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Thermal and packaging materials for enhancing the longevity and aroma integrity of fragrant rice during storage

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Abstract

Due to improper storage methods, a few months after storage the grain quality has gone to deteriorate. Retention of aroma for long time is the main bottleneck on the storage program of aromatic rice in Bangladesh. From this perspective the experiment was conducted at Agronomy Research Field and consecutively at Agronomy Laboratory of Sher-e-Bangla Agricultural University, Dhaka-1207 during the months from June 15 to November 25, 2022 (*Aman* season, a rice growing season) to evaluate the performance of storage temperature and packaging materials on aromatic rice. The experiment comprised three factors *viz*., factor:1; Two storage temperature (S₁= Cold storage: $4 \text{ °C} \pm 1 \text{ °C}$ and S₂= At room temperature: $25 \pm 2 \text{ °C}$); factor:2; Three packaging materials (P_1 - Vacuum plastic bags, P_2 - Polythene bags and P_3 - Jute gunny bags); factor:3; Two aromatic rice varieties (V_1 = Bangladesh Rice Research Institute (BRRI) dhan34 and V_2 = Tulshimala). The study was conducted as a Randomized Complete Block Design (RCBD) with four replications. The results revealed that the apparent amylose content, fat acidity and 2-acetyl-1-pyrroline (2-AP) content of aromatic rice grain was significantly impacted by storage temperature and/or packaging materials and/or variety. When examining apparent amylose content; among the 12 treatments combination $S_1P_1V_1$ treatment exhibited the highest (24.02 % and 23.12 %, respectively) amylose content at both 3 and 6 months after storage which was statistically ($p \le 0.05$ and $p \le 0.01$, respectively) similar with $S_1P_2V_1$ (23.92% and 23.07%, respectively) treatment; while, the lowest amylose content was found in $S_2P_3V_1$ and $S_2P_3V_2$

treatments. Regarding fat acidity, at both 3 and 6 month after storage, the $S_1P_1V_1$ treatment demonstrated a lower (7.010 mg 100g-1 and 8.220 mg 100g-1, respectively) fat acidity value compared to other treatment combination, and a statistical ($p \le 0.01$ and $p \le 0.05$, respectively) similarity with $S_1P_2V_1(7.210 \text{ mg } 100g^{-1}$ and $8.590 \text{ mg } 100g^{-1}$, respectively) treatment, while the $S_2P_3V_1$ and $S_2P_3V_2$ treatments showed a statistical higher fat acidity value. In terms of grain 2-AP content, at first 3 and 6 months after storage the $S_1P_1V_1$ treatment showed a higher (0.1230) μ g g⁻¹ and 0.0950 μ g g⁻¹, respectively) 2-AP content of grain compared to other treatment combination, which was statistically ($p \le 0.01$ and $p \le 0.01$, respectively) similar with $S_1P_2V_1$ $(0.1240 \,\mu g \,g^{-1})$ and $(0.0910 \,\mu g \,g^{-1})$, respectively) treatment, while the lowest 2-AP content was found in $S_2P_3V_1$ and $S_2P_3V_2$ treatments.

Introduction

Almost a billion people on the planet are dependent on rice (*Oryza sativa* L.) grown as a main crop,1 and fragrant rice is prized for its excellent grain quality, robust aroma, and delicious flavor.2 Bangladeshis enjoy flavorful rice on special occasions, particularly when it is cooked Asian style. It is steamed and used for making a variety of traditional recipes, including *firni*, *polau*, *payesh*, and *biriyani*. This is because aromatic rice has a pleasant aroma. Sometimes, for regular consumption, aromatic rice is preferred over plain rice.3 In Bangladesh, the trend for both production and demand for aromatic foods is rising, but the quality of the product cannot be maintained due to inadequate storage facilities. Throughout most of Bangladesh, food grains are kept in traditional household storage structures like *motka* (a local container) and jute sacks by farmers and people living in remote areas. These buildings offer scarce storage conditions and are not very sturdy. The wrong storage structure, farmers' and traders' ignorance of storage and poor management during the storage period are the main causes of the comparatively high rate of storage loss in our nation. Accordingly, after a few weeks of storage the grower smelt much less aroma from aromatic rice grain. Therefore, producers face challenges in meeting consumer demands for fragrant rice with a high intensity of aroma.⁴ According to Han *et al.*,⁵ the storage

