

Original Research Article

Comparative Analysis of Brewing Characteristics across Four Glutinous Rice Cultivars

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Received: 14.04.2025

Accepted: 21.05.2025

Published: 23.05.2025

Journal homepage:<https://www.easpublisher.com>**Quick Response Code**

Abstract: Owing to the heterogeneous climatic and geographical conditions across China, glutinous rice (*Oryza sativa* var. *glutinosa*) has evolved diversified cultivars through prolonged cultivation history. This cereal crop exhibits remarkable economic significance as a partial raw material for baijiu fermentation. The present investigation systematically examined four representative glutinous rice cultivars (designated as A, B, C, and D) to elucidate their compositional profiles and brewing characteristics. A comparative analysis was conducted to delineate the intrinsic disparities among these vinous-purpose cultivars, followed by comprehensive correlation analysis. Key findings demonstrated that (1) Significant compositional heterogeneity was observed among the four cultivars. Cultivars A and B displayed superior brewing suitability, characterized by elevated crude starch content (82.92–78.20 g/100 g), a high proportion of amylopectin (97.23–97.09%), moderate crude protein levels (6.74–6.60 g/100 g), and reduced lipid content (1.14–1.15 g/100 g). (2) All cultivars exhibited analogous trends in physicochemical parameter dynamics during fermentation. The moisture content of fermented grains increased rapidly prior to saturation, followed by a gradual ascent until stabilization. Acidity displayed an initial slow rise succeeded by a decline, while reducing sugars peaked before diminishing. Starch degradation proceeded swiftly in the early fermentation phase before stabilizing. Notably, cultivar B demonstrated the highest starch utilization efficiency and ethanol yield, with statistically significant differences ($p < 0.05$) relative to other cultivars. (3) Correlation analyses revealed that cultivars with higher amylopectin content exhibited enhanced starch utilization efficiency and ethanol yield. Ethanol yield showed significant positive correlations with starch utilization efficiency and crude starch content ($p < 0.05$), while displaying negative correlations with lipid content ($p < 0.05$) and protein content. Starch utilization efficiency exhibited a highly significant positive correlation with amylopectin content ($p < 0.01$), positive correlations with total starch and protein content, and a negative correlation with lipid content. Under standardized xiaoku baijiu fermentation protocols, cultivar B emerged as the optimal candidate due to its superior ethanol yield and starch utilization efficiency, demonstrating exceptional suitability for artisanal liquor production.

Keyword: Glutinous Rice, Compositional Content, Brewing Characteristics, Starch Utilization Efficiency, Xiaoku Baijiu.

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INTRODUCTION

As the principal constituent of glutinous rice (*Oryza sativa* var. *glutinosa*), starch serves not only as the primary energy source for human consumption but also as the critical nutritional substrate for microbial metabolism during fermentation [1]. Beyond fueling microbial growth, starch governs the formation of fermented grains (jiupei) and acts as a precursor for

flavor-active compounds. Starch comprises two macromolecular fractions: amylose and amylopectin. Amylose influences retrogradation properties, with elevated amylose content correlating to post-gelatinization hardening, which may impede microbial utilization of nutrients [2]. Conversely, the highly branched architecture of amylopectin enhances microbial proliferation, thereby facilitating the

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biosynthesis of diverse flavor metabolites during fermentation 3. Variations in amylose-to-amylopectin ratios among cultivars may induce differential starch utilization efficiencies during fermentation, thereby modulating brewing performance 4.

Despite the agricultural diversity of glutinous rice in China, comprehensive studies evaluating cultivar-specific compositional profiles and their brewing implications remain scarce. This research gap necessitates systematic investigation, as raw material selection critically determines the quality of artisanal baijiu 5. Chinese glutinous rice cultivars exhibit marked heterogeneity in starch, protein, lipid, and mineral content, as well as grain morphology. Optimal brewing requires cultivars with high starch (particularly amylopectin) content, moderate protein levels, and minimal lipid content to ensure both ethanol yield and the signature "mellow" flavor profile. However, increasing demand for diversified raw materials—driven by the need for blended or novel cultivars—introduces variability in liquor quality, fermentation kinetics, and flavor compound synthesis, ultimately affecting ethanol yield and organoleptic characteristics 69.

