

Full Length Research Paper

Effect of germination on structural and physicochemical properties of starch in glutinous brown rice

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This study compared the structural and rheological properties of native (GB0), 12 h (GB12), 24 h (GB24), 36 h (GB36), 48 h (GB48) and 72 h (GB72) germinated glutinous brown rice starch to improve the market value of glutinous rice through germination. The proportion of A chains (DP 6-12) increases and the proportion of B₁ chains (DP 13-24) decreased with germination time. Interestingly, we observed that the total proportion of B₂ and B₃ chains decreased, but was recovered after germination at 36 h. The effect of germination on the distribution of amylopectin length results in a decrease in relative crystallinity, gelatinisation temperature, gelatinisation enthalpy and pasting viscosities. In this study, we found that GB12 starch gel has the weakest thermal stability and its shear resistance is more difficult to retrogradation; while GB36 has the highest chain association (retrogradation) which is induced by cooling.

Key words: Glutinous brown rice, germination, starch, structural properties, rheological properties.

INTRODUCTION

Glutinous rice commonly referred to as sticky or waxy rice is characterised by its non-transparent surface and high amylopectin content. Such rice is widely processed into food products and has a high yield, particularly in China, where it is mainly used as material of traditional foods, such as Tangyuan, Zongzi, and Shaoxing wine, and as an added ingredient in other products. Glutinous rice starch is almost entirely amylopectin, with <2% amylose (Xu et al., 2013). The amylose-amylopectin ratio will influence the physicochemical properties of starch. Amylopectin exhibits poor syneresis and hard to

retrogradation. As a result, compared with the boiled rice from a normal-amylose cultivar, the low-amylose cultivar is stickier (Noda et al., 2003). The crystallization of amylopectin in high-amylopectin cultivars will also contribute to their resistance to enzymatic digestibility (Liu et al., 1999). Consequently, such cultivars result in indigestion and are not suitable as staple food, resulting in a much lower market value.

Compared with ordinary milled rice grains, brown rice grains are nutrient-rich, for example dietary fibres, vitamins, phytic acids, gamma aminobutyric acid, and

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vitamins, etc (Komatsuzaki et al., 2007). Because of the highly nutritional value of GBR, its products are popularity in health-conscious consumers in Asian. Despite its elevated nutritional content, brown rice is not acceptable as table rice because of its poor water absorbency, expansibility in cooking, and poor texture. The rich tough of out bran layer keeps off water into the endothecium of GBR, and leads to these shortcomings (Das et al., 2008). For this reason, the characters of brown rice have been improved by several new processing methods which developed in recent years, covering high hydrostatic pressure (Boluda-Aguilar et al., 2013; Xia et al., 2017; Wang et al., 2020), high-temperature fluidization (Jaisut et al., 2009), ultrasound (Cui et al., 2010), low-pressure plasma (Chen et al., 2016), and germination. During the process of germination, the degradation of high-molecular-weight polymers will result in generation of biofunctional substances and improved organoleptic qualities, resulting in a softened texture and increased flavour (Subba Rao and Muralikrishna, 2002). Starch accounts for about 90% of the total dry weight of rice, and the ease with which rice cooks and its overall taste is primarily affected by its starch properties. Amylopectin was the main component of most starches, and variations in the amylopectin fine structure will lead to different physicochemical properties. Many studies have examined the different characteristics between germinated and ungerminated brown rice starch (Xu et al., 2012; Musa et al., 2011). Pinkaew et al. (2016, 2017) only investigated the characteristics of the pre-germination stage of brown rice starches. Several researches have evaluated the impact of germination on the physiochemical properties of long-grain or round-grain brown rice starch (Wu et al., 2013; Li et al., 2017; Kalita et al., 2017). However, less research has been conducted to specifically examine germinated glutinous brown rice starch. The physicochemical properties of glutinous brown rice starch from germination at 36 h have only been evaluated by You et al. (2016), who examined the structural properties and the *in vitro* digestibility of native and 36-h germinated waxy brown rice starches, whereas other germination stages were not studied.

