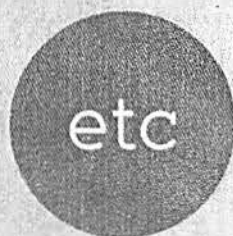
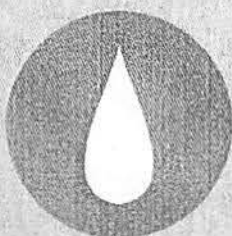
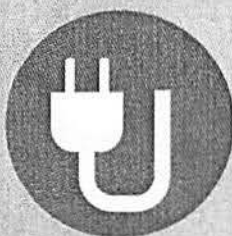




PROCEEDING ASEAN COSAT 2014

The Proceeding of ASEAN Conference on Science and Technology 2014
–9th ASEAN Science and Technology Week (ASTW-9)



Innovation for better ASEAN Community:
Science and Technology Innovation in Food, Energy,
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PROCEEDING ASEAN COSAT 2014

**THE PROCEEDING OF
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– 9TH ASEAN SCIENCE AND TECHNOLOGY WEEK (ASTW-9)**

**Innovation for better ASEAN Community:
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**Editor in Chief
Prof. Dr. Estiko Rijanto**

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PROCESS DESIGN OF PEPTONE PRODUCTION FROM PEANUT MEAL AS BYPRODUCT OF PEANUT OIL INDUSTRY USING CRUDE PAPAIN

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Abstract

Peptone is an important component in the microbial growth media which has function as the source of amino acids. Up until now, Indonesia still imports peptone which reaches \$17.84 million per year in the last five years. That fact is the reason to develop the research in peptone production by using protein source material that is available in Indonesia, such as peanut meal that can be obtained as by product of peanut oil industry. The objectives of this research was to design the production process of peanut peptone using enzymatic hydrolysis by crude papain. Design process was performed by determination of the best condition (time of hydrolysis, crude papain concentration and temperature of hydrolysis). Peanut peptone produced was characterized on its amino acids content and applied to be used as microbial growth media and compared with a commercial product. Crude papain powder was obtained from the sap of papaya fruit, through drying and milling process. The activity of crude papain used in this experiment was 5057.47 U/g min. Hydrolysis process was conducted on peanut meal that have been diluted in water with ratio of 1:2. The research showed that the best hydrolysis condition of peanut peptone was obtained by using 0.4% of crude papain for 4 h at temperature of 55 °C. The peptone product has appearance of brownish yellow color in liquid form. The yield peptone produced was 40.8%. The analysis of amino acids content showed that peanut peptone produced had high glutamic acid aspartic acid and arginine. Those 3 amino acids are very important for microbial growth. The result of growth test of *Escherichia coli* and *Staphylococcus aureus* by determining optical density (OD_{620nm}) and Total Plate Count showed that peanut peptone produced has similar performance with commercial peptone.

Key words: *Enzymatic hydrolysis, Peanut meal, Crude papain, Peanut peptone*

I. INTRODUCTION

Peptone is a protein hydrolysis product which is common to be used as nitrogen source in growth media of microorganisms. Up until now, Indonesia still imports peptone to fulfill its demand due to there is no peptone industry available in

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Indonesia. Imported peptone within the last two years increase by 3,296 tonnes with the value of US \$12,15 million in 2012 and become 5,102 tonnes with the value of US \$20,76 million in 2013 [1]. This facts lead to the need of preliminary research of development of peptone industry in Indonesia using raw materials which are available such as protein from plants.

Peanut is one of the protein source plants with the potential to be used as peptone raw material. Usually, peanut is used as materials for snacks, peanut butter and peanut oil. On the production of peanut oil, peanut meal will be produced as by products which contain 45–50% crude protein that can be utilized as raw material of peptone. Usually, by products of peanut oil industry is used for feed or traditional food, such as *oncom* which have low added value. By conducting this research, peanut meal is utilized as peptone raw material and applied to substitute imported peptone as microbial growth media. This research was aimed to design the production process of peanut peptone using enzymatic hydrolysis by crude papain. Design process was performed by determination of hydrolysis time, enzyme concentration and hydrolysis temperature and compared the peptone produced with commercial peptone as growth media of *Escherichia coli* and *Staphylococcus aureus*.

