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Research Article

Nutritional quality, sensory profile and textural improvement of mycoprotein nuggets derived from *Rhizopus oligosporus* with incorporation of various binding agents

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Abstract

Today's consumer awareness of reducing meat consumption is increasing for health and environmental reasons, driving to the exploration of alternative protein sources. Mycoprotein, one of which is *Rhizopus oligosporus*, demonstrates a potential as meat substitute in animal-derived product, such as nugget, due to its meat-like texture and excellent nutritional profile. However, mycoprotein nugget still lacks certain textural properties resembling meat; therefore, further improvement is necessary to perform for achieving the desired quality. The mycoprotein nugget was characterized and compared to vegan and chicken nugget from the nutritional and sensory aspects. For improving the textural properties of mycoprotein nugget, several binding agents were incorporated, including sodium tripolyphosphate, methylcellulose, and modified tapioca starch. The textural attributes of mycoprotein nugget were analyzed using Texture Profile Analyzer (TPA). The results showed that mycoprotein nugget exhibited a lower fat content, higher fiber and essential amino acid content, and better quality of fatty acid profile compared to vegan and chicken nugget. In terms of sensory profile, the overall liking of mycoprotein nugget was comparable to vegan nugget, but those still less preferred than chicken nugget. The TPA results showed that 10% of modified tapioca starch provides the most significant increase in hardness and chewiness of mycoprotein nugget. Further investigation of the mechanism underlying the functionalities of binding agents is required to discover new understandings in product development.

Keywords

Mycoprotein, nugget, nutrition, sensory, texture, binding agent

Abbreviations

GRAS – generally recognized as safe; ICP-OES – inductively plasma optical emission spectrometry; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; RDA – recommended dietary allowance; SFA – saturated fatty acid; TPA – texture profile analyzer; UPLC – ultrahigh-pressure liquid chromatography

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Introduction

Meatless Monday is a global campaign introduced to reduce meat consumptions for personal reasons and ecological well-being. This campaign has expanded to more than 40 countries, including the Philippines, Malaysia, Singapore, and others (Semba et al. 2024). Meat can be a nutritious food for some people. However, excessive meat consumption is associated with several health risks, such as colorectal cancer, cardiovascular disease, and diabetes (Godfray et al. 2018). Additionally, the meat industry is an important driver of greenhouse gas emissions and environmental degradation (Allen and Hof 2019). Therefore, an alternative protein source concerning diet quality and sustainability is required.

An increasing number of people are replacing their meat intake with alternative protein source, such as plant-based, edible insect, mycoprotein, and others. The popularity of plant-based meat is growing due to its higher content in dietary fiber and lower levels of saturated fat and cholesterol. However, plant-based meat contains some anti-nutrient that lower the bioavailability of nutrients. In addition, plant-based meat is deficient in certain essential amino acids (El Sadig and Wu 2024). Edible insects are also high in protein, but their acceptance by consumer is still low. This may be due to consumers' fear, disgust, and related feelings toward edible insects (Onwezen et al. 2021).

Mycoprotein produced from fungal biomass is a promising alternative protein source due to its nutritious content and environmentally friendly because it requires less land and emits lower greenhouse gasses (Hashempour-Baltork et al. 2020). It is low in fat, high in polyunsaturated fatty acids, low in saturated fatty acids, and rich in essential amino acids. The amino acid profile of mycoprotein is predominantly composed of glutamic acid which is responsible for its umami flavor (Wikandari et al. 2023). The best-known mycoprotein-based product is produced and commercialized by Quorn® using *Fusarium venenatum* A3/5, which has been thoroughly studied and selected from among >3000 soil fungi. It took decades of research and development to produce a nutritious and safe mycoprotein (Finnigan et al. 2019).

There are numerous other species of edible filamentous fungi that are commonly used as a starter culture in many food industries (Wang et al. 2023). However, their potential as a new source food, such as meat analogues has not yet been fully explored. One of these is *Rhizopus oligosporus*, which has been employed for making Asian fermented food and globally consumed for centuries. It is a prospective source to produce mycoprotein since it was considered generally recognized as safe (GRAS) and has favorable taste. *Rhizopus oligosporus* has been reported to contain high levels of protein, essential amino acids and polyunsaturated fatty acids with low fat content. It also has ability to be produced at low cost using the residual water of tempeh processing (Wikandari et al. 2023). The use of agro-industrial waste as a cultivation medium for mycoprotein production has been increasingly explored in recent years. Previous studies utilizing date waste as a substrate for *Fusarium venenatum* have reported favorable nutritional composition, including amino acid and fatty acid, as well as acceptable safety profiles (Hashempour-Baltork et al. 2020; Seyed Reihani and Khosravi-Darani 2018). However, *Rhizopus oligosporus* and *Fusarium venenatum* has different morphologhical properties, which may result in different filament structure. Therefore, further research is necessary to develop *Rhizopus oligosporus* into meat analogue product. One of the main challenges is that pure fungal biomass typically has a softer texture and is less firm than meat, which might affect the consumer acceptance. Nugget is a widely popular fast food originally made from minced meat that is breaded and battered, then deep-fried for providing crispiness and diverse sensory attributes (Barbut 2012). In contrast to our previous mycoprotein-based formulations developed as patty, the nugget represents a distinct structure that may offer different sensory perception (Wimbasara et al. 2025). Moreover, nugget is preferred by consumers, especially kids, due to their palatable taste and convenience (Sujarwanta et al. 2019). It is also recognized for its versatility so that it can be made with various ingredients in several ways of processing. The nutritional and sensory profile of mycoprotein nugget derived from *Rhizopus oligosporus* compared to vegan and chicken nugget has not yet been understood. This study was conducted to evaluate the nutritional and sensory profile of the mycoprotein profile.

