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A REVIEW : FISH PROTEIN HYDROLYSATES

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Abstract

The purpose of this review article is to examine the enzymatic production method for HPI and the factors that influence it, characteristics and application and development of HPI. Based on the results of a review of various articles published in journals and literatures, it can be concluded that the HPI manufacturing method consists of preparation, hydrolysis and drying stages. Factors that influence the hydrolysis process are temperature, time, pH, concentration and ratio of enzymes to protein, the type of protease used and the time of hydrolysis. The most important characteristic of HPI is the ratio of amino acids free nitrogen to total nitrogen which is called the degree of protein hydrolysis. HPI can be applied in the food sector (fortification and food additives) and non-food (pharmacy). The development of HPI is as a basic ingredient for making savory barbeque flavor.

Introduction

Fish is known as a food source of protein with a protein content ranging from 15-20%. The protein contained in fish is of very high quality, because it is composed of amino acids which are almost all needed by the human body and are easily digested and hydrolyzed (Dewita & Syahrul 2015). In addition to having high protein content, fish also have flavor or a distinctive taste (Nurhayati *et al.* 2007). This high protein content and easy to hydrolyze means that fish has the potential to be processed into raw materials for making protein hydrolyzate (Nurhayati *et al.* 2007).

Protein hydrolyzates are formed from the process of breaking down proteins using the help of enzymes, acids or bases and heat which produce simple peptides and amino acids through the hydrolysis process. Protein

hydrolysates can be liquids with 30% solids, and can be flour or hygroscopic pastes with 65% solids (Karnila 2012). Fish Protein Hydrolysate itself is a liquid product produced from fish by breaking down fish protein in the hydrolysis process with the addition of enzymes, acids or bases into a mixture of protein components such as short or simple peptides and amino acids (Witono *et al.* 2020). The process of making Fish Protein Hydrolysate is efficient enzymatically, because it produces free amino acids with varying short chains and allows to produce hydrolysis with flavor the strong one. In addition, it is safer because it produces protein hydrolysate which prevents amino acid damage (Kristinsson 2007).

The production of fish protein hydrolysate is one form of fish utilization which has the potential to provide a very high added value. Fish Protein Hydrolysate is a broad commercial product because it can be applied both in the food sector including functional food, as well as in and non-food such as the pharmaceutical sector (Wijayanti 2016). Fish Protein Hydrolysate product has high water solubility, good emulsion capacity, large swelling ability so that it has high water solubility, good emulsion capacity, range very broad application related to its functional properties or nutritional properties (Kunts 2009).

