Production of protein hydrolysate from tofu dregs using the crude extract of bromelain from pineapple core (*Ananas comosus* L)

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**Abstract.** Tofu dregs are one of the waste products from making tofu that still has the potential to be processed, because the protein content is relatively high, namely 23.62%. This waste has not been optimally utilized by the community unless it is used as animal feed. One alternative to utilizing tofu waste is processing it into specific products, namely producing protein hydrolysate using bromelain. The purpose of this study was to determine the best concentration of crude bromelain enzyme extract and hydrolysis time to obtain the degree of hydrolysis (DH) of protein hydrolysate in tofu dregs. DH of protein hydrolysate was determined with various concentrations of bromelain (8, 9, 10, 11, and 12%) (v/v) and variations in the hydrolysis time (120, 180, 240, and 300 minutes). The best degree of hydrolysis was 22.82% using 11% crude bromelain extract with a hydrolysis time of 120 minutes.

1. Introduction

Tofu is one type of food that is popular as a substitute for side dishes in Indonesia. The side product of making tofu produces solid waste in the form of dregs. Tofu dregs have not been fully utilized, and the public generally uses them as animal feed or fertilizer. The research results showed that tofu dregs contain quite high nutrients, such as protein 23.62%, fat 7.78%, and crude fiber 65%. [1]

Proteins when carried out by the hydrolysis process will produce short-chain compounds such as oligopeptides and amino acids. Protein hydrolysis can be done using enzyme or acid compounds. The hydrolysate produced in the hydrolysis stage is generally a mixture of oligopeptides and amino acids [2]. This hydrolysate in the food industry is used as an ingredient for flavoring, muscle building dietary supplements, supplements for indigestion sufferers, and free amino acid isolation intermediates for industry, food, and health [3].

Protein hydrolysate can be obtained enzymatically using bromelain enzyme with nyamplung (*Calophyllum inophyllum*) seed as a substrate. The protein content of the protein from the nyamplung seeds was 21.56% [4]. The best degree of hydrolysis was obtained using the bromelain enzyme concentration of 10% and the hydrolysis time of 240 minutes, with the value of the degree of hydrolysis of 6.43%. Also, it states that to hydrolyze protein, the best method is enzymatically when compared to hydrolysis with acids or alkalis. It is because the enzymes work specifically so that the peptide produced has a specific amino acid composition and sequence according to the type of protease used.
Protein hydrolysis to produce hydrolyzed compounds can be carried out using proteolytic enzymes, such as papain from papaya, bromelin produced by pineapples, as well as other types of protease enzymes. The type of protease enzyme that is widely used to produce protein hydrolysates is bromelin, because this type of enzyme is easily obtained in large quantities and is easily produced, both from cores and from other parts of pineapples such as skins and stems. Based on the results of the study, it was stated that the specific activity of bromelain from pineapple core was 7.215 U/mg [5].

The number of results from protein hydrolysis using proteases can be determined by determining the degree of hydrolysis. The degree of hydrolysis (DH) is the percentage (%) of free amino groups released during the hydrolysis process to the total nitrogen in the substrate. Factors that affect (DH) protein are the type of proteolytic enzyme, enzyme concentration, temperature, pH, and time of hydrolysis [6,7]. In this report, we have produced protein hydrolysate from tofu dreg by using crude bromelain from pineapple core.

2. Material and methods
The materials used are pineapple cores and tofu dregs, distilled water, phosphate buffer pH 7, NaOH (pa), H₂SO₄ (pa), HCl (pa), and Kjeldahl powder. The cobs of pineapple were crushed and homogenized by adding a cooled pH 7 phosphate buffer solution. The phosphate buffer is added with a 1:1 ratio slowly while stirring. Furthermore, the mixture was centrifuged at a speed of 3000 rpm for 15 minutes at a temperature of 150 °C. The supernatant was separated from the sediment and used for the hydrolysis process of tofu dregs.

2.1. Processing of tofu dregs flour
The tofu dregs are squeezed using gauze to remove the water contained in the tofu dregs. Furthermore, the sterilization stage was carried out using autoclave for 15 minutes and then dried at 60-700 °C. The tofu dregs were then blended and sieved using a 60 mesh sieve to obtain tofu dregs flour [8].