Cultivar selection is dictated by targeted liquor aroma types, process specifications, and compositional requirements. Morphological and biochemical disparities among glutinous rice varieties, including starch granule structure and protein-lipid ratios, directly influence fermentation dynamics and flavor development 1012. This study systematically explores the intrinsic linkages between raw material properties and liquor flavor chemistry, aiming to refine theoretical frameworks for baijiu production and provide empirical guidance for industrial practice 1314. Notably, physicochemical disparities among cultivars manifest distinctly during steaming—a critical pretreatment step requiring precise gelatinization to achieve "thorough cook without stickiness or residual rawness 1516. Suboptimal steaming compromises starch hydrolysis, accelerating retrogradation during cooling and impairing microbial degradation, thereby diminishing both fermentation efficiency and post-aging product quality. By analyzing fermentation indices (e.g., moisture, acidity, reducing sugars) across cultivars, this work identifies superior brewing materials, expands feedstock options for distilleries, and supports the sustainable advancement of China's glutinous rice and baijiu industries 1720.

1 MATERIALS AND METHODS

1.1 Samples

Four glutinous rice cultivars (A, B, C, D) were provided by the Sichuan Provincial Engineering Research Center for Baijiu-Specific Grains.

1.2 Physicochemical Analysis of Glutinous Rice

(1) Moisture Content

Samples were pulverized through a 40-mesh sieve and analyzed using the direct drying method specified in GB/T 5009.3—2016 (National Food Safety Standard: Determination of Moisture in Foods).

(2) Crude Starch Content

Ground samples (40-mesh) were subjected to acid hydrolysis following GB 5009.9—2016 (Determination of Starch in Foods).

(3) Crude Protein Content

Pulverized samples (40-mesh) were digested using a graphite digestion system and quantified via an automated Kjeldahl nitrogen analyzer in accordance with GB/T 5009.5—2016 (Determination of Protein in Foods).

(4) Crude Fat Content

Defatted samples were oven-dried at 105°C to constant mass. Fat content was determined via Soxhlet extraction (fat analyzer) as per GB 5009.6—2016 (Determination of Fat in Foods), calculated as the mass difference between pre- and post-extraction residues.

(5) Amylose and Amylopectin Content

Amylose levels were measured using GB/T 15683—2008 (Rice—Determination of Amylose Content). Amylopectin content was derived by subtracting amylose from total starch.

1.3 Brewing Experimental Procedures

Fermentation trials were conducted using laboratory-scale xiaoku (small starter) baijiu protocols in ceramic jars:

Material Preparation:

Defective grains were removed. A mixture of 2 kg glutinous rice and 50 g rice husks was soaked, drained, and steam-cooked in two stages: initial 10 min uncovered boiling, followed by 20 min covered steaming.

Secondary Steaming: Additional 30 min steaming in a separate vessel.

Inoculation:

Cooled substrates (~30°C) were mixed with 0.6% *Rhizopus* starter culture, transferred to saccharification tanks, and layered with pre-steamed husks.

Saccharification: Tanks were sealed with sterilized gauze and incubated at 28°C for 24 h.

Fermentation:

Saccharified mash was blended with husks, partitioned into Erlenmeyer flasks and ceramic jars, and fermented anaerobically at 30°C for 8 days.

Distillation:

Post-fermentation mash was distilled via steam stripping, with head and tail fractions discarded. Ethanol yield and flavor compounds were quantified.

Monitoring: Physicochemical parameters (moisture, acidity, reducing sugars) were measured every 48 h.

1.4 Physicochemical Analysis of Fermented Grains (Jiupei)

(1) Moisture Content

Approximately 10 g of jiupei was weighed into a pre-dried crucible, dried at 105°C to constant mass, and cooled in a desiccator. Moisture content was calculated based on mass differences before and after drying.

(2) Acidity

A 10 g sample was immersed in 50 mL deionized water for 15 min with intermittent stirring. The filtrate was titrated with standardized NaOH (0.1 mol/L) using phenolphthalein until a faint pink endpoint persisted for 30 seconds. Acidity was expressed as grams of lactic acid per 100 g sample.

(3) Reducing Sugars

The Lane-Eynon method was employed:

Standard Curve:

9 mL glucose standard solution was mixed with Fehling's A and B reagents (5 mL each) and titrated with glucose solution until the blue color disappeared.

Sample Analysis:

2 mL of sample extract was mixed with Fehling's reagents, heated to boiling, and titrated similarly. Reducing sugar content was calculated based on glucose equivalence.

