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The effects of wheat amylose ratios on the structural and physicochemical properties of waxy rice starch using branching enzyme and glucoamylase

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ABSTRACT

Based on our previous study, or wheat amylose (WA) can supply an appropriate donor for branching enzyme (BE) to generate more new α-1, 6 branch points in waxy rice starch (WRS), sequential glucoamylase (GA) and BE were used to modify the mixtures of WRS and WA in different amylose ratios. The effects of WA on sequential GA and BE catalysis were investigated and the structural and physicochemical properties of the GABE-modified WRS/WA samples were analysed. The results indicated that after GA→BE treatment, the degree of branching significantly increased with increasing ratios of WRS:WA (1:0.2–1:1.2). With increasing ratios of WRS:WA from 1:0.2 to 1:0.8, short chain length distribution (DP 6–12), solubility and loss tangent increased, whereas middle chain length distribution (DP 13–24), relative crystallinity, the rate of starch retrogradation, the gelatinization temperatures and enthalpy values, as well as storage modulus and loss modulus decreased. However, as the ratios of WRS:WA continued to increase (1:1.0–1:1.2), the above results were reversed. These data demonstrated that amylose ratios in WRS:WA mixtures was closely correlated with catalytic efficiency of sequential GA and BE. The outcome would provide a new pathway for controlling amylose ratios in WRS:WA mixtures to obtain the desirable physicochemical properties of waxy rice starch-based products via enzyme modification.

1. Introduction

Waxy rice has been used for puddings, thickened soups, and sauces because of its distinct viscosity (Bao, Sun, & Corke, 2004). Depending upon the processing method, waxy rice can be used as cooking, fried, and baking food. Cooking foods include traditional Chinese rice-pudding, glutinous cake, sweet dumplings, mochi, sweet green rice ball, and rice snacks in East and Southeast Asian countries, such as Taiwan and Japan (You, Lim, Lee, & Chung, 2014). Fried foods include glutinous rice balls, deep-fried expanded rice cake, and fried Nuomici. Baking foods are mainly glutinous rice crackers eaten in East Asia countries (Keeratipibul, Luangsakul, & Lertsatchayarn, 2008). Although waxy rice starch has high viscosity, it forms very weak gels, resulting in low shear resistance and weak gel hardness (Šárka & Dvořáček, 2017). This low anti-shear ability causes the viscosity of starch paste to drastically decrease during the processing of waxy rice-based foods, which leads to low cohesiveness and poor quality of waxy rice-based foods (Šárka & Dvořáček, 2017). Additionally, the high paste clarity of waxy starch renders the waxy rice-based foods transparent, resulting in prominent appearance quality (Craig, Maningat, Seib, & Hoseney, 1989). Moreover, waxy rice starch granules are not easy to dissolve in cold water. Thus, it is significant to improve the solubility, gel strength, and shear resistance of waxy rice starch to expand the industrial applications of starch.

Waxy rice starch (WRS) contains approximately 100% amylopectin. The branched structure of amylopectin has a significant impact on the unique stickiness of starch (Bean, Esser, & Nishita, 1984). It is feasible to change the branched structure of WRS to improve its physicochemical performance. Branching enzyme (BE) hydrolyses α-1,4-glycosidic linkage and then transfers the non-reducing ends of linear chains to the C-6

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hydroxyl position of an internal glucose residue through intra- or inter-molecular α-1,6-glucosidic linkages, generating new glucans with high branch density (Van der Maarel & Leemhuis, 2013). The thermostable BE from *Rhodothermus obamensis* can efficiently modify starch into highly branched glucans (Shinohara et al., 2001). In our previous study, the functional properties of WRS, such as solubility, gel strength, etc., have been significantly improved through sequential glucoamylase (GA) and branching enzyme (BE) modification with wheat amylose (WA) as the substrate since WA can supply an appropriate donor for BE to generate new α-1, 6 branch points (Guo, Li, Gui, Zhu, & Cui, 2020). What effects it has on the physicochemical performance of WRS in different wheat amylose ratios? So, in this study, the effects of WA on sequential GA and BE catalysis were investigated using the mixtures of WRS and WA in different wheat amylose ratios. Moreover, the structural and physicochemical properties of the GABE-modified WRS/WA samples were analysed. The study may provide a theoretical basis to better understand how wheat amylose at different ratios impact GA and BE catalysis in WRS and the structure and physicochemical properties of WRS.

2. Materials and methods

2.1. Materials

Waxy rice flour came from Yanzhifang Co., Anhui, China. WRS was prepared following a previously published method (Guo, Zhang, Hu, Li, & Du, 2015). Wheat starch, glucoamylase from *Aspergillus niger* (GA, 22, 000 U/mL) and isoamylase (120,000 U/mL) from *Pseudomonas amyloderamosa* were obtained from Sigma-Aldrich. BE from *Rhodothermus obamensis* (4800 U/mL) was provided by Novozymes, Denmark (Copenhagen, Denmark). WA was purified and its content was determined following our previous method (Guo et al., 2020). Herein, the purities of WRS and WA are (95.48 ± 0.03)% and (92.63 ± 0.02)%, respectively.

