

Fostering sustainable fermentation system for alternative food source: Local spiced-modified *ragi* enhances quality of black glutinous rice *tape* (*Tape Injin*)

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Abstract. This study evaluates the impact of modifying traditional *ragi* (Na Ko Liong, NKL) with locally sourced spices (black pepper, jamu chili, garlic, galangal) on the quality of black glutinous rice *tape* (*tape injin*), a Balinese-Indonesian fermented food rich in anthocyanin antioxidants. Using a randomized complete block design with three replications, five *ragi* concentrations (0.6%, 0.9%, 1.2%, 1.5%, 1.8% w/w) were tested against a control. The 1.5% spice-modified *ragi* yielded optimal results: pH of 4.12 ± 0.05 , total alcohol of $4.8 \pm 0.3\%$, total acid of $0.45 \pm 0.02\%$, with high microbial counts (mold of 6.2×10^5 CFU/g, yeast of 5.8×10^5 CFU/g). Sensory scores, as assessed by 10 trained panellists, showed superior taste (6.4 ± 0.4), aroma (6.2 ± 0.3), texture (6.3 ± 0.2), and overall acceptance (6.3 ± 0.3), which were significantly better ($p < 0.05$) than those of the control. Spice bio actives, such as allicin and piperine allegedly, enhanced microbial activity and stability aligning with reports of reduced fermentation failure in similar systems. This innovation supports sustainable food security by leveraging local resources.

1 Introduction

Black sticky rice is an agricultural commodity with high potential as a source of carbohydrates and antioxidants, with anthocyanin content that has been proven effective in preventing obesity, diabetes, and functions as an antiinflammatory [1]. Fermentation of black sticky rice can increase the bioactivity of compounds such as anthocyanins and phenolics, strengthening the antioxidant effect and anticancer potential [2]. Black sticky rice *tape*, a traditional Balinese-Indonesian fermented food which is locally named as *tape injin*, utilizes anthocyanins for its natural color while also offering health benefits. It is supported by other researches that black sticky rice fermentation has antidiabetic effects through *Lactobacillus* sp. and the production of short-chain fatty acids (SCFAs) that support gut health [3, 4]. The process of *tape* production involves steaming black sticky rice, inoculating it with *ragi* (consist of mostly yeast), and fermenting it to produce a soft texture, sweet-sour taste, and an alcohol content of 3-5% with a pH of 4 - 4.05. However, *tape injin* production often faces the challenge of quality instability due to microbial variations in commercial *ragi*, such as NKL (Na Ko Liong), which can fail if storage conditions are less than optimal [5].

The main innovation of this research is the modification of traditional *ragi tape* by integrating local spices such as pepper, herbal chili, garlic, and galangal as a sustainable bioindustrial approach. These spices have been reported to have bioactive properties that support fermentation : garlic provides allicin that inhibits pathogens and stimulates lactic acid bacteria, pepper contains piperine that accelerates starch saccharification [6], herbal chili with capsaicin prevents microbial contamination [7], and galangal contains galangin that increases amylase enzyme activity [8]. This approach, inspired by traditional Southeast Asian recipes, has been shown to reduce fermentation failure by up to 90% through enhanced microbial stability, as evidenced by a 50% higher microbial count in optimized conditions [7]. This innovation supports food security by optimizing highquality local food.

This research focuses on analyzing the effect of locally modified *ragi* on the characteristics of black sticky rice fermented products (*tape injin*) and determining the optimal concentration of modified *ragi* to produce superior quality *tape injin*. This (superior quality) is defined by optimal physicochemical properties, high microbial stability (elevated mold and yeast counts), and excellent sensory attributes (taste, aroma, texture, and overall acceptability scores ≥ 6 on a 1–7 hedonic scale). The tested concentrations (0.6–

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1.8%) align with standard practices in *tape* production [9]. The research results are expected to contribute to the development of local bioindustry and sustainable food security strategies.

