Manufacture of Toman Fish Protein (*Channa micropeltes*) Isolate with Different pH Methods using Star Fruit (*Averrhoa bilimbi*) Acid

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Abstract. Toman fish (*Channa micropeltes*) is a type of freshwater fish that belongs to the genus Channa which is the largest size compared to the others and has high protein. This study aims to obtain the isolate of toman fish protein (*Channa micropeltes*) by the method of pH regulation using star fruit acid. Wuluh star fruit acid is used as a substitute for HCl in making fish protein isolates to reduce pH. This research method uses a Completely Randomized Design (CRD). The treatments consisted of (P1 = pH 4, P2 = pH 5, P3 = pH 6). The results showed that star fruit acid can replace HCl as a protein precipitant and P1 (pH 4) obtained the most isolates, namely an average protein content of 91.64% (db), a moisture content of 1.67%, ash content of 2.06% (db), fat content of 2.74% (db), yield of 88.46%, and total amino acid composition of 61.30%, where the type of amino acid that dominates is glutamic acid (12.30%). Glutamic acid amino acid is the one that influences a lot in giving pH isoelectric point, in the treatment of P1 with pH 4 making isolates of toman fish protein to be the best treatment, because glutamic acid has an isoelectric point of 3.22 which is the closest to pH 4 plus also the pH of isoelectric point from other amino acids.

1. Introduction

Toman fish (*Channa micropeltes*) is the dominant freshwater fish caught in Riau's public waters. The catch of toman fish was in 2015 with a production volume of 520.9 tons [1]. Toman fish is included in the genus Channa which is the largest compared to the others. These large fish are usually less preferred to be cooked directly but are preferred if processed in a preserved form in the form of dried salted fish or wadi/bekasem [2].

The results showed that the nutritional content of fresh toman fish was quite high, namely; water (74.1%); protein (18.92%); fat (5.23%); and ash (0.94%) [2]. One of the uses of toman fish is by isolating its protein. The protein isolation method is the purest form of protein. Isolates were made by the process of removing non-protein components. The protein content is 90% (db) or more, and this product is almost free from carbohydrates, fiber, and fat so that its functional properties are much better than other forms of protein [3].

Protein isolates are made by precipitating protein in which heating and addition of acid are carried out to reach a certain pH (isoelectric pH), after clumping the precipitate (protein) obtained is separated from the liquid (starch). In the manufacture of protein isolates, the acid used can be a strong acid or a weak acid. The type and concentration of this acid will affect the amount of protein that can be extracted in the manufacture of protein isolates [4].

The manufacture of fish protein isolates using synthetic (inorganic) acid solvents based on chemicals causes the results of protein isolates to be less desirable to be directly applied to food products. The solvent commonly used in the manufacture of protein isolates is a strong acid...
solution of HCl, therefore it is necessary to replace synthetic acid-base solvents with natural or organic acids to make them more desirable.

Star fruit juice has acidic properties and has a pH value of 2 [5]. Belimbing wuluh is thought to be used in the manufacture of fish protein isolates as a substitute for HCl for the protein deposition process. Based on the explanation above, the authors are interested in researching on the use of natural acids in the form of star fruit in the manufacture of toman fish protein isolates with different pH methods to produce the best protein isolates and can be directly fortified into food products.

This study aimed were to determine: (1) to obtain the chemical composition (proximate) of meat and toman fish meal and to obtain the maximum acid pH of star fruit wuluh, (2) to obtain the best pH with the addition of acid star fruit juice to precipitate protein in the manufacture of isolates, chemical content, yield and amino acid composition of toman fish isolate.

2. Material and methods

The main material used in this research is toman fish (Channa micropeltes) obtained from the Teratak Reed Market and wuluh star fruit. proximate analysis such as total protein analysis using the Kjeldahl method, fat content using the Soxhlet method, ash content [6], moisture content, ash content [6], and carbohydrates (by difference). The tools used Soxhlet, thermometer, pH meter, stirrer bar, Kjeldhal flask, Erlenmeyer flask, fat flask, filter paper, porcelain dish, oven, desiccator, etc.

