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Effect of *Mosaccha* inoculum on fungal and yeast populations, sensory attributes, antioxidant activity, and β-glucan content in cowpea tempeh

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Abstract. *Rizal S, Kustyawati ME, Astuti S, Ristiani DA, Fatimah, Pratiwi LD, Hidayat R. 2025. Influence of* Mosaccha *inoculum on fungal and yeast populations, sensory profile, antioxidant level, and β-glucan content of cowpea tempeh. Biodiversitas 26: 2926-2935.* In addition to soybeans, cowpeas can also be used to make tempeh. *Rhizopus oligosporus* is the main fungus for fermentation, but the addition of *Saccharomyces cerevisiae* enhances the product's sensory qualities. This study aimed to evaluate the effects of *Mosaccha* inoculum, which combines *R. oligosporus* and *S. cerevisiae*, on the microbial, sensory, and chemical properties of cowpea tempeh. The experiment was conducted in a Complete Randomized Block Design (CRBD) with six inoculum concentrations (0.3%, 0.6%, 0.9%, 1.2%, 1.5%, and a control group with 0.2% Raprima inoculum), each replicated four times. Statistical analysis was done using Bartlett and Turkey tests, followed by ANOVA and LSD at a 5% significance level. The results indicated that the concentration of *Mosaccha* inoculum significantly influenced both fungal and yeast counts, as well as the sensory properties of the cowpea tempeh. The optimal inoculum concentration of 1.2% produced the highest quality tempeh, with a fungal count of 8.747 CFU/g and a yeast count of 8.570 CFU/g. The best sensory attributes included a white color with fully covered mycelium, a characteristic sweet aroma, a firm and easily sliced texture, and a taste that was well-accepted by panelists. The highest-quality *Mosaccha* cowpea tempeh meets the Indonesian National Standards for tempeh, contains 1.065% β-glucan, and has an antioxidant level of 60.74%. These findings suggest that *Mosaccha* inoculum at 1.2% is an effective starter culture for producing high-quality functional cowpea tempeh. Its application offers an alternative to soybean-based tempeh while enhancing sensory and nutritional profiles. This innovation may support tempeh diversification using locally available legumes.

Keywords: Cowpea, *Mosaccha* inoculum, *Rhizopus oligosporus*, *Saccharomyces cerevisiae*, tempeh

INTRODUCTION

Tempeh is a traditional fermented food made primarily from soybeans, which serve as a valuable source of plant-based protein. Soybeans are highly nutritious, containing approximately 34% protein, 34% carbohydrates, 19% fat, and 5% ash (Kanchana et al. 2016). While soybeans remain the most common ingredient for tempeh production, exploring alternative raw materials can diversify the food supply and reduce dependency on a single crop. Additionally, the use of alternative ingredients can enhance the nutritional value of tempeh and offer consumers greater variety.

Cowpea (*Vigna unguiculata* (L.) Walp.) is a resilient legume cultivated extensively in Indonesia, particularly in the Maluku region. The presence of ten local cowpea varieties on Lakor Island, Southwest Maluku District—characterized by a high level of diversity based on seed and pod morphology—has also been reported in West Southeast Maluku District (Afitu et al. 2016). Cowpea's adaptability to dry tropical climates and diverse local varieties highlights its agricultural significance. Cowpea, also known as black-eyed peas, is a legume that provides a rich source of proteins, vitamins, minerals, fiber, and fats (Jayathilake et al. 2018). Cowpea was selected for its nutritional profile, which is comparable to that of soybean. It contains protein ranging from 23.42% to 26.78%, ash content between 3.60% and 4.21%, crude fiber from 2.10% to 2.98%, carbohydrates between 56.10% and 59.59%, and fat content ranging from 1.98% to 2.28% (Animasaun et al. 2015). Its seeds vary in color, but the white variety is preferred for tempeh due to its visual appeal and better quality stability (Setyowati and Minantyorini 2016). Visual characteristics influence consumer acceptance, as darker beans may be associated with spoilage (Vital et al. 2018). Cowpea, a legume with high nutritional potential, has emerged as a promising alternative for tempeh production (Putri and Kartikawati 2022).

In tempeh fermentation, *Rhizopus oligosporus* is the primary fungus responsible for converting raw materials and enhancing the nutritional and functional properties of tempeh. Yeasts like *Saccharomyces cerevisiae* can interact synergistically with *R. oligosporus*, enhancing microbial growth and nutrient quality (Qibty et al. 2023). According to Rizal and Kustyawati (2019), adding liquid *S. cerevisiae* inoculum to the fermentation process results in tempeh with a more pleasant fragrance, reduced sourness, and a smoother taste. Additionally, *S. cerevisiae*-fermented tempeh has been shown to contain beneficial β-glucan compounds, which provide health benefits (Rizal et al. 2021). Rizal et al. (2023) developed a more practical *Mosaccha* inoculum, a powder form of *S. cerevisiae*, which adheres to SNI 3144: 2015 standards for sensory and chemical properties while also delivering health-enhancing β-glucans. The concentration of inoculum during fermentation significantly impacts the quality of tempeh, influencing its chemical properties, sensory characteristics, and nutritional content. Research indicates that optimal inoculum levels enhance microbial growth and the production of beneficial compounds, leading to improved tempeh quality (Rizal et al. 2024). Research by Rizal and Kustyawati (2019) highlighted that adding *S. cerevisiae* can improve the taste, aroma, and overall acceptance of tempeh. Aside from the growth of fungal and yeasts, the sensory profiles and the concentration of inoculum during fermentation had a significant impact on the quality of jack bean tempeh, such as the moisture and β-glucan content (Rizal et al. 2024). The interaction between *R. oligosporus* and *S. cerevisiae* alters the nutritional profile, increasing fat, ash, and moisture while reducing carbohydrates (Rizal et al. 2022a). Different inoculum types and concentrations influence fungal and yeast growth, sensory characteristics, and overall nutrition.

Using *Mosaccha* inoculum in cowpea tempeh represents an innovation in tempeh development, traditionally based on soybeans and *R. oligosporus;* however, there is limited research on its application to cowpea. Therefore, this study aimed to investigate the effect of *Mosaccha* inoculum on fungal and yeast counts and sensory characteristics of cowpea tempeh and to determine the optimal inoculum concentration to achieve the best product quality.

