CHARACTERIZATION OF FUNGAL BIOMASS *Rhizopus oligosporus* IN VARIATIONS OF C/N RATIO

(Bachelor Thesis)

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ABSTRACT

CHARACTERIZATION OF FUNGAL BIOMASS *Rhizopus oligosporus* IN VARIATIONS OF C/N RATIO

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The projected 50 percent increase in the global population, combined with growth in the global, is expected to lead to a more than 50 percent increase in food demand by 2050, with global demand for animal protein nearly doubling. Additionally, protein derived from plants and animals significantly contributes to greenhouse gas emissions and heavily relies on agricultural land. Notably, the livestock sector has a significant impact on greenhouse gas emissions and requires 30 percent of land use for grazing and feed crops. Recent research has focused on producing alternative protein sources that minimize environmental damage, such as single-cell protein foods like mycoprotein. Filamentous fungi have the advantage of being able to grow on various types of media including from food-safe agro-industrial liquid waste. Utilizing tapioca waste, soybean boiling water and others as substrates for filamentous fungi production has the potential to reduce waste and make use of available resources The research method employed a factorial Completely Randomized Design (CRD) with 4 treatments and 3 replications, specifically C/N ratios of 2/1, 4/1, 6/1, and 8/1 of medium growth.The data were processed using analysis of variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) for further analysis.C/N ratio affected the biomass yield, in which the highest yield was obtained at C/N ratio of C/N 2:1 and C/N 8:1 (0.275 g/L and 0.180 g/L) but are not significantly different from the C/N 4:1 and 6:1 (0.211 g/L and 0.208 g/L). C/N ratio had influence on protein and fiber content. that is C/N 2:1 15.78% and C/N 8:1 11.53% and crude fiber content C/N 2:1 5.89% and C/N 8:1 8.63%.Although C/N ratio affected the chewiness, hardness and cohesiveness of the sample according to texture profile analyzer, however this difference was not detected by panelist of sensory test

Keywords : Biomass,C/N Ratio,Protein,Texture

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I am Firhan Al Fariz, student identification number 2014051042.

I hereby declare that what is written in this work is my own original work based onthe knowledge and information I have obtained. This work does not contain material that has been previously published or, in other words, is not the result of plagiarism from other people's work.

This statement is made and can be accounted for. Should there be any dishonesty in this work in the future, I am prepared to take full responsibility.

Bandar Lampung, August 2024

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AUTOBIOGRAPHY

The author was born in Bandarlampung 4 October 2001 as the second of three children of Mr Chairul Effendi and Mrs Yayah Rohayah. The author completed elementary school at SDN 1 Tanjung Gading in 2013, junior high school at SMP Negeri 12 Bandar Lampung in 2016, and vocational high school at SMK SMTI in 2019. In 2020, the author was accepted as a student majoring in Agricultural Product Technology The Faculty of Agriculture, University of Lampung, uses the Joint Selection for Entrance to State Universities (SBMPTN).

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DEDICATION

Praise and gratitude are due to Allah SWT, for His blessings and gifts so that the author is able to complete the Thesis entitled "**CHARACTERIZATION OF FUNGAL BIOMASS *Rhizopus oligosporus* IN VARIATIONS OF CN RATIO**". as a requirement for obtaining a Bachelor's degree in Agricultural Technology from the University of Lampung.

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May Allah SWT always reward all the kindness of those who have been involved. The author sincerely hopes that this thesis is useful for readers and can be used as well as possible. The author realises that in writing this report there are still many mistakes and shortcomings due to the limited knowledge of the author, therefore, constructive criticism and suggestions are highly expected for future progress. Finally, the author would like to thank you and I hope that this report can be useful for the author in particular and readers in general.

