RESEARCH ARTICLE

Exploring the biological mechanism of quality changes in fragrant Japonica rice during storage by metabolomics and metagenomics

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Liaoningxiangjing 1396 (*Oryza sativa* L.) is a premium Japonica rice variety widely cultivated in Northeast China. This study investigated the dynamic changes in metabolites and microbial communities during the storage of Liaoningxiangjing 1396 rice grains, providing scientific insights for improving grain storage quality. The study employed a dynamic sampling strategy to integrate non-targeted metabolomics gas chromatography-mass spectrometry (GC-MS) with Internal Transcribed Spacer 1 (ITS1) metagenomic sequencing to investigate the co-evolutionary patterns of metabolites and fungal communities throughout the storage cycle. The results demonstrated that the fatty acid content in rice gradually increased from 12.24 mg/g to 17.63 mg/g during the early storage period (R20 - R12) followed by a slight decrease to 16.28 mg/g in the later period (R11, 16 months). A total of 263 metabolites were identified with carbohydrates (31 species) and lipids/lepidoids (24 species) constituting the largest proportions. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) revealed significant differences in metabolic profiles across storage periods with distinct clusters formed between R20 (initial control, 0 months) and R13 (8 months), and R14 (4 months) and R12 (12 months). Fungal community analysis indicated a marked increase in fungal diversity during the later storage period (R12) with *Pantoea* as the dominant genus, accounting for 38.19% of the total abundance. Differential metabolite analysis revealed a significant reduction in sugar compounds such as sucrose, fructose and ester compounds such as methyl 2,8-dimethyldecanoate during storage, while compounds such as 3-acetylbenzonitrile showed upregulation. This study elucidated the dynamic regulatory mechanisms of "fungus-metabolite" interactions during rice storage, providing a theoretical basis for targeted antimicrobial control and quality preservation.

Keywords: fragrant Japonica rice; storage quality; metabolomics; metagenomics; fatty acid value; fungal community; metabolites.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods globally with Liaoningxiangjing 1396 being a high-quality Japonica variety cultivated in Liaoning, China. The quality of stored rice grains deteriorates over time due to complex interactions between intrinsic biochemical processes and extrinsic environmental factors. Understanding the dynamic changes in metabolites and microbial communities during storage is crucial for developing effective grain preservation strategies [1]. Affected by storage and transportation environmental factors, rice is susceptible to fungal contamination, leading to lipid oxidation and hydrolysis, which are subsequently degraded into glycerol and fatty acids through lipase action. During mold growth,

free fatty acids accumulate and further oxidize into small-molecule substances such as aldehydes and ketones [2]. Compared to proteins and starch, lipids are more prone to decomposition [3]. Additionally, mycotoxins such as aflatoxins and deoxynivalenol secreted by fungi along with organic acid metabolites can elevate fatty acid values, cause mold growth, and pose food safety risks. Therefore, fatty acid values serve as a sensitive indicator for assessing rice quality and freshness. Recent studies have demonstrated that storage temperature, humidity, and gas composition are key factors driving fungal community succession and the synthesis of secondary metabolites [4-6].

With the advancement of omics technologies, metabolomics has increasingly demonstrated its role in quantitative detection and qualitative analysis of crop nutritional components [7, 8]. Multi-omics integrated technologies have provided novel perspectives for elucidating the mechanisms of rice grain deterioration. Non-targeted metabolomics enables systematic identification of dynamic changes in metabolites during storage such as the application of gas chromatography-mass spectrometry (GC-MS) for quantitative analysis of rice fatty acid degradation products [9]. Metagenomics reveals the structural succession patterns of fungal communities with Internal Transcribed Spacer 1 (ITS1) sequencing confirming that *Aspergillus* and *Fusarium* are dominant spoilage-causing fungi in stored rice [10]. Recent studies integrating multi-omics data have identified significant correlations between metabolic pathway enrichment and microbial abundance. Johnson *et al*. established a partial least squares discriminant analysis (PLS-DA) model, demonstrating a positive correlation between citric acid cycle dysfunction and *Aspergillus* abundance in rice [11]. Ross and Shepherd combined Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/) annotations to reveal the association between lipid oxidation pathways and *Fusarium* biomarkers [12]. However, existing studies predominantly rely on static time-point sampling, which fails to capture the continuous dynamics of "fungal-metabolite" interactions throughout the entire storage cycle [13, 14]. The temporal patterns of fungal community succession and metabolite accumulation remain unclear, making it difficult to identify critical turning points for quality deterioration [15]. Single-omics approaches struggle to decipher the multidimensional "fungal-metabolite-environment" network, thereby limiting the reliability of predictive models [16].

This study used Liaoningxiangjing 1396 as research subject and employed a dynamic sampling strategy to integrate non-targeted metabolomics GC-MS with ITS1 metagenomic sequencing to elucidate the co-evolutionary patterns of metabolites and fungal communities throughout the storage cycle, identify key metabolic pathways and functional microbial communities driving quality deterioration, establish a multidimensional "fungus-metabolite-environment" correlation model, and pinpoint critical regulatory thresholds in storage. The research provided molecular targets for targeted antimicrobial suppression and held significant practical value for reducing post-harvest losses and ensuring food security.

