Protein Profile from Catfish (*Pangasius hypophthalmus*) and Baung Fish (*Hemibagrus nemurus*) Muscle from South Kalimantan

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Received: 9 October 2017, Accepted: 6 November 2017
Published online: 25 February 2018

Abstract: The purpose of the study was to determine protein profile from catfish (*Pangasius hypophthalmus*) and baung fish (*Hemibagrus nemurus*) muscle from South Kalimantan, Indonesia. The protein was extracted with Tris-EDTA buffer, followed precipitation with 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, and 70-80% of ammonium sulfate. The protein concentrations were analyzing using Bradford reagent, then the molecular weight of protein was performed on SDS-PAGE. The highest concentration of protein from catfish (4.24±0.03 µg/µl) and baung fish (4.25±0.03µg/µl) were observed in the 60-70% and 70-80% saturation of ammonium sulfate. Twenty of protein bands from catfish were shown in 10% resolving gel and have molecular weight 24 to 146 kDa, respectively. Fourteen of protein bands from baung fish have molecular weight 25 to 123 kDa, respectively. The bands were characterized as C-protein, gelsolin, actin, tropomyosin, troponin and myosin light chain. These results allow a better understanding of the protein profile of catfish and baung fish and for the basis of the development of an immunoassay for the detection of fish muscle.

Keywords: protein; catfish; Baung fish; molecular weight.

1. Introduction

Catfish (*Pangasius hypophthalmus*) and Baung fish (*Hemibagrus nemurus*) belong to the Sub family Siluridae have highest potency economically in South Kalimantan than others freshwater species. The local people was consumed the meets in fresh condition to serves as special menu like *Gangan Asam Banjar* and *Pais/Pepes*. Catfish and baung fish meet was reported has a promising source of omega-3 fatty acids (EPA, DHA), low fat food and high content of protein [1], [2]. Consumption of fish meets is recommended as one way to prevent cardiovascular and other diseases [3]. In addition, fish meets are good food sources that have antimicrobial peptides useful in defense against human pathogens [4].

Fish meet in this context can play an important role because it is cheaper than others animal protein [5]. Therefore, there is a need to produce and document protein profile information of two species from Sub family Siluridae. Such documentation may be used to evaluate the morphometric and genetic diversity of freshwater fish species [6]. The protein profile generally defined as a method for identifying protein expression on specific tissues, under certain conditions and times.

Such information can be used to understand the mechanisms of individual response to environmental changes at the protein level. Fish protein profiles can thus provide important information about the taxonomy, phylogeny and ecology of a species [7]. Freshwater fish protein profiles that have been reported among others in Channa species from Indonesia [8], minnow Popovo fish of Serbian origin [7]; fish *Labeo rohita* and *Cirrhinus mirgala* from Saudi Arabia [9]; *Catla catla*, *Labeo rohita* and *Thalapia mosambica* from India [10]; and *Channa gachua* fish from India [11].
Accordingly, no reported studies were found about the catfish and baung fish protein profile. So the objective of this work was to determine protein profile of two species based on their concentration and separation on polyacrylamide gels. The identifying major protein bands in this result will provide valuable tool in the development of diagnostic markers for fisheries conservation and management.

2. Material & Methodology

2.1. Sample Collection

Five live catfish (*Pangasius hypophthalmus*) and Baung fish (*Hemibagrus nemurus*) were collected from either at the landing centers or the local fish markets of South Kalimantan, then were brought to the laboratory in ice. Fishes were cleaned and filleted, then stored in freezer at −20°C.

2.2. Protein Extraction

Protein extraction procedures were performed using 0.5 M Tris pH 8.3 with 1 mM EDTA [7]. One part of filleted fishes were homogenized with 3 part of buffer extraction using blender for 5 minutes. The mixed then was centrifuged at 10,000 rpm for 10 minutes at 4°C. Collected supernatants were kept for protein precipitation using ammonium sulfate [12]. Aliquots (1 mL) supernatants were added with ammonium sulfate with the six gradient % saturations: 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, and 70-80%. The mixed was centrifuged at 10,000 rpm for 30 minutes at 0°C. The pellet was stored at -20°C until used for protein quantitation.

2.3. Protein Quantitation

The determinations of the protein concentration samples were analyzed using Bradford reagent (Amresco) with 0.5 mg/mL of Bovine Serum Albumin/BSA (HiMedia) as standard. The standard BSA curve was used as a reference to determine the protein concentration of the samples. The protein concentrations of BSA and the samples were measured the absorbance at 595 nm using a plate reader (SPECTROstar Nano-BMG Labtech).