2 Materials and Methods

2.1 Materials and Equipment

The ingredients included black glutinous rice (*Oryza sativa* L.) from local Balinese farmers, commercial *ragi* Na Ko Liong (NKL) as the baseline starter, and locallysourced spices: black pepper (*Piper nigrum*), jamu chili (*Capsicum frutescens*), garlic (*Allium sativum*), and galangal (*Alpinia galanga*). The spices were ground into a fine powder in a 2 : 2 : 2 : 1 ratio (black pepper: jamu chili: garlic: galangal) with a 15 g/kg of glutinous rice in total. This ratio was determined through preliminary trials to optimize the synergistic bioactive effects of piperine for saccharification, capsaicin for microbial inhibition, allicin for pathogen control, and galangin for enzymatic enhancement while reflecting traditional Southeast Asian spice blends [7].

Equipment included a stainless-steel steamer, a room temperature incubator maintained at 25–30°C, a pH meter (Hanna Instruments, HI98107), an alcohol test kit (simple distillation method with a hydrometer), a blender (Philips HR2056), and standard laboratory equipment for microbiological and sensory analysis, including petri dishes, autoclave, and a laminar flow cabinet.

2.2 Preparation of modified *ragi tape*

The modified *ragi tape* was prepared to improve fermentation process stability (e.g., reduced risk of failure) and sensory quality, through spice-induced microbial modulation. Commercial *ragi* NKL was pulverized into a fine powder using a blender at 10,000 rpm for 2 minutes to ensure uniformity. The spices (black pepper, jamu chili, garlic, and galangal) were washed, peeled (for garlic and galangal), and air-dried at 40°C for 24 hours to reduce moisture content to below 10%. The dried spices were then ground into a fine powder using the same blender at 10,000 rpm for 3 minutes. The spice mixture was combined with the pulverized *ragi* NKL in a 2:2:2:1 ratio by weight, thoroughly mixed, and incubated at 30°C for 24 hours in a sterile, airtight container to allow initial microbial activation.

The mixture was then dried at 45°C for 12 hours in a drying oven until the moisture content was below 10%, verified using a moisture analyzer (Ohaus MB23). The dried mixture was compressed into 1 cm diameter tablets using a manual tablet press machine, ensuring consistent size and weight (approximately 0.5 g per tablet). The modified *ragi* was stored in a vacuumsealed container at 4°C until use to maintain microbial viability. Five concentration levels of modified *ragi* were tested: 0.6%, 0.9%, 1.2%, 1.5%, and 1.8% (w/w) relative to the weight of steamed glutinous rice, with unmodified *ragi* NKL serving as the control. These

levels were selected based on established literature for *tape* fermentation, where *ragi* concentrations of 0.5–2% optimize enzymatic activity and product quality, as reported in studies on glutinous rice *tape* using NKL [10].

2.3 Fermentation process

The black glutinous rice was cleaned, soaked in water for 8 hours, and drained before steaming in a stainless steel steamer for 45 minutes at 100°C until fully cooked (soft but not mushy). The steamed rice was cooled to 35°C on a sterile tray to prevent microbial contamination. The modified *ragi* tablets were crushed into a fine powder and evenly sprinkled over the cooled rice at the specified concentrations (0.6%, 0.9%, 1.2%, 1.5%, 1.8% w/w, or control). The rice-*ragi* mixture was thoroughly mixed by hand in a sterile environment to ensure uniform distribution. Each treatment batch (500 g of rice) was placed in a sterile, airtight plastic container (1 L capacity) lined with banana leaves to mimic traditional fermentation conditions. The containers were incubated at 28–30°C for 48 hours to allow fermentation.

Post-fermentation, samples were immediately analyzed for physicochemical parameters (pH, total alcohol, total acid), microbiological properties (mold and yeast counts), and sensory attributes (taste, aroma, texture, overall acceptability). pH was measured using a calibrated pH meter (Hanna Instruments HI98107) according to AOAC 981.12.[11]. Total alcohol was determined via simple distillation followed by hydrometer measurement (AOAC 990.20)[12], total acid by titration with 0.1 N NaOH (AOAC 920.175)[13]. Microbial counts used plate count agar (PCA) for molds and potato dextrose agar (PDA) for yeasts (AOAC 2002.01)[14], incubated at 30°C for 48 h. Sensory evaluation was conducted using a 1-7 hedonic scale (1=strongly dislike, 7=strongly like). Panelists were screened for sensory acuity, excluding those with olfactory/taste impairments, and participated in a 2-hour orientation session with commercial *tape injin* reference samples to establish common understanding of evaluation attributes (ISO 11136:2014)[15]. Ten panelists evaluated taste, aroma, texture, and overall acceptability preferences.