Materials and methods

Test materials and storage protocol

Liaonigxiangjing 1396, a rice cultivar harvested in October 2023, was obtained from Liaoning Provincial Rice Research Institute (Shenyang, Liaoning, China). The rice grain moisture content ranged from 21.2% to 24.5% measured post-harvest by using HR73 moisture analyzer (Mettler Toledo, Columbus, OH, USA) with impurities ≤ 0.3%. The samples were transferred to a storage bin of 1.1 × 1.1 × 1.8 m^3 (L × W × H) to simulate the storage environment for 16 months at the temperature of 17 – 25°C and relative humidity 45 – 75% through ventilation control. The grain pile height was set at 1.2 m, and the simulated storage was conducted according to the standard for actual storage bins. 150 g of samples were

collected every 4 months with the sampling stages being labeled as initial control (0 month) (R20), 4 months (R14), 8 months (R13), 12 months (R12), and 16 months (R11). The samples were divided into three parallel aliquots with 50 g of each and stored in sterile homogenization bags at 4°C.

Sample preparation

The rice was pulverized and ground through a 0.2 mm sieve to obtain a powdered sample. 50 mg of sample was mixed with 0.5 mL of methanol-water solution at the volume ratio of CH_3OH:H_2O = 4:1 containing 0.02 mg/mL of L-2-chlorophenylalanine as internal standard in a 2 mL grinding tube before adding a steel bead and 200 µL of chloroform. The mixture was grinded in a −20°C grinder at 50 Hz for 3 min twice. Ultrasonic extraction was then performed for 30 min followed by 30 min at −20°C. The sample was centrifuged at 13,000 rpm, 4℃, for 15 min. The supernatant was transferred to a glass derivatization bottle followed by drying under nitrogen. 80 µL of 15 mg/mL pyridine hydrochloride in 8-mercaptopurine was added to the derivatization bottle. After vortexing for 2 min, the sample was incubated at 37°C for 90 min to complete the oximation reaction. 80 µL of N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was then added to the reaction as the derivatization reagent followed by vortexing for 2 min and then reacting at 70°C for 60 min before standing at room temperature for 30 min and proceeding with omics analysis.

Determination of fatty acid value

The fatty acid value (FAV) was determined according to the Chinese National Standard GB/T 20569-2006. Briefly, rice samples were ground and passed through a 40-mesh sieve. 10 g sample was extracted with 50 mL anhydrous ethanol by shaking for 10 min. The extract was filtered, and 25 mL of the filtrate was titrated with 0.01 mol/L potassium hydroxide (KOH) standard solution using phenolphthalein as indicator until a faint pink color persisted for 30 s. FAV was calculated as milligrams of KOH required to neutralize free fatty acids in 100 g of dry sample and expressed as mg KOH/100 g.

Genome DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA of rice sample was extracted by using the FastPure Stool DNA Isolation Kit (Magnetic bead) (MJYH, Shanghai, China) following manufacturer's instructions. DNA fragmentation was conducted by using Covaris M220 (Gene Company, Hong Kong, China) following manufacturer's instructions. DNA fragments approximately 350 bp were used to construct a Paired-End library (PE library). The standard fungal ITS1 fragment (250 bp) was amplified using the primers of ITS5F (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS1R (5'-GCT GCG TTC TTC ATC GAT GC-3'). Magnetic beads were used to screen and remove adapter self-joining fragments, and PCR amplification was employed to enrich the library template. NEXTFLEX Rapid DNA-Seq PCR Kit (Bioo Scientific, Austin, TX, USA) was used for PCR reaction including 25 µL 2× PCR Master Mix, 10 µL DNA template (Adapter-ligated DNA), and 15 µL of specific primers. The PCR program was 95°C for 3 min followed by 8 cycles of 98°C for 20 s, 60°C for 15 s, 72°C for 30 s with a final extension at 72°C for 5 min. PCR products were recovered using magnetic beads, and the library was constructed using NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA) to obtain the final library. Metagenomic sequencing was performed using the Illumina NovaSeq™ X Plus (Illumina, San Diego, CA, USA) sequencing platform in Shanghai Meiji Biomedical Technology Co., Ltd.(Shanghai, China).

GC-MS analysis

Agilent 8890B-5977B GC-MS system (Agilent, Santa Clara, CA, USA) was employed for GC-MS analysis. The derivatized sample was injected into the system in split mode with an injection volume of 1 µL and a split ratio of 15:1. The sample was separated by a DB-5MS capillary column (40 m × 0.25 mm × 0.25 µm) (Agilent, Santa Clara, CA, USA) with the injection port temperature of 260°C, high-purity helium as the

carrier gas at a flow rate of 1 mL/min, a septum purge flow rate of 3 mL/min, and a solvent delay of 5.5 min. The heating program was set as initial temperature of 60°C for 0.5 min followed by a ramp to 310°C at a rate of 8°C/min and being maintained for 6 min. Mass spectrometry was set for electron impact ionization (EI) with transfer line temperature of 310°C, ion source temperature of 230°C, quadrupole temperature of 150°C, and electron energy of 70 eV. The scanning mode was set as full scan (SCAN) with a mass range of m/z 50 - 500 and a scanning frequency of 3.2 scans/s.

Data analysis

The preprocessed data matrix was subjected to principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) using the ropls package (Version 1.6.2) (https://bioconductor.org/packages/release/bioc/html/ropls.html) in R language. The stability of the model was evaluated through 7-fold cross-validation. Variable importance score (VIP) larger than 1 obtained from OPLS-DA and *P* value less than 0.05 from student t-test were used to select significantly different metabolites that were then annotated to identify metabolic pathways using KEGG database (https://www.kegg.jp/kegg/pathway.html). Pathway enrichment analysis was performed using the Python package scipy.stats (https://scipy.org). Fisher's exact test was employed to determine the most biologically relevant pathways associated with experimental treatments [17]. The amino acid sequences of non-redundant gene sets were aligned with the KEGG database using Diamond tool 2.0.13 (https://github.com/bbuchfink/diamond) with BLASTP alignment e-value of 1e-5 [18]. The KEGG functions corresponding to the genes were obtained, and the abundance of functional categories was calculated by summing the gene abundances corresponding to KEGG orthology (KO) pathway, enzyme commission number (EC), and module.