(4) Residual Starch

Samples were hydrolyzed with 1% HCl under reflux for 30 min, neutralized, filtered, and titrated using the same protocol as for reducing sugars.

1.5 Ethanol Yield Determination

Ethanol yield was calculated using the methodology established by Huang Zhijiu *et al.*, with alcohol content standardized to 57% vol (alcohol by volume). A: Ethanol Yield; B: Distillate Mass per Fraction; C: Alcohol Content in Fermented Grains (Current Fraction); D: Mass of Glutinous Rice Utilized.

$$A = \frac{\sum B \times C}{57 \times D} \times 100\%$$

1.6 Starch Utilization Rate

The starch content was measured after saccharification (Day 0) and after fermentation (Day 8).

The starch utilization rate was calculated using the following formula: Starch Utilization Rate (%) = (Starch Content on Day 0 – Starch Content on Day 8) / Starch Content on Day 0 × 100

1.7 Data Processing

Data were statistically analyzed using Excel 2019. Significance and correlation analyses were performed with IBM SPSS Statistics 27.0, and graphical representations were generated using GraphPad Prism 8.0. All measurements were conducted in triplicate.

2. RESULTS AND ANALYSIS

2.1 Analysis of Physicochemical Properties of Glutinous Rice

The compositional contents of different glutinous rice cultivars are shown in Table 1. In terms of moisture content, cultivar B has the highest moisture content at 14.74 g/100 g; cultivar C has the lowest moisture content at 13.37 g/100 g; the moisture content differences among the four cultivars are minimal. Regarding crude starch content, cultivar C shows the highest value at 82.92 g/100 g; cultivar D has the lowest crude starch content at 77.50 g/100 g. Cultivars A and B exhibit crude starch contents of 81.15 g/100 g and 78.20 g/100 g, respectively. The crude fat content between cultivars A and B is similar, at 1.14 g/100 g and 1.15 g/100 g, respectively. In contrast, significant differences exist between cultivars C and D, with crude fat contents of 1.45 g/100 g and 0.99 g/100 g, respectively.

In amylose content analysis, cultivar C contains the highest amylose content at 3.49%; cultivar D has the lowest amylose content at 2.34%. Cultivars A and B show amylose contents of 2.77% and 2.91%, respectively. The amylose content difference between cultivars C and D is minor, while other cultivars display notable disparities. For amylopectin content analysis, cultivar D exhibits the highest amylopectin content at 97.66%; cultivar C has the lowest amylopectin content at 96.51%. Cultivars A and B demonstrate amylopectin contents of 97.23% and 97.09%, respectively. The amylose content difference between cultivars A and B is negligible, while other cultivars show marked variations.

Regarding crude protein content, cultivar A has the highest crude protein content at 6.74 g/100 g; cultivar D shows the lowest crude protein content at 6.50 g/100 g. The crude protein contents of cultivars B and C are 6.60 g/100 g and 6.64 g/100 g, respectively, with no significant difference between cultivars C and D. Cultivar C has the highest crude fat content at 1.45 g/100 g; cultivar D has the lowest crude fat content at 0.99 g/100 g. No significant difference in crude fat content is observed between cultivars A and B.

