Tropical Small Island Agriculture Management (TSIAM)

Journal Homepage: https://ojs3.unpatti.ac.id/index.php/tsiam

Volume 5	Issue 1	June (2025)	DOI: 10.30598/tsiam.2025.5.1.1	Page: 1 - 16

Sensory Profile of *Canarium* Nut Tempeh Fermented with Varying Concentrations of *Rhizopus oligosporus*

Helen C. D. Tuhumury*, Gillian Tetelepta, Arthur F. A. Sopacua

Agricultural Product Technology Department, Faculty of Agriculture, Pattimura University, Jl. Ir. M. Putuhena, Kampus Poka Ambon, 97233, Indonesia

*Corresponding Author e-mail: helen.tuhumury@lecturer.unpatti.ac.id

Keywords:
Canarium nut; *Rhizopus oligosporus*; Tempeh; Sensory evaluation; Fermentation;

ABSTRACT

This study investigated the sensory characteristics of tempeh produced from canarium nuts (*Canarium indicum*) fermented with varying concentrations (0.5%, 1.5%, 2.5%, and 3.5% w/w) of *Rhizopus oligosporus*. Canarium nut tempeh presents a promising alternative to traditional soy-based tempeh, particularly for consumers seeking diverse textures and flavors or those with soy allergies. Sensory evaluation was conducted using hedonic and descriptive tests among 25 semi-trained panelists. The results demonstrated that the 0.5% inoculum concentration yielded the highest scores in aroma, taste, texture, and overall liking. Descriptive analysis revealed that lower inoculum levels preserved the distinctive canarium nut aroma and flavor while maintaining moderate fermentation intensity and balanced textural properties. In contrast, higher inoculum concentrations resulted in increased bitterness, excessive firmness, and masked nutty characteristics due to over-fermentation. These findings suggest that low inoculum levels are optimal for producing canarium nut tempeh with superior sensory appeal. This study supports the development of high-quality, non-soy tempeh products using underutilized local resources and provides insights into optimizing fungal fermentation processes.

INTRODUCTION

Fermented foods represent a fundamental element of global culinary heritage, rooted in ancient human practices that leverage controlled microbial activity and enzymatic processes to enhance food preservation, sensory qualities, and nutritional benefits. Historically, fermentation has played a vital role in extending the shelf life of seasonal produce, providing a solution to food scarcity across different civilizations (Campbell *et al*., 2022; Leeuwendaal *et al*., 2022). This technique can be applied to a vast range of food materials, giving rise to diverse fermented products that are deeply intertwined with cultural identities worldwide (Leeuwendaal *et al*., 2022; Tamang *et al*., 2016). Beyond their role in food preservation, fermented products are estimated to make up nearly one-third of global dietary intake, underscoring their importance in human nutrition (Campbell *et al*., 2022). One important fermented food product is tempeh.

Tempeh is a traditional Indonesian food product made by fermenting soybeans using the fungus *Rhizopus oligosporus*. This fermentation process transforms soybeans into a product rich in protein, fiber, and various essential nutrients. Tempeh is well known for its firm texture and distinctive slightly sour and umami flavor (Handoyo & Morita, 2006; Tamam, 2022). It is a nutritious food and an important source of plant-based protein for the population. Every 100 g of tempeh contains approximately 20.8 g of protein, 13.5 g of carbohydrates, and 0.19 mg of vitamin B1 (Puspitasari *et al*., 2022)

Additionally, tempeh offers a variety of significant health benefits. As a rich source of plant-based protein, tempeh supports tissue growth and repair as well as muscle health. Its probiotic content, derived from the fermentation process, promotes digestive health by maintaining a balanced gut microbiota and enhancing immune system function. Tempeh also contains isoflavones, which act as antioxidants and may help reduce the risk of cardiovascular disease and support bone health. Furthermore, its high fiber content helps regulate blood sugar levels and supports weight management by increasing satiety (Teoh *et al*., 2024).

Despite its health benefits, soy-based tempeh has several limitations that have led to the development of tempeh from alternative substrates. Some individuals are allergic to soybeans (Cabanillas *et al*., 2018), limiting the potential consumer base of soy tempeh. Soy also contains goitrogenic compounds that may interfere with thyroid function in certain individuals, particularly when consumed in large quantities (Domínguez-López *et al*., 2020). Moreover, the limited flavor and texture variations of traditional soy tempeh may be less appealing to consumers seeking diverse sensory experiences. Consequently, there has been growing interest in developing tempeh from other ingredients, such as legumes (Aliyah, 2024; Egounlety & Aworh, 2003) and plant seeds (Puspitasari *et al*., 2022; Sitompul *et al*., 2023). In this context, canarium nuts (*Canarium indicum*), a native nut species from Maluku, Indonesia, offer a promising alternative for diversifying tempeh's taste and texture, while potentially addressing soy-related allergies and goitrogenic effects in some consumers.

Canarium (*Canarium indicum*) is an endemic plant of the Melanesian region, including eastern Indonesia and the Maluku Islands (Tuhumury *et al*., 2023). Canarium nuts are considered a suitable substitute for soybeans in tempeh production due to their high nutritional content and functional properties similar to soy. They are rich in healthy fats, particularly omega-3 fatty acids, which support cardiovascular and brain health and have been shown to reduce cholesterol levels in animal studies (Mailoa *et al*., 2019). Canarium nuts also contain protein, fiber, B vitamins, and essential minerals such as magnesium and phosphorus (Bai *et al*., 2019). Their dense texture and rich nutrient profile make them a promising substrate for fermentation, yielding tempeh with a high nutritional value and appealing sensory variations.