Results and discussion

Changes in rice fatty acid content during different storage periods

The changes in rice fatty acid content during different storage periods demonstrated that samples R20 to R12 exhibited an upward trend, increasing from 12.24 mg/g to 17.63 mg/g. By the later stage of storage, R11 slightly decreased to 16.28 mg/g (Figure 1). Rice is rich in lipids and prone to the degradation and release of fatty acids, leading to elevated fatty acid values, which can reflect the degree of quality deterioration [19]. The results of this study were consistent with the previous studies that the increase in fatty acid values during storage was strongly negatively correlated with quality [20], the changes in fatty acid values were influenced by storage environment, gas composition, moisture, and other factors [21], and the fatty acid values of hybrid rice under different storage conditions initially increased and then stabilized in the later stage [22]. The quality of samples R20, R14, R13, and R12 showed a downward trend, while R11 stabilized. Consequently, samples R20, R14, R13, and R12 were selected for subsequent non-targeted metabolomics and metagenomics analyses.

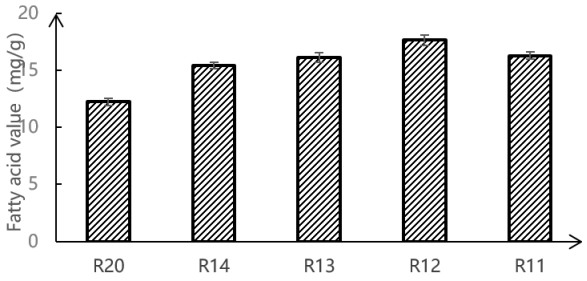

Figure 1. Changes in fatty acid values of rice during storage.

Identification and analysis of rice metabolites at different storage stages

A total of 264 cationic mass spectrometry peaks were detected, and through identification using databases, 263 highly credible metabolites were identified. Based on the HMDB hierarchical classification, the superclass level primarily

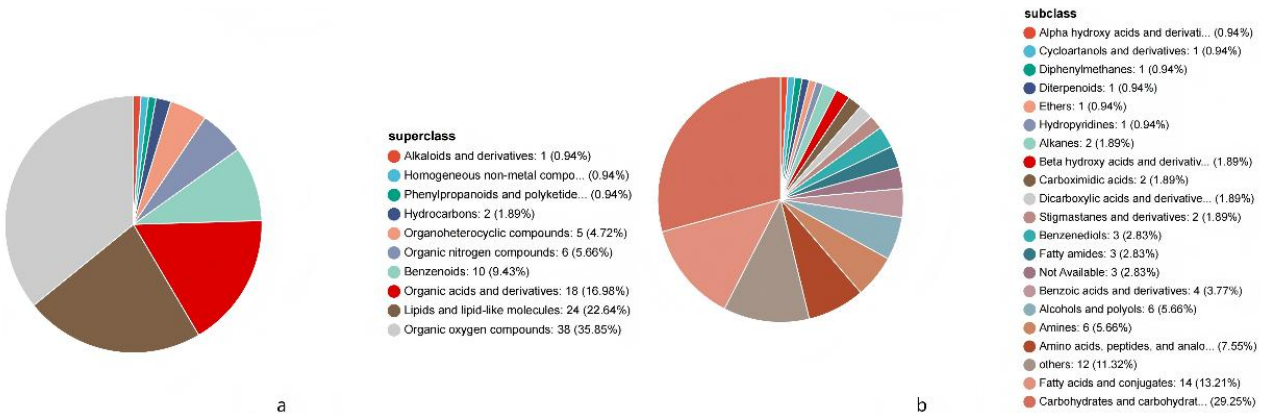

Figure 2. The counts of HMDB chemical taxonomy. **a.** Metabolite classification at the superclass level. **b.** Metabolite classification at the subclass level.