MATERIALS AND METHODS

Materials

The primary raw materials used in this study were local cowpeas purchased directly from farmers in Wetan Kali Hamlet, Balung Sub-district, Jember District, East Java, Indonesia, along with rice flour (Rose Brand by PT Budi Makmur Perkasa from Bumi Waras Group, Subang, Indonesia). Pure cultures of *R. oligosporus* FNCC 6010 and *S. cerevisiae* FNCC 3012 were obtained from the Inter-University Center for Food and Nutrition, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia, and Raprima brand tempeh inoculum as a control. The Raprima inoculum, commonly used in traditional tempeh production in Indonesia, contains a mixed culture of *R. oligosporus* and rice as a carrier. For the analysis, the materials included Potato Dextrose Agar (PDA, Oxoid, UK), Malt Extract Agar (MEA, Oxoid, UK), distilled water, NaCl, 70% alcohol, NaOH, H2SO4, HCl, phenolphthalein indicator, hexane solvent, 96% ethanol, acetic acid, Pb acetate, sodium oxalate, and phenol.

Research methods

The experiment was conducted in a Randomized Complete Block Design (RCBD) with a single factor consisting of six levels and four replications. The factor was the concentration of *Mosaccha* inoculum, which included the following levels: 0.3% (C1), 0.6% (C2), 0.9% (C3), 1.2% (C4), 1.5% (C5), and a control using 0.2% Raprima inoculum (C). Observations of *Mosaccha* cowpea tempeh in all treatments include total fungi (Rizal et al. 2020), total yeast (Rizal et al. 2020), and sensory characteristics by scoring test and hedonic test.

Procedures

Production of Mosaccha *inoculum*

The preparation of *Mosaccha* inoculum followed the method described by Rizal et al. (2023). First, rice flour was weighed and sterilized at 121°C for 15 minutes. Sterile distilled water was then added at a ratio of 1:5 (rice flour: water), and the mixture was homogenized until a dough-like consistency was achieved without being overly wet. The dough was inoculated with a 3% (v/w) mixture of *R. oligosporus* and *S. cerevisiae* at a 1:1 ratio and homogenized again. The inoculated dough was incubated for 96 hours at 28°C, then oven-dried at 37°C for 24 hours. Finally, the dried inoculum was ground into powder using a grinder.

Production of Mosaccha *cowpea tempeh*

The production of *Mosaccha* cowpea tempeh was carried out with modifications to the method described by Putri and Kartikawati (2022), which included roasting, soaking, dehulling, roasting, boiling, and fermentation. Modifications included changing the soaking time, replacing the roasting step with boiling, and using Mosaccha inoculum instead of Raprima inoculum. A total of 1.5 kg of cowpeas was boiled for 10 minutes to ease skin removal. After cooking, the beans were cooled to room temperature and soaked for 24 hours, with water changes every 6 hours, to achieve the ideal pH range of 4-5 for fungal growth. Once soaked, the skin of the cowpeas was removed, and the beans were washed. They were then boiled in a 1:4 water ratio for 10 minutes, steamed for 15 minutes, and cooled. The beans were inoculated with *Mosaccha* inoculum at varying concentrations (0.3%, 0.6%, 0.9%, 1.2%, 1.5%), while the control used 0.2% Raprima inoculum. Finally, the cowpeas were packed in perforated polypropylene plastic and fermented at room temperature (around 32°C) for 36-40 hours.

Total fungi and yeast count

Total fungi and yeast counts were analyzed to assess the microbial quality of tempeh. 1 g of sample was homogenized in 9 mL of 0.85% NaCl and serially diluted (10^{-1}-10^{-7}). From dilutions 10^{-5} to 10^{-7}, 1 mL was plated in duplicate on PDA for fungi and MEA for yeasts using the spread plate method. Plates were incubated at 32°C for 48 hours before colony enumeration.

Sensory evaluation

Sensory evaluation of cowpea tempeh was conducted using a scoring test (for color, texture, and aroma) by 20 trained panelists (ages 20-25 years) and a hedonic test (for taste and overall acceptance) by 50 untrained panelists (ages 20-25 years). The scoring test used a 5-point scale based on predetermined descriptive criteria, where a score of 1 represented the most desirable condition and 5 the least. For color evaluation, tempeh samples were graded based on their whiteness and the distribution of mycelium across the surface. A score of 5 represented a white, with mycelium covering the entire surface of tempeh, while a score of 1 indicated a yellowish, with no mycelium; texture assessment involved observing the sample's compactness and its resistance to being pressed with a finger. Scores ranged from 5 for very compact to 1 for very loose and prone to falling apart. In terms of aroma, panelists assessed the characteristic scent of tempeh, with scores ranging from 5 for a very fresh and typical tempeh aroma to 1 for a foul-smelling aroma. The hedonic test used a 5-point scale (1: dislike extremely, 5: like extremely). Approximately 25 g of each sample was served at room temperature, coded with three-digit random numbers, and presented in randomized order. Mineral water was provided as a palate cleanser. The evaluation was conducted on 29 February, 7 March, 14 March, and 21 March 2024, in accordance with ISO 8589:2007 sensory guidelines.

Analysis of moisture content

Moisture was determined using the thermogravimetric method (AOAC 2016). 2 g of sample were dried at 105-110°C in a pre-weighed crucible until constant weight. Moisture content was calculated as:

$$\text{Moisture (\%, wet basis)} = \frac{B - C}{A}$$

Where:
A : Sample weight (g)
B : Weight before drying (g)
C : Weight after drying (g)

The term wet basis indicates that moisture content is expressed relative to the initial (wet) weight of the sample.

