

Effect of pH and Extraction Time on Isolation Proteins from Red Kupang (*Musculita Senhousia*)

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Effect of pH and Extraction Time on Isolation Proteins from Red Kupang (*Musculita Senhousia*)

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Abstract. Red Kupang (*Musculista senhousia*) is one of the fisheries resources included in the bivalve class. Red Kupang is commonly found in the waters of Surabaya, Sidoarjo, Pasuruan, and the coast of the Madura Strait. The nutritional components contained in red mussel meat include 75.48% water content, 1.93% ash content, 15.02% protein, 6.17% fat, and 1.41% carbohydrate. The nutrient content of red mussels that can be utilized is protein. So that red mussels can be used as an alternative source of animal protein. For this reason, this research is carried out namely isolation of proteins against red mussels. Isolation can be carried out by means of extraction, where the pH and time of extraction affect this process. The research began with washing and drying the red mussels at 40°C until a constant weight (± 6 hours) was obtained. The second stage is making red mussel flour with 60 mesh particle size. The third step is the isolation of red mussel protein by extraction process that is 50 grams of red mussel flour homogenized in aquadest with a ratio of 1:10 w / v, then extraction is carried out at an alkaline pH (pH: 9, 10, 11, 12 and 13). at a temperature of 40 °C using a hot plate stirrer for 10, 20, 30, 40 and 50 minutes. The results obtained the best conditions at pH 11 and 50 minutes extraction time with a protein content of 72.70% ..

Keyword : Red Mussels, extraction, pH,

2 Introduction

Red Kupang (*Musculista senhousia*) is one of the fisheries resources included in the bivalve class. Red Kupang is commonly found in the waters of Surabaya, Sidoarjo, Pasuruan, and the coast of the Madura Strait. Red Kupang is used by the people of East Java, especially the area around Surabaya as a special food that is a complement of vegetable rice cake. In addition, red kupang are also processed into mussel broths, crackers and mussel paste [1]. Fresh Kupang contains many nutrients, especially protein. Nutritional components contained in red kupang meat as follows :

Table 1. Chemical Composition Yield of Red Kupang

	Wet Base (%)	Dry Base (%)
Water	75,48 \pm 0,01	-
Ash	1,93 \pm 0,01	7,86 \pm 0,01
Protein	15,02 \pm 0,03	61,24 \pm 0,04
Fat	6,17 \pm 0,04	25,16 \pm 0,03
Carbohydrates*	1,41 \pm 0,05	5,74 \pm 0,01
Meat + offal		22,80



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Shell	40,13
Yield Lost	37,07

The nutrient content of Red Kupang that can be utilize protein. Therefore that it can be used as an alternative source of animal protein [2]. Meanwhile the Pb content in it is 0.66 ppm, and the Hg content < 0.002 ppm. According to BPOM RI [3] and BSN [4] in SNI 7387: 2009 the maximum Pb limit in food is 1.5 ppm and Hg is 1.0 ppm. This is still on the verge of being safe for consumption in relatively small amounts. The levels of heavy metals in red mussel are not all the same, this is due to differences in habitat in mussel [1].

Protein is one of the important nutritional elements in food. The functional properties of protein for food include solubility, emulsification ability, foam formation, gel formation, water binding, curd formation, flavor binding and others. To obtain protein in high concentrations, protein can be isolated form protein isolates.

Protein isolation is in principle based on two main processes, namely extraction and coagulation. For this purpose, bases and acids are generally used, respectively, used for the process of extraction and clumping or deposition [5]. Protein isolate is the most refined form of protein that contains the purest protein content. The first protein isolate was produced in the United States around the 1950s [6].

In protein purification, the liquid solid extraction method (Leaching) is one method that can be used. In this method a base solution can be added to extract and dissolve protein from a material so that the protein can be separated from non-protein material. Generally this extraction method uses strong base types such as sodium hydroxide [7].

The principle of this extraction method is known as the salting-in event. Salting-in is an event where the presence of certain solutes that cause the solubility of the main substances in the solvent to be greater. The salting-in method is done by adding unsaturated salt or at low concentrations so that the protein becomes charged and dissolves in the saline solution. Protein solubility will continue to increase in line with an increase in salt concentration, if the salt concentration continues to increase, then the solubility of the protein will decrease, at a higher salt concentration, the protein will precipitate [7].

Precipitation of Proteins. In protein purification, the deposition stage is an advanced stage of the extraction method. To precipitate the protein one can use strong acids such as hydrochloric acid, because strong acids can completely dissociate in water so that equilibrium of positive and negative charges is quickly achieved [7]. In this process known as salting-out which is the event of the presence of certain solutes that have greater solubility than the main substance, so that it will cause a decrease in the solubility of the main substance or the formation of sediment due to chemical reactions.

