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Identification of Microflora Diversity and Its Influence on the Organoleptic Quality of the Traditional Dayak Food *Tempoyak* in Central Kalimantan

Sondra Swestyani[1*], Noor Hujjatusnaini[1]
[1]Pendidikan Biologi, Fakultas Tarbiyah dan Ilmu Keguruan, Institut Agama Islam Negeri Palangka Raya, Jl. G.Obos Komplek Islamic Centre Palangka Raya, Indonesia
**Corresponding author*: sondra.swestyani@iain-palangkaraya.ac.id

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Abstract: *Tempoyak* is a traditional fermented food of the Dayak ethnic group in Central Kalimantan. The production of *tempoyak* remains highly traditional and lacks innovation. Research on *tempoyak* is still limited, resulting in a lack of documentation of this food as part of Indonesia's local wisdom and cultural heritage. Therefore, exploring preferences and the appeal of *tempoyak*'s organoleptic qualities among the millennial generation is essential. This study employed a one-shot case study design. The variables examined include the diversity of microflora in *tempoyak* and its effect on organoleptic quality and the level of preference among millennials. Isolate identification was conducted using API-50CHL, biochemical tests, and physiological tests. Organoleptic quality was assessed using a hedonic scale. A total of 120 panelists participated in the study, consisting of individuals from Dayak, Madurese, Javanese, and Banjar ethnic groups. Data were analyzed using one-way ANOVA. The study identified 15 microflora isolates in *tempoyak* that influence its taste and organoleptic properties, including 8 lactic acid bacteria isolates: *Pediococcus acidilactici, Lactobacillus curvatus, Leuconostoc mesenteroides, Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus pentosus, Enterococcus faecium, and Weissella paramesenteroides*; 1 non-lactic acid bacterium isolate (*Micrococcus varians*); 2 yeast isolates (Saccharomyces cerevisiae and *Kluyveromyces marxianus*); and 4 mold isolates, including *Rhizopus, Monilia sitophila, and Mucor roxii*. All identified microflora could produce lactic acid, except *Aspergillus repens*. Statistical analysis showed a significant effect on all sub-variables of organoleptic quality—color, taste, aroma, and texture of *Tempoyak*. These findings suggest a preference for the color and texture, and a liking for the taste and aroma of *Tempoyak*. The study indicates potential for *Tempoyak* to be further developed in a more innovative and contemporary manner, making it more appealing to the millennial generation.

Keywords: *tempoyak*, organoleptic, microflora, Dayak

INTRODUCTION

Traditional food is an important part of a nation's cultural heritage and must be preserved (Barbara, 2019; Anneke et al., 2019; Tamang et al., 2020). Indonesia is rich in a variety of traditional foods originating from different ethnic groups, including the Dayak people of Central Kalimantan. According to Balia & Utama (2010), traditional food is a defining aspect of Indonesian cultural identity and heritage. Central Kalimantan has many traditional food products made from local plants, such as rattan, *cempedak, durian*, various types of ferns, and tubers of endemic plants. The preparation of traditional food in Central Kalimantan is generally very simple, with fermentation being one of the primary methods. One example of a fermented traditional food typical of the Dayak community is *tempoyak*.

Tempoyak is a traditional food made from the flesh of *durian* (Durio zibethinus). It is typically produced during the durian harvest season or from overripe or lower-quality durians (Amiza et al., 2006). The preparation of *Tempoyak* involves three stages. The first stage is the collection of durian pulp. The second stage is the addition of salt to the pulp, at a very low concentration. Ramdiah et al. (2014) emphasized that in *tempoyak* production, salt is added at a concentration of about 1.3%, primarily to enhance flavor and reduce the presence of pathogenic bacteria that may spoil the product. Such pathogens include proteolytic and spore-forming aerobic and anaerobic bacteria (Leisner, 2001), which can cause spoilage (Surono, 2003; Braghieri et al., 2015). The low amount of salt also supports the fermentation process and stimulates the growth of lactic acid bacteria (Claudia et al., 2017), ensuring that *tempoyak* is preserved and safe for consumption (Alcamo, 2001; Balia & Utama, 2017). The third stage is fermentation, which typically takes around 7 days, with a pH range between 3.8 and 4.6. This fermentation process influences the distinctive sour, salty taste and characteristic *durian* aroma of *tempoyak* (Ginajar, 2000).

