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The Variation of Rice Quality and Relevant Starch Structure during Long-Term Storage

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Abstract: The main substances of rice are starches, which vary their metabolism during storage. We conducted a series of tests including rice physicochemical properties, edible quality, starch content and chain length distribution along with starch structure variation to disclose the shift of rice quality by observing the changes of rice during storage. The results showed that: (1) the rice deterioration occurred as time passed, and the germination rate decreased from 70.8% to 29.4% during the storage; (2) fatty acid values increased significantly during long-term storage; (3) electrical conductivity increased as time passed; and (4) the two-year-storage rice showed significantly decreased viscosity and edible quality after sensory evaluation, decreased hardness and damaged surface area of starch granules as storage time passed. Additionally, the damaged surface area of starch granules increased with storage time. Fourier transform infrared spectroscopy (FTIR) showed that the short-range order and spiral degree of rice starch first decreased in the first year and then increased over the storage time. Furthermore, X-ray diffraction showed that the main starch of rice was A-type crystalline. Meanwhile, apparent amylose content increased from 31.00% to 33.85%, then decreased to 31.75%. The peak viscosity reduced from 2735.00 mPa·s to 2163.67 mPa·s and the disintegration value was brought down from 1377.67 mPa·s to 850.33 mPa·s. Based on the results, rice should not be stored for more than 2 years under suitable granary conditions to maintain it at a good quality.

Keywords: rice; starch structure; physicochemical quality; storage

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1. Introduction

Rice is a staple food for half of the world's population and plays an important role in daily diet [1]. The COVID-19 pandemic and the outbreak of conflict between Russia and Ukraine had disrupted world food production and supplication, making food storage more important than before [2]. The storage quality of rice is usually affected by temperature, humidity, atmosphere and storage time, so its physical, chemical and physiological characteristics will undergo a variation during long-term storage. When rice ages in storage, its color and flavor will change obviously [3]. Compared with fresh rice, aged rice usually turns dark and yellow and develops unpleasant odors due to the rancidity of fatty acids. Additionally, the processing characteristics of aged rice vary greatly, such as decreased viscosity and gelatinization temperature, along with increased hardness [4,5] Based on the effect of storage conditions and aging phenomenon on rice quality, it is quite important to control the aging rate of rice and develop methods to maintain the storage and edible quality of rice.

Starch, as the most important component of rice, accounts for about 80% of rice dry weight, which is closely related with rice aging and its quality variation [6]. Starch is a kind of polysaccharide polymer which is mainly composed of α -D-glucopyranosyl units linked by glycosidic bonds [7]. It contains linear amyloses linked by α-1, 4-glycosidic bond and highly branched amylopectins linked by α-1, 6-glycosidic [8]. The short side chains of branched starch are stacked in the double helix structure to form the crystalline region in starch. The alternative distribution of the crystalline region and the amorphous region constitutes the growth ring of starch granules [9]. The starch function usually changes accordingly with the structural transforms, like the straight-chain starch content is correlated with the pasting property of rice [10]. The higher straight chain amylose content, the harder the edible quality of the rice will be [11]. For the amylopectin, the longer branched chain can form a longer double helix structure in the semi-crystalline layer, leading to a higher gelatinization temperature of rice. The vice versa is also true. Meanwhile, the interaction between amylose and the outer chain of amylopectin in the crystal lamella also has a crucial impact on rice gelatinization [12]. Many studies have shown that the multi-scale structure and physicochemical property of starch have a significant influence on the texture, processing and nutritional properties of rice [13]. Starch retrogradation is the main reason for the hardening of rice after cooking and cooling [14]. When straight-chain starch content is increased, it will facilitate the starch retrogradation. What's more, the rice with higher straight-chain starch content and long-chain branched starch content tends to contain more slow-digesting starch (SDS) and resistant starch (RS) and possess more functional properties [15,16].

To improve the storage quality of rice, it is important to figure out the effect of the transformation of the starch structure and physicochemical properties on the taste quality of rice during storage. Although many research studies have explored this relationship in the laboratory conditions, there is still a lack of studies on the long-term storage at the granary level [17]. Compared with the previous related studies, in this paper, we mainly focus on the rice from granary and investigate the variation in the rice quality and starch structure and their interaction, so as to provide practical suggestions for maintaining rice with a desirable quality in granary.

