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Effect of Storage Conditions on the Stability of Rice Fragrance and Protein Content in Phka Rumdoul Rice Variety

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Abstract: *Rice (Oryza sativa L.) is a vital global staple, with aroma being a key factor in its market value. The compound 2-acetyl-1-pyrroline (2-AP) is the primary contributor to the fragrance of aromatic rice. However, storage conditions significantly impact the aromatic and nutritional quality of rice. This study evaluated the effects of temperature and duration on 2-AP retention, protein stability, and moisture content in Cambodia's fragrant Phka Rumduol rice over 12 weeks. Samples were stored at 4°C, room temperature (RT), and 40°C, and analyzed using near-infrared spectroscopy for protein and moisture content and gas chromatography–mass spectrometry for 2-AP quantification. Results revealed a significant decline in 2-AP content, particularly at 40°C, where 2-AP levels decreased by 67.44%, compared to losses of 46.71% at 4°C and 52.46% at RT. High temperatures also caused protein denaturation and increased moisture volatilization, while cold storage at 4°C effectively preserved 2-AP levels by minimizing these losses. Although storing rice at RT resulted in moderate 2-AP degradation, it remains a feasible and cost-effective option with appropriate packaging. The study underscores the necessity of optimizing storage temperature and duration to preserve the aromatic and nutritional quality of fragrant rice. Future research should investigate advanced storage technologies, particularly enhanced packaging, to further reduce quality deterioration during post-harvest.*

Keywords: Aromatic rice; Phka Rumdoul variety; 2-acetyl-1-pyrroline; Gas chromatography-mass spectrometry; Near-infrared spectrometry.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important staple foods [1]. Rice fragrance, also known as *aroma*, is a desirable trait in many rice varieties [2]. Rice aroma comes from volatile compounds and is a key trait affecting its market value [3]. In Cambodia, rice is the most important crop for farmers, with rice paddies occupying 75% of the total cultivated land [4]. Phka Rumduol is a popular fragrant long-grain rice variety widely grown in Cambodia. Developed and released by the Cambodian Agricultural Research and Development Institute (CARDI), this variety is highly valued for its pleasant aroma and soft texture [5]. The compound 2-acetyl-1-pyrroline (2-AP) was identified as the main aroma component of rice in 1982 by Ron Buttery and his team. This unstable chemical, found prominently in Basmati and Jasmine varieties, gives rice its distinctive, popcorn-like fragrance [6]. Fragrant rice has a mutation in the *BADH2* gene, which disables the corresponding enzyme. This [1] failure allows precursors, such as L-proline, to accumulate and form 2-AP, the compound responsible for rice's unique aroma [7]. A previous study showed that lower grain-filling temperature significantly increases the 2-AP level [8]. 2-AP levels in rice vary by variety and are influenced by environmental, microbial, and post-harvest factors like drying and storage [9]. Phka Rumduol rice has

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high 2-AP levels, 0.8 µg/g in CARDI samples and 0.38 µg/g in commercial rice [4].

Sample extraction is the first critical step in analyzing volatile compounds in rice, including 2-AP [10]. Aroma compound analysis involves extraction, concentration, separation, and quantification. Delays in detecting 2-AP were likely due to limitations in isolation methods [11]. Modern methods use HS-SPME or SHS for extraction, followed by GC with FID or MS to separate and identify aroma compounds [12]. Ethanol is ideal for extracting 2-AP because it is safe, moderately polar, and effective with sonication and mild heating. Its low boiling point also allows easy removal before analysis. [13]. Previous studies showed that ethanol combined with ultrasonic treatment at elevated temperatures provides significantly higher 2-AP recovery than other solvents [14]. Gas Chromatography-Mass Spectrometry (GC-MS) is a technique that is used to identify and separate various volatile compounds typically found in rice [15]. GC-MS is the main method used to analyze volatile compounds, including 2-AP in rice [16]. In addition, the protein content in Cambodian milled rice is typically 4–12% (microKjeldahl method). The protein can denature or degrade over time due to oxidative reactions or Maillard browning during improper storage [17]. Rice protein content varies with storage conditions and whether it's stored as paddy, brown, or polished rice [18]. Storing rice as brown rice preserves more protein, vitamins, and antioxidants than polished rice due to the intact bran layer [19]. Polishing reduces protein and vitamins in rice, making it more vulnerable to storage degradation. [20]. During storage, the rice loses free amino acids, causing browning, while enzyme activity shifts amylase decreasing and protease and lipase increasing [21]. The storage time and temperature have a major impact on the nutritional composition of rice. The temperature changes can alter enzyme activity, which in turn affects the levels of protein, starch, and lipids in the stored grains [22]. In addition, the moisture content (12–13%) is crucial in rice storage to prevent deterioration, microbial growth, and maintain quality.[23,24]. Researchers now aim to preserve whole grains' nutritional quality during storage to meet consumer demands [25].