included benzene ring compounds of 10 species, organic acids and their derivatives of 10 species, lipids and lipid-like molecules of 24 species, oxygen-containing organic compounds of 38 species, nitrogen-containing heterocyclic compounds of 6 species, hydrocarbons of 2 species, and alkaloids and their derivatives of 1 species (Figure 2a). Oxygen-containing organic compounds and lipids and lipid-like molecules together accounted for 23.6% of the total, which was consistent with previous report in geographically indicated rice [23], reflecting the core characteristics of the rice metabolome. Oxygen-containing organic compounds, predominantly sugars and alcohols, indicated that stored rice maintained basal metabolism to cope with stress. Lipids and lipid-like molecules, as core components of cell membranes, confirmed that lipids influenced anti-oxidation capacity by maintaining membrane structural stability [24], explaining the stable presence of these metabolites during storage. Organic acids and their derivatives and benzene ring compounds were functionally critical, where organic acids participated in core pathways such as the tricarboxylic acid cycle, and their stable presence suggested that energy pathways were not completely halted. Benzene ring compounds (mostly phenols) delayed lipid oxidation by scavenging reactive oxygen species (ROS), which was consistent with the protective effects of phenols [25]. Nitrogen-containing heterocyclic compounds and alkaloids with their derivatives might participate in nitrogen metabolic balance, providing a foundation for protein stability during storage [26]. Subclass-level statistics revealed that carbohydrates (31 types) were the dominated ones, which were consistent with the metabolic characteristics underlying the formation of superior rice flavor profiles. These metabolites including starch, oligosaccharides, and sugar alcohols not only served as primary energy sources but also exhibited composition-specific effects on rice gelatinization properties and taste scores. Oligosaccharide content positively correlated with viscoelasticity, while sugar alcohols enhanced textural refinement [27]. Fatty amides (3 types), as significant lipid derivatives, demonstrated higher stability than free fatty acids. They mitigated the formation of oxidative byproducts such as hexanal by inhibiting lipoxygenase activity, thereby delaying the "rancid flavor" [28]. Alcohols (6 types) and ether (1 type) might participate in osmotic pressure regulation and antioxidant defense mechanisms with their diversity reflecting the multi-pathway nature of metabolic regulation during storage (Figure 2b). The Venn diagram statistically analyzed the number of shared and unique metabolites across different storage periods. The results showed that R20, R14, R13, and R12 contained 12 shared metabolites with R20 having 11 unique compounds, R14 having 2, R13 having 9, and R12 having 3 (Figure 3). This

variation pattern aligned with the metabolic dynamics during storage. In the initial stage (R20), fresh rice grains were rich in specific metabolites accumulated during the growth phase such as antioxidant phenols and stress-resistant sugar alcohols, resulting in abundant unique metabolites. As storage progresses (R14), some specific metabolites underwent oxidative degradation or transformation, leading to a minimum in unique metabolite quantity. In the middle and late stages (R13), rice grains initiated stress metabolism, generating degradation products or new defensive metabolites, causing a rebound in unique metabolite quantity. In the final stage (R12), metabolic activity slowed down, retaining only a small number of stable unique metabolites. These results were consistent with the studies that metabolite types stabilized during the later storage period [29, 30].

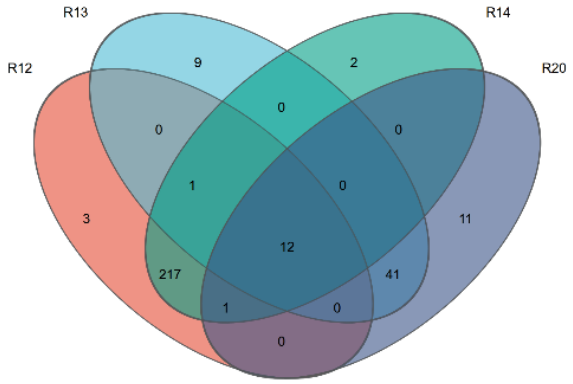

Figure 3. Venn diagram of compounds in rice samples.

Differences in rice metabolites across different storage periods

(1) Principal component analysis (PCA)

The results showed that the four sample groups were significantly dispersed on principal component 2 (PC2) with R20 and R13, R14 and R12 forming distinct clusters, indicating high similarity in metabolic profiles among corresponding groups. Principal component 1 (PC1) accounted for 42.8% of the variance, serving as the primary factor explaining metabolic differences between samples. All sample points were distributed within the 95% confidence ellipse, demonstrating good data reproducibility and compliance with metabolomics standards [31]. This clustering pattern aligned with the metabolic storage patterns of rice, where samples with similar metabolic characteristics tended to cluster together [32]. The combination of univariate and multivariate statistics effectively interpreted high-dimensional data, laying the foundation for comprehensive extraction of metabolic differences. Clear inter-group separation and intra-group clustering in PCA were essential prerequisites for screening differential metabolites [33]. The clustering of R20 and R13, R14 and R12 suggested that they might represent consecutive metabolic stages during storage with R20 (early stage) and R13 (mid-to-late stage) likely retaining conserved pathways that delayed quality deterioration, while R14 (mid stage) and R12 (late stage) might undergo similar metabolic transitions associated with decline [34]. This finding was consistent with the grain storage metabolomics clustering patterns [35].

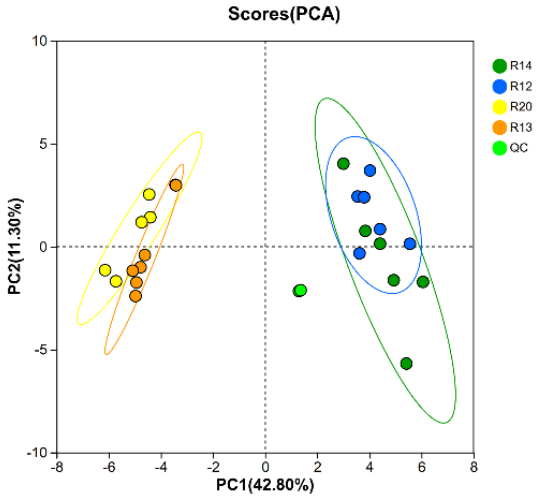

Figure 4. Principal component analysis (PCA) of aroma metabolites in rice at different storage periods.

