Optimization of Peptone Production from Soybeans

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Abstract. Soybeans are the most popular source of vegetable protein for the people of Indonesia, having protein content ranging from 35%–38%. In the protein analysis of 100 mesh size soybean powder using the Kjeldahl method with the Kjeltec 2100 Distilling Unit, the protein content is 35.95. The high protein content of soybeans can be used for the manufacture of Peptone by hydrolysis process using the papain enzyme. This study used a ratio of 1:2 substrate concentration, 0.3% enzyme concentration, pH 5–7, and a temperature of 40 °C–60 °C. The results of this study obtained the highest total dissolved nitrogen value of 14.7264%, at a temperature of 50 °C, pH of solution 5 and hydrolysis time of 7 h. The total dissolved nitrogen value was compared with the total nitrogen value of the material as a value indicating the result of enzymatic hydrolysis. Peptone-hydrolyzed soybeans are used as an alternative medium for the growth of Escherichia coli ATCC 25922 and Candida albicans ATCC 1023.

Keywords: Soybean · Peptone · Protein hydrolysis

1 Introduction

Food safety in Indonesia has applied the principles of GMP (Good Manufacturing Practices) but the supervision is not yet strict. Indonesia also faces various new food safety issues that are changing and different from other countries. Thus, it's very important to develop an Indonesian national food system that can guarantee the availability of food with a good level of safety, namely biological, chemical and other contaminants that can interfere and endanger human health.

Improving food quality and safety implements a combination of several sciences, especially biology, chemistry, and engineering. Utilization of biotechnology for soybean food security has 2 (two) aspects, namely aspects of increasing production through soybean cultivation and consumption aspects through soybean processing.

The development of Indonesia's soybean harvest in the 1980–2015 period fluctuated but tended to increase at an increasing rate of 0.62% per year [1]. Soybean as a functional food source can be viewed from the nutritional content of soybean seeds [2]. Soybean as a food ingredient is a source of fiber and a source of high quality protein composed of C, H, O, N, and S, which form amino acids. Determination of total protein content by

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calculating the element nitrogen (N%) using the Kjeldahl method through three stages, namely the process of destruction, distillation, and titration. The Kjeldahl method is a fairly accurate and specific method for determining the amount of protein by determining its nitrogen content [3]. Soybean protein content ranges from 47, 73% [4].

The way to increase the value of agricultural commodities is to link agriculture with the processing industry. If agriculture only stops as a cultivation activity, then the added value generated is relatively very small. However, agricultural added value will increase through further processing that produces various processed products.

Protein hydrolysate usually comes from animals; kerong fish can be produced through enzymatic hydrolysis using papain enzymes [5]. However, the novelty of this research is that the hydrolyzate is derived from soy vegetable protein. Produces high-quality peptone by taking soy protein isolate as the raw material for the production of raw materials [6]. Peptone commercial media is a source of nitrogen for bacterial growth at a fairly high price. Peptone is a protein hydrolysate containing amino acids, dipeptides, peptides and polypeptide mixtures obtained by hydrolyzing protein-containing materials through acid hydrolysis reactions or enzymatically. The use of peptone is very wide ranging from microbiology to biotechnology-based industries. Protein hydrolysate produced from vegetable raw materials produces peptone with halal qualifications that are in great demand by consumers [7].

Enzymes are classified as organic compounds that function to accelerate the course of metabolic reactions in living things without affecting the balance of reactions, because they are biocatalysts that accelerate the rate of these reactions. Enzymes convert substrate molecules into products whose molecules are different from those of the substrate. Enzymes are catalytic protein catalysts for chemical reactions in biological systems [8].

Enzymes consist of a group of proteins that regulate and carry out chemical changes in biological systems. Enzymes are produced by organs in animals and plants that catalytically carry out various reactions, such as hydrolysis, oxidation, reduction, isomerization, addition, radical transfer, and breaking of carbon chains [9]. From the enzymatic reaction process, the reaction rate increases with increasing substrate concentration, where after further increases the substrate concentration will reach a constant rate. The maximal velocity cannot be increased by increasing the substrate concentration, which is one of the parameters of the enzyme kinetics [10, 11].

As a biocatalyst for protein hydrolysis, papain enzymes are used, including protease enzymes obtained from papaya plant parts. Because of its ability to break down protein molecules, papain enzymes are used in the manufacture of peptones and amino acids needed in microbiological research and industry. The advantages of papain enzymes include resistance to temperature when compared to other proteolytic enzymes such as bromelain and lysine. The papain enzyme has an optimum temperature of 50 °C–65 °C, and an optimum pH of 5–7 [12]. Both of these materials are used as a source of raw materials for the manufacture of vegetable peptone which has a higher economic value.