life of brown rice is influenced by factors related to varieties, storage condition, and packaging. As per Sinija *et al.*,⁶ brown rice can have its storage life considerably increased by using storage conditions like freezers or refrigerators. In addition, rice quality cannot be increased while being stored; however, in an environment with regulated temperature and relative humidity, the grains can be kept until the right time to be sown without losing any of their quality.7 Hu *et al*.,8 demonstrated that biochemical reactions in rice were impacted by various storage conditions, including temperature, moisture, vacuum levels, and packaging. According to Hu *et al.*,⁸ also the intensity of aromatic volatile compounds is influenced by the intricate relationships that exist between rice varieties and storage factors during post-harvest management. The quality of rice can be significantly impacted by the various chemical, physical, and biological changes that stored rice will experience.⁹ The primary factors influencing the quality of stored rice are the long time of storage and the temperature.¹⁰ The best way to slow down the changes in rice properties caused by storage is generally thought to be to store it at 5°C.11 Wimonmuang *et al*.,12 discovered that within 7 days of storage, a high temperature (45ºC) causes a rapid decrease in 2 acetyl-1-pyrroline (2-AP). Okpala *et al*.,13 observed a drop in 2-AP content following a 6 to 9 month at room temperature during storage. According to Song,¹⁴ low temperature $(5{\text -}10^{\circ}C)$ storage condition of rice is noticeably better than other methods of rice storage. An increased respiratory process brought on by the grain mass's rising temperature speeds up the grain's metabolism, increasing the amount of dry matter it consumes and lowering its starch content.15 The hydrolysis and peroxidation of lipids in stored rice is caused by enzymatic action, which produces fatty acids and lipid peroxides, which change the aroma.16 The degree to which rice deteriorates during storage is indicated by the fatty acid value, an index of the free fatty acid content in grain. Biao *et al.*,¹⁷ reported an increase in the fatty acid value during storage. Significantly contributing to rice starch, amylose can also be used as a criterion to assess the quality of aromatic rice grains.13 According to Yehia and Khatab,18 cooked rice hardness and amylose content rose following storage. Although it is possible for 2-AP to form after harvest, it is unlikely to occur during the growth of non-fragrant rice. Post-harvest treatments have the potential to positively or negatively impact rice aroma. During storage the 2-AP content is lost from paddy and polished fragrant rice.19,20 The study of Kongpun *et al*.,4 also discovered that

during a six-month period of storage, the aroma quality of unpolished black aromatic rice was greatly impacted by both the manner of packaging and the length of storage. When rice is stored for the first few months, 2-AP content decreases quickly and by the second month, it is no longer noticeable in unpackaged rice. In comparison to polythene, gunny, and vacuum bags, Tananuwong and Lertsiri²¹ found that vacuum bag packaging increased the concentration of 2-AP in aromatic rice after storage. According to the aforementioned research findings, storage temperature and packaging conditions are crucial for preserving aromatic rice in order to enhance grain quality and aroma. However, there have not been many studies done on these topics in relation to Bangladeshi aromatic rice. So, we conducted an experiment to explore how variety, temperature, and packaging materials collectively influence the quality and aroma of aromatic rice, providing valuable insights for producers and stakeholders in the rice industry.

Materials and Methods

Site description of experiment (field and laboratory)

The experiment was conducted at Agronomy Research Field and Agronomy Laboratory of Shere-Bangla Agricultural University, Dhaka-1207, Bangladesh. The field experimental area was placed at 23o7'N latitude and 93oE' longitude at an altitude of 8.6 m above the sea level; research area belonged to the agro-ecological zone of "Madhupur Tract", AEZ-28. The experimental site is characterized by summer with a significant fluctuating climate under sub-tropical cropping zone during the months from June 15, 2022 to November 25, 2022 (*Aman* season) and then the laboratory research part was continued for next 6 months at an interval of 3 months. During field study period the data on temperature and relative humidity are provided in Table 1.