2.1. Material and Equipment

This research was carried out in two experimental stages, namely: 1) Preparation of materials (preparation of mashed meat, Toman fish flour, and starfruit), yield calculation, analysis of the chemical composition of meat and Toman fish meal, extracting star fruit juice, and checking the maximum pH of star fruit. 2) Production of toman fish protein isolate by extracting acid pH using natural acids in the form of star fruit, calculating the number of isolates, yield, analysis of chemical composition (proximate) of toman fish isolate, and total amino acid composition.

2.2. Method

The collected data from each treatment were analyzed using analysis of variance (ANOVA).

3. Results And Discussion

3.1. Sample preparation

The shape of the toman fish used as raw material can be seen in Figure 3.

Figure 1. Toman fish (Channa micropeltes)

The morphology of the toman fish in this study is that it has a long body, a slightly convex back, a flat ventral part, and a flat head. It has a dark brown almost black color, patterned with the deeper part of the belly the more white it is and loses its color. All fins are black. This fish is in the juvenile to adult phase as seen from the color of the fish which is dark brown to black and there is a black tinge that begins to fade and forms a new pattern in the back area. This fish has a size of 450-500 g.
The next sample preparation is the wuluh star fruit sample which is used to adjust the pH in the toman fish isolate maker. This preparation aims to obtain star fruit juice by crushing it using a blender and then filtering it so that the juice can be separated from the pulp, then checking the optimal acid pH.

Checking the optimal pH of star fruit acid using a pH meter shows the number 2 which is considered suitable as a pH regulating solution so that the pH in the manufacture of toman fish protein isolate can be adjusted in an acidic environment to pH 4, pH 5, and pH 6. The raw materials can be seen in Figure 4.

![Figure 2. Star fruit](image)

The star fruit used for extracting its juice is a ripe star fruit that is marked by its yellowish-green color. As stated by [7], that when ripe it is yellowish-green in color, has a lot of water and tastes sour. The selection of ripe star fruit is based on the amount of water produced from the young and the pH of ripe star fruit is suitable to be used as a pH regulator in the manufacture of toman fish protein isolate.

3.2. Yield and chemical composition of meat and toman fish flour

Yield is the percentage ratio of the number of products produced from materials that can be utilized. The results of the calculation of the yield of toman fish meal are presented in Table 1.

| Sample | Mashed meat (g) | Dried meat (g) | Fish flour (g) | Yield flour (%) |
|--------|-----------------|----------------|---------------|----------------|
| 1.     | 1410            | 300            | 280           | 93.33          |
| 2.     | 1380            | 320            | 290           | 90.62          |
| 3.     | 1440            | 310            | 290           | 93.54          |
| Average| 1410            | 306.67         | 268.66        | 92.49          |

Yield is calculated by dividing the total final product by the initial raw materials, then multiplied by one hundred percent. The results showed that the average yield produced was about 92.49%. The high or low yield is caused by drying and sieving. This yield is relatively high due to the drying temperature and drying time so that the water in the meat can evaporate properly and produce dry meat. Dried meat doesn’t leave too much meat that can’t pass the filter during sifting.

This is in accordance with the statement of [8], the high or low yield of fish meal products can be influenced by the handling at the time of milling, sieving and the level of freshness of the fish processed. The higher the yield, the more profitable from an economic point of view [3]. The appearance of the toman fish meat meal is as shown in Figure 3.
The flour characteristics are bright yellow in appearance, smooth texture, and the characteristic smell of dried fish. According to [9], the appearance of color in a fish meal has a yellow-brown color and the particle size can pass through a 40-60 mesh sieve. Chemical analysis of fish meat and toman fish meal. This study aims to determine the content of water, ash, protein, and fat used as test parameters in the manufacture of toman fish protein isolate.

Table 2. Results of chemical analysis of toman fish meat flour.

| Chemical composition       | Percent (%) |
|----------------------------|-------------|
| Moisture                   | 9.06        |
| Protein (db)               | 79.26       |
| Ash (db)                   | 7.51        |
| Fat (db)                   | 3.07        |
| Carbohydrate (by difference) (db) | 10.16 |

Based on the analysis of toman fish meat meal Table 2 shows that Toman fish meat meal is in quality I which refers to SNI 01-2715-1996 which has a moisture content (maximum) 10%, crude protein content (minimum) 65%, ash content (maximum) 20% and fat content (maximum) 8%.