Given that the storage time and temperature significantly influence rice's aroma and nutritional quality, this study aims to evaluate the quality of fragrant rice by investigating the impact of storage conditions (temperature, time) on fragrance retention and nutritional quality.

2. METHODOLOGY

2.1 Materials and chemicals

The Phka Rumdoul rice was collected from Preah Vihear Province. The 2-AP standard was obtained from the Ubon Ratchathani Rice Research Center, Thailand. The analysis was conducted using HPLC-grade ethanol (Fisher ChemAlart Guide, Korea). Helium (purity ≥ 99.999%) was used as carrier gas for the analysis of 2-AP by GC-MS (TVC Gas Solution, Vietnam).

2.2 Sample collection and experimental design

A total of one hundred kilograms of Phka Rumdoul rice was collected from two locations in Preah Vihear Province, Cambodia, in January 2025. Fifty kilograms were sourced from Tmat Paey, Choam Khsan district (13° 58′ 25.83″ N, 104° 53′ 1.27″ E). The remaining fifty kilograms were obtained from Dang Plet, Chhaeb District (13°51'49.0"N 105°16'19.6" E). After collecting samples from both locations, they were thoroughly mixed to make them uniform before starting the experiments (the research primarily focused on method optimization and observed changes in 2-AP during storage, which required a large quantity of samples, approximately 100 kg, which could not be obtained from a single farmer). The experiments were designed with two factors. The first factor consists of three storage conditions: 4°C (to inhibit microbial growth and enzymatic activity, commonly for preserving grains), room temperature (28±2°C), and elevated temperature at 40°C (to accelerate potential degradation processes, simulating warmer storage in warehouse or transportation conditions). The second factor involves periodic experimentation, with data collection every two weeks over 3 months (12 weeks). The samples were packed in zip-lock bags, each containing 100 g of material (individual samples for each time point and condition), which were then used throughout the study. This experimental design aimed to evaluate the impact of storage conditions on the quality and characteristics of Phka Rumdoul rice over time. For 2-AP and nutrition analysis, 100 g of rice seeds were initially prepared, and the husk was removed, followed by rice polishing to produce white rice.

2.3 Moisture and protein content analysis

The analysis of moisture and protein content (in dry mass) in rice samples, which used the NIR Rice Composition Analyzer Model AN-920, was conducted through a straightforward yet precise process. Initially, the rice sample was carefully prepared to ensure it was clean and free from contaminants. Typically, 80 g of white rice was used for analysis. Before the analysis was conducted, the analyzer was calibrated to ensure accurate measurements and reliable results for the specific rice variety being studied. Known reference standards were used during calibration. Once calibrated, the prepared rice sample was placed into the designated compartment of the analyzer, ensuring even distribution for optimal results. The analysis was then initiated by selecting the desired parameters, such as protein and moisture content. The NIR spectrometer scanned the sample and generated a near-infrared spectrum, which was processed by the software to quantify the composition of the

rice sample. After the analysis was completed, the software provided a detailed report, offering measurements for the protein and moisture content.

2.4 2-AP analysis

2.4.1 Sample extraction

White rice was then ground into a fine powder (model CT 293 Cyclotec™ was purchased from FOSS, Denmark) with an average particle size of 0.5 mm. Approximately 2 g of the powdered sample was weighed and transferred into a 15 mL Falcon tube. 2 mL of ethanol was added, and the tube was sealed with parafilm. The mixture was then shaken and vortexed. The tube was subsequently placed in an ultrasonic bath set to 80°C, where it underwent sonication at maximum speed for 30 minutes using an ultrasonic bath (Elmasonic Select 30, Elma Schmidbauer, Germany) with a 50/60 Hz frequency. The sample was vortexed during sonication every 2 minutes to maintain even dispersion. After the sonication process, the sample was stored at –20°C for 15 minutes to allow the aroma compounds to stabilize. Following this, the mixture was gently inverted to ensure complete homogenization. The sample was then centrifuged at 1,800 g for 5 minutes, and the liquid phase was carefully separated using liquid extraction. The liquid was passed through a syringe filter with a pore size of 0.45 µm and collected into a 2 mL vial, which was sealed with parafilm. Finally, the vial was stored at –20°C until it was ready for GC-MS analysis.