Analysis of protein content

Protein content was measured using the Kjeldahl method (AOAC 2016). 1 g of sample was digested with H_2SO_4, neutralized, distilled with NaOH, and titrated with 0.1 N NaOH. Protein content was calculated as:

$$\text{Protein (\%)} = \frac{(VA - VB) \times N \times 14.007 \times 5.71 \times 100}{W}$$

Where:
VA : HCl volume for sample titration (mL)
VB : HCl for blank (mL)
N : Normality of HCl
W : Sample weight (mg)

Analysis of fat content

Fat was extracted using the Soxhlet method (AOAC 2016). 2 g of sample were refluxed with hexane for 5-6 hours. The extracted fat was dried and weighed. Fat content was calculated as:

$$\text{Fat (\%)} = \frac{C - A}{B} \times 100$$

Where:
A : Empty flask weight (g)
B : Sample weight (g)
C : Flask + fat (g)

Analysis of crude fiber content

A defatted sample was boiled in H_2SO_4 and NaOH, filtered, dried, and weighed. Fiber content was calculated as:

$$\text{Crude fiber (\%)} = \frac{A - B}{W} \times 100$$

Where:
A : Sample + filter paper (g)
B : Filter paper (g)
W : Sample weight (g)

Analysis of β-glucan content

B-glucan was analyzed using the method of Kusmiati et al. (2007). 1 g of sample was hydrolyzed in NaOH and centrifuged at 10,000 rpm at 25°C for 30 minutes. The supernatant was removed, and the residue was washed with acetic acid, water, and ethanol, dried, and reacted with phenol-sulfuric acid. Absorbance was read at 490 nm using a UV-Vis spectrophotometer.

Analysis of antioxidant content

Antioxidant content was analyzed using the method of Padah and Dewi (2022). The antioxidant activity was determined using the DPPH radical scavenging method. The sample extract reacted with a DPPH solution, and the decrease in absorbance was measured to calculate the IC_{50} value, which indicates its free radical scavenging ability.

Determination of the best treatment

The best treatment of Mosaccha cowpea tempeh was determined using the effectiveness index method with weighted scoring (De Garmo et al. 1984), based on total fungi, total yeast, and sensory characteristics. The weights were assigned according to each parameter's priority in influencing product quality, with total fungi and yeast having the highest priority. The best treatment was indicated by the highest treatment score (NP), calculated from the weighted contribution of all parameters.

Data analysis

The data were analyzed for homogeneity using Bartlett's test, and the multiplicity of the data was evaluated with Tukey's test. Variance analysis was then conducted to assess the impact of the treatments, and if significant differences were found, the Least Significant Difference (LSD) test was applied at the 5% significance level.

RESULTS AND DISCUSSION

Total fungi and yeast

The effect of *Mosaccha* inoculum concentration on the number of fungi and yeast in cowpea tempeh is presented in Figure 1. One main aspect of *Mosaccha* tempeh production is the presence of both fungi and yeast since *Mosaccha* inoculum is composed of a mixed culture of these microorganisms that contribute synergistically to the overall fermentation process.

The total fungi count in *Mosaccha* cowpea tempeh ranged from 6.740 to 8.921 log CFU/g. The highest fungi count was recorded at 1.5% *Mosaccha* inoculum (C5), while the lowest occurred at 0.3% (C1). These findings suggest a strong positive correlation between inoculum concentration and fungal proliferation. This can be attributed to the higher availability of fungal spores and nutrient accessibility during the initial fermentation stage. However, the rate of increase in fungal growth began to plateau beyond 1.2%, possibly indicating the onset of environmental limitations such as nutrient depletion or accumulation of inhibitory metabolites. According to Juška (2015) and Schiraldi (2020), microbial growth typically follows a sigmoidal pattern, with an exponential phase transitioning into a stationary phase as resources become limited, balancing cell division and death.

Similarly, total yeast counts increased with higher inoculum concentrations, ranging from 6.390 log CFU/g (control) to 8.734 log CFU/g (C5). The presence of *S. cerevisiae* in the *Mosaccha* inoculum likely enhanced yeast growth in the medium, especially under conditions rich in fermentable sugars. As the concentration of *Mosaccha* inoculum increases, the number of viable *S. cerevisiae* cells introduced into the substrate also increases, thereby accelerating colonization and metabolic activity. This increase allows for faster adaptation and exponential growth during the early stages of fermentation. Furthermore, cowpeas are rich in carbohydrates, which serve as a readily available substrate for yeast metabolism, supporting higher cell proliferation. Yeast proliferation was significantly lower in the control (Raprima 0.2%) due to the absence of intentionally added *S. cerevisiae*, confirming the role of *Mosaccha* in improving yeast viability during fermentation.

This microbial enhancement plays a crucial role in the biochemical transformation of cowpeas during fermentation. *Rhizopus oligosporus* is known for its proteolytic and lipolytic activity, while *S. cerevisiae* contributes to the production of ethanol, organic acids, and aroma compounds (Rizal and Kustyawati et al. 2019; Lv et al. 2023). However, while a higher microbial count may indicate active fermentation, excessive microbial activity, especially at 1.5%, can result in undesirable sensory attributes, as confirmed by the low sensory scores in that treatment. Therefore, maintaining a balanced inoculum concentration, particularly at 1.2%, appears optimal for promoting microbial synergy without compromising product acceptability.

Sensory properties of *Mosaccha* cowpea tempeh

The sensory properties of cowpea tempeh were analyzed by a scoring test on a 5-point scale. Specific descriptions for each scale point were provided in the results section for each parameter, including color, texture, aroma, and then the hedonic test, including taste and overall acceptance. The performance of cowpea tempeh fermented with various concentrations of *Mosaccha* inoculum is shown in. Figure 2 illustrates that all cowpea tempeh, whether treated with *Mosaccha* inoculum or the control (Raprima commercial inoculum), were white, with the mycelium fully covering the surface of the tempeh. This observation suggests that both *Mosaccha* and commercial inoculum support sufficient fungal growth to form a complete mycelial layer. Further analysis and discussion of individual sensory parameters are presented in the following sections.

Color and texture of *Mosaccha* cowpea tempeh

According to Indonesian National Standards for tempeh (SNI 3144:2015), a high-quality tempeh should exhibit a uniformly white color across its surface. Based on the results of the 5% LSD test (Figure 3), all treatments met this standard, with mycelium covering most of the tempeh surface. The highest color score (4.280) was observed in the control group (Raprima inoculum), likely due to the commercial inoculum's high concentration of purified *R. oligosporus*, which is known to promote consistent and dense mycelial growth. Among the *Mosaccha* treatments, C5 (1.5%) achieved the highest color score, suggesting that increasing inoculum concentrations enhance fungal proliferation, which contributes to a more uniform white appearance.