(2) Partial least squares discriminant analysis (PLS-DA)

To further analyze the differences in metabolites R12, R13, R14, and R20, a PLS-DA model was constructed. Orthogonal comparison revealed

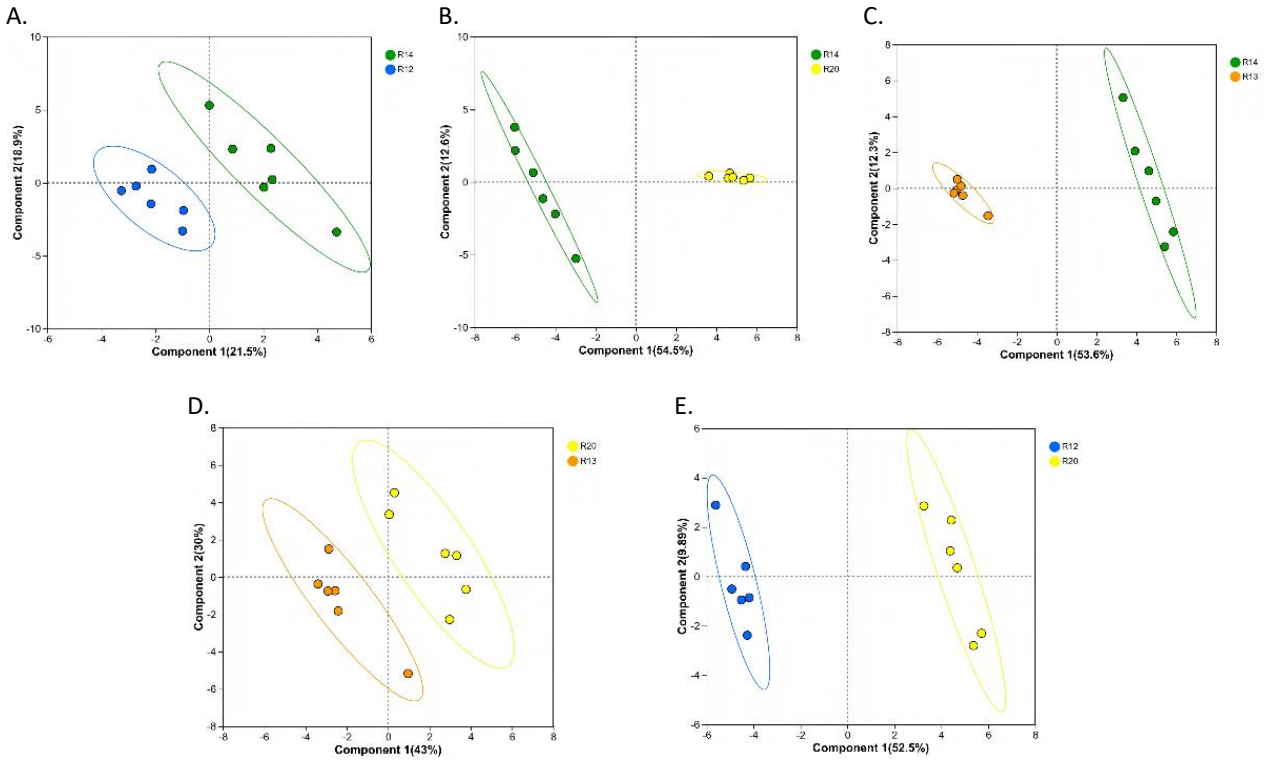

Figure 5. Distribution of PLS-DA metabolites in rice at different storage periods.

significant differences in the composition of aroma metabolites between R14 and R12, while R13 and R20 showed no distinct differentiation in their metabolic profiles, consistent with the PCA results (Figure 5). The permutation test (n = 200) results demonstrated that the regression line (Q2) and intercept on the Y-axis were < 0.05 (R^2 = 0.7221, Q^2 = 0.8972), indicating robust and reliable model performance without overfitting, making it suitable for differential metabolite screening [36] (Figure 6). PLS-DA enhanced the ability to distinguish between groups, which was crucial for identifying storage-stage-specific metabolic markers. The significant separation between R14 and R12 suggested that this interval might involve key metabolic transitions including core pathways such as lipid oxidation and carbohydrate degradation, which drove quality deterioration [37]. The metabolic profile similarity between R13 and R20 indicated that the R13 stage retained some early metabolic characteristics with a relatively slow rate of quality deterioration, which was consistent with the study of Johnson *et.al*. that antioxidant metabolites persistently delayed deterioration during the mid-storage period [38]. The clustering patterns observed in PCA and PLS-DA aligned closely with the theoretical framework regarding the dynamics of rice storage metabolism that cumulative effects such as oxidative stress and microbial activity during the later stages accelerated metabolic differentiation. [39].

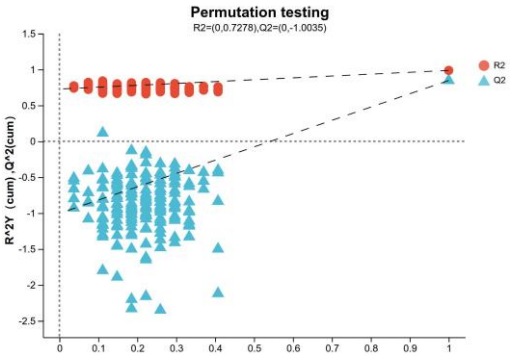

Figure 6. Permutation testing plots map of the PLS-DA.

