Differences in starch multi-layer structure, pasting, and rice eating quality between fresh rice and 7 years stored rice

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1. Introduction

Rice is the staple food of two-thirds of the world, providing people with more than 50% of the calories (Lampe, 1995). The rice eating quality is one of the most crucial properties, which refers to the sensory properties of rice. High eating quality rice is favored by people because of its low hardness after cooking. Rice is generally stored for a period of time after harvest before being eaten by people. Under the condition of long-term storage, the rice surface becomes yellow, and the peak viscosity and breakdown of rice are reduced (Xiao et al., 2020). Rice will produce a series of volatile aldehydes, ketones, and furans under high temperature and humidity conditions, which reduces the taste of rice (Biao et al., 2019). Previous studies have shown that stored rice generally has poor eating quality, hard texture, and an unpleasant taste, so it is not liked by people (Tran et al., 2005). Understanding the physical and chemical properties of stored rice is of great significance to changes in rice eating quality.

Starch occupies 70–80% of the chemical components in rice and is the number one chemical component (Juliano and Tua, 2019). Starch is composed of amylose and amylopectin, these two compounds constitute the multi-scale structure of starch, including micro-scale starch granules, nano-scale starch amorphous crystalline sheets, long-range crystalline structures, and short-range helical structures. The change in a starch multi-scale structure determines the starch pasting properties and eating quality of rice to a certain extent (Longvah and Prasad, 2020). Current studies have shown that the starch molecules of rice are degraded after one year of storage, and the change in the crystal structure of starch leads to lower gelatinization temperature and gelatinization enthalpy. Different rice varieties exhibit different aging effects (Gu et al., 2019). The proportion of short amylopectin chains in rice stored for one year is higher, and the average chain length of amylopectin is smaller (Wu et al., 2019). Differences in starch structure will lead to changes in rice eating quality.

Although a lot of research has been done on the changes in rice...
physicochemical properties and texture during rice storage. However, most of the current research focuses on the case of short-term storage, such as one year storage (Tananuwong and Malila, 2013; Wu et al., 2019; Zhou et al., 2010), 9 months storage (Patindol et al., 2005), 16 months storage (Zhou et al., 2007). These results all suggest that storage degrades the structure of starch and affects starch gelatinization properties, ultimately resulting in poor rice texture. High temperature storage has more adverse effects on the texture of rice. Rice is sometimes stored for a long time, sometimes for more than a few years. However, there are few studies on the eating quality of rice under long-term storage. The relationship between the eating quality of long-term storage rice and the multi-level structure of starch is unknown. In this study, the Huanghuazhan, a rice variety that is widely planted in China, is used as the material for this study. One is stored under conventional storage conditions for 7 years, and the other is fresh rice. We wanted to understand the reasons for the poor eating quality of long-term storage rice, to provide our suggestions for improving the rice eating quality.

2. Materials and methods

2.1. Materials

The 7 years stored rice was harvested on November 2, 2014, and the paddy rice was kept at room temperature for 7 years. Fresh rice was harvested in two different fields on November 10, 2020, and the paddy rice was stored in a 4 °C cold storage. The rice varieties of fresh rice and 7 years stored rice were Huanghuazhan, a rice variety widely grown in China. 4 °C was a suitable temperature for rice storage, and the physical and chemical properties of rice was hardly changed. The physicochemical properties of starch were determined in September 2021. All the rice samples were milled into polished rice using a rice mill (Satake, Tokyo, Japan), and then the polished rice was milled into powder and passed through a 100-mesh sieve.

2.2. Rice surface color

The color chroma meter (CR-400/410, Konica Minolta Sensing Inc., Tokyo, Japan) was used to measure the rice surface color. The L* values assessed the degree of lightness to darkness, a* values correlated with the degree of redness to greenness, and b* values measured the extent of yellowness to blueness.