Table 1. Total fungi and total yeast counts (log CFU/g) in *Mosaccha* cowpea tempeh at varying inoculum concentrations

Treatments (Concentrations)	Total fungi (log CFU/g)	Total yeast (log CFU/g)
C5 (Concentration of *Mosaccha* 1.5%)	8.921±0.114^{a}	8.734±0.145^{a}
C4 (Concentration of *Mosaccha* 1.2%)	8.747±0.188^{ab}	8.570±0.429^{a}
C3 (Concentration of *Mosaccha* 0.9%)	8.672±0.164^{b}	7.823±0.109^{b}
C (Control, Raprima 0.2%)	8.056±0.035^{c}	6.390±0.133^{c}
C2 (Concentration of *Mosaccha* 0.6%)	7.900±0.186^{c}	7.493±0.473^{b}
C1 (Concentration of *Mosaccha* 0.3%)	6.740±0.225^{d}	7.463±0.428^{b}

Note: Values are expressed as mean±standard deviation. Different superscript letters in the same column indicate significant differences (LSD test, α = 0.051)

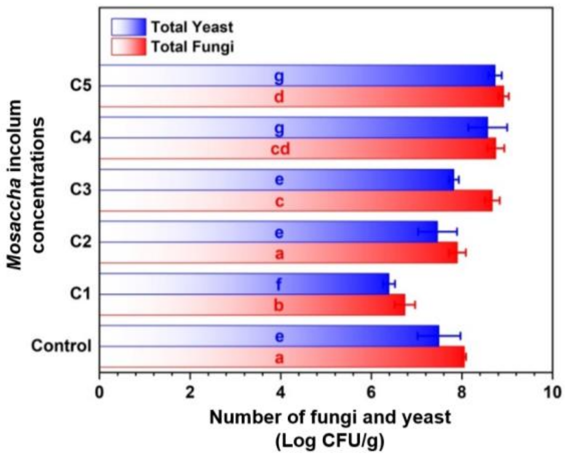

Figure 1. The influence of *Mosaccha* inoculum concentrations (C: Raprima 0.2%, C1: *Mosaccha* 0.3%, C2: *Mosaccha* 0.6%, C3: *Mosaccha* 0.9%, C4: *Mosaccha* 1.2%, C5: *Mosaccha* 1.5%) on the number of fungi and yeast in *Mosaccha* cowpea tempeh based on LSD test (The mean value followed by the same letter indicates no significant difference in the 5% LSD test; α = 0.214 for fungi; α = 0.495 for yeast)

Figure 2. The appearance of cowpea tempeh with different concentrations of inoculum. A. C: Control (Raprima 0.2%), B. C1: *Mosaccha* inoculum 0.3%, C. C2: *Mosaccha* inoculum 0.6%, D. C3: *Mosaccha* inoculum 0.9%, E. C4: *Mosaccha* inoculum 1.2%, F. C5: *Mosaccha* inoculum 1.5%

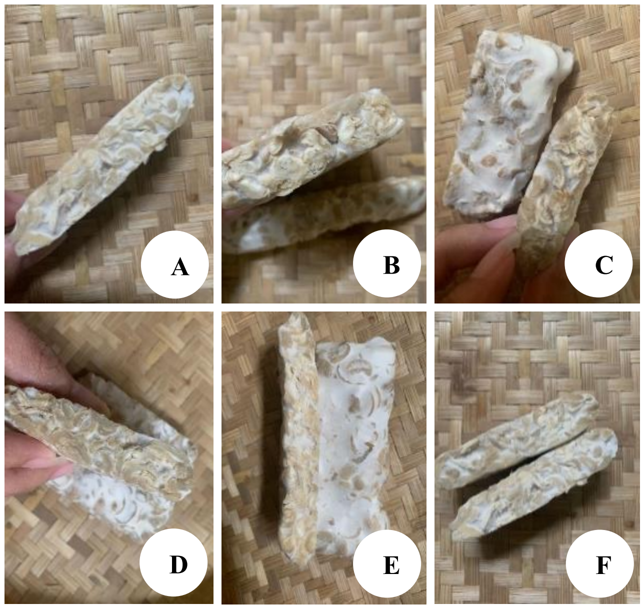

Figure 4. The appearance of sliced cowpea tempeh with different concentrations of inoculum. A. C: Raprima 0.2%, B. C1: *Mosaccha* 0.3%, C. C2: *Mosaccha* 0.6%, D. C3: *Mosaccha* 0.9%, E. C4: *Mosaccha* 1.2%, F. C5: *Mosaccha* 1.5%

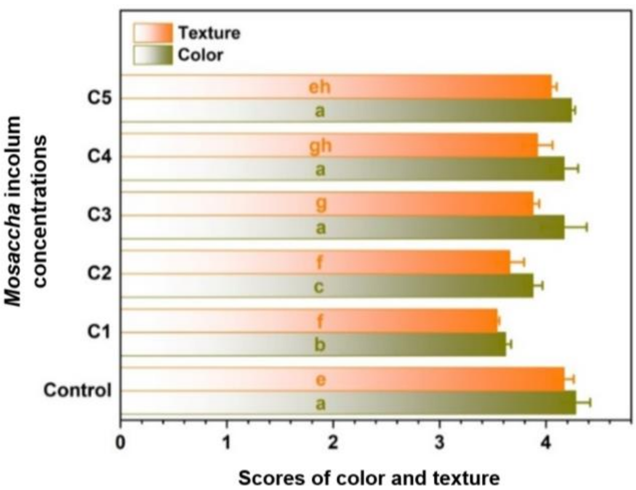

Figure 3. The effect of various *Mosaccha* inoculum concentrations (C: Raprima 0.2%, C1: *Mosaccha* 0.3%, C2: *Mosaccha* 0.6%, C3: *Mosaccha* 0.9%, C4: *Mosaccha* 1.2%, C5: *Mosaccha* 1.5%) on the score of color (1: Yellowish, no mycelium; 2: Yellowish white, mycelium slightly covers the surface of tempeh; 3: Yellowish white, mycelium covers part of the surface of tempeh; 4: White, mycelium almost covers the entire surface of tempeh; and 5: White, mycelium covers the entire surface of tempeh and texture of *Mosaccha* cowpea tempeh; The mean value followed by the same letter indicates no significant difference in the 5% LSD test, $\alpha = 0.184$) and score of texture (1: Not compact, easily crushed when sliced; 2: Not compact, somewhat crushed when sliced; 3: Somewhat compact, somewhat easy to slice; 4: Compact, easy to slice; and 5: Very compact, easy to slice; The mean value followed by the same letter indicates no significant difference in the 5% LSD test, $\alpha = 0.132$) of *Mosaccha* cowpea tempeh

