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Nutritional Characteristics of Protein Isolated from Snakehead (*Channa striata*) Using the pH-Shift Method

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Abstract. Protein isolate, the purest protein, can be produced through pH regulation so that the resulting amino acid content that can be used to formulate several food products for both human and animal consumptions. Thus, the objectives of present study were: 1) to determine the nutritional content of muscle and muscle flour of snakehead, 2) to obtain optimum alkaline and acid pH of protein isolates from snakehead, and 3) to determine nutritional of protein isolates from snakehead. The method of this research was experimental method by making protein isolates from snakehead by using different alkaline pHs (10, 11, and 12), furthermore, the best alkaline pH treatment followed by different acid pHs (4 and 5). The results showed the nutritional content (protein) of snakehead muscle was 73.32% dry basis (db) with a yield of 37.40%, while the protein content of snakehead muscle flour was 75.57% (db) with a yield of 82.30%. The best alkaline pH was pH 11 that nutritional characteristics were a yield of 9.56% and a protein content of 83.45% (db). Furthermore, pH 4 was the best acid pH that nutritional characteristics were a yield of 9.80%, protein content of 84.20% (db).

1. Introduction

Snakehead is found in Indonesia of inland openwater fisheries such as rivers, reservoirs and swamps. Snakehead production in Indonesia continued to increase every year [1].

The results showed that snakehead had high nutritional content, namely 76.9% (db) protein, 1.7% (db) fat, 5.96% (db) ash and 13.61% (wb) moisture. Furthermore it also contained Zn 0.31% mineral and Fe 0.44%. Albumin, one of the most important types of protein, can be used as a substitute for serum albumin which is usually used to accelerate postoperative wound healing [2].

Furthermore, snakehead also contains complete amino acids, especially essential amino acids that can be utilized by the body as neurotransmitters, non-polypeptide hormones and polypeptide hormones, such as insulin and glucagon.

Allegedly snak pead of amino acids in free form can be used for diabetics, because it can increase the stimulation of insulin secretion by pancreatic beta cells so as to reduce blood glucose levels [3]. In

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addition, snakehead also contain exogenous antioxidants that can help regenerate pancreatic tissue damaged by free radicals, thus it can be used as an alternative for healing diabetes mellitus (DM), given the prevalence of DM disease lately continues to increase significantly.

Embedding of snakehead protein isolates is thought to produce high enough free amino acids, because protein isolates are the purest form of protein, with a protein content of 90%. Determinations of the isoelectric point by adjusting the pH, causing the resulting isolate to contain specific free amino acids that can reduce blood glucose.

The production of snakehead protein isolates is expected to increase the presence of free amino acids, so that it can be consumed to accelerate the stimulation of insulin secretion. At present research that utilizes new cork fish meat is limited to the use of snakehead as a food source that has high protein, but there is no information about the types of amino acids making up snakehead protein and its activity in free form is unknown. Therefore, in the early stages of this study intended to determine the nutritional content of muscle and muscle flour of snakehead, get the optimal alkaline pH and acid isolates of snakehead protein, and determine the nutritional characteristics of snakehead protein isolates.

2. Materials and Methods

Snakehead obtained from fishermen around Pekanbaru with a size of 1 kg per a fish. Snakehead used measuring 40-60 cm with a weight of 900-1000 g / a fish. Preparation includes removal of the head, entrails, skin, washing, weeding, filing, weighing, milling muscle snakehead and flouring. The protein isolates extracted from snakehead was achieved through utilization of different pH values. The alkaline pH, such as 3 levels: P1 (pH 10), P2 (pH 11), P3 (pH 12), was evaluated using completely randomized design to determine the best alkaline pH. Furthermore, the best results on alkaline pH are used for the treatment phase of acidic pH with 2 levels of treatment namely (P4 = pH 4, P5 = pH 5). The acidic pH was evaluated using T-test method to determine the best treatment.

The nutritional parameters tested included nutritional content (proximate) muscle and muscle flour, protein content and isolate content at alkaline and acidic pH, and total amino acid content. The manufacture of protein isolates from snakehead using different pH based on the method of [4], [3], and [5] with a slight modification.

3. Results and Discussion

3.1. Raw Materials and Sample Preparation

The average muscle snakehead yield is 37.40%. The physical characteristics of muscle snakehead are white reddish with a smooth texture.

Furthermore, the results of proximate analysis of muscle snakehead as raw material in the manufacture of protein isolates include levels of protein, fat, moisture, ash and carbohydrates (by different) seen in Table 1. Protein levels of muscle snakehead are quite high at 73.32% db. This condition showed that sea cucumbers had good nutritional value as food.