Table 3-1: Compositional Profiles of Different Glutinous Rice Cultivars

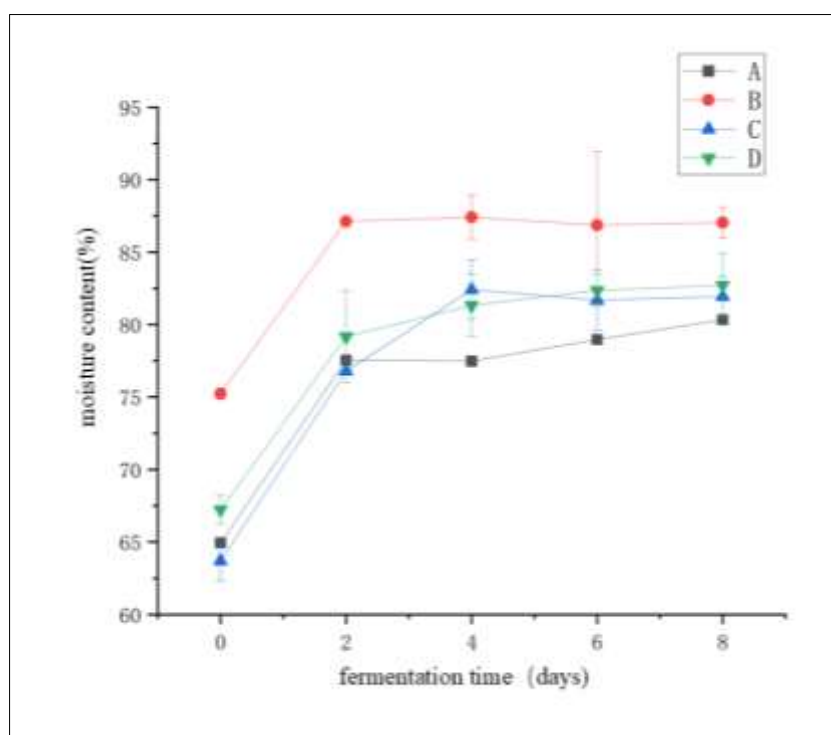
Component	A	B	C	D
Moisture(g/100g)	14.61±0.21a	14.74±0.30a	13.37±0.09b	13.52±0.18b
Crude Starch(g/100g)	81.15±0.59b	78.20±0.28c	82.92±1.23a	77.50±0.36a
Amylose(%)	2.77±0.18bc	2.91±0.09b	3.49±0.26a	2.34±0.20c
Crude Protein(g/100g)	6.74±0.01a	6.60±0.14ab	6.64±0.06b	6.50±0.06b
Crude Fat(g/100g)	1.14±0.11b	1.15±0.01b	1.45±0.10a	0.99±0.02c
Amylopectin (%)	97.23±0.18b	97.09±0.09b	96.51±0.26c	97.66±0.20a

2.2. Analysis of Fermented Grain Composition Results

2.2.1. Moisture Changes in Fermented Grains of Different Glutinous Rice Cultivars during Fermentation

The moisture content changes in fermented grains of different glutinous rice cultivars during fermentation are shown in Figure 1. From the figure, it can be seen that the trend of moisture content changes in fermented grains is basically consistent across different glutinous rice cultivars. Among them, the highest moisture content in fermented grains was observed in cultivar B at the initial fermentation stage (Day 0),

reaching 75.24%. Cultivar C had the lowest moisture content in fermented grains at 63.70%. During the early fermentation stage (0–4 days), the moisture content rapidly increased; in the late fermentation stage (4–8 days), the moisture content gradually stabilized. Among them, the moisture change trend of cultivar C was the steepest, while that of cultivar D was the most gradual. Starting from Day 2, the moisture content of cultivar B in the fermented liquor exceeded that of other cultivars. By the end of fermentation (Day 8), the moisture content of cultivar B reached the highest level at 86.87%, while that of cultivar A was the lowest at 80.31%.


Figure 1: Water content changes of different glutinous rice varieties during fermentation

2.2.2. Acidity Variations in Fermented Grains of Different Glutinous Rice Cultivars during Fermentation

The acidity changes in fermented grains of different glutinous rice cultivars during fermentation are shown in Figure 2. Overall, the acidity changes followed a consistent trend of initial increase followed by decline, though the magnitude of variation differed among cultivars. At the onset of fermentation (Day 0), cultivar B exhibited the highest acidity at 0.3810 mmol/10 g,

while cultivar C showed the lowest acidity at 0.1990 mmol/10 g. During the early fermentation stage (0–4 days), acidity gradually increased; in the late fermentation stage (4–8 days), acidity declined. Cultivar D demonstrated the most pronounced acidity variation, whereas cultivar B exhibited the most gradual change. By the end of fermentation (Day 8), the highest acidity was observed in cultivar C (0.7618 mmol/10 g), and the lowest in cultivar A (0.3139 mmol/10 g).

