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

(Bachelor Thesis)

By

Firhan Al fariz

2014051042

AGRICULTURAL PRODUCT TECHNOLOGY DEPARTMENT

FACULTY OF AGRICULTURE

LAMPUNG UNIVERSITY

2024

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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Name : Firhan Al Fariz

Student Identity Number : 2014051042

Major : Agricultural Product Technology

Faculty : Agriculture

ACKNOWLEDGE

1. Supervisory Committee

Prof. Dr. Ir. Udin Hasanudin, M.T.
NIP. 19640106 198803 1 002

Rachma Wikandari, S.T.P.,M. Biotech., P.hD
NIP. 19860126 201803 2 001

2. Chairman Department of Agricultural Product Technology

Dr. Erdi Suroso, S.T.P., M.T.A.
NIP. 19721006 199803 1 005

VALIDATE

1. Examination Committee

Chairman : Prof. Dr. Ir. Udin Hasanudin, M.T

Secretary : Rachma Wikandari, S.T.P.,M. Biotech., P.hD
(Department of Food and Agricultural Product Technology, Gadjah Mada University)

Examiner Non Advisor :Prof. Mohammad Taherzadeh
(Department of Resource Recovery, University of Boras)

2. Dean Faculty of Agriculture

Dr. Ir. Kuswanta Futas Hidayat, M.P.
NIP. 19641118 198902 1 002

Thesis exam passing date : 20th August 2024

STATEMENT OF ORIGINALITY

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.

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Bandar Lampung, August 2024

Firhan Al Fariz
SID 2014051042

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).

The author carried out Field Study and Community Service in January–February 2023 in Kalirejo Village, Wonosobo District, Tanggamus Regency. The author carried out internship at PT. Perkebunan Nusantara VII Unit Way Berulu in July 2023.

During his time as a student, the writer was an Assistant Lecturer for Chemistry in the 2022/2023, Starch Technology in the 2023/2024 and Micobiology in the 2023/2024 The author is also active in student activities, namely being the General Chair of the Ruang Pemimpi Indonesia (Indonesian Dreamers Space) which currently oversees the Provinces of Lampung and Central Java, a member of the Research and Technology Department of UKM U Science and technology Unila for the 2021/2022 period, and Chair of the Resource Management (HR) Division of UKM-U Saintek University of Lampung for the period 2022. The author also has the achievement of first place (Gold innovator) in the food sustainability category and third place (Bronze Innovator) in the aquaculture category held by ReachSci Indonesia by Cambridge University

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.

On this occasion the author would like to extend my thanks to: ;

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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
Author,

Firhan Al Fariz

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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: $\text{Fat (\%)} = \frac{\text{Fat Weight (g)}}{\text{Sample (g)}} x 100\%$

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.

IV. RESULT

4.1. Biomass Production

The results (Fig 1.) show the biomass growth pattern increases as the C/N ratio decreases.The amount of biomass produced at a C/N ratio of 2:1 yields the highest biomass and is significantly different from the other treatments. According to the research by Lonardo et al. (2020), a lower C/N ratio and higher N availability during the growth of Mucor hiemalis coincide with higher biomass production and growth efficiency.This could be due to limited amounts of nitrogen limit protein synthesis even though there is plenty of energy from carbon. This causes slow and less efficient growth because the fungus cannot synthesize cellular components fast enough. Yalemtesfa et al (2010) stated that the addition of $(NH_4)_2SO_4$ as a nitrogen source produced a higher protein level in *Chaetomium spp*. and *Aspergillus niger* .

On the other hand, findings made in the research of Halim et al (2022) showed that variations in C/N ratio on the growth of *rhizopus sp* mycelium did not have significant results and even experienced a decrease in the amount of biomass in higher C/N ratio. This might be caused by the addition of sugar disturbs the growth and ability to produced amino acids. Ahmed et al (2017) suggests that a balanced carbon to nitrogen ratio is the key to producing high-quality biomass. It meant that a higher amount of carbon and nitrogen did not guarantee higher biomass and protein content production (Halim et al 2022). There are many factors that must be considered which may have an influence apart from the nutrition of the media, namely the type of culture used, the conditions of the fermentation process, and others. .