The quality of tempeh is significantly influenced by the concentration of the fungal inoculum used during fermentation. *Rhizopus oligosporus* not only initiates fermentation but also drives enzymatic hydrolysis of macronutrients, contributing to the development of desirable texture, flavor, and aroma. Inoculum concentration is a key variable affecting fermentation rate, mycelial growth, and ultimately the sensory attributes of the final product (Ahnan-Winarno *et al*., 2021; Rizal *et al*., 2021; Seumahu *et al*., 2013). Previous studies on soybean and legume-based tempeh have shown that varying inoculum concentrations can lead to marked differences in product quality and consumer acceptance (Rizal *et al*., 2024). However, little is known about how such variations affect fermentation in oil-rich substrates like Canarium nuts, which differ significantly in their physicochemical and sensory properties from traditional substrates.

This study aims to address this gap by investigating the sensory characteristics of *Canarium nut* tempeh produced with different concentrations of *Rhizopus oligosporus*. By evaluating sensory attributes such as aroma, texture, taste, and appearance through structured sensory analysis, this research seeks to identify the optimal inoculum level for producing high-quality tempeh from Canarium nuts. The findings are expected to contribute to the development of novel fermented food products utilizing underutilized local resources, with potential implications for food security, local entrepreneurship, and functional food innovation.

METHODS

Materials

The primary materials used in this study were dried canarium nuts sourced from Ihamahu Village, East Saparua District, Central Maluku Regency, and tempeh starter culture (*Rhizopus oligosporus*- RAPIMA)

Methods

Canarium Nut Tempeh Production

A total of 2.5 kg of dried *Canarium indicum* nuts were soaked in water for 24 hours at room temperature to reduce fat content. After soaking, the nuts were thoroughly washed and subsequently boiled for 1 hour. Post-boiling, the nuts were drained and rinsed again to remove residual substances. The cleaned nuts were then air-dried for 30 minutes at ambient conditions. Following drying, 250 g of the prepared canarium nuts were allocated for each treatment group. The nuts were inoculated with *Rhizopus oligosporus* starter culture at four concentration levels: 0.5%, 1.5%, 2.5%, and 3.5% (w/w), and mixed thoroughly to ensure uniform distribution of the inoculum. These treatments were prepared in triplicate. The inoculated samples were placed into 1-kg polyethylene (PE) plastic bags, which had been perforated using a sterile toothpick to facilitate adequate air exchange during fermentation. The fermentation process was carried out in a dehydrator maintained at ambient temperature (±28°–30°C) for 48 hours.

Sensory Analysis

A sensory evaluation was conducted to assess consumer acceptability of canarium nut tempeh using a panel of 25 semi-trained participants. Panelists were selected based on their familiarity with fermented products and their ability to consistently provide individual sensory judgments. Each panelist evaluated the samples independently in a controlled environment. The evaluation included both hedonic and quantitative descriptive assessments. The hedonic test measured the degree of liking for five attributes: taste, appearance, aroma, texture, and overall acceptability. A 5-point numerical scale was used, where 1 = dislike very much and 5 = like very much (Table 1).

Table 1. Hedonic scale used for sensory evaluation of canarium nut tempeh

Score	Taste	Appearance	Aroma	Texture	Overall Acceptability
5	Like very much	Like very much	Like very much	Like very much	Like very much
4	Like	Like	Like	Like	Like
3	Slightly like	Slightly like	Slightly like	Slightly like	Slightly like
2	Dislike	Dislike	Dislike	Dislike	Dislike
1	Dislike very much	Dislike very much	Dislike very much	Dislike very much	Dislike very much

The quantitative descriptive analysis of canarium nut tempeh was evaluated by a panel of 25 semi-trained panelists using a structured sensory assessment. The evaluation focused on four main attributes: taste, texture, aroma, and appearance. Each attribute was rated based on specific sub-attributes using a five-point intensity scale, where 1 indicated the lowest intensity and 5 the highest. Definitions and descriptors for each scale point were provided to guide consistent scoring. The characteristics and descriptors used for the hedonic quality evaluation are presented in Table 2.

Table 2. Description of the quality atributes

Attribute	Sub-Attribute	Definition	Score	Descriptor
Taste	Canarium Nut Flavor	Intensity of the dominant canarium nut flavor	1	Very weak canarium nut flavor
			2	Weak canarium nut flavor
			3	Moderate canarium nut flavor
			4	Strong canarium nut flavor
			5	Very strong canarium nut flavor

Table 2. Description of … (Cont.)

Attribute	Sub-Attribute	Definition	Score	Descriptor
	Sourness	Perceived level of sourness	1	Not sour at all
			2	Slightly sour
			3	Moderately sour
			4	Sour
			5	Very sour
	Bitterness	Presence and intensity of bitter taste	1	Not bitter at all
			2	Slightly bitter
			3	Moderately bitter
			4	Bitter
			5	Very bitter
	Umami	Perceived savoriness	1	Not savory at all
			2	Slightly savory
			3	Moderately savory
			4	Savory
			5	Very savory
Texture	Hardness	Degree of firmness or chewiness	1	Very soft
			2	Slightly soft
			3	Moderately firm
			4	Firm
			5	Very firm
	Tenderness	How soft or tender the tempeh is when chewed	1	Very tough
			2	Slightly tough
			3	Moderately tender
			4	Tender
			5	Very tender
	Friability	Ease with which the tempeh crumbles under pressure	1	Very firm (not crumbly)
			2	Slightly crumbly
			3	Moderately crumbly
			4	Crumbly
			5	Very crumbly
Aroma	Fermentation Aroma	Intensity of aroma resulting from fermentation	1	No fermented aroma
			2	Slight fermented aroma
			3	Moderate fermented aroma
			4	Strong fermented aroma
			5	Very strong fermented aroma

Table 2. Description of … (Cont.)