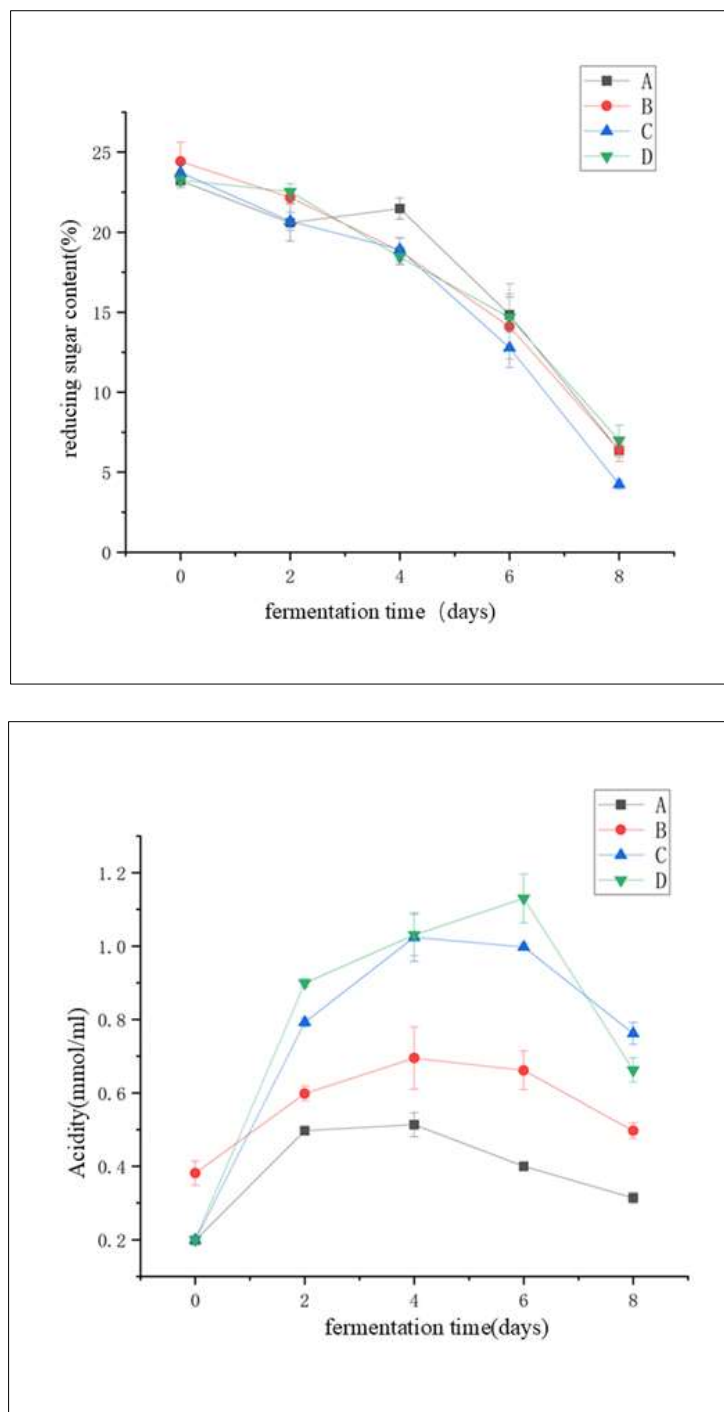


Figure 2: Changes in acidity of different glutinous rice varieties during fermentation

2.2.3. Reducing Sugar Variations in Fermented Grains of Different Glutinous Rice Cultivars during Fermentation

Figure 3 illustrates the changes in reducing sugar content during fermentation across glutinous rice cultivars. The variation pattern of reducing sugars was fundamentally consistent among cultivars. At the onset of fermentation (Day 0), cultivar B exhibited the highest reducing sugar content (73.25%), while cultivar C showed the lowest (69.62%). Except for cultivar A, the reducing sugar content in fermented grains declined

gradually during Days 0–2, followed by a sharp decrease from Days 2–8, reaching minimal levels by fermentation endpoint. Concurrently, acidity displayed a descending trend. Cultivar A demonstrated the steepest reduction in reducing sugars, whereas cultivar B exhibited the most gradual decline. The reduction amplitude of reducing sugars during the decline phase ranged between 16.26% and 19.48%. By the end of fermentation (Day 8), cultivar D retained the highest residual reducing sugar content (7.01%), while cultivar C had the lowest (4.24%).