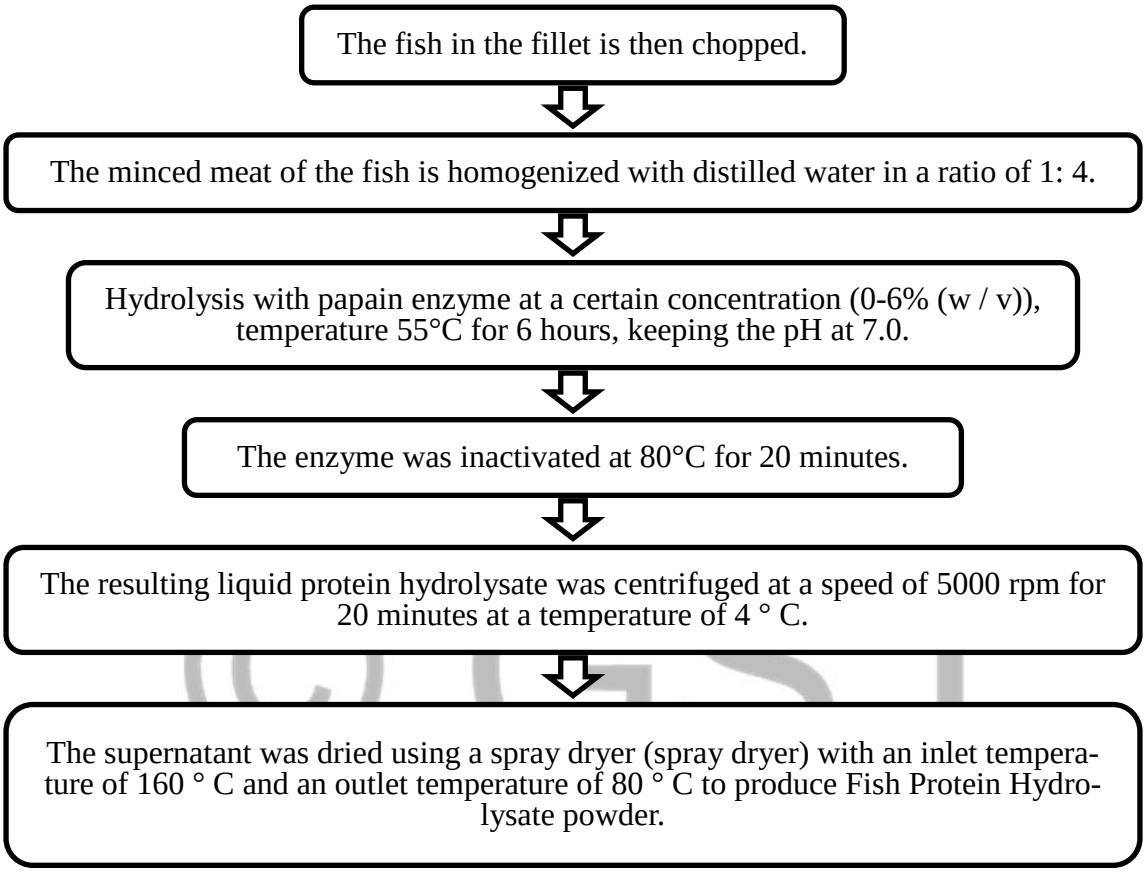
Fish Protein Hydrolysate in the food sector can be used as a source of amino acids and flavor enhancers in foodstuffs (Salamah *et al.* 2012). Protein hydrolyzate also functions as a fortification material to enrich the nutritional value of food products (Witono *et al.* 2016). Fish Protein Hydrolysate with quality below food quality can be used as a source of protein in feed, a source of nitrogen in plant fertilizers and media. growing bacteria (Kristinsson 2007). Fish Protein Hydrolysate in the pharmaceutical field can be used in the manufacture of dermatological products, such as facial cleansing creams and skin moisturizing creams. In addition, protein hydrolysate is also used for fortification into non-allergenic food formulations for babies, as an emulsifier and as a dietary supplement for people with digestive disorders (Pigot & Tucker 1990 *in* Purbasari 2008). The extent of the Fish Protein Hydrolysate application, it is very important to examine the enzymatic method of making Fish Protein Hydrolysate and the factors that influence it, the characteristics and application of Fish Protein Hydrolysate and its development.

Enzymatic Fish Protein Hydrolyzate Making Method

The process of making Fish Protein Hydrolyzate (HPI) is carried out in the following manner (Wijayanti *et al.* 2016):

1. Fish on fillet, then meat fillet chopped fish.
2. The minced meat of the fish is homogenized with distilled water in a ratio of 1:4.
3. After that, hydrolysis with papain enzyme is carried out at a certain concentration (0-6% (w / v), a temperature of 55°C using a water heater with a temperature control for 6 hours (The hydrolysis time used is 0-7 hours), maintaining the stability of the pH remains 7.0 during the hydrolysis process, CH3COOH is used as a regulator of the acidic atmosphere and NaOH as a regulator of alkaline conditions.

4. Furthermore, the inactivation or the enzyme is inactivated at 80° C for 20 minutes.
5. The resulting liquid protein hydrolysate was centrifuged at a speed of 5000 rpm for 20 minutes at a temperature of 4° C to obtain a solution fraction in the form of Fish Protein Hydrolysate.
6. The supernatant was dried using a spray dryer (spray dryer) with an inlet temperature of 160° C and an outlet temperature of 80° C to produce Fish Protein Hydrolysate powder.



