion temperature profile, screw speed, humidity, and HPF, evaluating response variables physicochemical characterization of protein, physical properties such as hardness, durability, density, buoyancy and expansion index. The hydrolyzed protein flour generated changes during the extrusion process related to its cohesiveness and plasticizer capacity. Regarding its cohesiveness, it was verified with the improvement in the physical properties of the pellet and its plasticizing capacity by reducing the average retention time. The results showed the techno-functional potential of hydrolyzed protein flour for its application in the animal feed industry, being a raw material that can be used as a regulating additive of the physical quality of the pellet. It is suggested to expand the study of the mass range of the peptides contained in hydrolyzed protein flour.
Keywords: Cohesiveness, Hardness, Expansion Index, Buoyancy, Pellet, Power Law.

Resumen

La cocción por extrusión es un método continuo de procesamiento de mezcla, cocción y formación. El objetivo principal del estudio fue determinar el efecto de la inclusión de residuos de ensilado químico de beneficio de trucha (*Oncorhynchus mykiss*) en el proceso de extrusión de una dieta alimenticia para peces. La evaluación de la inclusión de harina proteica hidrolizada (HPF) se realizó en una extrusora de tornillo simple, utilizando un diseño factorial 2k, con cuatro factores: perfil de temperatura de extrusión, velocidad del tornillo, humedad y HPF, evaluando variables de respuesta caracterización fisicoquímica de la proteína , propiedades físicas como dureza, durabilidad, densidad, flotabilidad e índice de expansión. La harina de proteína hidrolizada generó cambios durante el proceso de extrusión relacionados con su capacidad cohesiva y plastificante. En cuanto a su cohesividad, se comprobó con la mejora en las propiedades físicas del granulado y su capacidad plastificante al reducirse el tiempo medio de retención. Los resultados mostraron el potencial tecnofuncional de la harina proteica hidrolizada para su aplicación en la industria de la alimentación animal, siendo una materia prima que puede ser utilizada como aditivo regulador de la calidad física del pellet. Se sugiere ampliar el estudio del rango de masas de los péptidos contenidos en la harina de proteína hidrolizada.
Palabras clave: Cohesividad, Dureza, Índice de Expansión, Flotabilidad, Pellet, Ley de Potencia.

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1 Introduction

Extrusion cooking is a continuous mixing, cooking, and shaping processing method, which has the advantages of being low cost, versatile, and highly efficient in food processing (Cheng *et al*., 2020). Among the processed products are many products of different sizes, shapes, textures, and flavors for human consumption and animal feed (Alam *et al*., 2016).

Extruded foods are an essential product in manufacturing, packaging, handling, transportation, and storage capacity. Among the fundamental parameters is evaluating the moisture content in the equilibrium of the extruded product to know the drying and storage behavior. Therefore, the moisture content is the factor that most influences the changes in the quality of the extrudates in the processing and storage study (Sahu *et al*., 2021; Piñeros-Guerrero *et al*., 2021). On the other hand, ingredients and proportions during extrusion influence acceptability through the structural formation and body shaping of the expanded or low-density product.

In this way, the development of extruded products through locally accessible raw materials is considered good practice for enriching various nutrients in the final products (Sahu & Patel, 2020). In this way, materials have rheological properties that affect operating parameters, process system design, and energy use. In particular, the rheology of non-Newtonian fluids and soft solid foods influences the product's design, stability, and sensory properties (Stokes & Xu, 2019).

Among the different techniques to carry out rheological measurements, whether the determination is carried out off-line (off-line) or if the size is carried out online (in-line). In the first case, the experimental conditions can be applied mainly through capillary rheometers; These measurements are made with higher water content, so the determined viscosity is lower than that developed directly in an extruder (Nguyen *et al*., 2021; Monthe *et al*., 2019). In-line measurement systems, measurement is performed by attaching a device to the extruder outlet (Harper, 1981; Bhattacharya *et al*., 1992). Both the cylindrical type dies (rod die), and slot die (slot die) can be directly coupled to the extruder, achieving different cutting speeds with screw speed, achieving more comprehensive ranges of cutting speed. In the case of the technique used to determine the rheological behavior under extrusion firing, it has been