The increasing whiteness of the tempeh surface with higher *Mosaccha* concentrations can be attributed to the intensified activity of *Rhizopus* spp., which produces mycelium that forms a dense mat of hyphae over the beans (Vellozo-Echevarría et al. 2024). This dense mycelial growth not only improves appearance but also contributes to product protection and consistency. Although *S. cerevisiae* in *Mosaccha* inoculum does not directly affect color, its presence may support fungal development by modifying pH or providing growth-promoting metabolites during co-fermentation. This synergy potentially enhances *Rhizopus*'s colonization efficiency, resulting in better mycelial coverage.

For texture, scores ranged from 3.540 to 4.170, falling within the "somewhat compact" to "compact and easy to slice" categories. According to SNI 3144:2015, good tempeh texture is indicated by firmness and cohesiveness, where beans are tightly bound by well-developed mycelium. Treatment C1 (0.3%) showed the lowest texture score, likely due to sparse fungal growth that resulted in weak inter-bean binding. Lower inoculum concentrations may lead to insufficient mycelial development, producing a fragile texture with poor bean cohesion. This can cause structural instability and make the tempeh prone to breaking during slicing. In contrast, higher inoculum levels produced firmer and more cohesive structures, aligning with desirable textural characteristics. These findings demonstrate that optimizing *Mosaccha* inoculum concentration is essential not only for achieving a visually appealing product but also for ensuring structural integrity during handling and consumption. The appearance of whole and sliced *Mosaccha* cowpea tempeh is shown in Figure 4.

As the concentration of *Mosaccha* inoculum increases, the texture score of *Mosaccha* cowpea tempeh also improves. This may be because higher inoculum concentrations help optimize fungal growth. During the growth process, fungi require essential nutrients such as carbon (C), phosphorus (P), nitrogen (N), minerals, and vitamins (Zhang and Elser 2017). These nutrients are derived from the breakdown of complex components in the substrate (the beans), including carbohydrates, proteins, and fats. Cowpeas contain significant amounts of carbohydrates (56.6%) and proteins (24.4%), which are broken down by the amylase and protease enzymes produced by the fungi during fermentation to meet their nutritional needs. Adequate nutrition supports more efficient fungal growth, which in turn increases mycelium production. The greater the mycelial growth, the more effectively it binds the seeds together, resulting in tempeh with a dense, compact structure, a firm texture, and ease of slicing (Yusuf et al. 2021).

The compact texture of tempeh was the result of physical changes induced by the mycelium of fungi, particularly *R. oligosporus* and *S. cerevisiae*. The fungal spores develop into mycelium, a filamentous structure that envelops and intertwines the beans. As the mycelium expands, it spreads among the soybeans, binding them together. The mycelium acts as a natural binder, forming a cohesive mass and contributing to the white color of the tempeh (Putri et al. 2018).

Taste and aroma of *Mosaccha* cowpea tempeh

Based on the study results, cowpea tempeh fermented with *Mosaccha* inoculum concentrations of 0.3%, 0.6%, 0.9%, and 1.2% developed a typical tempeh aroma combined with a fragrant-sweet scent, scoring 3.640, 3.750, 4.030, and 4.070, respectively. This sweet aroma is attributed to *S. cerevisiae*, which converts carbohydrates into alcohol and aroma compounds like styrene during fermentation (Kustyawati et al. 2017). In contrast, treatment C5 received the lowest score of 2.420 due to an off-putting odor, suggesting that higher inoculum concentrations may lead to excessive ethanol and volatile compound production, resulting in a sharp yeast aroma. According to Kustyawati et al. (2017), the aroma of modified tempeh tends to be yeasty due to *S. cerevisiae*, which synthesizes specific volatile compounds during fermentation. These compounds, such as styrene—an aromatic hydrocarbon absent in regular tempeh—contribute to a sweet fragrance. However, at higher concentrations, this yeast aroma becomes overly intense and is perceived negatively by panelists.

The characteristic "*langu*" aroma of tempeh primarily originates from lipoxygenase (LOX) activity in soybeans. LOX catalyzes the oxidation of polyunsaturated fatty acids, generating volatile compounds that influence flavor. This reaction is activated when soybean skins are removed, exposing the beans to oxygen (Shi et al. 2020). The use of *Mosaccha* inoculum helps mask this "*langu*" aroma by producing a sweet fragrance that dominates the typical strong smell. This masking effect is due to the yeast's proteolytic and lipolytic activities, which break down proteins and fats into amino acids, esters, fatty acids, ethanol, and other aroma compounds (Kustyawati et al. 2017). Cowpea tempeh fermented with *Mosaccha* inoculum produced a more fragrant-sweet aroma, while tempeh fermented with commercial Raprima inoculum exhibited a slight "*langu*" odor. Therefore, *Mosaccha* offers a sensory advantage over conventional inocula.