Systematic Fish Protein Hydrolysate Manufacturing Process

Factors that Affect the Protein Hydrolysate Process

In the manufacture of Fish Protein Hydrolysate, there are several factors that will affect the speed of the hydrolysis process and the specificity of the hydrolysate produced, namely temperature, time, pH, concentration and ratio of enzymes to protein, the type of protease used and the time of hydrolysis. Meanwhile, the color, smell, taste, and level of amino acid damage are influenced by the purity of the protein from the starting material, the conditions and the hydrolyzing material used. Annisa *et al.* (2017) states, if the hydrolysis process is successful, a hydrolysate will be produced which consists of a mixture of 18-20 kinds of amino acids. Therefore, it is important to optimize some of these factors in order to obtain optimal fish protein hydrolyzate results. According to Perwitasari and Cahyo (2009), the factors that greatly influence the hydrolysis reaction include:

Temperature

Temperature greatly affects the process of running the hydrolysis reaction, especially in the constant

reaction speed, this follows the Atrhenius equation, that if the temperature used is high, the reaction rate constant will be greater so that the reaction will be faster (Sylvia *et al.* 2015). The longer the hydrolysis time is carried out, the better the hydrolysis results will be obtained, because it takes a long time to dissolve and break down protein. Using a higher temperature, the reaction time can be minimized (Wahyudi 2011). The use of high temperatures can also minimize the use of catalysts so that operating costs are more economical.

Catalyst

Braconnot in 1819 was the first person to carry out the hydrolysis reaction using a catalyst. The material being hydrolyzed is linen (cellulose) to ferment sugar using concentrated sulfuric acid. After it is found in the hydrolysis reaction, acid can be used as a catalyst to accelerate the reaction process, because it will affect the decrease in the activation energy so that the reaction runs quickly (Artati *et al.* 2013). Catalysts that can be used are acids, namely hydrochloric acid, sulfuric acid, sulfuric acid, nitric acid, or others. The more acid is used as a catalyst, the faster the hydrolysis reaction will be. The use of a catalyst with a small concentration (dilute solution) is preferred because it will facilitate mixing so that the reaction can run evenly and effectively. The use of a small catalyst concentration can reduce the reaction speed. However, this can be overcome by increasing the temperature of the reaction.

Time

According to Haslina (2004), one of the factors that affect the speed of hydrolysis and product characteristics when making hydrolyzate is the hydrolysis time. The reaction time will affect the resulting conversion. The longer the reaction time, the higher the resulting conversion. The longer the time will increase the number of collisions of reagents so that the molecules that react are more and more and multiply the results formed (Supranto 1998). In addition, the length of time for hydrolysis also determines the enzyme and substrate complexes (Restiani 2016).

Neutralization

The hydrolysis process ends by stopping the internal heating autoclave and neutralizing the acidic atmosphere so that the compound does not break out continuously (Perwitasari and Cahyo 2009).

Fachty *et al.* (2013) states that the factors that influence the hydrolysis process is the protein content of raw materials, hydrolysis pH, hydrolysis time, temperature, pressure and acid concentration. The reaction speed of the protein hydrolysis process will increase by the high acid concentration. The higher the acid concentration used in hydrolysis will increase the hydrolysis results obtained up to the optimum concentration. Usually the reaction rate will be proportional to the H + ion but at high concentrations the relationship is no longer visible (Artati *et al.* 2013).

The enzyme concentration will determine the degree of protein hydrolysis. This factor is one of the important factors that need to be considered in producing protein hydrolyzates with a high degree of hydrolysis. K

the availability of enzymes in large quantities so as to increase the speed of hydrolysis of the substrate in a fixed amount. The more enzymes added, the more peptide bonds can be hydrolyzed (Restiani 2016). In addition, the stirring speed also affects the hydrolysis reaction because it is related to the frequency of collisions (A) in the Arhenius equation so if there is stirring, the reaction speed will be higher (Artati *et al*. 2013).

Aplication Of Fish Protein Hydrolysate

Protein hydrolysate is a product resulting from the breakdown of fish protein into simple peptides and amino acids through the hydrolysis process by enzymes, acids or alkalis. Protein hydrolysis using enzymes is an efficient way because it can produce protein hydrolysates that avoid damage to certain amino acids, such as tryptophan and glutamine (Kristinsson 2007). Protein hydrolysate is a protein that undergoes hydrolytic degradation with amino acids and peptides.

Protein hydrolysates were first introduced in China and Japan around 1990 and are a byproduct of making Monosodium Glutamate (MSG). After the MSG crystallization process is complete, the remaining amino acids have been neutralized and dried. In the food sector, protein hydrolysates are used as food additives in soups, broths, flavorings, sausages, biscuits and crackers. Meanwhile, in the pharmaceutical sector, the use of protein hydrolysate is used to make peptone.