2. Materials and Methods

2.1. Materials

The samples belong to indica rice (Zhongzheyou 8), which are stored for 0, 1 and 2 years in Quzhou grain depot in Zhejiang Province under the temperature of 20–25 °C in summer and 10–15 °C in other seasons. The conditions were maintained by air conditioner to keep the rice at a quasi-low temperature. The rice stored for different years were set as different treatments. Each treatment was composed of five samples from five different silos, for a total of 15 samples in this study.

2.2. Physicochemical Properties' Variation of Rice

Rice germination rate was tested according to Hu's method with appropriate modifications [18]. One hundred pellets of rice seeds were soaked in 0.5% sodium hypochlorite for 30 s, and then rinsed 3 times using distilled water. The cleaned rice seeds were placed into a sterile germination box (20 × 20 cm), followed by being incubated under the condition of 60% relative humidity at 30 °C for 10 d. The germinated seeds were taken to calculate the germination rate. The rice conductivity was determined using Qu's method [19]. The determination of fatty acid values was carried out with Zhai's test method [20]. The protein content was determined by the method in Daiana deSouza's research [21].

2.3. Rice Edible Quality Evaluation

Rice edible quality was tested using a previous method with a slight modification [20]. Ten grams of cleaned rice seeds with different storage times were soaked for 30 min and cooked in a steamer for 40 min for a tasting test. The tasting test was conducted by artificial oral tasting combined with tasting apparatus (STA1B style, Sasake Co., Toshima, Japan).

2.4. Rice Starch Isolation

Rice was soaked overnight with five-time volumes of distilled water, after which it was pulped by a 200-mesh sieve and washed with distilled water. After centrifugation at

4000 rpm for 10 min, the suspension was collected by removing the upper layer of protein. Then, it was dried at 42 °C and filtrated through a 100-mesh sieve for the further analysis.

2.5. Starch Content Determination

2.5.1. Amylose and Total Starch Content Analysis

The straight-chain amylose content of rice starch was determined by iodine colorimetric method. It was calculated from the standard curves drawn with different proportions of straight-chain starch and branched-chain starch blends [22].

2.5.2. Distribution of Starch Chain Length

Based on Ren's method, the distribution of starch chain length in rice was tested [23]. The starch (10 mg) was dissolved in 5 mL water and then placed in a boiling water bath for 60 min. Afterwards, sodium azide solution (10 µL 2% *w*/*v*), acetate buffer (50 µL, 0.6 M, pH 4.4) and isoamylase (10 µL, 1400 U) were added to the starch dispersion, and the mixture was incubated at 37 °C for 24 h. The hydroxyl groups of the debranched glucans were reduced with 0.5% (*w*/*v*) of sodium borohydride under alkaline conditions for 20 h. Then, the solution was diluted with 570 µL of distilled water. The sample extracts were analyzed using high-performance anion-exchange chromatography (HPAEC) equipped with a CarboPac PA-200 anion-exchange column (4.0 × 250 mm; Dionex) and a pulsed amperometric detector (PAD; Dionex ICS 5000 system). Data were acquired on the ICS5000 (Thermo Scientific, Waltham, MA, USA) and processed using chromeleon 7.2 CDS (Thermo Scientific).

2.6. Starch X-ray Diffraction Analysis (XRD)

The crystal structure of rice starch was analyzed by a X'Pert3 Powder (PANalytical, Almelo, The Netherlands). The machine was equipped with a Cu-Kα target ray and the wavelength was 0.15406 nm. The starch was detected at the voltage of 40 kV and the tube flow was 200 mA. The diffraction ranged from 4° to 40° (2θ) with the 4°/min step length. The degree of relative crystallinity was calculated based on the two-phase hypohypothes according to the method reported by Lopez-Rubio et al. [24].

2.7. Starch Fourier Transform Infrared Spectroscopy Anlysis (FTIR)

The rice starch samples were ground with KBr at the ratio of 1:100. Then, the mixed power was tableted by a tablet machine to form flakes. The short-range structure was observed by FTIR (Brucker GMBH, Berlin, Germany) at the range of 4000–400 cm^{-1}. The operation was executed at the frequency of 4 cm^{-1} for 16 times.

2.8. Starch Rapid Viscosity Analysis (RVA)

The pasting property of starch flour was determined using a rapid viscosity analyzer and performed on the parboiled starch flour. The previously isolated starch was weighted around 2.58 g and mixed with distilled water to the total weight of 28 g. The suspension was maintained at 50 °C for 1 min, then it was heated to 95 °C at a rate of 12 °C/min and was held for 5 min. The rice paste was cooled to 50 °C at a rate of 12 °C/min and was maintained for 2 min. The pasting curve of rice stored for different times was obtained by RVA equipment (Newport Scientific Instruments inc. Fyshwick, Austrilia).

