Protein Profile of Pangasius (*Pangasius hypophthalmus*) with Variations Before and After Wet Salting Based on SDS-PAGE

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Abstract. Pangasius *(Pangasius hypophthalmus*) is a source of high animal protein and a freshwater commodity that is widely cultivated in Indonesia. One of the disadvantages of pangasius as a food ingredient is that it rots easily. To avoid spoilage, the fish is preserved using wet salting. The purpose of this study was to describe the protein profile of pangasius based on variations in wet salt concentration using the Gel Electrophoresis (SDS-PAGE) method. The samples used were five parts of pangasius which were treated for 12 hours without salting treatment, three parts for wet salting treatment with a salt solution concentration of 10%, 20%, and 30% w/v for 12 hours, and fresh fish meat. The type of research is experimental from profile protein and organoleptic tests. This research was a descriptive analysis by looking at the result of SDS-PAGE. The results showed that the highest concentration of protein is 27,92 µg/µL after 10% b/v of wet salting treatment compared to pangasius before salting treatment. The best protein profile was a concentration of 10% because the protein weights ranging from 249-48 kDa were almost the same as fresh pangasius. The conclusion is the best concentration after wet salting was the concentration of 10% because the protein concentration, organoleptic tests, and protein profile were almost the same as fresh pangasius.

Keywords: Pangasius, Protein Profile, wet salting, SDS-PAGE.

1. Introduction

Protein is one of the macronutrients needed for the growth and development of the human body, especially during the growing age period [1]. Protein has a function as a building and regulating substance. Based on the source, protein is divided into two, namely vegetable protein and animal protein. Vegetable protein comes from plants, for example nuts, tofu, tempeh, oncom and soy sauce. Animal protein comes from for example fish, chicken, beef, cheese, squid, shrimp, and eggs. Animal protein has high digestibility so the amount that

G. Setya Ayu Putri et al. (eds.), *Proceedings of the 2nd Lawang Sewu International Symposium on Health Sciences: Medical Laboratory Technology (LSISHS-MLT 2023)*, Advances in Biological Sciences Research 40,
https://doi.org/10.2991/978-94-6463-457-0_9

can be absorbed into the body is also high and the composition of amino acids is more complex than vegetable protein [2]. Fish is an animal protein that contains various substances and the absorption of fish protein is higher compared to other animal products such as beef or chicken because fish meat has shorter protein fibers than beef or chicken protein fibers [3]. Fish protein is also the largest group of animal protein, namely around 57.2% compared to other animal groups, one of which is pangasius (*Pangasius hypophthalmus)* [4].

Pangasius (Pangasius hypopthalmus) is one of the freshwater commodities that is widely cultivated in Indonesia, spread across parts of Sumatra, Kalimantan, and Java [5]. Pangasius production figures in Indonesia have increased from year to year, starting from 2015, it was 339,069 tons, in 2016 it increased to 437,110 tons, in 2017 it increased to 578,344, and in 2019 it increased to 1,149,400 tons, until now it continues increased quite significantly [6]. A fish fillet is a slice of meat without scales, bones, skin, gutted, and tail removed [7].

So that pangasius meat does not rot easily, efforts are made to maintain freshness and extend the shelf life of catfish by using preservatives by salting. Salting is an ancient process of preserving materials that is still used today. In general, there are three methods of salting, namely dry salting, wet salting, and mixed salting [8]. Salting in general is dry salting, but in this study, we tried wet salting because wet salting is economically cheaper and easier to obtain, and wet salting can contain a lot of fish and the salt absorbs more evenly into the fish flesh [9].

Salt is an ingredient used as a preservative because it can increase the osmotic pressure that causes it to occur plasmolysis in microbial cells, and unwanted microorganisms can be inhibited [10]. Protein denaturation is a process where proteins lose their tertiary structure and secondary structure by the application of external pressure or compounds, such as strong acids or weak acids, concentrated inorganic salts, organic solvents or heat. Most biological proteins lose their biological function when denaturation occurs [11].

The characteristics of the protein profile can be determined using the SDS-PAGE electrophoresis method (Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis), which is a type of electrophoresis used to separate polypeptide chains in proteins based on their ability to move in an electric current. The purpose of this research was to describe the protein profile of pangasiuss based on variations in wet salt concentration using the Gel Electrophoresis (SDS-PAGE) method.