Attribute	Sub-Attribute	Definition	Score	Descriptor
	Canarium Nut Aroma	Intensity of canarium nut aroma	1	No canarium nut aroma
			2	Slight canarium nut aroma
			3	Moderate canarium nut aroma
			4	Strong canarium nut aroma
			5	Very strong canarium nut aroma
Appearance	Color	Uniformity of tempeh color	1	Very uneven color
			2	Slightly uneven color
			3	Moderately uniform color
			4	Uniform color
			5	Very uniform color
	Nut Distribution	Homogeneity of canarium nut distribution in the tempeh matrix	1	Very uneven distribution
			2	Slightly uneven distribution
			3	Moderately even distribution
			4	Even distribution
			5	Very even distribution
	Surface Moisture	Visual moisture level on the surface of the tempeh	1	Very dry
			2	Slightly moist
			3	Moderately moist
			4	Moist
			5	Very moist
	Structural Clarity	Visibility of the internal structure, including mold patterns	1	Very unclear structure
			2	Slightly unclear structure
			3	Moderately clear structure
			4	Clear structure
			5	Very clear structure

Statistical Analysis

Data obtained from sensory evaluations were statistically analyzed using a completely randomized design. When significant ($p < 0.05$) or highly significant ($p < 0.01$) differences were observed, further analysis was performed using Tukey test with Minitab 2017 software.

RESULTS AND DISCUSSION

Color and Appreance

The results of sensory evaluation for canarium nut tempeh produced using varying concentrations of *Rhizopus* sp. (0.5%, 1.5%, 2.5%, and 3.5%) are presented in Table 3. The assessment included hedonic scores (color and appearance preferences) and Quantitative Descriptive Analysis (QDA) for color uniformity, nut distribution, surface moisture, and structural clarity. Sensory attributes were evaluated using a 5-point scale. No significant differences (p > 0.05) were observed across treatments for any of the attributes assessed.

Table 3. Hedonic and QDA scores for color and appearance of canarium nut tempeh with different *Rhizopus* concentrations

Rhizopus concentration	Hedonic		QDA			
	Color	Appearance	Color uniformity	Nut distribution	Surface moisture	Structural clarity
0.5%	3.33 [a]	3.47 [a]	3.21 [a]	3.32 [a]	3.21 [a]	3.63 [a]
1.5%	3.43 [a]	3.49 [a]	3.20 [a]	3.35 [a]	3.13 [a]	3.57 [a]
2.5%	3.47 [a]	3.41 [a]	3.20 [a]	3.33 [a]	3.03 [a]	3.55 [a]
3.5%	3.48 [a]	3.37 [a]	3.39 [a]	3.36 [a]	2.93 [a]	3.53 [a]

Note: Mean values within each column followed by the same letter are not significantly different (p > 0.05). Hedonic scale: 1 = dislike very much; 5 = like very much. QDA scale descriptions are provided in Methods.

Panelists rated the color of the tempeh samples between 3.33 and 3.48, indicating moderate to high liking across all *Rhizopus* concentrations. The highest color score was observed at 3.5% (3.48), while the lowest was recorded at 0.5% (3.33). Appearance scores ranged from 3.37 to 3.49, with the highest preference again found at 1.5% yeast concentration. However, these differences were not statistically significant, suggesting that variations in *Rhizopus* levels did not meaningfully affect consumer perception of tempeh color or appearance. These results align with previous findings that the visual attributes of tempeh are generally robust to moderate variations in inoculum concentration, provided the fermentation conditions support sufficient mold growth and substrate coverage(Nur *et al*., 2020; Rizal *et al*., 2021, 2022). The slight increase in appearance and color scores at higher inoculum concentrations (1.5–3.5%) may reflect improved surface mycelial coverage and fermentation uniformity, although the differences were not statistically significant.

Color uniformity scores ranged from 3.20 to 3.39 across treatments, placing all samples in the category of “moderately uniform” to “uniform” color. Although the 3.5% treatment showed the highest score (3.39), differences were not significant. Nut distribution, which reflects the evenness of canarium nut dispersion within the tempeh matrix, was rated between 3.32 and 3.36. All treatments achieved scores consistent with “moderately even” distribution, indicating effective ingredient mixing and mold binding across formulations. Surface moisture scores ranged from

2.93 to 3.21, indicating a tendency toward "moderately moist" textures, though a slight decline in moisture perception was noted as yeast concentration increased. The lowest moisture score at 3.5% yeast concentration (2.93) could suggest a drier surface, possibly due to increased fungal metabolism or extended fermentation, though the differences were again not statistically significant. Structural clarity, reflecting the visibility of mold growth and internal matrix structure, was consistently rated between 3.53 and 3.63. These values correspond to "moderately clear" to "clear" structure, with the highest clarity at 0.5% yeast. The relatively high scores across treatments confirm that structural development was sufficient at all inoculum levels to meet panelist expectations.

Overall, the sensory analysis demonstrates that varying the concentration of *Rhizopus* sp. inoculum from 0.5% to 3.5% does not significantly affect the color, appearance, or structural sensory attributes of canarium nut tempeh. All products were rated positively, indicating acceptability in terms of both hedonic preference and descriptive sensory characteristics. This suggests that a flexible range of inoculum levels can be employed in production without compromising consumer-perceived quality.