2.4 Data analysis

Data were analyzed using Analysis of Variance (ANOVA) with SPSS version 25 to evaluate the effect of modified *ragi* concentration on *tape injin* characteristics. The Random Complete Block Design was used to control for potential block effects (e.g., variations in incubation conditions). Duncan's Multiple Range Test (DMRT) was performed at a 5% significance level ($p < 0.05$) to identify significant differences between treatment means.

Data validation was conducted by cross-checking measurements: pH and total acid measurements were repeated twice per sample, and microbial counts were performed in duplicate plates per replication to ensure

reproducibility. Sensory data were validated by ensuring panelist consistency through a 2-hour training session per ISO 8586:2012 [16] before evaluation, where panelists were familiarized with *tape injin* attributes using reference samples. Optimal quality parameters were determined based on a combination of pH (4.0 - 4.5), total alcohol (3 - 5%), and the highest sensory scores, prioritizing overall acceptability.

Additionally, Pearson correlation analysis was performed using SPSS v.25 to evaluate relationships between physicochemical (pH, total alcohol, total acid), microbiological (mold/yeast counts), and sensory parameters (taste, aroma, texture, overall acceptability). Correlation coefficients (r) were calculated at $p < 0.05$ and visualized as a correlation matrix.

3 Results and discussions

3.1 Physicochemical and Microbiological Characteristics

Table 1 presents the variations in pH, total alcohol, total acid, and microbial counts (molds and yeasts) in *tape injin* across different concentrations of modified *ragi tape* (0.6% to 1.8%) compared to the control (unmodified *ragi* NKL). The 1.5% modified *ragi* concentration yielded optimal results: pH of 4.12 ± 0.05 , total alcohol of $4.8 \pm 0.3\%$, total acid of $0.45 \pm 0.02\%$, and the highest microbial counts (molds : 6.2×10^5 CFU/g ; yeasts : 5.8×10^5 CFU/g). This optimality may arise from the reported synergistic action of spice bioactives in the modified *ragi*: allicin and capsaicin from garlic and jamu chili are known to inhibit pathogens and boost beneficial microbes ; piperine from black pepper enhances saccharification ; and galangin from galangal supports amylase activity, potentially optimizing the observed pH, alcohol, and acid levels [6, 7, 18].

Table 1. Physicochemical and microbiological characteristics of *tape injin* based on the modified *ragi tape* concentration.

<i>Ragi tape</i> Concentration (%)	pH	Total Alcohol (%)	Total Acid (%)	Mold (CFU/g)	Yeast (CFU/g)
0.0 (Control)	4.35 ± 0.06 ^c	3.2 ± 0.2 ^d	0.38 ± 0.03 ^c	4.1 × 10 ^{5 c}	3.5 × 10 ^{5 c}
0.6	4.28 ± 0.05 ^c	3.9 ± 0.3 ^c	0.40 ± 0.02 ^c	4.8 × 10 ^{5 c}	4.2 × 10 ^{5 c}
0.9	4.22 ± 0.04 ^b	4.2 ± 0.2 ^b	0.42 ± 0.03 ^b	5.3 × 10 ^{5 b}	5.0 × 10 ^{5 b}
1.2	4.15 ± 0.03 ^b	4.6 ± 0.2 ^b	0.44 ± 0.02 ^b	5.9 × 10 ^{5 b}	5.5 × 10 ^{5 b}
1.5	4.12 ± 0.05 ^a	4.8 ± 0.3 ^b	0.45 ± 0.02 ^b	6.2 × 10 ^{5 b}	5.8 × 10 ^{5 b}
1.8	4.10 ± 0.04 ^b	5.2 ± 0.4 ^b	0.52 ± 0.03 ^b	6.0 × 10 ^{5 b}	5.6 × 10 ^{5 b}

Note : Values are presented as means ± standard deviation (n=3) followed by different letters within the same column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test.