For improving the textural properties of mycoprotein nuggets, various binding agents can be incorporated. The most common binding agent added into commercial mycoprotein-based products is albumen from egg white, which does not meet the requirement of vegan market. Hence, non-animal-based binding agents can be an alternative option, such as sodium tripolyphosphate, methylcellulose and modified tapioca starch (Kyriakopoulou et al. 2021). Sodium tripolyphosphate increase the pH value of the meat product system, thereby enhancing the water holding capacity and gel strength (Surjoseputro et al. 2004). Methylcellulose improves binding and water retention properties by forming a three-dimensional network (Bakhsh et al. 2021). Modified tapioca starch can interact with protein forming a strong gel matrix, improving the hardness and chewiness of meat-based product (Wei et al. 2023). However, the effect of addition those binding agents on sensory, especially texture, has not yet been studied. Therefore, this study aimed to investigate the effect of binding agents addition on texture of mycoprotein nugget.

Materials and Methods

Materials.

The mycoprotein was derived from a commercial tempeh starter Raprima (Toko Hasil Bumi Langgan, DIY, Indonesia). This fungus was produced from *Rhizopus microsporus* var. *oligoporus* and has identified similar to *R. microsporus* (CBS 631.82) (Hartanti et al. 2015; Yarlina et al. 2023).

Methods.

Mycoprotein production. The mycoprotein production was initiated through the preparation of a cultivation medium using residual boiling water from potatoes. An amount of 200 $g.L^{-1}$ of potatoes was subjected to boiling for 15 min. The residual boiling water was subsequently added with 10 $g.L^{-1}$ of sucrose and 5 $mL.L^{-1}$ of a 20% (v/v) lactic acid solution. The cultivation process was carried out using a 50 L stirred-tank reactor (Fermentor Custom CV. Multilab, DIY, Indonesia). A two-step sterilization (121°C, 15 min) was performed prior to inoculation, initially on the empty reactor, followed by a second sterilization after the addition of 40 L cultivation media into the reactor. The tempeh starter (10 $g.L^{-1}$) was inoculated on the reactor at 37°C for 48 h with agitation rates of 50 rpm and aeration rates of 0.25 vvm. The fungal biomass was harvested and separated from the cultivation medium. It was washed using distilled water three times and pressed to remove the excess water, then kept in the freezer (-18°C) until use.

Nugget formulation. Minced chicken meat (CK) and soy protein isolate meat substitute (VG) were used as the base of the nugget control. Formulations of controls and mycoprotein nuggets were mostly similar. The nugget formulation was prepared as described in Table 1. The frozen biomass was first thawed and pressed to remove water. The ingredients, other than mycoprotein and binding agents, were minced in a food processor (Philips Food Processor HR7627, Amsterdam, Netherlands). Subsequently, the mycoprotein and binding agents were added and mixed to homogeneity. The nugget mass was then formed in a tray (40 cm x 40 cm) and steamed for 15 min. The steamed nugget was coated with batter and breadcrumbs. Finally, the nugget can be fried (180°C) for 6 min or stored in freezer (-18°C) until analysis. For enhancing the textural quality, the mycoprotein nuggets were incorporated with several binding agents, including sodium tripolyphosphate (ST), methylcellulose (MC) and modified tapioca starch (MT) at concentration 0.1 and 0.3%; 2 and 4%; 7 and 10%, respectively.

Table 1. Nugget formulation

Ingredients	Content, %
Meat/mycoprotein	63-65
White bread	6-8
Onion	8-10
Tapioca flour	6-8
Sugar	1-3
Garlic	1-3
Corn starch	1-2
Mushroom seasoning	1-2
Salt	1-2
White food coloring (titanium dioxide)	0.4-0.6
Ground pepper	0.3-0.5
Nutmeg	0.1-0.2

Proximate analysis. The proximate composition of the nugget, including moisture, ash, protein, and fat was analyzed using official methods (AOAC 2005). The moisture and ash content were determined by thermogravimetry, the protein content was

determined by the micro-Kjeldahl method, and the fat content was determined by the Soxhlet method.

Determination of crude fiber content. The fiber content of the nugget was determined using acid and alkaline hydrolysis method (Sudarmadji 2003). As much as 1 gram of the sample was hydrolyzed using 1.25% H_2SO_4 for 1 h, then washed with water. It was then hydrolyzed again using 1.25% NaOH for 1 h. The hydrolyzed sample was dried in an oven (Memmert UN110, Schwabach, Germany) at 105°C until it reached a constant weight.

Determination of protein digestibility. The protein digestibility of the nugget was analyzed based on the method previously reported by Wikandari et al. (2023) with minor modifications. An amount of 0.2 g of the nugget was dissolved in 9 mL of 0.2 N walphole buffer solution (pH 2) and 1 mL of pepsin enzyme (Sigma-Aldrich, St. Louis, MO, USA). The mixture was incubated at 37°C for 6 h and then centrifuged at 3000 rpm for 20 min. Five mililiters of supernatant were added to 5 mL of 20% trichloroacetid acid (Sigma-Aldrich, St. Louis, MO, USA), then incubated for 16 h and filtered using Whatmann filter paper no. 41. The filtrate was further analyzed using the micro-Kjeldahl method to determine its protein content.

Determination of vitamin content. The vitamin content of the nuggets was determined using high-performance liquid chromatography (Waters Alliance e2695 HPLC, Milford, MA, USA) according to previous method (Shahbazpour et al. 2021). Five grams of the sample were mixed with 20 mL of 5% metaphosphoric acid in a vessel. The solution was homogenized and centrifuged at 3000 rpm for 5 min. Afterwards, the supernatant was double filtered using Albet no. 1305 filter papers and 0.45 µm Millipore filters, respectively. The sample was then transferred to vials and injected into HPLC.