2.9. Differential Scanning Calorimetry (DSC) Analysis of Starch

The thermal stability of starch was evaluated by DSC calorimeter (METTLER TOLEDO, Inc. New York, NY, USA). The rice starch (3 mg) and distilled water (1:3, *w*/*w*) were sealed in an aluminum crucible and then equilibrated overnight. The measurement temperature was raised from 30 °C to 130 °C at the ratio of 10 °C/min.

2.10. Scanning Electron Microscope Analysis of Starch (SEM)

The rice starch was fixed with 2.5% glutaraldehyde overnight and then eluted with gradient ethanol (30, 50, 70, 90, and 95%) for 20 min to preserve the rice morphology. After this treatment, a piece of fixed samples was coated with a thin layer gold and placed in the SEM to observe with the resolution of 2000× and 6000×.

2.11. Data Statistics and Analysis

The single treatment of each test had three replicates, and each test was conducted twice. Statistical analysis, one-way analysis of variance (ANOVA), Duncan's test and a post-hoc test were conducted using SPSS statistical software (Version 22.0, IBM, Armonk, New York, NY, USA). All the figures were plotted using Origin Pro software(Originlab 2020 student edition, Northampton, MA, USA).

3. Results and Discussion

3.1. Rice Physicochemical Properties Variation of Different Storage Time

Physical and chemical indexes are important characteristics of rice quality. Germination rate is a specific manifestation of seed vigor, which is usually affected by storage time. The longer the storage time, the lower the germination rate would be. In this study, as shown in Figure 1a, rice germination rate decreased significantly after being stored for two years, with a decrease of 41.4%, which means its vitality and nutritional quality decreased obviously. Additionally, fatty acid value is a key indicator of rice freshness and aging. The accumulation of excessive fatty acid can lead to rice rancidity, thus decreasing its edible quality [25]. Compared with fresh ones, the fatty acid value of rice became significantly higher after one year storage, whereas there was no difference after two years storage (Figure 1b), which may be due to the reduced lipase activity with longer storage time. Furthermore, cell membrane permeability is related to the nutrient supply of the rice embryo, so the rice cell membrane spoilage can be used to evaluate rice quality, which can be assessed by measuring conductivity [26]. The rice conductivity increased from 50 μs/(cm·g) to 73.6 μs/(cm·g) with storage time, indicating the rice quality decreased obviously (Figure 1c), which is consistent with the previous report [1]. However, the protein content is less affected by storage time, so it slightly decreased without evident difference in this study (Figure 1d).

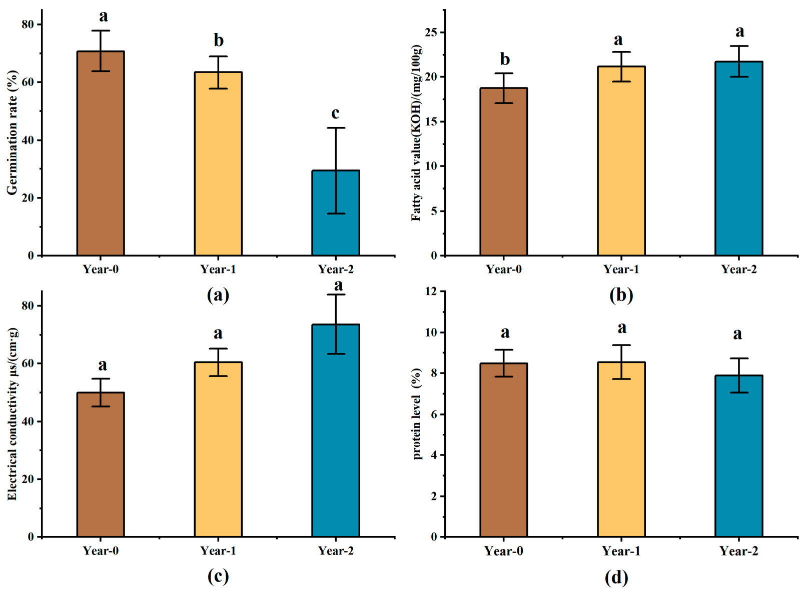


Figure 1. The changes in physical and chemical indexes of rice. (**a**) Germination rate, (**b**) fatty acid value, (**c**) electrical conductivity, (**d**) protein content. Different letters in this figure indicate there are significant differences among them. ($p < 0.05$).