The aim of the present study was to compare the structural and rheological properties of glutinous brown rice starch at 0-72 h germination stages. Figure 1 These findings can help to (1) understand the effects of different germination time on the structural and physicochemical properties of starch, and (2) improve the texture and organoleptic qualities of glutinous brown rice through germination, and (3) provide a basis for the development of glutinous brown rice, and a reference for more effective use of germinated glutinous brown rice as an additive.

MATERIALS AND METHODS

Glutinous brown rice was harvested in 2019 and provided by Anhui Guangming Huaixiang Industry and Trade Group Co., Ltd (Anhui

province, China), and stored at 4°C in a refrigerator.

Preparation of germinated glutinous brown rice

The glutinous brown rice grains (2000 g) were thoroughly rinsed with tap water and disinfected with 1% NaClO, followed by soaking in distilled water (pH=7, grain to water ratio 1:2 w/v) at 25°C for 12 h; the distilled water was changed every 2 h. The rice grains were placed between wet gauze and sponge on a tray after soaking, then germinated in a constant temperature incubator (DHP-9162, Yiheng technology Co., Shanghai, China) for 72 h at 35°C and 65% humidity. The sample was obtained every 12 h. After germination for regular time, the native and germinated glutinous brown rice were dried at 45°C for 24 h.

Isolation of starch

The starch of the native glutinous brown rice (GB0), 12 h germinated glutinous brown rice (GB12), 24 h germinated glutinous brown rice (GB24), 36 h germinated glutinous brown rice (GB36), 48-h germinated glutinous brown rice (GB48) and 72 h germinated glutinous brown rice (GB72) were isolated by soaking in 0.1% NaOH solution using the procedure proposed by Lim et al. (1999). The extracted starches were continuously dried at 45°C for 1 d, and ground into powder by using a mechanical grinder (DSY-9002, Jiushunying Chamber Commerce Co., Zhejiang, China) and then passed through a 100-mesh sieve.

Amylopectin branch chain length distributions

The branch chain length distribution of the starches was determined by high performance anion exchange chromatography-pulsed amperometric detector (HPAEC-PAD). Sample (9 mg) was dissolved in 100% DMSO (450 µL) and stirred in a boiling water bath for 12 h. The starch solution was diluted with ultrapure water (2250 µL) and 300 µL of sodium acetate buffer (0.1 M, pH 4.5). Next, 1 µL of isoamylase and 1 µL of pullulanase were added to the sample solution and then stirred (100 rpm) at room temperature for 12 h. The enzymatic reaction was terminated by heating in a boiling water bath. After debranching, the sample solution was centrifuged and filtered through 0.45 µm nylon syringe filter before being injected into the HPAEC system (ICS5000, Dionex, CA, USA); it was injected with Pulse ampere detector (PAD), using 150 mM NaOH and 500 mM sodium acetate in 150 mM NaOH at a flow rate of 1 mL/min to gradient elution separation.

X-ray diffraction

X-ray diffraction patterns were obtained by an X-ray diffractometer (X'Pert PRO, Philips Co., Almelo, Netherlands) which operated at 40 mA and 40 kV with scanning speed of 2.0°/min and scanning range of 3-60° (2θ). Using the Origin software (Version 8.0, Microcal Inc., Northampton, MA, USA) the two-phase method was followed to quantitatively calculate the relative crystallinity (Lopez-Rubio et al., 2008).

Differential scanning calorimetry

Using a differential scanning calorimeter (DSC8000, PerkinElmer Co., Norwalk, CT) to evaluate the thermal properties of the starches, 2 mg starch was weighed in aluminium crucibles. Then redistilled water (6 µL) was added with a pipette and mixed well with a fine needle. The crucibles had a sealing gland and were kept at