Determination of mineral content. The mineral content was assessed using inductively coupled plasma optical emission spectrometry (Agilent 700 Series ICP-OES, Santa Clara, CA, USA), which previously reported by Kalayci and Muhammet (2022). The sample (0.5 g) was placed into polytetrafluoethylene digestion vessel and added with 1.5 mL of HNO_3 and 2 mL H_2O_2, followed by incubation at room temperature for 6 h. The digestion vessel was closed for digestion process (170°C, 18 min) and left cooled overnight. The sample was subsequently transferred into tubes and diluted to 10 mL with ultrapure water for ICP-OES analysis.

Determination of amino acid profiles. The amino acid composition of nuggets were analyzed using ultrahigh-pressure liquid chromatography (Waters Acquity UPLC Photodiode Array, Milford, MA, USA) based on a previous method (Wikandari et al. 2023). The UPLC was equipped with photodiode array detector allowing the identification and quantification of the separated compounds. A total of 0.1 g of the sample was initially hydrolyzed using 6 M HCl at 110°C for 24 h. Afterwards, the hydrolyzed sample was transferred to a 50 mL calibrated flask and filtered with 0.45 µm filter paper. The filtrate (500 µL) was added to α-aminobutyric acid and double deionized water. Ten microliters of the mixture were dissolved in of AccQ-Fluor borate buffer, followed by incubation at 55°C for10 min) and injection into the UPLC. To quantify the amino acids, the peaks were compared to the amino acid standards.

Determination of fatty acid profiles. The analysis of fatty acid was performed using gas chromatography coupled with a flame ionization detector (FID) (Agilent 7890B GC System, Santa Clara, CA, USA) according to the previous method by Romulo and Sadek (2022). The sample (1 µL) was injected in split mode at 240°C. The oven temperature was set from 50°C and then continuously increased to 230°C. The data was interpreted according to the comparison of retention time with fatty acid standards (C_4–C_{24}).

Sensory analysis. The sensory characteristics of the nugget were evaluated by 80 untrained panelists at Gadjah Mada University. The panelists were served a tray containing six samples, water, and a cracker. The sensory evaluation was conducted using a 7-point hedonic scale, ranging from 'extremely dislike' (score = 1) to 'extremely like' (score = 7). Sensory attributes evaluated included color, taste, hardness, cohesiveness, springiness, chewiness, and overall liking.

Texture profile analysis. The texture profile of the nugget was analyzed according to the previous method (Hashempour-Baltork et al. 2023) with modifications. This analysis was done using Texture Profile Analyzer (Ametek Lloyd TA1

Series, Berwyn, PA, USA). The samples were cut into (2 × 2 × 1.5) cm^3 and placed onto the platform. The samples were then compressed to 50% of the sample height. The test speed was 0.50 $mm.s^{-1}$, the wait time was 0.50 s, the preload force was 1 N, and the preload speed was 300 $mm.min^{-1}$. Attributes such as hardness, cohesiveness, springiness, and chewiness were determined.

Statistical analysis. The experiment was conducted in two replicates. The obtained data were analyzed by one-way analysis of variance (ANOVA), followed by post hoc test Duncan's Multiple Range Test (DMRT) for multiple comparisons of means at 5% significance level. All tests were performed using IBM SPSS version 25.0 (International Business Machines Corporation, Armonk, NY, USA). The results were expressed as mean ± standard deviation (SD).

Results and Discussion

Nutritional quality of mycoprotein nugget. Mycoprotein has potential as an essential healthy diet due to its high value protein, fiber, and low fat content. Mycoprotein may offer several health benefits, such as regulating blood cholesterol and glucose levels, stimulating muscle synthesis, and increasing satiety (Finnigan et al. 2019; Majumder et al. 2023). The proximate composition of mycoprotein, vegan, and chicken nugget is presented in Table 2. This study revealed that mycoprotein nuggets contain lower level of fat than that of vegan and chicken nuggets. Hashempour-Baltork et al. (2023) obtained similar results that mycoprotein nugget made from *Fusarium venenatum* has lower level of fat content than chicken nugget. These align with several previous findings demonstrating that the fat profile of mycoprotein (1.5-13.0 g per 100 g dry matter) is lower than that of other protein sources (Derbyshire and Ayoob 2019; Finnigan et al. 2019; Wikandari et al. 2023). This suggests that mycoprotein can be a better choice of meat analogues for promoting healthier lifestyle.

However, mycoprotein nuggets exhibit lower contents of moiture and protein compared to vegan and chicken nuggets. A previous study by (Hashempour-Baltork et al. 2023) documented similar trends that mycoprotein nugget contains lower levels of protein compared to chicken nugget. The nutritional profile of mycoprotein, including protein, depends on several factors, such as fungal species, substrate composition, and cultivation conditions (Karimi et al. 2021). In general, the Recommended Dietary Allowance (RDA) of protein in a healthy adult is 0.80 $g.kg^{-1}$ body weight with average intake ranged from 47 g per day to 57 g per day (Richter et al. 2019). Thus, a 250-350 g servings of mycoprotein nugget is needed to meet the entire protein requirement. This amount of consumption is considerable since most people do not rely on single type of protein. For instance, a moderate intake of 100-150 g mycoprotein nuggets can cover 38-48% of daily protein needs.

