TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC QUY NHƠN

Nghiên cứu ảnh hưởng của bánh men thuần chủng đến chất lượng sản phẩm cơm rượu

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TÓM TẮT

Nghiên cứu này tập trung sử dụng các chủng vi sinh vật thuần chủng cho vào bánh men nhằm cải tiến chất lượng sản phẩm cơm rượu tại huyện Gò Quao, tỉnh Kiên Giang. Bánh men chứa các vi sinh vật quan trọng như nấm men, nấm mốc và vi khuẩn lactic, đóng vai trò quyết định trong quá trình lên men chuyển hóa tinh bột thành ethanol và tạo hương vị đặc trưng. Qua khảo nghiệm với các nồng độ bánh men thuần chủng (0,4%, 0,6%, 0,8%), kết quả cho thấy nồng độ 0,4% mang lại sản phẩm cơm rượu có chất lượng cảm quan tốt nhất, đồng thời duy trì các chỉ tiêu hóa lý như độ ẩm, pH, và hàm lượng acid lactic ở mức phù hợp, hàm lượng ethanol cao (khoảng 10% v/v), Ngoài ra, nghiên cứu còn khảo sát thời gian lên men trong vòng 2 đến 4 ngày, cho thấy quá trình lên men tối ưu trong 3 ngày, khi các chỉ tiêu như pH, độ cồn và acid lactic ổn định, tạo môi trường lên men lý tưởng đồng thời đảm bảo chất lượng sản phẩm, đồng thời không phát hiện các chất gây hại như methanol và furfurol. Nghiên cứu góp phần xây dựng cơ sở khoa học cho việc ứng dụng bánh men thuần chủng, nâng cao tính ổn định và chất lượng cơm rượu truyền thống, đồng thời hỗ trợ bảo tồn và phát triển nghề làm bánh men truyền thống địa phương.

Từ khóa: *Cơm rượu, Gò Quao, nấm men, nấm mốc, vi khuẩn lactic.*

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Study on the effect of pure fermentation starters (banh men) on the quality of fermented glutinous rice (com ruou)

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ABSTRACT

This study aimed to evaluate the impact of using pure microbial strains in fermentation starter (banh men) on the sensory, physicochemical, and microbiological quality of traditional fermented glutinous rice (com ruou) produced in Go Quao District, Kien Giang Province. Traditional banh men harbors essential microorganisms, including yeasts, molds, and lactic acid bacteria, which are responsible for the conversion of starch into alcohol and the development of characteristic flavors during fermentation. The study was conducted using pure fermentation starter (banh men) at concentrations of 0.4%, 0.6%, and 0.8%, with 0.4% yielding the most favorable sensory attributes while maintaining desirable physicochemical parameters such as moisture content, pH, lactic acid levels, and a high alcohol concentration (10.2% v/v). Fermentation time was also evaluated over a period of 2 to 4 days, with results indicating that optimal fermentation occurred after 3 days, when key indicators such as pH, alcohol, and lactic acid stabilized. Importantly, no harmful by-products such as methanol or furfural were detected. This study contributes to establishing a scientific basis for the application of pure fermentation starter in order to improve the stability and quality of traditional fermented glutinous rice (com ruou), while also supporting the preservation and development of the local starter culture production craft.

Keywords: *Fermented glutinous rice, Go Quao, yeast, mold, lactic acid bacteria.*

1. INTRODUCTION

Fermented foods constitute an important part of the culinary culture of many countries, including Vietnam. Among them, traditional fermented glutinous rice (com ruou) is a representative product closely associated with the daily life of the people. The production process of traditional fermented glutinous rice is influenced by starter cakes, which contain molds, yeasts, and lactic acid bacteria. These microorganisms initiate and sustain the transformation of starch into alcohol, while also contributing to the characteristic flavor of traditional fermented glutinous rice.

Starter cakes are typically produced using traditional methods, which involve mixing various medicinal herbs with rice flour and allowing the mixture to ferment under natural conditions. This process creates a favorable environment for the growth of diverse microorganisms, the most important of which are yeast species (e.g., *Saccharomyces cerevisiae*), molds (*Aspergillus*, *Rhizopus*, etc.), and lactic acid bacteria (including both lactic and acetic acid bacteria).