Aroma

The hedonic assessment of aroma showed a statistically significant ($p < 0.05$) difference among samples with different *Rhizopus oligosporus* inoculum concentrations (Table 4). The sample fermented with 0.5% inoculum received the highest mean score (3.95), indicating the most favorable aroma perception among panelists. This score was significantly higher than those of the 1.5%, 2.5%, and 3.5% inocula, which scored between 3.34 and 3.45 and were statistically similar to one another ($p > 0.05$). These findings suggest that a lower fungal inoculum yields a more acceptable overall aroma profile, potentially due to a better balance between the natural nutty aroma of canarium nuts and the mild fermentation notes.

Table 4. Hedonic and QDA Scores for Aroma of Canarium Nut Tempeh with Different *Rhizopus* Concentrations

Rhizopus concentration	Hedonic	QDA	
	Aroma	Fermentation Aroma	Canarium Nut Aroma
0,5%	3,95 ^{a}	2,91 ^{b}	3,69 ^{a}
1,5%	3,45 ^{b}	3,36 ^{ab}	3,24 ^{ab}
2,5%	3,34 ^{b}	3,24 ^{b}	3,25 ^{ab}
3,5%	3,44 ^{b}	3,81 ^{a}	3,16 ^{b}

Note: Mean values within each column followed by the same letter are not significantly different ($p > 0.05$). Hedonic scale: 1 = dislike very much; 5 = like very much. QDA scale descriptions are provided in Methods.

The QDA panel evaluated two aroma dimensions: fermentation aroma and canarium nut aroma. The intensity of fermentation aroma increased with higher inoculum concentrations, peaking at 3.81 in the 3.5% treatment, significantly higher than the 0.5% and 2.5% inocula ($p < 0.05$). This indicates that greater fungal biomass likely intensified the production of volatile compounds associated with fermentation, such as ethanol, esters, and short-chain fatty acids, which are typical by-products of *Rhizopus* metabolism (Dalile *et al*., 2019; Wang *et al*., 2023)

In contrast, the intensity of the canarium nut aroma was highest at the lowest inoculum level (3.69 at 0.5%) and significantly declined at higher inoculum concentrations, particularly at 3.5% (3.16, $p < 0.05$). This trend suggests that higher fungal activity may suppress or mask the inherent nutty volatiles of canarium nuts, possibly through enzymatic oxidation or metabolic competition during fermentation. Similar findings have been reported in tempeh made from high-fat substrates like peanuts and Koro nut (*Canavalia ensiformis*), where over-fermentation was found to diminish characteristic nutty aromas (Damayanti *et al*., 2025; Matsuo, 2006; Pangastuti *et al*., 2019; Zainal Abidin *et al*., 2020).

The relationship between hedonic aroma ratings and QDA results indicates a strong interplay between fermentation intensity and the preservation of intrinsic canarium nut aroma in determining consumer preference. The highest hedonic score for aroma was recorded in the tempeh inoculated with 0.5% *Rhizopus oligosporus* (mean score: 3.95), which also exhibited the highest canarium nut aroma intensity (mean score: 3.69) and a relatively lower fermentation aroma intensity (2.91). This suggests that the retention of the characteristic nutty aroma, combined with mild fermentation notes, was perceived as most pleasant by the panelists.

In contrast, samples with higher inoculum levels (1.5%–3.5%) showed lower hedonic scores (3.34–3.45) despite increased fermentation aroma intensity (peaking at 3.81 for 3.5%). Notably, these higher levels of fermentation aroma corresponded to a reduction in canarium nut aroma (3.24–3.16), suggesting that overpowering fermentation-derived volatiles may have masked the desirable nutty aroma, leading to reduced overall preference.

These findings align with prior research indicating that balanced aroma profiles, where native substrate aromas are preserved and fermentation-derived volatiles are present but not dominant, are generally more acceptable to consumers, for example in the fermented sweet potato (Sivakumar *et al*., 2010) and in fermented oat (Lee *et al*., 2016). In products like tempeh, excessive fermentation can lead to the accumulation of compounds such as ammonia, ethanol, and ketones, which may impart pungent or off-aromas (Ahnan-Winarno *et al*., 2021; Prameswari *et al*., 2021). This is particularly important in high-fat matrices like canarium nuts, where lipid oxidation can further alter volatile profiles under intense fungal activity.

The data suggest that the hedonic perception of aroma in canarium nut tempeh is more positively influenced by a strong canarium nut aroma and moderate fermentation aroma. Thus, the 0.5% inoculum level appears optimal, allowing for sufficient fermentation to develop structure and safety while maintaining the sensory identity of the base ingredient, an essential consideration for novel non-soy tempeh products aiming to retain cultural or regional flavor attributes.

The observed decrease in hedonic aroma ratings with increasing *Rhizopus* inoculum concentration aligns with previous reports that excessive fermentation intensity can produce overpowering or undesirable aromas in tempeh (Ahnan-Winarno *et al*., 2021; Nur *et al*., 2020; Pangastuti *et al*., 2019). Moderate levels of fermentation aroma are generally desirable, as they contribute to the characteristic "mushroom-like" or "earthy" scent of tempeh; however, excessive intensities, especially when combined with high enzymatic degradation, may introduce sour or ammonia-like notes that lower consumer acceptability (Nur *et al*., 2020).