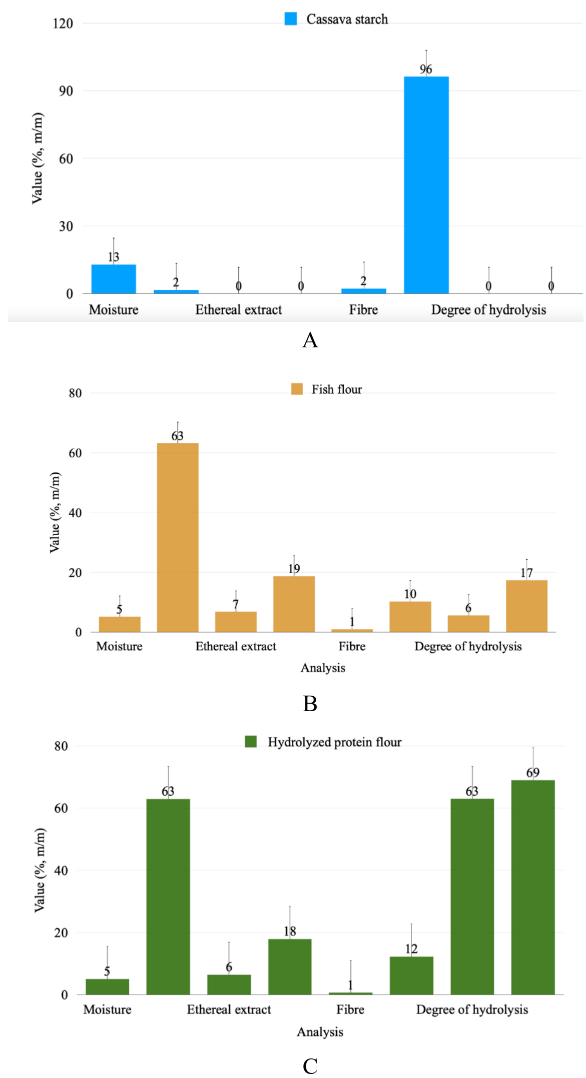

Fig 1. Chemical composition of raw materials on a dry basis (b.s): Cassava starch (A), fish flour (B), hydrolyzed protein flour (C).

established that the molten materials are essentially pseudoplastic whose rheological behavior can be adequately adjusted to a Power Law model depending on the temperature change of the Arrhenius type and an exponential sensitivity to change in water content (Decaen *et al*., 2020; Chen *et al*., 2020).

The objective of the present study is to evaluate the rheological behavior of an extruded fish diet that incorporates hydrolyzed protein meal processed by extrusion. The measurement was conducted online with a slit matrix rheometer, defining the rheological models and the parameters that fit the process. In addition, there are no reports on the use of Trout

(*Oncorhynchus mykiss*) residues through the extrusion process and optimized extrusion conditions to obtain a fish feed.

2 Materials and methods

2.1 Raw materials

The methodology described in previous works was followed (Hoyos-Concha *et al*., 2022). The raw materials used for the evaluation during the extrusion process were hydrolyzed protein flour, fish flour (Siquality SA, Guayaquil-Ecuador), and cassava starch (Sucre starch); their chemical composition is described in Figure 1. Additives such as vitamin core formulated for fish farming (Premex), Bentonite (Premex), calcium carbonate ($CaCO_3$) (analytical grade 99%, Carlo Erba), and sodium chloride (NaCl) (analytical grade 99%, Carlo Erba) were incorporated into the diet.

2.2 Diet formulation

Its incorporation was evaluated in a range between 15 and 35% as a substitute for fish meals. The starch source, vitamin nucleus, and minerals remained stable (Fig 2).

Isoprotein and isoenergetic diets were obtained, formulated for omnivorous fish in the fattening stage. The flours were sieved through a Tyler series No. 40 sieves and mixed in a Kitchen Aid mixer for 30 minutes. Later, they were packed in polyethylene bags and refrigerated for 24 hours to be evaluated.

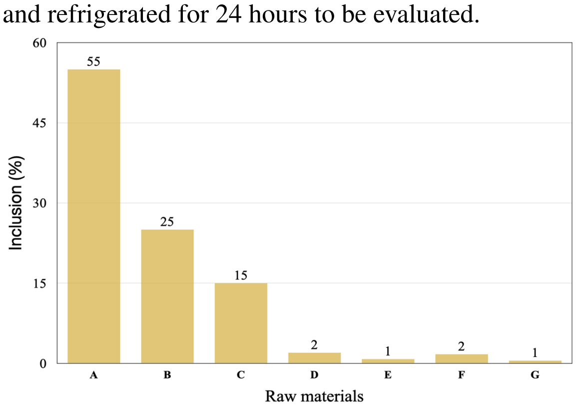

Fig 2. Raw materials required for the preparation of the diet. A: Cassava starch; B: Hydrolyzed protein flour; C:Fish flour; D:Vitamin nucleus; E:Calcium carbonate; F:Bentonite; G:Sodium chloride.

