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Fermentation of soybean meal-hydrolysates as the medium that treated by papain enzyme with *Saccharomyces cerevisiae* for biomass production

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Abstract. The purpose of this study was to determine the effect of soybean meal (SBM) media processing using the papain enzyme on the amino acid content and its effect on yeast biomass production. The study was divided into 2 experimental phases, first test: effect of papain enzyme hydrolysis test on amino acid in soybean meal that treated by boiling and non-boiling medium, second test: yeast biomass production was cultivated using soybean meal hydrolysates as medium with 3 treatments, namely control (- soybean meal), with soybean meal (-enzyme) and soybean meal (+enzyme). Data were analyzed using a completely randomized design with ANOVA and post hoc analysis using Tukey's multiple range test. From the analysis, it was found that the addition of papain enzymes supplemented on soybean meal medium was affected on crude protein and amino acids composition (L-Arginine, L-proline, L-methionine, and L-phenylalanine). Enzyme treatment on SBM as substrate was increased the yeast biomass compared to control ($P < 0.01$). In conclusion, enzyme treatment on soybean meal was improved the fermentation performance of yeast biomass production

1. Introduction

Yeast contains specific bioactive nutrients that have been widely used over the last two decades for feed supplements [1, 2]. The use of yeast in ruminants can play a role in stimulating the population of fiber-degrading microbes in the rumen, increasing the proportion of propionate [3], reducing lactic acid content, and regulating rumen pH [4]. One of the yeasts commonly used to produce single-cell protein biomass is *Saccharomyces cerevisiae* [4]. Cell wall biomass contains balanced amino acids, carbohydrate compounds, and several types of vitamins (especially vitamin B) [5].

During fermentation, yeast converts simple compounds into fermentation products such as ethanol, sugar, and biomass. The formation of yeast protein comes from soluble peptone compounds. Peptone is a protein hydrolysate that serves as a nitrogen source. The reaction resulting from protein breakdown by enzymes can increase the production of soluble proteins that yeast can use for cell wall protein synthesis [6, 7]. Lack of this compound will affect the fermentation process, inhibit yeast growth and reduce biomass production.

Some materials that contain high protein can be used for the production of peptone, among others, from animal and vegetable proteins. Soybean meal is one of the ingredients that contains very complete protein and amino acids. Optimization of the nutrient content of yeast media is carried out through pre-treatment to produce hydrolysates products that can increase the availability of nutrients for yeast growth. Media pre-treatment can be done in various ways, including physically, chemically, and enzymatically. Chemical hydrolysis of the substrate has the disadvantage that the product is not specific and requires more energy in the pre-treatment process [8]. While enzymatic hydrolysis has advantages such as not requiring high temperatures [9], this method can completely break down amino acids in the form of hydrolysates products. Papain enzyme is a type of enzyme capable of hydrolyzing proteins in amino acid groups and amide bonds



[10]. To evaluate the performance of the enzyme, it can be done by comparing it with physical pretreatment processing through heating, then testing the use of hydrolysates products as a yeast growth medium. The purpose of this study was to determine the effect of processing soybean meal using the papain enzyme on the composition of the amino acid content and its effect on yeast biomass production.

2. Materials and Methods

2.1. Microorganism

The microorganism used in this study was a *Saccharomyces cerevisiae* from the Feed Technology collection (Politeknik Negeri Jember, Indonesia) that was isolated from soil. The culture was stored in medium agar slants under freezing conditions (-18°C).

2.2. Enzyme preparation

Papain enzyme used in this experiment were provided by local commercial product with enzyme activity: 125.2 TU/mg.