2.2. Enzyme treatments of WRS/WA mixtures

Based on the degree of branching, the response surface methodology was used to determine the optimal conditions of GA or BE as follows:

The mixtures of WRS and WA in different amylose ratios, that is, 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1.0, or 1:1.2 (w/w), was dispersed in 0.02 mol/L citric acid-disodium hydrogen phosphate buffer, pH 5.5, to prepare 4% starch dispersion, respectively. Subsequently, GA (100 U/g dry weight of starch) was added to the dispersion and incubated at 53 °C for 4 h. The 3 mL of 1 mol/L sodium hydroxide solution was used to terminate the enzyme reactions. Next, the pH was adjusted to 6.5 with 0.02 mol/L sodium acetate buffer. The GA-treated starch solution was incubated with BE (500 U/g dry weight of sample) at 50 °C for 20 h to get GABE-modified starches. The 1 mol/L sodium hydroxide solution was used to terminate enzyme activity. The enzyme hydrolysate was dialysed through a dialysis membrane (molecular weight cut off: 3500 Da) in deionized water. Four volumes of 95% ethanol were added to the dialyzed hydrolysate to get starch precipitate. The deionized water was used to wash the precipitate four times and then lyophilized at −50 °C for 48 h.

2.3. Structural characteristics

2.3.1. Morphological characteristics

The appearance of the samples was observed using a scanning electron microscope (S-4800, Hitachi High-Technologies Corporation, Japan) at an accelerating potential of 10 kV. The samples were mounted on an aluminum stub and then coated with a thin film (20 nm) of gold.

2.3.2. Degree of branching and chain length distribution

The degree of branching was determined using proton nuclear magnetic resonance spectroscopy (^{1}HNMR). The 3 mg of sample was mixed with 1 mL of deuterium oxide. The mixed dispersion was heated in a boiling water bath for 30 min and then freeze-dried. Next, the freeze-dried sample was mixed with 1 mL of deuterium oxide again. The degree of branching was calculated by dividing the peak area at 5.0 ppm (α-1,6-glucosidic bonds) by the total peak area at 5.4 (α-1,4-glucosidic bonds) and 5.0 ppm (Lee et al., 2008).

According to our previous method (Guo et al., 2020), the chain-length distribution of debranched starch samples was determined using a high-performance anion-exchange chromatography equipped with a pulsed amperometric detector.

2.3.3. Crystalline features

An X-ray diffractometer (Bruker AXS, Rheinfelden, Germany) was used to analyse the crystalline features of the sample at 40 kV and 40 mA with Cu Kα radiation of 0.154 nm. The diffraction angle (2θ) ranged from 5 to 50°. The scanning rate was 2°/min, and the step size was 0.05°. The relative crystallinity (RC, %) was calculated with Jade 7.0 software.

2.4. Physicochemical characteristics

2.4.1. Water solubility

The 500 mg of the sample was added to deionized water (20 mL), with a constant agitation for 20 min. The suspension was then heated in a boiling water bath for 30 min, and then cooled to room temperature. The starch slurry was centrifuged at 7104 g for 10 min. The supernatant was gathered and subsequently dried to a constant weight. The solubility (%) was calculated by dividing the weight of dried supernatant by the mass of dry starch.

2.4.2. The turbidity of starch paste

The 100 mg of starch sample was mixed with deionized water to prepare 1% starch dispersion. The dispersion was heated in a boiling water bath for 30 min with constant stirring to obtain starch paste. Next, the starch paste was cooled to room temperature and subsequently the turbidity was determined at 600 nm after the paste was placed at 4 °C for 0, 24, 48, 72, and 96 h.

2.4.3. Gelatinization characteristics

A PerkinElmer DSC8000 was used to determine the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH). The 4.0 mg of the sample was placed into a pan. The deionized water was added to the pan to get a 25% suspension. The pan was sealed tightly and the sample was equilibrated at room temperature overnight. The sample was heated from 25 to 100 °C at 10 °C min^{-1}.

2.4.4. Dynamic rheological behavior

The dynamic rheology behavior of the sample was analysed by using a DHR-1 rotational rheometer with a 40 mm cone-plate geometry (TA Instruments, New Castle, USA). The 25% starch suspension was heated in a boiling water bath, with a constant stirring for 30 min. The hot slurry was then cooled to 30 °C. The storage modulus (G′), loss modulus (G″) and loss tangent (Tan δ = G″/G′) versus angular frequency (ω) ranging from 0.1 to 100 rad/s were determined.

2.5. Data analysis

All results were carried out in triplicate. The Origin 7.5 and Tukey's test was used to analyse the significance in the difference at a 0.05 level of confidence.

3. Results and discussion

3.1. Morphological characteristics

The appearance of the GABE-modified WRS/WA samples is depicted in Fig. 1. Through GA→BE action on substrates with different ratios of WRS:WA (1:0.2–1:1.2), many starch granules are adhered together to form the aggregations in different sizes and shapes due to the formation of stronger interaction force, such as hydrogen bonds, etc. Compared to the ratio of WRS:WA (1:0.2), the aggregation effect of starch granules becomes smaller and smaller, and more granules remain dispersed with increasing ratios of WRS:WA from 1:0.2 to 1:0.8 (Fig. 1ÃD). Surprisingly, more starch granules aggregate tightly as the ratios of WRS:WA continue to increase (1:1.0–1:1.2). These observations may imply that through GA→BE action on the mixed substrates of WRS and WA within a certain range of WA values (1:0.2–1:0.8), higher branch structure in amylose or amylopectin is not prone to chain realignment, preventing starch granules from aggregation (BeMiller & Whistler, 2009; Van der Maarel & Leemhuis, 2013). With continuously increasing WA ratios in the mixed substrates of WRS and WA (1:1.0–1:1.2), the aggregation effect of starch granules becomes more significant. The reason for this phenomenon will be confirmed (described later).