2.3 Rheological measurement using an in-line slit die rheometer

A Thermo Scientific (Germany) brand slit dies to type vertical line rheometer was used to measure the rheological properties of mixtures, including hydrolyzed protein flour, with an outlet opening of 1.5 x 20 mm, with ports for a melt temperature sensor and three pressure sensors (700, 500 and 300 bar), according to methodology of Hoyos-Concha *et al.* (2022).

The determination of the volumetric flow rate was carried out by quantifying the density of the melt. The measurement was carried out according to the ASTM D1238-2004c standard, in a Tinius Olsen model MP 1200 (Germany) plastometer, composed of a vertical cylinder, a die with a 2.095 mm hole diameter and 8 mm long. The test temperature was adjusted to 115 °C with 3.6 kg of weight on the piston, with a preheating time of 30 s. The travel meter was adjusted to 6.35 mm ± 0.25 mm, the plastome was loaded with an 8 g sample, the sample was made to flow through the die, and the weight was recorded for five examples for each material, obtaining the values of volumetric melt index, mass melt index and melt density. According to the following equations, the parameters evaluated were sheer speed, shear stress, and apparent viscosity (Gao *et al*., 2020; Hoyos-Concha *et al*. 2022).

$$\gamma_{app} = \frac{6 \cdot Q}{W \cdot H^2} \tag{1}$$

where is the apparent cutting velocity ($s\gamma_{app}^{-1}$), Q is the volumetric flow rate (m^3/s), is the width of the slit (m) and WH is the height of the slit (m) (Eq.1).

$$\tau = \frac{\Delta P \cdot H}{2 \cdot L} \tag{2}$$

τ is the shear stress (Pa), ΔP is the pressure variation in the capillary (Pa) and L the length of the cleft (m) (Eq. 2).

$$\gamma_{true} = \frac{1}{3} \cdot \gamma_{app} \cdot \left(2 + \frac{\partial \log \gamma_{app}}{\partial \log \tau}\right) \tag{3}$$

The correction of the cutting speed was performed using the Weissenberg-Rabinowitsch equation (Eq. 3).

$$\eta_{app} = \frac{\tau}{\gamma_{true}} \tag{4}$$

η_{app} is the apparent viscosity (Pa.s) quantified as the relationship between shear stress and true shear velocity (Eq. 4).

Table 1. Factorial design 3^3 applied to rheological evaluation.

Factors	Levels
Temperature (ºC)	115
	125
	135
Moisture (%)	20
	22
	24
Hydrolyzed protein flour (%)	15
	25
	35

The rheological behavior of the samples was represented by the Power Law , Bingham and Casson models (Eq. 5, 6, 7).

$$\tau = K_{lp}\gamma^{n} \tag{5}$$

$$\tau = \tau_{0bg} + K_{bg}\gamma \tag{6}$$

$$\tau^{0.5} = \tau_{0cs}^{0.5} + K_{cs}\gamma^{0.5} \tag{7}$$

Where K_{lp}, K_{cs}, K_{bg} are the consistency indices of the samples; corresponds to the minimum force to initiate the flow through the capillary τ_0.

The selected model was made according to the standard error (Eq. 8)

$$\frac{[\sum(X_m - X_c)^2/(N-2)^2]^{0.5}}{range} \times 1000 \tag{8}$$

Where X_m is the measured value; X_c is the calculated value; N is the number of data and range is the maximum value - minimum value of X_m.

2.4 *Statistical analysis*

A 3k factorial design was used where the factors temperature, humidity, and inclusion of HPF were evaluated, with three levels each, as described in Table 1.

The results of the factorial design were analyzed by analysis of variance with a value of p <0.05, and the data were processed in the Minitab V.16 software. A mean comparison analysis was performed with the Tukey test at p <0.05, for statistically significant data.

$$\eta = K_{lp} \cdot \gamma^{n-1} \cdot \exp\left[\frac{\Delta E}{R} \cdot \left(\frac{1}{T}\right) + b \cdot (MC)\right] \tag{9}$$

The experimental values obtained were adjusted to Harper's rheological regression model (Eq. 9). η is the apparent viscosity (Pa.s), K_{lp} is the consistency index (Pa.s^n), n is the flow behavior index, γ is the cutting speed (s^{-1}), ΔE is the activation energy (J/mol), R is the constant ideal gases (J/mol K), T is the temperature (K), MC is the mass fraction of water (b.h) and the parameter b relates the change in moisture content in the viscosity of the material.