2.2. Making of tofu dregs flour as protein hydrolysate
Tofu dregs flour was added with pH 7 phosphate buffer with the ratio of tofu dregs flour: phosphate buffer pH 7 of 1:10 (w/v). The pH of the suspension was adjusted to pH 7 using 1N NaOH solution and continued with pre-incubation in a thermoshaker for 20 minutes at 45 °C at 100 rpm. After that, bromelain enzyme extract was added that was dissolved in phosphate buffer pH 7 with various concentrations (8, 9, 10, 11, and 12%) and incubated for 180 minutes. DH values were determined after the hydrolysis process. The best-obtained DH values were then performed with time variations (120, 180, 240, and 300 minutes). The reaction of the hydrolysis process was stopped by heating at 100 °C for 15 minutes. The degree of hydrolysis (DH) was determined by the Kjeldahl method [4].

2.3. Analysis of degree of hydrolysis
Twenty mL of tofu dregs protein hydrolysate was added with 20 mL of 10% TCA solution. The mixture was left to stand for 30 minutes so that the protein coagulation process occurred, then centrifuged at a speed of 5000 rpm, for 15 minutes [9]. The supernatant was analyzed for nitrogen content using the Kjeldahl method [10]. The degree of hydrolysis was determined using the following formula:

\[
\text{Degree of hydrolyses (\%) } = \frac{\text{Dissolved N in TCA 10\%}}{\text{Total sample Nitrogen}} \times 100\% \tag{1}
\]

2.4. Protein level analysis with Kjeldahl
As much as 0.5 g of hydrolysate was put into the Kjeldahl tube, then 15 mL of concentrated H₂SO₄ and 2 g of Na₂SO₄·HgO mixture (20:1) was added. Next, the mixture was heated until the solution for 1.5 hours, then cooled for 30 minutes. After that, 35 mL of distilled water and 8.5 mL of NaOH-Na₂S₂O₃, 700°C.
were added then distilled further. Twenty-five mL of distillate was accommodated in 6.5 mL of 4% H$_3$BO$_3$ which has been added with the methyl red indicator. The distillate was titrated with 0.02 N HCl so that the color of the solution changed from yellow to red [10]. The protein content was calculated using the following formula:

$$\text{protein (\%) } = \frac{(V_{\text{sample}} - V_{\text{blank}}) \times N \times HCl \times f_k \times 14.01}{a} \times 100\%$$  \hspace{1cm} (2)$$

where Fk: conversion factor (6.25)
a: weight of the sample (mg)

3. Results and discussion

3.1 Effect of enzyme concentration on the degree of hydrolysis

The concentration of bromelain enzyme extract added to the substrate of tofu dregs for the hydrolysis process to produce hydrolysate greatly affects the degree of hydrolysis. As it is known that one of the factors that influence enzyme activity in enzyme concentration. The total protein content (total N) before the hydrolysis process was 38.26703%. The results of the nitrogen content determination of protein hydrolysate products using the Kjeldahl method were obtained, as shown in Figure 1.

![Figure 1](image-url)

**Figure 1.** Effect of bromelain crude extract concentration on DH of tofu dregs protein

In Figure 1, it can be seen that the DH increased significantly, along with the increase in the crude extract concentration of the enzyme bromelain. The increase in the crude extract concentration of the enzyme bromelain resulted in a linear relationship with the increase in DH [11]. The degree of protein hydrolysate with the addition of 11% bromelain enzyme extract was 7.23%. Bromelain enzymes can hydrolyze peptide bonds from a polypeptide chain in proteins into simpler amino acids so that it is easier for the body to digest [12]. In this case, the enzyme bromelain acts as a biocatalyst that accelerates the reaction of breaking down proteins into amino acids. Therefore, the higher the concentration of the enzyme added, the reaction speed will be higher as well [15].

Meanwhile, the enzyme concentration was 12%, the degree of hydrolysis was 6.79, where there was a decrease in the protein hydrolysate value. This decrease in value does not have a significant effect. The increasing number of enzymes added allows the interaction of the enzyme and the substrate to
increase so that the breakdown of the peptide chain is also greater. It, of course, results in the number of oligopeptides and amino acids produced.