2.3. Rice protein content and amylose content

The elemental analyzer (Elementar, Langenselbold, Germany) was used to determine the nitrogen content in the rice, and multiply it by 5.95 to determine the rice protein content. The amylose content was determined by iodometric method (Shi et al., 2022). Briefly, 0.01 g rice flour was weighed in a 15 ml centrifuge tube and 95% ethanol (100 μl) was added. The mixture was shaken well, then 1 mol/L NaOH (900 μl) was added, heated in a boiling water bath for 10 min, cooled to room temperature and then added with 9 ml of distilled water. Pipetted 500 μl of the solution from the centrifuge tube into a new centrifuge tube, then added 9.2 ml of distilled water and 1 mol/L acetic acid (100 μl). Finally, 200 μl of iodine solution (mixed 0.2 g of iodine and 2 g of potassium iodide and made up to 100 ml) was added and after standing for 10 min, the amylose content of rice was determined by measuring the absorbance of the solution at 620 nm. A standard curve was prepared for a mixed sample of amylose and amylopectin. Each sample was measured three times.

2.4. Rice eating quality

The rice taste analyzer (Satake, Hiroshima, Japan) was used to determine the eating quality of rice. Weighed 30 g of rice, washed it with water within 20 s, and placed it on a stainless-steel pot to ensure that the ratio of rice to water was 1:1.4 (Bergman, 2019), then soaked for 30 min, steamed in a rice cooker for 40 min, and kept warm for 10 min, and finally placed at room temperature for 1.5 h to determine the rice eating quality. Including the luster, taste, hardness, stickiness, and taste value of cooked rice. The luster and taste of each sample were graded with the highest score of 10. A higher taste value often indicated better rice eating quality. Each sample was measured three times.

2.5. Starch isolation

The starch was separated by the previous method (Li et al., 2020). Briefly, 100g of rice was mixed with 300g of water, then milled into a rice slurry by wet milling, and dried in an oven at 40 °C. Rice flour and 300 ml of NaOH solution (0.2%, w/v) were mixed and stirred for 1 h, centrifuged at 3500 r/min for 5 min (repeated 4 times), and the supernatant was discarded. 200 ml of distilled water was added to the residue and stirred for 1 h, neutralized with 0.1 mol/L HCl to pH 7.0, and centrifuged at 3500 r/min for 5 min (repeated twice). Finally, the residue was washed with 95% ethanol (200 ml), centrifuged at 3500r/min for 10 min, and dried at 40 °C overnight.

2.6. Scanning electron microscopy (SEM)

The scanning electron microscope (JSM-6390, NTC, Japan) was applied to measure the starch granule size distributions. The starch powder was placed into the reservoir containing distilled water, and the measurement was started when the shading value was between 10% and 20%. Each sample was measured three times.

2.7. Laser diffraction analysis

A Mastersizer 2000 laser-diffraction analyzer (Malvern, UK) was applied to measure the starch granule size distributions. The starch powder was placed into the reservoir containing distilled water, and the measurement was started when the shading value was between 10% and 20%. Each sample was measured three times.

2.8. Fourier transform infrared (FTIR) spectroscopy

Fourier infrared spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) was used to collect the FTIR spectrum. Made appropriate modifications according to our previous method (Chen et al., 2021). The resolution of the spectrum was 4 cm⁻¹. Air was scanned 64 times as background and each infrared spectrum was scanned 32 times. A half-band width of 19 cm⁻¹ and an enhancement factor of 1.9 was used to calculate the short-range order structure of starch. The OMNIC software was used to baseline correction and deconvolution. Each sample was measured three times.

2.9. X-ray diffraction (XRD)

D8 Advance X-ray powder diffractometer (XRD) (Bruker-AXS, Karlsruhe, Germany) was used to determine the crystal structure of starch, with a diffraction angle ranging from 3 to 40°, a sampling interval of 0.6 s, and a step size of 0.02°. Used MDI Jade v6.0 to calculate the relative crystallinity of starch. The relative crystallinity as the ratio of the area of the crystal peaks at 5.6°, 15°, 17°, 18°, 20°, and 23° at 2θ to the total diffraction area (Man et al., 2014). Each sample was measured three times.