As shown in Table 2, mycoprotein nuggets had higher levels of crude fiber than chicken nuggets and comparable to vegan nuggets. This could be due to the presence of cell walls containing glucans, chitin, chitosan, mannans, galactomannans, and glycoproteins (Bottin et al. 2016; Gow et al. 2017). Mycoprotein is a good source of various dietary fibers, which can be converted into short chain fatty acids in the gut. These considered as essential element in the regulation of immune system, metabolism, glucose levels and inflammatory response (Ahmad et al. 2022; Nogal et al. 2021). Moreover, the protein digestibility was also analyzed to determine the protein quality of mycoprotein nuggets. The digestibility value of mycoprotein nugget was comparable to chicken nugget, but lower than vegan nugget. Wang et al. (2023) evaluated the protein digestibility of five species filamentous fungi using INFOGEST in vitro digestion protocol, which was carried out by measuring the protein degree of hydrolysis (DH%). This study revealed that the DH% of filamentous fungi (43-62%) was comparable to or lower than that of common animal proteins, such as salmon, beef and chicken (59-67%).

Table 3 represented the vitamin and mineral profile of mycoprotein nuggets, which show similar composition with vegan and chicken nuggets. It was found that mycoprotein, vegan and chicken nuggets contain vitamin D3, but do not contain vitamin B9 and B12. This result corresponds to a prior studies by Derbyshire & Ayoob (2019), summarizing that mycoprotein provides vitamin D (1.4 $\mu g.100\ g^{-1}$), but lacks of B-complex vitamins.

Table 2. Proximate composition, crude fiber, and protein digestibility of mycoprotein nuggets made from *Rhizopus oligosporus* biomass

Sample	Moisture, %	Ash, %	Protein, %	Fat, %	Crude fiber, %	Protein digestibility, %
Mycoprotein nugget	50.17 ± 0.29^{c}	3.88 ± 0.52^{a}	18.50 ± 0.08^{c}	0.53 ± 0.04^{c}	1.73 ± 0.25^{a}	46.34 ± 1.77^{b}
Vegan nugget	52.39 ± 0.04^{b}	3.25 ± 0.10^{a}	22.58 ± 1.30^{b}	6.12 ± 0.83^{a}	1.40 ± 0.04^{a}	66.38 ± 0.91^{a}
Chicken nugget	53.95 ± 0.04^{a}	3.37 ± 0.26^{a}	25.72 ± 1.24^{a}	2.58 ± 0.05^{b}	0.20 ± 0.00^{b}	49.72 ± 0.83^{b}

$^{a-c}$*Mean ± SD. Different superscript letter within the same row represent significant differences ($p < 0.05$)*

However, the presence of vitamin D3 was found to be lower in mycoprotein nuggets than that of vegan and chicken nuggets. Considering the limited content of vitamin B, fortification strategy represents a promising practical approach to further improve the nutritional value of mycoprotein nugget. In additon, the implementation of shelf-life extension strategy could also strengthen its technological performance, supporting successful market penetration in regions with an established culture of tempeh consumption.

Table 3. Vitamins and minerals content of mycoprotein nuggets made from *Rhizopus oligosporus* biomass

Micronutrients	Mycoprotein nugget	Vegan nugget	Chicken nugget
Vitamin B9, µg.100 g^{-1}	n.d.	n.d.	n.d.
Vitamin B12, µg.100 g^{-1}	n.d.	n.d.	n.d.
Vitamin D3, µg.100 g^{-1}	1.43 ± 0.05^{c}	2.32 ± 0.04^{a}	1.61 ± 0.06^{b}
Potassium, mg.100 g^{-1}	137.40 ± 0.47^{a}	137.85 ± 0.11^{a}	86.91 ± 0.08^{b}
Phosphorus, mg.100 g^{-1}	150.01 ± 0.60^{a}	77.26 ± 0.16^{b}	77.74 ± 0.04^{b}
Sulfur, mg.100 g^{-1}	48.23 ± 0.21^{c}	85.73 ± 0.20^{b}	157.63 ± 0.44^{a}
Calcium, mg.100 g^{-1}	76.58 ± 0.29^{a}	11.93 ± 0.02^{c}	19.27 ± 0.03^{b}

$^{a-c}$*Mean ± SD. Different superscript letter within the same row represent significant differences ($p < 0.05$)*

It was also observed that mycoprotein contains a wide range of mineral compounds, such as potassium, phosphorus, sulfur and calcium. Mycoprotein nuggets contain phosphorus that are 2 times higher and calsium levels 4-6 times higher than those of vegan and chicken nuggets. The phosphorus content was comparable to 85 g of Australian lamb foreshank and 113 g of low-fat cottage cheese. Meanwhile, the calcium content was equivalent to a cup of toasted sunflower seeds (USDA 2018). Derbyshire & Delange (2021) have previously reported that mycoprotein from *Fusarium venenatum* was also rich in calcium, phosphorus, and magnesium. This may be related to the biochemical composition of the fungal cell wall. Safaei et al. (2016) indicated that filamentous fungi, especially zygomycetes, contain high polyphosphates, which are responsible for accumulating phosphorus. Meanwhile, calcium is a signaling element, which mainly found in vacuoles and cell wall of fungi (Dzurendova et al. 2021). The recommended intake of phosphorus and calcium in a healthy adult is 700 mg per day and 1000 mg per day, respectively (Meyers et al. 2006). A 250-300 g serving of mycoprotein nugget can provide approximately 53-64% and 19-23% of the daily phosphorus and calcium requirement, respectively.

Table 4 showed the amino acids composition of mycoprotein nuggets in comparison to chicken and vegan nuggets. Glutamic acid was found to be the most dominant amino acid in mycoprotein, vegan and chicken nuggets (Table 4). This component contributed to umami taste of food, which strongly correlated with consumer acceptance (Wikandari et al. 2021). Several amino acids, such as isoleucine, cystine, leucine, valine and threonine in mycoprotein nuggets exhibited significantly higher value than those of vegan and chicken nuggets. This indicates that mycoprotein is becoming increasingly feasible to play a key role in shifting trends of dietary protein.