3.2. Rice Taste Quality Eveluation

Sensory evaluation is the most classical and direct method to measure the taste quality of rice, which is also an important factor to influence consumers' purchase desire [27]. Rice characteristics, including hardness, stickiness, elasticity, color, odor and cold rice texture, are the expressions of rice taste quality. As shown in Table 1, the variation of rice sensory scores indicated that rice edible quality had significant decrease after being stored two years, especially rice luster, viscosity, elasticity and hardness. In a specific range, higher fat content of rice could make it more lustrous, so when the fat oxidation and decomposition happened in rice storage, its fatty acid value would increase, resulting in rice luster spoilage and the decreased flavor score [28]. The same phenomenon occurred in this study (Table 1). Additionally, firmness and stickiness are two key factors to assess the rice texture [29,30].

Table 1. Rice edible quality variation during different storage time.

Organoleptic Indicator	Year-0	Year-1	Year-2
Color	6.00 ± 0.00 ^{a}	6.00 ± 0.00 ^{a}	6.00 ± 0.00 ^{a}
Luster	5.20 ± 1.30 ^{a}	4.80 ± 0.84 ^{a}	4.60 ± 0.55 ^{b}
Grain integrity	3.00 ± 0.00 ^{a}	3.00 ± 0.00 ^{a}	3.00 ± 0.00 ^{a}
Viscosity	6.80 ± 0.45 ^{a}	6.20 ± 0.45 ^{a}	5.60 ± 0.55 ^{b}
Elasticity	6.20 ± 0.84 ^{a}	5.80 ± 0.84 ^{a}	5.60 ± 1.34 ^{b}
Hardness	7.20 ± 0.45 ^{a}	6.80 ± 0.84 ^{a}	6.60 ± 0.55 ^{b}
Taste	18.0 ± 1.73 ^{a}	17.00 ± 0.00 ^{a}	16.80 ± 0.45 ^{a}
Cold rice texture	3.00 ± 0.00 ^{a}	3.00 ± 0.00 ^{a}	3.00 ± 0.00 ^{a}

Notes: Different letter values in the same line were significantly different ($p < 0.05$).

The straight-chain starch content normally affects the hardness and viscosity of rice, which is on account of its leaching out and the formation of a web-like structure around the swollen granules during the cooking process. The long B-chain of branched starch can also form a double helix structure with straight-chain starch, which makes rice water absorption capacity decline and then results in rice hardness increasing [31]. Although the protein content did not have significant difference in this study, the increase in rice hardness indicated that the protein structure, especially the interaction between disulfide bonds, was strengthened [32]. Interestingly, the highest straight-chain amylose content was found in the rice stored for 1 year (Table 2), but the hardness score decreased significantly after 2-year storage. It manifested that rice hardness was not only affected by the straight-chain amylose content, but also by other factors, such as the soluble branched-chain starch content, molecular size and the interaction force between them [33]. As shown in Tables 2 and 3, rice stored for one year had the highest straight-chain starch content, but it didn't have an evident effect on rice viscosity. With the storage time extending, the straight-chain starch content decreased in the rice stored for two years, along with a declined viscosity, which might be due to the integrity breakdown of rice starch granules (Figure 2).

Table 2. Order degree of rice starch from different storage time.

Samples	Relative Crystalline (%)	995/1022	1047/1022
Year-0	34.23 ± 0.93 ^{a}	1.3871 ± 0.0139 ^{a}	1.3656 ± 0.0046 ^{a}
Year-1	34.95 ± 1.29 ^{a}	1.3192 ± 0.0117 ^{c}	1.3001 ± 0.0028 ^{c}
Year-2	35.25 ± 0.52 ^{a}	1.3616 ± 0.0136 ^{b}	1.3488 ± 0.0039 ^{b}

Notes: Values in the same column with different letters are significantly different ($p < 0.05$).

Table 3. Chain length distribution variation of rice amylopectin in storage.

Samples	AAC(%)	A(%)	B1(%)	B2(%)	B3(%)	ACL(DP)
Year-0	31.00 ± 0.73 ^{b}	24.67 ± 0.09 ^{a}	53.24 ± 0.06 ^{b}	11.16 ± 0.04 ^{b}	10.92 ± 0.10 ^{b}	20.15 ± 0.04 ^{b}
Year-1	33.85 ± 0.28 ^{a}	24.64 ± 0.02 ^{a}	51.08 ± 0.03 ^{c}	13.31 ± 0.03 ^{a}	11.00 ± 0.01 ^{b}	20.18 ± 0.01 ^{b}
Year-2	31.73 ± 0.48 ^{b}	23.63 ± 0.10 ^{b}	54.01 ± 0.05 ^{a}	11.19 ± 0.04 ^{b}	11.16 ± 0.04 ^{a}	20.32 ± 0.03 ^{a}

Notes: Apparent amylose content (AAC); A, B1, B2, B3 and average chain length (ACL) refer to DP ranges of 6–12, 13–24, 25–36, DP ≥ 37 and average chain length, respectively. Values in the same column with different letters are significantly different ($p < 0.05$).