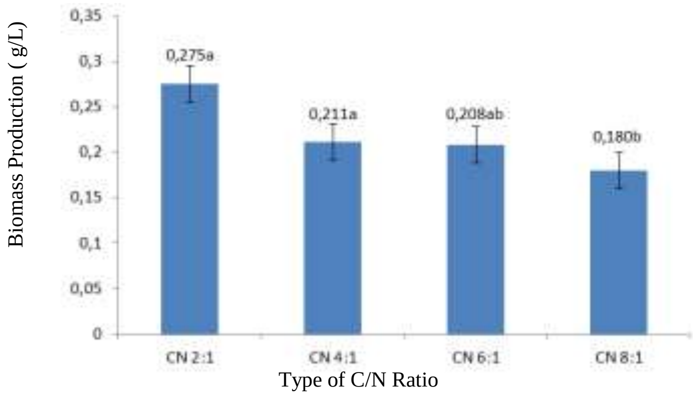

Figure 1 . Biomass Yield in each treatment C/N Ratio

In fermentation using *Rhizopus oligosporus*, the balance between carbon and nitrogen in the medium is very important to achieve optimal biomass growth. Carbon serves as the main energy source, while nitrogen is needed for the synthesis of proteins and nucleic acids which are important for cell growth. An optimal C/N ratio will ensure that the fungus has enough energy and raw materials for metabolic processes and cell growth. Utilizing by-products and food waste as growth media for edible fungi not only fosters innovation in food products but also helps reduce environmental pollution caused by the food industry. At the beginning of fermentation, the mold will start consuming simple carbohydrates in the media, but complex carbohydrates will also be digested once the simple carbohydrates are depleted.The use of cheap and complex nutrient substrates, i.e., agro-industrial waste materials, is common in industrial fermentation but its use depends on geographical location and accessibility. Agro-industrial waste is widely reported for biomass production (Arous et al, 2016). Research shows that tapioca waste is effective in increasing crude protein and cell mass production.

4.2 Proximate Content

4.2.1 Effect C/N Ratio to Protein Content

The variation of C/N ratio not only affects fungal growth but also can affect protein content in fungi. The effect of C/N ratio on fungal biomass protein content can be seen in Figure 2.

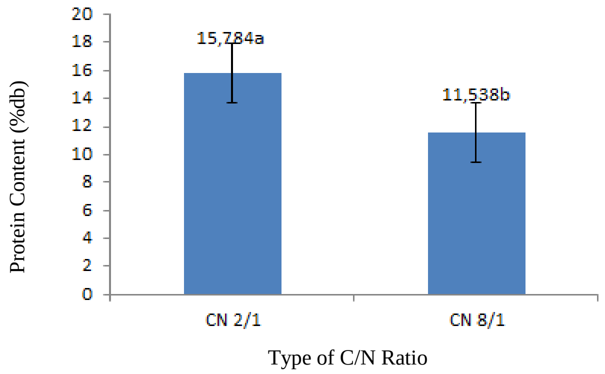

Figure 2. Effect C/N Ratio to Protein Content

For further evaluation regarding information on the nutritional value of the effect of C/N treatment, the ratios of the biomass yields are significantly different, namely C/N 2/1 and C/N 8/1 .The nutritional value of biomass is influenced by the rich nutritional value of the media used. Higher C/N ratio resulted in lower protein content (Figure 2). The ANOVA results showed that protein values were significantly different at the <0.05 level, indicating an influence on protein levels in biomass. Based on Sari and Suyono (2024), they explained in their research that the nitrogen concentration was high tends to increase biomass, and protein production while reducing accumulation of lipids, while low nitrogen concentrations tend to increases lipid and carbohydrate production while reducing protein production.The protein value obtained was lower than research conducted by Jin et al (2002) using starch processing wastewater as a medium substituted with yeast extract that can produce a protein value of up to 49% (db). The protein content of biomass is still below the protein content of several comertial food (Table 2). However, in general biomass contain nine types of essential amino acids with the highest content of the amino acid lysine (Wikandari et al, 2023).

4.2.2 Effect C/N Ratio to Fat Content

The variation of C/N ratio not only affects fungal growth but also can affect fat content in fungi. The effect of C/N ratio on fungal biomass fat content can be seen in Figure 3.