2. Material and Methods

2.1. Research Design

This research was conducted from April to May 2022. This research was carried out at the Molecular Biology Laboratory, Universitas Muhammadiyah Semarang. This research method is qualitative with an experimental design. The variable of this research is the protein profile of Pangasius with variations before and after wet salting at a concentration of 10%, 20%, and 30% for 12 hours. The protein profile of pangasiuss is the protein sub-units in pangasiuss obtained using the SDS-PAGE method.

The data collection technique used in this research is primary data and the data obtained is tabulated and then presented in the form of a descriptive narrative.

2.2. Tools and Materials

The tools used in this research is the type of research used analytical balances, mortar plates, vortex (VM-300), conical tubes, centrifuge, microtube, tip, micropipette, visible spectrophotometer, Electrophoresis ATTO WSE-1150 PageRunAce, dry bath, rotator, plastic press.

The materials used in this research were pangasius, table salt in the form of blocks made in solution form, Aquadest, BSA(Bovine Serum Albumin), BPA (Biorad Protein Assay), polyacrylamide 30%, TEMED, APS 10%, SDS 10%, 1,5 M Tris Ph 8,8 dan staining 0,1%, Coomassie Brilliant Blue (CBB) R-250, destaining, glacial acetic acid 10%, butanol, alcohol 70%, running buffer 1x, Dh2Sterile O, PBS Ph 7.4, sample buffer, and protein marker.

2.3. Salting Pangasius Fish Fillets

A total of 3 fresh pangasius fish (eyes clear, fish flesh feels fresh when pressed) were prepared. Next, the fish scales are cleaned; the gills and entrails are removed, and then washed with running water so that the fish is completely clean. Fish that have been washed too late are drained in a plastic basket. Then the fish is made into fillets, by splitting the fish from head to tail without causing the back to be cut. Fillets are made by slicing the ribs longitudinally to produce boneless meat. Pangasiuss were taken from the stomach area of the fish and then weighed ± 10 grams.

Pangasiuss that have been weighed 10 g are then soaked in 100 ml of salt solution with a concentration of 10%, 20%, and 30% w/v for 12 hours, and then labeled according to the concentration and duration of soaking in each soaking place. After 12 hours the fish fillet is removed and drained until completely drained of the salt solution.

2.4. Determination of Total Protein Content and Protein Separation of Pangasius using SDS-PAGE

Took 3 grams of catfish fillet samples that had been soaked in a salt solution and cut them into small pieces, ground them in a mortar cup, added PBS 1x then homogenized, put the sample in a conical tube and added PBS 1x more to the amount of ±7 ml. Then centrifuged at a speed of 3000 rpm for 15 minutes at a temperature of 4ºC, the sample that had been centrifuged was then taken from the supernatant, the supernatant was protein. Then the absorbance was read on a spectrophotometer ƛ 595 nm, then plotted on a protein standard curve.

SDS-PAGE procedure by manufacturing method resolving gel 12% is made and put in glass plate, and added with butanol to level the surface resolving gel. Furthermore, stacking gel entered above resolving gel quickly, and the comb was inserted on top. Wait for the gel until polymerization occurs. The comb is lifted from above stacking gel slowly.

Glass plate which already contains the gel is put into the ATTO electrophoresis chamber, the buffer electrode is inserted into it until the top and bottom of the gel are submerged, 10 µl of marker and the stock sample. Sample separation is made by using a stock sample, 1x PBS, 4 µl of buffer sample is pipetted according to The total calculation between the sample, PBS 1x, and sample buffer is 20 µl. A total of 20 µl was added to the wells on the gel and 10 µl of marker into the wells. then the ATTO WSE-1150 PageRunAce electrophoresis device was turned on and set to standard 2 for 160 minutes until the bromphenol blue reached the bottom of the stacking gel, after the bromphenol blue reached the bottom of the stacking gel, the electric current was turned off. The gel is removed from the printer slowly so that the gel does not tear.