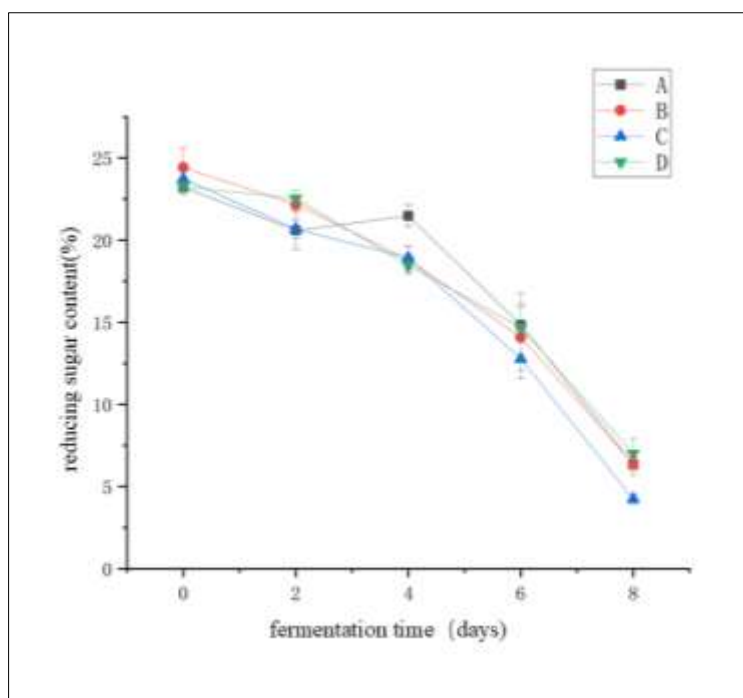


Figure 3: Changes in reducing sugar during fermentation of different glutinous rice varieties

2.2.4. Starch Variations in Fermented Grains of Different Glutinous Rice Cultivars during Fermentation

The starch changes in fermented grains of different glutinous rice cultivars during fermentation are shown in Figures 4. From the figures, it can be seen that the starch content trends in fermented grains were essentially consistent across cultivars, exhibiting an overall declining pattern. At the beginning of fermentation (Day 0), cultivar A had the highest starch content in fermented grains at 27.66%, while cultivar B

had the lowest at 25.09%. During Days 0–4, the starch content in fermented grains decreased substantially; from Days 4–8, the rate of decline decelerated. Cultivar A showed the steepest starch reduction trend, whereas cultivar B exhibited the most gradual decline. The reduction range of starch content during the declining phase was 16.11–18.07%. By the end of fermentation (Day 8), cultivar A retained the highest residual starch content at 10.78%, while cultivar B had the lowest at 8.98%.

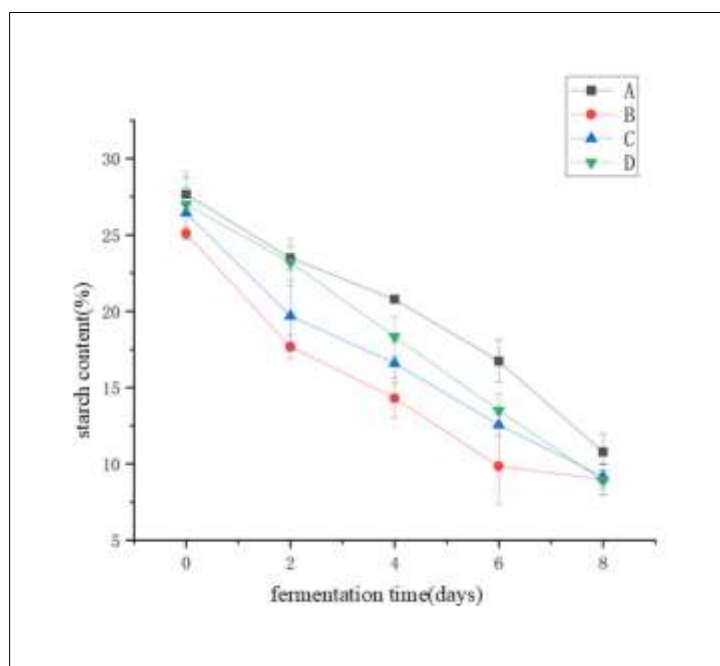


Figure 4: Changes of starch in different varieties of glutinous rice during fermentation

2.2.5. Comparative Analysis of Ethanol Yield across Glutinous Rice Cultivars

The ethanol yields of different glutinous rice cultivars are illustrated in Figure 5. As shown, cultivar B achieved the highest ethanol yield at 45.43%, followed

by cultivar A at 42.28%. The ethanol yield difference between cultivars A and B was minimal, with both exceeding 40%. In contrast, cultivar D exhibited the lowest ethanol yield at 34.74%.

