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Characteristics of Eel (*Monopterus Albus*) Protein Hydrolyzate with Bromelain Enzyme from Pineapple Extract (*Ananas Comosus*)

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A B S T R A C T

Eel is one type of freshwater fish that can be found in muddy rice fields and swamps and is well known by the public. Eels (Monopterus albus) are popular because of their delicious taste and high protein content. The use of eels and pineapples is only as food commodities. So it needs to be used as an alternative source of high protein. The purpose of this research is to make protein hydrolyzate with bromelain enzyme extract and to characterize protein hydrolyzate. The hope obtained through this research can produce protein hydrolyzate as a source of high protein. The output of this research is Scopus indexed International Journal. Based on the results of the research on Eel protein hydrolyzate, the best product was obtained by enzymatic hydrolysis with the addition of 15% pineapple extract concentration, which had yield value: 15.74±0.58%, degree of hydrolysis: 48.96 ± 0.80%, protein content : 80.73 ± 0.58% (Bk), moisture content: 6.70 ± 1.12%, and ash content 9.35 ± 1.40%. The highest type of amino acid is glutamic acid at 67,202.33mg/kg.

Keywords: eel, pineapple, bromelain enzyme, protein hydrolyzate, characterization

1. Introduction

Eel is one type of freshwater fish that can be found in muddy rice fields and swamps and is well known by the public. Eel (*Monopterus albus*) is popular because of its delicious taste and high protein content. The nutritional content in 100 grams of eel meat is 14 grams of protein, 27 grams of fat, 303 calories of calories, 20 mg of calcium, 200 mg of phosphorus, 1 gram of iron, Vitamin A 1600 SI, water content of 58 grams (Ulianty, 2002), and omega 3 worth 11.80 grams (Resiandini, 2013) and 10.9 grams of carbohydrates (Irianto and Soesilo, 2007).

Proteins are composed of twenty different amino acid monomers. Protein quality is assessed from the ratio of the amino acids contained in the protein (Winarno, 2008). Several types of amino acids contained in eels are glycine, valine, alanine, methionine, and glutamic acid. In addition, nucleotides from the types of IMP (inosine mono phosphate) and GMP (guanosine mono phosphate) also affect the characterization of taste, especially in the formation of the "umami" taste, which is a distinctive taste such as meat.(Subagio *et al*., 2004).

The protein content in eels can be utilized into several products with current technology, one of which is protein hydrolyzate from eels. Fish protein hydrolyzate is a product resulting from the decomposition of fish protein into short-chain compounds due to the hydrolysis process, either by enzymes, acids or bases (Bernadeta *et al*., 2012). Enzymatic hydrolysis is more efficient, inexpensive, produces fish protein hydrolyzate without losing essential amino acids, and avoids product changes or damage. According to Salamah *et al*. (2012), the optimum condition for the hydrolysis of fish meat is the use of papain enzymes with a concentration of 5% (w/v), pH 7.0 with a hydrolysis time of 6 hours.

Protein hydrolyzate is produced from the process of breaking down proteins into short chain compounds due to the hydrolysis process with the addition of enzymes, acids and bases. Protein hydrolysates in the food industry can be added to dietary supplements and as emulsifiers. In the pharmaceutical industry, protein hydrolyzate is used for the manufacture of dermatological products, such as facial cleansing creams and skin moisturizers. According to Nurilmala *et al*. (2018) that protein hydrolyzate made from eel can be used as a dietary supplement or can be added to food ingredients to improve the nutritional content of the food. Protein hydrolyzate will help increase protein intake for the human body.