Fish meat meal protein obtained by 79.26% the results decreased compared to fish meat protein which amounted to 82.98% this was due to the presence of protein that was dissolved in the water so that in the drying process it disappeared. The type of protein that dissolves in water is protamine. Protamine is the simplest type of protein compared to other types of protein, this protein is soluble in water and is not coagulated by heat [10].

3.3. Toman Fish Protein Isolate (Channa micropeltes)

The addition of star fruit juice was used to achieve the desired treatment pH to lower the pH. Toman fish protein isolates using sour star fruit require the addition of star fruit juice as much as 56 mL to achieve a pH of 4 at P1, P2 requires the addition of 40 mL to reach pH 5 and the P3 requires 20 mL to achieve a pH of 6.

The number of additions The acid of star fruit wuluh is adjusted to the pH value of fish meal, the more acidic the pH of the flour will be the less need for star fruit juice to achieve the desired pH in the manufacture of fish protein isolate. The addition of wuluh star fruit juice with different levels at each decrease in pH is one of the causes of the different characteristics of the protein isolates of toman fish produced. The physical appearance of the toman fish protein isolate can be seen in Figure 4.

Figure 3. Toman fish meat flour.
Figure 4. The results of protein isolate toman fish (*Channa micropeltes*) a) pH 4; b) pH 5; c) pH 6

The resulting isolate has a different physical appearance, the protein isolate of toman fish with first treatment is bright yellow-brown, has a slightly rough texture, and has a distinctive smell of dried fish coupled with the smell of star fruit. so that it smells fresh and not too fishy like the smell of the previous toman fish meat flour.

The characteristics of the pH 5 treatment were slightly darker than the pH 4 treatment, the texture was coarser and smelled the same as the pH 4 treatment, while in the pH 6 treatment the protein isolate was brown in color, had a rough texture and there were large clumping particles and a fishy smell. which is dried.

3.4. The yield of toman fish protein isolate

During the heating and pH adjustment process, clumping occurred which resulted in protein isolate. Precipitation of protein by acid occurs quite quickly in the presence of heat. First of all, precipitation will occur, namely the formation of precipitates or small particles that float in solution and can settle in a short time [11]. The results of toman fish protein isolate with different acid pH treatments can be seen in Table 3.

| Sample | Weight sample average (g) | Isolate amount average (g) | Yield (%) |
|--------|---------------------------|---------------------------|-----------|
| P₁     | 10.05                     | 8.89±0.30                 | 88.46     |
| P₂     | 10.10                     | 9.58±0.20                 | 94.82     |
| P₃     | 10.12                     | 10.07±0.08                | 99.45     |

This is because the final product used by the manufacture of toman fish protein isolate using an acid pH is precipitate. Proteins are coagulated by acid which results in protein deposition and low yields due to the condition of the solution pH which reaches the isoelectric point of most of the amino acids in toman fish meat meal. The high yield of toman fish protein isolates produced by P₂ and P₃ thought to be caused by the pH of the solution which was far from the isoelectric pH of most of the amino acids in toman fish meat flour.

[4], stated that the further the difference in pH from the isoelectric point, the more protein solubility will increase. The increased protein solubility causes the yield obtained to be even greater. This also occurs in the acidifying treatment using star fruit, that the yield value obtained decreases as the concentration of star fruit extract is added, the more the concentration of star fruit, the lower the resulting pH [12]. The results of the chemical composition of toman fish protein isolate with different acid pH treatments can be seen in Table 5.
| Treatment | Chemical composition |
|-----------|---------------------|
|            | moisture (%) | Ash (%db) | Fat (%db) | Protein (%db) |
| P₁         | 1.51±0.20ᵇ     | 1.49±0.20ᵇ | 91.41±0.08ᵇ | 2.80±0.14ᵇ    |
| P₂         | 1.70±0.17ᵇ     | 2.06±0.19ᵇ | 90.28±0.03ᵇ | 2.11±0.23ᵇ    |
| P₃         | 1.70±0.09ᵇ     | 2.81±0.20ᵇ | 86.63±0.18ᵇ | 3.69±0.30ᵇ    |

The results in Table 4 show that the use of star fruit acid as a substitute for HCl in the manufacture of toman fish protein isolate has a very significant effect on chemical content, especially on protein content. This happened because in treatment P1 the addition of star fruit juice was the most, which was 56 mL, and heating and stirring brought the protein to its isoelectric point state, where the pH was close to the isoelectric point of the amino acid glutamic acid which was most abundant in toman fish.