Furthermore, the masking of the canarium nut aroma at higher inoculum levels may be attributed to competitive dominance of fungal metabolites in the volatile profile. This phenomenon has been described in other fungal-fermented matrices, such as faba beans and lupines substrates, where native aroma compounds were suppressed due to fungal lipase activity and oxidative degradation (Ritter *et al*., 2022).

These findings highlight the importance of optimizing *R. oligosporus* inoculum concentration in non-soybean tempeh substrates, such as canarium nuts, to preserve the unique nutty aroma while ensuring sufficient fungal colonization. The 0.5% inoculum appeared optimal in balancing fermentation aroma with the native canarium nut characteristics while achieving the highest hedonic scores, making it a promising formulation for further product development.

Taste

The hedonic evaluation of taste in canarium nut tempeh varied with the concentration of *Rhizopus oligosporus* used in fermentation (Table 5). The highest mean hedonic score for taste (3.91) was observed in the 0.5% Rhizopus group, which was not significantly different ($p > 0.05$) from the 1.5% and 2.5% groups but was significantly higher than the 3.5% group (3.24). This suggests that lower inoculum levels may promote more acceptable taste profiles in canarium nut-based tempeh.

Table 5. Hedonic and QDA scores for taste of canarium nut tempeh with different *Rhizopus* concentrations

Rhizopus Concentration	Hedonic	QDA			
	Taste	Canarium Nut Flavor	Sourness	Bitterness	Umami flavor
0,5%	3,91 ^{a}	4,01 ^{a}	2,77 ^{a}	2,48 ^{c}	3,79 ^{a}
1,5%	3,44 ^{ab}	3,33 ^{b}	2,84 ^{a}	3,97 ^{b}	3,35 ^{ab}
2,5%	3,48 ^{ab}	3,47 ^{b}	3,16 ^{a}	3,35 ^{ab}	3,36 ^{ab}
3,5%	3,24 ^{b}	3,21 ^{b}	3,17 ^{a}	3,77 ^{a}	3,21 ^{b}

Note: Mean values within each column followed by the same letter are not significantly different ($p > 0.05$). Hedonic scale: 1 = dislike very much; 5 = like very much. QDA scale descriptions are provided in Methods.

Descriptive sensory analysis (QDA) revealed significant differences in several taste-related attributes that appeared to influence panelists' preferences. Tempeh fermented with 0.5% inoculum exhibited the strongest canarium nut flavor (4.01), the lowest bitterness (2.48), and highest umami perception (3.79), all of which are positively related with hedonic taste liking.

Conversely, samples fermented with higher inoculum levels (1.5%–3.5%) exhibited reduced canarium nut flavor intensity (3.21–3.47), increased bitterness (up to 3.97 at 1.5%), and lower umami (3.21 at 3.5%), potentially contributing to their lower hedonic ratings.

The presence of canarium nut flavor appears to be a key driver of taste preference. At low Rhizopus levels (0.5%), the dominant nutty flavor was retained and not masked by excessive fermentation by-products. This supports findings from other studies on nut-based fermented products, where excessive microbial activity may generate undesirable metabolites (e.g., phenolic degradation compounds, peptides, or bitter alkaloids), which reduce consumer acceptability (Ahnan-Winarno *et al*., 2021).

Bitterness showed a notable increase in the 1.5% and 3.5% groups, reaching values of 3.97 and 3.77 respectively, indicating a moderate to high level of bitter compounds likely formed through protein hydrolysis or lipid oxidation during more intense fungal activity (Ahnan-Winarno *et al*., 2021; Pangastuti *et al*., 2019). This increase in bitterness was inversely associated with hedonic ratings, suggesting it was perceived negatively.

Additionally, umami flavor, which contributes to the savory and palatable nature of fermented products, was highest in the 0.5% group (3.79) and declined in higher inoculum treatments. The generation of free amino acids and peptides responsible for umami may be optimal at moderate fermentation levels but degraded or masked by off-flavors at higher fungal activity levels (Ahnan-Winarno *et al*., 2021; Wihan & Anugrahati, 2023).

Sourness intensity increased slightly across treatments (2.77–3.17), though not significantly different ($p > 0.05$). However, even small increases in sourness and bitterness together may negatively affect flavor harmony if not balanced with umami or nutty sweetness.

Taken together, these results indicate that the preservation of canarium nut flavor, minimal bitterness, and higher umami intensity are essential for maximizing consumer preference. The 0.5% Rhizopus concentration was optimal for maintaining these sensory characteristics, whereas higher concentrations led to flavor degradation and off-taste development, diminishing hedonic acceptability.

Texture

The hedonic assessment of texture in canarium nut tempeh showed a significant influence of *Rhizopus oligosporus* concentration (Table 6). The highest texture preference was recorded in the 0.5% Rhizopus group with a mean score of 3.89, significantly higher ($p < 0.05$) than the 1.5% and 3.5% groups (3.35 and 3.43, respectively), while the 2.5% group (3.67) was statistically similar to all groups. This suggests that both lower (0.5%) and moderate (2.5%) inoculum levels may lead to better textural acceptability compared to higher concentrations.

Table 6. Hedonic and QDA scores for texture of canarium nut tempeh with different *rhizopus* concentrations

Rhizopus Concentration	Hedonic	QDA		
	Texture	Hardness	Tenderness	Friability
0,5%	3,89 [a]	3,05 [b]	3,24 [a]	2,96 [a]
1,5%	3,35 [b]	3,20 [ab]	3,00 [a]	2,87 [a]
2,5%	3,67 [ab]	3,23 [ab]	3,33 [a]	3,04 [a]
3,5%	3,43 [b]	3,64 [a]	3,20 [a]	2,92 [a]

Note: Mean values within each column followed by the same letter are not significantly different ($p > 0.05$). Hedonic scale: 1 = dislike very much; 5 = like very much. QDA scale descriptions are provided in Methods.