3.2. Degree of branching of the samples

The ^{1}HNMR spectra of the GABE-modified WRS/WA samples are indicated in Fig. 2. It shows that with increasing ratios of WRS:WA from 1:0.2 to 1:1.2, the percentages of α-1,6 glycosidic linkage increase from

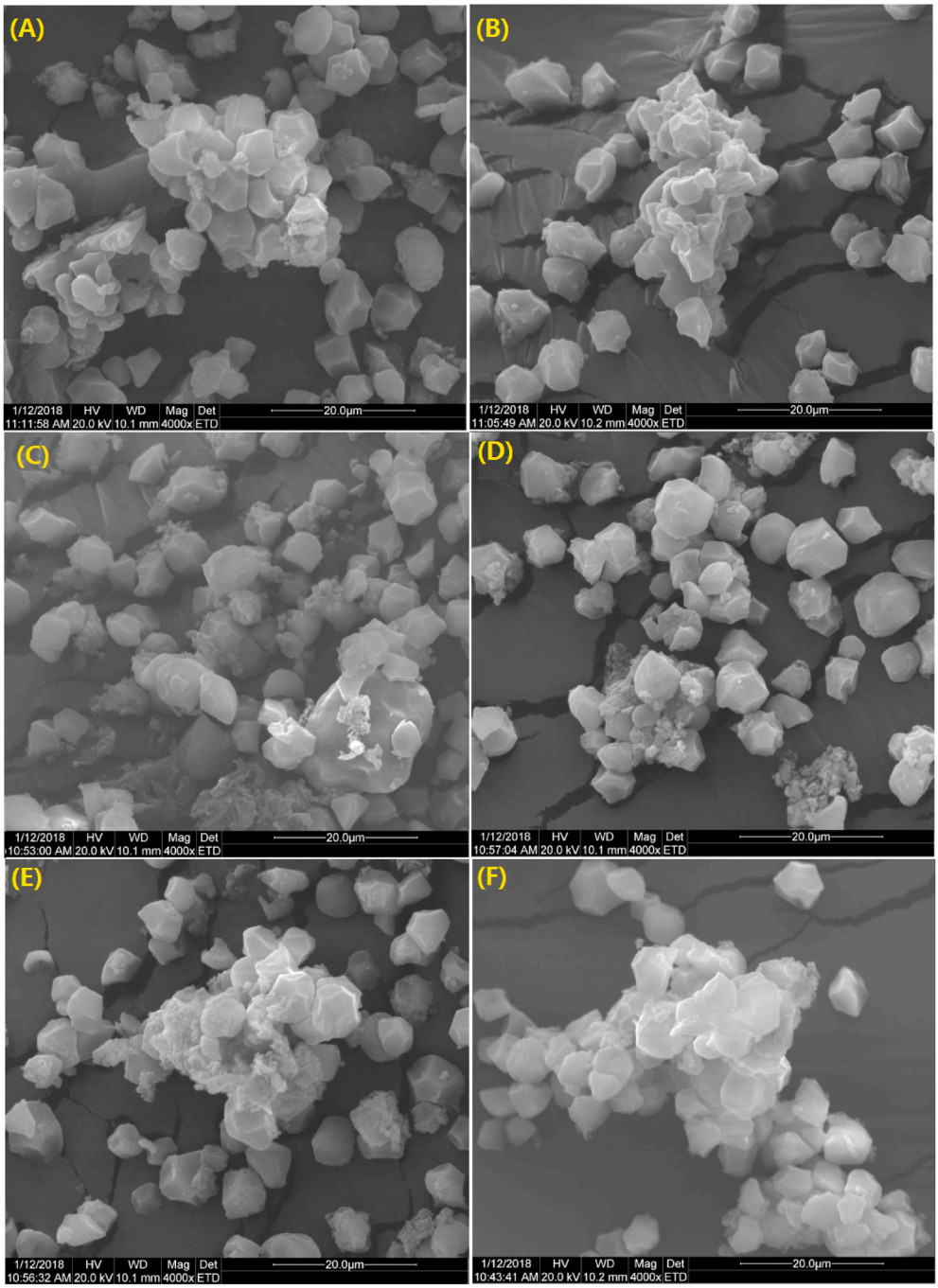

Fig. 1. SEM images of the GABE-modified WRS/WA: (A) 1:0.2; (B) 1:0.4; (C) 1:0.6; (D) 1:0.8; (E) 1:1.0; (F) 1:1.2.

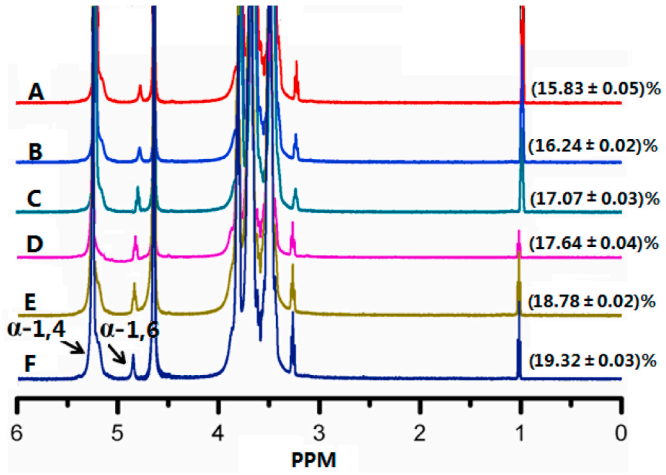

Fig. 2. The ^{1}HNMR spectra of the GABE-modified WRS/WA: (A) 1:0.2; (B) 1:0.4; (C) 1:0.6; (D) 1:0.8; (E) 1:1.0; (F) 1:1.2. Numbers at the right present the α-1, 6 glycosidic linkage ratio.