The availability of *tempoyak* in Indonesia remains limited, produced only during the peak durian season and through traditional fermentation, which hinders its mass-market distribution. However, this simple fermentation method allows *tempoyak* to have a relatively long shelf life due to the presence of lactic acid bacteria and the addition of salt during processing. A previous study by Leisner (2001) reported that Lactobacillus plantarum is the dominant lactic acid bacterium in *tempoyak*. The presence of fermentative bacteria significantly affects product quality. Until now, however, studies on the isolation and characterization of lactic acid bacteria in *tempoyak*, as well as determining optimal fermentation durations for improved organoleptic quality, are still lacking.

METHOD

This research used a qualitative descriptive analysis method through a naturalistic approach. The study design employed a one-shot case study, where a group is given a treatment and then observed to obtain results (Sugiyono, 2014; Ares, 2018). The aim of this research was to describe the level of preference

among panelists toward the organoleptic characteristics of *tempoyak*. The data were enriched by the results of isolation, characterization, and identification of lactic acid bacteria present in *tempoyak*, with the goal of contributing to the scientific understanding of this traditional food.

Isolation, Characterization, and Identification Bacteria in *Tempoyak*

The process of isolating, characterizing, and identifying lactic acid bacteria was conducted after traditional preparation and fermentation of *tempoyak*. Fermentation began with the collection of *durian* pulp. A total of 500 grams of durian pulp was mixed with 1.3% salt solution and incubated at room temperature for 24 to 72 hours. After fermentation, *tempoyak* samples were collected for microbial isolation. About 20 grams of *tempoyak* were diluted in 180 ml of sterile distilled water and serially diluted. One milliliter from each dilution series was plated using the pour plate method on various media: Glucose Yeast Peptone Agar (GYPA) for total plate count (TPC), De Man Rogosa Sharpe Agar (MRSA) for lactic acid bacteria, Sabouraud Dextrose Agar (SDA) for yeast, and Potato Dextrose Agar (PDA) for molds. All media were incubated for 48 hours at 35°C for bacteria and yeast, and at 30°C for molds.

Distinct single colonies were then purified using the streak plate method and transferred to slant agar for preservation, with lactic acid bacteria maintained on Bromocresol Purple slants. The isolation process followed the Indonesian National Standard (SNI ISO 6887-1:2012) (Supiana & Zainatur, 2016). Isolates grown on media after incubation were observed for colony morphology, including color, size (large, medium, small), shape (circular, irregular, spindle, filamentous, rhizoid), margin (entire, lobate, undulate, serrate, filamentous, curled), and elevation (flat, raised, convex, umbonate) (Zhenxiang et al., 2018).

Bacterial isolates were identified using the API 50 CHL test (API BioMérieux, France), which evaluates the ability of lactic acid bacteria to metabolize 49 different carbohydrates. Bacterial isolates were centrifuged and suspended before inoculating all carbohydrate wells on the API 50 CHL test strip, followed by incubation at 37°C for 48 hours. A positive reaction was indicated by a color change from blue to green, yellow, or black (Ozgun & Hasibe, 2011; Aswani & David, 2016).

Organoleptic Quality of *Tempoyak*

Organoleptic quality data were obtained using sensory evaluation tests. Organoleptic tests serve as indicators of preference level (Braghieri et al., 2014). The panelists in this study consisted of 120 students from IAIN Palangka Raya, representing four ethnic groups: Dayak, Madurese, Javanese, and Banjar. The hypothesis was developed based on theoretical frameworks rather than empirical observations (Sugiyono, 2014) and presented in Table 1.

Table 1. Research Hypothesis.

Hypothesis	Preference Dimension
1	There is a panelist preference for the **color** of *tempoyak*

2	Panelists express **liking** for the **taste** of *tempoyak*
3	Panelists express **liking** for the **aroma** of *tempoyak*
4	There is a panelist preference for the **texture** of *tempoyak*

The data collection technique used a preference questionnaire based on a hedonic scale (Table 2), which was transformed into a numerical scale for further statistical analysis. The research instrument was designed to explore the panelists' level of liking using a preference questionnaire sheet (Trias et al., 2020). The data were then analyzed statistically using One-Way ANOVA with SPSS version 22.

Table 2. Hedonic Scale for Preferences.

