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Physical and Functional Characteristics of Snakehead Fish Protein Concentrate Produced by Acid and Alkali Solubilization Methods

D Ikasari[1a)], Y Donny[2)]
[1)] Current Address : Research Center for Marine and Fisheries Product Processing and Biotechnology, Jakarta, Indonesia
[2)]University of Sultan Ageng Tirtayasa, Banten, Indonesia

Corresponding author : ikadiah263@gmail.com

Abstract. The increase consumption of Snakehead fish crude extract as supplement drink has left the fish meat residue which has been utilized yet. The study aims to investigate the physical characteristics of fish protein concentrate (FPC) produced from Snakehead fish (*Channa striata*) extraction by product using acid and alkali solubilization methods. The acid method was performed by adding HCl 0,1N until pH during extraction reached 2,3, and 4, while for alkali method using NaOH 0,1M with extraction pH of 10,11,12. The FPC was analyzed for its color, whiteness and functional properties. The results indicated that the highest whiteness was obtained from treatment of pH 4 e.g 69,9%. pH 11 resulted FPC with the highest L value, i.e 85.54 but low a* and b* values, i.e 0.33 and 19.1. Acid solubilization method resulted FPC with lower emulsifying activity but higher emulsifying solubility compared to alkali solubilization method. The acid solubilization method was considered more effective in producing FPC rather than alkali solubilization method due to higher physical and functional characteristics of FPC.

Keywords: Snakehead fish, fish protein concentrate, acid and alkali solubilization, functional properties

1. Introduction

Snakehead fish is one of popular fish species consumed by people due to its benefits during post-operation recovery process. This is because the fish is identified to contain albumin and several minerals such as Fe, Cu and Zn, which are essential for human body metabolisms [1]. For that aim, people are usually consumed the snakehead fish in the form of crude extract which is produced by water extraction process at 85 $^{\circ}$C for 30 minutes and continued by pressing process using filter press [2]. While people use the extract, the left meat residue is unutilized and discarded. The alternatives for utilizing the Snakehead meat by-product resulted from extraction process is converting into fish protein concentrate (FPC) which has higher economic value.

Fish protein concentrate (FPC) is a protein powder obtained from some process of fat and unwanted materials removal. The FPC is commonly used by food industry to improve product nutrition and characteristic. This is because FPC has certain physical and functional characteristics that influence the final product performance. The substitution of FPC into the food product is conducted during preparation, processing, storage and consumption [3].

Fish protein concentrate can be produced by several methods, including heating, dissolving of fish tissue in acid (pH $\leq$ 3.5) or alkali (pH $\geq$ 10.5) solution, or combining between acid-alkali solubilization

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and filtration [4]. Acid or alkali solubilization method is a method using pH shifting principal and generally used for protein isolation resulting in protein concentrate with preferred characteristics [5]. This method is conducted by bringing the materials into low or high pH, continued by precipitating the solution at isoelectric point where protein charges are opposite each other, causing differences in solubility [6,7]. The separation of soluble protein from residue (insoluble protein, skin, thorn, scale and fat) was conducted by using centrifugation process [8, 9]. Acid and alkali method is superior to other method because it produces high yield and higher protein quality of FPC, as well as has an effective process to separate impurities such as fat, membranes, skin and bones [10]

This study aimed to investigate the physical and functional characteristics of fish protein concentrate (FPC) produced from Snakehead fish (*Channa striata*) by-product using acid and alkali solubilization methods.

2. Materials and methods

2.1Materials

Material used in the experiment was Snakehead fish (*Channa striata*) with body size of 38,5 cm length, 4,4 cm wide and 4,4 cm thick, while the fish average weight was 475,6 g. The fish was obtained from Merauke district waters, Indonesia. Other materials used in the experiment were hydrogen chloride (HCl) 0,1 N, natrium hydroxide (NaOH) 0,1 N, and distilled water.

2.2Methods

2.2.1 Production of FPC using acid and alkali methods

The snakehead fish were killed, filleted and the fillet was extracted at temperature of 80ºC for 30 minutes. The left meat residue from extraction process was re-extracted using acid-alkali solubilization methods to produce FPC [8] as following steps: the meat residue was grinded using food processor and dissolved in cool distilled water (±4˚C) with ratio of 1 : 5. The solution was then homogenized for 10 minutes using Ultra Thurax homogenizer and solubilized with with HCl 0,1 N and NaOH 0,1 N for 20 minutes under pH of 2, 3, 4 (for acid) and 10, 11, 12 (for alkali). After solubilization process was completed, a separation process was performed by centrifugation at 9000 rpm for 20 minutes. The precipitated residue was brought to isoelectric pH i.e 5.5 and dried subsequently at temperature of 70ºC and 60ºC for 1 hour respectively, and at 50ºC for 24 hours to obtain protein concentrate powder.