Table 1. Results of analysis of nutritional content (proximate) fresh muscle snakehead.

Nutrient content	Percentage(%)	
Moisture (% db)	76,20±0,85	
Ash (% db)	7,57±0,92	
Proteint (% db)	73,32±0,77	

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Fat (% db)	9,66±0,63
Carbohydrates (% db)	9,45±0,51

The next preparation stage is the flouring process. The physical characteristics of the flour produced are bright yellow with a smooth texture. The greage yield of flour produced 82.30%. The results of the analysis of nutritional content (proximate) can be seen in Table 2.

Table 2. Results of nutrient content analysis (proximate) muscle flour from snakehead.

Nutrient content	Percentage (%)	
Moisture (% db)	5,25±0,88	
Ash (% db.)	8,34±0,32	
Proteint (% db	75,57±2,88	
Fat (% db)	3,85±0,25	
Carbohydrates (% db)	12,24±3,15	

Protein content in muscle flour from snakehead is 75.57% db and not much different from muscle protein, this showed that snakehead the potential to be used as a snakehead protein isolate.

3.2. The effect of different alkaline pH on the manufacture of protein isolates from snakehead The manufacture of protein isolates from snakehead using the main ingredient of muscle flour from snakehead. The process of making protein isolates from snakehead is done by extracting flour with 35% NaOH at different pHs namely pH 10, 11, and 12.

This different alkaline pH setting to dissolve snakehead of protein, then carried out the centrifugation and drying process. Dried sea cucumber protein isolates produced with open look less bright and brownish yellow, but after the process of flouring the shape becomes brighter and yellowish white in color.

The average yield of snakehead isolates was 9.56% with a protein content of 83.45%. Complete results of measurements of protein yields and levels of snakehead isolates at different basic pHs are shown in Table 3.

Table 3. Average yield and protein content of snakehead protein isolates at different alkaline pH.

Alkaline pH	Yield (%)	Protein Level (% db)
10	$9,40\pm0,44^{a}$	$78,24\pm3,45^{a}$
11	$9,56\pm0,52^{a}$	$83,45\pm2,18^{b}$
12	$9,58\pm1,33^{a}$	$83,47\pm3,74^{b}$

Results of variance ($\stackrel{\bullet}{\circ}$ NOVA) of yield showed no significant difference between different pHs. As for the protein content anova test results showed significant differences (p < 0.05). The pH 11 and 12 treatments were not significantly different, but compared with pH 10 showed a significant difference (P < 0.05). Based on the efficiency and effectiveness in making these isolates, pH 11 is the best pH that can be used for the next step, namely acidic pH.

3.3. The effect of different acid pH on the manufacture of cork fish protein isolates

The use of acidic pH ie pH 4 and 5 aims to precipitate proteins that have dissolved to alkaline pH. The basic pH used is the best pH in previous studies, namely pH 11. The average yield was 9.80% and the protein content was 84.20% db. Full results can be seen in Table 4.

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Table 4. Average yield and protein content of protein isolates from snakehead at different acidic pH.

Acidic pH	Yield (%)	Protein Level (% db)	
4	9,80 ^b	84,20 ^b	
5	7,60 ^a	$81,08^{a}$	

The T test results showed that the amendment value and protein content for pH 4 were significantly different from pH 5 (P < 0.05), therefore the pH 4 treatment was the best pH with the amendment and protein content higher than pH 5 (Table 4).

Although pH 4 is the best, it cannot yet be used as an isoelectric pH, due to the extremely low amendment value. The principle used to isolate proteins is the deposition of all fish proteins at their isoelectric point, namely the acidic pH where all proteins coagulate, due to a positive and negative charge of a balanced protein. At the isoelectric point, the total charge of each amino acid in the protein is equal to zero, meaning that there is a balance between the positive and negative charged groups. Electrostatic interactions between amino acids will be maximum because unbalanced loads tend to be tug-of-war, this can be observed with protein clumping [6].

4. Conclusions

The nutritional content of muscle and yield from snakehead is 73.32 db with a yield of 37.40%, while the protein content of muscle flour from snakehead is 75.57% db with a yield of 82.30%.

The protein content and the snakehead protein isolate yield at pH 10 was 78.24% db with a yield of 9.40%, for pH 11 83.45% db with a 9.56% yield, and pH 12 83.47% db with an amendment of 9.58%. Whereas at pH 4 the protein content was 84.20% db with a yield of 9.80%, while for pH 5 it had a protein content of 81.08% db with a 7.60% yield.

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