15.83 to 19.32%. It indicates that through GA→BE action on the mixed substrates of WRS and WA, the α-1,4-glycosidic linkages in amylose are broken down to produce many new linear chains. Next, these new linear chains either transfer to WRS molecules, making the branching degree of WRS increase, or transfer to amylose molecules, making amylose tend to amylopectin molecules (Sorndech et al., 2016). An higher WA ratios in WRS:WA mixtures offer a more optimal donor substrate for BE to form new branch points. It has been reported that the BE activity on waxy rice starch may be restricted by branching steric hindrance due to higher branching structure, hence resulting in lower catalytic efficiency (Sorndech et al., 2016; Tian et al., 2016). So, an increase in amylose content in waxy rice starch may contribute to accommodating in the BE catalytic subsites. The higher WA ratios is, the more BE catalytic subsites are accommodated. Moreover, with increasing WA ratios in WRS:WA mixtures, the inhibiting effect of steric hindrance in WRS on BE decreases, which causes BE to easily penetrate the linear structure of WA and to effectively catalyse acceptor chains during transfer reaction. Besides, higher WA ratios may accelerate the disproportion activity of amylose to further increase the degree of branching (Sorndech et al., 2016). So, GA→BE treatment more efficiently catalyse chain transfer in amylose than amylopectin to form new branch points. The ratio of amylose and amylopectin is crucial to catalytic efficiency of enzyme because BE catalyses the creation of new branch points via cleaving the α-1,4-glycosidic linkages in starch and generating α-1,6-glycosidic linkages within the linear α-1,4 segments (Roussel et al., 2013).

3.3. Chain length distribution of the debranched samples

Table 1 lists the chain-length distribution of debranched starch samples. It indicates that after GA→BE treatment, with increasing WRS:WA from 1:0.2 to 1:1.2, the proportion of A-, B1- and B2-chains greatly change. Compared to the ratio of WRS:WA (1:0.2), with increasing WRS:WA from 1:0.2 to 1:0.8, A chains (DP 6–12) increase by 5.47, 11.84 and 17.45%, respectively, whereas B1- and B2-chains (DP 13–36) decrease by 9.58, 23.12 and 32.73%, respectively. Conversely, as the ratios of WRS to WA continue to increase (1:1.0 and 1:1.2), short chains (DP 6–12) decrease by 3.43 and 8.37%, respectively, whereas middle and long chains (DP 13–36) increase by 2.66 and 10.69%, respectively. These data imply that appropriate amylose content is positively related to catalytic efficiency of enzyme. GA→BE treatment prefers appropriate amylose ratio (1:0.8) in WRS:WA mixtures as appropriate donor substrates. For the substrates with different ratios of WRS:WA (1:0.2–1:0.8), with increasing ratios of WRS:WA, the production of A chains (6-12) significantly increase, whereas the production of B1 chains (13–24)

Table 1
Chain length distributions of the debranched GABE-modified WRS/WA.

Samples	Chain length distributions (%)					Average chain length
	A-chains DP < 6	B1-chains DP 6–12	B2-chains DP 13–24	B3-chains DP 25–36	DP > 36	
1:0.2	0.90 ± 0.22^{e}	60.92 ± 0.25^{d}	32.67 ± 0.23^{c}	5.31 ± 0.33^{a}	0.20 ± 0.05^{a}	7.82 ± 0.22^{c}
1:0.4	1.23 ± 0.34^{d}	64.25 ± 0.24^{c}	30.62 ± 0.45^{d}	3.72 ± 0.27^{b}	0.18 ± 0.03^{b}	7.63 ± 0.21^{c}
1:0.6	2.52 ± 0.12^{b}	68.13 ± 0.04^{b}	27.51 ± 0.30^{e}	1.69 ± 0.34^{c}	0.15 ± 0.02^{c}	6.86 ± 0.19^{d}
1:0.8	2.87 ± 0.46^{a}	71.55 ± 0.57^{a}	24.62 ± 0.28^{f}	0.93 ± 0.42^{d}	0.03 ± 0.02^{d}	6.58 ± 0.24^{d}
1:1.0	2.14 ± 0.02^{c}	58.83 ± 0.03^{e}	38.85 ± 0.05^{b}	0.14 ± 0.03^{e}	0.04 ± 0.02^{d}	10.63 ± 0.32^{b}
1:1.2	2.12 ± 0.01^{c}	55.82 ± 0.08^{f}	41.97 ± 0.07^{a}	0.07 ± 0.03^{f}	0.02 ± 0.01^{d}	11.78 ± 0.15^{a}

Mean of three measurements ± SD values in the same column with different letters are significantly different ($p < 0.05$).