2.3 Experiment 1

Soybean meal was milled with a mesh size of 1 mm using a sample mill (IKA-Werke M20, Germany) to obtain a small and uniform soybean meal, then analyzed the chemical composition of the AOAC method [11]. The test was carried out with the following treatments: P1: Boiled soybean meal + papain enzyme 500 U, P2: Non-enzyme boiled soybean meal, P3: Non-boiled soybean meal + enzyme, P4: Non-enzyme non-boiled soybean meal. Weigh 5 g of ground soybean meal using a 100 ml beaker glass, then add 25 ml of the solution according to the treatment (P1-P4). The sample was then heated for 1 hour at a temperature of 70°C. Weigh the tube before centrifugation, then centrifuged at 10,000 rpm for 10 min. Next, weigh the weight of the precipitate and supernatant and record the weight and volume. The protein fraction was analyzed for protein in the precipitate using the Kjeldahl method, and amino acid test in the supernatant solution using (Liquid chromatography-mass spectrometry) (LC-MS) amino acid content compared with standard amino acids L-Arginine, L-proline, L-methionine, and L-phenylalanine (Sigma-Aldrich). The amino acid fraction of the supernatant was analyzed according to the method [12]. One ml of the solution was homogenized using an ultra turra blender and then stored at -20°C in a polyethylene tube. One ml was put in a centrifuge tube and vortexed for 2 min and centrifuged 5,000 g, 5 min at -5°C. The supernatant was then transferred to a clean vial, then filtered using a 0.45 m nylon syringe before being injected into LC-MS.

Table 1. Fragment characteristic and selected ion monitored by LC MS in amino acid analysis

Amino acid	Fragmen ion, m/z	Selected Ion, m/z
L- Arginin	175,129	175
L- Proline	116,70	116
L-Methionine	150, 133, 104	150
L-Phenylalanine	166, 149, 120	166

2.4. Experiment 2

The test was carried out with the following treatments P1: Medium made from standard peptone, P2: Medium made from non-enzyme soybean meal, P3: Medium made from soybean meal + enzyme. The medium used for this test has the same composition as the composition of the liquid medium with 1.5% peptone. Inoculation was carried out on 10 ml liquid media and incubated for 24 hours with 100 x g shaking, then transferred to 100 ml media in a 250 ml Erlenmeyer tube and

incubated for 48 hours. Yeast product collection was obtained by centrifugation at 10,000 x g for 5 min and dried in a dryer at 40°C. The number of yeast colonies was calculated by the total plate count (TPC) method at a temperature of 30°C with several dilutions. The amount of yeast that grew was calculated using a digital colony counter (Intech, Germany) as a quantitative growth value. The composition of the media for production consists of molasses, PDB (potatoes dextrose broth) as a carbon source [10], soybean meal, ammonium sulfate (NH₃SO₄), and a mixture of several minerals (mix minerals).

2.5 Statistic analysis

The Data were analyzed using a completely randomized design with ANOVA. The Minitab software package was used for static analysis (Minitab Inc, USA). The statistic models With the following models $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$. Where Y=dependent variables, μ : overall mean, α_i : the effect of treatment, ϵ_{ij} : random error. The post hoc Tukey's multiple ranges tests calculated and probability value less 0.05 were considered significant.

3. Results and Discussion

The results of the analysis showed that the protein content of soybean meal without enzymes was on average 40-41%, while with the addition of enzymes it was reduced by 34-35%. The difference is probably due to the reduced protein content in the precipitate into dissolved protein. The reduced protein content in the precipitate was caused by the increase in the hydrolysis process of the protein thereby reducing the protein fraction on the sediment. A previous study by Pantaya et al [13] reported that enzyme treatment could increase the dissolved protein content on incubation for 4 hours at 60°C on soybean meal. Papain enzymes including proteolysis enzymes can break down protein fractions into simple components. This simulation study proves that enzyme treatment can increase the soluble protein fraction.

There is no significant effect on the boiling treatment of the substrate, this is because boiling at a temperature of 60°C has not affected protein binding so it does not affect the released amino acid content. According to Jiang et al [14], the peak point of cooking loss usually occurs at a temperature of 90-100°C, so that to increase dissolved protein, an increase in heating or boiling temperature is required. This study shows that the use of papain enzymes is more effective for protein hydrolysis in producing amino acid compared to boiling system. The use of enzymes works at low temperatures and does not require high temperatures. Enzymes can hydrolyze protein compounds in specific chains. The advantages of the enzymatic process are that it does not require high temperatures, the hydrolysis process takes place specifically, and can conserve all amino acids.