Hedonic Scale	Numerical Scale
Extremelly likes	6
Very likes	5
Likes	4
Somewhat likes	3
Neutral	2
Disliked	1

RESULT AND DISCUSSION

Microflora in Tempoyak

A total of 15 microflora isolates were successfully isolated, characterized, and identified from *tempoyak*, as presented in Table 3.

Table 3. Results of Isolation, Characterization, and Identification of Microflora in Tempoyak.

Isolate	Colony Characteristic
TTF-1	Milky white colony, medium size, circular shape, entire margin, convex elevation
TTF-2	Milky white colony, medium size, circular shape, entire margin, raised elevation
TTF-3	Milky white colony, small size, irregular shape, undulate margin, convex elevation
TTF-4	Translucent white colony, medium punctiform size, circular shape, undulate margin, raised elevation
TTF-5	Translucent white colony, small size, circular shape, undulate margin, convex elevation
TTF-6	Translucent white colony, small size, irregular shape, undulate margin, droplet-like elevation
TTF-7	Irregular-shaped colony, some appear round and others rod-like, non-motile
TTF-8	Translucent white colony, coccus shape, undulate margin, non-motile
TTF-9	Translucent white colony, coccus shape, entire margin, non-motile
TTF-10	Yellowish-white colony, clustered, round to elliptical-cylindrical shape, circular margin, glistening surface, convex elevation
TTF-11	Translucent white colony, entire margin, raised elevation, glistening surface
TTF-12	Grayish-brown colony with single sporangium and hyphae formation
TTF-13	Long septate hyphae with oval conidia, likely formed from fragmentation of fertile hyphae branches

TTF-14	White aseptate hyphae with spherical to oval sporangiospores, dark-colored spores, no visible stolon or rhizoid
TTF-15	Visible structures include conidiophores, vesicles, primary and secondary sterigmata, and round conidia in chain-like formation

Based on the API 50CHL test, eight isolates were identified as lactic acid bacteria, as shown in Table 4.

Table 4. Results of Lactic Acid Bacteria Analysis in Tempoyak.

Isolate	Scpecies Indentification	Test API 50CHL
TTF-1	*Lactobacillus curvatus*	91.1%
TTF-2	*Leuconostoc mesenteroides*	90%
TTF-3	*Lactobacillus plantarum*	97.3%
TTF-4	*Lactobacillus pentosus*	92.6%
TTF-5	*Streptococcus thermophilus*	90.1%
TTF-6	*Pediococcus acidilactici.*	90%
TTF-7	*Weissella paramesenteroides*	91%
TTF-9	*Enterococcus faecium*	99.3%

Based on the results of the API 50CHL test, Enterococcus faecium showed the highest estimated similarity at 99.3%. The bacterial isolate Micrococcus varians, characterized by its clear white colonies, coccus shape, undulate edges, and non-motile nature, was found in the smallest quantity. This bacterium does not play a significant role in fermentation and is not classified as a lactic acid bacterium.

The TTF-10 isolate, based on its morphological features, did not resemble typical bacteria but rather appeared to belong to the yeast group. Its colony was yellowish-white, clustered, with a round to ellipsoidal or cylindrical shape, circular edges, a glistening surface, and a convex elevation. A similar morphological profile was observed in the TTF-11 isolate, which had clear white colonies, entire edges, raised elevation, and a shiny surface. These isolates were subsequently cultured using SDA (Sabouraud Dextrose Agar) medium. Isolates with similar characteristics were grouped based on morphological, biochemical, and physiological features, as presented in Table 5.

Table 5. Results of Biochemical and Physiological Analysis of Microflora.

Isolate	Species identification	*Thermo tolerant test* (^{0}C)	*Ethanol tolerant test* (%)	*Ethanol Product* (% b/v)
TTF -10	*Saccharomyces cereviciae*	48	15	2.47
TTF -11	*Kluyveromyces marxianus*	40	13	2.51

Based on Table 5, the isolates demonstrated the ability to withstand relatively high temperatures, including Saccharomyces cerevisiae at 48°C and Kluyveromyces marxianus at 40°C. According to Choudhary et al. (2011), such characteristics can be interpreted as an indication of thermotolerance. In addition, both isolates also exhibited ethanol tolerance, with TTF-11 tolerating up to 15% ethanol and TTF-12 up to 13%.