3 Results

The raw material used for the experimental development corresponded to trout viscera, highlighting in the characterization carried out the high fat and protein content, close to 90% on a dry basis. By applying chemical silage as a method for processing rainbow trout viscera, it facilitated the exit of fat trapped in the tissues, achieving greater extraction, and obtaining a defatted hydrolyzed protein meal. Authors reported that fish has a high nutritional value due to the high level of nutrients, high protein content, high quality lipids and low cholesterol levels, in which essential fatty acids, such as omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have important functions in the human body, including reducing the risk of cardiovascular diseases. In addition, DHA improves brain cognitive processes, such as memory function and the proper functioning of neurons (Al-Ghannami *et al*., 2019; Fujii *et al*., 2021; Do Souza *et al*., 2022).

In its macro components, hydrolyzed protein meal (HPF) and conventional fish meal do not present differences once the proximal analysis has been performed, adjusting to NTC 646 for fish meal. However, when evaluating the characteristics of the protein with additional tests, Differences were observed in the degree of hydrolysis, water-soluble nitrogen, and digestibility in pepsin. Among the results obtained, a more excellent digestibility in pepsin of HPF stands out, which gives it differential nutritional characteristics to other protein raw materials for its application in animal nutrition.

Once the HPF was obtained and characterized, it was evaluated as a raw material in elaborating an extruded fish feed, corresponding to one of the four factors studied in the extrusion process: temperature, humidity, screw speed, and HPF. The results obtained from the variation of the factors described above within the evaluated experimental plane affected the system's parameters in the process, the physical and microstructural properties of the extruded pellet.

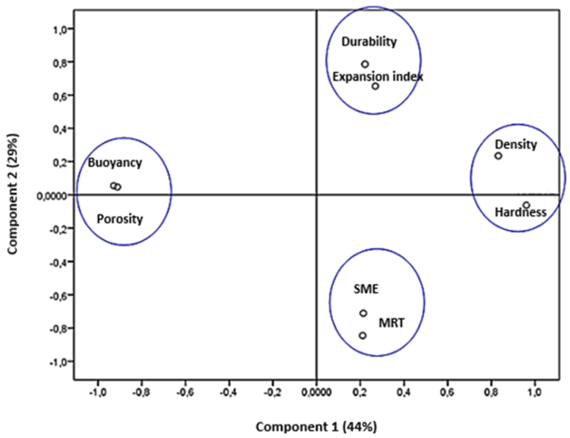

Fig 3. Principal component analysis. Component 1 refers to the variables of density, hardness, buoyancy, and porosity. Component 2 refers to the variables SME (Specific Mechanical Energy and MRT (Mean Retention Time).

The objective of the present study is to evaluate the rheological behavior of an extruded fish diet that incorporates hydrolyzed protein meal processed by extrusion. The measurement was conducted online with a slit matrix rheometer, defining the rheological models and the parameters that fit the process. In addition, there are no reports on the use of Trout (*Oncorhynchus mykiss*) residues through the extrusion process and optimized extrusion conditions to obtain a fish feed.

In order to reduce the dimension of the number of response variables processed in the research, the information obtained was analyzed by the principal components method (Figure 3), where components 1 and 2 explain the total variance of the experiment. The group of response variables grouped in circles shows a positive correlation with each other and a negative with opposite response variables on the same axis. Bartlett's sphericity test presented a p-value <0.05, indicating that statistical analysis can be applied.

The hydrolyzed protein flour was the factor with the highest incidence in the evaluation of the extrusion process, obtaining the highest values in the estimation of F for the regression analysis and representing in the diet formulation the raw material to be evaluated during the experiment. Therefore, the discussion around its incidence in the obtained results was carried out.