remarkably reduces. While for the substrates with different ratios of WRS:WA (1:1.0–1:1.2), higher amylose ratios accelerate the production of more B1 chains (13–24), while A-chains (DP 6–12) decreases. We speculate it may be attributed to two reasons: (1) Amylose contents in WRS:WA mixtures may exceed the activity of added enzymes when amylose ratios are larger than 1:1, which prevents amylose from being completely cleaved into shorter linear chains to some extent. As a result, longer linear chains (DP 13–24) increase; (2) Higher amylose ratios contribute to higher the degree of branching, or, the production of more number of branching chains. Among these branching chains, some branching chains may possess higher DP (13–24). Through high-performance anion-exchange chromatography, longer linear chains (DP 13–24) may release from the branching chains debranched with isoamylase and pullulanase, resulting in an increase in B1 chains (13–24). Table 1 shows that compared to the ratio of WRS:WA (1:0.2), with increasing WRS:WA from 1:0.2 to 1:0.8, the average chain lengths of the GABE-modified WRS/WA slightly decrease from 7.82 to 6.58. However, the chain lengths significantly increase from 7.82 to 11.78 as the ratios of WRS to WA continue to increase (1:1.0 and 1:1.2).

3.4. FTIR spectroscopy of the samples

The original and deconvoluted FTIR spectra of the GABE-modified WRS/WA samples are depicted in Fig. 3. It shows that the absorption peaks at 3100-3600 cm^{-1} of enzyme-treated starches move to higher wavenumbers decreasing from 3390 to 3380 cm^{-1} with increasing the ratios of WRS:WA from 1:0.2 to 1:1.2. It suggests that the intensity of hydrogen bonds enhances after GA→BE treatment due to the formation of more intra- or inter-molecular hydrogen bonding of the hydroxyl groups. The absorption peaks at 1047 and 1022 cm^{-1} are closely associated with the crystalline and amorphous regions, respectively. Thus, the changes in the ratios of 1047/1022 represent the changes in the crystalline structure. The higher ratios manifest the more proportion of long-range ordered structure, or crystalline structure (Liu et al., 2020, p. 242116332; Van Soest, Tournois, de Wit, & Vliegenthart, 1995). As is shown in Fig. 3, with increasing ratios of WRS:WA from 1:0.2 to 1:0.8, the ratios of 1047/1022 gradually decrease. It indicates that after GA→BE treatment, an increase in degree of branching in amylose or amylopectin prevents chain realignment, hence resulting in a lower ordered structure (Donth et al., 1996). However, the ratios of 1047/1022 increase as the ratios of WRS:WA continue to increase (1:1.0 and 1:1.2). A plausible explanation may be ascribed to higher degree of branching and more uniform middle branch chain lengths (DP 13–24), as is shown in Fig. 2 and Table 1. It indicates that higher amylose ratios (≥1:1.0) in WRS:WA mixtures contribute to the development of symmetric middle chain branches with similar chain length (DP 13–24) that are more prone to entangle each other, facilitating the formation of the

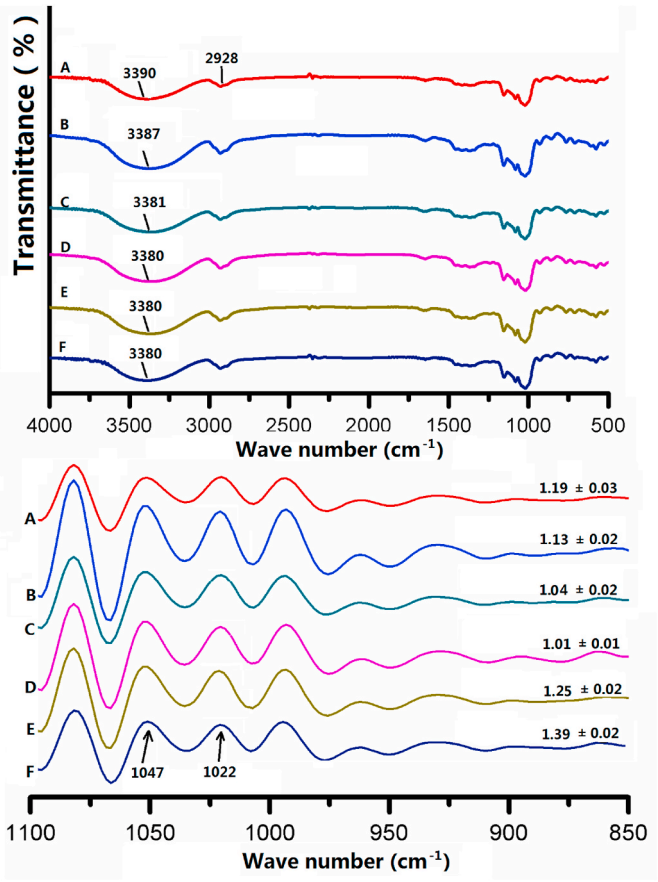

Fig. 3. The original (top) and deconvoluted (bottom) FTIR spectra of the GABE-modified WRS/WA: (A) 1:0.2; (B) 1:0.4; (C) 1:0.6; (D) 1:0.8; (E) 1:1.0; (F) 1:1.2. Numbers are the ratio of 1047 cm^{-1} to 1022 cm^{-1}.

ordered structure of starch.