Table 4. Amino acids composition of mycoprotein nuggets made from *Rhizopus oligosporus* biomass

Amino acids, g.100 g^{-1}	Mycoprotein nugget	Vegan nugget	Chicken nugget	RDA for adults*, mg.kg^{-1}
L-Histidine	0.19 ± 0.00^{a}	0.14 ± 0.00^{c}	0.16 ± 0.00^{b}	14
L-Isoleucine	0.34 ± 0.00^{a}	0.07 ± 0.00^{c}	0.11 ± 0.00^{b}	19
L-Leucine	0.53 ± 0.00^{a}	0.33 ± 0.00^{c}	0.38 ± 0.00^{b}	42
L-Lysine	0.33 ± 0.00^{b}	0.25 ± 0.00^{c}	0.34 ± 0.00^{a}	38
L-Methionine	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	19
L-Tryptophan	0.07 ± 0.00^{c}	0.15 ± 0.00^{a}	0.08 ± 0.00^{b}	5
L-Valine	0.38 ± 0.00^{a}	0.16 ± 0.00^{b}	0.16 ± 0.00^{b}	24
L-Phenylalanine	0.38 ± 0.00^{a}	0.29 ± 0.00^{b}	0.24 ± 0.00^{c}	33
L-Threonine	0.32 ± 0.00^{a}	0.14 ± 0.00^{b}	0.16 ± 0.00^{b}	20
L-Alanine	0.33 ± 0.00^{c}	0.36 ± 0.00^{b}	0.38 ± 0.00^{a}	-
L-Arginine	0.48 ± 0.00^{a}	0.38 ± 0.00^{c}	0.39 ± 0.00^{b}	-
L-Aspartic acid	0.48 ± 0.00^{c}	0.70 ± 0.00^{a}	0.62 ± 0.00^{b}	-
Glycine	0.37 ± 0.00^{b}	0.47 ± 0.00^{a}	0.33 ± 0.00^{c}	-
L-Glutamic acid	1.48 ± 0.00^{c}	1.72 ± 0.00^{b}	1.76 ± 0.00^{a}	-
L-Cystine	0.43 ± 0.00^{a}	0.25 ± 0.00^{b}	0.11 ± 0.00^{c}	-
L-Proline	0.43 ± 0.00^{a}	0.37 ± 0.00^{b}	0.36 ± 0.00^{c}	-
L-Serine	0.35 ± 0.00^{b}	0.39 ± 0.00^{a}	0.32 ± 0.00^{c}	-
L-Tyrosine	0.19 ± 0.00^{c}	0.21 ± 0.00^{a}	0.20 ± 0.00^{b}	-

*$^{a-c}$Mean ± SD. Different superscript letter within the same row represent significant differences ($p < 0.05$) *Data from Meyers et al. (2006)*

This current study also revealed that mycoprotein nuggets contain higher content of total essential amino acids in comparison to vegan and chicken nuggets (Fig. 1). Similar result was obtain by Shahbazpour et al. (2021) that all of essential amino acids present in mycoprotein sausage, with threonine as the most abundant amino acid. Other earlier studies have reported that the amino acid profile of mycoprotein were characterized by a high content of essential amino acid with glutamic acid as the abundant component (Hashempour-Baltork et al. 2020; Seyed Reihani and Khosravi-Darani 2018; Wikandari et al. 2023). These findings were also in good agreement with the study by Monteyne et al. (2020), which mentioned that mycoprotein was a superior source of essential amino acids. Essential amino acids cannot be synthesized by human body and must be obtained through dietary protein. These play various regulatory roles that are important for the body's growth, maintenance and repair (Meyers et al. 2006). A 100 g serving of mycoprotein nuggets are sufficient to meet or even exceed the daily requirement of all essential amino acids for adults with a body weight of 50-60 kg, except for methionine.

The fatty acid profile of mycoprotein nuggets was presented in Table 5. The results showed that the fat

of mycoprotein nugget was mainly composed of unsaturated fatty acids, comprising 0.72% polyunsaturated fatty acids (PUFAs) and 1.44% monounsaturated fatty acids (MUFAs). Mycoprotein nugget was also rich in omega 9 fatty acid, palmitic acid, omega 6 fatty acid and linoleic acid. It has been reported that mycoprotein contained higher levels of unsaturated fatty acids (UFAs) (Hashempour-Baltork, Khosravi-Darani et al. 2020; Shahbazpour et al. 2021). UFAs has potenstial healt benefits, such as lowering the risk of cardiovascular disease, some cancers, asthma and diabetes (Milićević et al. 2014).

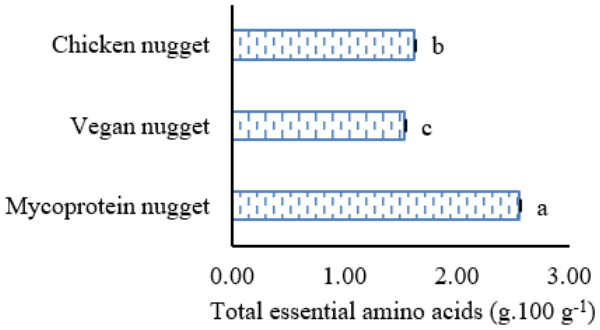


Fig. 1. Total essential amino acids of mycoprotein nuggets made from *Rhizopus oligosporus*

Table 5. Fatty acids composition of mycoprotein nuggets made from *Rhizopus oligosporus* biomass

