So sánh ảnh hưởng của men truyền thống và men phân lập đến chất lượng, đặc tính cảm quan và an toàn vi sinh của cơm rượu.

TÓM TẮT

Nghiên cứu này được thực hiện nhằm đánh giá ảnh hưởng của men truyền thống và men phân lập đến các chỉ tiêu hóa lý**,** cảm quan và an toàn vi sinh của sản phẩm cơm rượu. Cơm rượu được sản xuất theo quy trình truyền thống tại làng nghề Trung Thạnh (thành phố Cần Thơ), sử dụng men truyền thống và men phân lập. Các chỉ tiêu phân tích bao gồm hàm lượng ẩm, pH, °Brix, hàm lượng ethanol, acid lactic, mật số nấm mốc, nấm men, vi khuẩn lactic, vi sinh vật gây bệnh và đánh giá cảm quan. Kết quả cho thấy men truyền thống có ưu thế về khả năng đường hóa và sinh tổng hợp acid lactic, góp phần hình thành hương vị đặc trưng của sản phẩm. Trong khi đó, men phân lập thể hiện tính ổn định cao hơn về chất lượng sản phẩm, hiệu suất tạo ethanol và khả năng kiểm soát các vi sinh vật chỉ thị, đặc biệt là *Escherichia coli*. Điểm cảm quan của sản phẩm sử dụng men phân lập cao hơn có ý nghĩa thống kê so với sản phẩm sử dụng men truyền thống. Phân tích an toàn thực phẩm cho thấy methanol, furfurol không được phát hiện ở cả hai loại men, trong khi hầu hết các vi sinh vật gây bệnh đều nằm trong giới hạn cho phép. Kết quả nghiên cứu khẳng định tiềm năng ứng dụng của men khởi động phân lập như một giải pháp cải tiến, góp phần nâng cao độ an toàn và tính ổn định của sản phẩm, đồng thời vẫn bảo tồn và phát huy giá trị cảm quan của sản phẩm nếp lên men truyền thống.

Từ khóa: *Cơm rượu, bánh men truyền thống, bánh men phân lập, an toàn vi sinh, chất lượng cảm quan*.

Comparison of the effects of traditional starter culture and isolated starter culture on the quality, sensory attributes, and microbiological safety of fermented glutinous rice.

ABSTRACT

The study was conducted to evaluate the effects of traditional starter culture and isolated starter cultures on the physicochemical quality, sensory attributes, and microbiological safety of fermented glutinous rice. Fermented glutinous rice was produced following the traditional process in Trung Thạnh craft village (Can Tho City), using traditional starter culture and isolated starter cultures. Analytical parameters included moisture content, pH, °Brix, ethanol content, lactic acid, counts of molds, yeasts, lactic acid bacteria, pathogenic microorganisms, and sensory evaluation. The results showed that traditional starter culture had advantages in saccharification and lactic acid production, contributing to characteristic flavor formation, whereas isolated starter cultures exhibited higher stability in product quality, ethanol yield, and control of indicator microorganisms, particularly *Escherichia coli*. Sensory scores of products using isolated starter cultures were significantly higher than those using traditional starter culture. Food safety analysis indicated that methanol, furfurol was not detected in either starter type, while most pathogenic microorganisms were within permissible limits. The findings confirm the potential application of isolated starter cultures as an improved starter, ensuring product safety and stability while preserving and inheriting the sensory values of traditional fermented glutinous rice.

Keywords: *Fermented glutinous rice, traditional starter, isolated starter culture, microbiological safety, sensory quality.*

1. INTRODUCTION

Fermented cereal-based foods have long been used in many Asian cultures, not only as energy-rich foods but also as functional food systems due to the involvement of microorganisms during processing.[1] Within this group, fermented glutinous rice is a characteristic product of Vietnam, formed through the combination of starch hydrolysis and alcoholic fermentation, providing distinct sensory and biological values compared with unfermented glutinous rice products.

The nature of fermented glutinous rice fermentation is the simultaneous activity of multiple groups of microorganisms, mainly including molds producing starch-hydrolyzing enzymes, yeasts converting sugars into ethanol, and lactic acid bacteria involved in organic acid formation.[2,3] The composition and population density of these microorganisms depend closely on the type of starter culture used, thereby determining fermentation rate, physicochemical characteristics, and flavor of the final product. Therefore, the starter culture is considered the central factor governing fermented glutinous rice quality.

