Selected quality attributes of paddy rice as affected by storage temperature history

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**ABSTRACT**

Five experimental storage conditions were designed to study the effect of storage temperature on paddy rice. Three of the conditions were designed with varied order of temperature but with the same overall exposure. The five conditions were: (i) 15°C for 12 months (low-temperature condition, LC); (ii) 30°C for 12 months (high-temperature condition, HC); (iii) 15°C for 6 months followed by 30°C for an additional 6 months (low–high-temperature, LH); (iv) 30°C for 6 months followed by 15°C for an additional 6 months (high–low-temperature, HL); and (v) alternating 2-month intervals at 15°C and at 30°C for a total of 12 months (alternating low–high-temperature, ALH). The resultant quality attributes of the stored rice samples were measured and compared at intervals over the 12 months. For all samples, fat acidity increased during storage, while germ cell volume and cell gap decreased. The changes were greater after exposure to high temperature than after exposure to low temperature. However, the overall results for the samples stored at varied-temperature conditions showed only slight differences in fat acidity, as well as in germ cell volume and cell gap. In addition, the overall results for these samples yielded only slight differences in polyphenol oxidase activity, peroxidase activity, pasting parameters, and cooked rice quality attributes (water uptake ratio, volume expansion ratio, hardness, and springiness). These results suggest that order changes of high- and low-temperature conditions can affect the intermediate quality of the stored rice but have limited influence on the final quality.

**Introduction**

Rice (*Oryza sativa* L.) is a staple grain for more than half of the world’s population.\(^1\) As a dietary source of carbohydrates and protein, rice plays an important role in meeting the energy and nutrient requirements of people.\(^2,3\) In 2019, the total yield of rice exceeded 700 million tons.

Rice plants take 3–6 months to grow from seed to mature plants, depending upon the variety and environmental conditions. However, the harvested rice grains are consumed year-round. This necessitates the storage of rice for certain period of time. In addition, in some countries, considerable amounts of rice are stored as reserve grain for 2–3 years. This stabilizes the rice market and ensures the safety of the economy,\(^4\) for example, by ensuring the rice supply during the global COVID-19 pandemic. In recent decades, increased social division of labor has led to a high-speed growth in the international rice trade.\(^5\) As a traded commodity, it is necessary for rice to be stored in transit warehouses and transportation facilities, especially for cross-continental trade.

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Change in rice quality during storage has attracted increasing attention and has been extensively studied. It has been found that fat is hydrolyzed by the endogenous lipase to free fatty acid, which then undergoes peroxidation by the lipoxygenase, giving rise to an undesirable off-flavor.\(^{6-8}\) As a living organism, rice grain gradually loses its vitality when kept in unsuitable storage conditions (for example, high moisture or temperature).\(^{9,10}\) Poor storage conditions can also result in a change in texture with increased hardness of the cooked product.\(^{11-13}\)

Rice quality during storage is affected by many factors. Temperature is considered a critical factor.\(^{14-16}\) To date, most storage research has been conducted at fixed temperatures. However, storage temperature of rice varies with the atmospheric temperature, with the variation amplitude and frequency being more obvious for rice traded across geographic regions. The main objective of this study was to investigate the characteristics of rice after storage at temperature-varied conditions. Three temperature-varied conditions were designed to store rice samples. Two temperature-constant conditions were included in the study as controls. Quality attributes of stored samples were compared to investigate the influence of different storage temperatures on rice quality.

**Materials and methods**

**Materials**

Paddy rice (variety Yuenongsimiao), harvested in Hubei, China, was obtained from a local farmer (Hubei, China). Chemicals were of analytical grade unless otherwise stated.

**Storage of paddy rice**

Five paddy rice samples (5 kg each) with a moisture content of 13.5% were placed in capped glass bottles and stored in incubators (SPX-250B-Z, Shanghai Boxun Industry & Commerce Co., Ltd., Shanghai, China). Each sample was placed in one of five temperature conditions and stored for 12 months. The five conditions were: (i) 15°C for 12 months (low-temperature condition, LC); (ii) 30°C for 12 months (high-temperature condition, HC); (iii) 15°C for 6 months followed by 30°C for an additional 6 months (low–high-temperature, LH); (iv) 30°C for 6 months followed by 15°C for an additional 6 months (high–low-temperature, HL); and (v) alternating 2-month intervals at 15°C and at 30°C for a total of 12 months (alternating low–high-temperature, ALH).