Experimental treatments and design

The experiment comprised three factors *viz*., factor: 1. Two storage temperature $(S_1 = Cold$ storage: 4° C \pm 1°C and S₂= At room temperature: $25 \pm 2^{\circ}$ C); factor: 2. Three packaging materials (P₁=Vacuum plastic bags, P₂= Polythene bags and P₃= Jute gunny bags); factor: 3. Two aromatic rice varieties (V₁= Bangladesh Rice Research Institute (BRRI) dhan34 and V₂= Tulshimala). The study was conducted as a Randomized Complete Block Design (RCBD) with four replications. So, there were 48 total bags in this study where unit bag size was 1.0 Kg weight.

Description of rice varieties

Tulshimala, a native cultivar, was obtained from a farmer in Bangladesh's Sherpur district, while seeds of BRRI dhan34 were obtained from the Plant Genetic Resource Division of the Bangladesh Rice Research Institute (BRRI) in Joydebpur, Gazipur. According to BRRI rice varieties information BRRI dhan34 rice has a 135-day lifecycle and is small-grained, similar to chinigura or kalojira rice. It is ideal for making *polao* (a traditional Bangladeshi food). According to the information from local farmers, the life cycle of Tulshimala rice is 140 days, has a pleasant aroma, and is about the same size as chinigura rice.

Field crop and laboratory management

The seeds were chosen using the specific gravity method, and after soaking in a bucket of water for a full day, they were ready to be stored in bags. After being sown, the seeds took 48 h to germinate and 72 h to sprout. Depending on the needs of the soil, nutrients were seeded on a meter-wide spacing. The bed was seeded with 70 g m-2 of germination-ready seeds on June 15, 2022. Twenty-five (25) day old seedlings were uprooted to transplant in main field. Initially, each hill received 3 seedlings. Crop management ought to make use of all nutrients that are available, according to BRRI.22 Plots received regular irrigation, weeding, and treatment with fungicides and insecticides. On November 25, 2022, harvest maturity was deemed to have occurred when about 80% of the seeds turned golden yellow. Separate, clearly labeled bundles containing the collected goods were delivered to the threshing floor. For every plot $(10 \text{ m} \times 10 \text{ m})$, divided into two separate plots for cultivating two varieties), grains were washed, dried, and weighed. After sun drying, the weight was modified to reflect the 12% increase in moisture content. The dried

grains were then kept and arranged in lab research room in accordance with the design of study packed with different packaging materials and stored at different temperature condition. We keenly observed the temperature in the laboratory at both room temperature and cold storage.

Data collection

The following traits have been collected under present study just after drying of grains (0 month), at three (3) month after storage (MAS) and at six (6) MAS.

Apparent Amylose Content (AAC)

The method of Juliano *et al*.23 was used to measure the amount of amylose in rice samples. Boiling water was added to a volumetric flask containing 100 mg of milled rice, 1 mL of 1 M NaOH, and 1 mL of 95% ethanol in order to gelatinize the starch. Subsequently, 5 mL of starch extract were added to a 100 mL volumetric flask with 1 mL of 1 Normal acetic acid and 2 mL of iodide solution, making a total volume of 100 mL with distilled water. After shaking the mixture, it was left to stand for 20 minutes. Subsequently, absorbance was measured at 620 nm using a Cary 60 UV-VIS spectrophotometer (Agilent Technologies, CA, USA) and the amylose content (expressed as a percentage) was determined using a potato amylose standard curve.

Fat acidity (mg 100 g-1)

The fat acidity of rice samples was assessed using the colorimetric method of Ohtsubo *et al*.,24 Samples of rice flour were used 2 h after grinding. The sample was precisely weighed in 2 g, and 6 mL of toluene was used to extract the fat for 1 h at 30°C. The extract was put into a tube with 4 mL of chloroform and 2.5 mL of copper reagent, which was made by vigorously shaking a mixture of 50% (v/v) 6.45 % copper nitrate solution, 5% (v/v) 1 M acetic acid, and 45% (v/v) 1 M triethanolamine. Finally, 3 mL of the chloroform layer was mixed with 0.1 % diethyldithiocarbamate isobutanol solution (0.05 mL), and the absorbance at 440 nm was recorded. The amount of potassium hydroxide (mg/100 mL) required to neutralize the fatty acid released from 100 g of rice flour was used to express the fat acidity.