In practice, traditional starter cultures are still widely used due to their availability, low cost, and ability to generate characteristic flavors. However, many studies have shown that the microbial communities in traditional starter culture often fluctuate greatly over time, environmental conditions, and production techniques, leading to quality instability and potential food safety risks.[4,5] In response to increasing demands for standardization and food safety, the development of controlled starter cultures has become a general trend in fermented food technology.

Isolated starter culture, produced from selected and pure-cultured microbial strains, allow better control of the fermentation process and minimize the presence of undesirable microorganisms. This approach has been successfully applied in many fermented food systems such as wine, beer, and rice-based products in Asia, improving product uniformity and reproducibility.[6,7] However, for Vietnamese fermented glutinous rice, direct comparative studies between traditional and isolated starter culture remain limited, particularly in the context of simultaneous evaluation of physicochemical, sensory, and microbiological safety parameters.

Based on this context, the present study was conducted to compare the effects of traditional starter culture and isolated starter culture on fermented glutinous rice quality. The results not only clarify the role of each starter type in the fermentation process but also provide a scientific basis for developing isolated starter culture that inherit indigenous microbial resources, serving the production of fermented glutinous rice that is stable, safe, and aligned with the modernization of traditional foods.

2. MATERIALS AND METHODS

2.1 Materials and chemicals

2.1.1 Sample preparation

- This study was conducted at Ms. Non's household in Trung Thạnh Commune, Co Do District, Can Tho City. Fermented glutinous rice was produced using two types of starters: traditional and isolated. After 72 h of fermentation, samples were placed in zip bags, stored in an ice box, and transported to the laboratory of Kien Giang University for analysis. The products were stored frozen to prevent microbiological changes prior to analysis.

- The glutinous rice used was locally available at Ms. Non's household, representing Trung Thanh craft village.

- Starter culture was produced in the laboratory of Kien Giang University with mold counts of 10^7 CFU/g, yeast counts of 10^7 CFU/g, and lactic acid bacteria counts of 10^8 CFU/g. Microorganisms in the starter culture included strains isolated from traditional starter culture in Trung Thanh, Kien Giang, and identified by molecular biological techniques: the yeast *Saccharomyces cerevisiae*, the mold *Rhizopus arrhizus*, and the lactic acid bacterium *Lactiplantibacillus plantarum*. These strains were preserved and propagated at the Microbiology Laboratory, Kien Giang University.

2.1.2 Chemicals and culture media

Chemicals used in the study included 0.85% NaCl solution, Buffered Peptone Water (Himedia, India), and NaOH (China). Microbiological media used for isolation and enumeration included PCA (Himedia, India), YPD (Himedia, India), PDA (Himedia, India), MRS agar (Himedia, India), TBX agar (Himedia, India), VRBL agar (Himedia, India), Baird-Parker agar (Himedia, India), MYP agar (Himedia, India), DG18 agar (Himedia, India), and TSC agar (Himedia, India).

2.2 Methods

2.2.1 Physicochemical properties, sensory attributes, methanol, furfural, and microbial enumeration

Moisture content (% w/w):

The moisture content of fermented glutinous rice was determined using 10 g of sample analyzed with a moisture analyzer MOC-63u (Shimadzu, Japan), based on the gravimetric drying principle.

Total soluble solids (°Brix):

A homogenized mixture of fermented glutinous rice and 10 mL of distilled water was prepared, and the soluble solid content was measured using a Master-3T refractometer (Atago, Japan).

pH measurement:

The pH value was measured directly in the rice–water mixture using a MI150 pH meter (MARTINI, Romania) after thorough homogenization.

Ethanol content (% v/v):

Alcohol concentration was determined by the alcohol recovery distillation method using a distillation unit (Model 2505200, Witeg, Germany). The ethanol content of the distillate was measured using an alcohol meter CHG-3C33 (China) and corrected to 20 °C.[8]

Titratable acidity (expressed as lactic acid, mg/mL):

Ten milliliters of fermented glutinous rice mixture were diluted with 20 mL of distilled water, followed by the addition of 1–2 drops of phenolphthalein indicator. The solution was titrated with 0.1 N NaOH until a persistent pale pink color appeared.[9] Titratable acidity was calculated as degrees Thörner (°T) using the formula:

$$°T = V_{NaOH} \times 10$$

Lactic acid concentration (mg/mL) = °T × 0.009

where V_{NOH} is the volume (mL) of 0.1 N NaOH consumed, and 1 °T corresponds to 9 mg of lactic acid.