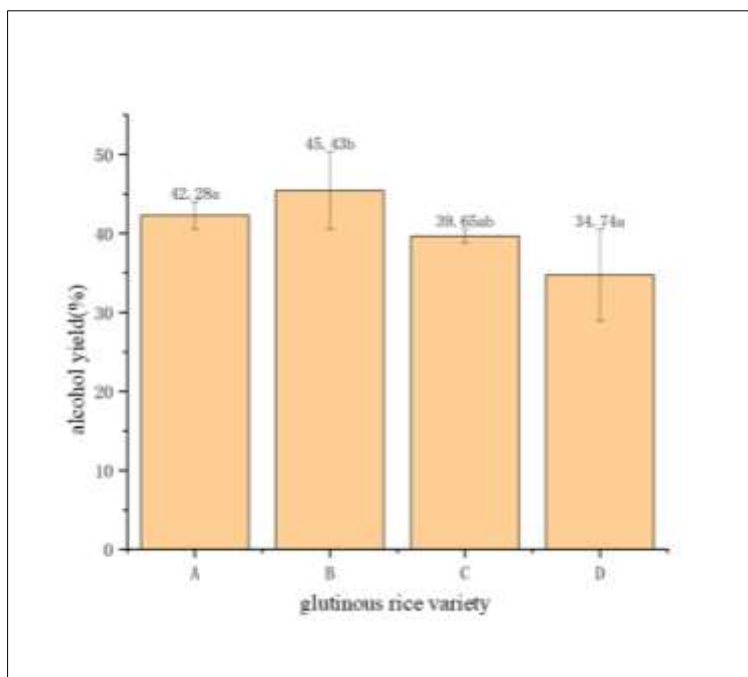


Figure 5: Alcohol yield of different glutinous rice varieties

2.2.6. Comparative Analysis of Starch Utilization Efficiency across Glutinous Rice Cultivars during Fermentation

The starch utilization efficiency of different glutinous rice cultivars during fermentation is depicted in Figure 6. As shown, cultivar D exhibited significant differences in starch utilization efficiency compared to

other cultivars, achieving the highest value at 66.98%. Excluding cultivar D, the variation range of starch utilization efficiency among the remaining three cultivars was 61.02–65.32%. Cultivar A displayed the lowest efficiency at 61.02%. Notably, all cultivars (A, B, C, D) demonstrated starch utilization efficiencies exceeding 60%.

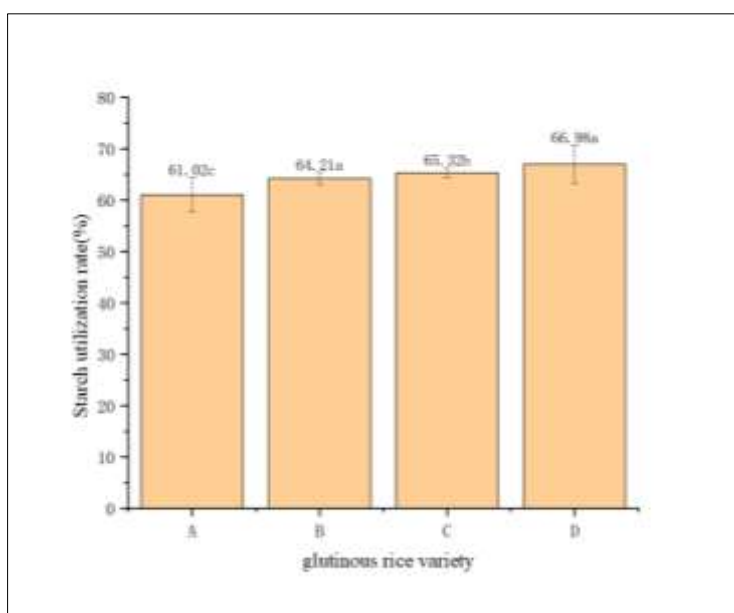


Figure 6: Starch utilization of different glutinous rice varieties

2.2.7. Correlation between Starch Utilization Rate, Ethanol Yield, and Component Content in Glutinous Rice

Table 2 shows the correlations between starch utilization rate, ethanol yield, and component content in different glutinous rice cultivars. From the analysis, it can be observed that the correlations between starch utilization rate, ethanol yield, and total starch content, amylopectin, fat, and protein content are as follows:

Table 2: Correlation between utilization rate of glutinous rice starch and yield rate and component content

Parameter	Total Starch	Protein	Fat	Amylopectin
Starch Utilization Rate	0.237	0.221	-0.113	0.842**
Ethanol Yield	0.382*	-0.130	-0.069*	0.905*

Note: * and ** are significant ($P < 0.05$) and extremely significant ($P < 0.01$), respectively

ethanol yield has a highly significant positive correlation with crude starch content and amylopectin content, and a significant negative correlation with fat content, while showing a negative correlation with protein content. Starch utilization rate exhibits a highly significant positive correlation with amylopectin content, a positive correlation with total starch content and protein content, and a negative correlation with fat content.