**Fat acidity**

Fat acidity was determined according to the ISO 7305:1998 method with slight modification. Briefly, rice grains were unhusked with a hulling machine (THU358, Satake Co., Ltd., Riichi, Japan) and then milled with a hammer mill (JXFM, Shanghai Jiading Grain & Oil Instrument Co., Ltd., Shanghai, China). The brown rice flour was passed through a 40-mesh sieve. Ten g of each flour was extracted with 50 ml of anhydrous ethanol for 10 minutes with constant stirring. The mixture was then filtered through Whatman No. 1 filter paper. Fifty mL of distilled water was added to 25 mL of the filtrate. The mixture was titrated with 0.5 mol/L potassium solution (KOH), using phenolphthalein as an indicator. The fat acidity of the rice was expressed as mg KOH per 100 g of sample.

**Germination rate**

The germination rate of stored rice was determined following the procedure of Genkawa et al.\(^{10}\) with slight modification. Grains were washed four times with distilled water. They were then placed on wet filter paper in a Petri dish. The Petri dishes were placed in an incubator at 25°C. The ratio of germinated grains to total grains was counted after seven days.
**Peroxidase and polyphenol oxidase activities**

The peroxidase (POD) activity of rice was determined by the guaiacol method. One gram of brown rice flour was homogenized in 10 mL of 50 mM potassium dihydrogen phosphate (KH₂PO₄). The homogenate was then centrifuged at 4000 g for 10 minutes at 4°C. The volume of the supernatant was adjusted to 10 mL with KH₂PO₄ to prepare the enzyme solution. Fifty-six μL of guaiacol was added to 100 μL of 0.1 M phosphate buffer (pH 6.0). The solution was heated to 40°C for 20 minutes and subsequently cooled to room temperature. Then, 38 μL of 30% hydrogen peroxide (H₂O₂) was added to prepare the POD mixed reaction solution. To determine POD activity, 3 mL of the POD mixed reaction solution and 1 mL of enzyme solution were added to the sample cuvette. A cuvette containing POD mixed reaction solution and KH₂PO₄ was used as a blank control. The absorbance was read at 470 nm.\[^{[17]}\] POD activity was expressed as U·g⁻¹. One unit of polyphenol oxidase (PPO) activity was defined as a 0.01 unit change of absorbance value per minute.

The PPO activity of rice was determined by the catechol method. Three g of brown rice flour was homogenized in 10 mL of 0.1 M KH₂PO₄ and then centrifuged at 4000 g for 10 minutes at 4°C. The supernatant was adjusted to 10 mL with the KH₂PO₄ solution to prepare the enzyme solution. One mL of enzyme solution was added to 2 mL of 0.08 mM catechol. The mixture was placed in a 37°C water bath for 5 minutes. Subsequently, the absorbance of the reaction mixture was measured at 420 nm.\[^{[18]}\] PPO activity was expressed as U·g⁻¹. One PPO unit (U) was defined as a 0.01 unit change of absorbance at 420 nm per minute.

**Transmitting electron microscopy**

Midsection samples of 0.25–0.5 mm thickness were cut from rice germ and incubated in fixative solution (4% glutaraldehyde, v/v) for 2 weeks. After fixation, the germ midsection was rinsed four times for 15 minutes each with 0.2 mol/L phosphate-buffered saline (pH 7.2; PBS), and immersed in post-fixative solution (1% osmic acid, w/v) for 90 minutes. After post-fixation, the germ midsection was rinsed three times for 15 minutes each with PBS, followed by immersion for 15 minutes each in a series of acetone solutions (30%, 50%, 70%, 90% and 100%, v/v) for dehydration. Subsequently, the germ midsection was immersed for 120 minutes each in a series of EPON 812 resin/acetone solutions (1:2, 1:1, 2:1, and 1:0) for embedment. The embedded germ midsections were oven-dried for 12 hours each at 37°C, 45°C, and 60°C. Plastic molds were trimmed down, and an 80-nm section was prepared with a rotary microtome. The section was stained in uranyl acetate for 25 minutes, and then in lead citrate for 20 minutes. Stained samples were viewed and photographed using a JEM-1200EX transmitting electron microscope (JEOL Ltd., Tokyo, Japan).