Peptone produced from hydrolysis of soybean protein is water soluble and doesn't agglomerate in the presence of heat. Peptone production is carried out by enzymatic hydrolysis using proteolytic enzymes, which has the advantage of not requiring high temperatures. So that the hydrolysis process takes place specifically, and can conserve the existing amino acids. Hydrolyzed vegetable peptone can be used as a medium for bacterial growth because its composition is in the form of certain nutrients needed to grow and study the nature of bacteria. The nutritional composition of the complete media contains sources of carbon, nitrogen, sulfur, phosphate, micro metals, vitamins, fertilizers, NaCl, and water [13]. While the classification of bacterial growth is based on the source of nutrition, physical form, chemical components, differences in bacterial growth, whether or not bacteria can select and grow bacteria [14]. To determine growth, microorganisms were isolated using diffusion methods with different media; (TSA) Tryptone Soya Agar, (SDA) Sabouraud Dextrose Agar, with the results of the study that the fungus *C. albicans* was the most effective for growth as indicated by the number of colonies growing on the media [15].

Nutrient Agar media was used as a comparison on the growth of *E. coli* bacteria. Meanwhile, to show that peptone is used as a source of nutrition in *C. albicans* growth media with control medium (SDA) Sabouraud Dextrose Agar [16].

To see the effect of various variables in this study using the response surface method, and is a useful experimental strategy to determine the response of several factors and the purpose of the experiment is to find the midpoint and travel of the star arms. Response Surface Methodology includes the selection of an appropriate experimental design for optimization and a factor space search method to find the optimum area.

The use of this method serves to optimize the process of determining the optimum formulation, especially in the areas of design, development and formulation of new products, as well as improvement of existing product designs. The method used is statistics to see the relationship between one or more treatment variables. The response surface method is a set of mathematical methods that are carried out quantitatively with a response variable that aims to optimize the response in an experiment.

2 Experimental

2.1 Materials

Materials used in this research are Glycine max (L) Merr from Bringin Kabupaten Semarang, enzyme papain, Escherichia coli ATCC 25922, Candida albicans ATCC 1023, boric acid 40%, H_2SO_4, HCl 0.1 N, hydrogen peroxide 30%, NaOH 40%, all chemicals used were of analytical grade.

2.2 Tools

The tools used in this research are autoclave, oven, destruction unit FOSS tecator, FOSS scrubber, kjeltec 2100 distilling unit, water bath, petri dish, pH meter, hot plate stirrer, scales, incubator, laminar air flow, 100 mesh sieve.

2.3 Preparation of Peptone Hydrolyzed

Analyzing equipment such as petri dishes, erlenmeyer, micro-pipette, finntips, measuring cups, and test tubes were sterilized in an autoclave at 121 °C for 20 min. Furthermore, the equipment is dried in an oven at 160 °C for 2 h. Soybeans are sorted to remove stones and irregular shapes, then soaked for 4–5 h, removed from the husk, dried for 2 days, then roasted to reduce moisture content, and milled so that it passes through a 100 mesh sieve.

2.4 Determination of Total Nitrogen and Protein

The protein content of soybean powder was analyzed using the Kjeldahl method by multiplying the soybean conversion factor by 5,75.

$$\%Nitrogen = \frac{(Vs - Vb) \times N \times 14,007 \times 100}{Sample\ weight \quad (mg)} \tag{1}$$

$$\%Protein = \%Nitrogen \times 5,75 \quad (convertion\ factor\ of\ sovbean) \tag{2}$$

2.5 Hydrolized Peptone

Hydrolyzed peptone from soybean powder 100 mesh soybean powder in a ratio of 1:2, with a papain enzyme concentration of 0.3% and a pH variation of 5–7, then hydrolyzes at various temperatures (40–60 °C), and time variations (6–7 h). Then inactivate the enzyme at 85 °C for 15 min, then the sample is deposited for 24 h at 4 °C. The hydrolyzed liquid peptone was filtered using 300 mesh nylon to test the total dissolved nitrogen content.

2.6 Microbiology Analysis

Peptone effectiveness test was carried out by comparing the growth of *E.coli* bacteria on peptone from soybeans and standard media. The samples were then incubated at 37 °C ± 1 °C for 72 ± 3 h. Peptone effectiveness test was also carried out on *C.albicans* with an incubation temperature of 25 °C ± 1 °C for 5 × 24 h. Total Plate Colony (TPC) method was applied to growth performance of soy peptone in media.