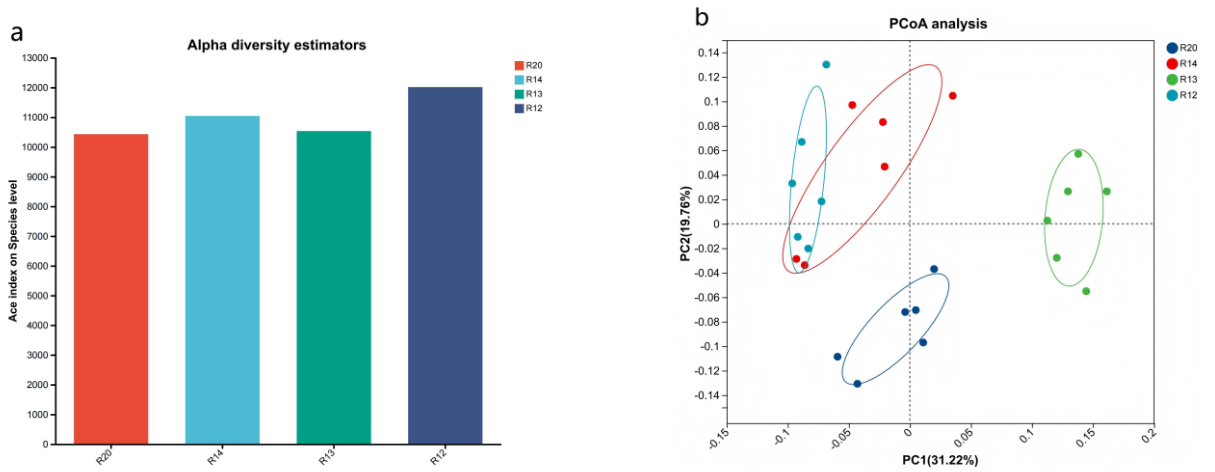

Figure 7. Comparison of fungal communities in rice stored at different periods. **a.** α-diversity analysis. **b.** Principal component analysis (PCA) score plot.

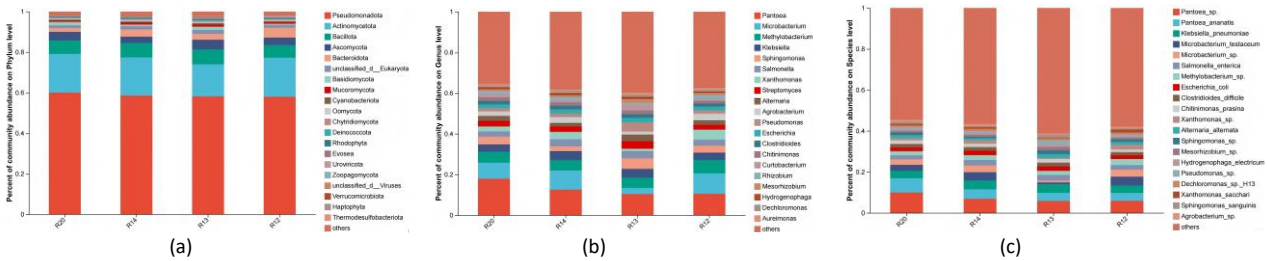

Figure 8. The microbial community composition of rice samples at the phylum (a), genus (b), and species (c) levels.

Differences in fungal community structure composition of rice at different storage periods

The PCA analysis revealed significant separation of fungal community composition among the four sample groups with R20 showing the most pronounced distinction from other stages, indicating a unique early community structure (Figure 7a). α-diversity analysis demonstrated that R12 exhibited the highest ACE index and the richest fungal diversity, consistent with the pattern of fungal proliferation and increased species richness observed with prolonged storage duration [40] (Figure 7b). The high fungal diversity in R12 during the later storage period might be attributed to favorable conditions created by changes in rice moisture and nutrient degradation such as starch hydrolysis providing sufficient carbon sources for monosaccharide formation [41]. The phylum-level community composition showed the dominant phyla in descending abundance as *Pseudomonadota*, *Actinomycetota*, *Bacillota*, *Ascomycota*, and *Bacteroidota*. Pseudomonadota was the absolute dominant phylum. Compared to R20, R12 showed significantly increased abundance of *Actinomycetota* and *Bacteroidota*, reflecting their proliferative dominance during the later stage [42]. The absolute dominance of *Pseudomonadota* aligned with the widespread distribution of Gram-negative bacteria in grain storage environments. Some species of the *Pseudomonas* genus possessed extracellular enzyme activity, which could degrade rice proteins, lipids, and other nutrients, potentially accelerating quality deterioration [43] (Figure 8a). At the genus level, *Pantoea* was the dominant genus with a relative abundance of 38.19%, while *Aeromonas* exhibited extremely low abundance (Figure 8b). The species-level analysis revealed that *Pantoea* sp., a dominant