The highest hardness score was observed at 3.5% Rhizopus concentration (3.64), significantly firmer than the 0.5% group (3.05, $p < 0.05$). This trend indicates that higher fungal loads may lead to denser tempeh structure, potentially due to overgrowth of mycelium that binds the substrate more tightly. While some firmness is desirable in fermented foods, excessive hardness can reduce consumer acceptability, as suggested by the lower hedonic score in the 3.5% group.

Tenderness values ranged from 3.00 to 3.33 across all samples, with no significant differences ($p > 0.05$). All samples were generally perceived as moderately tender, which may explain why tenderness did not emerge as a strong discriminator of preference in this study. However, the slightly higher tenderness score in the 2.5% group (3.33) may have contributed to its relatively high liking score.

Friability, referring to the crumbliness or disintegration of tempeh under pressure, also showed no significant differences across treatments ($p > 0.05$), ranging from 2.87 to 3.04. All samples were rated as moderately crumbly, which is typical of tempeh products and suggests structural stability. This attribute, although not statistically different, may still contribute subtly to mouthfeel and the perception of textural integrity.

The high texture liking in the 0.5% Rhizopus group appears to be driven by a balance of moderate firmness (hardness = 3.05) and acceptable tenderness (3.24), resulting in a pleasing chewiness without being overly hard. The combination of lower hardness and moderate friability (2.96) likely contributed to a texture that is perceived as easy to chew and break apart, aligning with consumer expectations for traditional tempeh products.

In contrast, the highest fungal concentration (3.5%) led to significantly higher hardness (3.64), which may have negatively impacted chewability, reducing its hedonic score despite acceptable tenderness and friability. This supports findings from similar studies where excessive fungal colonization results in dense or rubbery textures that are less preferred (Magdalena *et al*., 2022).

Moderate inoculum levels (2.5%) showed relatively high hedonic scores (3.67), likely due to an optimal balance among QDA attributes, neither too soft nor too firm, highlighting that controlled fungal growth can enhance texture quality in canarium nut-based substrates.

These findings emphasize the importance of optimizing inoculum concentration to control texture formation in tempeh-like products. The combination of moderate hardness, adequate tenderness, and moderate friability, as observed in the 0.5% and 2.5% treatments, was most favored. Over-fermentation (as in the 3.5% group) can lead to excessive compaction and reduced textural appeal. Understanding the link between fungal activity and physical structure is therefore essential for maintaining consumer-acceptable product profiles.

Overall Likeness

The overall sensory acceptability of canarium nut tempeh was significantly influenced by the concentration of *Rhizopus oligosporus* used during fermentation (Table 7). The hedonic scores for overall likeness ranged from 2.83 to 4.01 on a 5-point scale, where 1 represents "dislike very much" and 5 indicates "like very much".

Table 7. Hedonic for Overall Likeness of Canarium Nut Tempeh with Different *Rhizopus* Concentrations

Rhizopus Concentration	Hedonic
	Overall Likeness
0,5%	4,01 ^{a}
1,5%	3,37 ^{b}
2,5%	3,33 ^{b}
3,5%	2,83 ^{c}

Note: Mean values within each column followed by the same letter are not significantly different ($p > 0.05$). Hedonic scale: 1 = dislike very much; 5 = like very much.

The tempeh produced using 0.5% *Rhizopus* inoculum achieved the highest overall liking score (4.01), which was significantly different ($p < 0.05$) from all other concentrations. This indicates that the panelists generally liked the 0.5% sample the most, classifying it as "liked." In contrast, the lowest liking score was recorded at the highest inoculum level (3.5%), with a mean score of only 2.83, positioned between "neutral" and "dislike", and significantly different from all other groups. The 1.5% and 2.5% inoculum treatments received intermediate scores of 3.37 and 3.33, respectively. These values were not significantly different from each other ($p > 0.05$) but were both significantly lower than the 0.5% treatment and significantly higher than the 3.5% treatment.

The observed trend suggests that increasing the concentration of *Rhizopus* beyond a certain point negatively affects the overall acceptability of the product. This may be attributed to several cumulative sensory effects related to aroma, taste, and texture: At low concentrations (0.5%), fungal growth may be sufficient to induce desirable fermentation changes, such as mild umami development, balanced canarium nut flavor, and optimal textural firmness, without overpowering flavors or excessive structural changes. As a result, panelists perceived the tempeh as well-balanced and more palatable. At moderate levels (1.5–2.5%), sensory defects may begin to emerge. These could include increased sourness or bitterness, or a firmer, less desirable texture (as indicated in previous texture and taste QDA results). While these changes were not severe enough to drastically lower acceptability, they may have contributed to a less favorable perception overall. At high concentrations (3.5%), over-fermentation likely led to excessive development of off-flavors (such as bitterness and strong sour notes), as well as a firmer and denser texture. Additionally, increased enzymatic activity at high inoculum loads can cause degradation of substrate components, altering flavor balance and aroma, which was less preferred by the panelists.

These findings align with prior research on inoculum optimization in fungal fermentation, where lower inoculum levels often produce milder and more acceptable organoleptic properties, while higher levels accelerate fermentation beyond the ideal point for consumer preference (Rizal *et al*., 2022; Starzyńska-Janiszewska *et al*., 2012).