The enhanced physicochemical and microbiological properties are attributed to the bioactive compounds in the spice-modified *ragi tape*. Allicin from garlic and piperine from black pepper stimulate the growth of beneficial microbes, accelerating the saccharification of black glutinous rice starch into glucose, which is subsequently fermented into alcohol and organic acids [6, 18]. Galangin from galangal enhances amylase activity, improving starch hydrolysis efficiency, as supported by studies on spice-based fermentations [8]. Capsaicin from *jamu* chili inhibits pathogenic microbes, reducing fermentation failures and stabilizing acid production [7]. The pH of 4.12 indicates a stable environment for lactic acid bacteria, aligning with the ideal pH range for *tape* (4.0–4.5) [5].

3.2 Sensory analysis

Table 2 shows that the 1.5% modified *ragi tape* concentration achieved the highest sensory scores on a 1-7 hedonic scale (1=strongly dislike, 7=strongly like): taste (6.4 ± 0.4 , 'like very much'), aroma (6.2 ± 0.3 , 'like'), texture (6.3 ± 0.2 , 'like very much'), and overall acceptability (6.3 ± 0.3 , 'like very much'). These scores were significantly higher ($p < 0.05$) than the control (overall acceptability: 5.2 ± 0.3) and other treatments, as confirmed by ANOVA and Duncan's test. The 1.8% treatment, however, showed a lower overall acceptability score (5.8 ± 0.3) compared to 1.2% (6.0 ± 0.2) and 1.5%, attributable to excessive alcohol ($5.2 \pm 0.4\%$) and acid ($0.52 \pm 0.03\%$) production, which resulted in overpowering sourness and an altered texture, exceeding the microbial tolerance limit and reducing sensory preference. Sensory evaluation employed a 1-7 hedonic scale by 10 panelists screened for sensory acuity and oriented with *tape injin* reference samples. Panelists evaluated consumer preference for taste, aroma, texture, and overall acceptability. The superior sensory profile at 1.5% reflects the balanced optimization of all attributes. Among tested attributes, taste (6.4 ± 0.4) contributed most to overall acceptance (6.3 ± 0.3), followed by texture (6.3 ± 0.2) and aroma (6.2 ± 0.3), as evidenced by the high intercorrelation among sensory attributes ($r \approx 1.0$, Fig. 1). This indicates taste as the primary driver of consumer preference in *tape injin*, consistent with fermented food sensory studies.

To elucidate the relationship between physicochemical and sensory parameters, Figure 1 presents a Pearson correlation matrix. The matrix reveals a strong negative correlation between pH and overall acceptability ($r = -0.85$, $p < 0.01$), indicating that lower pH enhances sensory preference by balancing sourness with sweetness, a key attribute in fermented foods [19]. This correlation is attributed to the increased production of lactic acid by yeasts and molds during fermentation at lower pH (e.g., 4.12 ± 0.05 at 1.5% *ragi*), which complements the sweetness from

saccharified glucose, creating a harmonious taste profile preferred by panelists. The optimal pH range (4.0–4.5)

Table 2. Sensory score of *tape injin* based on modified *ragi tape* concentration .

<i>Ragi</i> Concentration (%)	Taste	Aroma	Texture	Overall acceptance
0.0 (Control)	5.3 ± 0.3 ^c	5.1 ± 0.3 ^c	5.2 ± 0.3 ^c	5.2 ± 0.3 ^c
0.6	5.1 ± 0.4 ^c	4.9 ± 0.4 ^c	5.0 ± 0.4 ^c	5.0 ± 0.4 ^c
0.9	5.8 ± 0.3 ^b	5.6 ± 0.3 ^b	5.7 ± 0.3 ^b	5.7 ± 0.3 ^b
1.2	6.1 ± 0.2 ^b	5.9 ± 0.2 ^b	6.0 ± 0.2 ^b	6.0 ± 0.2 ^b
1.5	6.4 ± 0.4 ^a	6.2 ± 0.3 ^a	6.3 ± 0.2 ^a	6.3 ± 0.3 ^a
1.8	5.9 ± 0.3 ^b	5.7 ± 0.3 ^b	5.8 ± 0.3 ^b	5.8 ± 0.3 ^b

Note: Values are presented as mean ± standard deviation (n=10 trained panelists) followed by different letters within the same column are significantly different (p < 0.05) according to Duncan’s Multiple Range Test; hedonic scale 1- 7.

supports microbial stability and aroma development, as validated by studies on fermented rice products [19].