The analysis of variance showed that *Mosaccha* inoculum concentration significantly affected the flavor of cowpea tempeh ($\alpha = 0.05$), with panelist scores ranging from 2.910 (somewhat like) to 4.000 (like). The 5% LSD test results for flavor scores are presented in Figure 5. Treatment C3 achieved the highest flavor score (4.000), which was not significantly different from the Control (C) but differed significantly from the C1, C2, C4, and C5 treatments. The favored tempeh displayed a distinctive taste derived from the fermentation of carbohydrates, proteins, and fats by the fungi. According to Witono et al. (2015) the flavor of tempeh is influenced by the protein content of its raw materials. Cowpea, which contains 23.42-26.78% protein (Animasaun et al. 2015), contributes to the development of a savory taste through the breakdown of protein into amino acids, especially glutamic acid. Panelists preferred tempeh flavors that were not sour. The lowest taste score (2.910) was found in C5 (1.5% *Mosaccha*), likely due to the development of sourness. Rizal and Kustyawati (2019) also noted that tempeh fermented with *S. cerevisiae* tends to develop a sour taste. Additionally, Lv et al. (2023) found that *S. cerevisiae* produces ethanol and various Volatile Organic Compounds (VOCs), along with free amino acids and esters, during fermentation. These byproducts can lower the pH and contribute to sour flavors in fermented foods. Overall, cowpea tempeh fermented with *Mosaccha* inoculum concentrations of 0.9% and 1.2%, as well as the control (0.2% Raprima), was preferred by panelists. This suggests that *Mosaccha* inoculum can produce cowpea tempeh with aroma and flavor qualities comparable to those of tempeh made with commercial inocula while also offering the benefit of reducing the "*langu*" aroma commonly associated with soybean tempeh.

Overall acceptance of *Mosaccha* cowpea tempeh

Overall acceptance is a sensory observation parameter that includes color, texture, aroma, and taste parameters in cowpea tempeh treated with the addition of *Mosaccha* inoculum with different concentrations. The results of the analysis of variance of the overall acceptance sensory test showed that the addition of *Mosaccha* inoculum concentration had a significant effect at $\alpha = 0.05$ on the overall acceptance score of cowpea tempeh. The results showed that the overall acceptance score of cowpea tempeh ranged from 3.555 (Somewhat like) to 3.855 (Like). The effect of various *Mosaccha* inoculum concentrations and the results of the 5% LSD test on the overall acceptance score of cowpea tempeh with various concentrations of *Mosaccha* inoculum are shown in Figure 6.

According to SNI 1344:2015 (Indonesian National Standards for tempeh), high-quality tempeh should have a uniform white color, a compact texture that holds together when sliced, and a characteristic tempeh aroma without any ammonia smell. Cowpea tempeh fermented with 0.9% *Mosaccha* inoculum received the highest overall acceptance score of 3.855, which was not significantly different from the control tempeh fermented with Raprima inoculum (3.845). Panelists preferred this tempeh because it had a white color, with mycelium covering the entire surface, a compact texture that was easy to slice, and a typical tempeh aroma with a sweet fragrance. The addition of *S. cerevisiae* during fermentation promotes fungal growth. It influences both the appearance and flavor of the tempeh by breaking down carbohydrates into alcohol and other aroma compounds, including esters, organic acids, and carbonyl compounds, which have a great impact on the flavor of fermented food products (Kustyawati et al. 2017).

The best treatment

The best treatment for *Mosaccha* cowpea tempeh with the addition of *Mosaccha* inoculum was determined using the effectiveness index method, which uses weighted procedures to evaluate parameters such as total fungi, total yeast, and sensory characteristics. Table 2 summarizes the analysis results for total fungi, total yeast, and sensory tests of cowpea tempeh with different inocula, along with the effectiveness test results for the *Mosaccha* inoculum concentrations using the effectiveness index method.

The optimal treatment, as indicated by the effectiveness index, is determined by the highest effectiveness value, which reflects the combined influence of all parameters that contribute to product quality. Table 2 shows that treatment C4 (1.2% *Mosaccha* inoculum) achieved the highest Effectiveness Value (EV) of 0.815. Cowpea tempeh fermented with 1.2% *Mosaccha* inoculum yielded a total fungal count of 8.747 log CFU/g, total yeast of 8.570 mCFU/g, and exhibited sensory characteristics such as a white color with mycelium covering the entire tempeh, a compact and sliceable texture, a typical tempeh aroma with a sweet fragrance, and a taste and overall acceptance that the panelists favored.

Nutritional composition of the best *Mosaccha* cowpea tempeh

Cowpea tempeh fermented with the optimal concentration of *Mosaccha* inoculum, specifically treatment C4 (1.2% *Mosaccha* inoculum), was further analyzed for moisture content, protein content, fat content, crude fiber content, β-glucan content, and antioxidant activity. The nutritional composition of cowpea tempeh with the best *Mosaccha* inoculum addition was compared to that of cowpea tempeh fermented with Raprima inoculum, soybean tempeh fermented with *Mosaccha*, and soybean tempeh fermented with Raprima, as presented in Table 3.

Moisture content

The moisture content of cowpea tempeh produced with the best treatment (*Mosaccha* inoculum concentration of 1.2%) in this study, which was 53.40%, aligns with the standards set by SNI 3114:2015. Table 3 shows that the use of *Mosaccha* inoculum results in higher moisture content in tempeh compared to the commercial inoculum (Raprima), both in cowpea and soybean tempeh. This may be because *S. cerevisiae* in the *Mosaccha* inoculum can contribute to the moisture content of tempeh through its respiration process. When oxygen is available during fermentation, *S. cerevisiae* undergoes respiration, converting sugar into carbon dioxide (CO_2) and water. This water, along with water produced by mold (*R. oligosporus*) metabolism, is believed to increase the moisture content of tempeh (Kustyawati 2018).

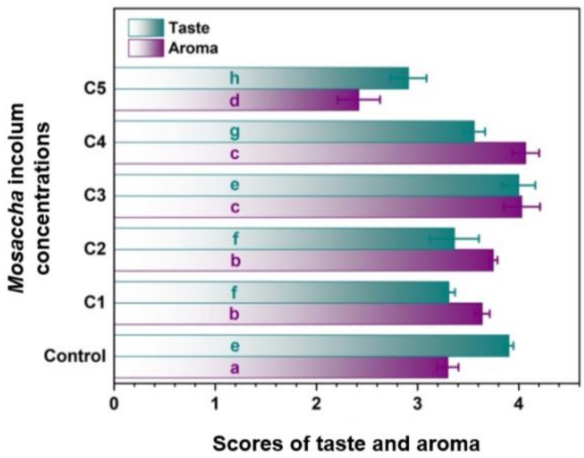

Figure 5. The effect of various *Mosaccha* inoculum concentrations (C: Raprima 0.2%, C1: *Mosaccha* 0.3%, C2: *Mosaccha* 0.6%, C3: *Mosaccha* 0.9%, C4: *Mosaccha* 1.2%, C5: *Mosaccha* 1.5%) on the score of aroma (1: Foul smelling; 2: typical of tempeh, deviant smell; 3: Typical of tempeh, languorous smell and slightly sweet-smelling; 4: Typical of tempeh and sweet-smelling; and 5: Very typical of tempeh, sweet-smelling; The mean value followed by the same letter indicates no significant difference in the 5% LSD test, α = 0.192) and the score of taste (1: Strongly dislike; 2: Dislike; 3: Somewhat like; 4: Like; and 5: Strongly like; The mean value followed by the same letter indicates no significant difference in the 5% LSD test, α = 0.194) of *Mosaccha* cowpea tempeh