Grain 2-AP content (µg g-1)

The grains were ground with a mortar and pestle prior to analysis in accordance with Huang et al.,25 to determine the 2-AP content of grains. For continuous steam distillation, a 500 mL roundbottom flask was connected to an extraction head. Roughly 10 g of grains and 150 mL of filtered water were mixed and added equally. In an oil pot, the mixture was cooked at 150°C. The other head of the continuous steam distillation apparatus was connected to the extraction solvent. It was composed of a 500 mL round-bottom flask holding 30 mL of dichloromethane. The flask was then heated in a water pot to 53°C. The continuous steam distillation extraction system was connected to a cold water circulation unit in order to maintain a temperature of 10°C. The extraction procedure took approximately 35 minutes to complete. Four (4) g of anhydrous sodium sulfite were added to the extract in order to absorb the water. The dried extract was filtered using an organic needle filter, and the 2-AP content was ascertained through Gas Chromatography/Mass Spectrometry (GC/MS), by using GCMS-QP 2010 Plus (Shimadzu corporation, Tokyo, Japan). High purity helium served as the carrier gas at a flow rate of 2 mL min−1. The gas chromatography oven's temperature gradient was set as follows: 40°C for 1 min, increased at a rate of 2°C min−1 to 65°C, maintained at 65°C for 1 min, further increased at a rate of 10°C min−1 to 220°C, and maintained at 220°C for 10 min. The 2-AP retention time was verified as 7.5 min. Three replications were performed per sample, and 2-AP content was expressed in μ g g⁻¹.

Statistical analysis

Statistical analysis utilized the three-way ANOVA technique and the computer-based program Statistix-10 (Analytical Software, Tallahassee, FL, USA); the data acquired for the various parameters had their means corrected by Least Significant Difference (LSD) at the (0.05) 5% level of significance. To examine the relationship between grain quality attributes, correlation

graph was presented utilizing Microsoft (MS) Excel spread sheet in conjunction with the mean values of the parameters under investigation after 6 month of storage (MAS).

Results

Apparent Amylose Content (AAC) (%)

When evaluating the cooking and eating qualities of rice, amylose content is a crucial factor. Variations in temperature and packaging material treatments had a notable impact on the apparent amylose content of aromatic rice varieties (Table 2). At 0 month of storage, no variation (p=Not Significant - NS) was found among the treatment combination except varietal difference $(p \le 0.01)$. Amylose content was decreased with the increases of storage intervals. At both 3 and 6 months after storage, the highest (24.02 % and 23.12 %, respectively) amylose content was exhibited in $S_1P_1V_1$ treatment which was statistically (p≤0.05 and p≤0.01, respectively) similar to $S_1P_2V_1$, whereas the lowest amylose was determined in $S_2P_3V_1$ and $S_2P_3V_2$ treatment at both 3 and 6 months after storage.

Fat acidity (mg 100 g-1)

Fat acidity is the crucial factor of storage quality and aroma of fragrant rice which control the rancidity in stored grains.15 Notable distinctions in fat acidity were seen among temperature, packaging materials and variety (Table 3). At 0 month of storage, no variation (p=NS) was found among the treatment combination (three combined factors) but we found a variation in variety as single effect ($p \le 0.01$); packaging materials as single effect ($p \le 0.01$); variety and storage temperate as combination ($p \leq 0.05$). Fat acidity increased with the increases of storage intervals. At both 3 and 6 months after storage, the lowest $(7.010 \text{ mg } 100 \text{ g}^{-1}$ and $8.220 \text{ mg } 100 \text{ g}^{-1}$, respectively) fat acidity value was exhibited in $S_1P_1V_1$ treatment which was statistically similar with $S_1P_2V_1$, whereas the highest fat acidity value was determined in $S_2P_3V_1$ and $S_2P_3V_2$ treatment at both 3 and 6 months after storage.