2.10. Small-angle X-ray scattering (SAXS)

The NanoSTAR system (Bruker, Germany) was used to determine the layered structure of starch. In short, a starch slurry with a water content of 60% was kept at about 26 °C for 4 h and then placed on the sample stage, using empty cells of water as a background. 0.015 < q < 0.15 Å⁻¹ was used as the result of SAXS. The scattering vector q = 4πsin θ/λ(20),
the scattering angle). The average thicknesses of semi-crystalline (d_a), crystalline (d_c), and amorphous (d_m) lamellae were calculated using the previous method (Kuang et al., 2017).

2.11. Rapid Visco analyzer (RVA) analysis

A rapid viscosity analyzer RVA (Newport, Warri wood, Australia) was used to determine the pasting of starch. About 3g of rice starch was mixed with 25 ml of water and then put on the aluminum can. The RVA program first heated at 50°C for 1 min, then heated to 95°C in 3.75 min, and then heated at 95°C for 2.5 min. It was then cooled to 50°C in 3.75 min and held for 1.4 min. The peak viscosity, hold viscosity, pasting temperature, and final viscosity were determined from RVA profiles. Breakdown (peak viscosity - hold viscosity) and setback (final viscosity - peak viscosity) were also used. Each sample was measured three times.

2.12. Differential scanning calorimeter (DSC)

Approximately 3 mg of starch was weighed in an aluminum crucible and then 3 times the sample mass of water was added. The crucible was placed in a 4°C refrigerator to equilibrate for 12 h. The onset temperature, peak temperature, end temperature and gelatinization enthalpy were then obtained using a differential scanning calorimeter (DSC-8500, PE, USA) from 25°C to 100°C at a rate of 10°C/min.

2.13. Data analysis

The SPSS 20.0 software (Chica Granule morphology go, IL, USA) and Origin 2021 (Northampton, MA, USA) were used for the analysis of variance (ANOVA) and drawing. Significant differences were deemed to occur at P < 0.05.

3. Results and discussion

3.1. Rice protein content, amylose content, and color

It was found in Fig. 1 that the 7 years stored rice and fresh rice showed different colors, and the 7 years stored rice had a yellowish surface. From the color analysis in Table 1, it could be known that the b* value of rice stored for 7 years was significantly higher than that of fresh rice, which was the same as the result in Fig. 1. A recent study showed that phenylpropane and flavonoids were the most up-regulated metabolites in the process of rice yellowing, which may be the direct cause of rice yellowing (Li et al., 2021).

During storage, the protein content of rice generally did not change (Zhou et al., 2002), the difference in protein content should be attributed to the difference in environmental conditions during the growth process (Kashiwagi and Munakata, 2018). Some studies on rice storage indicated that starch was degraded due to the action of amylase during storage. There are a lot of starch-degrading enzymes in rice, such as α-amylase, β-amylase, and α-glucosidase. Moreover, the optimum temperature of starch degrading enzymes in the endosperm of rice was higher than that of other parts (Awazuha et al., 2000). Starch is mainly located in the endosperm of rice. In the process of rice storage, these enzymes can degrade rice starch through combined action and degrade starch into some small molecular sugars. A similar situation occurred during the storage of corn (Setiawan et al., 2010). The amylose was more susceptible than amyllopectin because it was restricted to the amorphous structure of starch. Changes in enzymatic reactions and other chemical reactions made amylose easier to degrade (Patindol et al., 2005). In Table 1, it could be found that the amylose content of 7 years stored rice was 13.29%, which was significantly different from the amylose content of fresh rice. In some studies, it has been reported that the amylose content of Huanghua was generally between 16.78% and 23.61%(Shi et al., 2021), so long-term storage for 7 years reduced the amylose content of rice.

3.2. Rice eating quality

It could be found in Table 2 that 7 years stored rice had a lower luster, taste and taste value than fresh rice. The hardness of the 7 years stored rice was higher and the stickiness was lower than fresh rice, indicating that the eating quality of the stored rice was lower, which may be due to the difference in rice protein content and starch structure. Similar results were found in previous studies, long-term storage of rice tended to have poorer eating quality (Matsue et al., 1991). Generally speaking, rice with lower amylose content tends to have a softer texture and higher eating quality (Li et al., 2009). Later studies found that the structure of amylose and amyllopectin also had an important effect on the eating quality of rice (Li et al., 2016; Takeda et al., 1989). In our study, 7 years stored rice had lower amylose content and harder texture. This may be related to the structure of amylose and amyllopectin. Some