2.4.2 Gas chromatography-mass spectrometry analysis

The GC-MS analysis was performed using a Shimadzu GC-MS-TQ8040 coupled to a GC-2010 Plus. This instrument was equipped with an AOC-20S autoinjector and -20i autosampler, manufactured by Shimadzu (Japan). The injection mode was splitless, with an injection volume of 1 µL. The capillary column was DB-5ms (30 m x 0.25 mm inner diameter, 0.25 µm film thickness, Agilent, USA). The oven temperature programmed was initially set at 40°C with a holding time of 1 min and then increased to 310°C (8°C.min^{-1}) with a holding time of 4 mins. The injector and interface temperatures were 250°C and 270°C, respectively. Helium (purity ≥ 99.999%) was used as a carrier gas with a flow rate of 50 mL.min^{-1}. For MS, the electron impact energy (EI) was 70 eV. The ion source and interface temperatures were 200°C and 300°C, respectively. The sample was analyzed in full-sim mode within the range of m/z 45-600 to confirm in the spectral library search the presence of 2-AP compounds. The 2-AP was identified by a data treatment system and computer that calculated the monoisotopic mass and predicted the structural formula of the compound, and then compared it using the MS database.

2.5 Statistical analysis

All statistical analyses were conducted using R Studio (version 4.4.2). Descriptive statistics, including means and standard deviations, were calculated for 2-AP, protein, and moisture content across different storage temperatures and time points. A two-way analysis of variance (ANOVA) was performed for each parameter to assess the main effects of storage temperature and storage time, as well as their interaction. The assumption of homogeneity of variances was evaluated using Levene's test. Post hoc comparisons were carried out using Tukey's Honestly Significant Difference (HSD) test to identify specific group differences. Statistically significant differences ($p < 0.05$) were indicated using compact letter displays (CLD). Data manipulation and visualization, including the generation of line graphs with error bars, were performed using the dplyr and ggplot2 packages. The car package was utilized for Levene's test, while the agricolae package facilitated ANOVA and Tukey HSD tests with compact letter displays. The ggpubr package was employed for plot arrangement.

3. RESULTS AND DISCUSSION

3.1 Effect of storage conditions on the moisture content of rice

Moisture content is a critical parameter in rice storage, as it directly impacts grain deterioration and susceptibility to microbial growth [23]. Monitoring moisture content is essential as it regulates the rate of enzymatic and oxidative reactions during storage [27,28]. Moisture loss at high temperatures promotes 2-AP volatilization [29,30] and can induce structural changes in rice proteins [31,32], thus indirectly affecting both aroma and nutritional quality. Rice is typically dried to a moisture content of 12–14% before milling. For long-term storage, this is further reduced to a range of 9–12%, which is simplified by NIR, to minimize spoilage and maintain quality [32]. In this study, the moisture content was measured to define the correlation between moisture to the rice protein and aroma during storage in Phka Rumdoul rice.

As shown in **Table 1**, the moisture content in Phka Rumdoul rice changed during storage at different temperatures. At low and room temperatures, the moisture content showed a similar pattern within 12 weeks of storage. In contrast, the high-temperature storage showed a statistically significant difference from the low and room temperatures after 6 weeks of storage. All experimental conditions started with an initial moisture of 11.43±0.05%. Within 12 weeks of storage, the moisture content decreased by about 9.32%, 7.5%, and 16.3% of the initial moisture for the storage temperatures at low, room, and high temperatures,