The gel that has undergone electrophoresis is then placed in a staining solution, then incubated while shaking for approximately 2-3 hours until the protein bands are stained. Next, to remove the color from the gel which does not contain protein, it is given a destaining solution. The destaining solution was changed 3-4 times until the gel looked clean. When the gel is clean, the washing is stopped and the destaining is replaced with a 10% glacial acetic acid solution. Then gel it on a press using plastic and dry it for 48 hours in a dark room.

Determination of the molecular weight of the desired protein is calculated using Rf and plotted on a logarithm graph of the Rf of the protein marker whose molecular weight is not yet known. In this study, gel analyses Electrophoresis SDS uses GelAnalyzer software, so lines and bands can be detected automatically, and get more accurate Rf values and molecular weights.

3. Result and Discussion

In this study, the spectrophotometric method was used to determine the concentration of pangasiuss before and after wet salting with concentrations of 10%, 20%, and 30% for 12 hours. The results of spectrophotometer observations are shown in Table 1.

Table 1. Total protein of Pangasius.

No.	Salting concentration (% w/v)	Salting time (hours)	Total protein (µg/µl)
1	Fresh	0	31,75
2	Without salting	12 jam	24,80
3	10	12 jam	27,92
4	20	12 jam	25,40
5	30	12 jam	19,34

Based on measurements of the total protein of pangasiuss using a spectrophotometer with a wavelength of 595 nm, it was found that the highest value was for pangasiuss fresh or before wet salting at 31.75 µg/µl when compared to pangasiuss that were given 10% wet salting. 20%, and 30% w/v with a salting time of 12 hours of 27.94 µg/µl, 25.40 µg/µl, and 19.34 µg/µl. Meanwhile, the total protein of pangasiuss without treatment or left for 12 hours was 24.80 µg/µl. So it can be concluded that pangasiuss that have been treated with varying concentrations of wet salting and without treatment or left for 12 hours have a lower total protein concentration when compared to the total.

Table 2. Assessment of Pangasius Organoleptic Test.

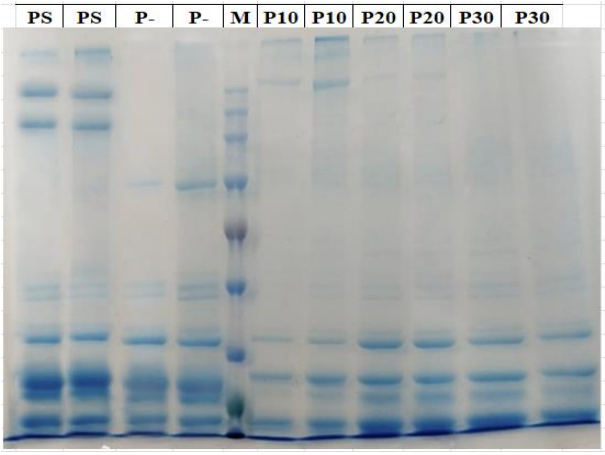
Concentration (% v/v)	Organoleptic		
	Appearance	Texture	Smell
0 (fresh)	Whole, clean, brilliant cut of meat	Very fresh, specific type of freshwater fish	Solid, compact, supple
0 (24 hours incubation)	The cut of meat is not intact, clean, less bright, lots of damage	The smell is not fresh, the smell is starting to rot	Starting to become soft, not compact, less elastic
50	The incision is intact, clean, slightly pale in color, slightly damaged	Salt neutral	Dense, less compact, not chewy (hard)
75	Creamy meat incision, pale color, clean, much damaged	Salt neutral	Solid, compact, not chewy (hard)

100	Creamy cutlet, very pale color, clean, lots of damage	Salt neutral	Dense, not compact, not chewy (hard)

The organoleptic test, which examines the look of the meat slices intact, clean, bright, and possessing a distinct fresh fish fragrance, as well as their compact, dense, and chewy texture, was conducted on fresh pangasius or before to wet salting in this study. In the meantime, the pangasius total protein content decreased after they were left for 12 hours without being salted. The meat slices were no longer whole, clean, or bright, and their fragrance had started to deteriorate. Additionally, their texture had become softer and less elastic. Thus, it is evident that organoleptic tests for fish fillets that are either untreated or left for 12 hours decrease. Dead fish are left unattended; they can rot rapidly and cause protein degradation, which is indicated by the development of a rotten odor. This is because sulfur-containing amino acids transform into hydrogen sulfide [3].

