Study on Gelatinization Behavior of Aging Rice Starch Granules at High Temperature

Rui Song[a]
School of Biological and Food Engineering, Anhui Polytechnic University, Wuhu, China

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Abstract: Rice starch is one of the most important nutrition sources worldwide. In the current study, we prepared, characterized and evaluated gelatinized glutinous rice starch. The main purpose of this study was to investigate the effect of 95℃ slurry on the separation and functional properties of rice endosperm cells granules aged for 12 months. Our results showed that during high temperature treatment, particle size analysis observed that the degree of separation for endosperm cells granules decreased, morphology examination suggested that the protein distribution was uneven, and starch granules was more difficult to swelling. Morever, The texture of cooked aged rice was higher hardness, chewiness and gumminess while lower adhesiveness, also higher setback value whereas lower breakdown value. Thus, different methods have been proposed to classify that why the n vitro weight loss rate diminished for aged rice starch granules at at high temperatures. These results provide a new basis for understanding aging mechanisms from the pasting behavior of rice flour particles at high temperatures.

1 INTRODUCTION

More than half of the world's population consumes rice and rice products, rich in starch, as their main staple food (Lamberts, De, Vandeputte, 2007). To product flour for various food items, the quality of aging rice needs to be increased. Aging is the most common phenomenon in rice storage, but its mechanism is still not fully understood. Starch is the main ingredient of rice, since it is its most important component. Rice products are mainly processed by starch entities such as cooking, gelatinization, and retrogradation properties, which are important factors to consider when commercializing rice products (Likitwattanasade, Hongsprabhas, 2010)

At present, most rice products, such as rice cakes, baby food, and instant rice milk, are made from rice noodles. Thus, there are many domestic and overseas countries to improve the quality of rice products through heat-moisture treatment, like the superheated steam modifying wheat flour (Hu, Guo, Liu, 2018), food crop starches (L, Y, X, 2019) and potato starches (Hu, Guo, Liu, 2018), add functional polysaccharides and enzyme preparations, etc (Heo, Jeon, Lee, 2014). And China mainly from the improvement of production process, delaying aging, improving nutrition and other aspects of rice flour (Advances on fermentation in rice noodle production, 2013). During the storage process, the microcrystal bundle structure of starch strengthens, the molecular weight decreases, the content of insoluble amylose increases. Due to these changes, water molecules are contained in the cell so that water absorption expansion force is too weak for the aging starch granules to disperse, reducing the amount of dissolved starch molecules and consequently the texture. The current study investigates the influence of high temperature on its morphology and functional properties to validate the theory and observations.

The aim of this paper is to clarify the separation of high temperature starch granules by using inner rice flour for endosperm cells aggregates. The purpose is to study the gelatinization behavior under the effect of high temperature gelatinization treatment on the separation of new and aged rice starch granules as well as the changes in functional properties of aging rice, for understanding the mechanism of rice aging and improve the process of commercial manufactures of rice products.

[a] https://orcid.org/ 0000-0002-1911-5292

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2 MATERIALS AND METHODS

2.1 Materials

This study used freshly milled non-waxy japonica rice harvested in Jiangsu Province, China with an initial water content of 14.30±0.01%. All chemicals and reagents used in the study were of analytical grade.

2.2 Preparation of Endosperm Cell

The rice was first divided into two samples and sealed in a bottle, with one sample stored at 4℃ as a control and the other in a 37℃ incubator for 12 months to obtain the aged rice sample for further testing (Zhou, Robards, Helliwell, 2010). The rice samples were then milled using a fine rice machine. Rice flours of both new and aged rice grains were obtained by grinding the inner layer of rice grains, which accounted for 15% in the whole rice grains. 20 g of kernel rice was crushed in the shredder for 20s The crushed powder further went through a 150-mesh sieve, and then passed a120-mesh sieve. The sieved powder (approximately with sizes finer than 100-125μm) were stored in a self-sealed pocket at 4℃ for later use.