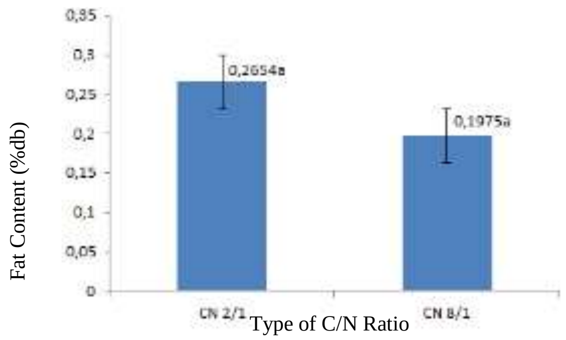

Figure 3 Effect C/N Ratio to Fat Content

The resulting fat content is very low, only 0.26% and 0.20% (db), not significantly different at a significant level of <0.05. In this study, no influence was found on the C/N ratio on fat content. This result is lower than the panel research carried out by Jin et al (2002) which used R. oligosporus culture, namely 1.1%. Depletion of nitrogen in cultivation medium causes a decrease in growth with concomitant increases in d lipid productivities (Vooren et al,2012). According to theory in the article Sizwe et al (2021) Nutrient imbalances, most typically the excess of carbon source and a limitation in other vital nutrients such as nitrogen, triggers several physiological and metabolic changes leading to the channeling of the carbon flux toward lipid synthesis. Therefore, it is necessary to know more about the balance and imbalance of carbon to nitrogen which causes differences in the amount of fat in filamentous fungi.In accordance with European Commission (2008), nutrition claims that can be made for mycoprotein contains no more than 3 g of fat per 100 g of solids and low in saturated fat does not contain more than 1.5 g of saturated fatty acids per 100 g of solids.

4.2.3 Effect C/N Ratio to Fiber Content

The variatio of C/N ratio not only affects fungal growth but also can affect fiber content in fungi. The effect of C/N ratio on fungal biomass fiber content can be seen in Figure 4.

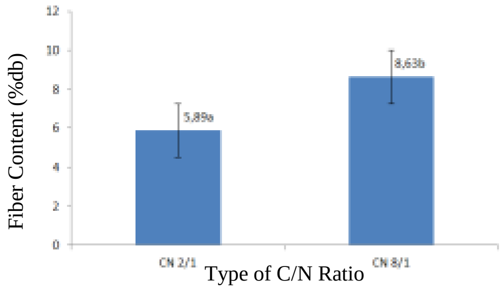

Figure 4. Effect C/N Ratio to Fiber Content

The higher C/N Ratio resulted ini higher fiber significantly different ANOVA results ($p<0.05$). The effect of the C/N ratio is limited nitrogen limits the synthesis of proteins and other nitrogen-containing components, so that more carbon allocated to fiber production tends to synthesize more carbon-rich structural components, such as fiber (cellulose and chitin). The fiber present in fungal biomass is naturally occurring, and typically one-third of this is chitin (poly n-acetyl glucosamine) and two-thirds is β-glucan (Denny et al,2008). One of the interesting anabolic processes in *Rhizopus* is the synthesis of chitosan in its cell walls. Chitosan is a polysaccharide obtained through deacetylation of chitin. The chitosan formed is then integrated into the cell wall(Pellis et al,2022). Denardi et al., (2018) that regarding nutrition, the beneficial relationship between carbon sources and nitrogen sources in. The ideal growth media range is 4:1 to 10:1 during the fermentation process by *Rhizopus oryzae*. Increasing carbon sources with including the provision of a nitrogen source will have an impact increase in chitosan biomass Linear β-glucans (from cereals) and branched β-glucans (fungi; yeast) have been shown to have immunostimulating effects and participate in physiological processes related to the metabolism of fats in the human body (Kudrenko et al,2009)

4.2.4 Comparison Nutritional Content between Biomass this Research and Several Comercial Food

Table 2. shown based on the products from this research, when compared with other products, the protein value is lower than others. however these results are superior based on the attributes of lower fat and higher fiber.

Table 2. Proximate content of fungal biomass in this research and several comertial food

Nutrition Parameter	C/N 2/1	C/N 8/1	Quorn vegan burger*	Real Beef*	Soybean tempeh*
Crude Protein %db (Nx6.25)	15.78±0.11[a]	11.53±0.03[b]	21	20	19
Fat %db	0.26 ±0.20[a]	0.19±0.45[a]	14	17	12
Carb by difference	69.42	75.23	-	-	9
Crude Fiber %db	5.89±0.24[a]	8.63±0.2z[b]	3.1	-	1.4
Ash %	1.09±0.23[a]	2.06±0.53[b]	-	-	1.62

*by refference (Godschalk-Broers et al. 2022) and (USDA,2019)