However, since the traditional production of starter cakes relies heavily on folk experience and lacks scientific and technical control, fermentation efficiency is often inconsistent, alcohol yield is low, and contamination risk is high. This underscores the urgent need for

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research and improvement in the production process.

Previous studies have demonstrated significant potential in isolating and applying indigenous microorganisms from starter cakes. Specifically, Ngo Thi Phuong Dung developed and applied defined starter cultures in the fermentation of black glutinous fermented glutinous rice, using *Amylomyces rouxii* and *Saccharomyces cerevisiae* as inoculum strains in the production of defined fermented glutinous rice starter.[1] Similarly, Nguyen Van Hue investigated the antioxidant and antibacterial properties of plant leaf extracts and applied them in the production of herbal starter cakes for fermented glutinous rice fermentation.[2] His findings showed that fermented glutinous rice produced with lab-made starter cakes containing leaf extract had alcohol concentrations comparable to wine fermented using traditional starter cakes obtained from local producers in Da Ban commune.

In addition, Doan Thi Kieu Tien and colleagues isolated *Saccharomyces cerevisiae* from Giac fruit wine (*Antidesma bunius*) and assessed its fermentation performance and storage conditions for Giac fruit wine.[3] However, practical application studies of these strains under production conditions remain limited.

In the Mekong Delta region, which boasts a rich culinary culture and abundant agricultural resources, traditional starter cake villages are facing multiple challenges-from the loss of traditional knowledge to the pressure of competition from industrial products. In this context, conducting scientific research to evaluate and standardize starter cake production processes is a necessary direction. Such efforts not only help preserve and develop traditional crafts but also enhance product value, meeting the increasing demands for food safety and product quality.

From the issues outlined above, this study aims to investigate the production process of fermented glutinous rice using isolated starter cultures, with key objectives including the application of selected microbial strains in a pilot fermentation process and evaluation of fermentation performance using local raw materials

2. MATERIALS AND METHOD

2.1. Materials and chemicals

Glutinous rice was procured from Vinh Tuy commune, Go Quao district, Kien Giang province.

Pure fermentation starters (banh men) were produced in the laboratory of Kien Giang University with the following microbial densities: molds at 10^7 CFU/g, yeasts at 10^7 CFU/g, and lactic acid bacteria at 10^8 CFU/g. Microorganisms present in the starter culture were isolated from traditional fermentation starters collected in Go Quao district, Kien Giang province. Molecular identification techniques revealed the presence of the yeast *Saccharomyces cerevisiae*, the filamentous fungus *Rhizopus oryzae*, and lactic acid bacterium *Lactiplantibacillus plantarum*. These strains were preserved and propagated in the Microbiology Laboratory of Kien Giang University.

Chemicals: 3.5-Dinitrosalicylic acid (China), NaOH 0.1N (Cemaco, Vietnam).

Culture media used for microbial propagation: YPD (Yeast extract Peptone Dextrose) medium, PDA (Potato Dextrose Agar) medium, MRS Broth medium, and various synthetic media from Himedia (India).

2.2. Methods

+ **Moisture (% w/w)**: 10 g of fermented glutinous rice was determined for moisture content using a moisture balancer MOC-63u (Shimadzu, Japan).

+ **Brix**: 10 mL of water and fermented glutinous rice mixture, then measure Brix with Master-3T refractometer (Atago, Japaen).

+ **The pH value**: Using 10 mL of water

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and fermented glutinous rice mixture, dip the electrode of the pH meter MI150 MARTINI (Romania) into the solution to determine the pH.

+ **Alcohol content (% v/v)**: Determine alcohol content by alcohol recovery distillation method, using alcohol distillation kit 2505200 (Witeg, Germany), then measure alcohol concentration CHG-3C33 (China) and convert to 20°C.[4]

+ **Titratable acidity (as %lactic acid)** (mg/mL): Put 10 mL of water and fermented glutinous rice mixture into a test tube. Add 20 mL of distilled water. Add 1-2 drops of phenoltaphlein. Shake well to mix the substances evenly. Titrate the solution with 0.1N NaOH until the solution turns pink, then stop.[5]

Lactic acid concentration is calculated in degrees Therner:

$^oT = V_{NaOH}*10$

% acid lactic (mg/mL) = $^oT * 0.009$

In which: ^oT: degrees Therner, 1 ^oT corresponds to 9 mg of lactic acid. V_{NaOH}: volume of 0.1N NaOH consumed (mL)

Formula for calculating lactic acid concentration (mg/mL) = $V_{NaOH}*10*0.009$

+ Methanol content (%) Using gas chromatography method according to TCVN 8010:2009

+ Furfurol content (%) Using gas chromatography method according to TCVN 7886:2009

- The density of yeast, mold and lactic acid bacteria density by live count method on culture media:

The fermented glutinous rice samples were homogenized by placing 10 g of sample in a stomacher bag containing 90 mL of 0.85% NaCl solution, and the sample was ground for 1 min at 260 rpm. The samples were diluted to the appropriate concentration.

+ **Determining the density of yeast:** 0.1 mL of the diluted sample at a dilution of 10^{-5} was spread on plates containing YPD agar and incubated in a UN110 incubator (Memmert, Germany) at 30 °C for about 48 h.6

+ **Determining the density of mold:** 0.1 mL of the diluted sample at a dilution of 10^{-5} was spread on plates containing PDA and incubated in a UN110 incubator (Memmert, Germany) at 37 °C for about 72 h.7

+ **Determining the density of lactic acid bacteria (LAB):** 0.1 mL of the diluted sample at a dilution of 10^{-5} was spread on plates containing MRS agar and incubated in a UN110 incubator (Memmert, Germany) at 37 °C for about 48 h.8

Formula for calculating density: CFU/g = |a x n x 10 In which a: Number of colonies in 0.1 mL of sample; n: dilution.

+ **Sensory evaluation:** The numerical rating scale method was applied according to Vietnamese Standard TCVN 3215-79. The fermented glutinous rice samples were evaluated by a panel of 10 assessors, including lecturers and students from the Faculty of Food Science and Health. The evaluation was conducted using a 20-scores scale (0-20 scores) based on the following criteria: color, aroma, sweetness, mild sourness, and softness. Each attribute was equally weighted. The final result was calculated as the average score given by all panelists.

2.3. Data analysis

The results were processed and graphed using Microsoft Excel 2013 software (Microsoft Corporation, USA). The data were processed and statistically analyzed using Statgraphics Centurion XIX software (Statpoint Technologies Inc., USA).

3. RESULTS AND DISCUSSION

Sticky rice was finely ground and dried at 100°C for 24 hours to reduce moisture content and stabilize the raw material. The rice flour was then mixed with a microbial consortium consisting of mold, yeast, and lactic acid bacteria. These microorganisms were previously propagated in

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suitable media: yeast in YPD, mold in PDA, and lactic acid bacteria in MRS broth, each incubated at 30°C for 24 hours.

After mixing the rice flour with the microbial cultures, sterilized water was added to achieve the desired moisture content. The mixture was thoroughly blended and formed into pellets. These pellets were incubated at 30°C for 24 hours to increase the microbial population. Following incubation, the fermented mass was dried at 42°C until the moisture content reached approximately 15%.

Traditional Fermented glutinous rice (Com ruou) Production Process (for reference):

Sticky rice → First wash → Drained → First steaming (30 min) → Cooled → Second wash → Second steaming (30 min) → Cooled → Shaped into balls (hands moistened with salt water, salt/water ratio = 1:4) → Starter preparation → Starter ground finely → Evenly sprinkled over rice balls → Wrapped in banana leaves → Fermented in a plastic basket covered with PE film at room temperature → Collected fermented glutinous rice and extract. The final fermented glutinous rice product (com ruou) was analyzed for various quality parameters, and the specific results are presented as follows:

3.1. Effect of the proportion of pure fermentation starters on the quality of fermented glutinous rice (Com ruou)

To evaluate the quality of com ruou using isolated fermentation starters at three different concentrations - 0.4%, 0.6%, and 0.8% (w/w based on cooked glutinous rice) - a traditional local production method was followed. The procedure included washing the rice, steaming twice (30 minutes each), cooling, shaping into balls with hands moistened in saltwater (ratio 1:4), sprinkling with finely ground fermentation starter, and fermenting the rice balls wrapped in banana leaves and placed in PE bags for 3 days to collect the sweet liquid extract. After fermentation, the samples were transported to the laboratory at Kien Giang University for quality analysis. The parameters assessed included pH, Brix value, alcohol content, mold, yeast and lactic acid bacteria counts, as well as sensory evaluation. The results aim to improve product quality and consistency for future commercialization. The findings are presented in Table 1.