Methanol and furfural determination:

Methanol and furfural contents were quantified by gas chromatography following Vietnamese standards TCVN 8010:2009 and TCVN 7886:2009, respectively.

Microbial enumeration:

For microbial analysis, 10 g of fermented glutinous rice was homogenized with 90 mL of sterile 0.85% NaCl solution in a stomacher bag and processed for 1 min at 260 rpm. Serial decimal dilutions were prepared for plate counting:

Yeast count: 0.1 mL of the appropriate dilution (10^{-5}) was spread onto YPD agar plates and incubated at 30 °C for 48 h using a UN110 incubator (Memmert, Germany).[10]

Mold count: 0.1 mL of diluted sample (10^{-5}) was plated on PDA medium and incubated at 30 °C for 72 h.[11]

Lactic acid bacteria (LAB): 0.1 mL of the diluted sample (10^{-5}) was inoculated on MRS agar and incubated at 37 °C for 48 h.[12]

Microbial density was calculated using the formula:

CFU/g = a × n × 10

where *a* is the number of colonies counted in 0.1 mL and *n* is the dilution factor.

Sensory evaluation:

Sensory quality was assessed using the numerical rating scale method in accordance with Vietnamese Standard TCVN 3215-79. A panel of 10 assessors, consisting of lecturers and students from the Faculty of Food Science and Health, evaluated the samples using a 20-point scale (0–20) based on color, aroma, sweetness, mild sourness, and softness. All attributes were equally weighted, and the final score was calculated as the mean value of all panelists' evaluations.

2.2.2 Pathogenic microbiological parameters

Fermented glutinous rice samples corresponding to the two product types were stored in an ice box and transported to FAO Company (Can Tho City) for analysis of pathogenic microorganisms. For each analytical parameter, 25 g of sample was accurately weighed and homogenized in 225 mL of Buffered Peptone Water to obtain an initial suspension with a 10^{-1} dilution. From this suspension, subsequent decimal dilutions were prepared following standard procedures. Analytical results were expressed as colony-forming units (CFU/g sample) and calculated.

- Total aerobic microorganisms: determined by the pour plate method on PCA and incubated at 30 °C for 72 h (ISO 4833-1).

- *Escherichia coli*: enumerated by the spread plate method on TBX agar, incubated at 44 °C for 18–24 h (ISO 16649-2).

- Coliforms: determined by the pour plate method on VRBL agar, incubated at 37 °C for 24 h (ISO 4832).

- Coagulase-positive *Staphylococcus aureus*: determined on Baird-Parker agar, incubated at 37 °C for 24–48 h, and confirmed by the coagulase test (ISO 6888-1).

- *Bacillus cereus*: enumerated on MYP agar, incubated at 30 °C for 24 h (ISO 7932).

- Total yeasts and molds: enumerated by the surface spread method on DG18 agar, incubated at 25 °C for 3–5 days (ISO 21527-1).

- *Clostridium perfringens*: determined by the pour plate method on TSC agar and incubated under anaerobic conditions at 37 °C for 24 h (ISO 15213-2).

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

3.1 Effects of traditional starter culture on fermented glutinous rice quality

The study was conducted at Ms. Non's fermented glutinous rice production facility (Trung Thanh Commune, Cờ Đỏ District, Can Tho City), using traditional starter culture at three concentrations of 0.4%, 0.6%, and 0.8% (based on glutinous rice weight). The production process followed the local traditional method: glutinous rice was washed, steamed twice (30 min each), cooled, and shaped into balls, with hands moistened using a saline solution (1:4, w/v) to limit undesirable microorganisms. The starter culture were finely ground, evenly sprinkled onto the rice balls, then wrapped in banana leaves and incubated anaerobically in PE bags with collection of syrup for 3 days. After fermentation, samples were analyzed for pH, °Brix, ethanol content, mold, yeast, and lactic acid bacteria counts, and sensory evaluation; results are presented in Table 1.