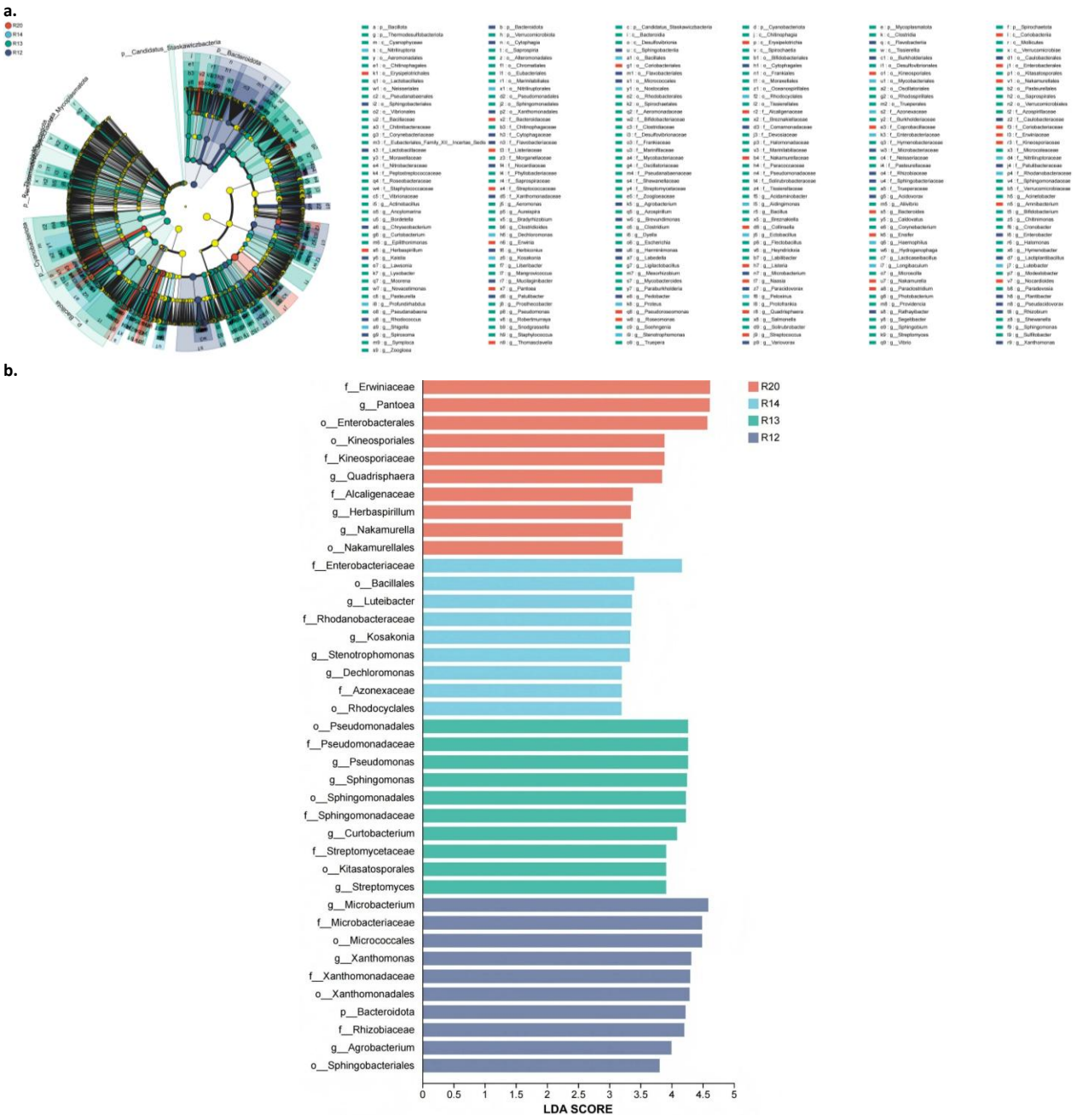

Figure 9. Comparison of fungal communities in rice stored at different periods. **a.** LEfSe analysis. **b.** LDA score plot.

species (36.17%), was identified as a core biomarker of fungal communities during rice storage with its abundance changes serving as a key indicator for characterizing the storage phase [44] (Figure 8c). Some species of *Pantoea* were endophytic fungi, while others might become pathogenic under storage conditions. The sustained increase in their abundance might be associated with elevated mold risk [45]. Previous Study indicated that metabolites produced by *Pantoea* sp. could influence grain flavor, and their biomarker properties provided potential targets for developing rapid early-warning technologies for storage quality [46]. Kruskal-Wallis sum-rank test was used to screen differences in species abundance for multi-group comparisons, while

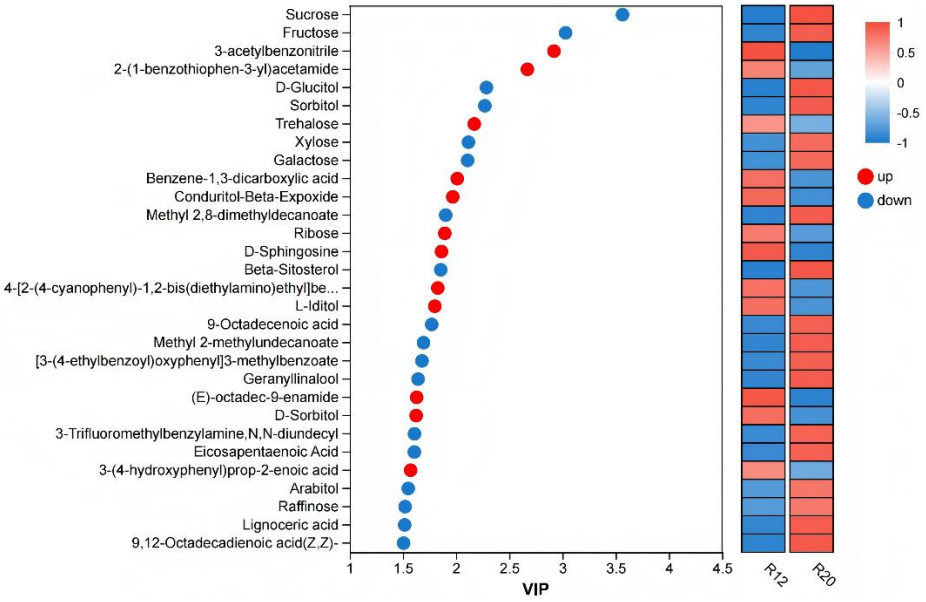

Figure 10. Variable importance in projection (VIP) scores of rice samples R12 and R20.