Table 1. Effect of pure fermentation starters on the quality of fermented glutinous rice.

Concentration	Moisture (% w/w)	pH	Brix	Alcohol	Titratable acidity (mg/ml)
0.4%	47.5 ^{b} ± 1.8	3.98 ^{b} ± 0.06	40.33 ^{b} ± 0.57	10.2 ^{b} ± 0.4	0.75 ^{a} ± 0.05
0.6%	44.8 ^{a} ± 0.72	3.71 ^{a} ± 0.04	31.67 ^{a} ± 0.3	9 ^{a} ± 0.2	0.98 ^{b} ± 0.02
0.8%	43.76 ^{a} ± 0.76	3.91 ^{b} ± 0.08	32.67 ^{a} ± 0.5	8.8 ^{a} ± 0.2	0.89 ^{b} ± 0.1

Data are the mean values of triplicates. In the same column, different letters (a, b) indicate statistically significant differences at the 95% confidence level (statistical significance level $p < 0.05$).

Based on Table 1, the concentration of the isolated starter culture significantly influenced the moisture content of the fermented glutinous rice. Increasing the starter culture concentration from 0.4% to 0.6% and 0.8% resulted in a corresponding decrease in moisture content

from 47.5% to 44.8% and 43.76%, respectively. The 0.4% sample exhibited the highest moisture content, which was statistically significantly different from the other two samples, while no significant difference was observed between the 0.6% and 0.8% samples. The moisture content of all samples ranged from 43% to 47% w/w. Moisture content reflects the water content in the food matrix, affecting quality, stability, and shelf life.[9] Overall, the fermented glutinous rice in this study had moisture content below 50%, lower than that reported for Khao-Mak (50.76 - 53.04%).[10,11] Table 1 also shows that the total soluble solids (TSS) of the finished fermented glutinous rice ranged from 31 to 40 °Brix. During fermentation, microorganisms secrete enzymes that metabolize starch into sugars, alcohol, and acids.[12,13] In the initial phase, molds produce enzymes that degrade starch into soluble sugars. However, as fermentation progresses, the total soluble solids decrease due to the utilization of sugars as substrates by yeasts and other microorganisms.[9] A similar decreasing trend has been observed during the production of Jiu Niang.[14]

Alcohol content of the fermented glutinous rice samples ranged from 8% to 10% v/v, with statistically significant differences observed among them. Saccharification by molds and alcoholic fermentation by yeasts convert glucose into alcohol.[7] A small amount of alcohol can also be produced by lactic acid bacteria during fermentation at temperatures above 30 °C in the presence of maltose.[15] Compared to previous studies, alcohol levels reported here are relatively high. For example, Khao-Mak from Thailand reached only 2.15 - 2.58% v/v after 3 days of fermentation,[10] while other studies reported alcohol concentrations below 0.5% v/v at day 3, gradually increasing to 1 - 2% v/v by day 7.[16] This discrepancy may be attributed to differences in raw materials.[16]

The lactic acid content in the fermented glutinous rice samples ranged from 0.75 to 0.98 mg/mL. An inverse correlation was observed between alcohol and lactic acid concentrations: alcohol accumulation inhibits acid production, thereby helping to control acidity and reduce spoilage.[15] The 0.4% starter culture sample exhibited the lowest lactic acid concentration (0.75 mg/mL), whereas the 0.6% sample had the highest (0.98 mg/mL), similar to the lactic acid content reported in Khao-Mak fermentation after 3 days.[2] Jiu Niang also contains various organic acids, primarily lactic acid, along with malic, fumaric, acetic, tartaric, citric, and succinic acids, with total concentrations increasing over time and slightly decreasing after 60 hours.[14] This phenomenon is related to the complex activity of the microbial community in the starter culture or possible contamination.[17]