In the ethanol productivity test conducted in YP medium with 10% (w/v) glucose at a fermentation temperature of 48°C, TTF-11 produced 2.47% ethanol, while TTF-12 produced 2.51%. These biochemical and physiological data complement the morphological characterization. According to Hasanudin (2010), TTF-11 and TTF-12 showed circular colonies with clear white color, convex elevation, entire margins, smooth configuration, and dull appearance. Their cells were round to semi-round with a multilateral budding pattern—morphological traits similar to those of Saccharomyces cerevisiae.

Isolate TTF-11 also displayed features typical of the yeast group and shared similar characteristics with Kluyveromyces marxianus. In contrast, isolates TTF-12, TTF-13, TTF-14, and TTF-15 did not exhibit typical bacterial or yeast morphology. Instead, their features more closely resembled those of filamentous fungi. These isolates were further cultured on PDA (Potato Dextrose Agar) medium to confirm their classification as fungal microflora, as presented in Table 6.

Table 6. Results of Analysis on PDA Medium.

Isolate	Colony Characteristic	Species
TTF -12	Colonies appeared grayish-brown, with single sporangiophores and hyphae formation.	*Rhizopus*
TTF -13	The hyphae were long and septate, with oval-shaped conidia. These conidia were presumed to result from fragmentation of fertile hyphae at the upper branches.	*Monilia sitophila.*
TTF -14	White-colored hyphae were non-septate, containing nearly oval-shaped sporangiospores and black-colored spores, with no visible stolons or rhizoids.	*Mucor roxii*
TTF -15	Structures resembling conidiophores, vesicles, primary and secondary sterigmata, and round-shaped conidia were observed. These conidia appeared in chains emerging from the sterigmata.	*Aspergillus repens*

Isolates TTF-12, TTF-13, TTF-14, and TTF-15, characterized by the presence of hyphae, were confirmed as fungal groups. Table 6 shows that isolate TTF-12 possessed hyphae with single sporangiophores, where rhizoids were observed growing at the base of the spore stalk. These characteristics, observed on fermentative media, suggest classification under the Rhizopus genus.

Microscopic observation of TTF-13 revealed longer hyphae with visible septa and oval-shaped conidia. These morphological traits suggest a resemblance to *Monilia sitophila*, a microscopic fungus found to be the most dominant among the isolates. Isolate TTF-14 displayed morphological characteristics indicative of Mucor, especially due to its prominent white mycelium. Microscopically, its hyphae were non-septate, with no observable rhizoids or stolons. These features are more specifically aligned with *Mucor roxii*, a species known from several studies to be a potential fungus for lactic acid fermentation.

Another isolate with similar morphological traits was TTF-15, which exhibited conidia and secondary sterigmata. Microscopically, structures resembling conidiophores were observed, with round conidia emerging in chains

from sterigmata, indicating characteristics typical of the Aspergillus genus. One such fungal species capable of growing optimally in sugar- and salt-rich fermentative media is *Aspergillus repens*.

Research Subject Profile

Organoleptic quality was evaluated using a hedonic test, based on the perception and preference levels of the panelists. Most of the panelists were aged between 19 and 22 years, categorized as early adulthood, with age 20 being the most common (35.8%). In terms of ethnicity, the panel consisted of Dayak (41.7%), Madurese (6.7%), Javanese (29.2%), and Banjar (22.5%) respondents, totaling 120 participants, as shown in Table 7.

According to the recapitulated data, 25% of the panelists reported rarely or never consuming *Tempoyak*. The majority (45.8%) reported that their knowledge of *Tempoyak* came from family sources. A cumulative 50% of panelists reported either rarely or never consuming *Tempoyak*, reflecting that this traditional fermented food is less favored, especially among young people or the millennial generation (ages 19–22). This is supported by the fact that most knowledge of *Tempoyak* as a traditional food from the Dayak community in Central Kalimantan was inherited through family, indicating its limited familiarity among the younger generation. Therefore, innovations in both the processing and packaging of *Tempoyak* are needed to make it more appealing and relevant to modern tastes, while also ensuring its preservation as part of Central Kalimantan's cultural heritage and local wisdom.

Organoleptic Quality of Traditional Food: *Tempoyak*

The organoleptic profile of Tempoyak was assessed based on color, taste, aroma, and texture indicators by 120 panelists, as presented in Table 7.