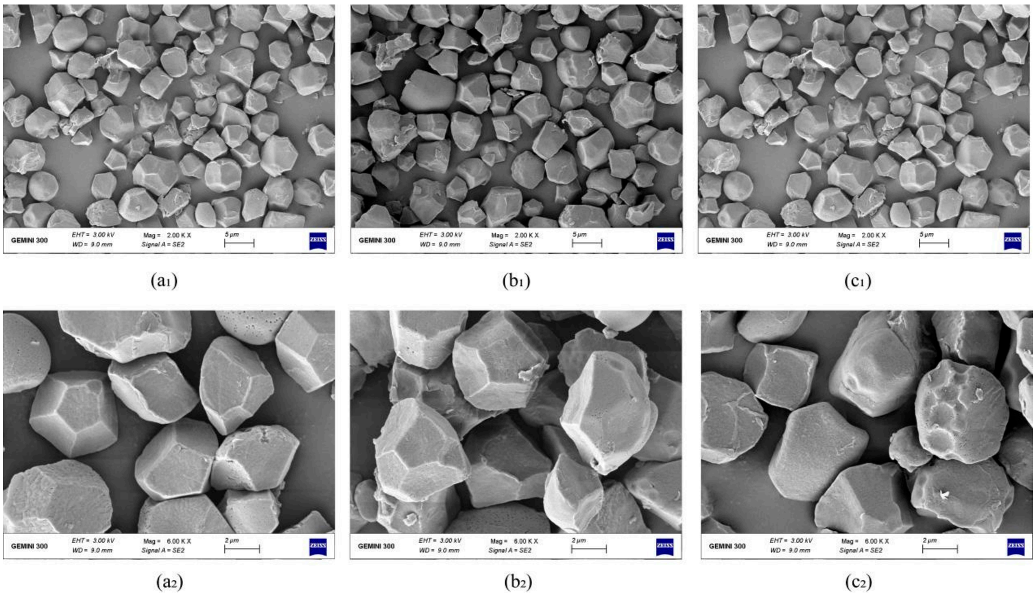


Figure 2. Scanning electron microscope photos of rice starch granules with different storage times; (**a1**,**a2**) are starch granules under storage year-0 2K, 6K lens; (**b1**,**b2**) are starch granules under storage year-1 2K, 6K lens; (**c1**,**c2**) are starch granules under storage year-2 2K, 6K lens.

3.3. Rice Starch Structure Variation of Different Storage Time

Natural starch granules mainly have three types of structure, including A, B and C. A-type crystalline structure has characteristic diffraction peaks at 2θ around 15.33°, 17.33°, 18.15° and 23.22°; B-type crystalline structure has characteristic diffraction peaks at 2θ around 5.59°, 17.2°, 23.2° and 24°; C-type crystalline structure has characteristic diffraction peaks at 2θ around 5.73°, 15.3°, 17.3°, 18.3° and 23.3°. Meanwhile, the main characteristic diffraction peaks of V-type crystalline structure are at 2θ of 7.36°, 13.1° and 20.1°. The rice starch structure generally belongs to A-type crystal texture [34].

As shown in Figure 3a, the starch isolated from rice had specific monomorphic diffraction peaks at 15.33° and 23.5°. The starches from rice with different times all belonged to A-type crystal structure, indicating that the storage time had no effect on rice crystal configuration, whereas the peak intensities were different at 2θ of 18.15°, 20.17° and 23.22°. The peak intensity decreased after 1 year of storage but increased after 2 years, which was consistent with the variation of A-type starch crystals. According to the results in Figure 3a and Table 3, the straight-chain starch content in rice samples stored for 2 years decreased significantly, which suggested that the free short straight-chain starch was bound to form a double-helix structure and this led to an increase in A-type crystals. The peak intensity at 20.1° represents the decreased content of V-type crystals, which are usually composed of single-helix straight-chain starch and lipids [35]. The tendency was related to the fat oxidative decomposition, which promoted the increase in straight-chain starch content. However, the B-type crystals gradually increased based on the diffraction intensity up to 23.22°. The composition of B-type crystals is often related to the content of branched starch B-chains. As shown in Table 3, the content of longer B-chains (B2, B3) increased with storage time extending, which was the principal reason for the B-type crystals' accession.