2.3 Particle Size Analysis

Using a laser light scattering particle size analyzer, we determined the particle size distribution of rice flour particles. Then 0.2 g of samples was added to the tube, followed by 10 mL of distilled water vortexing for 1 minute to prevent the sticky wall from dispersing the samples, which then pasted in a 95℃-water bath for 5 min. The sample was removed and placed in a bath for 50℃ to prevent aging. Particle size analysis was performed 5 times and averaged using an ultrasound dispersion using water as the dispersant at 1800 r/min, and the shading between 10%-20% (Odeku, Itiola, 2007).

2.4 Light Microscope Observation

After completely dispersed using a vortex for 1 min, rice flour particles were dispersed in distilled water and heated in a water bath at 95 °C for 5 min. Then the sample was kept in 50 °C before further testing. After removed from water bath, the samples were briefly vortexed for 1 min, dripped on slides with 0.02% iodine fluid, and covered with cover glass. The particles were then observed and imaged under a light microscope to observe its morphological properties.

2.5 Scanning Electron Microscope Observation

Following the same sample preparation method as above, the sample was vortexed for 20s and set still for 2 min. Supernatant was removed using a pipette and the residues were washed three times through a 5 mm filter film using 10 mL of 50℃ distilled water. The filter residue on the filter membrane was dispersed into a petri dish. After the sample freeze-dried, it was grinded and bagged, followed by mounting to a circular aluminum stub with double-sided sticky tape. After coating with gold to form a thickness of 10 nm, the sample was examined and photographed using a cold field emission scanning electron microscope at an accelerating voltage of 15 kV.

2.6 Determination of the Textural Properties

We implemented similar textual analysis method similar to Huo et al., etc (Huo, Yuan, Tang, 2019) methods with slight modifications. 2,000 g samples were taken with 8 mL distilled water supplemented to form a rice slurry suspension of 20%. The mixture was stirred evenly in a 40 mm diameter small aluminum box, heated at a constant temperature to 95℃ water bath for 15 min, and then cooled to room temperature and stored in an upper 4℃ refrigerator for 12 h. Each starch gel sample in the canister was pressed to form distances of 30 mm (trigger force = 5.0 g) with a cylinder probe with 0.5 inch in diameter at the speed of 1.0 mm/s during two replication. The bite speed, pre-speed and post-speed were all set at 1.0 mm/s, compressing the original sample to 50% and residence time of 4s.

2.7 Determination of the Pasting Properties

A 0.5g sample of rice flour was added to 4mL of distilled water and stirred ten times quickly to prevent sticking to the wall, and the starch pasting viscosity measurement instrument (FDV-E) was used to set the programmed temperature control: 50-50℃, 5min; 50-95℃, 6min; 95-95℃, 4min; 95-51℃, 6min; 51-51℃, 5min. peak viscosity (PV), final viscosity (FV), Holding strength (HS) and pasting temperature (PaT).

2.8 Determination of the in Vitro Mass Loss Rate

We following the produced according to Li et al (LI, YANG, XU, 2019) with slight modifications. 200 ± 0.1mg rice paste sample was added to 10ml of distilled water in centrifuge tubes, heated up to 95°C for 5min, added 6mL of artificial gastric juice, and then digested at a constant temperature of 37°C for 4 hours. After 10 min- centrifugation at 10,000 r/min, the supernatant was discarded, and the precipitation was washed three times with anhydrous ethanol. The precipitation was dried at 105°C, and the the mass loss rate of artificial gastric juice was calculated as W1:

$$W1 = \frac{m1-m2}{m1}\times 100\%$$

The above digested samples were combined with 6mL of artificial intestinal fluid and digested at a constant temperature for 5h. Then the mixer was centrifuged at 10,000 r/min for 10min, and the supernatant was discarded, washed three times with anhydrous ethanol and dried at 105°C. The mass loss rate of artificial intestinal fluid, W2, was measured and calculated as the following:

$$W2 = \frac{m1-m3}{m1}\times 100\%$$

3 RESULTS AND DISCUSSION

3.1 Starch Particle Size Analysis

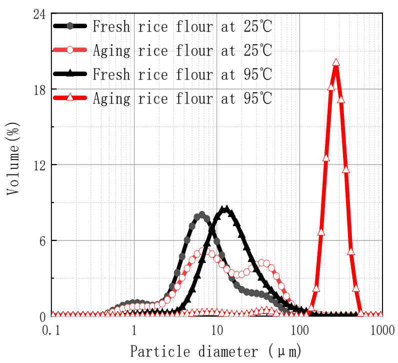

Figure 1: Starch granule particle size distribution of native and gelatinized starch at 25°Cand 95°C. (Black curve: fresh rice; red dashed curve: aging rice).

It is obvious from the above figure that the new rice particle distribution after 95°C compared that with room temperature. Three particle size peaks are shown in the figure. The peak of middle particles with most of it concentrating near 7 um, which belongs to the range of single starch (JANE, KASEMSUWAN, LEAS, 2010). The peaks of small particle sizes are concentrated around 1 um and fall within the range of protein bodies. (COFFMAN, JULIANO, 1987) Large particle size peaks are concentrated in the range of 40 um and found in endosperm cells (PAN, ZHAO, LIN, 2017). At room temperature, in addition to the easy dissociation between the endosperm cells, the starch granules also disaggregated easily between the fresh rice; while the aged rice only dissociated easily between the endosperm cells, but it was difficult to disaggregate between starch granules, which was reflected by the higher peak of the endosperm cells than the starch granules in the aged rice, indicating that aging had little effect on the dissociation between the cells, but mainly affected the tightness of the bond between the starch granules. When aged rice is pasted at 95°C, the small particle size peak decreases to a certain extent, while the large particle size peak increases. Moreover, fresh rice peak position shifted to the right. It appears that even the native rice has difficulties in endosperm cells, and endosperm cells between starch granules separate more completely,

Rice flour granules can be sorted into small starch granules, swelling, and unbroken rice flour particles. Aged rice becomes difficult to separate between starch granules within endosperm cells, which decreases the volume fraction of small particle size peaks. In aged rice, gelatinization at high temperatures inhibits the depolymerization of starch granules while promoting slightly their swelling. Due to uneven water absorption and swelling of rice noodle particles, the edge of larger particles scatters and falls off, and then the internal particles absorb water and dissolve. Water is more easily absorbed and swollen by thinner particles. Consequently, the amount of small and middle particle granules decrease while glued at 95°C, and the amount of larger particle gradually increases.

3.2 Light Microscope

The new rice disaggregated more smaller particles at 95°C than the old rice. There is less cracking on the aged rice starch granules, because the edges of the rice flour particles, are dissociated, and there are fewer granules in the cell wall. There is high edge dissociation in rice flour particles, a small swelling of starch granules, and the boundaries between the cell walls is. The degree of fresh rice flour is uniform, most of which is higher than that of old rice. the starch granules are larger and the cell wall boundary is clear and thick. The aging rice shows more protein body in the grain rim, meaning that the body has expanded; the protein body in the new rice is evenly distributed throughout the rice flour particles, indicating that it is more likely to dissolve.

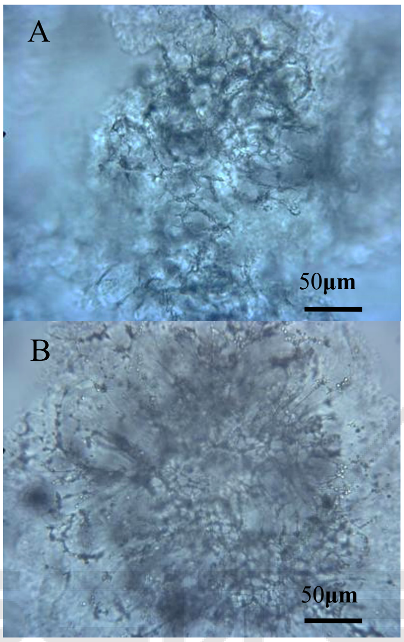

Figure 2: Light microscope images of fresh (A) vs. aged rice particles (B) at 400× resolution.