Bandar Lampung, August 2024
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I. INTRODUCTION

1.1. Background

The projected 50 percent increase in the global population, combined with growth in the global, is expected to lead to a more than 50 percent increase in food demand by 2050, with global demand for animal protein nearly doubling (Henchion et al., 2017). One in nine people worldwide is still undernourished, and current trends indicate that global nutritional deficiencies are increasing (FAO, 2019). Additionally, protein derived from plants and animals significantly contributes to greenhouse gas emissions and heavily relies on agricultural land. Notably, the livestock sector has a significant impact on greenhouse gas emissions and requires 30 percent of land use for grazing and feed crops (Herrero et al., 2016). Recent research has focused on producing alternative protein sources that minimize environmental damage, such as single-cell protein foods like mycoprotein.

Mycoprotein is a food ingredient gaining increasing attention in the food industry due to its potential to be a sustainable and protein-rich alternative. Mycoprotein is known as protein produced from fungi. The production process involves fermenting these microorganisms on nutrient-rich substrates (Istianah et al., 2018). The resulting fermentation product is then processed into food items that can serve as meat substitutes in various dishes. The high protein content and low fat levels in mycoprotein make it an attractive choice for individuals concerned with health and environmental aspects. Moreover, mycoprotein allows for innovation in creating more diverse plant-based food products .

Filamentous fungi have the advantage of being able to grow on various types of media including from food-safe agro-industrial liquid waste. Utilizing tapioca waste, soybean boiling water and others as substrates for filamentous fungi production has the potential to reduce waste and make use of available resources. Therefore, there have been many previous studies producing filamentous mushrooms using agro-industrial liquid waste. Several related studies such as pea processing waste can produce biomass using the fungus *A. Oryzae* with a protein yield of 43.13% wet weight (Souza et al., 2018). Bread processing waste can produce biomass using the fungus *A. Oryzae* with a protein yield of 43.8% wet weight (Hashemi et al., 2021). Starch processing liquid waste can produce biomass using the fungus *A. Oryzae* with a protein yield of 43.13% wet weight and the fungus *R. Oligosporus* with a protein yield of 49.7% wet weight (Jin et al., 2002).

Based on that research, the media used have different nutritional contents, one of which is the difference in the C/N ratio value. The carbon source in fungi is useful as energy to form cell structures. Nitrogen is part of proteins, nucleic acids and coenzymes which have physiological functions for microbes for their growth process, so that they can maximize the work of these microbes during the fermentation process. However, the final result of filamentous fungi is significantly influenced by the C/N ratio (Lonardo et al,2020) . Research on the effects of the C/N ratio in agro-industrial waste on the characteristics of mycoprotein is crucial to optimizing the production process and obtaining high-quality products. These factors can affect the nutrition, texture, firmness, taste, and success of mycoprotein as a competitive food alternative.

1.2.Purpose

The purpose of this research are :

1. Investigating the effect of C/N ratio on the quantity and quality of fungal biomass production
2. Investigating the effect of C/N ratio on texture profile of fungal biomass

1.3. Framework

The fungus *R. oligosporus* has a complex enzyme system that can utilize various substrates such as agricultural by-products. Utilizing agricultural by-products to cultivate fungal biomass can reduce raw material costs, lessen the environmental impact of agricultural by-products, and convert these low-value materials into high-value products. Pea processing waste can produce biomass using the fungus *A. oryzae* with a protein yield of 43.13% wet weight (Souza et al., 2018). Bread processing waste can produce biomass using the fungus *A. oryzae* with a protein yield of 43.8% wet weight (Hashemi et al., 2021). Starch processing liquid waste can produce biomass using the fungus *A. oryzae* with a protein yield of 43.13% wet weight and the fungus *R. oligosporus* with a protein yield of 49.7% wet weight (Jin et al., 2002). Protein significantly affects the sensory attributes of food. The fibrous structure of fungal protein can be utilized as an analog to meat, having a texture similar to that of animal meat (Hong et al., 2022). Therefore, understanding the variation in the C/N ratio on the characteristics of mycoprotein from tapioca waste is crucial to optimizing the production process and obtaining high-quality products. In addition to carbon sources, the presence of nitrogen in the culture medium is essential for the optimal growth of zygomycetes and the final product yield. Various inorganic and organic nitrogen sources have been used. Typically, inorganic nitrogen such as ammonium sulfate and ammonium nitrate has been used, but organic nitrogen sources like peptone, urea, yeast extract, corn steep liquor, or even fish protein hydrolysate can also be utilized.