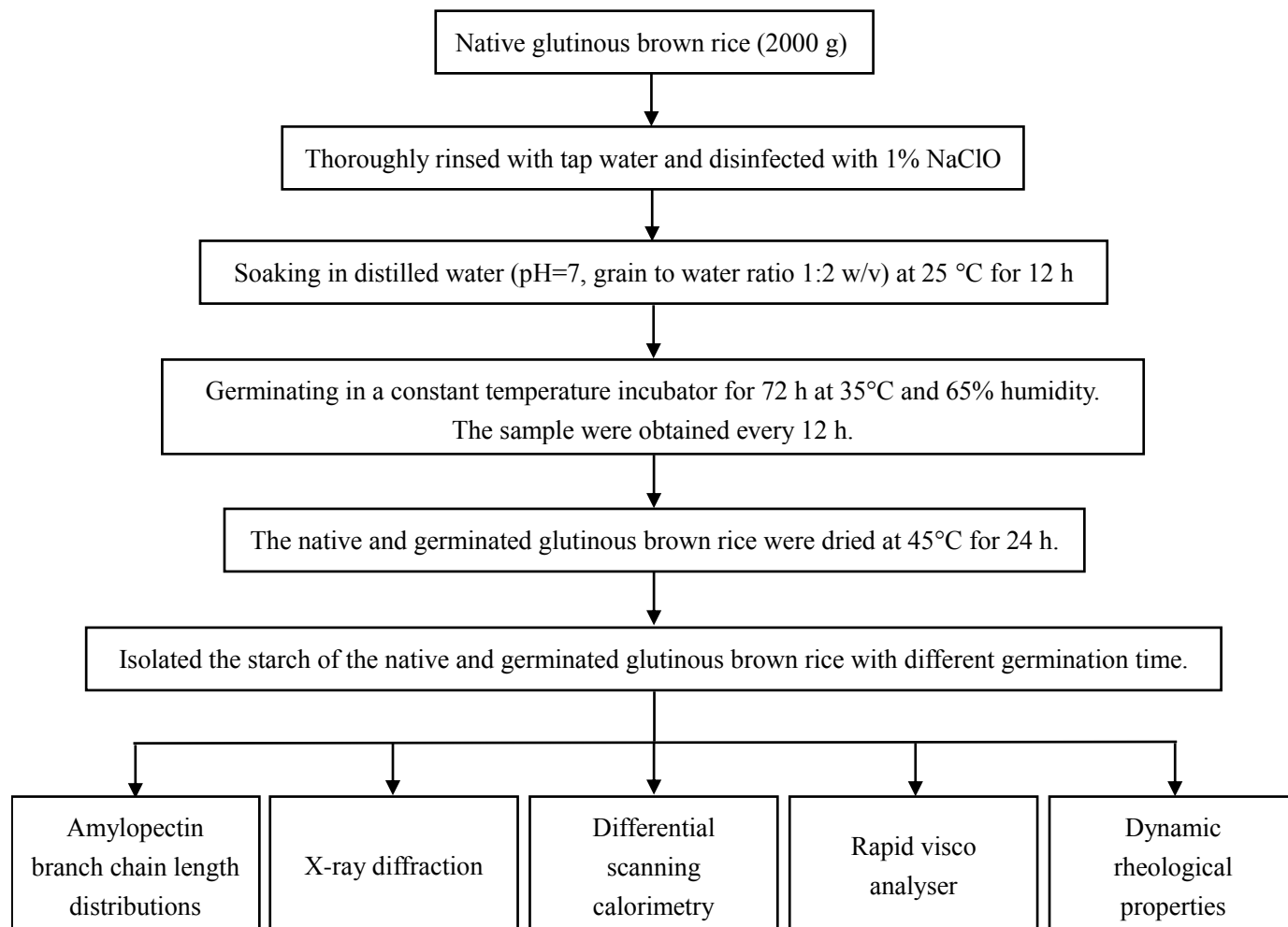


Figure 1. Flow chart.

room temperature to equilibrate moisture for 24 h. The crucibles with a heating rate of 10°C/min were heated from 30 to 130°C. The blank control was used as an empty aluminium crucible. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures, as well as the enthalpy of gelatinization (ΔH) were determined from the data recording software.

Rapid Visco Analyser

A Rapid Visco Analyser (RVA-TecMaster, Warriewood, Australia) was used to analyze the pasting properties of the starches. The starch suspension liquid was prepared (7% w/w, 30 g of total weight), and the temperature program was set as: held at 30°C for 1 min, heated to 95°C at a rate of 6°C/min, held at 95°C for 5 min, cooled to 30°C at 6°C/min, and finally held at 30°C for 2 min.