According to Bernadeta *et al*. (2012) that protein hydrolyzate can be made chemically and enzymatically. However, making enzymatic hydrolyzate is more efficient, inexpensive, produces protein hydrolyzate without loss of essential amino acids, and avoids changes or damage to non-hydrolytic products. The enzymes used to hydrolyze proteins are protease enzymes. There are several types of protease enzymes commonly used to hydrolyze proteins, one of which is the bromelain enzyme. According to Nur *et al*. (2017) that the bromelain enzyme is a sulfhydryl protease enzyme capable of hydrolyzing peptide bonds in proteins into smaller molecules, namely amino acids. Bromelain is in the form of amorous powder with clear white to yellowish color, characteristic odor, partially soluble in: Acetone, Ether, and $CHCl_3$, stable at pH 3.0 – 5.5. The optimum temperature for the bromelain enzyme is 50^0C - 80^0C. The bromein enzyme is most commonly found in ripe pineapples.

2. Materials and Methods

2.1 Protein Hydrolyzate Production

The manufacture of protein hydrolyzate using the enzymatic hydrolysis method refers to Nurhayati *et al*. (2018) modified using Eels. The first step in making protein hydrolyzate is eel added with distilled water in a ratio of 1:2. The mixture is then blended until smooth and homogeneous. Furthermore, pineapple extract was added with concentrations of 5%, 7.5% and 10% (v/v). The hydrolysis process uses a water batch with a temperature of 65^0C for 6 hours. Next, the hydrolyzate was heated at 80^0C for 20 minutes in order to inactivate the enzyme. The sample was then centrifuged for 20 minutes at 3000 rpm in order to separate the supernatant and the nathan. Furthermore, the protein hydrolyzate was dried using mechanical drying to become powder. The protein hydrolyzate in the form of powder was then analyzed further.

2.2 Yield (AOAC, 1995)

The yield of the sample was obtained from the comparison of the dry weight of the resulting sample with the weight of the leather material. The yield is obtained by the formula:

Sample Yield (%) = (final weight)/(initial weight) x 100%

2.3 Hydrolysis Degree Measurement

The calculation of the degree of hydrolysis refers to the research of Amiza *et al*. (2012). A total of 20 mL of protein hydrolyzate was added with 20 mL of TCA 20% (w/v). The mixture was then allowed to stand for 30 minutes for precipitation to occur, then centrifuged (7800 x g speed, for 15 minutes). The supernatant was analyzed for nitrogen content using the Kjeldahl method (AOAC, 2005). The degree of hydrolysis was calculated using the formula:

Degree of Hydrolyzate (%) = [(10% dissolved nitrogen)/(total sample nitrogen)] x 100%

2.4 Water Content Analysis

The porcelain dish was dried in the oven for 30 minutes, then the dish was left in a desiccator for 15 minutes. Then the cup is weighed until it shows a constant weight. Furthermore, a sample of 2 grams was weighed in a cup and dried in an oven at 105ºC for 3 hours or until the weight was constant. The cup and its contents were then cooled in a desiccator and weighed until a constant weight was obtained. The calculation of water content can be seen as follows:

Moisture content (%) = (B-C)/(B-A) 100%

Information :

A = weight of empty cup (grams)

B = weight of the cup + initial sample (grams)

C = weight of the cup + dry sample (grams)

2.5 Ash Content Analysis

The ashing dish was dried in the oven for one hour at 105ºC, then cooled for 15 minutes in a desiccator and weighed until a constant weight was obtained. The sample that has been weighed as much as 5 g is put into an ashing cup and incandescent over a Bunsen flame until it no longer smokes. After that, it was put into an ashing furnace at a temperature of 600ºC for 1 hour, then put in a desiccator to room temperature, then weighed until a constant weight was obtained. Ash content is determined by the formula:

Ash Content (%) = (C-A)/(B-A) 100%

Information :

A : weight of empty porcelain cup (grams)

B : weight of cup with sample (grams)

C : weight of the cup with the sample after drying (grams)