The degree of hydrolysis produced when compared to the total sample protein was much smaller. It showed that the activity of crude extract of the bromelain enzyme extracted from pineapple hump was small. If the number of enzymes is small, the activity will be small too. Moreover, the enzymes used were extracts which not concentrated by salting out. The protease content in the hydrolysis process is not up to 11%, in addition to other enzymes and other organic materials. It is possible that if the salting-out process is carried out, the results obtained tend to be greater because the amount of enzymes is more. When the substrate concentration is fixed, and the number of enzymes increases, an enzymatic reaction rate will increase in proportion to the rise in the enzyme concentration \([14]\). A study reported using a fat-free meal and hydrolyzed its protein using 2% bromelain enzyme with a hydrolysis time of 300 minutes resulting in DH of 20 %\([15]\). Meanwhile, the results of other studies using nyamplung seed substrate with a bromelain concentration of 10% and 240 minutes of hydrolysis time obtained the degree of hydrolysis of 6.43% \([4]\). Although using the same type of enzyme, each hydrolysis process produces different DH. It is due to are different types of protein contained in the substrate, as well as the source of the bromelain used.

The results of the SPSS analysis in the ANOVA table show that the concentration of the enzyme bromelain from pineapple cores has a significant effect on the degree of hydrolysis of tofu dregs protein. It can be seen from the significant value, namely 0.001, less than 0.05 (Sig. <0.05). Based on this analysis, there are three different subsets, wherein the treatment the enzyme concentration of 8% is significantly different from the concentration (9%, 10%, 11%, and 12%), while in the treatment the enzyme concentration (10%, 11%, 12%) is insignificantly different.

### 3.2 The effect of hydrolysis time on the degree of hydrolysis

Another factor that affects enzyme activity is the hydrolysis time. The longer the interaction between the enzyme and the substrate, the longer the reaction process will be, so that the amount of peptide and amino funds produced will also increase. In the research, the time of hydrolysis using the highest activity produced hydrolysate, namely the enzyme concentration of 11%. The results of the Kjedhal test on the protein hydrolysate product at the variation of the hydrolysis time are as shown in Figure 3.

Figure 2 showed that the degree of hydrolysis (DH) produced by the variation of hydrolysis time minutes was 22.82%, 7.64%, 4.46%, and 2.86%, respectively. These results indicated that the DH obtained experienced a significant increase in the hydrolysis time of 120 minutes. Then it decreased at the hydrolysis time of 180, 240, and 300 minutes. The high amount of DH in 120 minutes was probably due to the enzyme used was still fresh, so peptide bonds' cutting works optimally. When the hydrolysis time of 180 minutes to 300 minutes has decreased, the enzyme may be damaged due to environmental conditions. Besides, other factors can inhibit the action of enzymes in carrying out hydrolysis. So that the amount of oligopeptide and amino acids produced was less.

This study's results have a higher degree of hydrolysis compared to the results of research conducted by Restiani \([4]\). In his study, it was obtained a DH value of 6.9% using the enzyme bromelain from pineapple cores with a concentration of 10%; the hydrolysis time was 180 using a protein substrate from nyamplung seeds. After that, the degree of hydrolysis decreased at 240 minutes of hydrolysis. It also proves that different types of protein are used as substrates; the ability of bromelain to carry out hydrolysis is also different.
Bromelain is a proteolytic enzyme belonging to the sulphhydryl protease group. The active center is an amino acid with a sulfur functional group such as cysteine compounds and other amino acids, such as histidine. Both types of amino acids play an important role in the activity of these enzymes. So this enzyme specifically cuts peptide bonds in carbonyl groups such as those found in arginine or aromatic amino acids, namely phenylalanine or tyrosine [16]. This bromelain enzyme hydrolyzes the peptide bond in the middle of the peptide chain, so it is classified as an endopeptidase enzyme [17]. If the protein substrate contains a lot of amino acids, as mentioned above, the cutting side of the peptide chain will be better; this results in the amount of hydrolysate obtained. Like tofu dregs derived from soybeans, many of the amino acids cysteine are compared to nyamplung seeds so that the degree of tofu dregs hydrolysis is higher than the hydrolysis degree of nyamplung seeds.

Based on the results of the SPSS analysis in the ANOVA table, it is known that the significance value of the effect of hydrolysis time on DH tofu dregs protein is 0.006 less than 0.05 (Sig. <0.05), so it means significant differences between the treatments used.

4. Conclusion
The optimum concentration of crude extract of the bromelain enzyme in this study was 11%, with the resulting DH value of 7.23%. The optimum hydrolysis time in this study was 120 minutes, with the resulting DH value of 22.82%. It can be concluded that tofu dregs are quite potential for the source of hydrolysate protein by using bromelain as biocatalyst.

Acknowledgement
We cannot express enough thanks to the Department of Chemistry Faculty Mathematics and Natural Sciences Tadulako University for providing facilities and the laboratory of the Faculty of Agriculture for technical assistance.

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