3.5. XRD spectra of the samples

The XRD spectra of the GABE-modified WRS/WA samples are depicted in Fig. 4. It indicates that the GABE-modified WRS/WA samples show an A-type model with diffraction peaks at 2θ of 15.2°, 17.2° and 23.5° (BeMiller & Whistler, 2009). With increasing the ratios of WRS:

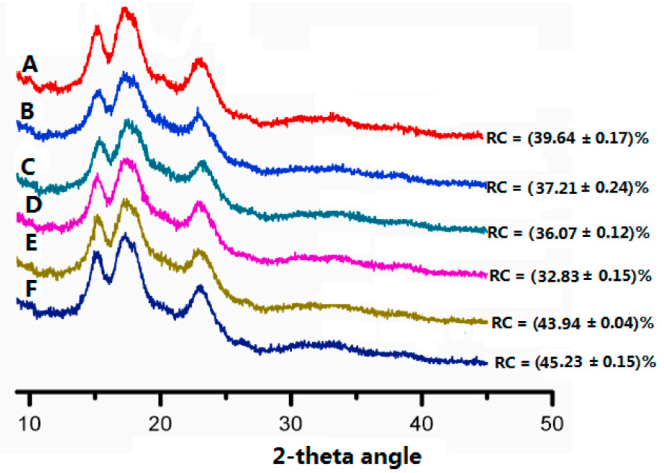

Fig. 4. XRD plots of the GABE-modified WRS/WA: (A) 1:0.2; (B) 1:0.4; (C) 1:0.6; (D) 1:0.8; (E) 1:1.0; (F) 1:1.2. RC means relative crystallinity.

WA from 1:0.2 to 1:0.8, the relative crystallinity of the GABE-modified WRS/WA samples decreases from 39.64 to 32.83%. It is explained by the fact that the crystalline structure was damaged after GA→BE treatment. Moreover, increased degree of branching and more short branch chains (DP ≤ 12) that are not prone to intertwine each other largely restrain chain rearrangement, hence resulting in lower crystallinity (Hizukuri, 1985). As the ratios of WRS:WA continue to increase to 1:1.0 and 1:1.2, the relative crystallinity significantly increases to 43.94 and 45.23%, respectively. It may be attributed to the two reasons: (1) after GA→BE treatment, the branched degree in amorphous domain increases and the distance between branch points decreases, hence resulting in the spontaneous recrystallization of amylopectin (Hanashiro, Tagawa, Shibahara, Iwata, & Takeda, 2002); (2) With increasing ratios of WRS:WA, more symmetric middle chain branches (DP 13–24) produced after enzyme treatment are easier to entangle each other, resulting in the formation of the ordered crystalline structure.

3.6. Solubility of the samples

The solubility of the GABE-modified WRS/WA samples are displayed in Fig. 5. It shows that with increasing ratios of WRS:WA (1:0.2–1:0.8), the solubility of the GABE-modified WRS/WA samples gradually increases. It may be attributed to chain depolymerization, an increase in short chains, etc. after enzyme treatment (Takata, Takaha, Okada, Takagi, & Imanaka, 1996). After GA→BE treatment, the glucosidic bonds in starch molecules cleave and then more short chains create, hence resulting in an increase in the solubility. However, the solubility slightly decreases as the ratios of WRS:WA continue to increase (1:1.0–1:1.2). It may be because higher amylose ratios (≥1:1.0) facilitate the formation of symmetric middle chain branches (DP 13–24), accelerating the entanglement between chains, hence resulting in the slight reduction in the solubility. It can be clearly seen from the picture in Fig. 5 that the clarity of the enzyme-treated samples increases with increasing ratios of WRS:WA (1:0.2–1:0.8), especially for the ratio of

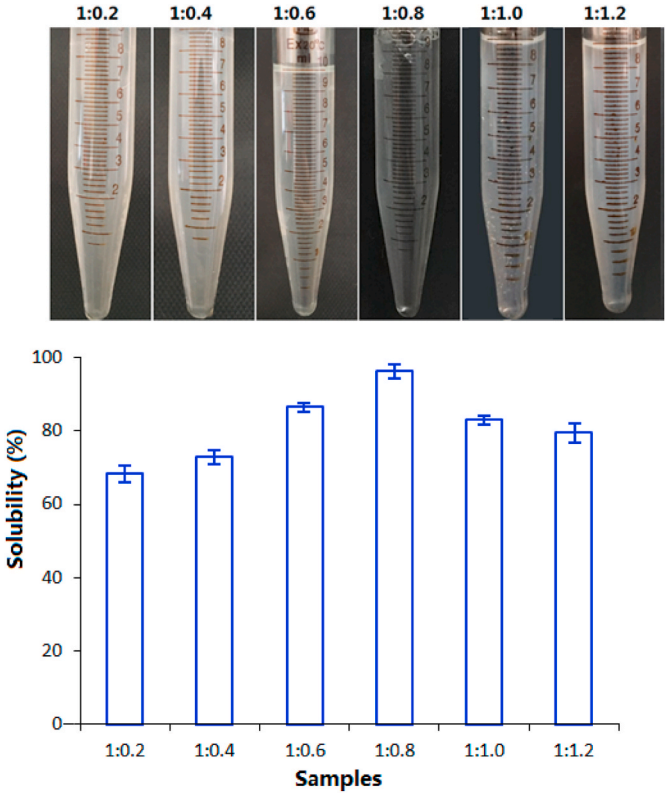

Fig. 5. Solubility of the GABE-modified WRS/WA samples.

WRS:WA (1:0.8). However, the clarity decreases as the ratios of WRS:WA continue to increase (1:1.0–1:1.2).