respectively (**Table 3**). At 4°C, the moisture content stayed pretty steady from week 0 to week 6, and slightly decreased from week 6 to week 12. This means not much water was gained or lost in this storage temperature. However, at 40°C, the moisture content changed more, often showing a slight decrease between week 0 (11.43±0.05%) to week 12 (9.6±0.17%). This is because water evaporates faster at higher temperatures. The main reason for moisture changes in stored rice is the exchange of water vapor between the rice and the air around it. If the air is drier than the rice, water will leave the rice, and if the air is wetter, the rice will absorb water. This exchange depends on how much water vapor is in the air compared to the sample [26]. Higher temperatures make water evaporate much faster from the rice into the air [33]. Significant differences in moisture content were found between high temperature (40°C) and the lower temperatures, especially after many weeks of storage. The stable moisture content observed at both 4°C and room temperature indicates that these conditions are effective for maintaining the desired water content in rice. Notably, selecting room temperature storage presents a more cost-effective solution compared to refrigerated conditions (4°C).

Table 1. Variation of moisture and protein content over time, depending on storage temperature.

Storage Time (Weeks)	Moisture content (%)			Protein content (%)		
	4°C	RT	40°C	4°C	RT	40°C
0	11.43 ± 0.06^{Aa}	11.43 ± 0.06^{Aa}	11.43 ± 0.06^{Aa}	8.43 ± 0.12^{Aa}	8.43 ± 0.12^{Aa}	8.43 ± 0.12^{Aa}
2	11.43 ± 0.06^{Aa}	11.30 ± 0.10^{Bb}	11.17 ± 0.06^{Cb}	7.47 ± 0.06^{Cf}	8.10 ± 0.00^{Ab}	7.73 ± 0.06^{Bb}
4	11.07 ± 0.07^{Ab}	11.07 ± 0.06^{Ac}	10.83 ± 0.23^{Bc}	7.70 ± 0.00^{Bb}	7.80 ± 0.00^{Ac}	7.60 ± 0.00^{Cc}
6	10.97 ± 0.25^{Ac}	10.80 ± 0.10^{Bd}	10.30 ± 0.10^{Cd}	7.67 ± 0.06^{Ac}	7.70 ± 0.00^{Ad}	7.57 ± 0.06^{Bd}
8	10.63 ± 0.06^{Ad}	10.27 ± 0.25^{Bg}	10.10 ± 0.00^{Ce}	7.63 ± 0.06^{Ad}	7.67 ± 0.06^{Ad}	7.53 ± 0.06^{Be}
10	10.70 ± 0.00^{Ad}	10.67 ± 0.12^{Ae}	9.67 ± 0.12^{Bf}	7.67 ± 0.06^{Ac}	7.60 ± 0.00^{Be}	7.53 ± 0.06^{Ce}
12	10.37 ± 0.12^{Be}	10.57 ± 0.06^{Af}	9.60 ± 0.17^{Cg}	7.60 ± 0.00^{Ae}	7.53 ± 0.06^{Bf}	7.40 ± 0.00^{Cf}

Values are expressed as mean ± standard deviation of triplicate analysis.

$^{A-C}$ Values bearing different superscript uppercase letters within the same rows are significantly different (Tukey HSD post hoc test, $p > 0.05$)

$^{a-g}$ Values bearing different superscript lowercase letters within the same columns are significantly different (Tukey HSD post hoc test, $p > 0.05$)

3.2 Effect of storage conditions on protein content of rice

Protein content in rice is a crucial nutritional parameter, contributing significantly to dietary nitrogen intake. However, like other nutritional values, rice protein is susceptible to degradation during storage [27,28]. Protein stability during storage also affects the availability of precursors involved in 2-AP biosynthesis, particularly through amino acid pathways such as proline and GABA [7, 8]. Although rice protein is not the main protein for daily intake, it could be one of the markers to measure the effect of temperature and storage time on the nutritional value of rice. Therefore, this study also evaluated the impact of storage conditions on the proximate value of protein in Phka Rumdoul rice using NIR as described in Section 2.3. Protein content in Cambodian milled rice is typically 4% to 12%, which was analyzed by the microKjeldahl method [35]. Another study showed that protein content in rice samples ranged from 5.90% to 14.50% by using near-infrared diffuse reflectance spectroscopy (NIDRS) [36].