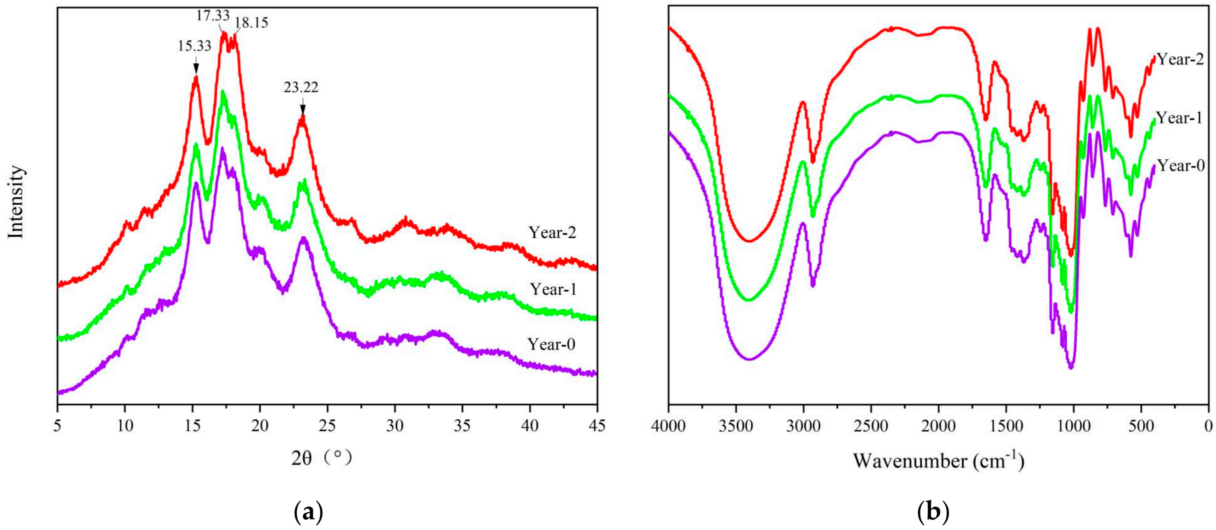


Figure 3. Rice starch variation during storage. (**a**) X-ray diffraction patterns of rice starch at different storage periods; (**b**) Fourier spectra of rice starch at different storage periods.

In addition to the variation of crystal types, we also calculated the relative crystallinity of starch isolated from rice and evaluated its helical structure and short-range ordering. As shown in Figure 3b, there was no new peaks appearing in rice with different storage times, indicating that the storage can only affect the physical structure of starch. As shown in Table 3, there was no significant difference in the relative crystallinity. The light transmission of freshly harvested rice starch was at 3410 cm^{-1}, which indicated the hydrogen bonds between starch molecules were the most solid helical structure. When the storage time was extended, the light transmittance decreased, manifesting that the quantity of hydrogen bonds decreased and the interaction force between starch molecules became weak. Besides, the density ratios of peaks at 1047 cm^{-1}/1022 cm^{-1} and 995 cm^{-1}/1022 cm^{-1} were used to determine the degree of short-range orderliness and double helix structure in starch, respectively [36,37]. As shown in Table 3, the starch of fresh rice had the highest 1047/1022 ratio and 995/1022 ratio, with 1.39 and 1.37, respectively. This means that the starch of fresh rice had the most stable helical structure and the highest short-range orderliness. With the storage process prolonging, the helical structure and short-range orderliness started to decrease and then increase. The decrease in helical structure and orderliness was mainly related with the disruption of branched chains in branched starch, and the increase in helical structure might be due to the fact that apparent amylose was involved in the amorphous region and then formed a new double helical structure, which was supported by the previous results in Table 2.