CONCLUSION

This study demonstrates that careful adjustment of *Rhizopus oligosporus* inoculum is essential for optimizing the sensory quality of canarium nut tempeh. Using a low starter concentration (0.5%) best preserves the inherent nutty character and creates a balanced texture, yielding consistently high consumer approval. In contrast, higher inoculum levels can lead to overly intense fermentation notes and excessive firmness, which diminish overall appeal. By adopting the optimal inoculum rate, producers can streamline culture usage, reduce costs, and deliver a novel, high-quality tempeh alternative. Future work should investigate how fermentation time, temperature, and packaging further influence shelf life and sensory stability of this promising plant-based product.

REFERENCES

Ahnan-Winarno, A.D., Cordeiro, L., Winarno, F.G., Gibbons, J., & Xiao, H. (2021). Tempeh: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. *Comprehensive Reviews in Food Science and Food Safety*, *20*(2), 1717–1767. https://doi.org/https://doi.org/10.1111/1541-4337.12710

Aliyah, N. (2024). Pembuatan tempe kacang tunggak (Vigna unguiculata) dengan variasi lama fermentasi menggunakan inokulum tempe. *Joiurnal of Comprehensive Science*, *3*(1), 62–78.

Bai, S. H., Brooks, P., Gama, R., Nevenimo, T., Hannet, G., Hannet, D., Randall, B., Walton, D., Grant, E., & Wallace, H.M. (2019). Nutritional quality of almond, canarium, cashew and pistachio and their oil photooxidative stability. *Journal of Food Science and Technology*, *56*(2), 792–798. https://doi.org/10.1007/s13197-018-3539-6

Cabanillas, B., Jappe, U., & Novak, N. (2018). Allergy to peanut, soybean, and other legumes: recent advances in allergen characterization, stability to processing and IgE cross-reactivity. *Molecular Nutrition & Food Research*, *62*(1), 1700446. https://doi.org/https://doi.org/10.1002/mnfr.201700446

Campbell, R., Hauptmann, A., Campbell, K., Fox, S., & Marco, M.L. (2022). Better understanding of food and human microbiomes through collaborative research on inuit fermented foods. *Microbiome Research Reports*, *1*(1), 1–7. https://doi.org/10.20517/mrr.2021.06

Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature Reviews Gastroenterology & Hepatology*, *16*(8), 461–478. https://doi.org/10.1038/s41575-019-0157-3

Damayanti, A., Bahlawan, Z.A.S., Winaningsih, I., Ramadhani, R.R., Bancin, W.A.P., & Yuliono, B. (2025). A quality analysis of different types of peanuts tempeh wrap as food security. *AIP Conference Proceedings*, *3166*(1), 20030. https://doi.org/10.1063/5.0236745

Domínguez-López, I., Yago-Aragón, M., Salas-Huetos, A., Tresserra-Rimbau, A., & Hurtado-Barroso, S. (2020). Effects of dietary phytoestrogens on hormones throughout a human lifespan: A review. *Nutrients*, *12*(8), 1–25. https://doi.org/10.3390/nu12082456

Egounlety, M., & Aworh, O.C. (2003). Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Ha). *Journal of Food Engineering*, *56*(2), 249–254. https://doi.org/https://doi.org/10.1016/S0260-8774(02)00262-5

Handoyo, T., & Morita, N. (2006). Structural and functional properties of fermented soybean (Tempeh) by using rhizopus oligosporus. *International Journal of Food Properties*, *9*(2), 347–355. https://doi.org/10.1080/10942910500224746

Lee, S. M., Oh, J., Hurh, B.-S., Jeong, G.-H., Shin, Y.-K., & Kim, Y.-S. (2016). Volatile compounds produced by lactobacillus paracasei during oat fermentation. *Journal of Food Science*, *81*(12), C2915–C2922. https://doi.org/https://doi.org/10.1111/1750-3841.13547

Leeuwendaal, N. K., Stanton, C., O'Toole, P.W., & Beresford, T.P. (2022). Fermented foods, health and the gut microbiome. *Nutrients*, *14*(7), 1527. https://doi.org/10.3390/nu14071527

Magdalena, S., Hogaputri, J.E., Yulandi, A., & Yogiara, Y. (2022). The addition of lactic acid bacteria in the soybean soaking process of tempeh. *Food Research*, *6*(3), 27–33. https://doi.org/10.26656/fr.2017.6(3).304

Mailoa, M., Widyaningsih, T.D., Putri, W.D.R., & Harijono. (2019). Fresh and roasted Canarium nut (*Canarium vulgare*) altering the lipid profile of hypercholesterolemic rats (Rattus norvegicus). *Eurasia J Biosci*, *13*, 231–238. https://www.proquest.com/docview/2234975066?pq-origsite=gscholar&fromopenview=true

Matsuo, M. (2006). Preparation and preferences of peanut-tempeh, peanuts fermented with *Rhizopus oligosporus*. *Food Science and Technology Research*, *12*(4), 270–274. https://doi.org/10.3136/fstr.12.270

Nur, N., Meryandini, A., Suhartono, M.T., & Suwanto, A. (2020). Lipolytic bacteria and the dynamics of flavor production in indonesian tempeh. *Biodiversitas*, *21*(8), 3818–3825. https://doi.org/10.13057/biodiv/d210850

Pangastuti, A., Alfisah, R.K., Istiana, N.I., Sari, S.L.A., Setyaningsih, R., Susilowati, A., & Purwoko, T. (2019). Metagenomic analysis of microbial community in over-fermented tempeh. *Biodiversitas*, *20*(4), 1106–1114. https://doi.org/10.13057/biodiv/d200423