Table 7. Preference Level of the Millennial Generation for the Traditional Fermented Product (TFP) *Tempoyak.*

Category	Fermentation Duration	Preference Level (%)					
		1	2	3	4	5	6
Color	$F_{1\ (24\ jam)}$	0.00	0.00	10.00	50.00	30.83	9.17
	$F_{2\ (48\ jam)}$	0.00	0.00	14.17	48.33	29.17	8.33
	$F_{3\ (72\ jam)}$	0.00	0.00	29.17	35.00	22.50	13.33
Flavor	$F_{1\ (24\ jam)}$	0.00	0.00	19.17	35.00	31.67	14.17
	$F_{2\ (48\ jam)}$	0.00	0.00	3.33	45.00	30.00	21.67
	$F_{3\ (72\ jam)}$	0.00	0.00	0.00	45.83	30.83	23.33
Aromatic	$F_{1\ (24\ jam)}$	0.00	0.00	0.00	47.50	31.67	20.83
	$F_{2\ (48\ jam)}$	0.00	0.00	0.00	46.67	32.50	20.83
	$F_{3\ (72\ jam)}$	0.00	0.00	0.00	45.00	33.33	21.67
Texture	$F_{1\ (24\ jam)}$	0.00	0.00	0.00	46.67	30.00	23.33
	$F_{2\ (48\ jam)}$	0.00	0.00	0.00	42.50	33.33	24.17
	$F_{3\ (72\ jam)}$	0.00	0.00	0.00	41.67	34.17	24.17

The panelists' responses presented in Table 8 provide an overview of the millennial generation's opinions regarding the organoleptic quality of

Tempoyak, showing a fairly diverse distribution. Notably, no panelists expressed "disliked" or "somewhat liked" ratings across any of the indicators—color, taste, or texture. Some panelists selected "neutral" for the color indicator, particularly at 24 hours of fermentation (19.17%) and 48 hours (3.33%), while none selected "neutral" at 72 hours of fermentation. There was a clear tendency for panelists to "like" the taste after 24 hours of fermentation (35%), and to "very much like" it after both 48 hours (45%) and 72 hours (45.83%). The correlation between color, taste, aroma, and texture quality and the overall level of preference was statistically analyzed using a one-way ANOVA test, as shown in Table 8 below

Tabel 8. *One Way Anava*

Hypothesis	Mean	SD	sig.(2-tailed) 0.05	Conclusion
There is a panelist preference for the color of *Tempoyak*	4.392	0.791	0.023	Reject
There is a panelist liking for the taste of *Tempoyak*	4.625	0.880	0.003	Reject
There is a panelist liking for the aroma of *Tempoyak*	4.744	0.783	0.031	Reject
There is a panelist preference for the texture of *Tempoyak*	4.797	0.800	0.022	Reject

The organoleptic test results for the color sub-variable of Tempoyak (Table 7) indicate that fermentation duration significantly affects organoleptic quality, with a significance value (2-tailed) of 0.023 < 0.05. Therefore, the null hypothesis (H0) is rejected, and it can be interpreted that there is a panelist preference for the color of Tempoyak.

For the taste sub-variable, a significance value of 0.003 < 0.05 was obtained, leading to the rejection of H0 and indicating that panelists liked the taste of Tempoyak. Similarly, the aroma sub-variable showed a significance value of 0.031 < 0.05, meaning that H0 is rejected and the panelists expressed liking for the aroma. For the texture sub-variable, the significance value was 0.022 < 0.05, again rejecting H0, and indicating a preference for the texture of Tempoyak.

These results suggest a strong potential for Tempoyak to be liked and accepted by the millennial generation, especially when innovations are made in processing traditional food products. More than 50% of panelists expressed positive responses—ranging from "likes" to "very likes" and "extremely likes"—toward the color, taste, aroma, and texture of Tempoyak at all fermentation durations (24h, 48h, and 72h). Conversely, only 10–29% gave lower ratings such as "somewhat likes," regardless of fermentation time (Table 8).

The change in the base color of Tempoyak is influenced by lactic acid bacteria during fermentation. Non-enzymatic reactions between sugars and amino acids result in a color change from bright yellow to a more brownish-yellow and sour tone (Ramdiyah et al., 2014). The flavor compounds responsible for the distinct durian aroma that persists in Tempoyak after fermentation include ethyl 2-methyl butanoate and ethyl propanoate (Neti et al., 2011; Monica

et al., 2018). The unique taste characteristics of Tempoyak make it a distinctive traditional food (Andrestian, 2009; Ramdiyah et al., 2014).

