Influence of pH in the enzymatic hydrolyzate of concentrates from the fishmeal industry

Influencia del pH en el hidrolizado enzimático de concentrados de la industria de harina de pescado

Influência do pH no hidrolisado enzimático concentrados da indústria de farinha de peixe

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Abstract

The fishmeal concentrate is a byproduct that has an important amount of components useful for the food industry. However, if the fishmeal concentrate is not processed, it could cause an imbalance of the environment in which the waste is discharged. The aim of this research was to evaluate the influence of the pH in the enzymatic hydrolysis of the fishmeal industry for the production of protein concentrates from stickwater as a primary commodity. Thus, the pH values (5.32, 5.94 y 6.33) were examined, also a proximal analysis to hydrolysate and soluble fish was carried out. In order to determine protein, moisture, fats and ashes the following methods were used, Kjeldhal method for protein, rapid thermobalance method for moisture, Soxhlet methos for fats, and official INEN 0467 method for ashes. Protein concentration analyzes were performed by the Bradford method, and subsequently, the hydrolysis approximation was calculated and the amino acid composition was determined by the reference method Waters UPLC. The results showed that the pH of 6.33 allowed to achieve a better hydrolysis because a higher hydrolysis approximation was obtained, thus also the results obtained from the amino acid composition in the final product demonstrate its potential use as a food additive.

Resumen

El concentrado de la industria de la harina de pescado es un subproducto que contiene una gran cantidad de compuestos utilizables en la industria alimentaria. Sin embargo, en el caso de no ser procesados podría causar un desequilibrio en las propiedades físicas, químicas y biológicas del entorno en el cual se realiza la descarga de los desechos. El objetivo del estudio fue evaluar la influencia del pH en el hidrolizado enzimático de la industria de harina de pescado para la elaboración de concentrados proteínicos a partir de muestras de agua de cola como materia prima. Para la ejecución de esta investigación se evaluaron tres valores de pH (5,32; 5,94 y 6,33). Se realizó el análisis proximal al hidrolizado y al soluble de pescado. La determinación de proteína se realizó por el método Kjeldahl, humedad por el método rápido de la termobalanza, grasas por el método Soxhlet y cenizas por el método oficial de la INEN 0467. Se realizaron análisis de concentración de proteína por el método de Bradford, posteriormente, se calculó la aproximación de hidrólisis y se determinó la composición de aminoácidos mediante el método de referencia Waters UPLC. Los resultados mostraron que el pH de 6,33 permitió alcanzar una mejor hidrólisis debido a que se obtuvo una aproximación de hidrolisis más elevada, así también, los resultados obtenidos de la composición de aminoácidos en el producto final demuestran su potencial uso como aditivo alimenticio.

Palabras clave: Proteína, agua de cola, aminoácidos, aditivo alimenticio, hidrolizado proteico.

Resumo

O concentrado da indústria da farinha de peixe é um subproduto que contém uma grande quantidade de compostos utilizáveis na indústria alimentar. Contudo, se não for processado, poderá causar um desequilíbrio nas propriedades físicas, químicas e biológicas do ambiente em que os resíduos são descarregados. O objectivo deste estudo foi avaliar a influência do pH no hidrolisado enzimático na indústria de farinha de peixe para a produção de concentrados de proteínas a partir de amostras de água de cola como matéria-prima. Três valores de pH (5,32, 5,94 e 6,33) foram avaliados para a execução desta investigação. Foi realizada uma análise proximal do hidrolisado de peixe e peixes solúveis. A proteína foi determinada pelo método Kjeldahl, a humidade pelo método de termobalanço rápido, a gordura pelo método Soxhlet e as cinzas pelo método oficial INEN 0467. A análise da concentração de proteínas foi realizada pelo método de Bradford, depois a aproximação da hidrólise foi calculada e a composição de aminoácidos foi determinada pelo método de referência Waters UPLC. Os resultados mostraram que o pH de 6,33 permitiu alcançar uma melhor hidrólise porque foi obtida uma maior aproximação à hidrólise. Além disso, os resultados obtidos a partir da composição de aminoácidos no produto final demonstram a sua potencial utilização como aditivo alimentar.