3.7. Turbidity of the sample pastes

The turbidity of the GABE-modified WRS/WA pastes in different time at 4 °C is listed in Table 2. It has been reported that the turbidity of starch pastes at 4 °C presents the degree and rate of starch retrogradation. The higher the turbidity is, the larger the retrogradation degree. Table 2 indicates that compared to the ratio of WRS:WA (1:0.2), the turbidity of the GABE-modified WRS/WA pastes decreases with increasing the ratios of WRS:WA from 1:0.2 to 1:0.8. It may be explained by the fact that after GA→BE treatment, the degree of branching of WRS increases. The more branch chains are not easy to entangle each other, resulting in lower rate of starch retrogradation. However, the turbidity increases as the ratios of WRS:WA continue to increase (1:1.0–1:1.2). It may be because more middle chain branches (DP 13–24) produced after enzyme treatment are more liable to accelerate chain entanglement and chain realignment to form a more organized crystalline structure, hence facilitating starch retrogradation (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008).

3.8. Gelatinization characteristics of the samples

The DSC plots of the GABE-modified WRS/WA are displayed in Fig. 6. It indicates that compared to the ratio of WRS:WA (1:0.2), the gelatinization temperatures of the GABE-modified WRS/WA reduce from 72.71 to 70.86 °C with increasing ratios of WRS:WA from 1:0.2 to 1:0.8. Corrspondingly, the gelatinization enthalpy values decrease from 15.32 to 13.05 J/g as the ratios of WRS:WA increase. It may be because the increase in short chains (DP ≤ 12) contribute to the formation of a great number of shorter double helices that are lower heat resistance. Moreover, an increase in degree of branching in amylose or amylopectin prevents chain realignment, hence resulting in lower ordered structure. However, the gelatinization temperatures and enthalpy values increase as the ratios of WRS:WA continue to increase (1:1.0–1:1.2). It further confirms that with increasing amylose ratios, more middle chain branches (DP 13–24) produced facilitate the formation of more long-range ordered structure, which promotes a more intensely intermolecular interaction, resulting in higher gelatinization temperature and enthalpy. Besides, more amounts of linear chains (DP 13–24) released from redundant amylose after enzyme treatment are be more liable to accelerate molecule mobility, alignment and chain rearrangement to form a more organized crystalline structure (Lopez-Rubio et al., 2008). Moreover, as is shown in Fig. 3, with increasing amylose ratios, GA→BE treatment accelerates the formation of more new intra- or inter-molecular hydrogen bonding, increasing the ordering and stability of double helical structures, hence higher energy required to destroy double helices (Cooke & Gidley, 1992). The results are in agreement with the results from FTIR and XRD.

Table 2
Turbidity of the GABE-modified WRS/WA pastes in different time at 4 °C.

Samples	0 h	24 h	48 h	72 h	96 h
1:0.2	0.486 ± 0.022^{c}	0.509 ± 0.014^{b}	0.562 ± 0.013^{b}	0.583 ± 0.022^{c}	0.594 ± 0.016^{b}
1:0.4	0.369 ± 0.024^{d}	0.427 ± 0.017^{c}	0.442 ± 0.032^{c}	0.469 ± 0.017^{d}	0.474 ± 0.028^{c}
1:0.6	0.337 ± 0.017^{de}	0.366 ± 0.023^{cd}	0.382 ± 0.015^{d}	0.392 ± 0.033^{e}	0.396 ± 0.021^{d}
1:0.8	0.302 ± 0.024^{e}	0.352 ± 0.017^{d}	0.362 ± 0.021^{d}	0.372 ± 0.016^{e}	0.392 ± 0.033^{d}
1:1.0	0.551 ± 0.019^{b}	0.574 ± 0.023^{b}	0.620 ± 0.012^{a}	0.687 ± 0.022^{b}	0.729 ± 0.014^{a}
1:1.2	0.611 ± 0.027^{a}	0.632 ± 0.012^{a}	0.648 ± 0.028^{a}	0.702 ± 0.034^{a}	0.753 ± 0.016^{a}

The mean of three measurements ± SD values in the same column with different letters are significantly different ($p < 0.05$).

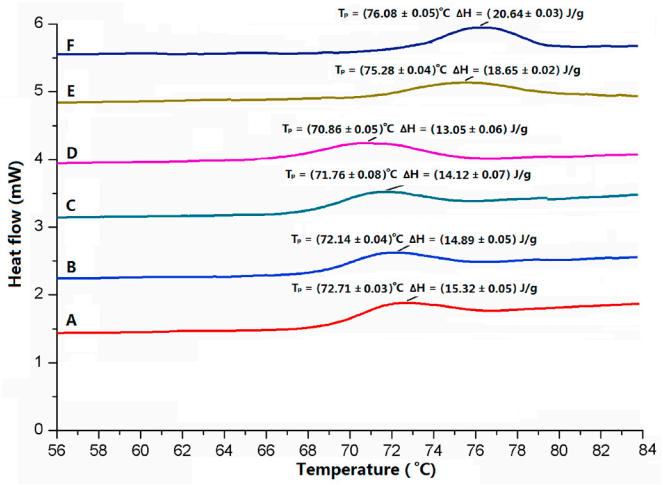

Fig. 6. DSC plots of the GABE-modified WRS/WA: (A) 1:0.2; (B) 1:0.4; (C) 1:0.6; (D) 1:0.8; (E) 1:1.0; (F) 1:1.2.