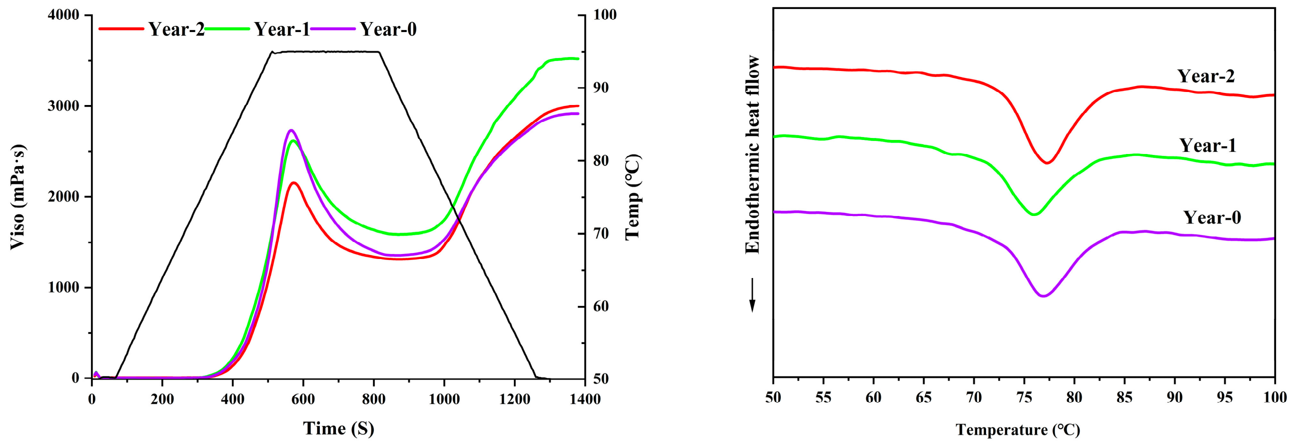
3.4. Pasting and Thermal Properties Variation of Rice in Storage

Generally, the variation of starch granules can cause different rice pasting and thermal properties. Here, we evaluated these two properties to uncover the quality transformation of rice, in which pasting refers to the process of turning the starch structure from ordered to disordered. The rapid viscosity analysis (RVA) is usually used to test rice pasting property, including the expansion, destruction and reorganization of starch [38]. As shown in Table 4 and Figure 4a, compared with the starch in fresh rice, the peak viscosity of stored rice starch continued to decrease prominently during the storage, especially in the second year, which is consistent with the results of the sensory evaluation (Table 1). Unlike other studies that showed a positive correlation between the peak viscosity and the straight-chain starch content [39], the results in this study indicated that there was not simply a positive relationship between them. The straight-chain starch content increased, whereas the peak viscosity decreased after two years of storage. Combined with the SEM images of starch granules (Figure 2), the starch granules were severely damaged at the same time. The incompleteness of starch granules limited the starch expansion, leading to the decline of peak viscosity. Starch pasting is the water blending in the starch cluster crystallization area under heating, which disassembles the intermolecular state of starch and causes starch molecules to lose their original arrangement; for example, the hydrogen bonds between the ordered (crystalline) and disordered (amorphous) molecules of starch granules are broken and dispersed in water to become a colloidal solution [40].

Table 4. Rice pasting properties variation in storage.

Samples	PV (mPa·s)	TV (mPa·s)	BD (mPa·s)	FV (mPa·s)	PT (°C)	SB (mPa·s)
Year-0	2735.00 ± 84.18 [a]	1357.33 ± 27.93 [b]	1377.67 ± 59.65 [a]	2918.00 ± 37.36 [a]	83.83 ± 0.78 [ab]	1560.67 ± 32.65 [c]
Year-1	2622.00 ± 24.27 [a]	1586.33 ± 13.65 [a]	1035.67 ± 26.54 [b]	3519.00 ± 83.47 [b]	82.62 ± 0.28 [b]	1932.67 ± 91.48 [a]
Year-2	2163.67 ± 88.79 [b]	1313.33 ± 37.07 [b]	850.33 ± 51.73 [c]	3003.00 ± 45.90 [a]	84.63 ± 0.81 [a]	1689.67 ± 23.71 [b]

The letters PV represent the peak viscosity; TV represents the trough viscosity; BD represents the breakdown viscosity; FV represents the final viscosity; SB represents the setback viscosity; PT represents the pasting temperature. Values in the same column with different letters are significantly different ($p < 0.05$).



(**a**) (**b**)

Figure 4. Rice pasting and thermal property variation during storage. (**a**) Rice starch RVA diagram; (**b**) Rice DSC diagram.