Palavras-chave: Proteína, água de cola, aminoácidos, aditivo alimentar, hidrolisado de proteínas.

Introduction

Currently the demand for fishmeal has increased rapidly, especially in some of the emerging aquaculture countries in Asia, such as China, which is the largest producer and exporter of aquaculture and also the largest. fishmeal consumer worldwide (Han *et al*., 2018; Li *et al*., 2019).

Fishmeal is a naturally balanced food ingredient, rich in protein, energy and minerals (Jannathulla *et al*., 2019), however, during the fishmeal production process the raw material is subjected to a process of pressing, obtaining a mass in which the suspended solids and fish oil are separated by a centrifugation process, producing an effluent commonly called tail water (Wu & Bechtel, 2012), if the by-product is not used again in the plant, is usually discarded in the environment, causing serious damage to the local fauna and flora (Babazadeh *et al*., 2014). For this reason, it is necessary to find an efficient method to reuse the by-product and in turn give it added value, making the most of its properties. Tail water evaporated to a thick syrup can provide valuable amounts of protein, soluble solids, vitamins and minerals (Mahdabi & Hosseini-Shekarabi, 2018). Currently, the by-products of fishmeal production are considered a raw material with a high potential for the production of products instead of being considered a waste (Mahdabi *et al*., 2018).

According to Wisuthiphaet *et al*. (2015), one way to use fishmeal by-products is for production of fish protein hydrolyzate. Although this method is more time consuming and has a higher production cost, the fish protein hydrolysates obtained by this method are more nutritional and offer a wide range of applications including animal nutrition or food additives.

The objective of this study was to evaluate the Influence of pH in the enzymatic hydrolyzate of concentrate from the fishmeal industry for the elaboration of protein concentrates from tail water samples of the marine species *Thunnus albacares*, *Katsuwonus pelamis* and *Opisthonema libertate*.

Materials and methods

Raw material

The raw material (tail water obtained from the scrap of tuna from the marine species *Thunnus albacares*, *Katsuwonus pelamis* and *Opisthonema libertate*), was collected from companies producing fishmeal in the city of Jaramijo-Manabí 0°58'34.9" South and 80°38'14.9" West.

Enzyme

The proteolytic enzyme Granozyme ACC® was used for enzymatic hydrolysis. The details of the proteolytic enzyme used in the present study are presented in table 1.

Table 1. Description of the Granozyme ACC® enzyme.

Specifications	
Effective pH range	6 to 7,5
Effective temperature range	45 to 60°C
Shape	Liquid
Smell	Typical of the enzyme
Density	1.15 g.mL^{-1}
Minimal Activity	840 UHb.g^{-1}

Source: (Granotec, 2017)

Preparation of the enzymatic hydrolyzate

To obtain the enzymatic hydrolyzate, the tail water was passed through a concentration process in a triple effect evaporating plant, obtaining a liquid with a high concentration of organic matter called concentrate or soluble fish, which must have 40 °Brix. Subsequently, the soluble concentrate passed to a reactor where an 85 % potassium

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hydroxide solution was added, which allowed it to increase the pH of the concentrate to a value that ranged between 5.5 and 7, (Hanna HI99121 pH-meter). The selected enzyme was added when the temperature in the reactor was 60 °C. Once the enzyme was added, one (1) hour was waited, giving rise to the enzymatic process, in which the proteins were hydrolyzed into peptides and amino acids of lower molecular mass. Simultaneously with the hydrolysis, the stabilizer (Xanthan Gum) was added in a dose of 3.5 g.kg⁻¹ of product and potassium sorbate was used as preservative, in a dose of 2000 mg.kg⁻¹ of product, to achieve a better homogenization of these with the hydrolyzed product. The Granozyme ACC® enzyme was activated to perform its function and at the end of the reaction it must be inactivated, either by temperature or pH. For which, the entry of steam was opened to the jacket of the reactor to raise the temperature of the hydrolyzate to 90 °C, maintaining it for 15 min. Subsequently, the hydrolyzate was cooled to room temperature. Phosphoric acid was added to the reactor, in a dose of 4 to 5 %, obtaining a finished product with a pH lower than 4.5. Finally, the product was unloaded in properly cleaned and sealed containers.