Prameswari, H.A., Nursiwi, A., Zaman, M.Z., Ishartani, D., & Sari, A.M. (2021). Changes in chemical and sensory characteristics of gunungkidul's lamtoro (*Leucaena leucocephala*) tempeh during extended fermentation. *IOP Conference Series: Earth and Environmental Science*, *828*(1), 012001. https://doi.org/10.1088/1755-1315/828/1/012001

Puspitasari, D., Nasir, M., & Azmin, N. (2022). Uji organoleptik tempe dari biji asam (*Tamarindus indica*) berdasarkan waktu fermentasi. *JUSTER : Jurnal Sains Dan Terapan*, *1*(1), 8–14. https://doi.org/10.55784/juster.vol1.iss1.12

Ritter, S.W., Gastl, M.I., & Becker, T.M. (2022). The modification of volatile and nonvolatile compounds in lupines and faba beans by substrate modulation and lactic acid fermentation to facilitate their use for legume-based beverages—A review. *Comprehensive Reviews in Food Science and Food Safety*, *21*(5), 4018–4055.

https://doi.org/https://doi.org/10.1111/1541-4337.13002

Rizal, S., Kustyawati, M.E., Murhadi, & Hasanudin, U. (2021). The growth of yeast and fungi, the formation of β-Glucan, and the antibacterial activities during soybean fermentation in producing tempeh. *International Journal of Food Science*, *2021*(1), 6676042. https://doi.org/https://doi.org/10.1155/2021/6676042

Rizal, S., Kustyawati, M.E., Murhadi, Sari, R.K., & Hidayat, R. (2024). Microbiological, sensory, and chemical properties of high-quality tempeh made with instant Mosaccha tempeh inoculum powder. *Food Science and Technology International*, *21*, 10820132241264444. https://doi.org/10.1177/10820132241264443

Rizal, S., Kustyawati, M.E., Suharyono, A.S., & Suyarto, V.A. (2022). Changes of nutritional composition of tempeh during fermentation with the addition of *Saccharomyces cerevisiae*. *Biodiversitas*, *23*(3), 1553–1559. https://doi.org/10.13057/biodiv/d230345

Seumahu, C.A., Suwanto, A., Rusmana, I., & Solihin, D.D. (2013). Bacterial and fungal communities in tempeh as reveal by amplified ribosomal intergenic sequence analysis. *HAYATI Journal of Biosciences*, *20*(2), 65–71. https://doi.org/https://doi.org/10.4308/hjb.20.2.65

Sitompul, S.R., Eliska, & Tarigan, A.A. (2023). Uji daya terima dan kandungan gizi tempe biji karet (*Hevea brasiliensis*). *Jurnal Kesehatan Masyarakat Indonesia*, *1*(1), 35–45. https://doi.org/10.1016/j.jnc.2020.125798

Sivakumar, P.S., Panda, S.H., Ray, R.C., Naskar, S.K., & Bharathi, L.K. (2010). Consumer acceptance of lactic acid-fermented sweet potato pickle. *Journal of Sensory Studies*, *25*(5), 706–719. https://doi.org/https://doi.org/10.1111/j.1745-459X.2010.00299.x

Starzyńska-Janiszewska, A, Stodolak, B, Duliński, R, & Mickowska, B. (2012). The influence of inoculum composition on selected bioactive and nutritional parameters of grass pea tempeh obtained by mixed-culture fermentation with *Rhizopus oligosporus* and *Aspergillus oryzae* strains. *Food Science and Technology International*, *18*(2), 113–122. https://doi.org/10.1177/1082013211414771

Tamam, B. (2022). Tempe: Pangan lokal unggul (Superfood) khasanah budaya bangsa. *Indonesian Red Crescent Humanitarian Journal*, *1*(1), 41–48. https://doi.org/10.56744/irchum.v1i1.14

Tamang, J.P., Watanabe, K., & Holzapfel, W.H. (2016). Review: Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology*, *7*, 1–28. https://doi.org/10.3389/fmicb.2016.00377

Teoh, S.Q., Chin, N.L., Chong, C.W., Ripen, A.M., How, S., & Lim, J.J.L. (2024). A review on health benefits and processing of tempeh with outlines on its functional microbes. *Future Foods*, *9*, 100330. https://doi.org/https://doi.org/10.1016/j.fufo.2024.100330

Tuhumury, H.C.D., Souripet, A., & Pattiwael, K.J. (2023). Production of canarium (*Canarium indicum* L) butter with different sugar concentrations. *Journal of Applied Agricultural Science and Technology*, *7*(2), 130–141. https://doi.org/10.55043/jaast.v7i2.138

Wang, K., Wu, H., Wang, J., & Ren, Q. (2023). Microbiota composition during fermentation of broomcorn millet huangjiu and their effects on flavor quality. *Foods*, *12*(14), 2680. https://doi.org/10.3390/foods12142680

Wihan, L.A., & Anugrahati, N.A. (2023). Utilization of partially purified papain enzyme in mallika black soybean tempeh hydrolysate as umami seasoning. *Caraka Tani: Journal of Sustainable Agriculture*, *38*(2), 215–228. https://doi.org/10.20961/carakatani.v38i2.71093

Zainal Abidin, N.A., Mohd Zin, Z., Abdullah, M.A.A., Rusli, N.D., & Zainol, M.K. (2020). Physicochemical Properties and Sensory Acceptance of Canavalia ensiformis Tempeh Energy Bar. *Food Research*, *4*(5), 1637–1645. https://doi.org/10.26656/fr.2017.4(5).150