3.3 Scanning Electron Microscope

In scanning electron microscope (SEM) observations, fresh rice flour after swelling has a loose structure, the particles are small, and aging rice has a small swelling degree. as Along with the overall swelling, compact structure, which is consistent with those of the particle distribution analysis for fresh rice paste starch compound or cell loss left diameter larger pit. Moreover, it can be seen that the starch shed inner wall has a smaller and shedding pit, and the protein body is more concentrated in the edge and more exposed. Despite this, the starch particle shedding pit is small and shallow, its diameter is large, the inner wall is smooth, not as thick, and partially buried in the starch.

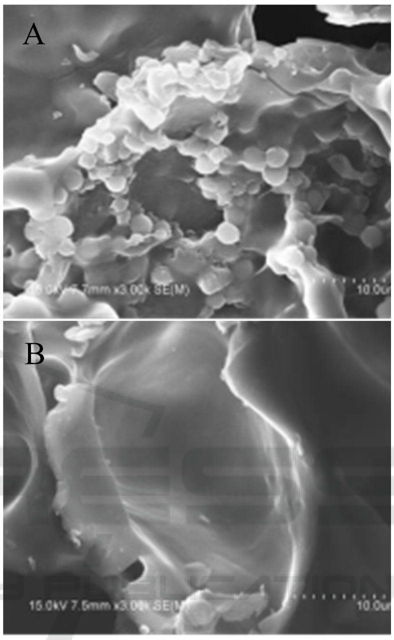

Figure 3: Electron microscope images of fresh (A)vs. aged rice granules (B) at 3000× resolutions.

3.4 Gel Texture

Table 1: Measured hardness, adhesiveness, chewiness, and gumminess of fresh and aged rice flour.

Type	Hardness/N	Adhesiveness/N	chewiness/N	gumminess/N
Fresh	1.507±0.0084	0.373±0.031	1.326±0.081	1.328±0.081
Aging	1.840±0.016	0.548±0.064	2.101±0.218	1.537±0.070

Rice is often been characterized by the texture of it (such as hardness, chewiness and adhesiveness). Changes in starch gel hardness are related to the starch colloid structure. According to studies, rice hardness is positively related to rice protein content (Zhu, Wu, Cheng, 2020). It can be seen that when under 95℃ the four texture parameters of aged rice flours are significantly higher than that of native rice flours, higher hardness and chewing are the typical characteristics of aging. (Table 1). It is possible that the central part of fresh starch granules becomes fully gelatinized during cooking because increased soluble solids, resulting in a softer texture (low hardness). Aged rice slurry has significantly improved hardness and chewiness compared with new rice. their because fresh endosperm cells are relatively brittle, easy to rupture and form a sticky state. When aged rice undergoes oxidation, the rice protein located around the starch granules becomes cross-linked, preventing them from absorbing water and swelling, resulting in insufficient gelatinization. Additionally, the disulfide bond increases during heating, and the stronger binding effect of protein and starch promotes the formation of gel network structures. Aged rice becomes harder, gummier, and chewier as the gel strength and force needed to grow stronger when chewing. For rice grains, the hardness is increased after the aging, but its adhesiveness is reduced; and for rice glue, the hardness and adhesiveness of the aged increase (Wu, Li, Bai, 2019), maybe since the aged rice particles in glue are large and the clearance space is small, the surface solids of the unit particles are relatively large, or the small amount of aged starch particles dissociated are more likely to rupture.

3.5 Determination of the Pasting Properties

Table 2: Measured PaT, PV, HS, and FV of fresh and aged rice flour.