Wilcoxon rank-sum test for two-group comparisons. The consistency of differential species within subgroups was further validated by Wilcoxon rank-sum test combined with linear discriminant analysis effect size (LEfSe) analysis for biomarker identification (Figure 9a). A total of 38 clearly annotated differential biomarkers excluding unannotated species were identified with LDA scores ranging from 0 to 4.61. Among these, 19 high-influence biomarkers with LDA scores > 4 played a critical role in distinguishing fungal communities at different storage stages [47] (Figure 9b). The accumulation of amino acid metabolites during late storage might promote the proliferation of *Bacteroides*, while lipid oxidation products provided nutrients for *Actinomycetes* [48]. This "metabolite-microorganism" interaction collectively drove changes in rice storage quality [49].

Correlation analysis between dominant bacterial communities and major volatile compounds

The expression patterns of metabolites in the differential groups across samples and their contribution to classification (VIP value) showed that a total of 30 major differential metabolites with VIP > 1 were identified in R12 and R20. Upregulated metabolites in R12 included 3-acetylbenzocyanonitrile, 2-(1-benzothiophene-3-yl) acetamide, trehalose, phenyl-1,3-dicarboxylic acid, condurito β-epoxide, ribose, ceramide, 4-[2-(4-cyanophenyl) - 1,2-bis (2-ethylamino) ethyl] benzocyanonitrile, edulitol, (E)-octadecen-9-enamide, D-sorbitol, and 3-(4-hydroxyphenyl) prop-2-enoic acid. Downregulated metabolites in R20 included sucrose, fructose, sorbitol, sorbitan, xylose, galactose, 2,8-dimethyldecanoate, phytosterol, 9-octadecenoic acid, 2-methylundecanoate, 3-[(4-ethylbenzoyl) oxyphenyl] 3-methylbenzoate, linalyl camphorol, 3-trifluoromethyl benzylamine, N,N-dodecyl, eicosapentaenoic acid, arabinitol, raffinose, lignotaric acid, and 9,12-octadecadienic acid (Z, Z) (Figure 10). Among them, sucrose dominated the flavor profile of rice grains, contributing the most to the sweetness of high-quality Japonica rice varieties such as Liaoxing and Daohuaxiang No.2. Its taste activity value (TAV) > 1 was identified as the primary sweetener [50]. Sucrose not only directly contributed to sweetness but also participated in the starch-sucrose metabolic pathway, positively correlating with gelatinization temperature and indirectly affecting the smoothness of rice texture [51]. Studies have confirmed that significant sucrose

reduction under high-temperature or long-term storage conditions leads to diminished rice sweetness and a bland flavor profile. Xylose and galactose, as auxiliary sweeteners with sweetness approximately 1/5 that of sucrose, primarily influenced flavor by modulating the "sweetness perception" and reducing stickiness. Their content decreased with prolonged storage, synchronizing with carbohydrate metabolic disorders and potentially leading to a monotonous flavor profile [52]. Raffinose with the sweetness approximately 22 - 30% of sucrose did not strongly contribute to sweetness but enhanced the "soft and dense" texture of rice, improving its mouthfeel [53]. A decline in its content resulted in a shift from "soft and fluffy" to "dry and hard" texture, affecting fullness. 2,8-Dimethyldecanoate, 2-methylundecanoate, and 3-[(4-ethylbenzoyl)oxyphenyl]3-methylbenzoate were ester compounds. The fruit aroma contribution of branched fatty acid methyl esters was a crucial component of rice fragrance. 2,8-Dimethyldecanoate and 2-methylundecanoate exhibited strong volatility, imparting fresh fruit aromas such as "apple" and "pear" to rice grains [54], serving as characteristic aromatic components of high-quality fragrant rice and playing a pivotal role in distinguishing variety-specific aroma profiles [55]. This study confirmed that these ester compounds were prone to volatilization during storage, resulting in a shift from "rich" to "mild" aroma [56]. The structure of 3-[(4-ethylbenzoyl)oxyphenyl] 3-methylbenzoate was complex and had low volatility, suggesting a potentially limited direct contribution to flavor profile [57]. Phytosterols exhibited potent antioxidant properties, inhibiting lipid oxidation and reducing the formation of "rancid" flavors [58]. Studies indicated that phytosterols influenced cell membrane integrity by modulating membrane fluidity, and their increased content helped maintain cellular structural stability under drought stress, indirectly protecting flavor compounds [59]. During storage, the decline in their content weakened lipid antioxidant capacity, accelerating flavor deterioration. Linalool, a key compound that distinguished fragrant rice varieties, possessed strong "lily" and "rose" aromas as a terpene, serving as the characteristic fragrance for varieties like jasmine and rice flower [60]. It exhibited high aroma activity value (OAV) and significant contribution [61]. This study confirmed its easy loss during storage, leading to the weakening of "floral characteristics" in rice grains and reduced recognizability [62].

Conclusion

This study elucidated the biological mechanisms underlying quality deterioration in Japonica rice during storage through an integrated analysis of metabolomic and metagenomic profiles. The results demonstrated that rice quality degraded significantly over time, characterized by distinct shifts in metabolic signatures and fungal community structures. The observed decline in key flavor-related metabolites directly impacted sensory attributes, while the dynamic interplay between microbial ecology and metabolic pathways revealed a complex regulatory network. These insights provided a theoretical foundation for developing targeted strategies to suppress microbial activity and preserve grain quality. Future research should expand to include diverse rice genotypes to establish broader applicability and inform the selection of storage-resistant cultivars.

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