Proteins composed of amino acids contain carboxyl groups (acids) and amine groups (bases) which make amino acids acidic and basic or amphoteric [8]. If both groups are ionized, then in an amino acid molecule there will be two different ions, namely COO⁻ and NH₃⁺. Figure 1. shows the reaction of zwitter ion formation on amino acid molecules:

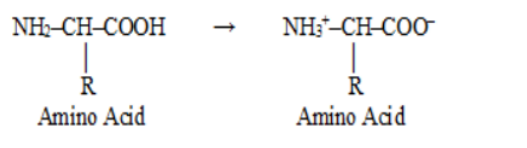


Figure 1. Reaction of zwitter ion formation on amino acid molecules

When a state is reached where a neutral or uncharged protein is present the pH is said to reach the isoelectric point. pH above the isoelectric point, then the amino acid will be negatively charged which results in being drawn towards the positive electrode when electrolyzed and vice versa, if the pH is

below the isoelectric point then the amino acid will be positively charged and drawn towards the negative electrode [7].

The amino group titrates at a much higher pH, and the charge of this group changes with an increase in pH. Thus, amino acids that carry charges only in the α -carboxyl group and α -amino groups have positive overall charges at low pH, positive and negative charges at physiological pH (around 7.4) and negative charges at higher pH [8].

The isoelectric point (pI) is a pH state where proteins have zero electrostatic charge. In pI, proteins do not dissolve in water because the hydrophobic pull of proteins is greater than the electrostatic attraction of water-proteins resulting in isoelectric precipitation. On the other hand when the pH shifts away from the protein pI, then attraction between protein-water and isoelectric repulsion between proteins is stronger so as to produce isoelectric dissolution [9].

Protein isolation is very closely related to the isoelectric point. The basic principle of protein isolation is to use a low concentration of salt by adjusting the pH at its isoelectric point. In an acidic atmosphere protein molecule will form positive ions, whereas in an alkaline atmosphere will form negative ions. At the isoelectric point the protein has the same positive and negative charges, so it does not move towards the positive or negative electrodes when placed between the two electrodes [8]. Therefore, the solubility of the protein at this isoelectric point is very low, resulting in turbidity and the protein contained in the solution can be precipitated and obtained protein with high purity.

The nature of soluble proteins makes protein easily isolated, according to [10], proteins have water-soluble properties, saline, acidic, basic, and ethanol solutions. The longer the extraction time, the longer the contact between the solute and the solvent is so that more protein is taken, this causes the protein content to increase [11]. However, after exceeding the optimum time limit of extraction, protein levels can be reduced because the condition of the solvent is saturated with solutes. The method used in liquid solid extraction is determined by the number of constituents to be dissolved, the distribution of the constituents in solid, the nature of the solid and the particle size. If the constituents to be dissolved are spread evenly on the solid, then the existing constituents on the surface will dissolve into the solvent first. As a result, the remaining solid will become porous. Furthermore, the solvent must penetrate the solution layer on the surface of the solid to reach the constituents underneath, consequently the extraction rate will decrease due to the difficulty of penetrating the layer. But if the constituents to be dissolved constitute a large portion of the solid, then the porous solid residue will immediately break into a smooth solid and will not prevent permeation of the solvent into the deeper layers. Therefore, it requires a relatively long time so that the extraction process can run well and more dissolved protein [12].

In the stage of isolating proteins, solubility is the key so that the separation of protein from non-protein material can work well. Protein solubility is significantly affected by pH and generally has a minimum value at isoelectric pH. Changes in pH will affect the ionization of the protein functional groups so that the total charge of the protein changes. In this condition, the protein molecule is uncharged and is unable to interact with the ions in the solution so that it easily forms aggregates or deposits. Most proteins have an isoelectric pH in the range of 4-6 [7]. In the study of [13-15] the best isoelectric pH used in isoelectric protein precipitation is pH 5.5. The extraction time is one important factor in separating proteins in a material at the extraction stage. The length of time of extraction

2. Research Methods

2.1. Materials

The materials used in this research are red kupang peel (*Musculitas senhausia*) taken from Pasuruan, Aquadest, Hydrochloric acid (HCl) and Sodium hydroxide (NaOH). The equipment used in this research is a series of extraction tools, centrifuges and pH meters.

2.2. Procedures of Research

The research conducted at three steps:

The first step is washing and drying red mussel. First peeled red peel washed with clean water. Then dried in an oven at 40°C until a constant weight is obtained (estimated for ± 6 hours 40 °C). The second stage is making red mussel flour. Red Kupang peel that has dried, mashed until it becomes red kupang flour. Sift the red kupang flour using a 60-mesh sieve.

The next step is protein isolation. Begins with extraction. Red gram kupang flour is homogenized in aquadest at a ratio of 1:10 w / v. Given an alkaline pH treatment (pH: 9, 10, 11, 12 and 13) by adding 2 M NaOH solution to the desired pH. Heated at 40 °C while stirring using a hot plate stirrer for 10, 20, 30, 40 and 50 minutes. Then, centrifuged to form two layers namely sediment and supernatant (centrifugation 1). Next is the coagulation process. Supernatant was separated and added 2 M HCl so that the pH became 5.5. Then centrifuged for 40 minutes (centrifugation 2). The precipitate obtained was dried in an oven at 40°C for ± 6 hours.