The more concentration of H⁺/acid added, the closer the filtrate is to the pH of the isoelectric point of the protein, where the amino acids will be in the most dipolar state or zwitter ions. In this situation the protein solution in water is the smallest so that the protein will clot and settle [4].

Treatment P1 by adjusting the pH 4 being the best treatment in the manufacture of toman fish protein isolate because the protein produced reaches more than 90% (db). According to [3], the protein content of isolate is 90% (wk) or more, and this product is almost free from carbohydrates, fiber, and fat so that its functional properties are much better than other forms of protein.

3.5. Amino Acid Analysis
The results of the analysis of the levels and types of total amino acids in toman fish protein isolate are presented in Table 5.

| No. | Amino acid group | Amino acid types | Toman fish protein isolate (%) |
|-----|------------------|------------------|--------------------------------|
| 1.  | Arginin          |                  | 1.68                           |
| 2.  | Histidin         |                  | 2.95                           |
| 3.  | Isoleusin        |                  | 3.78                           |
| 4.  | Leusin           |                  | 6.04                           |
| 5.  | Amino esensial   | Lisin            | 4.95                           |
| 6.  | Metionin         |                  | 1.64                           |
| 7.  | Penilalanin      |                  | 2.69                           |
| 8.  | Treonin          |                  | 1.72                           |
| 9.  | Valin            |                  | 3.42                           |
| Total |                  |                  | 28.87                          |
| 10. | Amino non esensial | Asam aspartat  | 4.27                           |
| 11. | Amino glutamat   |                  | 12.30                          |
| 12. | Alanin           |                  | 1.68                           |
| 13. | Glisin           |                  | 5.04                           |
| 14. | Prolin           |                  | 4.29                           |
| 15. | Serin            |                  | 1.69                           |
| 16. | Sistein          |                  | 1.41                           |
| 17. | Tirosin          |                  | 1.76                           |
| Total |                  |                  | 32.44                          |
| Total |                  |                  | 61.30                          |

The results of the amino acid analysis showed that the amino acid glutamic acid was the most influential in giving the isoelectric point pH, in treatment P1 with pH 4 the manufacture of toman fish protein isolate was the best treatment because glutamic acid had an isoelectric point of 3.22 which was closest to pH 4 plus the isoelectric point pH of the other amino acids. According with [13] that the isoelectric point of albumin is at pH 4.55-4.90 which is according with the treatment of making the best Toman protein isolate P1(pH 4).
At a certain pH called the isoelectric point (range 4-4.5), the charge of the free amino and carboxyl groups will neutralize each other so that the molecule has a zero charge. Each type of protein has a different isoelectric point. The fastest deposition occurs at this isoelectric point, and this principle is used in processes of separation and purification of proteins [14]. The isoelectric point is the moment at which the amino acid pH is in an amphoteric form (zwitter ion), and at this isoelectric point the protein solubility decreases and reaches the lowest number, the protein will be precipitated.

4. Conclusion
Based on the research results, the chemical composition of toman fish meat In this study, the water content was 80.25%, protein 82.98% (db), ash 4.68% (db), fat 4.59% (db) and carbohydrates by difference 7.75% (db). The chemical composition of toman fish meal In this study, water was 9.06%, protein 79.26% (db), ash 7.51% (db), fat 3.07% (db), carbohydrates by difference 10.16% (db). The star fruit used in this study has a maximum pH of 2 which can be used in the process of increasing the pH in the manufacture of toman fish protein isolate.

Based on the results of the study, pH 4 was the best treatment in making toman fish protein isolate using star fruit juice with an average protein content of 91.64% (db), water content 1.67%, ash content 2.06 % (db), fat content 2.74% (db), 88.46% yield, and total amino acid composition of 61.30%.

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