2.4. Protein Molecular Weight Determination

Molecular weight determination of the protein samples was performed on SDS-PAGE according to Laemmli (1970) [13]. Protein bands were separated on 1 mm thick slab gel consist of 10 % resolving gel and 5 % stacking gel using Maxi Vertical Electrophoresis System, MV-20DSYS (Major Science). Each sample was mixed with 5x sample buffer, then 10 µl loaded in each wells. The samples were run on MV-20DSYS about 4 hours at 120 volt and 40 mA. The gel was stained with Coomassie Brilian Blue R-250 (HiMedia) for 30 minutes, and then continued with destaining solution for 30 minutes with shaking at room temperature. The gel was washed twice with deionized water until the bands of protein visible. The molecular weight (MW) of each band was determined by plotting the log MW vs. relative migration distance (Rf) of the MW Chromatein Prestained Protein Ladder (PR0602-Vivantis).

3. Results and Discussion

3.1. Result

This study was examined protein profile content of catfish and Baung fish. The protein concentrations from two fishes are measured using Bradford reagent, then purified by 6 ammonium sulfate fractions (Figure 1). The highest concentration of protein from catfish (4.24±0.03 µg/µl) and Baung fish (4.25±0.03µg/µl) were observed in the 60-70% and 70-80% saturation of ammonium sulfate. The proteins are separated on denaturing SDS-PAGE with 10% resolving gel. The protein bands are prestained and the protein patterns are compared (Figure 2.). Twenty of protein bands from catfish were show in 10% resolving gel and have molecular weight 24 to 146 kDa respectively. Fourteen of protein bands from Baung fish have molecular weight 25 to 123 kDa respectively.
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Figure 1. Comparison of protein content (µg/µl) in catfish (Pangasius hypophthalmus) and Baung fish (Hemibagrus nemurus) muscle with 6 gradient ammonium sulfate saturation (%)

Figure 2. Molecular weight (kDa) of protein muscle from catfish (Pangasius hypophthalmus) and Baung fish (Hemibagrus nemurus). L = Ladder protein; 1 = catfish; 2 = Baung fish

3.2. Discussion

The determination of protein profile from catfish (Pangasius hypophthalmus) and baung fish (Hemibagrus nemurus) muscle from South Kalimantan was done. The buffer extraction has been used in this study is 0.5 M Tris pH 8.3 with 1 mM EDTA. According to [7], protein from fish muscle can separate perfectly in Tris/EDTA buffer. This buffer solution gives great screening result, which the sarcoplasmic proteins had molecular weight higher than 34 kDa could appear on polyacrylamide gel electrophoresis.
Conventional purification methods with ammonium sulfate (AS) was performed, to obtain what is the % saturation give the highest protein concentration on each sample. The quantity of protein sample was measured using Bradford reagent. The highest concentration of protein from catfish (4.24±0.03 µg/µl) was found precipitate on 60-70 % AS, while for baung fish (4.25±0.03µg/µl) on 70-80% AS. According to [13], about 20-30% AS could precipitate unwanted protein, while higher saturation 60-70% will precipitate a lot of desired protein.

Polyacrylamide gels electrophoresis with 10% resolving gel have been studied to identification major protein of catfish and baung fish muscle. About six bands of protein called myosin light chain (15-25 kDa), troponin (30 kDa), tropomyosin (35 kDa), actin (42 kDa), gelsolin (90 kDa), and C-protein (140 kDa) were found. Sixth protein which appeared is numerous proteins are required for muscle contraction. According to [14], C-protein has function as myosin breakdown product, gelsolin as fragments actin filaments, actin as forms filaments, tropomyosin as strengthens actin filaments, troponin as regulates contraction, and myosin light chain as slide actin filament. Three major groups of muscle proteins which differ in functions and solubility properties are sarcoplasmic, myofibrillar and stromal proteins [7]. Myofibrillar proteins are the major proteins of fish muscle which found in this study, because they include myosin, actin and regulatory proteins tropomyosin, and troponin [15]. According to [16], catfish have protein contain actin, which if consumed works for muscle contraction, helps healing wound-injured, bone builder, and skin.

4. Conclusion

The highest concentration of protein from catfish (4.24±0.03 µg/µl) was found precipitate on 60-70 % AS, while for baung fish (4.25±0.03µg/µl) on 70-80% AS. About six bands of protein called myosin light chain (15-25 kDa), troponin (30 kDa), tropomyosin (35 kDa), actin (42 kDa), gelsolin (90 kDa), and C-protein (140 kDa) were found. Sixth protein which appeared is numerous proteins are required for muscle contraction.

Acknowledgement. This research is fully supported by Ministry of Research, Technology and Higher Education of the Republic of Indonesia 2017.

References

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