pH values of fermented glutinous rice samples in this study ranged from 3.71 to 3.98, reflecting the level of organic acid accumulation during fermentation. There was an inverse relationship between pH and titratable acidity. as lactic acid, produced by lactic acid bacteria, accumulated, the pH decreased. Although the pH variations were relatively small, even slight changes could significantly affect the sensory attributes and stability of the final product. This reflects the relationship between pH and the ionization strength of acids. In the case of Khao-Mak made from black rice, the pH decreased from 6.00 to 4.00–4.27 after 3 days of fermentation.[18] Yeasts and molds participate in the conversion of sugars into alcohol and acids, contributing to the pH reduction and creating an antimicrobial environment that ensures food safety.[17] Overall, the quality of fermented glutinous rice varies depending on the source of the starter culture, traditional processing methods, environmental conditions, and fermentation duration.

In addition to physicochemical parameters such as moisture content, pH, acid concentration, Brix value, and alcohol content, the fermented glutinous rice product was also subjected to sensory evaluation to assess consumer preference. The results of the sensory evaluation are presented in Table 2.

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Table 2. Effect of pure fermentation starters concentration on the sensory quality of fermented glutinous rice product.

Concentration	Average Sensory Scores	Methanol (%)	Furfurol (%)
0.4%	18.41 ^{b} ± 0.27	ND	ND
0.6%	16.41 ^{a} ± 0.34	ND	ND
0.8%	16.0 ^{a} ± 0.25	ND	ND

Note: "ND: no detected"; *Data are the mean values of triplicates. In the same column, different letters (a, b) indicate statistically significant differences at the 95% confidence level (statistical significance level $p < 0.05$).*

The average sensory score at a starter culture concentration of 0.4% was 18.41 ± 0.27, which was statistically significantly different from those at 0.6% (16.41 ± 0.34) and 0.8% (16.00 ± 0.25). No significant difference was observed between the sensory scores of the 0.6% and 0.8% samples. These results indicate that the product achieved the highest sensory acceptance at a starter culture concentration of 0.4%.

Moreover, the analysis showed that two undesirable compounds, methanol and furfurol, were not detected in the fermented glutinous rice product. These compounds are known to cause headaches and have neurotoxic effects on consumers. Therefore, the absence of these substances in the experimental samples is highly significant for future commercialization.

In addition to physicochemical and sensory parameters, microbiological characteristics of the fermented glutinous rice samples were also analyzed, including determining the density of molds, yeasts, and lactic acid bacteria. This provided an overview of the microbial populations present in the product. The results of microbial enumeration are presented in Table 3.

Table 3. Effect of pure fermentation starters concentration on microbial population in the product.

Concentration	Molds (Log_{10} CFU/g)	Yeasts (Log_{10} CFU/g)	Lactic acid bacteria (Log_{10} CFU/g)
0.4%	7.27 ^{c} ± 0.02	7.28 ^{c} ± 0.01	8.22 ^{a} ± 0.01
0.6%	6.22 ^{a} ± 0.02	7.27 ^{b} ± 0.01	8.27 ^{b} ± 0.02
0.8%	6.92 ^{b} ± 0.01	7.20 ^{a} ± 0.01	8.24 ^{a} ± 0.01

Data are the mean values of triplicates. In the same column, different letters (a, b) indicate statistically significant differences at the 95% confidence level (statistical significance level $p < 0.05$).

The analysis results presented in Table 3 indicate that the fermented glutinous rice sample with 0.4% isolated starter culture had the highest yeast and mold counts, at 7.27 log_{10} CFU/g and 7.28 log_{10} CFU/g, respectively, while the lowest counts were observed in the 0.6% sample, with yeast and mold counts of 6.22 log_{10} CFU/g and 7.27 log_{10} CFU/g, respectively. These findings confirm the presence of both molds and yeasts in the fermented glutinous rice samples. These two groups of microorganisms play key roles and exhibit principal activities during saccharification and alcoholic fermentation processes.[19,20]

Similarly, a study on Khao-Mak fermentation[6] reported comparable results, where at the end of the fermentation period (day 3),

total mold and yeast counts were 7.00×10^2 CFU/g and 5.40×10^5 CFU/g, respectively.[11] Additionally, lactic acid bacteria were also detected. Besides molds, certain bacteria are capable of producing enzymes that hydrolyze starch in raw materials into sugars, which yeasts can subsequently utilize for alcohol production.[21]