Dynamic rheological properties

Dynamic rheological properties were evaluated using an HAAKE RheoStress 6000 (Thermo Scientific, USA) with parallel plate system (4 cm plate diameter); the gap size was set at 1 mm, and measured with 0.5% of strain and 1 Hz of frequency. The dried

starches were disperse in distilled water (6% dry solids) by heating in a boiling water-bath for 30 min with stirring. The gelatinized sample was immediately transferred on the test board, and preheated to 95°C for the analysis. The free surface of the sample edges was covered with silicone oil to reduce evaporation losses during measurements. The storage (G') and loss (G'') moduli were measured while the paste was cooled to 4°C at a rate of 1 °C /min. The dynamic viscoelastic properties, including elastic modulus (G'), viscous modulus (G''), and complex modulus, $G^* = (G'^2 + G''^2)^{1/2}$ were recorded as a function of frequency.

Statistical analysis

The SPSS statistical software version 21.0 (SPSS Inc., Chicago, IL, USA) was employed to analyze the significant differences among means ($P < 0.05$) through Duncan's multiple range test.

RESULTS AND DISCUSSION

Structural characterization

Figure 2 shows the normalized HPAEC-PAD chromatogram of the amylopectin branch chain length

distribution of the starches isolated from GB0, GB12, GB24, GB36, GB48 and GB72. Researchers divided the normalized amylopectin into four parts: A chains (DP 6–12), B₁ chains (DP 13–24), B₂ chains (DP 25–36), and B₃ chains (DP 25– (Hanashiro et al., 1996). After germination, the proportion of A chains increased significantly; in contrast, the proportion of B₁ chains decreased gradually. The total proportion of chain A and chain B₁ increased. According to the starch cluster model of amylopectin, A and B₁ chains formed double helix structure in starch granules, and was the most part external (Tester et al., 2004). Therefore, part of them are degraded to oligosaccharides to provide energy for the growing germs, which is due to the activation of hydrolases during germination (Mohan et al., 2010). Interestingly, we observed that the total proportion of B₂ and B₃ chains decreased before germination at 36 h; it rose again at 36 h, and then continued to decrease with the extension of germination time. The enzymatic degradation at the beginning of germination may be the cause of this trend. The maximum limit of the amylopectin chain length was approximately 100. Nevertheless, in our HPAEC system, the super longer chains (DP > 78) could not be detected, but they can actually form the whole amylopectin macromolecules by connecting with each other into clusters. So, the super longer chains can degrade during germination and lead to the rise in the proportion of B₂ and B₃ chains of GB36 starch in this study (Hizukuri, 1985).

Crystalline structure

The starches isolated from GB0, GB12, GB24, GB36, GB48, and GB72 all exhibited the typical A-type diffraction pattern with individual peaks at 15° and 23° and unresolved peaks at 17° and 18°. From Figure 3 we could not find any significant changes in the X-ray diffraction pattern of starches after germination. The A-type diffraction pattern reflects a compact packing of amylopectin double helices (Khunae et al., 2007). Compared with the GB0 starch, the relative crystallinity of GB12 starch and GB24 starch had no significant difference.

This shows that the hydrolysis during germination primarily attacked the amorphous area of the starch granules before germination at 24 h. Nevertheless, when germination occurred at 36 h, germination did cause a lower relative crystallinity, and the relative crystallinity was decreased with the germination time. Relative crystallinity is possibly connected with the interaction of double helices (Jane et al., 1999). (Cooke and Gidley, 1992) confirmed that the existence of short amylopectin chains (DP < 10) leads to the instability of the double helix. The proportion of DP 10–13 is negatively correlated with crystallinity which is confirmed by Cheetham and Tao (Cheetham and Tao, 1998). Therefore, a high-proportion of A chains in the starch of GB36, GB48 and GB72 may

cause the decrease in the relative crystallinity.