2.6 Protein Content Analysis

There are three stages of protein analysis, namely destruction, distillation, and titration. Measurement of protein content was carried out using the microkjeldahl method. The sample was weighed as much as 1 gram, then put into a 100 ml Kjeldahl flask, then added 0.25 grams of selenium and 3 ml

of concentrated H_2SO_4. The sample was destroyed at 410ºC for approximately 1 hour until the solution was clear and then cooled. After cooling, 50 ml of distilled water and 20 ml of 40% NaOH were added to the Kjeldahl flask, then distilled at a distillation temperature of 100ºC. The distillation results were collected in a 125 ml Erlenmeyer flask containing a mixture of 10 ml of 2% boric acid (H_3BO_3) and 2 drops of pink bromcherosol green-methyl red indicator. After the distillate volume reaches 40 ml and is bluish green, the distillation process is stopped. The distillate was then titrated with 0.1 N HCl until a pink color change occurred. The titrant volume is read and recorded. The blank solution was analyzed as an example. With this method, the total nitrogen content is calculated. Protein content is calculated by the following formula:

N (%) = ((S-B)x NHCL x 14)/(W x 1000) 100%

Information:

S : Volume of sample titrant (ml)

B : Volume of blank titrant (ml)

W : Weight of dry sample (mg)

% Protein Content: % Nitrogen x conversion factor

Description: Protein contains an average of 16% nitrogen

Conversion factor = (100%)/(16%) = 6.25

2.7 Fat Content Analysis

A total of 2 grams of the sample was spread on a cotton pad lined with filter paper and rolled into a thimble, then put into a Soxhlet flask. Samples were extracted for 6 hours with 150 ml of fat solvent in the form of hexane. The extracted fat was dried in an oven at 100ºC for 1 hour. Fat content is calculated by the formula:

Fat content (%) = (Sample dry weight-(B-A))/(Sample weight) x 100%

3. Results and Discussions

3.1 Yield

The process of calculating the yield value of eel protein hydrolyzate can be done by comparing the meat raw materials used with the final product of the protein hydrolyzate produced. The yield value of eel protein hydrolyzate is presented in Table 1.

Table 1. Yield of Eel Protein Hydrolyzate

Consentration of Pineapple Extract (%)	**Hidrolisat Weight (gr)**	**Yield (%)**
0%	$6,27 \pm 0,50^a$	$3,14 \pm 0,25^a$
5%	$20,75 \pm 2,00^b$	$10,37 \pm 1,00^b$
10%	$27,08 \pm 2,30^c$	$13,54 \pm 1,15^c$
15%	$31,49 \pm 1,17^d$	$15,74 \pm 0,58^d$

Information:

1. Data is the mean of 3 replications ± standard deviation
2. Data followed by different lowercase letters showed a significant difference ($p<5\%$)

The results of the normality and homogeneity test of the yield of eel protein hydrolyzate with the addition of pineapple extract with different concentrations showed a sig value > 0.05. This means that the yield of eel protein hydrolyzate with the addition of pineapple extract has normal and homogeneous data. Furthermore, the data was analyzed using analysis of variance (ANOVA). The results of the ANOVA test on the treatment of different concentrations of pineapple extract obtained F count (123,996) > F table (3.8, 0.05) = 4.07. The results of the analysis showed that the addition of pineapple extract had a significantly different effect on the yield of eel protein hydrolyzate. The effect of differences in the concentration of pineapple extract on the yield value of eel protein hydrolyzate can be further tested such as the Honestly Significant Difference Test (HSD). The results of the HSD test with a test level of 95% can be concluded that the different concentrations of pineapple extract resulted in significantly different yields of eel protein hydrolyzate in each treatment.

3.2 Protein Content Results

The results of the analysis of protein contenct of eel protein hydrolyzate with different concentrations of pineapple extract can be seen in Table 2.