The profile of the enzyme hydrolysis results as shown in Figure 2. The amino acid content of L-Arginine released was the highest compared to L-methionine, L-phenylalanine, and L-proline, which was 58 ppm. Enzyme treatment in a higher concentration of amino acids than without the enzyme. The papain enzyme is effectively used in hydrolyzing the crude protein fraction into amino acids. Crude protein is a complex compound composed of amino acids. The detected amino acid concentration indicates the profile of the amino acid content in soybean meals. The concentration indicates the specific character of the amino acids contained in a protein. The concentration of each amino acid varies in protein depending on its solubility. This increase in amino acids indicates the availability of amino acids that could be used for yeast growth media.

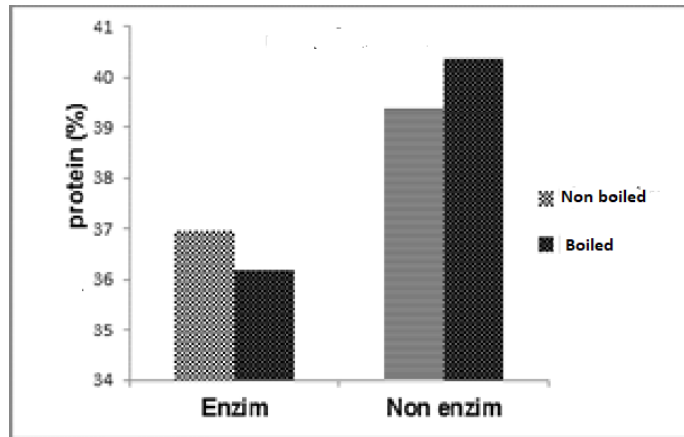


Figure 1. Protein profile in soybean meal residue (n=3)