1.4. Hypothesis

1. Differences in the C/N ratio affect mycoprotein yield.
2. Differences in the C/N ratio affect the chemical and sensory characteristics of mycoprotein.

II. LITERATURE REVIEW

2.1.Mycoprotein

Mycoprotein is a type of protein that is becoming increasingly popular in the world of vegetarian and vegan foods. It is also known as "mushroom protein" because it is derived from filamentous fungi, primarily the species known as *Fusarium venenatum* (Derbyshire and Ayoob, 2019). The production process involves fermenting this fungus with nutrient-rich substrates. The result is a white product with a texture similar to meat, making it a common meat substitute in vegetarian and vegan dishes. Mycoprotein is high in protein, low in fat, and contains dietary fiber that is beneficial for digestive health (Table 1). Mycoprotein is also an attractive alternative for those looking to reduce meat consumption while still obtaining adequate protein intake.

Table 1 shows that mycoprotein contains 11.5 g of protein per 100 g, including all essential amino acids, making it a high-quality protein that is nearly perfectly digestible. Mycoprotein also contains a beneficial fatty acid profile and makes up about 6% of the total protein in food. The cell walls of the hyphae are a source of dietary fiber (a polymer of N-acetylglucosamine with beta 1,3 and 1,6 glucans), the cell membrane is a source of PUFA (Polyunsaturated Fatty Acids), and the cytoplasm is a source of high-quality protein (Ahangi et al., 2008). This relatively high fiber content, combined with low fat and saturated fat levels and excellent protein quality, makes mycoprotein a valuable food for a healthy diet

Tabel 1. Nutrition Fact of Mycoprotein (wb)

Contain	Amount
Energy (Kcal)	86
Protein (g)	11.5
Carb Total	1.7
Sugar	0.8
Fat Total (g)	2.9
Saturated Fat	0.6
Monounsaturated Fatty Acid	0.5
Polyunsaturated Fatty acid	1.8
Dietary Fiber (g)	6.0
Sodium(mg)	4

Source : Finnigan,2011

2.2.Nutrition Growth

A medium is a blend that includes macronutrients, micronutrients, elements, growth factors, vitamins, and minerals essential for microbial growth. In addition to fostering microbial growth, the medium can be utilized for isolation, proliferation, testing physiological properties, and quantifying microbes. Carbon is an essential element that carbon is used to produce ATP (adenosine triphosphate), which is the main energy molecule used by cells to carry out various biochemical processes and fungal cells use carbon to synthesize new cellular components necessary to increase the size and number of cells. Nitrogen is a key macronutrient essential for microbial growth, as it constitutes cell plasma and plays a critical role in protein synthesis.

Different carbon and nitrogen sources can lead to varying SCP (Single Cell Protein) production outcomes. According to Ahmed et al. (2017), a reasonable carbon-to-nitrogen ratio results in high-quality biomass. Research indicates that C/N ratios of 6:1, 8:1, and 25:1 affect the growth of Candida and Rhodotorula species for SCP production (Zheng et al., 2005). the protein content produced in

that study increased from C/N 25:1 to 10:1 and remained stable thereafter. In the study by Halim et al. (2022), the growth of *Rhizopus spp*. biomass with C/N substrate ratios of 20:1, 20:2, and 40:2 resulted in biomass yields of 0.57g, 0.60g, and 0.5g. The results indicated that the influence of C:N ratio concentration on sporulation and fungal growth. Therefore, consideration for the complexity of nutrient requirements is essential for improving yields of fungal .