Component 1, which represents 44% of the variability of the experiment and corresponds to the x-axis, shows the relationship between the response variables density, hardness, buoyancy, and porosity. A positive correlation is observed between the variables buoyancy and porosity, where the high content of HPF in the formulation improved the porosity of the pellet with the consequent positive effect on buoyancy. During the extrusion cooking process, the available starch and low molecular weight protein are converted into a melt, in which links and networks of high elasticity were possibly formed, capable of retaining water vapor for a longer time at its disposal-extruder outlet. Finally, the empty spaces formed will be the cells that constitute the internal structure of the pellet (Oterhals *et al*., 2019; Cobos & Farías, 2020; García-Reyes *et al*., 2017), generating thin-walled cells (Turchini *et al*, 2019), which improved buoyancy.

The hardness and density showed a positive correlation with each other and a negative one for the porosity and buoyancy parameters. The proportion of HPF included in the mixture decreased the hardness of the pellet due to its high degree of hydrolysis; a result attributed to high expansion and low density. Commonly, low hardness values result in low durability values, a result contrary to that obtained in the present study, a condition related to the formation of a cohesive structure due to the addition of low molecular weight protein (Turchini *et al*., 2019), which for the case study was hydrolyzed protein flour. The decrease in HPF and increase in a conventional fish meal in the formulation generated compact structures, resulting in pellets with high densities.

The SME and MRT variables presented a positive correlation, observed in component 2. In the analysis of the extrusion process, it was observed that the higher the content of HPF, the SME, and the MRT is reduced, which is directly related to the plasticizing effect of protein, which in turn is a source of low molecular weight peptides and even amino acids which have a plasticizing effect (Offiah & Falade, 2019; Gautam, Choudhury, & Gogoi, 1996; Oterhals *et al*., 2019), facilitating mobility and fluidity of the mixture as it passes through the extruder (Ahmad *et al*., 2019). In contrast, the durability and expansion index values present a positive correlation and a negative correlation with SME and MRT. Therefore, the lower the durability and expansion index, the higher the SME and MRT. This behavior occurs at a higher concentration of fish meal (high molecular weight protein). The latter is capable of absorbing and retaining water with greater force, limiting the efficient distribution of available water, which implies a decrease in plasticizer in the mixing and an increase in MRT (Goswami *et al*., 2021; Oterhals *et al*., 2019;

Sukumar & Athmaselvi, 2019; Treviño-Garza *et al*., 2020).

The results observed for the response variables such as SME, density and hardness, show an increase in the evaluation midpoints (125ºC, 220 rpm, HPF 20%, humidity 20%). This behavior is related to the type and concentration of protein present. Protein plays a regulatory role in the availability of water during the extrusion process, and its ability to capture water, to unfold during the thermomechanical process, and the formation of aggregates will depend on the size of the molecules and their interaction to form hydrogen bonds, bonds, hydrophobic, ionic bonds and cysteine bonds. The concentration relationship between hydrolyzed protein meal (low molecular weight protein) and conventional fish meal (high molecular weight protein) in the midpoints generated a more significant number of interactions due to cross-linking effect, which was reflected mainly in response variables of physical quality, such as high hardness, high density, low buoyancy, and low porosity. However, at the extreme (axial) points of the central composite design, particularly for the temperature factor (115ºC and 135ºC); An opposite behavior was observed (low density, high buoyancy, high porosity), related to the decrease in the viscosity of the material due to the fragmentation generated at high temperatures and with the increase of the MRT when it is processed at low temperatures.

According to the established experimental conditions, the molten material presented a pseudoplastic behavior, with a decrease in apparent viscosity as the shear rate increased, as indicated by the value of n in the Power Law model (Hoyos-Concha *et al*, 2021; Hilliou & Covas *et al*., 2021; Padmanabhan & Bhattacharya, 1993; Chisenga *et al*., 2019; Nguyen *et al*., 2021; Chen *et al*., 2020; Singh & Smith, 1999). In addition, the ANOVA carried out showed the concentration of HPF in the mixture as the factor that most influenced the experimental development, followed by temperature and humidity.