Thermal properties

Table 1 presents the thermal properties of the starch samples obtained from GB0, GB12, GB24, GB36, GB48, and GB72. In this study, the gelatinisation temperature of the glutinous brown rice starch decreased slightly following germination. Similarly, You et al. (2016) also declared the gelatinization temperature decreased after germination. The molecular structure of crystalline regions which are related to the amylopectin branch chain length distributions can influence gelatinization temperatures. Thus, low gelatinization temperatures may have resulted from the high proportion of A chain and a less amount of long chain amylopectin (Pinkaw et al., 2017). In addition, the starch exhibited lower gelatinisation enthalpy during the germination. Similarly, Xu et al. (2012) and Li et al. (2017) also found the gelatinization enthalpy of germinated rice starches were lower than the native rice starches. The reduced gelatinization enthalpy after germination can confirm the fracture of the hydrogen bonds linking adjacent double helices (Chung et al., 2008). The damage of double helical order in crystalline and non-crystalline regions can normally be represented by gelatinization enthalpy (Cooke and Gidley, 1992). Gidley and Bulpin (1987) confirmed that the gelatinization enthalpy can also be impacted by the amylopectin branch chain length distributions. The lower proportion of long branch chains constitutes a shorter order of double helical and leads to smaller gelatinization enthalpy (Li et al., 2017)

Pasting properties

The differences of pasting properties between the starch of native and germinated glutinous brown rice are given in Figure 4. The behaviour exhibited was the same as that classically encountered in aqueous starch dispersions. In the present study, gradual decreases were observed in the pasting temperature and viscosities during the germination. Li et al. (2017) suggested that more rigid crystalline structure cannot swell easily. Chung et al. (2011) proposed that keeping the swollen granules in perfect condition needs strong interaction which could not be provided by the high proportion of short chains (DP 6–12) and resulted in lower pasting temperature and pasting viscosities. The higher proportion of A chains (DP 6–12) after germination may result in the decrease in pasting temperature. During germination, the α -1,4-glycosidic linkages of the starch molecules were broken by activated α -amylase which might explain the high dissolubility and easy saccharification of starch gel, leading to a lower peak viscosity (Zhu et al., 2010).

The breakdown of the glutinous brown rice starches was increased after germination. Keeping the swollen granules in perfect condition needs strong interaction