3. Discussion and Result

3.1. Discussion

The raw material in this study is the red kupang obtained from collectors of kupang fishermen in Balongdowo Village, Candi District, Sidoarjo Regency. The initial conditions of raw material in the form of mussels which have been peeled. Red Kupang is then made into flour by drying it using an oven to reduce water content. After constant mussel weight, dry mussel is mashed and sieved with a 60-mesh sieve. The results of the flour are then performed proximate analysis at the Jember Polytechnic food analysis laboratory with the following results:

Table 2. Proximate Analysis of Raw Materials

Analysis	Analysis Results (%)
Water Content	9,05
Abu Content	6,12
Fat	4,79
Protein	65,20
Carbohydrates	14,84
TOTAL	100,00

The results of the proximate analysis of the initial ingredients revealed that the protein content in red mussel flour was 65.2%. It shows that the raw material proximate analysis of red kupang flour a value that was almost the same as that done by Jacob [1] which obtained protein content in red mussel flour by 61.24%. The difference in the red mussel habitat causes differences in the content of marine biota which is a nutrient for marine animals such as different mussels.

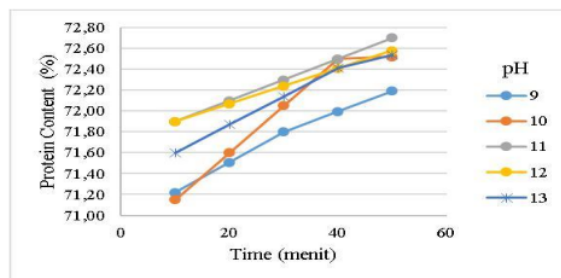


Figure 2. Relationship between Dissolution Time and Protein Isolate Levels of Kupang Red Protein at Various Dissolving pH

Based on Figure 2. It is known that the length of time of protein extraction can increase the percentage of protein content. The results obtained are in accordance with research conducted by Kurniati [16] about the relationship between extraction time and protein concentration of old winged bean seed concentrate, that the longer the extraction time, the greater the protein content obtained. Extraction time has an optimum time limit, but if the addition of time exceeds the optimum time limit, the addition of time is meaningless because the solvent is saturated with the solute. This can be seen in the graphs at the 10th minute to the 50th minute, where at the 50th minute at pH-11 the highest protein content was obtained, amounting to 72.70%. The average percentage of the highest protein content was obtained in the 40-th minute and 50-th minute where in all variations the pH of the protein content obtained was above 72%.

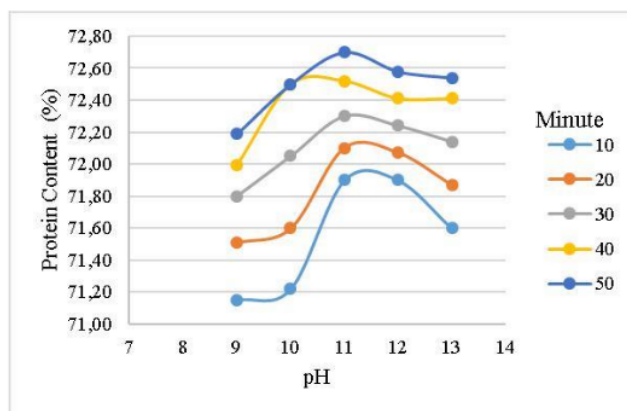


Figure 3. Effect of pH of Dissolution to Protein Levels of Red Kupang Protein Isolates at Various Extraction Times

The graph of the relationship between dissolving pH and protein levels of red mussel isolates at extraction times showed that the optimum levels of extracted protein were obtained at pH-11. In this study the lowest protein content was obtained at pH-9 compared to pH-10 and pH-11. This is because the condition of the solution is less alkaline, so the ability of OH⁻ ions to bind H⁺ ions to proteins contained in red mussels is reduced. However, at pH-12 the protein content obtained is lower than that at pH-11.

The more alkaline the extraction conditions, the greater the concentration of OH⁻ ions which are able to bind H⁺ ions to NH₃⁺. This can be seen in Figure 4. at pH-11 the protein content obtained is greater than at pH-10, as well as at pH-10 the protein content obtained is greater than pH-9.

In Figure.4 it can be seen that the protein content obtained at pH-12 is lower than pH-11. This is caused by protein denaturation. Denaturation is the change or modification of secondary, tertiary, and quaternary structures of protein molecules. Protein denaturation can occur by several factors including heating, extreme acid or base conditions, chemicals, mechanics and so on. Very extreme changes in pH due to conditions that are too alkaline will damage the ionic interactions between OH⁻ and H⁺ in the NH₃⁺ group [17].

Surasani [18] conducted a study by extracting protein from pangasius fish meat with a pH of 1-12. In this study, the best conditions were extraction in an alkaline atmosphere at pH-12. Whereas at pH-13 experience decreased the effectiveness of extraction. The phenomenon that occurs in this study can support the research we do.

3.2. Result

The results of research and discussion that has been obtained, conclusions can be taken, namely the alkaline pH and the length of time of extraction affect the isolation of red mussel protein. With an initial protein level of 65.20% it increased to 72.70% at pH 11 during the 50-minute extraction time.

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