Table 1. Effects of traditional starter culture on Fermented glutinous rice quality

Concentration	**Moisture (% w/w)**	**pH**	**Brix**	**Ethanol (% v/v)**	**Lactic acid content (mg/mL)**
0.4%	$41.73^{a} \pm 1.25$	$3.77^{a} \pm 0.03$	$33.67^{a} \pm 4.16$	$4.5^{a} \pm 1.5$	$0.87^{a} \pm 0.05$
0.6%	$44.6^{b} \pm 0.79$	$4.06^{b} \pm 0.06$	$43.33^{b} \pm 1.52$	$5.4^{b} \pm 0.2$	$0.99^{ab} \pm 0.01$
0.8%	$41.07^{a} \pm 0.25$	$3.74^{a} \pm 0.03$	$31.67^{a} \pm 4.04$	$4.4^{a} \pm 0.52$	$1.05^{b} \pm 0.05$

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 results in Table 1 show that the moisture content of Fermented glutinous rice samples ranged from 41.07–44.6% (w/w), in which the sample using 0.6% starter culture had the highest moisture content (44.6% w/w), significantly different from those using 0.4% and 0.8%, while the latter two did not differ significantly. Moisture content reflects the water content of the product and directly affects quality, stability, and shelf life.[13] Overall, the moisture content in this study was lower than that of Khao-Mak (50.76–53.04%) reported previously.[14,15]

Soluble solids content ranged from 31.67–43.33 °Brix, reflecting the balance between starch saccharification by mold enzymes and sugar consumption by yeasts during fermentation,[13,16,17] similar to trends reported for Jiu Niang.[16] Ethanol content ranged from 4.4–5.4% (v/v), with the highest value in the 0.6% sample (5.4% v/v) and the lowest in the 0.8% sample (4.4% v/v). Ethanol is mainly formed from glucose fermentation by yeasts and partly by lactic acid bacteria under suitable conditions,[18,19] and was higher than that reported for some fermented glutinous rice products.[14,20]

Table 2. Effects of traditional starter culture on the sensory quality of fermented glutinous rice

Concentration	**Mean sensory score**
0.4%	$15.87^{a} \pm 0.53$
0.6%	$17.3^{b} \pm 0.41$
0.8%	$15.17^{a} \pm 1.01$

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 $p < 0.05$).

Lactic acid content ranged from 0.8–1.05 mg/mL and showed an inverse relationship with pH (3.74–4.06). The 0.8% starter culture sample had the highest acid content, while the 0.4% sample showed lower values. The accumulation of organic acids and pH reduction contributes to the inhibition of undesirable microorganisms, enhancing product safety and stability,[21,22] consistent with studies on Khao-Mak and Jiu Niang.[23,24] In addition to physicochemical parameters, sensory attributes including color, flavor, and texture were evaluated; the results are presented in Table 2.

Fermented glutinous rice was prepared following the traditional process of Trung Thanh craft village and produced directly at the site. After processing, samples were transported to the laboratory for monitoring and sensory evaluation after 3 days of fermentation. Sensory criteria included aroma, taste, color, and texture, characterized by mild sweetness, slight alcoholic pungency, no sourness or bitterness; uniform opaque white color without mold; characteristic aroma of glutinous rice and alcohol; intact grains without disintegration. Scoring results (Table 2) showed that the sample using 0.6% starter culture achieved the highest sensory score (17.3 points), significantly different from the 0.4% and 0.8% samples, in which increasing starter concentration resulted in a stronger alcoholic taste and reduced structural quality. This trend is consistent with reports showing that moderate yeast populations yield optimal sensory quality by balancing desirable aroma compounds and undesirable by-products.[25]

In addition to sensory evaluation and physicochemical parameters, fermented glutinous rice samples were analyzed for microbiological parameters to assess safety and microbial characteristics of the traditional product, including counts of molds, yeasts, and lactic acid bacteria. Microbiological results are presented in Table 3, providing a scientific basis for evaluating the quality and stability of traditional fermented glutinous rice currently available on the market.

Table 3. Effects of traditional starter culture on yeast, mold, and lactic acid bacteria counts in fermented glutinous rice

Concentration	Molds (Log_{10} CFU/g)	Yeasts (Log_{10} CFU/g)	LAB (Log_{10} CFU/g)
0.4%	$6.83^{b}\pm 0.02$	$7.20^{b}\pm0.02$	$8.26^{c}\pm0.02$
0.6%	$7.25^{c}\pm 0.01$	$7.24^{c}\pm0.01$	$8.22^{b}\pm0.01$
0.8%	$6.10^{a}\pm 0.50$	$7.17^{a}\pm0.01$	$8.12^{a}\pm0.01$