For this reason, research on the production of Tapai-a traditional Indonesian product similar to Vietnamese fermented glutinous rice has explored supplementation with *Lactobacillus plantarum* strains alongside starter cultures. The results demonstrated that Tapai fermented with *L. plantarum* exhibited slightly different characteristics compared to traditional Tapai and received higher acceptance scores from sensory panelists.[22]

3.2. Effect of fermentation time on the quality of fermented glutinous rice

The fermentation process of fermented glutinous rice exhibited significant changes in physicochemical parameters over time. Table 4 presents data on Brix value, pH, alcohol content, and sensory scores at fermentation time of 2, 3, and 4 days.

Table 4. Effect of fermentation time on the quality of fermented glutinous rice.

Parameter	Fermentation Time (days)		
	2	3	4
Brix (^{o}Brix)	$39.4^{c} \pm 0.52$	$38.1^{b} \pm 0.26$	$35.6^{a} \pm 0.2$
pH	$3.82^{a} \pm 0.02$	$4.05^{c} \pm 0.04$	$3.91^{b} \pm 0.01$
Alcohol (% v/v)	$8.0^{a} \pm 0.2$	$10.67^{b} \pm 0.58$	$11.02^{b} \pm 0.57$
Sensory Evaluation (Scores)	$14.50^{a} \pm 0.32$	$17.44^{c} \pm 0.14$	$16.25^{b} \pm 0.26$
Moisture (% w/w)	$44.3^{a} \pm 0.67$	$47.5^{b} \pm 0.05$	$49.2^{c} \pm 0.22$
Titratable acidity (mg/mL)	$0.87^{a} \pm 0.05$	$0.99^{ab} \pm 0.01$	$1.05^{b} \pm 0.05$
Methanol (%)	ND	ND	ND
Furfurol (%)	ND	ND	ND

Note: "ND: no detected"; *Data are the mean values of triplicates. In the same row, different letters (a, b) indicate statistically significant differences at the 95% confidence level (statistical significance level $p < 0.05$).*

The study results demonstrated that fermentation time significantly influenced the physicochemical and sensory parameters of the product. The Brix value gradually decreased from 39.4 °Brix on day 2 to 38.1 °Brix on day 3 and further to 35.6 °Brix on day 4. This reduction was statistically significant and reflects the conversion of sugars into alcohol and other by-products. The pH of the samples showed fluctuations, with a slight increase from 3.82 to 4.05 on day 3, followed by a decrease to 3.91 on day 4. These changes may be associated with the formation and degradation of organic acids during fermentation, although the pH remained within the weak acid range appropriate for fermented food products.

Alcohol content in the product increased from 8.0% on day 2 to 10.67% on day 3, reaching 11.02% on day 4. The difference between day 2 and the subsequent time points was statistically significant; however, the variation between days 3 and 4 was not significant, indicating that the rate of alcohol biosynthesis peaked around day 3. The sensory score rose sharply from 14.50 on day 2 to 17.44 on day 3, followed by a slight decrease to 16.25 on day 4. The sample fermented for 3 days was rated as having the best sensory quality, reflecting a balanced combination of flavor, alcohol content, and acidity.

The moisture content of the product gradually increased over time, from 44.3% to 49.2%, reflecting the breakdown and dissolution of compounds during raw material hydrolysis. Concurrently, the total acid content also showed a slight increase from 0.87 to 1.05 mg/mL, consistent with the accumulation of organic acids due to microbial activity. Notably, neither

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malcohol nor furfural was detected in any of the samples, confirming the safety of the fermentation process.

In addition to physicochemical and sensory parameters, microbiological indicators of the fermented glutinous rice samples were also analyzed, including the enumeration of molds, yeasts, and lactic acid bacteria. This provided an overall overview of the microbial populations present in the fermented glutinous rice product. The results of the microbial counts are presented in Table 5.

Table 5. Effect of fermentation time on microbial population in the product.