2-AP content of grain (µg g-1)

Stronger-smelling rice varieties are preferred by customers because of the lovely perfume produced by the concentration of 2-AP in grain when fragrant rice boils.3 As to 2-AP content, notable distinctions were seen among temperature, packaging materials and variety (Table 4). At 0 month of storage, no variation (p=NS) was found among the treatment combinations except varietal performances ($p \le 0.01$). The 2-AP content of grain decreased with the increases of storage intervals. At both 3 and 6 months after storage, the higher 2-AP content of grain was exhibited in $S_1P_1V_1$ treatment (0.1230 µg g⁻¹ and 0.0950 µg g⁻¹, respectively) which was statistically ($p \le 0.01$ and $p \le 0.01$, respectively) similar with $S_1P_2V_1$, whereas the lower 2-AP content of grain was found in $S_2P_3V_1$ and $S_2P_3V_2$ treatment for both of 3 and 6 months after storage.

Pearson correlation (r)

We examined the correlation between different traits of aromatic rice under present study and found significant relation between them. In details, according to Figure 1, a strong and negative correlation ($r = -0.85**$, p-value=0.01) was exhibited between apparent amylose content and fat acidity of aromatic rice. So, the fat content is increased with the decreasing of amylose content of rice grain. A strong and positive correlation ($r= 0.83**$, p-value=0.01) was found between apparent amylose content and 2-AP content of aromatic rice grain (Figure 2). According to Figure 3, a strong and negative correlation ($r = -0.97**$, p-value=0.01) was exhibited between fat acidity and 2-AP content of fragrant rice grains.

Discussion

In the present study a remarkable effect of temperature and packaging materials was found on the features of two Bangladeshi aromatic rice cultivars. The amylose content was found to decrease as the storage temperature increased and a gradual decrease in amylose content of fine rice varieties was found with the increases of storage duration from initiation of the study to 4 months.26 Low temperature during grain storage has also been reported to improve the retention

of amylose content of aromatic rice.27, 28 Our results are in conformity with these findings indicating that amylose content of rice decreased as the storage period increased. Our result revealed that the amylose content decreased both under high and low temperature, but in case of low temperature there was lower decrease of amylose. We speculated that this may be due to the storage in vacuum plastic bags and polythene bag rather than jute gunny bag. Also, the decrease in amylose content may be due to the fractional changes in its molecular weight which decreased during storage. Kim Loan *et al.*,²⁹ explained that for the initial days of storage, rice germ of grains retains maturing stage and contains a considerable content of simple sugars involving continual metabolic activity. However, during storage, its germ is stabilized, while simple sugars are exhausted, implying rice grains are forced to use amylose in its germ for metabolic reaction causing a slight decline of amylose content.

Lipid content is one of the most important components of rice.16 The deterioration degree of rice quality during storage can be reflected by the change in fatty acid value, which is one of the important indices to measure the freshness and aging degree of rice. Too much accumulation of fatty acids during storage will lead to rice rancidity and seriously degrades its edible quality.30 The influence of temperature on fatty acid value is mainly due to its influence on enzyme activity. A previous study reported that, higher temperature strengthens the enzyme activity which leads to a faster decomposition of fat and a faster increase in fatty acid value.³¹ Park *et al*.,32 reported that the fat acidity increased during storage and it is evident that the fat acidity values of the milled rice stored at higher temperatures (30°C) were higher than those of rice stored at lower temperatures (4°C). They also described that, although a gradual increase in fat acidity was observed in rice stored at 4°C, much higher values were obtained at 30°C after 4 months of storage. Yoshihashi *et al.*,³³ also reported that the high temperature (35^oC) increased fat acidity during storage (4-7 weeks) of grains, but an exception was found when stored at 5°C. So, from these citations it is clear that the high temperature range (30-35°C) decreased the fat acidity rapidly than low temperature range (4-5°C) during storage periods. We found that, just after drying there was no variation among the treatments combination but at 3 and 6 months after storage fat acidity increased. We also observed that the fat acidity increased under both temperature regimes but there was found a slow increasing nature at low temperature than at

high temperature. It may be due to the storing of grains in vacuum and polythene bag and these packaging materials may induce the oxidation process very slowly compared to jute gunny bag.34 So, the possible reason for this observation was that the activity of rice fatty acid synthases was limited by the low temperature but only a few parts of them were active.15