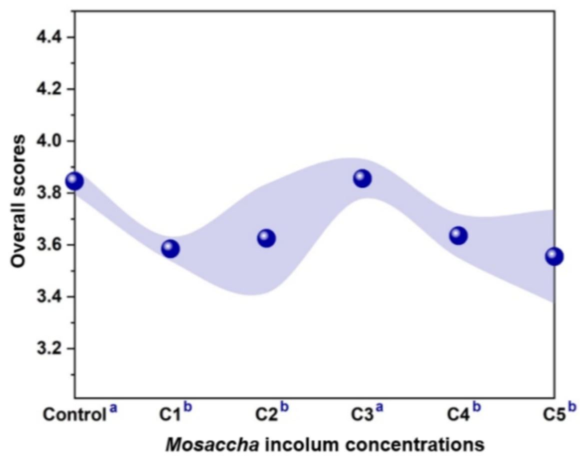

Figure 6. The effect of various *Mosaccha* inoculum concentrations (C: Raprima 0.2%, C1: *Mosaccha* 0.3%, C2: *Mosaccha* 0.6%, C3: *Mosaccha* 0.9%, C4: *Mosaccha* 1.2%, C5: *Mosaccha* 1.5%) on the score of overall acceptance of *Mosaccha* tempeh (The mean value followed by the same letter indicates no significant difference in the 5% LSD test; α = 0.181; score 1: Strongly dislike; 2: Dislike; 3: Somewhat like; 4: Like; and 5: Strongly like)

Table 2. The recapitulation of determining the best *Mosaccha* cowpea tempeh treatment with different concentrations of inoculum

Parameters	Treatments											
	C	EV	C1	EV	C2	EV	C3	EV	C4	EV	C5	EV
Total fungi	8.056	0.151	6.740	0.000	7.900	0.133	8.672	0.221	8.747	0.230	8.921	0.250
Total yeast	6.390	0.000	7.463	0.098	7.493	0.101	7.823	0.131	8.570	0.199	8.734	0.214
Color	4.280	0.179	3.620	0.000	3.880	0.070	4.170	0.149	4.170	0.149	4.240	0.168
Texture	4.170	0.143	3.540	0.000	3.660	0.027	3.920	0.086	3.880	0.077	4.050	0.116
Aroma	3.300	0.057	3.640	0.079	3.750	0.086	4.030	0.105	4.070	0.107	2.420	0.000
Taste	3.905	0.065	3.310	0.026	3.365	0.030	4.000	0.071	3.560	0.043	2.910	0.000
Overall acceptance	3.845	0.035	3.585	0.004	3.625	0.008	3.855	0.036	3.635	0.010	3.555	0.000
Total		0.629		0.207		0.456		0.799		0.815		0.748

Note: EV: Effectiveness value, *Mosaccha* tempeh inoculum (C1: 0.3%; C2: 0.6%; C3: 0.9%; C4: 1.2%; C5: 1.5%) and C: A control group with 0.2% (w/w) Raprima inoculum

Table 3. Nutritional composition of cowpea tempeh and soybean tempeh with the addition of *Mosaccha* and Raprima inoculum

Nutritional components	Cowpea tempeh with *Mosaccha* inoculum (C4)(*)	Cowpea tempeh with Raprima inoculum	Soybean tempeh with *Mosaccha* inoculum	Soybean tempeh with Raprima inoculum
Water content (%; Max. 65)	53.40	42.61(1)	65.74(2)	60.45(3)
Protein content (%; Min. 15)	23.09	26.01(1)	17.40(2)	28.87(3)
Fat content (%; Min. 7)	8.07	2.11(1)	8.23(2)	8.20(3)
Crude fiber content (%; Max. 2.5%)	4.36	0.34(1)	10.80(4)	2.10(5)
β-glukan content (%)	1.06	0.34(*)	0.58(2)	0.29(2)
Antioxidant level (%)	60.74	59.66(3)	66.36(6)	56.66(3)

Source: *: Primary data of present research, 1. Putri and Kartikawati (2022), 2. Rizal et al. (2022a), 3. Dewi et al. (2014), 4. Rizal et al. (2024), 5. Animasaun et al. (2015), 6. Rizal et al. (2022b)

The use of cowpeas in the current study led to a lower moisture content in tempeh compared to soybeans due to the difference in the moisture content of the raw materials. Soybeans have a higher moisture content of 15.72% (Adipa et al. 2022), while cowpeas have 10.10% (Animasaun et al. 2015). Besides the initial moisture content of the raw materials, moisture content is also influenced by water absorption into the seed matrix. According to (Oyetunji et al. 2023) cowpeas' skin is strongly bound to the cotyledons due to the gum layer, making it more difficult to separate than soybean epidermis. This structure allows soybeans to absorb water more easily during soaking and boiling, leading to a higher moisture content.

Protein content

The protein content of soybean tempeh fermented with Raprima was found to be highest at 28.87% (Table 3). Soybean tempeh contains more protein than cowpea tempeh because raw soybeans have a higher protein content than cowpeas (Dewi et al. 2014). However, cowpea tempeh fermented with 1.2% *Mosaccha* inoculum had a high protein content of 23.09%, which was not much lower than that of soybean tempeh and well above the minimum quality requirement of 15% set by SNI 3114:2015. This demonstrates that cowpeas have potential as a raw material for tempeh production and should be explored further. The protein content of cowpea tempeh fermented with *Mosaccha* inoculum is lower than tempeh fermented with Raprima inoculum. *S. cerevisiae* produces proteases that break down proteins into smaller peptides and amino acids, thus increasing protein digestibility while potentially reducing the total protein content in tempeh. In addition, because yeast uses nitrogen sources from the substrate for growth, this can reduce the nitrogen available for protein synthesis in tempeh (Rizal et al. 2020).