2.2.2 Analysis of FPC sample

The FPC was then analysed for its color and whiteness index, as well as functional properties. The functional properties being observed were including water holding capacity (WHC), water absorption capacity (WAC), fat absorption capacity (FAC), emulsion activity (EA) and emulsion stability (ES).

2.2.3 Color and whiteness index

The color of fish protein concentrate was measured with ColorFlex colorimeter. The sample was placed into the colorimeter dish and read for L, a* and b* values. The whiteness index was measured with whiteness meter Novasina. The standard was placed into the chamber and read, then the samples was read and recorded as whiteness index.

2.2.4 Functional properties

2.2.4.1 Water Holding Capacity (WHC) [11]

One g of sample was weighed and put into centrifuge tube which was known for its weight. 9 mL aquades was added into the centrifuge and homogenized using vortex mixer. The centrifuge tube was

covered with aluminum foil then incubated at 0 ºC for 15 minutes. The tube was then centrifuged at 3000 rpm for 20 minutes with a temperature of 20 ºC. The supernatant and unscathed liquids were separated, then the remaining liquid was measured again. The remaining water mixed with the supernatant was the volume of water absorbed.

$$\text{WHC} = \frac{\text{Volume of absorbed water (ml)}}{\text{Weight of sample (g)}}$$

2.2.4.2 Water Absorption Capacity (WAC) [12]
A g of sample was suspended in 10 mL distilled water, then homogenized with a vortex mixer for 1 minute. The solution was then centrifuged at 4000 rpm for 10 minutes at 26ºC. The supernatant was separated and the tube was weighed.

$$\text{WAC} = \frac{W2-W1\ (g)}{W0\ (g)}$$

Note :
W0 = Weight of dry sample (g)
W1 = Weight (Tube + Dry Sample) (g)
W2 = Weight (Tube + Residue) (g)

2.2.4.3 Fat Absorption Capacity (FAC) Analysis [12]
1 g of sample was weighed and suspended in 10 mL corn oil, then homogenized with a vortex mixer for 1 minute. The solution was then centrifuged at 2000 rpm for 5 minutes at 26ºC. The supernatant was separated and the tube was weighed.

$$\text{FAC} = \frac{W2-W1\ (g)}{W0\ (g)}$$

Note :
W0 = Weight of dry sample (g)
W1 = Weight (Tube + Dry Sample) (g)
W2 = Weight (Tube + Residue) (g)

2.2.4.4 Emulsifying Activity (EA) and Emulsifying Stability (ES) Analysis [13]
The sample was weighed 0.2 g, and it was suspended in 10 mL deionized distilled water (DDW) 25ºC, and 10 mL corn oil then homogenized with a vortex mixer for 1 minute. The solution was then separated into 10 mL each tube. One tube centrifuged at 6000 rpm for 30 minutes, while others were heated in the water bath at 80ºC for 30 minutes then cooled in the freezer for 30 minutes until the temperature reached 15ºC. The solution was centrifuged at 6000 rpm for 30 minutes. The height of the formed emulsion layer was compared with the height of overall emulsion (both heated or non-heated tube).

$$\text{EA} = \frac{\textit{Height of emulsion layer}}{\textit{Height of overall emulsion}} \text{ X } 100$$

$$\text{ES} = \frac{\textit{Height of emulsion layer after heated}}{\textit{Height of overall emulsion after heated}} \text{ X } 100$$

2.3 Analysis of data

The study was using Completely Randomized Design of experiment with two variables, namely acid and alkali solubilization methods and pH levels, three replicates were run for each variable. The obtained data were then statistically analyzed using ANOVA (One-way Analysis of Variance) at 5% level of significance ($p<0,05$) and continued with Tukey's test when a significant different was identified between samples.

3. Results and Discussion

3.1 Color and whiteness

Result of color measurement showed that the brightness value (L) of snakehead fish protein concentrate were (+) 84.43-85.54 with a* : (+) 0.2-1.8 and b* : (+) 19.17-24.74 (Table 1). Treatment using pH 11 resulted fish protein concentrate with the highest L value, i.e 85.54 but low a* and b* values, i.e 0.33 and 19.17, respectively. Based on color diagram measurement using Hunterlab ColorFlex EZ (Figure 1), L value shows level of brightness, while a*(+) shows red color and b*(+) shows yellow color. Thus, fish protein concentrate resulted in the study was detected to have a brownish yellow color with a high brightness level.