Proximal analysis

The proximal analysis of the enzymatic hydrolyzate and the soluble fish was carried out using the following methods: moisture was determined by the rapid thermobalance method, according to the procedure described in the Official Mexican Standard for the determination of moisture in food (NMX, 1982), the percentage of proteins was calculated using the Kjeldahl method according to the methodology described by the Association of Official Analytical Chemists (AOAC, 2005), fats were determined using the Soxhlet method described in the Ecuadorian Technical Standard 0466 (INEN, 1980a) and ashes were determined by means of the Ecuadorian Technical Standard 0467 (INEN, 1980b).

Determination of protein concentration and hydrolysis approximation

The protein concentration was determined by the method of Bradford (1976), the reading was carried out with a microplate reader (iMark Microplate Absorbance Reader-catalog # 168-1130) at an absorbance of 595 nm. Protein concentration was calculated using the standard curve for bovine serum albumin. The results were expressed in mg.mL⁻¹.

The hydrolysis approximation was determined by the following formula:

$$\text{Hydrolysis approximation} = (1 - (A / B)) \times 100 \qquad \text{Equation 1}$$

Where A: is the protein concentration after hydrolysis and B: is the protein concentration before subjecting the concentrate to the hydrolyzing action of the enzyme.

Composition of amino acids

The amino acid composition was determined using the Waters UPLC reference method for amino acid analysis (Waters, 2021), using ultra-performance liquid chromatography (UPLC), for which an ultra-high-performance liquid chromatograph (Waters Acquity UPLC), equipped with a Waters AccQ-TagTM Ultra column (2.1 mm x 100 mm), a mobile phase A AccQ-TagTM Ultra Eluent A1, a mobile phase B AccQ-TagTM Ultra Eluent B and the flow rate was 0.7 mL.min⁻¹. The sample and column temperatures were 20 and 55 °C, respectively. The amino acids determined were: arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine and valine. These amino acids were selected because they are within

the nutritional requirements of essential amino acids necessary for the marine species to which the product is directed (shrimp).

Experimental design and statistical analysis

A completely randomized design (DCA) was used with three treatments and three repetitions for each one. Each treatment consisted of a pH value with a different degree of alkalization at the beginning of each hydrolyzate (5.32, 5.94 and 6.33). For statistical analysis the mean values of the Bradford analysis and the hydrolysis approximation were obtained from three replicates and were used for statistical analysis. The difference between the means of the treatments was calculated using Tukey's multiple range test ($p \le 0.05$) using the statistical software SPSS version 23.0 (IBM SPSS Statistics, 2015). Data were expressed as means ± standard deviation (SD).

Results and discussion

Proximal composition of fish soluble and hydrolyzate

The results of the proximal composition of the tail water (AC) and the hydrolyzate with each of the different pH´s are shown in tables 2 and 3.

Table 2. Proximal composition of the fish soluble.

	Fish soluble			
pH	Humidity (%)	Protein (%)	Ash (%)	Fat (%)
5.32	54.08 ± 1.35	32 ± 0.02	10.98 ± 0.11	1.70 ± 0.22
5.94	54.44 ± 1.31	33 ± 0.01	10.25 ± 0.23	1.55 ± 0.32
6.33	53.98 ± 1.29	32 ± 0.04	10.66 ± 0.15	1.57 ± 0.06

The data represent the mean ± SD

Table 3. Proximal composition of the hydrolyzated.