II. MATERIALS AND METHODS

A. Materials

Materials that were used were peanut meal, papaya sap, Sodium metabisulphite, NaCl, yeast extract, Bacto™ peptone from BD (Beckton Dickinson), BactoAgar (BD), aquadest, *Escherichia coli*, *Staphylococcus aureus* and Nutrient Broth (BD).

B. Methods

Enzyme Preparation

Enzyme preparation was obtained by mixing papaya sap with 1.4% sodium metabisulphite and 0.3% NaCl in proportion of 1:1. The mixture was then gently stirred, filtered and dried in 50–55°C, and milled. The crude enzyme was characterized by analyzing its enzyme activity using casein as substrates. Yield of papain enzyme obtained was 19.8% with protease activity of 5,057.47 U/g min.

Determination of Hydrolysis Time and Enzyme Concentration

Peanut meal was added with aquadest of 1:2 (b/v) and papain enzyme of 0.2%; 0.4%; and 0.6%. The mixture was mixed well and hydrolyzed for 4, 6 and 8 h. Hydrolysis was performed at temperature of 60°C in incubator. This step was stopped by enzyme inactivation by heating the samples at 85°C for 15 minutes in water *bath*. The samples were then filtered in 225 meshes and centrifuged at

4,000 rpm for 15 minutes to obtain soluble fraction. Hydrolysis performance was determined by analyzing ratio of Total of Soluble Nitrogen to Nitrogen Total of material using Kjeldhal method.

Determination of Hydrolysis Temperature

After hydrolysis, time and enzyme concentration were obtained and the research was continued by determining hydrolysis temperature. Hydrolysis was carried out in temperature of 50, 55 and 60 °C. Performance of hydrolysis was measured by determining ratio of Total of Soluble Nitrogen to Nitrogen Total of material using Kjeldhal method.

Determination of Amino Acid Content in Peptone Produced

The peptone produced was analyzed by HPLC to determine the amino acids content.

Application of Peptone Produced as Bacterial Growth Media

The peptone produced was analyzed to be used as growth media of bacteria and compared with commercial peptone (Bacto™ peptone, BD). The bacteria used were *Escherichia coli* and *Staphylococcus aureus*.

Liquid medium for growing bacteria was prepared by mixing 0.5% yeast extract, 1% peptone, and 1% NaCl. The using of peptone samples that were produced from peanut meal hydrolysate was set up by equalizing the total of nitrogen samples with total of nitrogen commercial peptone. Medium was sterilized at 121 °C for 15 minutes. Bacteria were inoculated to 10 ml of medium containing peptone produced and then incubated at 37 °C for 24 h and the optical density (OD 620 nm) was measured as indicator of bacterial growth.

Bacterial growth as Total Plate Count was also measured by plating bacteria on agar plate medium using peptone produced and compared with commercial peptone. The composition of media was the same as liquid medium but it was added by 1.5% Bacto Agar. After inoculation, the agar plates were incubated at 37 °C for 48 h. The number of colony was counted by using *Colony Counter Quebec*.

Experimental Design

Experimental design used in this research was Randomized Factorial Design which performed in two steps namely Randomized Factorial Design two factors to determine hydrolysis time and enzyme concentration; and Randomized Factorial Design one factor to determine the effect of hydrolysis temperature. The results that showed significant different value was analyzed further using Duncan test.

III. RESULTS AND DISCUSSION

Hydrolysis Time and Enzyme Concentration

The best hydrolysis condition was determined based on ratio of Total of Soluble Nitrogen (NTT) to Nitrogen Total of Material (NTB). Higher Ratio of NTT/NTB meant that hydrolysis process was optimally occurred [2].