In general, protein hydrolysates are used to improve the characteristics of various food products, as a flavoring, as a continuation for the isolation of amino acids, as well as for treatment. In addition, protein hydrolysate can also be included as a menu for sufferers of indigestion. The application of fish protein hydrolysate products, among others, is used in the processing of additional food ingredients with the aim that in addition to adding protein sources that are rich in amino acids it also improves the taste of the product (Kirk and Othmer 1953 *in* Purbasari 2008).

Protein hydrolysates have an important role in the fortification of food and beverages to enrich protein and nutritional value of food, due to high levels of solubility and digestibility. Fish protein hydrolysate is also useful as a fortification ingredient to enrich the nutritional value of supplementary food products, especially for children and a substitute for egg albumin in the process of making ice cream, gelatin, and functionally it can be said to be an emulsifier, developer, and filler (Pigot and Tucker 1990 *in* Purbasari 2008).

During its development, protein hydrolysate was also used as a special medical diet in cases of pancreatitis, bowel syndrome, Crohn's disease, and food allergies. It hoped that protein hydrolysate will later be developed to replace cow's milk protein which in some people or babies causes allergies (Schimidi *et al.* 1994). In general, protein hydrolysate is used as a food additive because of high nutritional value from amino acids, as a flavor enhancer, cosmetics, and health. The peptides contained in protein hydrolysates are also reported to be bioactive, including as antioxidants, antibacterial, anticancer, and antihypertensives (Castro and Sato, 2015).

As mentioned in the research of Rosa Putri (2020), the bioactive peptides contained in protein hydrolysates from fish waste have shown potential as anti-hypertension by inhibiting the work of Angiotensin Converting Enzyme (ACE) (Ahn *et al* 2012). Angiotensin Converting Enzyme is one of the enzymes that regulate blood pressure and ACE inhibition has been shown to be the most active in the use of hypertension treatment (Kim *et al* 2016). High blood pressure drugs with an ACE inhibitory mechanism are currently found in the market such as benazepril, captopril, enalapril, perindopril, randolapril, quandapril, ACEon, and univasc. However, these drugs have side effects such as coughing, increased blood potassium levels, low blood pressure, skin rashes, headaches, fatigue, fetal abnormalities, numbness. Therefore ACE inhibitors with natural ingredients such as hydrolysates from fish waste can be synthetic ACE inhibitors because they are more stable and can minimize side effects caused by using synthetic drugs (Lee and Hur 2017). Besides being able to be used as the use of protein hydrolysate hypertension drugs, it can also be developed as a nutaceutical because they can prevent and treat several diseases including cardiovascular, cancer and inflammation (Ahn *et al* 2012)

According to Purbasari (2008), protein hydrolyzate products with low fat content are generally more stable. and durable when compared to hydrolyzate products which have a high fat content. In addition, the low levels of fat in hydrolyzate products can be used as dietary ingredients, namely foods with a fat content of less than 5% and as a supplement in the manufacture of white bread and baby food.

The research conducted by Wulandari *et al* (2019) on the process of making natural MSG was carried out from the hydrolysate of fish protein by fermentation. The easier fermentation method does not require high operating pressure so that production costs can be managed or economical and the materials used are not difficult because of the abundant availability of raw materials in Indonesia, so the continuity of natural MSG production is easy to find as well as the tools. The advantages of this fermentation method are minimized costs and a fairly simple process. The industrial process for making protein hydrolysate uses an enzymatic process, which is considered more suitable and less expensive. The processing is faster and provides protein hydrolysate without losing much of the essential amino acids.

Characteristics of Fish Protein Hydrolysate

The results of hydrolysis include free α-amino nitrogen which is generally used to determine the degree of completeness of the hydrolysis process. The ratio between α-amino free nitrogen and total nitrogen was used to determine the quality of protein hydrolysate. A high ratio indicates a high quality of protein hydrolysate (Hidayat 2005). The dissolved protein can be measured in the hydrolysate product. Based on the analysis, the protein content of the protein content was 5.3% (wet basis) or 66.17% (dry basis). When compared to raw materials that have a protein content of 15.61% (wet basis) or 64.7% (dry basis), it can be said that all the protein in the raw material is converted into dissolved protein. This means that the protein hydrolysis process is complete. Research by Iskandar and Desi (2009) showed that the total nitrogen content of lemurum soy sauce

increased after the pineapple bromelain enzyme was increased from 6% to 8%, but decreased after 10% enzyme was added.