The research results showed that the protein content was still lower than the comparison product. Due to its relatively high protein value, increased fiber, and low saturated fatty acid content, mycoprotein is important for a healthy diet. Mycoprotein may provide several nutritional benefits, including increasing satiety and controlling blood sugar and cholesterol levels, according to experimental studies. Mycoprotein must have high protein quality if used in main meals. Mycoprotein contains all the essential amino acids. For the most part, mycoproteins contain less than 1.5 g of long and short chain saturated fatty acids per 100 g of solids. This mushroom protein is rich in mono- and poly-unsaturated fatty acids (Saeed et al,2023). Ash content at a significance level <0.05 indicates results that are not significantly different. Ash content indicates the minerals contained in the material. Further research is needed to determine the mineral profile of this research product. According to nutritional claims made by the European Commission, mushroom protein is a powerful source of various mineral components, including zinc, phosphorus, calcium, iron, potassium, and more.

4.3 Sensory Evaluation

Sensory evaluation was carried out to determine differences in variations in the C/N ratio in the resulting texture profile compared with commercial products.The sensory evaluation was conducted using a scoring method with parameters of texture including springiness, cohesiveness, adhesive hardness, and chewiness. The results of the sensory evaluation were based on the perceptions of semi-trained panelists (n=60) regarding the texture attributes of the product (Fig 2). It is estimated that differences in the nutritional values of the growth media and the nutritional content of mycoprotein may influence the variation in texture produced. However, literature by Godschalk-Broers et al. (2022) does not indicate a specific relationship between composition and texture values in analog burger samples. Sensory evaluation results, as shown in the figure, were tested at a significance level of <0.05, revealing no significant differences between treatments, although both treatments differed significantly from the control. The chewiness, hardness, and springiness values of both samples were lower than those of the control, indicating that panelists found it easier to deform the samples compared to the control. Kamani et al. (2019) found that mycoprotein nuggets required less force to chew compared to chicken samples and optimized samples. This is likely due to the weaker mycoprotein network, which results in reduced resistance of the product to compression. Non-meat proteins can retain more water and fat, which may fill the intercellular spaces in the protein matrix and reduce elasticity (Kamani et al., 2019).

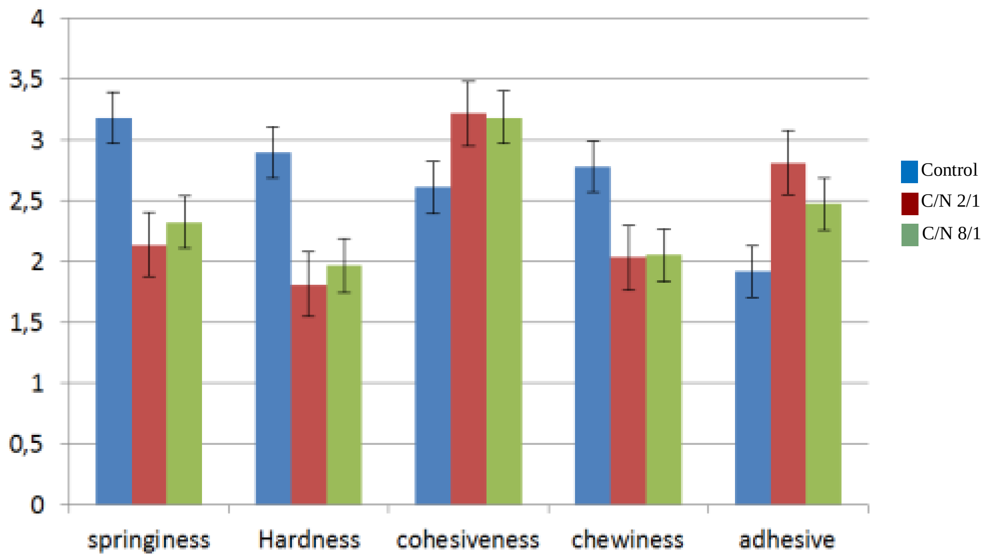

Figure 2. Scores of the sensory attributes (scale: 0-5) assessed for the meat analogues studied in the present research (n = 60).