Table 2. Result of Protein Content of Eel Protein Hydrolyzate

Consentration of Pineapple Extract (%)	Protein Content (%)
0%	74,84 ± 1,82[a]
5%	80,66 ± 2,47[b]
10%	78,14 ± 1,36[ab]
15%	80,73 ± 0,58[b]

Information:

1. Data is the mean of 3 replications ± standard deviation
2. Data followed by different lowercase letters showed a significant difference (p<5%)

The results of the normality and homogeneity test of eel protein hydrolyzate protein content with the addition of pineapple extract with different concentrations showed sig>0.05. This proves that the variety of protein content data on protein hydrolyzate is normal and homogeneous. Furthermore, a significant difference test was carried out with the ANOVA test. The results of the ANOVA test on the addition of pineapple extract with different concentrations obtained a value of sig <0.05 which indicates the effect of adding pineapple extract with different concentrations.

3.3 Water Content Results

The results of the water content of the eel protein hydrolyzate with the addition of pineapple extract with different concentrations can be seen in Table 3.

Table 3. Results of Eel Protein Hydrolyzate Water Content

Consentration of Pineapple Extract (%)	Water Content (%)
0%	4,64 ± 0,28[a]
5%	9,32 ± 1,11[b]
10%	5,56 ± 1,01[a]
15%	6,70 ± 1,12[a]

Information:

1. Data is the mean of 3 replications ± standard deviation
2. Data followed by different lowercase letters showed a significant difference (p<5%)

The results of the normality and homogeneity test of the water content of the eel protein hydrolyzate with the addition of pineapple extract with different concentrations showed a sig value > 0.05. This means that the water content of the eel protein hydrolyzate with the addition of pineapple extract has normal and homogeneous data. Furthermore, the data was analyzed using analysis of variance (ANOVA). The results of the ANOVA test on the treatment of different concentrations of pineapple extract obtained F count (8.56) > F table (3.8, 0.05) = 4.07.

3.4 Ash Content Results

The results of the ash content of Eel protein hydrolyzate with the addition of pineapple extract with different concentrations can be seen in Table 4.

Table 4. Results of Eel Protein Hydrolyzate Ash Content

Consentration of Pineapple Extract (%)	Ash Content (%)
0%	9,17 ± 0,21[a]
5%	6,16 ± 0,26[b]
10%	5,39 ± 0,54[b]
15%	9,35 ± 1,40[a]

Information:

1. Data is the mean of 3 replications ± standard deviation
2. Data followed by different lowercase letters showed a significant difference (p<5%)

The results of the normality and homogeneity test of the ash content of the eel protein hydrolyzate with the addition of pineapple extract with different concentrations showed a sig value > 0.05. This means that the ash content of the eel protein hydrolyzate with the addition of pineapple extract has normal and homogeneous data. Furthermore, data analysis was carried out using analysis of variance (ANOVA). The results of the ANOVA test on the treatment of differences in concentration of pineapple extract obtained F count (14.93) > F table (3.8, 0.05) = 4.07.

3.5 Hydrolysis Degree Yield

The results of the analysis of the degree of hydrolysis of eel protein hydrolyzate with the addition of pineapple extract with different concentrations can be seen in Table 5.

Table 5. Results of the Degree of Hydrolysis of Eel Protein Hydrolyzate

Consentration of Pineapple Extract (%)	**Degree of Hydrolysis (%)**
0%	$27,84 \pm 0,53^{a}$
5%	$45,12 \pm 0,80^{b}$
10%	$46,45 \pm 0,78^{b}$
15%	$48,96 \pm 0,80^{c}$

Information:

1. Data is the mean of 3 replications ± standard deviation
2. Data followed by different lowercase letters showed a significant difference (p<5%)

The results of the normality and homogeneity test of the degree of hydrolysis of the eel protein hydrolyzate with the addition of pineapple extract with different concentrations showed a sig value > 0.05. This means that the degree of hydrolysis of the eel protein hydrolyzate with the addition of pineapple extract has normal and homogeneous data. Furthermore, data analysis was carried out using analysis of variance (ANOVA). The results of the ANOVA test on the treatment of different concentrations of pineapple extract obtained F count (56.81) > F table (3.8, 0.05) = 4.07. The results of the analysis showed that the addition of pineapple extract had a significantly different effect on the degree of hydrolysis of eel protein hydrolyzate.