2.7 Statistical Analysis

This experimental research in a laboratory by providing treatment with a factorial completely randomized design with three factors, three levels, and 3 repetitions and Response Surface Methodology includes the selection of an appropriate experimental design for optimization.

3 Results and Discussion

Soybeans used for this hydrolysis have a protein content of 35.95% and a nitrogen content of 6.25%, making them a source of protein that can be used to produce peptone. The hydrolysis process using enzymes is generally influenced by the hydrolysis time factor, 0.3% enzyme concentration, substrate concentration ratio 1:2, pH 5–7, and temperature 40 °C–60 °C. The results of hydrolysis of protein sources are shorter peptide chains and amino acids that make up proteins. Determination of the best hydrolysis time, pH, and temperature in the given treatment is known from the total nitrogen value, which is the total dissolved nitrogen value tested from the liquid resulting from dissolved protein or peptide using the Kjeldahl method. The total dissolved nitrogen value was compared with the total nitrogen value of the material as a value indicating the result of enzymatic hydrolysis.

Table 1 shows that the hydrolysis time, pH, and temperature influence the total nitrogen value of the material. The highest total dissolved nitrogen value was 14,7264%, at a temperature of 50 °C, pH of solution 5 and hydrolysis time of 7 h. Thus, these conditions can be selected as the best hydrolysis conditions to produce soybean peptone with commercial papain enzymes. When hydrolysis will reduce the molecular weight of the protein and increase the number of polar groups. Hydrolysis of proteins can cause proteins that are initially insoluble to become soluble proteins which are then hydrolyzed by the enzyme papain into amino acids, so that the amount of soluble protein decreases during hydrolysis. The lowest total dissolved nitrogen value was 4.0656%, at a temperature of 40 °C, a solution of pH 6 and a hydrolysis time of 5 h. This operating condition was in accordance with the research of [4, 14] which showed that the hydrolysis temperature of 50 °C had the highest dissolved protein content.

Peptone of kerong fish may be used as a nutrient in the growth of bacteria in the growth medium. Peptone from the stomach contents of cinch fish was hydrolyzed by autolysis using proteolytic enzymes using 12 N HCl at operating conditions of pH (1, 2, 3 and 4), hydrolysis temperature of 40 °C for 24 h. The results showed that the peptone of kerong fish had a total nitrogen content of 12.66% and could be used as a growth medium for *E.coli* bacteria ATCC 8739 and *Staphylococcus aereus* 6538 [4].

The experimental results of dissolved nitrogen levels showed that the hydrolysis temperature from 40 °C (Fig. 1) to 50 °C (Fig. 2) experienced an increase in dissolved nitrogen content, but at an increase in temperature of 60 °C (Fig. 3) the dissolved nitrogen content decreased.

The hydrolysis temperature affects the dissolved nitrogen content of the protein hydrolysis process from soybean powder with the help of papain enzymes. The temperature change factor in the protein hydrolysis process affects the dissolved protein product with an optimal temperature of 50 °C, with the higher the 60 °C temperature the lower the % dissolved nitrogen produced. The decrease in the value of % dissolved nitrogen may be due to a change in protein conformation; the higher the temperature will cause protein denaturation, this study found. Temperature is generally shown through a complex mechanism involving stimulation and activation phenomena. The degradation of the peptide bond will increase with increasing temperature at a certain point, enzyme inactivation will occur, which is marked by a decrease in hydrolysis products [4, 14].

Table 1. Analysis of Total Nitrogen Dissolved Peptone Variations in Hydrolysis Time, pH, and Temperature

Treatment			Nitrogen (%)	
Temperature °C	pH	Time (Hour)		Total Dissolved Nitrogen (%)
40	**5**	**5**	0,5278	8,4448
40	**5**	**6**	0,5467	8,7472
40	**5**	**7**	0,7311	11,6976
40	**6**	**5**	0,2541	4,0656
40	**6**	**6**	0,4154	6,6464
40	**6**	**7**	0,6983	11,1728
40	**7**	**5**	0,7857	12,5712
40	**7**	**6**	0,8108	12,9728
40	**7**	**7**	0,8628	13,8048
50	**5**	**5**	0,8368	13,3888
50	**5**	**6**	0,8265	13,224
50	**5**	**7**	0,9204	14,7264
50	**6**	**5**	0,9189	14,7024
50	**6**	**6**	0,8859	14,1744
50	**6**	**7**	0,87	13,92
50	**7**	**5**	0,8757	14,0112
50	**7**	**6**	0,86	13,76
50	**7**	**7**	0,8552	13,6832
60	**5**	**5**	0,6686	10,6976
60	**5**	**6**	0,4874	7,7984
60	**5**	**7**	0,4896	7,8336
60	**6**	**5**	0,5413	8,6608
60	**6**	**6**	0,4122	6,5952
60	**6**	**7**	0,4613	7,3808
60	**7**	**5**	0,3987	6,3792
60	**7**	**6**	0,3988	6,3808
60	**7**	**7**	0,4016	6,4256