The results of organoleptic testing on pangasius soaked in a 10% salt solution showed that the incision was clean, unbroken, and appeared somewhat pale. It also smelled fresh and dirty, had a dense texture, and was less compact and rigid. When pangasius were immersed in a 20% salt solution, organoleptic testing revealed that the incision was in a clean, unbroken, and had a pale hue. It also smelled fresh and slightly muddy and had a dense texture that was less compact and firm. Organoleptic examinations of pangasius immersed in a 30% concentration of salt solution revealed that the incision appeared intact, clean, extremely pale in color, fresh and odorless, dense, less compact, and extremely rigid.

Analysis of the protein profile of pangasius before and after wet salting at concentrations of 10%, 20%, and 30% for 12 hours.



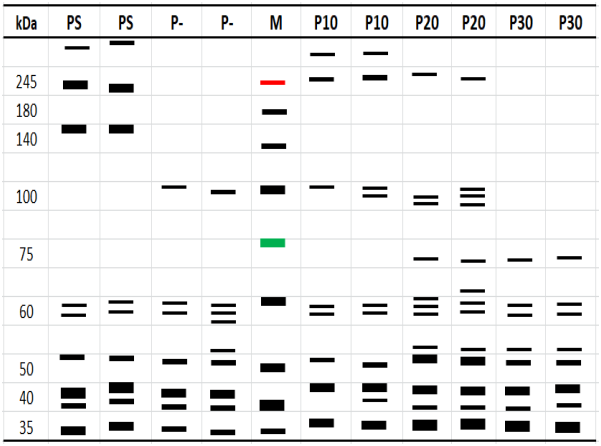


Fig. 1. Results of electrophoresis SDS-PAGE and visualization of protein profiles in pangasiuss of the same size and size of 10 grams were taken from the stomach of the fish before and after wet salting with varying concentrations of 10%, 20%, and 30% w/v for 12 hours.

M : Marker protein
PS : Control (fresh pangasius)
P- : Pangasiuss are left for 12 hours
P10: Pangasius soaked in 10% salt solution for 12 hours
P20: Pangasius soaked in 20% salt solution for 12 hours
P30: Pangasius soaked in 30% salt solution for 12 hours

Pangasiuss that were salted, unsalted (fresh), and left for 12 hours were analyzed for total protein with 12% SDS-PAGE along with determination of protein molecular weight by inserting photos or pictures gel SDS-PAGE electrophoresis results containing protein bands into the GelAnalyzer Application.

The Rf value and Molecular Weight (BM) of pangasius samples that have been treated before and after wet salting with varying concentrations of 10%, 20%, and 30% w/v for 12 hours will be automatically known using GelAnalyzer software, The following are known major and minor bands as shown in the table below.

Table 3. Molecular weight of pangasius protein before and after wet salting

Code	Pita Protein		Molecular Weight (kDa)	
	Mayor	Minor	Mayor	Minor
PS	6	3	245, 175, 48, 45, 44, 43	400, 55, 53
P-	4	4	47, 45, 44, 43	97, 58, 55, 53, 49
P10	4	7	249, 48, 44, 43	347, 104, 97, 55, 53, 49, 48

P20	4	9	47, 45, 44, 43	266, 108, 100, 95, 62, 57, 54, 52, 48
P30	3	5	47, 45, 43	63, 54, 52, 48, 44

4. Discussion

Finding out the *pangasius* (*Pangasius hypophthalmus*) protein profile both before and after wet salting was the goal of this study. The pangasius protein may become denatured as a result of the wet salting procedure. A spectrophotometer and SDS-PAGE can be used to measure the protein concentration in order to determine the protein profile in this research sample. Pangasius has a weakness in that it rots easily and must be preserved by salting wet in order to prevent spoiling. Despite having relatively high protein levels, pangasius contains all the essential amino acids and higher levels of lysine and arganine than protein from meat and milk [12].