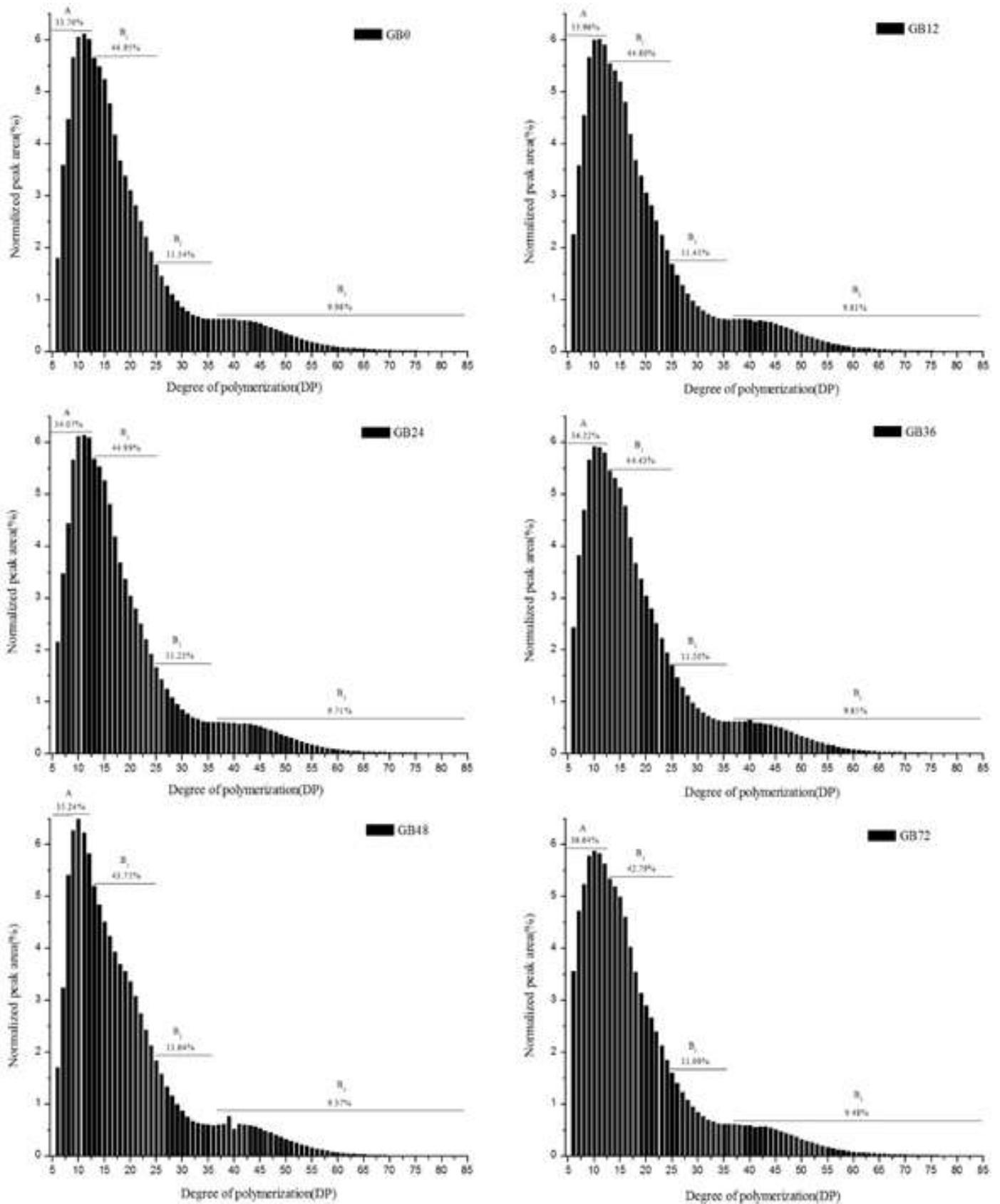


Figure 2. High-performance anion-exchange chromatography normalized chromatogram of native and germinated glutinous brown rice. GB, germinated glutinous brown rice starch; 0, 12, 24, 36, 48, 72, germinated time.