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 results in Table 3 show that yeast and mold populations were present in all fermented glutinous rice samples, in which the sample using 0.6% starter culture exhibited the highest counts (yeasts: 7.24 log_{10} CFU/g; molds: 7.25 log_{10} CFU/g), whereas the 0.8% sample showed the lowest counts (6.10 and 7.17 log_{10} CFU/g). This confirms the dominant role of molds and yeasts in saccharification and alcoholic fermentation.[26,27] However, quantitative results did not allow clear discrimination between beneficial and contaminant microorganisms. This trend is consistent with reports on Khao-Mak after 3 days of fermentation.[19,28]

In addition, lactic acid bacteria were detected with statistically significant differences among starter concentrations, increasing with higher starter levels and decreasing at the highest level. Lactic acid bacteria, together with molds and yeasts, contribute to starch-hydrolyzing enzyme production and formation of flavor compounds in fermented products,[29,30] similar to studies on Tapai with supplementation of *Lactobacillus plantarum*.[31] Overall integration of microbiological, physicochemical, and sensory results (Tables 1 and 2) indicated that the sample using 0.6% traditional starter culture achieved optimal quality and differed significantly from the 0.4% and 0.8% levels; therefore, this concentration was selected for comparison with the isolated starter culture in the subsequent section.

3.2 Effects of isolated starter culture on fermented glutinous rice quality

In traditional fermented glutinous rice production, folk starter cultures are commonly used due to their availability and characteristic fermentation, imparting traditional flavor to the product. However, because the microbial composition of traditional starters is unstable and easily affected by storage conditions and production processes, fermented glutinous rice quality often varies among batches. To enhance stability, fermentation control, and suitability for large-scale production, the application of isolated starter culture is a promising approach. Therefore, this study conducted experiments using isolated starter culture for comparison with the traditional method to evaluate their applicability in fermented glutinous rice production. The results are presented in Table 4.

Table 4. Effects of isolated starter culture on fermented glutinous rice quality

Concentration	Moisture (% w/w)	pH	Brix	Ethanol (% v/v)	Lactic acid content (mg/mL)
0.4%	$44.8^{a} \pm 0.72$	$3.71^{a} \pm 0.04$	$31.67^{a} \pm 0.3$	$4.0^{a} \pm 0.2$	$0.98^{c} \pm 0.02$
0.6%	$47.5^{b} \pm 1.0$	$3.98^{b} \pm 0.06$	$40.33^{b} \pm 0.57$	$5.9^{b} \pm 0.1$	$0.75^{a} \pm 0.05$
0.8%	$43.76^{a} \pm 0.76$	$3.91^{b} \pm 0.08$	$32.67^{a} \pm 0.5$	$4.8^{a} \pm 0.2$	$0.89^{b} \pm 0.01$

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 results in Table 4 show that the concentration of isolated starter culture significantly affected the physicochemical parameters of fermented glutinous rice. When the starter concentration increased from 0.4% to 0.6% and 0.8%, the moisture content increased from 44.8% to 47.5% and then decreased to 43.76% (w/w), in which the 0.6% sample showed a significantly higher moisture content, while the 0.4% and 0.8% samples did not differ significantly. Moisture content ranged from 43–47% (w/w), lower than that reported for Khao-Mak fermented glutinous rice,[14,15] and plays an important role in product quality and shelf life.[13]

Soluble solids content ranged from 31–40 °Brix and was clearly influenced by starter concentration. At 0.6%, the highest °Brix value was recorded (40.33 ± 0.57 °Brix), significantly different from the 0.4% and 0.8% levels, while the latter two did not differ significantly. This variation reflects the balance between starch saccharification by mold enzymes and sugar consumption by yeasts during fermentation,[13,16,17] similar to trends reported for Jiu Niang.[23]

Ethanol content ranged from 5–6% (v/v), with the highest value observed in the 0.6% sample (5.9 ± 0.1% v/v), significantly higher than those of the 0.4% and 0.8% samples. Lactic acid content ranged from 0.75–0.98 mg/mL and showed an inverse relationship with pH (3.71–3.98), in which the 0.4% sample exhibited the highest lactic acid content and the lowest pH. The accumulation of organic acids contributes to improved safety and stability of fermented products.[19,21,22] Overall, the results indicate that a starter concentration of 0.6% is a suitable condition to optimize ethanol content and maintain the quality of the final fermented glutinous rice product.

Table 5. Effects of isolated starter culture on the sensory quality of fermented glutinous rice

Concentration	Mean sensory score
0.4%	$16.41^{a} \pm 0.34$
0.6%	$18.41^{b} \pm 0.27$
0.8%	$16.0^{a} \pm 0.25$

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 $p < 0.05$).