### Table 1

| Sample | Protein content(%) | Amylose content(%) | L* | a* | b* |
|--------|-------------------|-------------------|----|----|----|
| Fresh  | 5.97 ±            | 23.61 ±           | 72.09 ± | -0.27 ± | 6.78 ± |
| rice 1 | 0.15c             | 0.43a             | 0.01a | 0.02a | 0.01b |
| Fresh  | 7.56 ±            | 22.96 ±           | 71.78 ± | -0.6 ± | 6.77 ± |
| rice 2 | 0.16a             | 0.62a             | 0.60a | 0.08b | 0.09b |
| 7 years| 6.52 ±            | 13.29 ±           | 71.36 ± | -0.39 ± | 10.32 ± |
| rice 1 | 0.09b             | 0.33b             | 0.73a | 0.16a | 0.59a |

Different letters in the same column indicate significant differences (P < 0.05).

### Table 2

| Sample | Luster | Taste | Hardness | Stickiness | Taste value |
|--------|--------|-------|----------|------------|-------------|
| Fresh  | 8.73 ± | 8.47 ± | 1.38 ±   | 0.19 ±     | 79.67 ±     |
| 1      | 0.06a  | 0.06a | 0.17b    | 0.04a      | 1.15a       |
| Fresh  | 8.23 ± | 7.97 ± | 1.48 ±   | 0.18 ±     | 73.33 ±     |
| 2      | 0.06b  | 0.06b | 0.28b    | 0.02a      | 0.58b       |
| 7 years| 6.47 ± | 6.87 ± | 1.97 ±   | 0.10 ±     | 68.33 ±     |
| rice   | 0.06c  | 0.06c | 0.23a    | 0.03b      | 0.58c       |

Different letters in the same column indicate significant differences (P < 0.05).

Fig. 1. Surface picture of rice.
previous studies presented a similar view, with two types of rice with 15.91% and 16.01% amylose content of 80.67 and 61.67 in taste values (Peng et al., 2021). Amylose can entangle with amylopectin in the crystalline lamellae, affecting starch expansion during cooking, and resulting in a harder texture (Li and Gilbert, 2018).

3.3. Granule features

The scanning electron microscope of starch was shown in Fig. 2. It was found that the three kinds of rice all showed similar starch granule morphology, and the starch presented a polygonal irregular morphology, which was consistent with previous studies (Pérez and Bertoft, 2010). Some small holes were found in fresh starch 2, which may be related to enzymes in the starch formation process (Buléon et al., 1998). It was worth noting that the polyhedral edges and corners of some 7 years starch granules seem to be abraded, indicating that the storage process for 7 years may cause some starch granules to degrade (Wang et al., 2022).

3.4. Granule size distribution

According to previous studies, starch could be divided into three types, small starch granules (<1 μm), medium starch granules (1–5 μm), and large starch granules (>5 μm) (Wang et al., 2020). The particle size distribution of starch was shown in Fig. 3. With the increase of starch particle size, the particle size ratio of fresh rice starch showed a consistent law. When the particle size was about 10 μm, there were more starch particles, when the particle size was greater than 15 μm, no starch granules were seen. 7 years starch still existed when the particle size was greater than 15 μm, indicating that long-term storage was prone to producing more large starch granules. It could be found in Table S1 that the 7 years starch had fewer small starch granules (<1 μm) and medium starch granules (1–5 μm), and more large starch granules (>5 μm). A similar rule was also found during the storage of sweet potato starch, small starch granules may be more easily degraded due to the larger relative surface area of small starch granules (Niu et al., 2019). The volume mean diameter of 7 years starch was more than 4 times that of fresh rice starch, indicated that long-term storage greatly changed the grain size of rice starch.