As shown in **Table 1**, there was not much difference in the change of protein after two weeks of storage at three different temperatures. The data indicate that protein content exhibited relatively stable levels at lower temperatures (4°C and RT) throughout the storage period. In comparison, a slight decrease or alteration was observed at 40°C, particularly towards the later weeks. The significant protein loss at week 2, particularly at 40°C, likely reflects early-stage degradation due to rapid denaturation and protease activity [22,31]. Early changes, like protein bonding and clumping, make the proteins harder to dissolve and reduce the accuracy of NIR detection [31,32]. After week 2, the rate of protein loss slowed, likely because the more reactive protein fractions had already degraded, while the remaining proteins became structurally stabilized and less susceptible to further breakdown under continued storage conditions [31,37]. At week 12, protein content at 40°C (7.4±0.00%) was significantly lower than at 4°C (7.6±0.00%) and room temperature (7.53±0.05%). High temperatures can accelerate protein denaturation and aggregation, leading to reduced solubility and changes in functional properties [37]. During

storage, especially when temperatures are high, rice protein changes by losing free sulfhydryl groups and gaining disulfide bonds. This process causes the proteins to clump and compact, making them harder to digest and negatively affecting the rice's texture [30]. Even though the total protein amount doesn't change, the structural changes during storage, like proteins clumping and tightening, make the rice harder and stickier, reducing its cooking and eating quality [34]. The decline and alteration observed at 40°C showed that high temperatures damage the integrity of rice proteins, causing a quality loss known as the "hardening" or "aging" effect.

3.3 Effect of storage conditions on 2-AP content in rice

The distinct fragrance of aromatic rice, primarily attributed to 2-AP, was previously studied. The concentration of 2-AP in rice samples extracted with ethanol by the ultrasound-assisted solvent extraction (UASE) method ranged from 0.0194 to 0.1240 µg/g [38]. Phka Rumduol rice has high 2-AP levels, 0.8 µg/g in CARDI samples and 0.38 µg/g in commercial rice [4]. In this study, a method for the extraction of 2-AP by ethanol was developed, as described in the method session, and the impact of storage conditions on the 2-AP content in Phka Rumdoul rice was evaluated.

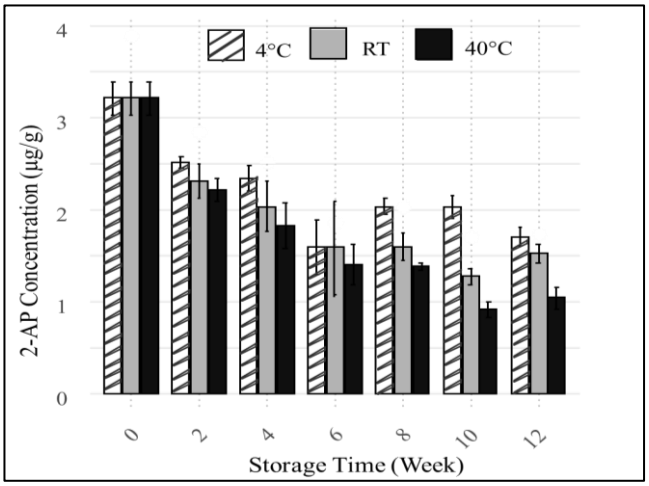

Fig. 1. Variation in 2-AP content over time, depending on different storage temperatures. The bars in the graph represent the mean ± standard deviation of the triplicate analysis.

Table 2. Variation of 2-AP content over time**,** depending on storage temperature.

Storage Time (Week)	2-AP Content (µg/g)		
	4°C	RT	40°C
0	3.21 ± 0.17^{Aa}	3.21 ± 0.17^{Aa}	3.21 ± 0.17^{Aa}
2	2.52 ± 0.06^{Ab}	2.31 ± 0.18^{Bb}	2.21 ± 0.12^{Cb}
4	2.34 ± 0.13^{Ac}	2.04 ± 0.27^{Bc}	1.83 ± 0.25^{Cc}
6	1.59 ± 0.29^{Af}	1.59 ± 0.51^{Ad}	1.41 ± 0.21^{Bd}
8	2.03 ± 0.08^{Ad}	1.60 ± 0.14^{Bd}	1.38 ± 0.03^{Cd}
10	2.03 ± 0.12^{Ad}	1.27 ± 0.08^{Bf}	0.92 ± 0.08^{Cf}
12	1.71 ± 0.10^{Ae}	1.52 ± 0.10^{Be}	1.04 ± 0.11^{Ce}

Values are expressed as mean ± standard deviation of triplicate analysis.