3. DISCUSSION

Analyzed the physicochemical indices during the fermentation of Fengxiang-type baijiu fermented grains 212324. The results showed that the moisture content in the fermented grains first increased rapidly and then stabilized; the reducing sugar content exhibited an initial rise followed by a decline during fermentation; and the starch content first decreased and then stabilized during fermentation 2526. The trends in the changes of fermented grain indices observed in this study are largely consistent with the above patterns. The moisture in fermented grains primarily originates from three sources: first, water consumed and produced through microbial physiological and biochemical reactions during grain saccharification and fermented grain fermentation; second, the moisture content of fermented grains is closely related to microbial growth and reproduction; third, the moisture content of fermented grains affects microbial growth, reproduction, and community structure, while microbial growth and metabolism in turn regulate the moisture content of fermented grains 2728.

The changes in acidity of fermented grains are due to vigorous microbial metabolism and substantial organic acid production during the early fermentation stage 2930. As fermentation progresses, acidity decreases due to buffering capacity and organic acid decomposition. Except for cultivar D, the acidity of fermented grains from other glutinous rice cultivars peaked on Day 4. After Days 4–6, the acidity of fermented grains began to decline slowly 3132. This is attributed to the stabilization of temperature and moisture in the late fermentation stage, as acid-producing bacteria gradually adapt to the nutrient and physicochemical conditions of the fermented grains through metabolic reactions. The increase in reducing sugar content may result from incomplete saccharification of steamed glutinous rice after 24 hours of saccharification, with saccharifying enzymes continuing to break down starch into reducing sugars during the early fermentation stage 33. The reduction phase of reducing sugars is mainly due to microbial consumption to sustain their growth, metabolism, and reproduction, coupled with a lower production rate of

reducing sugars compared to their utilization rate during this stage 3435.

4. CONCLUSION

1. The four glutinous rice cultivars exhibit significant differences in compositional content. In terms of component content, cultivars A and B possess higher crude starch content (82.92–78.20 g/100 g), a larger proportion of amylopectin (97.23–97.09%), moderate crude protein content (6.74–6.60 g/100 g), and lower crude fat content (1.14–1.15 g/100 g).
2. During fermentation, the physicochemical indices of the four glutinous rice cultivars follow similar trends: moisture content increases rapidly before saturation, then rises slowly and stabilizes; acidity in fermented grains first rises slowly and then declines; reducing sugar content in fermented grains initially increases before decreasing; starch content declines rapidly in the early fermentation stage and then stabilizes. Cultivar B demonstrates the highest starch utilization rate and ethanol yield, showing significant differences from other cultivars and superior brewing characteristics.
3. Correlation analysis indicates that cultivars with higher amylopectin content exhibit higher starch utilization rates and ethanol yields. Ethanol yield shows significant positive correlations with starch utilization rate and crude starch content, a significant negative correlation with fat content, and a negative correlation with protein content. Starch utilization rate exhibits a highly significant positive correlation with amylopectin content, positive correlations with total starch and protein content, and a negative correlation with fat content.

In summary, the four glutinous rice cultivars differ significantly in compositional content and brewing characteristics. Comprehensive analysis of brewing

traits, component content, and physicochemical properties of fermented grains reveals that, under identical xiaoqu brewing conditions, cultivar B achieves the highest ethanol yield, high starch utilization efficiency, and the second-highest amylopectin content. Thus, cultivar B demonstrates optimal compositional properties and brewing performance, making it the most suitable choice for xiaoqu baijiu production.

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Cite This Article: Fei Kang, Lijuan Gong, Bowen Lin (2025). Comparative Analysis of Brewing Characteristics across Four Glutinous Rice Cultivars. *EAS J Nutr Food Sci*, 7(3), 100-109.