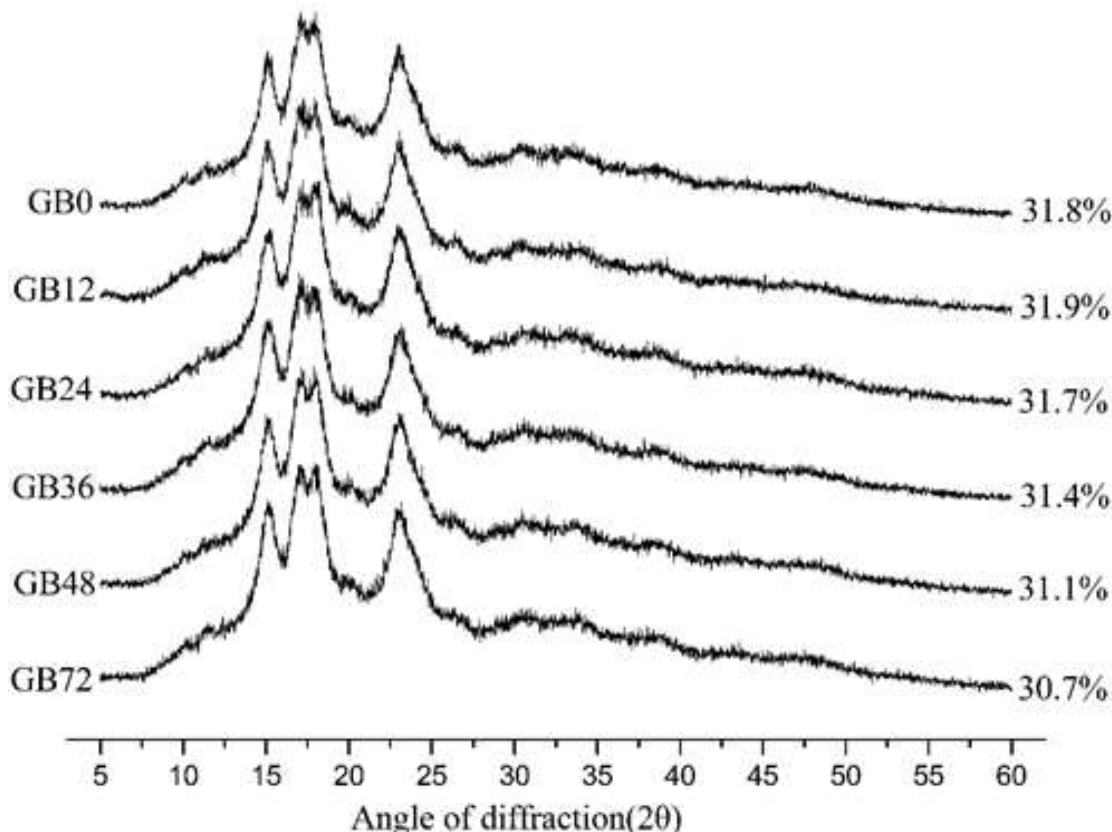


Figure 3. X-ray diffractogram of isolated native and germinated glutinous brown rice. GB, germinated glutinous brown rice starch; 0, 12, 24, 36, 48, 72, germinated time.

Table 1. Thermal properties of native and germinated brown rice ^A.

Starch	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
GB0	60.20±0.42 ^a	65.88±0.06 ^a	73.24±0.45 ^a	12.72±0.06 ^a
GB12	58.38±0.44 ^b	66.26±0.52 ^a	72.10±0.40 ^{bc}	12.35±0.12 ^{ab}
GB24	58.05±0.19 ^{bc}	65.75±0.25 ^a	71.71±0.33 ^{cd}	12.46±0.40 ^{ab}
GB36	57.38±0.42 ^c	65.87±0.14 ^a	72.28±0.21 ^{bc}	12.29±0.44 ^{ab}
GB48	57.51±0.47 ^{bc}	65.88±0.00 ^a	72.83±0.69 ^{abc}	11.87±0.32 ^b
GB72	56.28±0.09 ^d	65.68±0.18 ^a	71.02±0.27 ^d	11.95±0.37 ^{ab}

GB, germinated glutinous brown rice starch; 0, 12, 24, 36, 48, 72, germinated time; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy. ^A Values followed by the different superscripts in the same column are significantly different (P < 0.05).

which could not be provided by the short branch chains, so the higher proportion of A chains (DP 6-12) may be due to the larger breakdown (Cheetham and Tao, 1998). The highest breakdown of GB12 indicates that the shear resistance and thermal stability of the glutinous brown rice starch gel in the present study decreased after germination, and the GB12 starch gel has the weakest thermal stability and shear resistance. The final viscosity and setback decreased after germination, which might be due to the degradation of starch structure during germination in which the gelatinized starch molecules

have a low tendency of aggregation during cooling (Li et al., 2017). GB12 also has the highest value of setback during germination; it might indicate that GB12 starch is more difficult to retrogradation.

Dynamic rheological properties

Figure 5 shows the complex modulus of the starch gel of GB0, GB12, GB24, GB36, GB48, and GB72. In dynamic oscillatory measurements, the complex modulus (G*) was redrawn as the logarithm of the temperature. In the first

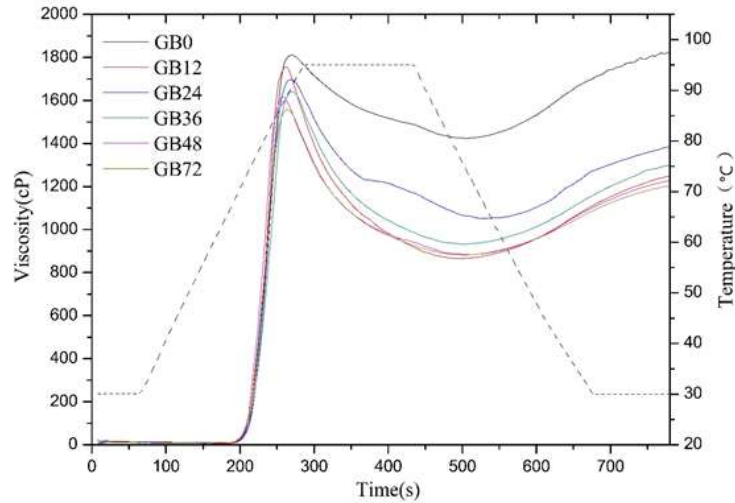


Figure 4. Comparison of the pasting profiles of the native and germinated glutinous brown rice starch. GB, germinated glutinous brown rice starch; 0, 12, 24, 36, 48, 72, germinated time.