Type	Fresh rice flour	Aging rice flour
PaT/°C	71.467±0.702	72.667±1.457
PV/cP	1424.167±37.942	1080.833±27.651
HS/cP	639.167±5.774	685.000±17.321
FV/cP	1395.000±70.134	1415.833±13.769

From Table 2, it can be seen that aging increases the FV, the PV decreases significantly, and the HS does not change much and increases slowly a little. The breakdown (BD) value decreases and the setback(SB) value increases. BD value is the most sensitive indicator in pasting properties, the decrease in BD value indicates that the less starch particles are released in heating. The smaller the decreases, the smaller the taste (Zhang, Xu, Jiang, 2021) The SB value represents the stability and aging trend of starch cold paste, the larger value, the more prone to aging, the worse the quality of eating. Moreover, the increase of pasting temperature indicates that the pasting ability of starch is inhibited, the degree of water absorption and dissociation and dispersion of starch gradually decreases, and the energy required for gelatinization increases. It can be seen that aging increases the degree of swelling of rice flour but it is difficult to break the granules, the number of remaining starch granules is smaller, the viscosity and the eating quality decreases.

3.6 Determination of the in Vitro Weight Loss Rate

Studies reveal that the degree of gelatinization of rice flour is closely related to its in vitro digestibility (Molecular disassembly of rice and lotus starches during thermal processing and its effect on starch digestibility, 2016). Investigate the influence of rice flour particle size on the degree of gelatinization using in vitro digestibility studies. In Figure 5, which simulates in vitro digestion of artificial gastric juice and artificial intestinal juice. It is shown that gelatinized rice at high temperatures is also digestible in artificial gastric juice and artificial intestinal juice, and new rice noodles can be digested at higher temperatures than old rice noodles. Therefore, after high-temperature gelatinization, rice starch granules are more prone to absorbing water, swelling and can be more easily hydrolyzed by digestive enzymes. Leading to the increase of the rate of the digestibility in aged rice starch granules expand with temperature. The higher the aged rice paste, the greater the degree of gelatinization of aged rice noodles, the greater the degree of swelling in aging rice flour. Moreover, compared with new rice starch, the old losses less weight in vitro.is still decline. It is possible that aged rice flour particles are difficult to dissolve and highly gelatinized under high temperatures, which impedes enzymes from entering the particles and slow digestion. Therefore, the digestion rate of new rice noodles is affected by both rate dispersion and rate of gelatinization. furthermore, aging rice slurry are mainly affected by gelatinization due to their difficulty in dispersing.

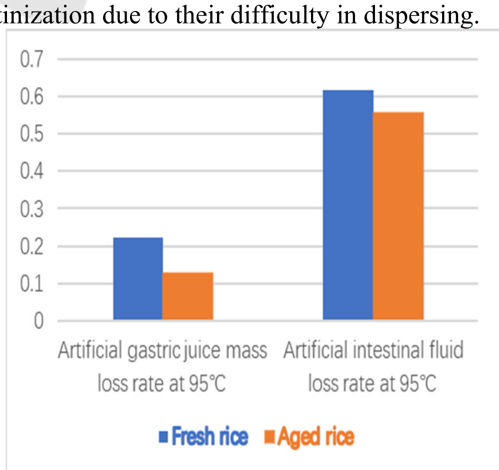

Figure 3. Calculated simulated gastric juice mass loss rate and artificial intestinal fluid loss rate of fresh vs. aged rice.

4 CONCLUSIONS

In this study, we investigated the effect of high temperature on the gelatinization behavior and functional properties of fresh and aged rice in non-waxy rice flour. After gelatinization at 95 ℃. Aging endosperm cells are harder to separate into individual starch and protein bodies. Aged rice starch particles has difficulty swelling and dissociation, and dispersion outside the particles. Morphological observation showed aged rice starch granules contain more tightly bound protein and starch. TPA indicated higher hardness, chewiness and gumminess, while lower adhesiveness. FDV revealed higher SB whereas lower BD. These can lead to starch-protein complexes forming, which may be difficult to disperse due to insufficient friction between granules or surface polymerization force, and low gelation and low degree of digestion. The quality deterioration after high temperature gelatinization of aging rice starch granules can be explained by owing to the increasing gel strength of starch-protein complexes, as well as decline of low water absorption ability swelling, and permeability, contributing to starch granules difficult to dissociation, which interfere with the absorption of internal starch. This will provide a basis for discovering the mechanism of rice aging, and then regulating its edible quality.

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