3.5. Crystalline structure

The crystal structure of starch was generally divided into 3 types, A type, B type, and C type (Cheetham and Tao, 1998). It could be found in Fig. 4(A) that the rice starch presented single diffraction peaks at 15° and 23°, and undivided double diffraction peaks at 17° and 18°. The rice starch presented a typical A-type crystal structure. This was consistent with the results of previous studies, the crystal type of starch often depended on different plant sources and had nothing to do with variety (Wang et al., 2012). 7 years starch had similar FTIR spectra.

The FTIR spectrum of starch was shown in Fig. S1(A). The characteristic peaks around 3420 cm⁻¹ and 2930 cm⁻¹ were related to the vibration of O–H stretching and the C–H deformation of the glucose unit. The absorbance at 1650 cm⁻¹ was related to the O–H bending vibration of water absorbed by the amorphous region of the starch. Three characteristic peaks belonging to C–O stretching vibration were observed between 1020 cm⁻¹ and 1160 cm⁻¹ (Kizil et al., 2002). Fresh starch and 7 years starch had similar FTIR spectra.

The Fourier deconvolution spectrum of starch was shown in Fig. S1 (B). The 1047 cm⁻¹, 1022 cm⁻¹, and 995 cm⁻¹ bands in the FTIR
spectrum were sensitive to the crystalline and non-crystalline regions of starch. Therefore, 1047/1022 cm$^{-1}$ and 1022/995 cm$^{-1}$ could be used to express the short-range ordered structure of starch (van Soest et al., 1995).

It could be seen from Fig. S1(C) that the 7 years starch had the highest ratio of 1022/995 cm$^{-1}$ and the lowest ratio of 1047/1022 cm$^{-1}$, indicating that the short-range ordered structure of long-term storage starch was lower. The starch structure of rice gradually dissociated after storage, which affected the short-range order structure of starch. Our research results were consistent with previous studies (Wang et al., 2022). The long-term storage of starch could cause the dissociation of the starch aggregation structure due to the action of enzymes and ultimately led to the reduction of the short-range order structure of starch.

3.7. Lamellar structures

The Double-logarithmic SAXS patterns of starch were shown in Fig. S2(A). All samples showed obvious scattering peaks at a q value of 0.065 Å$^{-1}$. According to Bragg’s law $D = 2n/q$, the layer thickness of starch was approximately 8–9 nm (Li et al., 2020). The intensity of the starch scattering peak was determined by the number of semi-crystalline structures and the electron density in the crystalline and amorphous regions (Yuryev et al., 2004). It could be seen from Fig. S2(A) that the peak intensities of the three rice starches showed different differences. The peak intensity of fresh starch 1 was the largest, and the peak intensity of fresh starch 2 was the smallest, indicating that the difference in electron density between the two types of lamellae of fresh starch 1 was the largest. In order to make the scattering peak clearer, the SAXS after Lorentz correction was shown in Fig. S2(B), from which we could also find similar results. The one-dimensional correlation function was shown in Fig. S2(C). One-dimensional correlation function could be used to analyze the structural parameters of the layered structure of starch, including starch thickness of crystalline ($d_c$), amorphous ($d_a$) region of the lamella and long period distance ($d_{lp} = d_a + d_c$) (Kuang et al., 2017). The specific calculation results were shown in Table S2. We found that 7 years starch had lower $d_c$ and higher $d_a$, which may be related to the amylose content. A recent study showed that amylose contributes to the crystal structure of starch, and the increase in amylose content will lead to an increase in $d_c$ (Zhong et al., 2021). In some previous studies on short-term storage, it was found that with the increase of storage time, the thickness of the crystalline lamellae of starch first increased and then decreased (Wang et al., 2022). During long-term storage, starch-degrading enzymes may cleave α-1,4- and α-1,6-glycosidic bonds, thereby reducing the thickness of the crystalline lamellae (Wang et al., 2022). Our results also suggested that long-term storage led to severe hydrolysis of the crystalline lamellae.