Fermentation Time (days)	Molds (Log_{10} CFU/g)	Yeasts (Log_{10} CFU/g)	Lactic acid bacteria (Log_{10} CFU/g)
2	$7.01^{c} \pm 0.05$	$7.03^{c} \pm 0.01$	$8.14^{a} \pm 0.01$
3	$6.13^{b} \pm 0.03$	$7.15^{b} \pm 0.01$	$8.23^{b} \pm 0.02$
4	$5.92^{a} \pm 0.02$	$7.6^{a} \pm 0.01$	$8.34^{c} \pm 0.01$

Data are the mean values of triplicates. In the same row, different letters (a, b) indicate statistically significant differences at the 95% confidence level (statistical significance level $p < 0.05$).

During the fermentation process, significant changes in the population densities of microbial groups were observed over time. The mold count gradually decreased from 7.01 log_{10} CFU/g on day 2 to 6.13 log_{10} CFU/g on day 3, reaching the lowest level of 5.92 log_{10} CFU/g on day 4. This reduction was statistically significant, as indicated by different letter notations on the mean values ($p < 0.05$). In contrast, yeast populations increased significantly over time, from 7.03 log_{10} CFU/g on day 2 to 7.15 log_{10} CFU/g on day 3 and 7.60 log_{10} CFU/g on day 4, with the increase also showing statistical significance. Similarly, lactic acid bacteria exhibited strong growth trends, with counts rising from 8.14 log_{10} CFU/g to 8.23 and 8.34 log_{10} CFU/g on days 2, 3, and 4, respectively. Mean values with different letter notations confirmed significant differences between days ($p < 0.05$).

These results indicate that the fermentation process created favorable conditions for the proliferation of yeasts and lactic acid bacteria-two beneficial microbial groups-while substantially inhibiting mold growth, thereby contributing to the quality and microbiological safety of the product. This trend aligns with international reports on alcoholic fermentation. According to Fleet,[23] during wine fermentation, *Saccharomyces cerevisiae* is the dominant yeast species, which grows vigorously and produces a harsh environment (alcohol and low pH) that suppresses undesirable microorganisms such as molds and aerobic bacteria. Yoon similarly reported approximately 4.6×10^{7} CFU/mL of *Saccharomyces cerevisiae* present in fermented glutinous rice water after 48 hours of fermentation.[24]

Collectively, these findings suggest that a fermentation period of 3 days is optimal, yielding high alcohol content, favorable sensory qualities, and satisfactory safety parameters. This duration is recommended for practical application to maximize product quality and food safety.

Thus, the optimal fermentation time for fermented glutinous rice is 3 days using a 0.4% starter culture concentration, ensuring ideal quality parameters such as pH, Brix, alcohol concentration, acid content, and counts of molds, yeasts, and lactic acid bacteria. Additionally, the final product received positive sensory evaluations from consumers.

4. CONCLUSIONS

The study applied pure microbial strains isolated from traditional fermentation starters collected in Go Quao district, Kien Giang province, for the production of fermented glutinous rice. The results indicated that the ratio of fermentation starter used and fermentation time were two critical factors affecting the sensory, physicochemical, and microbiological quality of the final product. Among the tested concentrations of pure fermentation starter (0.4%, 0.6%, and 0.8%), the 0.4% level yielded optimal results in terms of sensory quality and fermentation performance, with a high ethanol content (10.2% v/v), appropriate moisture and pH values, balanced lactic acid levels, and no detection of harmful compounds such as methanol or furfural. Additionally, samples at this concentration showed high yeast and mold counts, indicating effective fermentation.

Regarding fermentation time, three durations (2 - 4 days) were evaluated, with 3 days determined to be optimal, showing a balance of physicochemical parameters (pH, Brix, moisture, ethanol, acid), favorable sensory characteristics, and viable counts of beneficial microorganisms. Notably, the simultaneous increase in yeast and lactic acid bacteria populations and the decline in mold counts reflected the establishment of a stable microbial community, suitable for the production of safe fermented products.

The findings provide a scientific basis for standardizing the use of pure starter cultures in terms of ratio and fermentation time, while enhancing the stability and sensory quality of traditional fermented glutinous rice. More importantly, this research contributes to the preservation and sustainable development of traditional starter-making practices-a valuable cultural heritage of the Mekong Delta-through a scientific, safe, and commercially viable approach.

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