As shown in Figure 4b, the pasting temperature showed a tendency of first decreasing and then increasing during storage. There was no significant difference between the pasting temperature of fresh rice and one-year stored rice, but it increased significantly in the second year. The thermal stability was related to the double helix structure, which was consistent with the trend of pasting temperature variation. The disintegration value reflects the stability of the starch, like shearing resistance and heating resistance. The larger branched starch molecules will be more likely to intertwine with each other, allowing to maintain the integrity of the starch granules [41]. The results mentioned above showed that the proportion of longer branched starch increased with storage time extending, which led to the improved disintegration values of starch. It was reported that the decrease in the solubility of denatured protein resulted in the limitation of starch swelling and the enhancement of starch granule integrity, which might be another reason for the decrease in starch disintegration value [42]. When the dextrinized starch solution is cooled slowly, the starch molecules will automatically get together and form insoluble crystal bundles through the intermolecular hydrogen bond interaction, which is recognized as retrogradation [43]. The results showed that rice starch retrogradation value increased from 1560.67 mPa·s to

1932.67 mPa·s after being stored for one year, whereas it decreased to 1689.67 mPa·s after 2-year storage. It is consistent with our findings in the straight-chain content variation.

The pasting parameters of starch includes the onset temperature (To), peak temperature (Tp), resultant temperature (Tc) and enthalpy (ΔH). According to Figure 4b and Table 5, the To of starch from freshly harvested rice was about 71.78 °C, which became lower after one-year storage, indicating that the thermal stability of rice starch declined after being stored for the same time. This tendency was caused by the decrease in the content of the branched chain starch, the disruption of the double helix structure and the decline of the short-range ordering (Tables 2 and 3). The To increased with the storage time increasing to 2 years, indicating that long storage time could make rice possess high thermal stability. The results are consistent with the increase in short-range ordering and the decrease in straight-chain starch content. The ΔH value reflects the heat energy associated with crystal melt and the quantity of double helices in starch. The enthalpy of starch decreased after 1 year storage and then kept level off in the rest of storage time, whereas its thermal stability first increased and then decreased during storage.

Table 5. Gelatinization parameters of rice starch during storage.

Samples	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Year-0	71.78 ± 0.15 ^{b}	76.28 ± 0.12 ^{b}	81.82 ± 0.28 ^{a}	3.29 ± 0.16 ^{a}
Year-1	70.55 ± 0.25 ^{c}	75.40 ± 0.10 ^{c}	80.67 ± 0.23 ^{b}	3.23 ± 0.07 ^{a}
Year-2	72.44 ± 0.17 ^{a}	76.72 ± 0.09 ^{a}	81.53 ± 0.16 ^{a}	3.39 ± 0.08 ^{a}

Notes: The letter ΔH represents the endothermic enthalpy; To represents the onset temperature; Tp represents the peak temperature; Tc represents the conclusion temperature. Values in the same column with different letters are significantly different ($p < 0.05$).

3.5. Morphological Structure Variation of Rice Starch

The effect of long-term storage on the starch granules morphology was investigated by scanning electron microscopy (SEM). The morphological characteristics of starch granules from fresh and stored rice are shown in Figure 2. The fresh rice starch granules were uniform in size, polygonal in shape, regular in shape and smooth on the surface, which is agreeable with other studies [44]. The stored rice starch granules were polygonal, irregular in shape and rough on the surface. There was no significant difference between fresh rice starch and rice stored for 1 year, but the proportion of broken starch granules increased after 2-year storage. Besides, the areas of wrinkles and roughness increased significantly, and the surface of starch granules showed obvious pits. It indicated that starch granules were damaged, thus leading to the declined integrity with the extension of storage time. Furthermore, the moisture is more likely to make water blend in the starch granules from the broken area, which provides more chances to contact with the double helix structure of branched starch. As a result, it is easier to destroy the orderly structure of starch under the heating condition and then lead to decreasing the pasting temperature.

4. Conclusions

In this paper, we investigated the effect of the long-term storage on the multiscale structure and physicochemical properties of rice under granary conditions. The results showed that the fatty acid value and the electrical conductivity of rice increased, whereas its germination rate decreased significantly. The straight-chain starch content first increased and then decreased with the storage time extending. The same tendency occurred in the long-branched chain of branched starch, the short-range orderliness and the degree of double helix structure. However, the pasting temperature had an opposite trend. Simultaneously, the starch granule suffered from breakage, along with the peak viscosity and disintegration value of rice starch decreasing. Some other indicators, such as relative crystallinity, enthalpy, hardness, viscosity and luster, also exerted the corresponding variation. All the results indicates that the structure of rice starch changed significantly after 2-year storage, accompanied by the transformed physicochemical properties. Therefore, to maintain rice with a fair quality, it should be stored under suitable granary conditions for no more than two years. However, most of the results are the description of rice quality and starch variation; they are not the deep mechanism. Thus, if we want to know the reason of the transformation, the changes of their biochemicals, genes and chemical bonds should also be studied in the next studies.

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