In this investigation, the total protein content of pangasius that had been left for 12 hours without being salted was 24.80 µg/µl, whereas the total protein content of fresh pangasius, or before wet salting, was 31.75 µg/µl. Thus, it is evident that the fish fillets' overall protein content has dropped after being left untreated or for 12 hours. The presence of microorganisms that can break down protein causes fish protein that has died quickly to deteriorate, lowering the protein content of fresh protein fish [3]. After the fish was left for 12 hours, the amount of total protein decreases. Following salting, it was discovered that pangasius soaked in a 10% salt solution had a total protein content of 27.94 µg/µl. When compared to other salting concentrations, it has the highest protein concentration. The pangasius's total protein content was decreased at the highest concentration of the salting process. This demonstrates that the concentration decreases with increasing salt concentration used to soak pangasiuss [13]. According to the study's findings, using salt at a 30% concentration is not advised since it significantly lowers the levels of total protein.

The amount of salt used and the length of the salting period can have an impact on the fish's texture, appearance, and smell because the longer the salting period and the higher the concentration of salt used, the less water the fish flesh contains, giving the fish a more distinct smell. The fish will vanish, the texture will get rougher, and the appearance will turn pale. Thus, the study's findings indicate that the pangasius that results from salting has a harder texture a higher the concentration of the salt solution [14].

In this study, fresh pangasius yielded six major bands and three minor bands when tested for protein molecular weight; pangasiuss left for 12 hours without salting produced four major bands and four minor bands. As a result, the protein bands in the fish fillets have shrunk even after a 12 hour rest or treatment. When microorganisms that break down protein are present, the fish protein that has perished rapidly deteriorates. This causes protein damage, which is indicated by the development of an unpleasant odor because sulfur-containing amino acids have converted to hydrogen sulfide. After that, the pangasiuss were wet salted, and the number of protein bands four major bands, and seven

minor bands of the fish fillets soaked in a 10% concentration of salt solution was determined. Four major bands and nine minor bands were identified among the protein bands of pangasiuss soaked in a salt solution with a 20% concentration of salt solution. Three major and five minor protein bands were identified after pangasius were soaked in a salt solution with a 30% concentration. Therefore, it is evident that pangasius that are wet salted at concentrations of 10%, 20%, and 30% have thinner and smaller protein bands.

The salting process causes a decrease in protein solubility. This occurs due to the formation of cross-links from disulfides, causing protein solubility to decrease. Using the right salt level will bind the protein so that solubility does not increase [15]. The salt content used of 10% can prevent protein damage in the salting process, so the greater the salt concentration, the lower the protein content. Salt affects the stability of protein structures. This is due to the ability of salt to bind water strongly by changing the hydration properties of proteins. At low concentrations, the salt stabilizes the protein structure due to increased protein hydration and is weakly bound to the protein. On the other hand, salt can also cause instability in protein structures because it reduces protein hydration yields and binds strongly to proteins [16].

The effect of salt to stabilize or destabilize protein structures is related to its concentration and effect on water. The increase in protein stability at low salt levels is caused by increased hydrogen bonding between water molecules. On the other hand, at high concentrations, salt denatures proteins because it damages the water structure so that water becomes a good solvent for nonpolar protein residues [16]. So, for wet salting, a concentration of 10% w/v is recommended because the protein contained in the fish has a slight change in protein bands. This is supported by research conducted by Suardi, et al [9] which states that the higher the salt concentration and the longer the salting time, the higher the level of protein denaturation and the smaller the protein molecular weight, indicated by the thinning of the major protein bands into minor protein bands. . So it is important to use the right level or concentration of salt to avoid protein denaturation.

5. Conclusion

Based on the research results, it can be concluded that wet salting at a concentration of 10% for 12 hours is the most recommended because the protein contained in the fish changes slightly in the protein content. Meanwhile, wet salting at a concentration of 30% for 12 hours is not recommended because many major protein bands have been denatured into minor protein bands. The higher the salt concentration and the longer the salting time, the higher the level of protein denaturation which is characterized by the thinning and disappearance of the protein profile bands.

Authors' Contributions. Meutia Srikandi Fitria carried out the determination of molecule weight and compiled a publication manuscript. Roma Dhona Beauty Zakia performed

protein isolation and sample treatment. Aprilia Indra Kartika carried out the measure of the protein concentration. Aditya Rahman Ernanto performed SDS-PAGE.

Acknowledgments. This research received Dosen Pratama Grant from LPPM Universitas Muhammadiyah Semarang, Indonesia.

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