Figure 1 showed the effect of hydrolysis time and enzyme concentration on ratio of NTT/NTB. Based on statistical analysis, it was determined that hydrolysis time and enzyme concentration significantly affected ratio of NTT/NTB ($p < 0.05$). The highest ratio of NTT/NTB was obtained at 4 h of hydrolysis time with enzyme concentration of 0.4%. Duncan test result showed that 4 h hydrolysis time was significantly different with 6 h and 8 h of hydrolysis time. Enzyme concentration of 0.4% was significantly different with enzyme concentration of 0.2% but not significantly different with enzyme concentration of 0.6%.

Hydrolysis time is one factor that affects the enzyme stability, which tends to decrease along with hydrolysis time [3]. In addition to that, [4] stated that hydrolysis rate and nitrogen recovery will increase along with the increasing of enzyme concentration. Ratio of NTT/NTB which relatively low with increasing

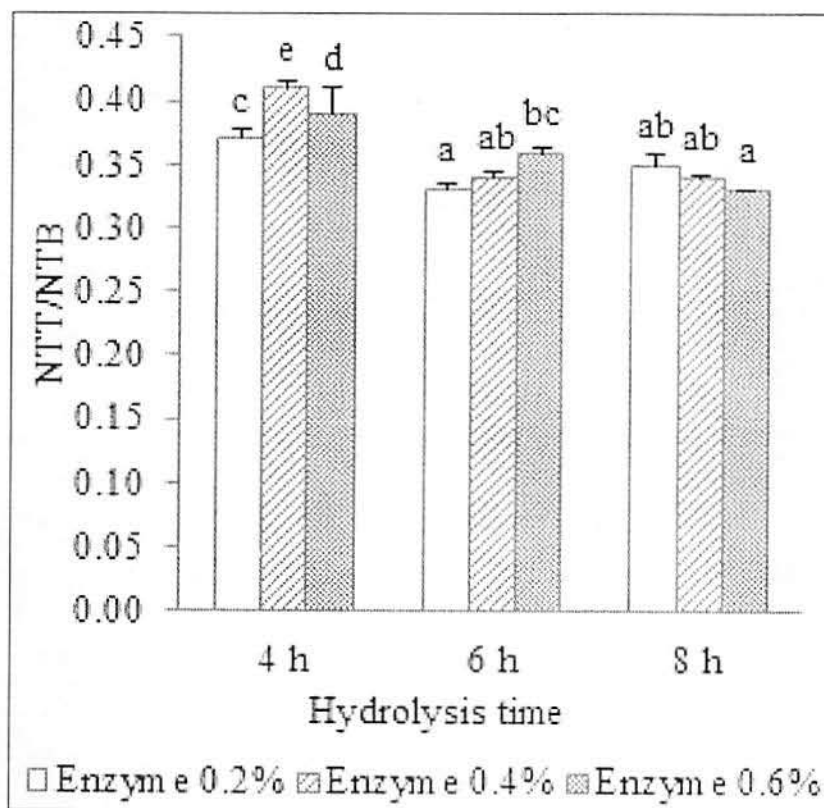


Figure 1. The effect of hydrolysis time and enzyme concentration on ratio of NTT/NTB. Different superscript indicated significant difference ($p < 0.05$).

hydrolysis time can be affected by decreasing enzyme stability. Besides that, [5] stated that enzymatic hydrolysis rate decrease and reaches stationary phase when there is no more hydrolysis process occurred. Soluble protein hydrolysate will be obtained in early stage of hydrolysis, but although an amount of enzyme is added on stationary stage of hydrolysis process, there will be no increase of hydrolysate yield. On that condition, product inhibition will be occurred. More over, the decrease of enzymatic reaction rate can be occurred by decreasing specific peptide bond, enzyme inactivation and competition between substrates with peptide obtained from hydrolysis process [6].

Hydrolysis Temperature

Figure 2 showed that hydrolysis temperature affected ratio of NTT/NTB. The highest ratio of NTT/NTB was obtained on hydrolysis temperature of 55 °C. Statistical analyzes followed by Duncan test showed that hydrolysis temperature of 55 °C was significantly different with hydrolysis temperature of 50 °C and was not significantly different with hydrolysis temperature of 60 °C. Therefore, hydrolysis temperature of 55 °C was decided as the best temperature for peanut meal peptone production using crude papain concentration of 0.4% for 4 h.