	Hidrolizated			
pH	Humidity (%)	Protein (%)	Ash (%)	Fat (%)
5.32	49.05 ± 1.27	34.90 ± 0.05	14.55 ± 0.46	1.20 ± 0.34
5.94	49.60 ± 1.25	35.00 ± 0.07	14.20 ± 0.53	0.90 ± 0.68
6.33	50.30 ± 1.29	35.60 ± 0.11	13.55 ± 0.22	1.05 ± 0.01

The data represent the mean ± SD

It can be seen that in all three cases there was a decrease in the percentage of fat and moisture, and an increase in the percentage of ash and protein (component of greater nutritional interest). The results obtained in the hydrolyzate are different from those reported in previous studies carried out by Nilsang *et al*. (2005), Souissi *et al*. (2007); Ovissipour *et al*. (2009); See *et al*. (2011); and Taheri *et al*. (2013). These variations could be due to the source of the raw material used, the production process or even the season in which the fish were caught. In this regard, Šližyte *et al*. (2014) indicated that this aspect can be an influencing factor in the quality of the final product.

Protein concentration and hydrolysis approximation

The results of the protein concentration and hydrolysis approximation are shown in table 4. The statistical analysis showed that there are no statistically significant differences ($p \le 0.05$) in the protein concentration and in the hydrolysis approximation, at values of pH 5.32 and 5.94. However, it can be observed that there is a significant difference ($p \le 0.05$) for a pH of 6.33, these results suggest that the pH has an important effect at the time of hydrolyzing. These results agree with the studies carried out by Baez-Suarez *et al*. (2016)

and Zapata *et al*. (2019). In addition, it can be observed that the hydrolyzate prepared with a pH of 6.33 had a higher hydrolysis approach, generating a reduction in concentration, which indicates that the hydrolysis with this treatment was more effective.

Table 4. Protein concentration and hydrolysis approximation.

pH	Protein concentration mg.mL^{-1}	Hydrolysis approximation (%)
5.32	2.38 ± 0.12[a]	46 ± 2.50[a]
5.94	2.64 ± 0.20[a]	40 ± 4.40[a]
6.33	1.22 ± 0.14[b]	72 ± 3.10[b]

[a], [b] Different letters symbolize statistically significant differences (p <0.05). The data represent the mean ± SD

Amino acid composition

The results of the composition of essential amino acids (EAA) of the enzymatic hydrolyzate using the soluble fish as raw material for its elaboration are shown in table 5. It can be seen that the hydrolyzate using the Granozyme ACC® enzyme had a lower EAA composition than that obtained by Bhaskar *et al*. (2008) and Ovissipour *et al*. (2012). These results are as expected, because these studies have used viscera or blood that contain a higher percentage of protein to obtain the hydrolyzate. However, the composition of amino acids obtained meets all the nutritional requirements of EAA of the marine species *Marsupenaeus japonicus* (Teshima *et al*., 2002), which is a promising result for the use of this type of by-products in the supplement industry. nutritional.

Table 5. Essential amino acid composition of the hydrolyzate.

EAA	Hidrolizated g.100g^{-1}	Bhaskar et al. (2008) g.100g^{-1}	Ovissipour et al. (2012) g.100g^{-1}	EAA request (*Marsupenaeus japonicus*) g.100g^{-1}
Arginine	1.80	10.82	8.81	1.40-1.80
Histidine	2.32	2.06	8.45	0.50-0.70
Isoleucine	1.40	3.6	6.93	1.10-1.40
Lisine	1.92	7.07	1.87	1.70-2.00
Methionine	1.36	2.02	1.48	0.60-0.80
Phenylalanine	1.36	3.53	3.85	1.30-1.60
Threonine	1.52	4.02	5.9	1.10-1.40
Valine	1.53	4.79	8.93	1.20-1.50

EAA: essential amino acids; sample number: 25

Conclusions

The enzymatic hydrolysis of the soluble fish had a better performance at a pH of 6.33; With the implementation of this value, the performance in obtaining large amounts of protein with this by-product as raw material could be improved, generating added value to it.

Soluble fish has proven to be a promising source of nutritional supplements for marine species to take advantage of a by-product that would otherwise be discarded causing environmental pollution. However, future studies in various marine species are necessary to determine its efficacy as a food additive or animal nutrition.

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