According to the Indonesian National Standard for tempeh (SNI 3114:2015), the minimum fat content in tempeh is 7%. The best cowpea tempeh fermented with 1.2% *Mosaccha* inoculum meets the SNI quality standard, with a fat content of 8.07%. As shown in Table 3, the fat content of soybean tempeh was higher than that of cowpea tempeh. This may be due to the higher fat content in soybeans (16.7%) compared to cowpeas (2.10%) as raw materials (Animasaun et al. 2015). The fat content of cowpea tempeh is higher than that of raw cowpeas, which aligns with the findings of Sahara et al. (2016), who found that fermentation increases the degree of unsaturation of fat, resulting in an increase in fat content in tempeh. This process leads to a rise in Polyunsaturated Fatty Acids (PUFAs), such as oleic and linoleic acids, while reducing the levels of palmitic acid and linoleic acid.

Fiber content

The fiber content of the best cowpea tempeh, fermented with 1.2% *Mosaccha* inoculum, was 4.36%, which did not meet the SNI 3144:2015 quality standard, which specifies a maximum of 2.5%. However, the fiber content of cowpea tempeh (4.36%) was lower than that of soybean tempeh, which had a fiber content of 10.80%. This difference is due to the lower crude fiber content in whole cowpeas (1.6%) compared to whole soybeans (3.2%) (Ministry of Health, Republic of Indonesia 2017). The higher crude fiber in tempeh compared to the initial raw materials results from the fermentation process, during which the mycelium of the tempeh fungi increases and binds the seeds together. The cell walls of *Rhizopus* sp. fungi mycelium are primarily composed of cellulose and chitin, which are considered crude fiber in tempeh (Kurniati et al. 2017).

The use of *Mosaccha* inoculum, a mixture of *R. oligosporus* and *S. cerevisiae*, during tempeh fermentation results in higher crude fiber levels compared to using Raprima inoculum. As shown in Table 3, the crude fiber content of soybean tempeh and cowpea tempeh fermented with *Mosaccha* inoculum was 10.80% and 4.36%, respectively. In comparison, the crude fiber content of soybean tempeh and cowpea tempeh fermented with Raprima inoculum was 3.24% and 0.34%, respectively. According to (Cedro et al. 2024), the cell walls of *R. oligosporus* hyphae are primarily composed of polysaccharides. These polysaccharides include significant amounts of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN), which are indicative of chitin and chitosan presence in the cell wall structure. As the amount of polysaccharides increases, the crude fiber content also increases. Additionally, *S. cerevisiae* has a cell wall primarily made of β-glucans, which are considered fiber and belong to the non-starch polysaccharide group (Singla et al. 2024). This contributes to the higher crude fiber content in cowpea tempeh fermented with *Mosaccha* inoculum, which exceeds the established maximum limit.

β-glucan content

The β-glucan analysis results in Table 3 indicate that the highest β-glucan content was found in cowpea tempeh fermented with 1.2% *Mosaccha* inoculum, yielding a content of 1.06%. The β-glucan levels in cowpea tempeh, whether fermented with *Mosaccha* inoculum (1.06%) or Raprima inoculum (0.34%), were higher than those in soybean tempeh fermented by Raprima (0.29%) (Rizal et al. 2022a). This difference is likely due to the higher carbohydrate content in raw cowpea (56.6%) compared to soybean (24.9%). During fermentation, yeast breaks down these carbohydrates into simple sugars, which serve as an energy source for yeast growth (Stewart 2017). Kusmiati et al. (2007) explain that *S. cerevisiae* achieves optimal biomass production when grown in environments rich in sugars and nitrogen sources.

Antioxidant levels

Table 3 shows the antioxidant levels in cowpea and soybean tempeh fermented with either Raprima or *Mosaccha* inoculum. In cowpea tempeh, *Mosaccha* produced slightly higher antioxidant levels (60.74%) than Raprima (59.66%). A similar pattern appeared in soybean tempeh, with *Mosaccha* yielding 66.36%, compared to 56.66% with Raprima. This indicates that *Mosaccha* enhances antioxidant activity in both types of tempeh. According to Fujita et al. (2015), fermentation produces aglycones through β-glucosidase activity, which increases antioxidant potential. *Rhizopus oligosporus* converts isoflavone glucosides (daidzin, genistin) into aglycones (daidzein, genistein), while the addition of *S. cerevisiae* in *Mosaccha* likely boosts this process. Meanwhile, cowpea tempeh's high antioxidant levels, regardless of inoculum, result from the formation of ferulic and *p*-coumaric acids during fermentation, known for their strong antioxidant properties (Dewi et al. 2014).

In conclusion, the findings of the present study indicate that the concentration of *Mosaccha* inoculum significantly influences the total fungal and yeast counts, as well as the sensory attributes of cowpea tempeh, including color, texture, aroma, taste, and overall acceptance. The optimal concentration of 1.2% *Mosaccha* inoculum resulted in the highest quality cowpea tempeh, with a total fungal count of 8,747 log CFU/g, a total yeast count of 8,570 CFU/g, and sensory characteristics that were not just preferred by the panelists but also appreciated for their high quality, such as a white color with mycelium covering the entire tempeh, a typical tempeh aroma accompanied by a fragrant, sweet scent, a compact texture (easy to slice), taste and overall acceptance. The best cowpea tempeh produced in this study had a moisture content of 53.40%, a protein content of 23.09%, a fat content of 8.07%, a crude fiber content of 4.36%, and a β-glucan content of 1.06%. These findings suggest that *Mosaccha* inoculum at 1.2% is an effective starter culture for producing high-quality functional cowpea tempeh. Its application offers an alternative to soybean-based tempeh while enhancing sensory and nutritional profiles. This innovation may support tempeh diversification using locally available legumes.

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