Fatty acids	Mycoprotein nugget	Vegan nugget	Chicken nugget
Saturated fat, %	1.03 ± 0.00^{b}	7.50 ± 0.07^{a}	7.65 ± 0.04^{a}
Unsaturated fat, %	2.16 ± 0.00^{c}	9.02 ± 0.07^{b}	9.90 ± 0.04^{a}
Polyunsaturated fat, %	0.72 ± 0.00^{c}	2.07 ± 0.03^{a}	2.00 ± 0.01^{b}
Monounsaturated fat, %	1.44 ± 0.00^{c}	6.95 ± 0.04^{b}	7.89 ± 0.04^{a}
Omega 3 fatty acid, %	0.02 ± 0.00^{c}	0.06 ± 0.00^{a}	0.05 ± 0.00^{b}
Omega 6 fatty acid, %	0.69 ± 0.00^{b}	2.01 ± 0.03^{a}	1.95 ± 0.01^{a}
Omega 9 fatty acid, %	1.31 ± 0.00^{c}	6.90 ± 0.04^{b}	7.78 ± 0.04^{a}
Lauric acid, %	0.01 ± 0.00^{c}	0.05 ± 0.00^{b}	0.08 ± 0.00^{a}
Palmitic acid, %	0.80 ± 0.00^{b}	6.54 ± 0.07^{a}	6.53 ± 0.05^{a}
Palmitoleic acid, %	0.09 ± 0.00^{a}	0.02 ± 0.00^{c}	0.08 ± 0.00^{b}
Stearic acid, %	0.14 ± 0.00^{c}	0.69 ± 0.00^{b}	0.76 ± 0.00^{a}
Oleic acid, %	1.31 ± 0.00^{c}	6.90 ± 0.04^{b}	7.78 ± 0.04^{a}
Linolenic acid, %	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	0.04 ± 0.00^{b}
Linoleic acid, %	0.66 ± 0.00^{c}	2.01 ± 0.03^{a}	1.94 ± 0.01^{b}

$^{a\text{-}c}$*Mean ± SD. Different superscript letter within the same row represent significant differences ($p < 0.05$)*

Achieving a balanced fatty acid intake is necessary as an effective strategy to promote health and reduce the risk of cardiovascular disease. The recommended ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) should be above 0.4 (Milićević et al. 2014). Mycoprotein nuggets exhibit a better balanced fatty acid with PUFA/SFA ratio 0.70 in comparison to vegan and chicken nuggets (Fig. 2). Previous studies have reported that the PUFA/SFA ratio of mycoprotein was about to 1.4-1.5 (Hashempour-Baltork et al. 2020; Seyed Reihani and Khosravi-Darani 2018). This suggested that mycoprotein nugget can be a prospective source of balanced fatty acids. Compared to animal and plant proteins mycoprotein production needs limited resources and land, and emits lower emissions (Majumder et al. 2023).

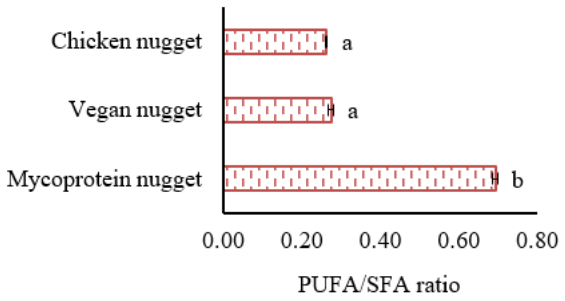


Fig. 2. PUFA/SFA ratio of mycoprotein nuggets made from *Rhizopus oligosporus*

Compared to animal and plant proteins, mycoprotein production needs limited resources and land, and emits lower emissions (Majumder et al. 2023). It also provides excellent nutritional profile, such as essential amino acids and unsaturated fatty acids. This suggests that mycoprotein can be a promising and sustainable alternative protein source, which can substitute meat in meat-based product, such as nugget. Additionally, mycoprotein consumption has been demonstrated to have various benefits on metabolic markers (Hashempour-Baltork, Khosravi-Darani et al. 2020).

Sensory characteristics of mycoprotein nugget. Sensory attributes, including color, taste and texture seem to be important aspects that drive consumer acceptance of food products, such as meat analogue. Generally, meat analogue are created through various processing techniques to closely resemble the physical, nutritional, and organoleptic properties of real meat. One commercial meat analogue is mycoprotein-based products, which have been marketed as minced meat, nugget, sausage, etc. Mycoprotein naturally contains bundled fibers, giving a meat-like texture for the final products. Compared to plant protein, mycoprotein has more fibrous structure and improved eating qualities. As consumer expect meat analogue to mimic the characteristics of conventional meat, sensory perception must be considered as a crucial parameter for the acceptability of meat (Abdullah et al. 2024).

To understand the consumer acceptance regarding the sensory properties of mycoprotein nuggets, hedonic test was performed. The results revealed that the hedonic scores of mycoprotein nugget for each attribute were comparable to vegan nugget, but significantly lower than those of chicken nugget (Fig. 3). A past study by Godschalk-Broers et al. (2022) noted similar trends that mycoprotein-based meat analogue has comparable scores on look, texture, flavor, and overall liking with those of several plant-based meat analogues, but lower than real chicken nugget (Fig. 3).