From the experimental results of various hydrolysis times with conditions (5 h, 6 h, and 7 h) showed a sharp increase in the amount of dissolved nitrogen at the beginning of the hydrolysis reaction until optimal operating conditions occurred at 7 h. Peptone produced from hydrolysis for 7 h contains a mixture of small molecular weight peptides

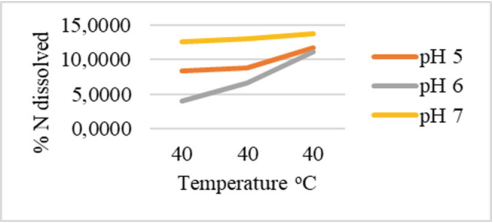


Fig. 1. Comparison of 40 °C hydrolysis temperature with %N dissolved

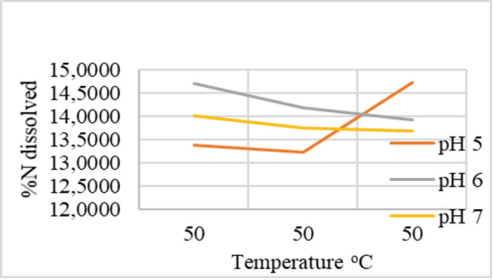


Fig. 2. Comparison of 50 °C hydrolysis temperature with %N dissolved

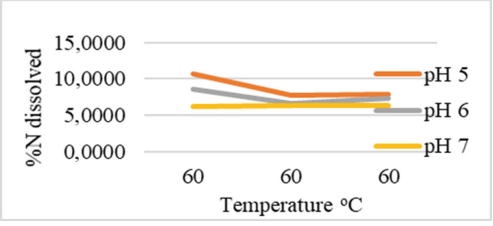


Fig. 3. Comparison of 60 °C hydrolysis temperature with %N dissolved

more than the process for 5 h. Thus, theoretically, hydrolysis for 7 h can produce peptone, which can be used as a medium for bacterial growth.

From the experimental results at various initial pH conditions (5.0; 6.0; and 7.0) it turns out that the optimal pH for peptone hydrolysis is at pH 5.0. This is indicated by the total dissolved nitrogen of 14.7264% with the hydrolysis temperature treatment. 50 °C, while the lowest average pH at the hydrolysis temperature treatment of 60 °C. This is because the longer the incubation time, the longer the papain enzyme works, and the more carboxylate groups are released during the hydrolysis process. The hydrolyzed protein solution will experience a decrease in pH, because when the protease enzyme breaks the peptide bond, the carboxylate group is released and a number of hydrogen ions will be released.

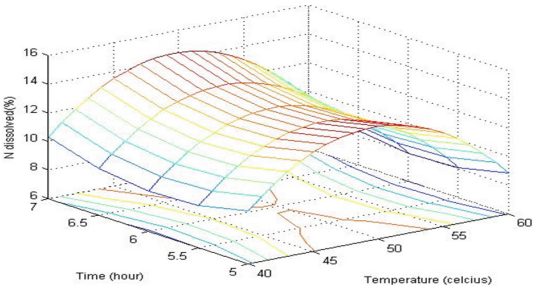


Fig. 4. RSM graph of the relationship between time, temperature, and % N dissolved at a solution pH of 5.0

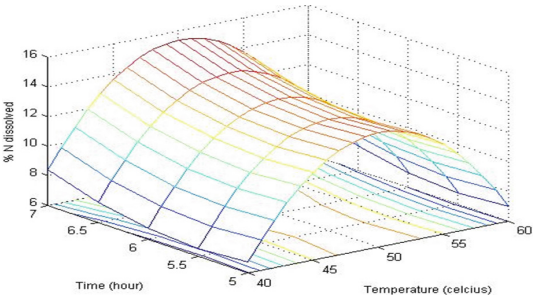


Fig. 5. RSM graph of the relationship between time, temperature, and % N dissolved at a solution pH of 6.0

3.1 Response Surface Method Test at pH 5

RSM analysis (Fig. 4) shows that the longer the hydrolysis time, the higher the value of % N dissolved, the hydrolysis temperature from 40 °C to 50 °C experienced an increase in %N dissolved. But at an increase in temperature of 53 °C, the dissolved nitrogen content decreased. The decrease in the value of % dissolved nitrogen may be due to a change in protein conformation; the higher the temperature will cause protein denaturation. Based on the RSM graph, it shows that the maximum amount of %N dissolved occurs at a temperature of 50 °C with a hydrolysis time of 7 h, while the minimum amount of %N dissolved occurs at 60 °C with a hydrolysis time of 6 h.