The most important component in the hydrolysate product is protein. The quality level of the hydrolysate product is largely determined by the solute content, especially protein. This shows that the type of enzyme and also the concentration is very influential on the hydrolysis product. As explained by Eed (2013) that enzyme activity is influenced by several factors, including enzyme concentration, temperature and pH and each enzyme has a different preference for these factors. During hydrolysis there is a conversion of insoluble proteins into soluble nitrogen compounds, which then break down into compounds that are more easily absorbed by the body. If the hydrolysis is complete it will produce a hydrolysate consisting of a mixture of 18-20 amino acids. Essential amino acids cannot be produced by the body so they must be supplied through food, while non-essential amino acids can be produced in the body (Sitompul 2004). Protein quality can be determined based on the constituent essential amino acids (Wu et al. 2010). Protein which can provide essential amino acids in a composition almost the same as human needs, is a high quality protein. protein hydrolysate products have advantages because of their high solubility and stable conditions.

Free nitrogen amino acid levels can determine the success of the hydrolysis process. The content of the compound in the hydrolysate product is 0.06%. This value is higher than the hydrolysate product which is produced in making this product run more perfectly. According to Anwar and Rosmawati (2013), the percentage of the amount of hydrolyzed product produced against the volume of raw materials before hydrolysis is called the yield of hydrolyzate products. The yield value can describe the economic value of a material. The higher the yield value, the higher the economic value because the higher the amount that can be utilized from the material.

Fish Protein Hidrolyzed with a low fat content value will be more stable and durable. According to Roslan, et al. (2014), several studies have reported that the fat content of various HPIs is <5%. According to Purbasari (2008), protein hydrolysate products with low fat content are generally more stable and durable when compared to hydrolysate products that have high fat content.

There are 17 types of amino acids formed from hydrolysis, namely aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cystine, isoleucine, leucine, phenylalanine, and lysine. 2,007%. These data indicate that the hydrolysis process is close to perfect. According to Cholifah (2014), hydrolysis that runs perfectly will produce a hydrolysate consisting of a mixture of 18-20 kinds of amino acids. According to Kurniawan, *et al*. (2012), all proteins that are hydrolyzed will produce amino acids, but there are some proteins which in addition to producing amino acids also produce protein molecules that are still bound. However, when compared with the total protein content (5.3%), the amount of amino acids formed was lower (2.007%). It is suspected that other dissolved proteins are still in the form of

peptides. Among the detected amino acids, essential amino acids needed for humans, namely lysine 0.117%, leucini0.105%, isoleucine 0.067%, threonine 0.077%, 0.085% phenylalanine, 0.075% valine, methionini 0.120%, arginine 0.094% iidanihistidine 0.097%. Protein content increases with increasing enzyme concentration. The increase in protein value indicates an increase in the total amount of nitrogen in HPI because the analytical method used is the Kjeldahl method which uses the amount of nitrogen as a conversion in the calculation of protein content.

The amino acids contained in the three HPIs consist of essential amino acids and non-essential amino acids. Essential amino acids cannot be produced by the body such as histidine, arginine, threonine. valine, methionine, isoleucine, leucine, phenylalanine, and lysine. Non-essential amino acids can be produced by the body such as aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine. According to Jacoeb, *et al*. (2012) stated that essential amino acids cannot be produced in the body so they must be added in the form of food, while non-essential amino acids can be produced by the body. According to Widadi (2011), the quality of protein can be determined based on the essential amino acid content which compiles it which almost matches human needs.

Conclusion

Based on the results of a review of various articles published in journals and other literature, it can be concluded that the method of making HPI consists of the stages of preparation, hydrolysis and drying. Factors that influence the hydrolysis process are temperature, time, pH, concentration and ratio of enzymes to protein, the type of protease used and the time of hydrolysis. The most important characteristic of HPI is the ratio of amino acids free nitrogen to total nitrogen which is called the degree of protein hydrolysis. HPI can be applied in the food sector (fortification and food additives) and non-food (pharmacy). The development of HPI is as a basic ingredient for making savory barbeque flavor.

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