**Pasting properties**

A Rapid Visco Analyzer (Newport Scientific Pty, Ltd., Warriewood, Australia) was used to determine the pasting temperature of the milled rice flour. Viscosity profiles were recorded using rice flour suspensions (12%, w/w). Using a programmed heating and cooling cycle, the suspensions were maintained at 50°C for 1 minute, and then heated to 95°C at a rate of 12°C/min, maintained at that temperature for 2.5 minutes, and finally cooled to 50°C at a rate of 12°C/min.

**Cooking quality**

Brown rice was milled with a rice milling machine (TM05C, Satake Co., Ltd.). Milled rice was cooked in a steam cooker (CFXB40B2T-65, Supor Co., Ltd., Hangzhou, China), and the hardness and springiness of cooked rice were determined using a Cooked Rice Taste Analyzer (STA1B, Satake Co., Ltd.).
**Statistical analysis**

Data were presented as mean results ± standard deviation (SD). Statistically significant differences between means were determined by Duncan’s multiple range. A significance level was set at 0.05. All statistical analyses were performed using the commercial statistical package (SPSS, Inc, Chicago, IL, USA).

**Results and discussion**

**Fat acidity**

Fat acidities of rice stored at different temperature conditions are shown in Figure 1. During storage, there was an upward trend in fat acidity of the rice stored under all five temperature conditions. Among the five samples, rice stored at LC showed the slowest trend and rice stored at HC showed the fastest trend in the rise of fat acidity, suggesting that an increase of storage temperature can accelerate the aging of rice. These results are consistent with those found by Park et al. Rice stored at the three temperature-varied conditions (LH, HL, and ALH) showed different increments of fat acidity at different storage periods. The increase in fat acidity of rice stored at LH slowed after 6 months of storage, while the opposite trend was found for rice stored at HL. Rice stored at ALH showed a small increase in fat acidity after storage at a temperature of 15°C and then a larger increase at 30°C. After 12 months of storage, rice stored at LH, HL, and ALH yielded only slight differences in fat acidity. These results suggest that for the same storage durations of high and low temperature, the order of high and low temperature did not have significant influence on changes in fat acidity of rice.

**Germination rate**

The germination rates of rice stored at different temperature conditions are shown in Figure 2. Rice stored at LC showed a high germination rate (88.0–95.0%) for the whole storage duration. Rice stored at HC did not have an obvious change in germination rate after 2 months of storage, but then...
exhibited a dramatic decrease in the rate from the second to the sixth month, which thereafter decreased to zero. These results suggested that storage at high temperature deteriorated the vigor of rice grains.\textsuperscript{20,21} For rice stored at LH, the germination rate began to decrease as the storage temperature changed from 15°C to 30°C and dramatically decreased to almost zero, with the maximum decrease happening at the tenth month. Rice stored at HL had a low germination rate after 6 months of high-temperature storage, and its germination rate gradually decreased to near zero over the next 6 months. Rice stored at ALH showed a trend in the germination rate like rice stored at LH, but a dramatic decrease occurred when the rice went through the second high-temperature period at the eighth month. The small differences in the final germination rates of rice stored at LH, HL, and ALH suggested that changes in the order of high and low temperatures did not relieve the deterioration of high-temperature storage on the vigor of rice grains.

**Germ microstructure**

The germ slice images of rice stored for 12 months are shown in Figure 3. Germ of unstored rice showed normal cell morphology and organelles, exhibiting dense cell wall and flat cell membrane. Among the five stored rice samples, rice stored at LC showed the best maintenance of the germ structure of original rice, although there was a decrease in the gap among germ cells. For rice stored at HC, the cell volume of the germ decreased as did the gap among the cells; additionally, plasmolysis occurred. These results suggested that the cell structure of the germ was more affected at high storage temperature. The rice samples stored at the three temperature-varied conditions (LH, HL, and ALH) exhibited a germ structure like that of rice stored at HC. This was consistent with the results for germination rate.

**PPO and POD activities**

PPO and POD activities of rice stored for 12 months are shown in Table 1. After 12 months of storage, PPO and POD activities of the rice samples stored at all five conditions decreased. This result was
consistent with the data of Chen and Chen,[22] which suggested that enzyme activity can reveal the freshness of rice. Our data indicate that rice aged under all five temperature conditions studied. After storage, decrease in activities of the two enzymes was different for the five samples, with rice stored at LC and HC showing the lowest and the highest decreases, respectively. Rice stored at HL and ALH did not show significant difference in the activities of these two enzymes. Rice stored at HL (or ALH) and LH showed slight significant differences in both PPO and POD activities. These results suggested that order changes of high- and low-temperature conditions had only limited influence on the freshness of the stored rice.