Table 1. The value of L, a*, b* of Snakehead fish protein concentrate produced by various pH treatment

treatment	L*	a*	b*	*yie
pH 2	84.43	1.80	24.74	47.24
pH 3	85.28	1.51	22.40	42.95
pH 4	84.98	1.16	21.73	41.64
pH 10	84.74	0.27	20.94	39.63
pH 11	85.54	0.33	19.17	36.25
pH 12	84.82	1.16	21.71	41.67

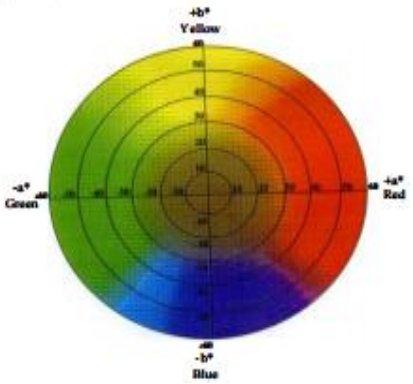


Figure 1. L*,a*,b* value reference for color test using ColorFlex Hunterlab

The result of whiteness showed that there was no significant difference between the treatment of pH (Table 2). The whiteness of the fish protein concentrate resulted in the study were ranging from 67.7-69.9%. According to the whiteness standard used in the measurement which revealed 85.4 as a standard value, it indicates that the whiteness of samples was still below of the standard. The highest value was obtained from treatment of pH 4 with whiteness 69.9%.

Table 2. Whiteness Index (WI) of Snakehead fish protein concentrate from various pH treatment

Treatment	Whiteness Index (WI) (%)
pH 2	68.75[a]± 0.63
pH 3	68.73[a]±1.89
pH 4	69.93[a]±1.83
pH 10	67.73[a]±1.41
pH 11	68.50[a]±1.35
pH 12	68.35[a]±1.48

3.2 Functional properties

Several functional properties of Snakehead fish protein concentrate being observed were water holding capacity, water absorption capacity, fat absorption capacity, emulsion activity and emulsion stability. Results of functional properties of Snakehead fish protein concentrate at various treatments are presented in Table 3.

Table 3. Functional properties of Snakehead fish protein concentrate at various pH treatments

Treatment	**Water Holding Capacity (%)**	**Water Absorption Capacity (%)**	**Fat Absorption Capacity (%)**	**Emulsion Activity (%)**	**Emulsion Stability (%)**
pH 2	6.90[a]±0.17	2.38[a]±0.32	1.15[a]±0.09	13.50[a]±3.78	56.43[b]±4.92
pH 3	6.73[a]±1.14	2.37[a]±0.20	1.12[a]±0.04	16.24[a]±1.99	58.12[b]±4.76
pH 4	6.87[a]±0.12	2.41[a]±0.04	1.12[a]±0.06	15.19[a]±0.33	57.14[b]±4.76
pH 10	6.50[a]±0.30	2.62[a]±0.28	1.15[a]±0.07	30.92[b]±1.01	30.16[a]±2.75
pH 11	6.90[a]±0.17	2.89[a]±0.84	1.06[a]±0.13	30.04[b]±1.27	32.37[a]±1.67
pH 12	5.33[a]±1.33	2.34[a]±0.12	1.10[a]±0.02	26.92[b]±3.85	35.38[a]±0.86

Based on the data above, water holding capacity values between treatment pH was not significantly different. Similarly, the water absorption capacity and fat absorption capacity were also not significantly different between treatments. Meanwhile, the emulsion activity of alkali pH treatments was significantly higher compared to acid pH treatments. On the contrary, the emulsion stability of acid pH treatments was significantly higher compared to alkali pH treatments. Protein emulsion activity is the ability of protein to take a part in the formation of emulsion and to stabilize emulsion that has been formed. Emulsion stability is capability of emulsion droplets. Emulsion stability is capability of emulsion droplets to keep dispersed without experiencing coalescence, flocculation and creaming. Several factors can affect protein emulsion properties including protein concentration, pH value, ionic charge (the present of salt that reduces emulsion stability) and heat treatment.

4. Conclusions

The results indicated that the whiteness index obtained from acid treatments were ranging from 68.7 to 69.9%; while for alkali treatments were from 67.7 to 68.50%. pH 11 resulted in FPC with the highest L value, i.e 85.54 but low a* and b* values, i.e 0.33 and 19.1, respectively. Acid solubilization method resulted in FPC with lower emulsifying activity but higher emulsifying solubility compared to alkali solubilization method. Both acid and alkali solubilization method can be used to produce FPC with insignificantly different physical and functional characteristics.

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