[A-C] Values bearing different superscript uppercase letters within the same rows are significantly different (Tukey HSD post hoc test, $p > 0.05$)

[a-f] Values bearing different superscript lowercase letters within the same columns are significantly different (Tukey HSD post hoc test, $p > 0.05$)

Table 3. Change of moisture, protein, and 2-AP content during 12 weeks of storage of rice.

Storage Temperature (°C)	Moisture content (%)	Protein content (%)	2-AP content (%)
4	9.33^{b}	9.88^{c}	46.71^{c}
RT	7.58^{c}	10.67^{b}	52.46^{b}
40	16.03^{a}	12.25^{a}	67.44^{a}

[a-c] Values bearing different superscript lowercase letters within the same columns are significantly different (Tukey HSD post hoc test, $p > 0.05$)

Fig. 1 illustrates the dynamic changes in 2-AP content in rice across different storage temperatures (4°C, room temperature, and 40°C) over 12 weeks. As observed, 2-AP content generally exhibited a declining trend with increasing storage duration, particularly noticeable at higher temperatures. This is primarily attributed to the volatile nature of 2-AP, which facilitates its loss through diffusion and evaporation from the rice matrix, especially when not stored in airtight conditions [28]. The rate of 2-AP degradation was highly dependent on the specific storage temperature conditions. For instance, rice stored at 40°C showed the most rapid decrease in 2-AP, with statistically significant differences observed as early as week 2 (2.21±0.12 µg/g) compared to storage at 4°C and room temperature (2.52±0.06 µg/g and 2.31±0.18 µg/g, respectively). High temperatures accelerate the rate of chemical reactions that degrade 2-AP and also increase its vapor pressure, leading to faster volatilization [39]. Additionally, increased lipid oxidation at elevated temperatures can generate off-flavors that mask or contribute to the perceived loss of 2-AP, even if some 2-AP remains [29]. Conversely, storage at 4°C effectively preserved the 2-AP content, maintaining significantly higher levels even at week 12 (1.71 ± 0.1 µg/g) compared to storage at room temperature (1.52 ± 0.1 µg/g) and 40°C (1.04 ± 0.1 µg/g) (**Table 2**). This corresponds to an approximate decrease in 2-AP content of 46.71% and 52.34% for storage at low (4°C) and room temperature, and 67.29% for storage at high temperature (40°C) (**Table 3**). Lower temperatures reduce molecular mobility and slow down enzymatic and chemical

reactions, thereby minimizing 2-AP loss [28]. The preservation of 2-AP at lower temperatures underscores the importance of cold storage in maintaining the characteristic aroma of aromatic rice varieties. These findings highlight the importance of cold storage to preserve the rice quality during post-harvest [32]. However, setting up and running a full cold storage system for a large amount of rice is usually too expensive [40]. Therefore, room temperature storage is chosen mainly because it's cheaper and easier to manage for large-scale rice operations. Critically, the moderate degradation observed at room temperature can be commercially acceptable for the product's intended market and shelf life, especially when the negative effects are mitigated by advanced packaging like vacuum-sealed, high-barrier films, which block the oxygen and light that drive 2-AP depletion [41].

4. CONCLUSIONS

This study investigated the effects of different storage conditions, low temperature (4°C), room temperature, and high temperature (40°C) on the moisture, protein, and 2-AP content of Phka Rumdoul rice over 12 weeks, demonstrating that storage conditions significantly impact the rice's aromatic and nutritional quality. The results showed that low-temperature storage (4°C) most effectively preserved all quality parameters, including moisture, protein, and 2-AP content, although its higher operational costs may limit practicality for large-scale applications. Room temperature storage served as a practical alternative with moderate losses, as it effectively preserved moisture and protein content despite a gradual decline in 2-AP levels over time. In contrast, high-temperature storage (40°C) caused substantial moisture evaporation, protein degradation, and a significant decrease in 2-AP content by over 50%, compromising rice's texture, nutritional value, and aroma. These findings highlight the importance of considering storage temperature, duration, packaging methods, and materials to preserve the desirable attributes of the aromatic rice. Future studies should extend storage duration and investigate the effects of humidity and microbial activity on rice aroma and nutritional quality, thereby guiding the development of cost-efficient and effective post-harvest preservation strategies for aromatic rice.

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