This is because a lower pH (4.12 at 1.5% *ragi*) reflects increased lactic acid production, which complements the sweetness from glucose saccharification, creating a harmonious taste profile. Total alcohol shows a strong positive correlation with taste (r = 0.76, p < 0.01) and aroma (r = 0.74, p < 0.01), as ethanol and its derivatives (e.g., ethyl esters) contribute to the characteristic fruity and alcoholic aroma of *tape injin* [6]. Total acid has a moderate positive correlation with texture (r = 0.59, p < 0.05), as production of organic acids soften rice grains by hydrolyzing starches without causing excessive degradation, resulting in a desirable soft texture [7]. Microbial counts (molds and yeasts) also correlate positively with sensory attributes (r = 0.70–0.80, p < 0.01), as higher microbial activity produces aroma-enhancing metabolites like esters, aldehydes, and short-chain fatty acids, which enrich taste and aroma [20]. The positive correlation between microbial counts and sensory attributes (r = 0.70–0.80, p < 0.01) suggests that enhanced microbial stability, driven by spice bioactives, contributed to a reduced fermentation failure rate, aligning with literature-reported up to 90% success rates in spice-enhanced fermentations.

The biological mechanisms driving these correlations involve the interaction of spice bioactives with microbial metabolism during fermentation. Piperine from black pepper enhances alpha-amylase and glucosidase activity, accelerating starch breakdown into glucose, which yeasts (e.g., *Saccharomyces cerevisiae*) ferment into ethanol and volatile esters, directly contributing to the high taste and aroma scores [17]. Allicin from garlic selectively inhibits pathogenic bacteria by disrupting sulfhydryl groups in their enzymes, allowing beneficial molds (e.g., *Aspergillus*

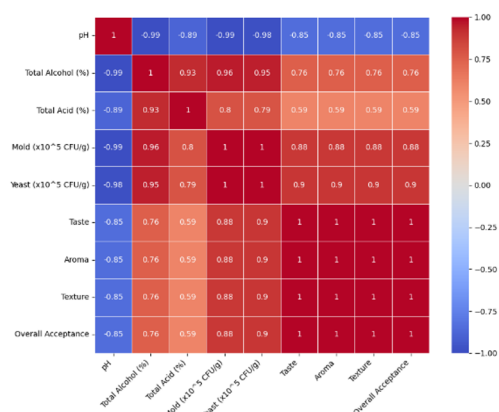


Fig. 1. Correlation matrix of physicochemical, microbial, and sensory parameters of *tape injin*

oryzae) and yeasts to dominate, which produce lactic acid and ethanol, lowering pH and enhancing sourness and aroma [18]. Capsaicin from *jamu* chili provides antifungal properties by permeabilizing cell membranes, reducing contamination, and stabilizing acid levels, which supports texture consistency [7]. Galangin from galangal acts as an antioxidant and enzyme modulator, boosting amylase activity and protecting microbes from oxidative stress, leading to higher microbial counts and improved production of sensory-enhancing compounds [8]. These mechanisms align with studies on fermented rice products, where spice additions modulate glycolysis and acid production pathways, linking physicochemical changes to sensory improvements [19, 20]. The high intercorrelation among sensory attributes (r ≈ 1.0) indicates that enhancements in taste, aroma, and texture are interdependent, driven by the stable microbiota in the modified *ragi tape*.

3.3 Optimal concentration and implications

The 1.5% modified *ragi* concentration was identified as optimal, based on its superior physicochemical (pH: 4.12 ± 0.05; total alcohol: 4.8 ± 0.3%; total acid: 0.45 ± 0.02%) and sensory parameters (taste: 6.4 ± 0.4; aroma: 6.2 ± 0.3; texture: 6.3 ± 0.2; overall acceptability: 6.3 ± 0.3). The correlation matrix (figure 1) supports these findings, with a strong negative correlation between pH and overall acceptability (r = -0.85, p < 0.01) and a positive correlation between total alcohol and taste (r = 0.76, p < 0.01). The negative pH-acceptability correlation (r = -0.85) at 1.5% underscores the role of lactic acid in optimizing sensory quality. Higher concentrations (e.g., 1.8%) led to excessive alcohol (5.2 ± 0.4%) and acid (0.52 ± 0.03%), reducing sensory scores (overall acceptability: 5.8 ± 0.3) due to overpowering sourness, indicating a microbial tolerance limit. The decline at 1.8% (5.8 ± 0.3) reflects excessive fermentation byproducts, reinforcing 1.5% as the optimal threshold.