Lactic acid bacteria in spontaneous fermentation influence flavor development (Leisner et al., 2001; Yuliana, 2004; Amin et al., 2004; Amiza et al., 2006). In addition to lactic acid bacteria, other microflora identified included Saccharomyces cerevisiae and Kluyveromyces marxianus (Table 5), both belonging to the yeast group. Saccharomyces cerevisiae is known to ferment various sugars, producing ethanol and CO_2 (Nurcholis et al., 2020). While their direct role in the fermentation of Tempoyak remains unclear, these yeasts are commonly found in other fermented foods like sauerkraut (Viander et al., 2003; Khalid, 2011; Nurcholis et al., 2019) and contribute sulfurous volatile compounds from methionine in cheese (Yuliana & Dizon, 2011).

Fungi such as Rhizopus, Monilia sitophila, and Mucor roxii (Table 7) are also known to produce lactic acid, potentially affecting the taste of Tempoyak. Meanwhile, Aspergillus repens—also found in this study—has the ability to saccharify sugars, although it is not recognized as a lactic acid-producing fungus (Hasanuddin, 2010). The acids produced by fermentative bacteria in Tempoyak not only influence its color but also contribute to its unique taste and aroma (Trias et al., 2020). Panelist responses to the aroma indicator reflect the millennial generation's liking for Tempoyak's distinct aroma. More than 50% expressed positive responses across all fermentation durations (24h, 48h, 72h), suggesting that the aroma is familiar across different ethnic groups.

To broaden the understanding beyond the Dayak community, this study included only 33% Dayak panelists, while the rest were from Madurese (18%), Javanese (25%), and Banjar (25%) ethnic backgrounds. 1.3% (w/v) salt suspension used during fermentation alters the fruit's texture (Yuliana & Garcia, 2009). Durian pulp, which is initially soft, becomes mushier after fermentation (Trias et al., 2020). This change is due to the loss of turgor pressure when exposed to hypertonic saline conditions. High water content in the pulp increases cell turgidity, but under hypertonic conditions, cells lose water and pressure, leading to plasmolysis (Ingeborg et al., 2014; Janice, 2019). The protoplasm detaches from the cell wall, creating intercellular gaps and cavities.

The biochemical and physiological states of the cells influence the organoleptic quality of Tempoyak. Statistical results show significant differences in all sub-variables—color, taste, aroma, and texture—indicating panelist preferences and likings across these attributes. The exploratory findings in this study reveal promising opportunities for modern innovations to make Tempoyak more appealing and accepted by the millennial generation.

Developing traditional foods is essential to ensure consumer acceptance (Pawiroharsono, 2007; Anja et al., 2016), as perception and psychological responses are critical in product marketing (Sarkar & Costa, 2008; Atila et al., 2019). Consumers play a major role in driving innovation in the traditional food industry. The findings of this study offer insights into the organoleptic quality of Tempoyak—a traditional food of the Dayak community in Central Kalimantan—and serve as a benchmark for millennial generation acceptance.

CONCLUSION

A total of 15 microflora isolates were identified in Tempoyak. Among them, 8 isolates were classified as lactic acid bacteria that influence the organoleptic quality of Tempoyak, including Pediococcus acidilactici, Lactobacillus curvatus, Leuconostoc mesenteroides, Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus pentosus, Enterococcus faecium, and Weissella paramesenteroides. One bacterial isolate, Micrococcus varians, was identified as non-lactic acid and unrelated to fermentation, suggesting contamination. Two isolates were identified as yeasts: Saccharomyces cerevisiae and Kluyveromyces marxianus. Four isolates were identified as fungi: Rhizopus, Monilia sitophila, and Mucor roxii. Overall, most microflora found in Tempoyak are capable of producing lactic acid, except Aspergillus repens. Statistical tests showed significant effects on all organoleptic sub-variables—color, taste, aroma, and texture—indicating panelist preferences for color and texture, and likings for taste and aroma. These findings suggest that Tempoyak holds potential for further development through innovative, modern approaches, making it more appealing to the millennial generation.

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