The increase of temperature affects chemical reaction rate due to the increase of kinetic energy between reactant. This phenomenon also occurred in enzymatic reaction which involves substrates and enzymes. However, because an enzyme is protein, the increase of enzymatic reaction will be performed on certain temperature. If hydrolysis temperature is too high, protein enzyme can be denaturated, tertiary enzyme structure can be disrupted and the enzyme catalytic activity will decrease [7].

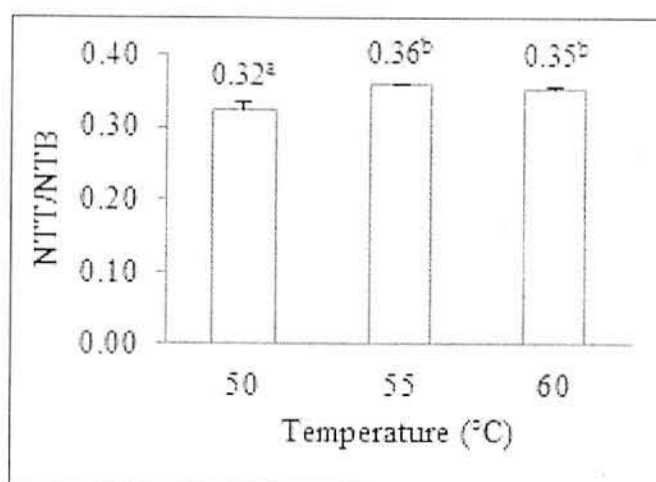


Figure 2. The effect of hydrolysis temperature on Ratio of NTT/NTB. Different superscript indicated significant difference ($p < 0.05$).

Amino Acid Content in Peptone Produced

The concentration of amino acids in peptone produced was different with commercial peptone (Bacto™peptone). Peanut peptone contained three highest amino acids, namely 1.63% glutamic acid, 0.87% aspartic acid and 0.78% arginine while the lowest amino acid was methionine (0.06%). Commercial peptone contained the highest amino acid namely glycine amounted 2.03%. The different of amino acid content is caused by the difference of raw material used in peptone production. [8] stated that Bacto™peptone was produced by using of animal protein as raw material and hydrolyzed by pancreatic enzyme.

Application of Peptone Produced as Bacterial Growth Media

Escherichia coli

Testing result of peptone produced on formulation of liquid media showed that peanut peptone produced had the higher ability for growing of *E. coli* than Bacto™peptone. This can be showed by the results of turbidity determination of the culture (*OD*_{620 nm}) which relatively higher than commercial peptone (Figure 3a).

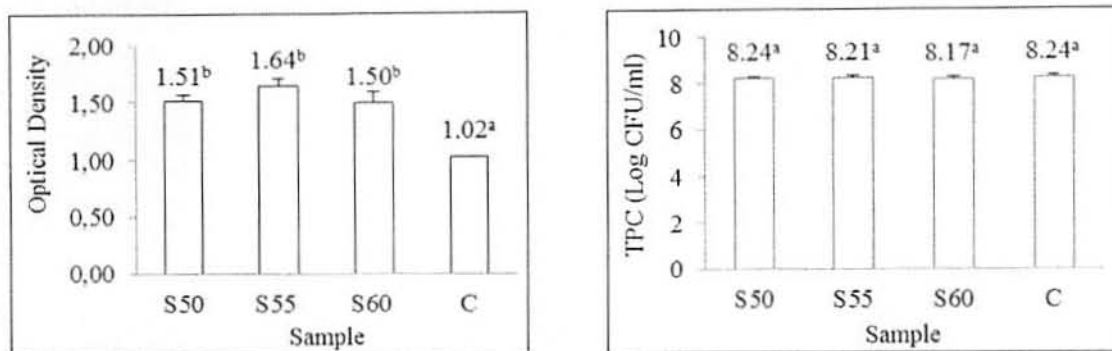
Statistical analysis showed that *OD*_{620 nm} of culture broth using peanut peptone produced was significantly different with commercial peptone. Different with liquid media, application of peanut peptone produced in solid media showed that the growth ability was not significantly different with commercial peptone (Figure 3b).