In addition to physicochemical parameters (moisture, pH, acid content, °Brix, and ethanol content), fermented glutinous rice products were also evaluated sensorially to assess consumer preference. Sensory evaluation results are presented in Table 5.

The results in Table 5 show that the mean sensory scores of fermented glutinous rice differed markedly according to starter concentration. The sample using 0.6% starter culture achieved the highest score (18.41 ± 0.27), significantly different from the 0.4% (16.41 ± 0.34) and 0.8% (16.00 ± 0.25) samples, while the latter two did not differ significantly (ANOVA, LSD, $p < 0.05$). The high sensory score at 0.6% reflects superior quality in aroma, taste, texture, and overall acceptability.

The decline in sensory scores at higher starter concentrations indicates over-fermentation, resulting in a strong starter odor, less harmonious taste, and unstable product structure. The results confirm that a starter concentration of 0.6% is an appropriate condition to optimize the sensory quality of fermented glutinous rice. In addition to physicochemical and sensory parameters, fermented glutinous rice samples were analyzed for microbiological indicators (mold, yeast, and lactic acid bacteria counts) to comprehensively assess the microbial community present in the product; the results are presented in Table 6.

Table 6. Effects of isolated starter culture on microbial counts in fermented glutinous rice

Concentration	Molds (Log_{10} CFU/g)	Yeasts (Log_{10} CFU/g)	LAB (Log_{10} CFU/g)
0.4%	$6.22^{a} \pm 0.02$	$7.27^{b} \pm 0.01$	$8.27^{b} \pm 0.02$
0.6%	$7.27^{c} \pm 0.01$	$7.28^{c} \pm 0.01$	$8.18^{a} \pm 0.01$
0.8%	$6.92^{b} \pm 0.01$	$7.20^{a} \pm 0.01$	$8.24^{a} \pm 0.01$

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 results in Table 6 show that yeast and mold populations were present in all fermented glutinous rice samples produced using isolated starter culture, in which the 0.6% sample exhibited the highest counts (yeasts: 7.27 log_{10} CFU/g; molds: 7.28 log_{10} CFU/g), while the 0.4% sample showed the lowest counts (6.22 and 7.27 log_{10} CFU/g). This confirms the dominant role of molds and yeasts in saccharification and alcoholic fermentation.[26,27] However, quantitative data did not allow clear differentiation between beneficial microorganisms and contaminants. This trend is consistent with reports on Khao-Mak after 3 days of fermentation.[10,28] In addition, the presence of lactic acid bacteria indicates a complex microbial community involved in starch-hydrolyzing enzyme production and flavor compound formation in fermented products,[29,30] consistent with studies on Tapai supplemented with *Lactobacillus plantarum*.[31]

Integration of physicochemical (Table 4), microbiological (Table 6), and sensory (Table 5) results shows that the sample using 0.6% isolated starter culture achieved optimal quality, with the highest sensory score and statistically significant differences compared with the 0.4% and 0.8% levels ($p < 0.05$). Increasing starter concentration did not improve but could reduce sensory quality due to changes in enzyme activity and microbial density, affecting sugar metabolism and flavor formation. Therefore, a 0.6% isolated starter concentration was selected as the optimal condition and used as the basis for comparison with the traditional starter culture in subsequent experiments.

3.3 Comparison of fermented glutinous rice production using isolated and traditional starter culture

In fermented glutinous rice production, the type of starter culture plays a decisive role in fermentation and product quality. Two common starter types are traditional starter culture, with diverse microbial communities, and isolated starter culture, using selected and controlled microbial strains. Comparing the quality of fermented glutinous rice produced from these two starter types aims to evaluate the advantages and limitations of each approach and to guide process improvement; the results are presented in Table 7.

Table 7. Effects of starter type on fermented glutinous rice quality

Starter type	Moisture (% w/w)	pH	Brix	Ethanol (% v/v)	Lactic acid content (mg/mL)
Isolated	$47.5^{b} \pm 1.0$	$3.98^{a} \pm 0.07$	$40.33^{a} \pm 0.57$	$5.9^{b} \pm 0.1$	$0.75^{a} \pm 0.05$

Traditional	44.6^{a}± 0.7	4.06^{a}±0.06	43.33^{b}± 1.52	4.4^{a}±0.2	0.99^{b}±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 $p < 0.05$).