2.3 Rhizopus oligosporus

Rhizopus oligosporus is a saprophytic fungus belonging to the phylum *Zygomycota*. This fungus is commonly found in soil, decaying organic matter, and ripe fruits. Rhizopus oligosporus plays a crucial role in ecosystems as a decomposer of organic materials and as a biological control agent. R. oligosporus grows optimally at temperatures between 30-35°C, with a minimum growth temperature of 12°C and a maximum of 42°C. The growth characteristics of R. oligosporus include brownish-gray colonies with a height of 1 mm or more. The sporangiophores can be single or grouped, with smooth or slightly rough walls, measuring over 1000 µm in length and 10-18 µm in diameter. The sporangia are globose, turning blackish-brown when mature, with a diameter of 100-180 µm. Chlamydospores are numerous, either single or in short chains, colorless, containing granules, and formed on hyphae, sporangiophores, and sporangia. The chlamydospores are globose, ellipsoidal, or cylindrical, measuring 7-30 µm or 12-45 µm x 7-35 µm (Dewi and Aziz,2011).

R. oligosporus has been used in Indonesia to produce soybean tempe (tempe kedele) since ancient times, and interest in this food increases worldwide (Nout & Kiers 2005). During incubation with R. oligosporus, the soybeans are bound together by the white mycelium, forming a cake, and enzymes released by the fungus makes the protein-rich product more digestible to humans. R. oligosporus has high proteolytic activity and does not produce toxins (Rauf et al. 2010). Proteases can break down complex protein molecules composed of amino acids bonded in peptides. Proteases from microbes such as *Rhizopus sp*. can also be

used as supplements to aid in food digestion. The advantage of using enzymes from fungi is that only a small dose is usually required, with a broad pH range of 5.2-8. Digestive enzymes in animals, such as pancreatic enzymes, can work synergistically with enzymes derived from molds.

2.4 Texture

The texture of fungus-based food products can be controlled in various ways depending on the growth mode of the fungal microorganisms. First, the texture can be modified through the addition of certain chemicals such as albumin protein, which is used in the production of Quorn mycoprotein. The production of albumin gel and fiber bonds resulting from the combined effects of albumin addition, steaming, and freezing is thought to provide "fibrous" to the Quorn material(Finnigan,2011). Another factor that influences product texture is mold morphology. The size of pellets grown in liquid culture is influenced by mass transfer and mechanical effects that can influence the density and porosity of the pellets. For example, the interior of a mushroom pellet that is too large will be very low in oxygen, causing anaerobic conditions that may result in autolysis within the pellet, creating a hollow texture with low biomass density (Espinosa-Ortiz et al,2016). In contrast, when the pellet grows to a very small diameter, the entire pellet section can be more filled with fungal biomass due to better oxygen transfer capabilities across the width of the pellet. Other methods for modifying the texture of mushroom-based food products can be carried out after cultivation and involve mechanical processes such as pressing, extrusion, and others.

Texture is usually measured using the texture profile analysis (TPA) method. This methodology uses special equipment that measures the force intensity and deformation of the sample during two compression cycles using appropriate probes (Brenee, 1975). Texture encompasses many aspects of a material's force profile and compressibility and can be expressed by parameters such as hardness, brittleness, cohesiveness, elasticity, tackiness, chewiness, and resilience. Hardness is defined as the maximum force from the first compression, while stickiness

measures how well the product survives the second deformation. Durability is measured by how well the product returns to its original height after compression.

III. METHODS

3.1. Time and Place

This research was conducted from November 2023 to February 2024. Biomass production was carried out at the Biotechnology Laboratory of FTP UGM, chemical testing was conducted at the Food and Nutrition Laboratory of FTP UGM, and sensory testing was conducted at the Sensory Testing Laboratory of FTP UGM.

3.2.Methods

This research utilized the fungus *R. oligosporus* with an inoculum quantity of 10^5 spores/mL. The research method employed a factorial Completely Randomized Design (CRD) with 4 treatments and 3 replications, specifically C/N ratios of 2/1, 4/1, 6/1, and 8/1 of medium growth to obtain data on mycoprotein biomass yield. The yield data from the highest and lowest treatments were further analyzed for proximate composition, amino acid profile, and sensory evaluation. The data were processed using analysis of variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) for further analysis.