According to [9], amino acid serine, aspartic acid, and glutamic acid are amino acids which are very important for bacterial growth. Peanut peptone produced contained higher glutamic acid and aspartic acid than commercial peptone, so those could support better cells growth of bacteria.

Staphylococcus aureus

Figure 4a showed testing result of peanut peptone produced for growing of *Staphylococcus aureus*. The result showed that peptone produced had higher ability to support growth of *S. aureus* than commercial peptone. Statistical analysis showed that the growth response of peanut peptone produced was significantly different with commercial peptone.

The same with its response to *E. coli*, application of peanut peptone in solid media for growing *Staphylococcus aureus* showed that they were not significantly different (Figure 4b). By testing the peanut peptone produced for growing of *E. coli* and *S. aureus* both in solid and liquid media, generally, peanut peptone produced has similar ability with commercial peptone Bacto™peptone from BD.

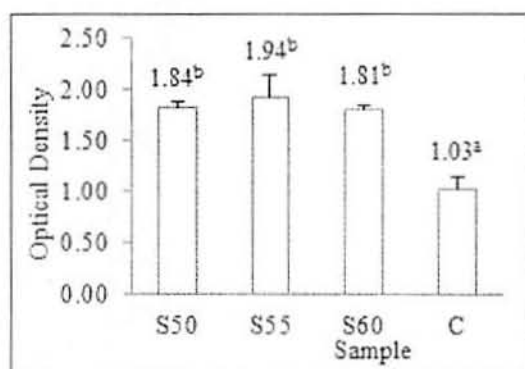


*Peptone on hydrolysis temperature: 50°C (S50); 55°C (S55); 60°C (S60); and commercial peptone (C)

(a)

(b)

Figure 3. Growth for *Escherichia coli* in (a) liquid media and (b) solid media. Different superscript indicated significant difference ($p < 0.05$).



*Peptone on hydrolysis temperature: 50°C (S50); 55°C (S55); 60°C (S60); and commercial peptone(C)

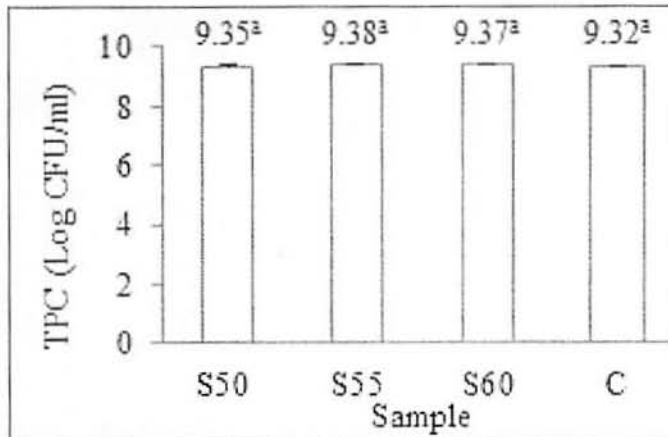
Figure 4a. Growth of *Staphylococcus aureus* in liquid media. Different superscript indicated significant difference ($p < 0.05$).

IV. CONCLUSION

Peanut peptone can be produced by hydrolysis of peanut meal using crude papain enzyme. Production process of the peptone can be performed by hydrolysis condition using 0.4% of crude papain for 4 hat temperature of 55 °C. The yield peptone produced was 40.8%. Amino acids content in peanut peptone that was obtained had high glutamic acid, aspartic acid and arginine. Generally, peanut peptone produced has similar ability with commercial peptone Bacto™peptone for growing of *E. coli* and *Staphylococcus aureus* in liquid and solid media.

V. ACKNOWLEDGMENT

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*Peptone on hydrolysis temperature: 50 °C (S50); 55 °C (S55); 60 °C (S60); and commercial peptone (C)

Figure 4b. Growth of *Staphylococcus aureus* in solid media. Different superscript indicated significant difference ($p < 0.05$).

VI. REFERENCES

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