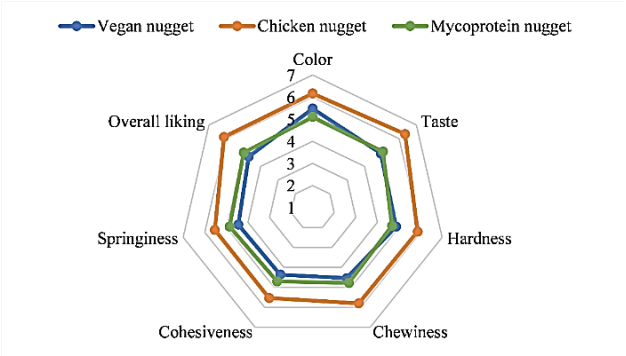


Fig. 3. Sensory evaluation mycoprotein nuggets made from *Rhizopus oligosporus*

A past study by Godschalk-Broers et al. (2022) noted similar trends that mycoprotein-based meat analogue has comparable scores on look, texture, flavor, and overall liking with those of several plant-based meat analogues, but lower than real chicken. Meat analogues still lacked in hardness, chewiness, cohesiveness, fibrousness, juiciness, and meaty flavor (Godschalk-Broers et al. 2022). Meat has organized and aligned structures resulting in unique sensory profile, making it challenging to mimic. This sometimes appears to be a limitation for the acceptance of meat analogues. Particularly, textural properties of meat have long been identified as the main driver of consumer acceptance since the first perception of meat is mostly associated with hardness. Spesific sensory attributes, such as fibrousness, juiciness, and meaty flavor may also contribute to the sensory profile of meat analogue. However, these attributes were not individually evaluated in this present study. Therefore, the improvement of sensory quality is necessary to obtain a desirable meat-like product, which can fully satisfy the consumers. To more effectively improve the sensory quality, future investigations could integrate quantitative descriptive analysis (QDA) to clarify the key sensory attributes, thereby facilitating targeted optimization of texture and flavor. Abdullah et al. (2024) suggested that mimicking physical properties of meat could be more feasible than reformulating the meat taste and flavor.

Effect of various binding agents incorporation on mycoprotein nugget texture using Texture Profile Analyzer (TPA). Texture is a chief parameter determining consumer acceptance of food. Several textural attributes are significant for meat-based products, including hardness, cohesiveness, springiness, and chewiness (Abdullah et al. 2024; Godschalk-Broers et al. 2022). Mycoprotein nugget was still found lacking certain textural attributes mimicking real meat nugget. The texture of mycoprotein nugget can be enhanced by incorporating several binding agents to achieve the desirable and acceptable textural quality. Fig. 4 represents the hardness, cohesiveness, springiness, and chewiness of mycoprotein nuggets with the addition of various binding agents. Hardness refers to force required to compress food into pieces at the first bite of molar teeth (Godschalk-Broers et al. 2022). The addition of methylcellulose successfully increased the hardness of mycoprotein nuggets. It was reported that the addition of 2% methylcellulose increased the hardness of plant-based patties, made from texturized vegetable protein (Sakai et al. 2021). Methylcellulose can form a strong gel possibly due to polymer associations resulting from hydrophobic interaction between its exposed hydrophobic segments (Bakhsh et al. 2021). However, the increasing concentration of methylcellulose even decreased the hardness of mycoprotein nuggets. Similar results was reported by Pérez-Mateos and Montero (2000) in blue whiting gel. The reason might be due to interference by steric hindrance in the gel formation.

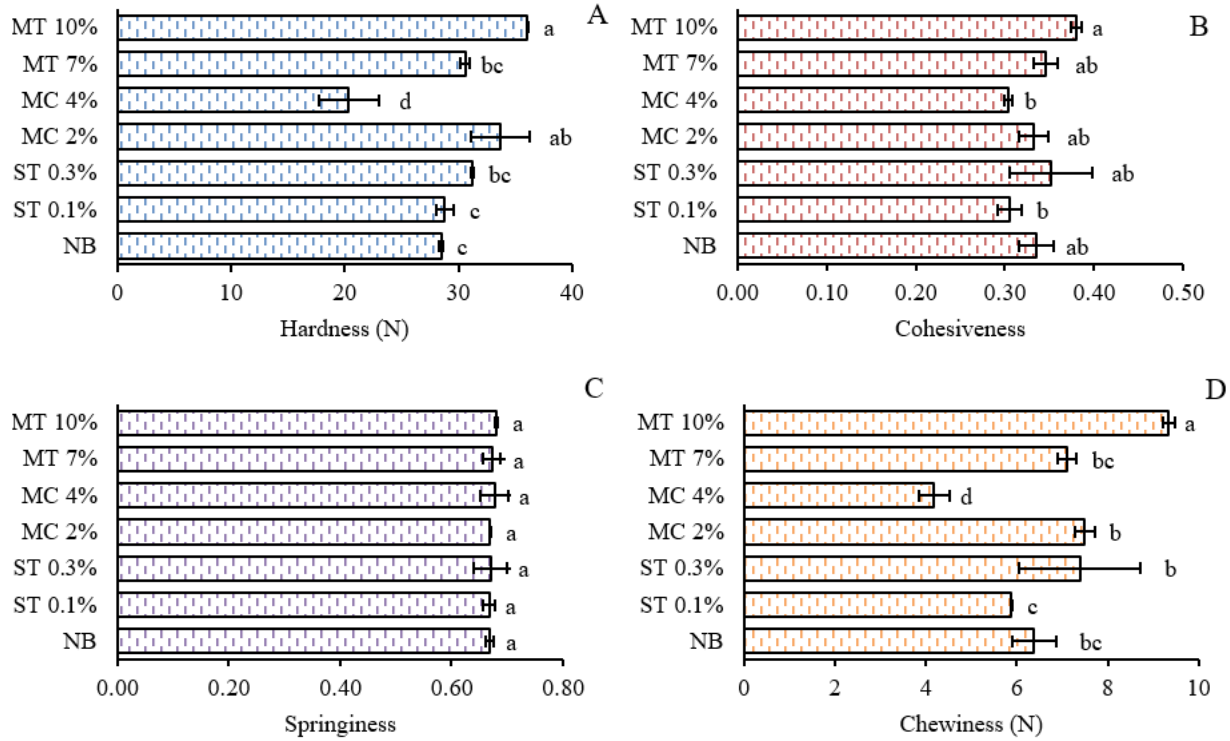


Fig. 4. The hardness (A), cohesiveness (B), springiness (C), and chewiness (D) of mycoprotein nuggets made from *Rhizopus oligosporus* with incorporation of various binding agents

The incorporation of 10% modified tapioca starch also significantly increased the hardness of mycoprotein nuggets. The increasing concentration of modified tapioca starch can elevate the hardness of mycoprotein nuggets. Wei et al. (2023) observed similar results that the addition of modified tapioca starch at 4-8% improve the hardness of chinese meatball (kung-wan). This textural enhancement might be attributed to filling effect of starch in the protein matrix. Modified tapioca starch can gelatinize during heating and interact with protein of the food matrix resulting in the formation of gel network. Furthermore, the water binding ability of starch helps to strengthen the protein gel network. The effect of modified starch addition on textural properties depends on the amount incorporated (Wei et al. 2023; Wimbasara et al. 2025).