Figure 4 presents a synthesis of the interactions between the evaluated factors (temperature, humidity, HPF), with the flow behavior index (n) as the response variable. The interaction of the humidity and temperature factors showed the expected behavior during the monitoring of the rheological properties, observing an increase in the value of n, obtaining values that tend to 1 (Newtonian behavior) with the increase in temperature and humidity as individual factors and interaction. The increase in moisture content combined with the increase in temperature

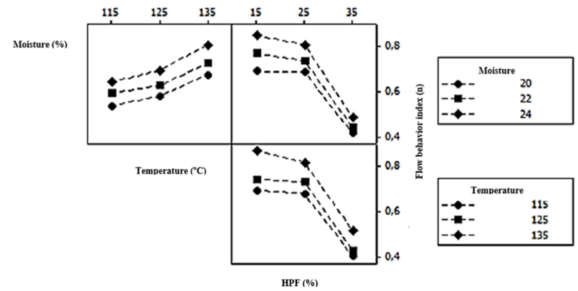

Figure 4. Interaction of factors with respect to the flow behavior index (n) in relation to temperature, humidity and HPF.

facilitated mobility in the extrusion barrel (Yagci *et al*., 2022), increasing molecular degradation and increasing mobility (Hilliou & Covas, *et al*., 2021; Padmanabhan & Bhattacharya, 1993; Jiang *et al*., 2020 ; Cheng *et al*., 2021). The results observed for the response variables such as SME, density and hardness, show an increase in the evaluation midpoints (125ºC, 220 rpm, HPF 20%, humidity 20%). This behavior is related to the type and concentration of protein present. Protein plays a regulatory role in the availability of water during the extrusion process, and its ability to capture water, to unfold during the thermomechanical process, and the formation of aggregates will depend on the size of the molecules and their interaction to form hydrogen bonds, bonds, hydrophobic, ionic bonds and cysteine bonds. The concentration relationship between hydrolyzed protein meal (low molecular weight protein) and conventional fish meal (high molecular weight protein) in the midpoints generated a more significant number of interactions due to cross-linking effect, which was reflected mainly in response variables of physical quality, such as high hardness, high density, low buoyancy, and low porosity. However, at the extreme (axial) points of the central composite design, particularly for the temperature factor (115 oC and 135ºC); An opposite behavior was observed (low density, high buoyancy, high porosity), related to the decrease in the viscosity of the material due to the fragmentation generated at high temperatures and with the increase of the MRT when it is processed at low temperatures.

Figure 4 shows that the content of hydrolyzed protein in interaction with humidity and temperature showed the lowest n values of the experiment, moving away from Newtonian behavior with the increase in the percentage of inclusion of HPF. This behavior indicates interaction of the macromolecules present in the mixture, such as protein-protein interactions, protein carbohydrates that influenced the pseudoplastic behavior, forming networks of intermolecular bonds such as hydrogen and ionic bonds that align in the direction of flow as they are increases sheer speed (Wang *et al*., 2021; Turchini *et al*., 2019; Fajardo-Espinoza *et al*., 2020). Due to the low molecular weight of the peptides from hydrolyzed protein flour, they tend to be located between the starch chains, acting as plasticizers and crosslinking agents between the different extrudate fractions (free sugars, dextrins, peptides of different molecular weights) (Wang *et al*., 2021), forming a three-dimensional network that can be established by the terminal groups of the molecules present, increasing the viscosity of the melt.

The hydrolyzed protein flour contains peptides that promote the sliding of starch chains acting as plasticizers and peptides with exposed amino acid residues to react with terminal groups of dextrins and sugars, which act as crosslinking agents, affecting molecular mobility and increased viscosity.

Conclusions

The hydrolyzed protein meal obtained from chemical silage can be considered a substitute for fish meal. Regarding its cohesiveness capacity (crosslink), it was verified with the improvement in the physical properties of the pellet such as buoyancy, expansion index, porosity, hardness, and durability. The plasticizing capacity generated a reduction in the mean retention time in the extruder barrel and a decrease in the specific mechanical energy. Although HPF has a cohesive action, the increase in mass flow due to the plasticizing effect impacts SME, reducing its value. Durability and toughness are response variables that generally show a positive correlation. For the present study, the behavior was the opposite, achieving pellets with high durability due to the cohesiveness of the hydrolyzed protein flour, but at the same time with low hardness due to the high porosity achieved. Porosity was one of the response variables in which the addition of hydrolyzed protein flour presented the highest incidence. Therefore, the techno-functional potential of hydrolyzed protein flour for its application in the animal feed industry is verified. It is recommended to verify the interaction with protein of vegetable origins such as soybean cake and its effect on the physical properties rheological. Similarly, it is suggested to carry out *in vivo* digestibility studies and tests of the bioactivity of hydrolyzed protein flour. It is necessary to expand the study of the mass range of the peptides contained in hydrolyzed protein flour to determine the proportion and type of proteins that act as plasticizing agents and crosslinking agents. Clarifying this condition can improve the precision of the application of the peptides contained in the flour.

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