### Pasting property

Rapid Visco Analyzer parameters of rice stored for 12 months are shown in Table 2. Pasting temperature (PT) of rice stored at HC was higher than that of rice stored at LC and the original sample. These results were consistent with those of Zhou et al.[23] Storage at high temperatures
accelerated the aging of rice grains, leading to a decrease in the hydrophilicity of the starch granule surface and the enhancement of the granule hardness, which limited granule hydration during heating. Rice stored at both HL and ALH showed no significant change in PT. Rice stored at LH showed very slight significant change in PT.

Peak viscosity (PV), trough viscosity (TRV), and final viscosity (FV) of all rice stored under all five conditions increased. This might be attributed to the formation of the starch-lipid complex and the decline of amylase activity. There was no difference in viscosity parameters among rice samples stored at the variable temperature conditions. These results suggested that order changes of high- and low-temperature conditions did not have significant influence on the final pasting property of the stored rice.

Properties of cooked rice

The quality of cooked rice is related to the freshness of the rice. The properties of the cooked rice stored at the five different temperature conditions for 12 months are shown in Table 3. The water uptake ratio (WUR) and volume expansion rate (VER) of all five rice samples increased after storage. Cooked rice showed an increase in water absorption ability and volume after storage. This was due to the formation of stronger connections and networks in rice starch and protein regions. After storage, all five rice samples exhibited significant increases in hardness and decreases in springiness, suggesting that aging worsened the texture quality of rice. This is consistent with Park et al. Changes in WUR, VER, and texture parameters of rice stored at HC were greater than those of rice stored at LC. Cooked rice of the samples stored at the three temperature-varied conditions presented WUR, VER, and texture parameters between those of samples stored at HC and LC. These results indicated that a reduction in the total duration of high-temperature storage can inhibit the deterioration of cooked rice quality. The comparison of quality parameters of cooked rice prepared from rice stored at LH, HL, and ALH reveals that order changes of high- and low-temperature conditions cause only slight differences in some quality attributes of cooked rice. This result was consistent with the results of fat acidity, germination rate, germ structure, PPO, and POD activities. As discussed above, order changes of high- and low-temperature conditions had limited influence on the freshness of rice, thus yielding only slight differences in quality attributes of the cooked rice.

Table 3. WUR, VER, hardness and springiness of cooked rice prepared from rice stored for 12 months.

| WUR (%) | VER (%) | Hardness (g) | Springiness (mm) |
|---------|---------|-------------|-----------------|
| Original rice | 2.96 ± 0.01 | 3.80 ± 0° | 1.16 ± 0.01 | 0.888 ± 0.001 |
| LC | 3.28 ± 0.01 | 4.13 ± 0.01 | 1.81 ± 0.01 | 0.800 ± 0.003 |
| HC | 3.98 ± 0.01 | 4.92 ± 0.01 | 2.29 ± 0.02 | 0.720 ± 0.002 |
| LH | 3.79 ± 0.02 | 4.60 ± 0.01 | 2.01 ± 0.02 | 0.769 ± 0.002 |
| HL | 3.73 ± 0.02 | 4.58 ± 0.01 | 2.07 ± 0.01 | 0.758 ± 0.002 |
| ALH | 3.78 ± 0.02 | 4.77 ± 0.01 | 1.94 ± 0.02 | 0.772 ± 0.002 |

Data were expressed as means ± SD. Means within a column that had the same letter were not significantly different (α = 0.05). WUR = water uptake ratio; VER = volume expansion ratio.
Conclusion
Fat acidity of rice stored at three temperature-varied conditions increased with the prolongation of storage. The increment of change was greater at the high-temperature storage periods for all three conditions. Rice stored at LH, HL, and ALH began to show great change in germination rate at the tenth, fourth, and eighth months, respectively. These samples showed only slight differences in final fat acidity and germination rate after 12 months of storage. After storage, the volume of germ cell, as well as the gap among cells, of rice stored under all three temperature-varied conditions decreased. Only slight differences were found in PPO activity, POD activity, pasting parameters, WUR, VER, and texture parameters of the cooked rice prepared from rice stored at the three temperature-varied conditions. These results suggest order changes of high- and low-temperature conditions have only limited influence on the final quality of the stored rice. In the future, more environmental factors will be considered to conduct systematic research.

Declaration of interest statement
The authors declare that they do not have any conflict of interest.

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