3.2 Response Surface Method test at pH 6

RSM analysis (Fig. 5) shows that the longer the hydrolysis time, the higher the value of % N dissolved, the hydrolysis temperature from 40 °C to 50 °C experienced an increase in %N dissolved. But at an increase in temperature of 53 °C, the dissolved nitrogen content decreased with increasing hydrolysis time. Based on the RSM graph, it shows that the maximum amount of % N dissolved occurs at 50 °C for 5 h, while the minimum amount of % N dissolved occurs at 40 °C for 5 h.

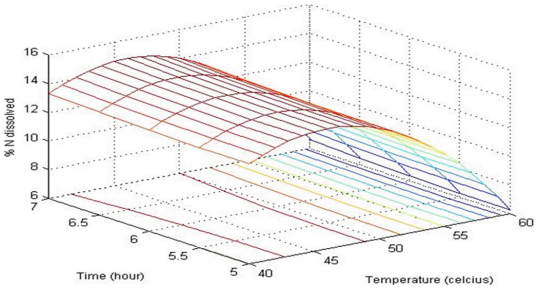
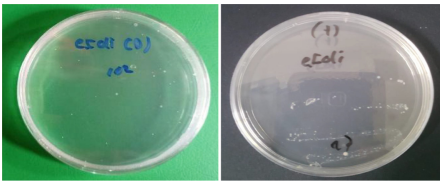


Fig. 6. RSM graph of the relationship between time, temperature, and % N dissolved at a solution pH of 7.0.



E. coli (P) *E. coli* (CM)

Fig. 7. Colony growth of *Escherichia coli* on soybean peptone media and commercial media P = Peptone hydrolyzed by papain, CM = Commercial media

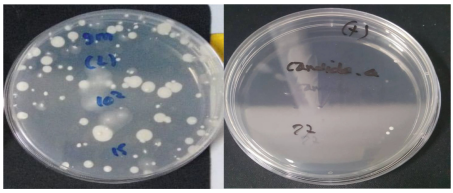
3.3 Response Surface Method Test at pH 7

RSM analysis (Fig. 6) shows that the longer the hydrolysis time, the higher the value of % N dissolved, the hydrolysis temperature from 40 °C to 50 °C increased in %N dissolved. But at an increase in temperature of 53 °C the dissolved nitrogen content decreased with increasing temperature and hydrolysis time. Based on the RSM graph, it shows that the maximum amount of % N dissolved occurs at 50 °C for 5 h, while the minimum amount of % N dissolved occurs at 60 °C for 5 h.

3.4 Peptone Effectiveness of Bacterial Growth

The growth of *E.coli* ATCC 25922 showed that the bacterial growth pattern on soybean peptone media was similar to that of Tryptone Glucose Yeast Agar media (Fig. 7). To see the quality of bacterial growth, 1 mL of liquid sample was planted which was incubated at 30 °C ± 1 °C for 72 ± 3 h in Tryptone Glucose Yeast Agar media with soy peptone substitution and on Tryptone Glucose Yeast Agar media. From the form of colonies growing on Tryptone Glucose Yeast Agar media, soy peptone substitution was less than commercial media due to peptone content (Fig. 7).

The growth of *C.albicans* ATCC 10231 also showed that the fungal growth pattern on soybean peptone media was similar to that of Yeast Extract Agar (Fig. 8). To see the quality of the growth of the bacteria, 1 mL of liquid sample was planted which was incubated at 25 °C ± 1 °C for 5 days. Thus, it can be concluded that the growth



C. albicans (P) *C. albicans* (CM)

Fig. 8. Colony growth of *C. albicans* on soybean peptone media and commercial media P = Peptone hydrolyzed by papain, CM = Commercial media

of *C.albicans* on peptone media produced from this study was similar to commercial media (Fig. 8).

4 Conclusion

Based on the results of the RSM analysis and protein test, it was concluded that soybeans could be used as a source of peptone for microbiological media. Peptone production using papain from soybeans obtained optimal dissolved %N of 14.7264% with optimal conditions of hydrolysis temperature of 50 °C, hydrolysis pH of 5, and hydrolysis time of 7 h. Peptone produced has a quality similar to commercial peptone which can be used as a growth medium for *E.coli and C.albicans.*

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