Table 7 presents a comparison of physicochemical characteristics and fermentation performance of fermented glutinous rice produced using isolated starter culture (containing selected yeast, mold, and lactic acid bacteria strains) and traditional starter culture from Trung Thạnh craft village. The results show that moisture content of fermented glutinous rice produced with isolated starters was higher than that of traditional starters but remained below 50%, lower than that reported for Khao-Mak fermented rice (50.76–53.04%).[14,15] The pH values of the two starter types did not differ significantly, ranging from 3.98–4.06, suitable for lactic acid bacteria activity and inhibition of spoilage microorganisms.

Soluble solids content was significantly higher in the traditional starter (43.33 °Brix) than in the isolated starter (40.33 °Brix), reflecting more efficient starch-to-sugar conversion due to the mixed microbial community.[22] However, the isolated starter produced higher ethanol content (5.9% v/v) than the traditional starter (4.4% v/v), while lactic acid content was significantly lower (0.75 vs. 0.99 mg/mL), indicating better control of acid production due to the selected microbial composition.

Overall, the results indicate that traditional starter culture has advantages in saccharification and acid production, whereas isolated starter cultures are superior in stability, microbial control, and ethanol production efficiency. In addition to physicochemical parameters, fermented glutinous rice products were also evaluated sensorially to assess consumer preference; the sensory evaluation results are presented in Table 8.

The results in Table 8 show that the mean sensory score of fermented glutinous rice produced with the isolated starter culture reached 18.53 ± 0.22, higher than that of the product using the traditional starter culture (17.0 ± 0.41), with a statistically significant difference ($p < 0.05$). The higher sensory score and lower standard deviation of the isolated starter sample reflect more stable and uniform sensory quality, indicating that consumers were able to clearly distinguish differences between the two products within the scope of evaluation. This result is consistent with the biological characteristics of pure isolated yeast strains, which exhibit stable growth, efficient fermentation, and production of compounds that enhance flavor and product structure.

Table 8. Effects of starter type on the sensory quality of fermented glutinous rice

Starter type	Mean sensory score
Isolated	18.53^{b} ± 0.22
Traditional	17.0^{a}± 0.41

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 findings confirm the potential application of isolated starter culture in improving uniformity and quality control of fermented glutinous rice, while also indicating a higher level of consumer preference compared with products using traditional starter culture. However, the preservation and use of traditional starter culture remain important for maintaining the identity and flavor diversity of fermented products. In addition to physicochemical and sensory parameters, fermented glutinous rice samples were further analyzed for microbiological indicators (mold, yeast, and lactic acid bacteria counts) to comprehensively evaluate the microbial community present in the product; the results are presented in Table 9.

Table 9. Effects of starter type on microbial counts in fermented glutinous rice

Starter type	Molds (Log_{10} CFU/g)	Yeasts (Log_{10} CFU/g)	LAB (Log_{10} CFU/g)

Isolated	$7.27^{a} \pm 0.01$	$7.28^{b} \pm 0.01$	$8.18^{a} \pm 0.01$
Traditional	$7.25^{a} \pm 0.01$	$7.24^{a} \pm 0.02$	$8.22^{b} \pm 0.01$

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 $p < 0.05$).

Table 9 presents the microbial counts ($\log_{10}$ CFU/g) of molds, yeasts, and lactic acid bacteria. Microbiological analysis showed that the populations of molds, yeasts, and lactic acid bacteria in products using isolated and traditional starter culture differed significantly ($p < 0.05$). Mold counts in the isolated starter product (7.27 $\log_{10}$ CFU/g) were slightly higher than those in the traditional starter product (7.25 $\log_{10}$ CFU/g), indicating that production using isolated starters provided more favorable conditions for mold growth. Similarly, yeast counts in the isolated starter product were more stable and significantly higher, reflecting the uniform growth capacity of the selected yeast strains.

In contrast, the traditional starter product exhibited higher lactic acid bacteria counts (8.22 $\log_{10}$ CFU/g) than the isolated starter product (8.18 $\log_{10}$ CFU/g), indicating that the natural fermentation environment favored the growth of lactic acid bacteria—microorganisms that play an important role in flavor development and characteristic fermentation properties. Differences in microbial communities clearly reflect the influence of production methods: traditional starters contribute to distinctive flavor formation, whereas isolated starters enable better microbial control, enhancing product stability and safety.