However, the addition of sodium tripolyphosphate did not affect the hardness of mycoprotein nuggets. A past study by Wimbasara et al. (2025) also obtained similar result that incorporation of sodium tripolyphosphate did not significantly influenced the texture of mycoprotein patty from *Rhizopus oligosporus*. Glorieux et al. (2017) also pointed out that incorporating sodium tripolyphosphate did not affect the hardness of pork sausage. This may be attributable to sodium tripolyphosphate's ability to break down the protein matrix, increasing the material sensitivity to compression.

Incorporation of binding agents did not significantly influence the cohesiveness of mycoprotein nuggets (Fig. 4B). This trend was in correpondence with the previous findings by that the addition of binding agents did not change the cohesiveness of mycoprotein patty (Wimbasara et al. 2025). Several studies also demonstrate that meat analogue products were generally perceived as less cohesive when compared to meat-based products (Abdullah et al. 2024; Godschalk-Broers et al. 2022). The presence of air bubble or air pores was reported can break the structure of mycoprotein-based product, which may affect the cohesiveness (Wimbasara et al. 2025). Cohesiveness is related to the intermolecular interactions among the matrix elements, which are influenced by the material structure and ingredients (Sharima-Abdullah et al. 2018; Wijono and Estiasih 2021).

Springiness corresponds to the extent to which a deformed food material returns to its initial form after the force is removed (Zhang et al. 2022). The result showed that the addition of binding agents did not significantly affect the springiness of mycoprotein nuggets, ranging between 0.67-0.68 (Fig. 4C). The springiness value of chicken analogue pieces obtained in the previous study ranged between 0.65-0.91 (Godschalk-Broers et al. 2022). Various studies performing TPA revealed that mycoprotein-based product showed a lower springiness than meat product (Abdullah et al. 2024; Hashempour-Baltork et al. 2023; Shahbazpour et al. 2021). Non-meat protein can retain more water and fat compared to meat protein, which subsequently fills the interstitial space within the protein matrix and results in lower springiness (Kamani et al. 2019).

Chewiness represents the force required to chew a solid food to a suitable form for swallowing (Godschalk-Broers et al. 2022). Fig. 4D showed that the addition of 10% modified tapioca starch significantly increased the chewiness of mycoprotein nuggets. Similar results obtained in a study by Wei et al. (2023), reporting that the incorporation of modified tapioca starch (at a concentration of 2-8%) increased the chewiness of kung-wan. This could be attributed to the starch ability to fill the protein matrix and bind the water. However, the addition of 7% modified tapioca starch did not enhance the chewiness of mycoprotein nuggets. It may be due to the low concentration of the starch, preventing it from adequately filling the gel structure and damaging the protein matrix (Wei et al. 2023). The result of this present study also in line with Wimbasara et al. (2025) that modified tapioca starch gave significant increase in chewiness of mycoprotein patty, which also may be attributable to the high value of hardness since it correlates positively with chewiness. Microstructural analysis using scanning electron microscopy (SEM) demonstrated that mycoprotein-based patty added with modified starch has smooth surfaces with small and uniform air bubbles, explaining the observed increases in hardness and chewiness. It was further explained, based on fourief transform infrared spectroscopy (FTIR) results, that the incorporation of 10% modified tapioca starch increased hydrogen bonds and hydrophobic interactions, which may stabilize the internal structure of mycoprotein patty (Wimbasara et al. 2025).

As previously mentioned, texture is an important parameter in meat-based product, which can influence the consumer acceptance. According to the TPA results, modified tapioca starch has potential as binding agent, increasing the hardness and chewiness of mycoprotein nuggets. Starch has been known for its utilization as both a filler and binder in meat products, which is mainly attributed to its water binding ability. The effect of starch incorporation varies depending on the type and amount of starch. Therefore, a wide range of texture can possibly achieved with modified tapioca starch, allowing for several adjusments based on the desired quality of the final products (Bühler et al. 2022).

Conclusions

Mycoprotein nugget was found to be rich in fiber and essential amino acids, with low content of fat and nutritionally valuable fatty acids. The overall liking of mycoprotein was comparable to vegan nugget, but remained lower than chicken nugget. Concerning the improvement of mycoprotein nuggets quality, this current study investigate the effect of binding agents addition on texture of mycoprotein nuggets. Based on the TPA results, modified tapioca starch was observed to provide the most significant increase in hardness and chewiness of mycoprotein nuggets. This indicates that modified tapioca starch has the potential to elevate the texture quality of mycoprotein-based product. Future work should focus on optimizing the nugget formulation and investigating the mechanism underlying the functionalities of binding agent.

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Author Contributions

Conceptualization, R.W.; methodology, R.W.; formal analysis H.N.; investigation, H.N.; writing—original draft preparation, H.N.; writing—review and editing, R.W. and A.D.S.; supervision, R.W. and A.D.S.; funding acquisition, R.W. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The experimental protocol for the involvement of human subjects was approved by Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada, protocol number KE/1629/11/2023, dated December 12nd 2023.

Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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