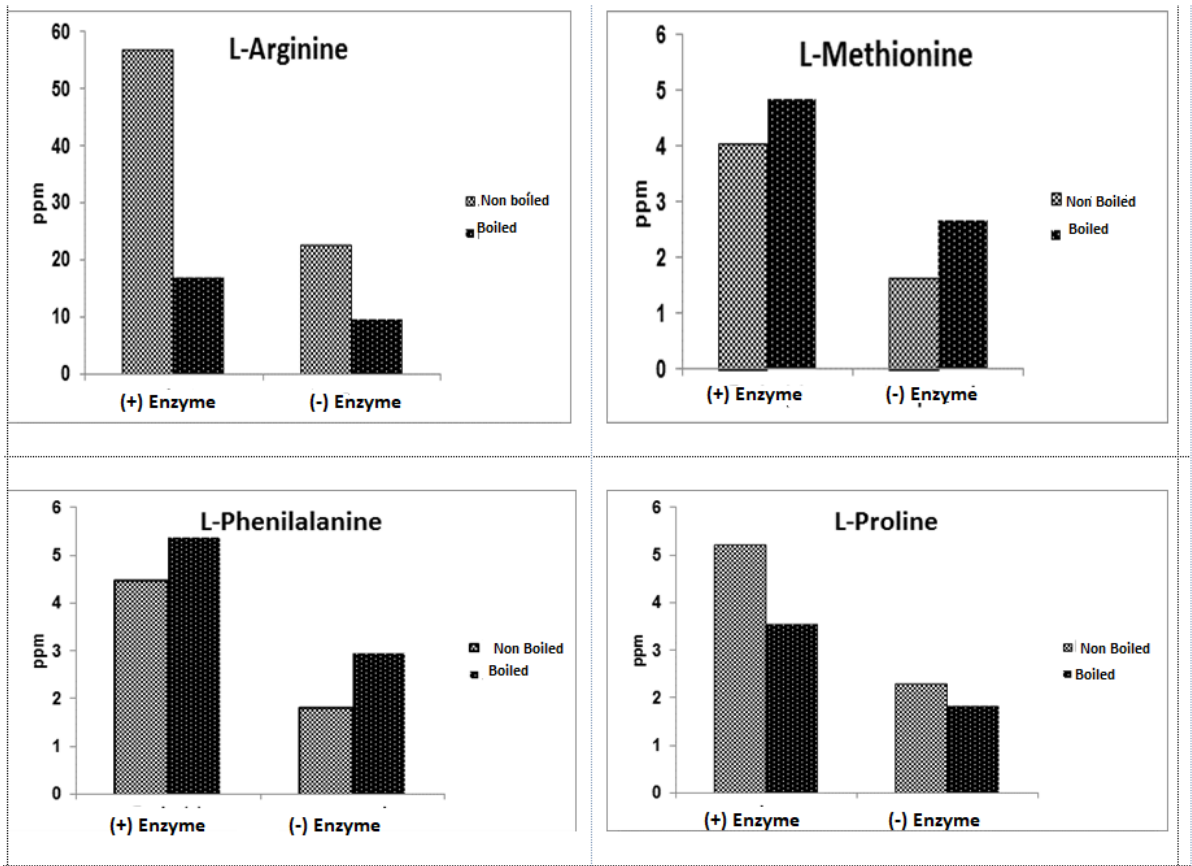


Figure 2. Influence of heating and papain enzyme of amino acid profile in the substrate based soybean meal (n=3)

Yeast grows on all mediums and produces biomass. The results showed the characteristics of yeast biomass production using soybean meal peptone as a medium (Table 2). The statistical analysis results demonstrated that the addition of enzymes had a significant effect on biomass production and Colony forming Unit (CFU/g) ($P < 0.05$). In this study, the hydrolyzed media substrate can increase yeast biomass. These results showed that a positive correlation between amino acid concentration and biomass production.

Amino acids are components that play a role in the formation of proteins, value of concentrations of amino acids cause sufficient availability of cell wall formation. According to the opinion of Saenz [15] which states that amino acids are used as materials for protein synthesis, thus affecting biomass production. The amino acid content detected in the substrate was included in the essential amino acids, namely L-phenylalanine, L-methionine, while the conditionally essential amino acids were L-arginine and L-proline [6]. The transfer of amino acids in plasma through two systems, namely NCR (nitrogen catabolic repression) which regulates the permeases process, and NCI (nitrogen catabolic inactivation) which regulates the import of unfavorable nitrogen sources. The increase in biomass production is due to the increased use of nitrogen and amino acids [16] for anabolic processes [17]. The results demonstrated that L-phenylalanine, L-methionine, L-arginine and L-proline in medium have a positive effect on biomass production.

Table 2. Yeast biomass production using a papain enzyme treatment in soybean meal culture (n=4)

Parameters	Biomass production g/100 ml medium	Dry matter of Biomass (g)	CFU Log10/g
Control	6.30 ± 0.12 ^a	1.53±0.06 ^a	9.07±0.09 ^a
SBM (-) Enzyme	6.31 ± 0.02 ^a	1.60±0.10 ^a	9.22±0.06 ^a
SBM (+) Enzyme	7.10 ± 0.06 ^b	2.00±0.06 ^b	11.20±0.08 ^b

CFU: Colony-forming unit

SBM: Soybean Meal

From these results, it is obvious that enzymatic treatment can increase dissolved protein so that it has a positive effect on increasing biomass production. The use of enzymes can increase the concentration of amino acids in the supernatant and can increase the production of yeast biomass.

4. Conclusion

The use of enzymes in the preparation of the medium resulted in ideal yeast biomass production. Enzymatic treatment using papain could increase the amino acid content resulting from protein hydrolysis of the media and had a positive effect on increasing yeast biomass production.

5. Acknowledgments

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