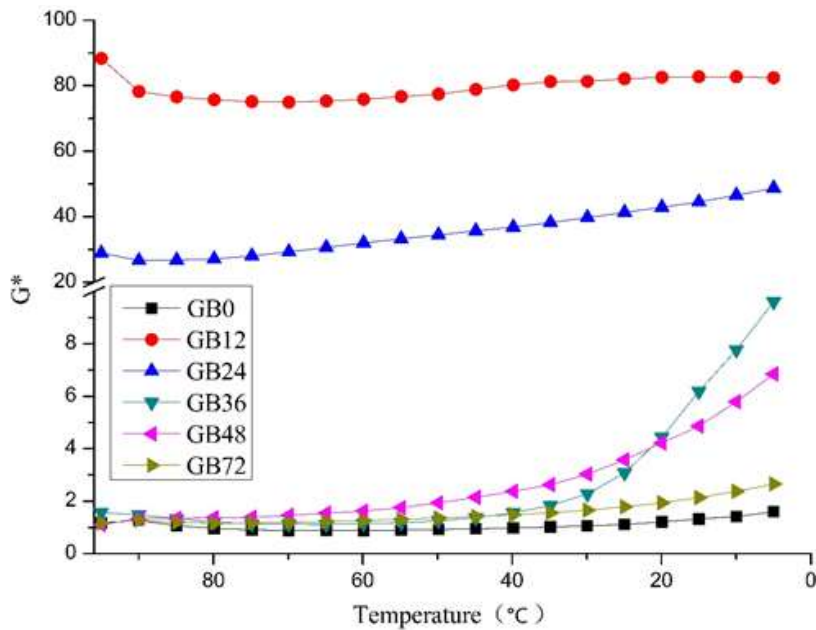


Figure 5. Complex modulus (G^*) change versus temperature in log scale during the cooling of the native and germinated glutinous brown rice starch paste (95-4°C). GB, germinated glutinous brown rice starch; 0, 12, 24, 36, 48, 72, germinated time.

stage, the G^* increased, and the starch chains in the paste maintained a high fluidity above 50 °C, so the response to the oscillation strain was not obvious; In the second stage, with the further decrease of temperature, the increase of intermolecular association due to the poor mobility of amylopectin to oscillatory response becomes obvious. Therefore, the higher G^* can be the evidence of

the overall chain association in the second stage (Chung et al., 2008).

The temperature ($T_{intercept}$) at the intersection point between the first and the second stages of G^* increase may express the onset temperature for chain association (Chung et al., 2008). After germination, $T_{intercept}$ increased, and the $T_{intercept}$ of GB36 is the lowest in all the germinated

glutinous brown rice starch. This indicates that germination leads to the chain association become harder. Among the second stage in the test, the rising slope of GB36 starch paste was the highest and the GB12 starch paste was the lowest; this indicates that GB36 starch is easy to get into chain association (retrogradation) but GB12 starch is difficult to retrogradation induced by cooling. GB36 has the highest proportion of long B chains, and the long B chains have more possible contact points which are easier to associate between chains (Chung et al., 2008).

Conclusions

This study evaluated the impact of different germination stages on the structural and rheological properties of the glutinous brown rice starch. The proportion of A chains was increased significantly; in contrast, the proportion of B₁ chains decreased gradually. The total proportion of chain A to chain B₁ reduced. Interestingly, we observed that the total proportion of B₂ and B₃ chains decreased before germination at 36 h, and rose again at 36 h, and then continued to decrease with the extension of germination time. The change of chain length distribution has a direct effect on the physicochemical properties of glutinous brown rice starch. Germination leads to a decrease in the degree of the relative crystallinity, gelatinisation temperature, gelatinisation enthalpy and pasting viscosities. In all the germinated glutinous brown rice starches, GB12 starch gel has the weakest thermal stability and its shear resistance is more difficult to retrogradation; the T_{intercept} of GB36 is the lowest and is easier to associate between chains (retrogradation). The effect of germination treatment on the fine structure and *in vitro* digestibility of glutinous brown rice starch has not been studied systematically. Further research is needed to provide theoretical basis for the development of glutinous brown rice starch.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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