3 Effects of fermented glutinous rice starters on food safety

Two fermented glutinous rice samples representing the two starter types (traditional and isolated), with a sample weight of 500 g each (including rice and liquid), were stored under cold conditions and transported to FAO Company (Can Tho City) for analysis of pathogenic microorganism contamination. The determined parameters included total aerobic microorganisms (at 30 °C), *Escherichia coli*, Coliforms, coagulase-positive *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*, in accordance with current regulations of the Ministry of Health (Consolidated Document No. 09/VBHN-BYT, 2023; Circular No. 17/2023/TT-BYT). The results are summarized in Table 10.

Table 10. Food safety quality of fermented glutinous rice samples

Microbiological parameters	Limits according to 09/VBHN-BYT:2023 (CFU/g)	Traditional (CFU/g)	Isolated (CFU/g)
Total aerobic microorganisms (30 °C)	$\leq 1.0\times10^{4}$	2.5×10^{7}	3.3×10^{7}
Escherichia coli	≤ 3	< 10	≤ 3
Coliforms	≤ 10	< 10	< 10
Staphylococcus aureus (coagulase-positive)	≤ 10	< 10	< 10
Bacillus cereus	≤ 10	< 10	< 10
Total yeasts and molds	$\leq 1.0\times10^{2}$	1.9×10^{7}	2.4×10^{7}
Clostridium perfringens	≤ 10	< 10	< 10
Methanol (%)	ND	ND	ND

Furfurol (%)	ND	ND	ND

Note: ND = Not Detected

The microbiological safety test results of fermented glutinous rice produced using traditional and isolated starter culture showed that total aerobic microorganisms (30 °C) and total yeasts–molds were both high, ranging around 10^7 CFU/g. These values exceeded the reference limits of Consolidated Document No. 09/VBHN-BYT:2023; however, this is a common characteristic of traditional fermented products as “living systems,” in which yeasts and molds play a central role in saccharification and alcoholic fermentation. This finding is consistent with reports on fermented glutinous rice produced in the Mekong Delta, in which total aerobic microorganisms and yeasts–molds were recorded at 8.46–9.46 log CFU/g, reflecting high microbial counts that remain compatible with the biological nature of traditional fermented products.[13] A similar trend has also been reported for Khao-Mak, a comparable Thai product.[14,15]

For indicator and pathogenic microorganisms, most parameters met regulatory requirements for both starter types, including Coliforms, coagulase-positive *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*. Notably, *Escherichia coli* showed differences depending on the starter type: fermented glutinous rice produced with the isolated starter met the permissible limit (≤3 CFU/g), whereas the traditional starter sample exceeded the reference threshold. This result demonstrates the clear advantage of isolated starter culture in limiting microbial contamination through the use of selected and controlled microbial strains. This observation is consistent with findings showing that isolation and control of starter microorganisms are key factors in stabilizing product quality and enhancing the safety of fermented glutinous rice compared with fully natural traditional starters.[13]

Another notable point is that methanol was not detected in either sample, indicating that the fermentation process did not pose a significant risk of volatile toxic compounds. This result agrees with recent studies on rice-fermented products, which indicate that methanol is not a typical by-product when microbial systems and fermentation conditions are properly controlled.[22]

4. CONCLUSIONS

The study shows that both traditional and isolated starter culture facilitate fermented glutinous rice production, yielding products with mildly acidic pH and ethanol and lactic acid contents consistent with the characteristics of glutinous rice–based fermented foods. Traditional starter culture has advantages in saccharification and acid production, contributing to the characteristic flavor of fermented glutinous rice; however, product quality tends to be variable and poses potential risks of indicator microbial contamination.

Overall, traditional starter culture demonstrate prominent cultural and sensory value due to their diverse microbial communities, contributing to the characteristic flavor and local identity of fermented glutinous rice. In contrast, isolated starter culture show clear advantages in stability, control of indicator microorganisms, and assurance of food safety. Accordingly, the findings suggest the potential application of isolated starter culture as an improved starter culture that inherits indigenous microbial resources while being standardized in composition and population density, meeting the requirements for stable, safe production and aligning with the industrialization trend of traditional fermented foods.[13]

In contrast, isolated starter culture exhibit higher stability in physicochemical parameters, ethanol yield, and control of undesirable microorganisms, while achieving significantly higher sensory scores. Food safety analysis indicates that methanol was not detected in either starter type and that most pathogenic microorganisms were within permissible limits.

Overall, the results confirm the potential of isolated starter culture as an improved starter culture for fermented glutinous rice production, enhancing safety, uniformity, and quality control while preserving the traditional values of Vietnamese fermented glutinous rice.

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