3.6 Amino Acid Profile Analysis Results

The results of the analysis of amino acid profile from eel protein hydrolyzate can be seen in Table 6.

Table 6. Analysis Results of Amino Acids Contained in Eel Protein Hydrolyzate

Parameter	**Unit**	**0%**	**5%**	**10%**	**15%**
Essential Amino Acids					
L-Lisin	mg/kg	42.393,15	51.073,37	50.547,78	42.214,61
L-Arginin	mg/kg	37.151,36	**56.420,97**	49.927,1	40.923,03
L-Fenilalanin	mg/kg	31.711,64	29.863,33	27.720,82	22.799,64
L-Leusin	mg/kg	25.609,52	47.114,78	47.068,48	40.096,6
L-Treonina	mg/kg	24.827,26	38.251,66	36.058,11	31.183,89
L-Valin	mg/kg	17.019,17	30.649,74	29.932,93	26.666,83
L-Isoleusin	mg/kg	14.140,42	23.995,15	24.666,47	21.517,17
L-Histidin	mg/kg	**9.098,08**	18.407,06	17.523,81	14.371,04
Total Essential Amino Acids		**201.950,6**	**295.776,06**	**283.445,5**	**239.772,81**
Non Essential Amino Acids					

L-Glisin	mg/kg	**106.971,3**	86.026,41	65.052,88	67.202,33
L-Asam Glutamat	mg/kg	71.884,45	98.720,67	96.275,27	81.826,14
L-Asam Aspartat	mg/kg	46.988,57	61.361,12	50.747,43	43.958,17
L-Alanin	mg/kg	45.964,01	50.970,62	45.065,07	43.035,25
L-Prolin	mg/kg	44.831,31	42.019,78	34.698,74	35.971,01
L-Serin	mg/kg	25.734,75	34.739,72	31.872,61	27.501,43
L-Tirosin	mg/kg	**8.005,83**	17.093,71	17.701,52	13.441,92
Total Non Essential Amino Acids		**350.380,2**	**390.932,03**	**245.138,25**	**312.936,25**
Total Amino Acids		**552.330,8**	**686.708,09**	**528.583,75**	**552.709,06**

Conversion of the amount of amino acids produced from the process of making eel protein hydrolyzate with the addition of pineapple extract is shown in Table 7.

Table 7. Conversion of the Amount of Amino Acids Produced from the Process of Making Eel Protein Hydrolyzate with the Addition of Pineapple Extract

Treatment	Protein Hydrolysate Weight (kg)	Amino Acid Content (mg/kg)	Total Number of Amino Acids (mg)
0%	0.00627	552.330,8	3.463,11
5%	0.02075	686.708,1	14.249,19
10%	0.02709	528.583,8	14.319,33
15%	0.03149	552.709,1	17.404,80

4. Conclusions

The conclusions that can be drawn from the results of the analysis of the quality of eel protein hydrolyzate with different concentrations of addition of pineapple extract (*Ananas comosus*) are as follows:

1. Eel protein hydrolyzate can be produced by enzymatic hydrolysis using the addition of pineapple extract which has a bromelain enzyme purity level of 15%. The addition of different concentrations of pineapple extract had a significantly different effect (P<5%) on the yield value, degree of hydrolysis, protein content, water content, ash content and amino acid profile.
2. Based on the research results of Eel protein hydrolyzate, the best product was obtained by enzymatic hydrolysis with the addition of 15% pineapple extract concentration, which had yield value: 15.74±0.58%, degree of hydrolysis: 48.96 ± 0.80%, protein content: 80.73 ± 0.58% (Bk), water content: 6.70 ± 1.12%, and ash content 9.35 ± 1.40%. The highest type of amino acid was glutamic acid at 67,202.33mg/kg.

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