3.8. Thermal characteristics

The changes in starch gelatinization temperature represented the interaction of multiple factors within the starch, including the ratio of amylose and amyllopectin in the starch, the granule size of the starch, and the long-range and short-range order structure of the starch, etc. The thermograms of 7 years starch and fresh starch were shown in Fig. S3. The peak temperatures of fresh starch 1 and fresh starch 2 were 66.70°C and 64.67°C, respectively. The peak temperature of 7 years starch was 69.23°C. 7 years starch had a higher peak temperature, probably due to differences in starch granule size. Larger starch granules are more difficult to gelatinize than small starch granules and therefore require higher peak temperatures (Zhang et al., 2013). Higher gelatinization enthalpy in starch represented more crystallinity and more ordered double helix. From the results of gelatinization enthalpy, the gelatinization enthalpy of 7 years starch was the lowest (1.68 J/g), and the fresh starch had higher gelatinization enthalpy (9.47 J/g and 10.92 J/g). Long-term storage may cause the crystalline structure of starch to gradually begin to dissociate and eventually lead to instability of the crystalline structure of starch. However, fresh starch 2 and 7 years starch had similar crystallinity and different gelatinization enthalpy. This has happened similarly in some other studies, and the gelatinization enthalpies of two rice starches with a crystallinity of 45.74% and 46.02% were 123 J/g and 255.63 J/g, respectively (Govindaraju et al., 2022). This may be related to the chain length distribution of amyllopectin. The crystalline region wrapped by long amyllopectin was more stable, thus requiring more thermal energy to break the crystal structure. Long-term storage may lead to the gradual dissociation of long amyllopectin, which ultimately affects the stability of the starch crystal structure (Wang et al., 2022; You et al., 2014). The gelatinization enthalpy of starch was related to the eating quality of rice, and some studies have found that lower gelatinization enthalpy was often associated with rice varieties with poor eating quality (Peng et al., 2021). Therefore, the eating quality of 7 years stored rice was poor.

3.9. Pasting properties

The structural changes of starch could affect the gelatinization properties of starch. It could be found in Fig. S4 that the 7 years starch had the highest peak viscosity and the lowest final viscosity, while the two fresh starch had similar RVA curves. The breakdown and setback of starch were shown in Table S3. The breakdown of 7 years starch was the highest and the setback was the lowest. Peak viscosity was a measure of the maximum viscosity reached by gelatinized starch when heated in water. It indicated the water binding capacity of starch samples. Previous studies have shown that large granular starch could increase the water binding capacity of starch (Bhat and Riar, 2016). Therefore, 7 years starch showed a higher peak viscosity due to more large starch...
granules. On the other hand, the presence of amyloide could inhibit the expansion of starch granules and maintain the integrity of starch granules (Peng et al., 2021). 7 years starch showed a higher peak viscosity due to lower amylose content. Generally speaking, rice with higher peak viscosity and breakdown had better eating quality (Yang et al., 2020). However, the eating quality of 7 years stored rice was the worst, which may be attributed to the difference in pasting temperature (Zhang et al., 2016). A previous study suggested that lower pasting temperatures may be the key to good eating quality rice (Chun et al., 2015). 7 years starch had the highest pasting temperature, indicating that higher temperatures were required to gelatinize the starch. Therefore, long-term storage of rice showed poor eating quality.

4. Conclusion

In order to understand the reasons for the poor eating quality of rice stored for a long time, we explored the differences in starch multilayer structure and pasting properties between 7 years stored rice and fresh rice. Our results indicated that 7 years stored rice had lower amylose content. From the perspective of starch structure, 7 years starch had more large starch granules and volume mean diameter, lower starch short-range order structure and thickness of crystalline lamellae. In particular, the volume mean diameter of 7 years starch was more than 4 times that of fresh starch. Long-term storage affected the stability of starch crystal structure. More large starch granules and unstable crystal structure affected starch gelatinization, which eventually resulted in higher pasting temperature and lower gelatinization enthalpy of 7 years stored starch. Therefore, 7 years stored rice had higher hardness and the poorer eating quality.

CRediT authorship contribution statement

Shijie Shi: Conceptualization, Software, Formal analysis, Writing – original draft. Keqiang Pan: Methodology, Writing – review & editing. Ming Yu: Methodology, Writing – review & editing. Lina Li: Writing – review & editing. Jichao Tang: Methodology. Bo Cheng: Writing – review & editing. Juan Liu: Writing – review & editing. Cougui Cao: Investigation. Yang Jiang: Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.08.013.

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