Yoshihashi *et al.*,³³ depicted that with increased (35^oC) storage temperature the content of 2acetyl-1-pyrroline decreased even faster. Cold storage at 5°C in sealed low-density polyethylene (LDPE) bags preserved the contents most effectively. They also reported no significant change in 2-AP content of rice grains, after 7 weeks of storage at room temperature. During storage of rice cultivars, 2-AP content decreased with time and appeared to be significantly affected by the temperature.32 It was demonstrated that higher 2-AP concentrations were obtained with the shortest storage time of 3 months and the lowest storage temperature of 4°C.35 We observed that the content of 2-AP varied in two rice cultivars just at starting of storage and no variation was found among other treatments at that stage. Our results also showed significant decrease in 2-AP content, at 3 and 6 months after storage at room temperature. So, based on our findings, we can speculate that 2-AP evaporated gradually during grain storage. However, we found that under cold storage temperature ($4^{\circ}C \pm 1^{\circ}C$) there was lower decrease of 2-AP than that at room temperature storage. We also speculated that this may be due to storing in vacuum and polythene bag; thus, these packaging may slow down the 2-AP volatilization process but in case of jute gunny bag there may happen a rapid volatilization loss of 2-AP due to higher respiration. Guo *et al*.,36 reported a negative trend of correlation between fat content and amylose content in rice. So, the fat content is increased with the decreasing of amylose content of rice grain. Yoshihashi *et al*.,33 also found a similar trend of relation between fat acidity and 2-acetyl-1-pyrroline content showing to be inverse at different stage of storage.

Conclusions

Based on our findings we can conclude that storage temperature and packaging materials significantly influenced the amylose content, fat acidity and 2-AP content of two aromatic rice cultivars at 3 and 6 months after storage. In our present study, the cold storage ($4^{\circ}C \pm 1^{\circ}C$) performed better over room temperature $(25 \pm 2^{\circ}C)$ for all the characters of aromatic rice. Among 3 packaging materials, vacuum plastic bags and polythene bags performed better over

jute gunny bag for all the characters studied in the present research. The variety BRRI dhan34 exhibited the better results for traits compared to Tulshimala. The present study suggests that the Bangladeshi aromatic rice growers can cultivate BRRI dhan34 to retain higher grains aroma for 3-6 months under cold storage condition packaged with vacuum plastic bags/polythene bags instead of traditional storage methods. From the correlation study, it was also found that different characters of aromatic rice grain were correlated either positively or negatively. Finally, a future study is recommended to do to evaluate the exact storage time for how long Bangladeshi aromatic rice growers can maintain their good grain quality and aroma intact.

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Table 1. Monthly temperature and relative humidity data recorded during the experimental period.

Source: Metrological Centre (Climate Division), Agargaon, Dhaka, Bangladesh.

Table 2. Effect of storage temperature, packaging material and variety on apparent amylose content of fragrant rice grains.

The results with the same letter are statistically similar, whereas those with different letters differ significantly at the 0.05 probability level. *: indicates significance at the 5% (0.05) probability level; **: indicates significance at the 1% (0.01) probability level; NS: indicates non-significant. S₁, Cold storage: $4^{\circ}C \pm 1^{\circ}C$ and S₂, At room temperature: $25 \pm 2^{\circ}C$. P₁, Vacuum plastic bags, P₂, Polythene bags, P₃, Jute gunny bags. V₁, BRRI dhan34, V₂, Tulshimala.

Table 3. Effect of storage temperature, packaging material and variety on fat acidity of fragrant rice grains.

The results with the same letter are statistically similar, whereas those with different letters differ significantly at the 0.05 probability level. *, indicates significance at the 5% (0.05) probability level; **, indicates significance at the 1% (0.01) probability level; NS, indicates non-significant,

S₁, Cold storage: 4° C \pm 1°C and S₂, At room temperature: $25 \pm 2^{\circ}$ C. P₁, Vacuum plastic bags, P₂, Polythene bags, P₃, Jute gunny bags, V₁, BRRI dhan34, V₂, Tulshimala.

The results with the same letter are statistically similar, whereas those with different letters differ significantly at the 0.05 probability level. **, indicates significance at the 1% (0.01) probability level; NS, indicates non-significant. S₁, Cold storage: $4^{\circ}C \pm 1^{\circ}C$ and S₂, At room temperature: 25 $\pm 2^{\circ}$ C. P₁, Vacuum plastic bags, P₂, Polythene bags and P₃, Jute gunny bags. V₁, BRRI dhan34, V2, Tulshimala.