Based on the results of the sensory test, variations in the C/N ratio had no effect on all the attributes tested. The results indicate that panelists did not find specific differences between the two tested treatments but could distinguish the control treatment, which used a control vegan burger patty made from Campagnion. The cohesiveness dan adhesive values of treatments C/N 2/1 and C/N 8/1 were higher than those of the control. Higher cohesiveness values are correlated with their fibrous structure (Godschalk-Broers et al., 2022). A high adhesive value indicates that the material or product has significant sticky properties. In foods, this can mean a smoother and thicker texture (Nishinari et al,2013). It is suspected that the fat content during the cooking process includes the addition of oil so that fat often increases the sticky properties of the product, thus increasing the adhesive value (Szczesniak,2002)

The results of this study's sensory evaluation suggest that the mycoprotein tested is not yet fully similar to commercial vegan patties. However, some parameters showed significant differences from the control, and these differences have not been statistically related to panelists' preferences or dislikes. As previously mentioned, the organoleptic quality of meat products requires further research, especially in processed meat products where perceived texture attributes, such as bite, chewiness, and tenderness, are crucial to consumers. Maintaining sensory and textural quality presents a significant challenge in producing meat analog products. Texture and flavor (especially tenderness and palatability) are highly valued by consumers, whereas non-meat ingredients may lack the same sensory attributes (Elzerman et al., 2011).

4.4 Texture Profile

The results of the Texture Profile Analysis test are presented in table 3. It shows that several parameters have significant results between treatments and between controls. The hardness parameter C/N 2/1 is significantly lower than the control and C/N 8/1 is not significantly different from the control. Higher values for hardness indicate a harder texture (Schreuders et al, 2021). The control treatment used a vegan burger sample made from champignion mushrooms. The chewiness value of C/N 2/1 was significantly lower than the control and C/N 8/1 was not significantly different from the control. The Quorn Burger matched the real beef burger the most on hardness, chewiness, cohesiveness, and fibrousness (Godschalk-Broers et al, 2022). the TPA test on Quorn Burger produced a hardness value of 199.78 N, springiness 0.70 mm, cohesiveness 0.37 mm and chewiness 65.52 N. The high hardness value can be attributed to the compactness of the sample, such as Quorn having a dense structure (Godschalk-Broers et al, 2022).The results of this research are the test results whose values are the most similar to real meat. The expected characteristic of analog meat is that it can be chewed with good elasticity (Hendartina, 2014).

Table 3. Instrumental properties measured for the meat analogues studied in the present research. Different letters refer to statistical differences for the different parameters in the column.

Parameter	Control	C/N 2/1	C/N 8/1
Hardness	45.9015 ± 5.64^{a}	14.1075 ± 1.79^{b}	31.7960 ± 7.33^{a}
Chewiness	10.8745 ± 0.07^{a}	2.608 ± 0.39^{b}	8.7040 ± 1.98^{a}
Springiness	0.7092 ± 0.01^{a}	0.6340 ± 0.01^{b}	0.6750 ± 0.21^{ab}

Parameter	Control	C/N 2/1	C/N 8/1
adhesive	0.2629±0.36[a]	0.3420±0.43[a]	0.2540±0.14[a]
cohesiveness	0.3356±0.03[a]	0.2910±0.02[a]	0.4139±0.01[b]

The hardness and hardness values were directly proportional and were not significantly different from the chewiness between treatments. This is because the relationship between the hardness value and the chewiness value in meat products is the energy value needed to chew food, the harder it is to chew the food, the higher the energy required. The lower hardness value in C/N 2/1 can be attributed to the high protein value, namely the nature of the protein itself, namely as a emulsifier which can bind more water and fat so that the texture becomes loose and soft. This can be shown in research by Youseff and Barbut (2009) who stated that more aggregates and less homogeneous protein matrix structures were observed in meat emulsions with higher protein content.

Parameter springiness is the rate at which a deformed material goes back to its undeformed condition after deforming force is removed. It is a measurement of elastic recovery. The results showed that the C/N 2/1 treatment showed a significant difference ($p<0.05$) to the control but was not significantly different from the C/N 8/1 treatment. In terms of springiness, there tends to be no relationship between the composition of analog meat growth media or other analog meat formulations and changes in springiness values in analog meat products. In Baksh et al's (2021) study, cohesiveness and springiness varied according to patty type, but the differences were not statistically significant. This is also supported by tests carried out by Godschalk-Broers et al (2022) who tested 15 types of analog burger patties to produce 9 types of patties whose springiness values were not significantly different from each other with values ranging from 0.4-0.69 and likewise with cohesiveness values there were 10 types of patties. which are not significantly different from each other, range in value between 0.28-0.36. No real differences were found in the adhesive attribute in this study, but in Wee et al's (2018) research, high protein and fat values had a negative correlation with food products, while carbohydrate values did not have a significant correlation with adhesive values .

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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