3.3.Materials

The materials used included the culture of the fungus *R. oligosporus*. The pure fungal culture was obtained from the Faculty of Agricultural Technology at UGM. The medium used to grow the pure culture was PDA (Potato Dextrose Agar). The raw materials used in the mycoprotein production process were tapioca

supernatant liquid waste separated from tapioca starch filtrate using a separator, soybean boiling water directly from the cooking furnace, and cheese whey from curd filtration obtained from PD Semangat Jaya in Lampung dan UD Super Dangsul in Bantul and mazaraat cheese factory. Other materials used included K_2SO_4, HgO, H_2SO_4, NaOH-$Na_2S_2O_3$, H_3BO_3, BCGMR indicator, HCl, and Tween 80.

3.4. Implementation of Research

3.4.1. Preparation Inoculum

The fungal culture used was an isolate of *R. oligosporus* grown on PDA medium. Spores were harvested from the surface of the slanted agar by adding 100 mL of 0.05% Tween 80 solution. This suspension contained 10^5 spores/mL.

3.4.2. Preparation Medium

The liquid waste medium was varied based on the C/N ratio from several waste sources, namely tapioca supernatant liquid waste dan soybean boiling and cheese whey wastewater with C/N ratios of 2/1, 4/1, 6/1, and 8/1. in a 250 mL Erlenmeyer flask under sterile conditions.

3.4.3. Production Biomass

Biomass production was carried out using the fungus *R. oligosporus* on a liquid waste substrate. A 10 mL spore solution of R. oligosporus containing 10^5 spores/mL was inoculated into a 250 mL Erlenmeyer flask containing 100 mL of medium. The mixture was then incubated in a shaker bath at 30°C and 110 rpm for 48 hours (Wikandari, 2023).

3.4.4. Evaluation

The harvested biomass was analyzed for proximate composition, amino acid profile, fatty acid profile, texture, and sensory properties.

3.4.4.1.Protein

Protein testing is based on SNI 01-2891-1992. Initially, the sample is weighed into a Kjeldahl flask, followed by the addition of 1.9 ± 0.1 g K_2SO_4, 40 ± 10 mg HgO, and 2.0 ± 0.1 mL H_2SO_4. The solution is then heated for 1-1.5 hours until it becomes clear.After cooling and diluting with distilled water, the sample is distilled with the addition of 8-10 mL NaOH-$Na_2S_2O_3$ solution. The distillate is collected in an Erlenmeyer flask containing 5 mL H_3BO_3 and 2-4 drops of BCGMR indicator. The obtained distillate is then titrated with 0.02 N HCl until the color changes from green to gray. The result represents the total nitrogen content, which is used to calculate the protein content in the mycoprotein, using a conversion factor of 4.38 for fungal samples. The protein content of the sample is determined using the following formula:

$$\text{Protein (\%)} = \frac{(\text{Y} - \text{Z})\text{x (N x 0.014 x 6.25)}}{\text{W}} x100\%$$

Explanation:

Y = mL of HCl used to titrate the blank

Z = mL of HCl used to titrate the sample

W = Weight of the sample (g)

N = Normality of HCl (N)

3.4.4.2. Fat Content

The flask is dried in an oven at 105°C for 15 minutes. A sample weighing 1-2 grams is placed into a filter paper sleeve, which is then inserted into a Soxhlet extractor with a condenser positioned above it and the fat flask placed below. The fat flask is filled with a sufficient amount of hexane solvent. Reflux is conducted for at least 6 hours until the solvent dripping into the fat flask becomes clear again.

Afterward, the solvent in the fat flask is distilled and collected. The fat flask containing the extracted fat is then heated in an oven at 105°C until a constant weight is achieved and cooled in a desiccator. Finally, the flask along with the fat is weighed to determine the fat content. The calculation formula is as follows: $\text{Fat (\%)} = \frac{\text{Fat Weight (g)}}{\text{Sample (g)}} x100\%$

3.4.4.3.Carbohydrate

Carbohydrate testing based on *by difference*

3.4.4.4. Ash Content

A sample weighing 2-3 grams is placed into a pre-weighed porcelain crucible and dried. The sample is then charred over a flame, followed by ashing in an electric furnace at a maximum temperature of 550°C until the ashing process is complete. Occasionally, the furnace door is slightly opened to allow oxygen to enter. The porcelain crucible containing the ash is cooled in a desiccator and weighed until a constant weight is achieved.