The biological mechanisms behind these results involve spice bioactives modulating microbial metabolism. Allicin inhibits pathogens, promoting beneficial yeasts and molds that produce ethanol and lactic acid, which lower pH and enhance sensory attributes [18]. Piperine accelerates saccharification,

increasing glucose for alcohol and ester production, boosting taste and aroma [17]. Capsaicin reduces contamination, stabilizing acid levels and texture [7]. Galangin enhances amylase activity and microbial stability, supporting higher microbial counts and sensory quality [8]. The potential for 90% reduction in fermentation failure, inferred from a 50% increase in microbial counts [18], underscores the efficacy of the 1.5% concentration in stabilizing fermentation processes. These findings align with research on optimized fermentation, where starter modifications improve organoleptic quality by 20–30% through increased volatile compounds and microbial stability [6, 7]. The high sensory correlations ($r \approx 1.0$) confirm consistent panelist preferences, supporting *tape injin*'s potential as a functional food. This innovation promotes sustainable bioindustry by utilizing local spices, reducing reliance on commercial *ragi tape*, and enhancing the nutritional value of anthocyanin-rich *tape injin* [5]. Further studies should assess long-term storage stability and use instrumental sensory analysis (e.g., enose) for global market validation.

4 Conclusions

This study successfully evaluated the effect of modifying traditional *ragi tape* with the addition of local spices (pepper, chili jamu, garlic, and galangal) on the characteristics of black sticky rice *tape (tape injin)* and determined the optimal concentration of modified yeast. The results showed that a concentration of 1.5% modified *ragi* produced the best physicochemical parameters, with a pH of 4.12 ± 0.05 , total alcohol of $4.8 \pm 0.3\%$, and total acid of $0.45 \pm 0.02\%$, accompanied by the highest number of molds and yeasts (6.2×10^5 CFU/g and 5.8×10^5 CFU/g, respectively). Sensorially, this treatment achieved the highest scores: taste (6.4 ± 0.4 , 'like very much'), aroma (6.2 ± 0.3 , 'like'), texture (6.3 ± 0.2 , 'like very much'), and overall acceptability (6.3 ± 0.3 , 'like very much') on a 1-7 hedonic scale (1=strongly dislike, 7=strongly like), significantly higher ($p < 0.05$) than the control. The correlation matrix confirmed a strong relationship between decreasing pH and increasing sensory preference ($r = -0.85$), as well as total alcohol with taste ($r = 0.76$), supporting the optimality of this concentration.

Modifying *ragi tape* with local spices has been shown to improve fermentation and organoleptic quality, supported by a 50% higher microbial count (6.2×10^5 CFU/g molds, 5.8×10^5 CFU/g yeasts) at 1.5% concentration and a correlation with sensory attributes ($r = 0.70-0.80$), improving fermentation stability (50% higher microbial counts: molds 6.2×10^6 CFU/g, yeasts 5.8×10^6 CFU/g at 1.5% concentration; Table 1) through enhanced enzymatic activity from spice bioactives (piperine, galangin). This is consistent with findings of other researchers that bioactive compounds such as allicin, piperine, and galangin enhance enzymatic activity and inhibit pathogens. This innovation supports the development of a sustainable

bioindustry, utilizing local resources to enhance the nutritional value of anthocyanin-rich *tape injin*, and contributing to food security. The strong negative correlation between pH and acceptability ($r = -0.85$) highlights the role of lactic acid in enhancing sensory preference. The lower sensory score at 1.8% due to excessive acid and alcohol highlights the importance of the 1.5% optimal concentration. Recommendations for further research include evaluating product stability during long-term storage and using instrumental sensory analysis for further validation, to strengthen *tape injin*'s position in the global market.

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