3.4.4.5. Moisture Content

empty crucible is dried in an oven for 15 minutes at a temperature of 103°C ± 2°C, then cooled in a desiccator and weighed. A sample weighing 1-2 grams is placed into the pre-weighed crucible and dried in the oven at 103°C ± 2°C for 3 hours. The crucible containing the sample is then transferred to a desiccator, cooled, and weighed again. Repeat the drying process until the difference between two consecutive weighings does not exceed 0.005 grams.

3.4.4.6.Crude Fiber

A sample of 1 g of defatted material is added to 100 ml of 0.255 N H_2SO_4. Then, it is boiled for 30 minutes with a reflux condenser. After that, 100 ml of 0.313 N

NaOH is added and boiled again for 30 minutes with a reflux condenser. The next step is filtration using filter paper of known weight. The filter paper is washed with 10% K_2SO_4, boiling water, and 15 ml of 95% ethanol. This washing process is intended for the separation of ash and silicates. The filter paper is then dried at 105°C for 2 hours, cooled, and weighed. The determination of crude fiber content is calculated using the following formula:

$$\text{Fat (\%)} = \frac{\text{Fat Weight (g)}}{\text{Sample (g)}} x100\%$$

3.4.4.7.*Teksture Profile Analysis* (TPA)

The biomass results from each experiment are followed by an analysis of the physical characteristics of the mycoprotein using a texture analyzer. The analysis includes three parameters: hardness, springiness, and chewiness. The conditions used for the analysis are as follows(Hendartina,2014):

Probe : silinder 3.5 cm (SMS P/35)

Mode : texture

Profile Analysis Option : return to start

Pretest speed : 5 mm/s

Test speed : 0.5 mm/s

Posttest speed : 5 mm/s

Distance : 30%

Trigger type : auto

Trigger force : 5 g

The determination of the three parameters is conducted as follows:

- Hardness: The maximum force (peak value) during the first compression.
- Springiness: The distance traveled by the sample during the second compression to reach the maximum force (L2) compared to the distance traveled during the first compression to reach the maximum force (L1), calculated as L2/L1.
- Chewiness: The product of springiness and the ratio of the area under the curve from the second compression (A2) to the area under the curve from the first compression (A1).

3.4.4.8.Sensory Evaluation

Sensory evaluation of the mycoprotein was conducted using a scoring test to assess parameters such as hardness, springiness, adhesiveness, chewiness, and cohesiveness. The sensory testing samples were prepared as food products in the form of burger patties, made using a standard recipe with branded ingredients and used in accordance with BPOM safety limits. The scoring test involved 60 panelists, who were semi-trained individuals from the Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Gadjah Mada University.

V. CONCLUSION

5.1 Conclusion

The conclusions of this research are as follows:

1. C/N ratio affected the biomass yield, in which the highest yield was obtained at C/N ratio of C/N 2:1 and C/N 8:1 (0.275 g/L and 0.180 g/L) but are not significantly different from the C/N 4:1 and 6:1 (0.211 g/L and 0.208 g/L).

2. C/N ratio had influence on protein and fiber content. that is C/N 2:1 15.78% and C/N 8:1 11.53% and crude fiber content C/N 2:1 5.89% and C/N 8:1 8.63%.

3. Although C/N ratio affected the chewiness, hardness and cohesiveness of the sample according to texture profile analyzer, however this difference was not detected by panelist of sensory test.

5.2 Sugestion

1. It is necessary to carry out further research on all factors that influence the growth of fungal biomass

2. Further research is needed regarding the correlation between growth composition, culture type, nutritional composition and additional ingredients to obtain analog meat products that can outperform or resemble real meat.

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