3.9. Dynamic rheological behavior of the sample gels

The dynamic rheological properties of the GABE-modified WRS/WA gels are depicted in Fig. 7. It displays a relatively high dependence of storage modulus (G′) and loss modulus (G″) on angular frequency (ω). G′ (solid-like property) and G″ (liquid-like behavior) exhibit frequency dependence behavior, or G′ and G″ increase with increasing frequency. It implies that the molecular chain mobility becomes relatively high due to the formation of more short linear chains (DP 6–12) through GA→BE treatment (Lopes-Da-Silva, Santos, Freitas, Brites, & Gil, 2007). Moreover, the G′ values are higher than G″ values indicating a solid-like behavior (Fig. 7A and B). With increasing ratios of WRS:WA from 1:0.2 to 1:0.8, G′ and G″ of the sample gels generally decrease since shorter branch chain lengths and higher degree of branching decrease the number of junction zones within the gel network, hence resulting in lower viscoelasticity (Li et al., 2017). However, G′ and G″ values of the GABE-modified WRS/WA gels increase as the ratios of WRS:WA continue to increase (1:1.0–1:1.2), resulting in higher viscoelasticity. It may be attributed to the three reasons: (1) Higher amylose content may contribute to a chain rearrangement in amylose and amylopectin, generating a stronger network structure of the gels (Donth et al., 1996); (2) Higher amylose accelerates starch gelation and increases the solid behavior (Ning et al., 2020; Tsai, Li, & Lii, 1997); (3) More linear chains (DP 13–24) released after GA→BE treatment are more prone to entangle and orderly arrange in the gels, forming a stronger network structure of the gels. Loss tangent (Tan δ) is defined as the ratio of G″ to G′ of the gels (Hesarinejad, Koocheki, & Razavi, 2014). Fig. 7 shows that Tan δ values of all samples increase with the increase in frequency, indicating the predominance of viscous property in gels and reduction in elastic property at higher frequencies. With increasing ratios of WRS:WA from 1:0.2 to 1:0.8, Tan δ values increase, indicating a more liquid-like behavior with increasing amylose ratio in the mixtures. However, Tan δ values decrease as the ratios of WRS:WA continue to increase (1:1.0–1:1.2), suggesting that the mixtures with higher amylose ratios tend to have higher G′ and lower G″ and tan δ, and vice versa.

4. Conclusions

This research indicates that after GA→BE treatment, with increasing ratios of WRS:WA from 1:0.2 to 1:1.2, the percentages of α-1,6 glycosidic linkage significantly increase, indicating higher WA ratios in WRS:WA mixtures offer a more optimal donor substrate for BE to form more

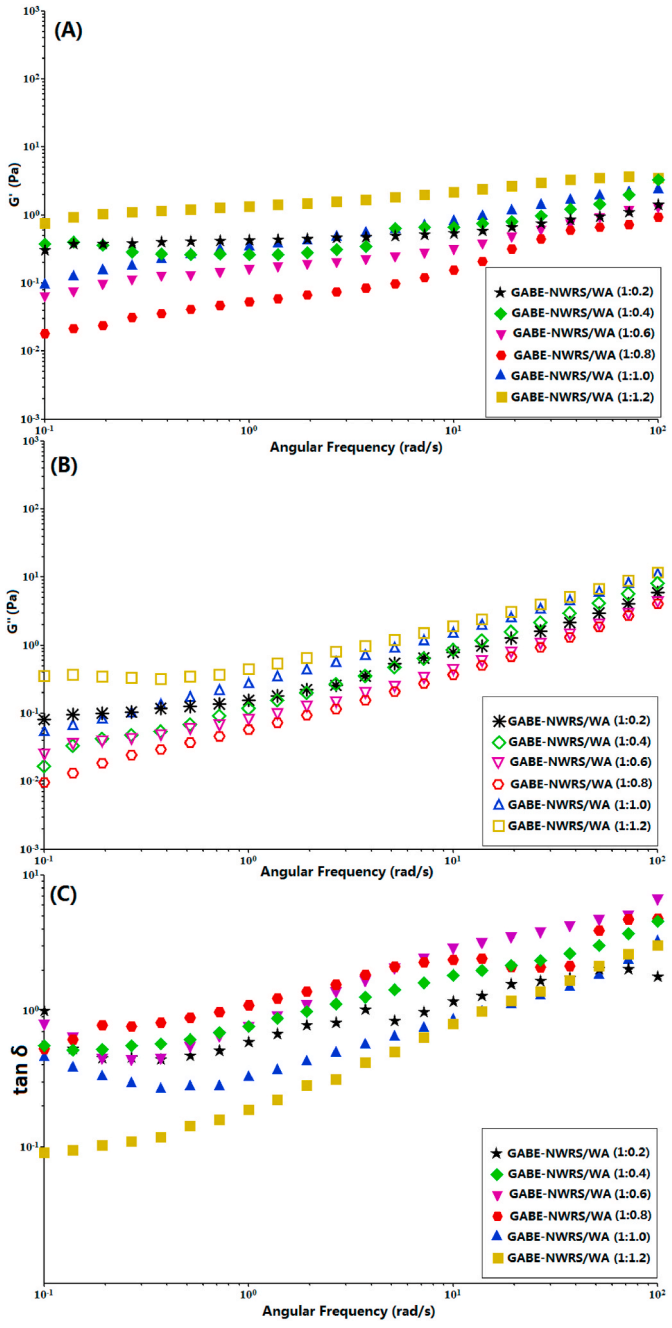

Fig. 7. The variation of G′, G″and Tan δ as a function of angular frequency.

branch points. With increasing ratios of WRS:WA from 1:0.2 to 1:0.8, short chain proportions (DP 6–12) increase and middle chain proportions (DP 13–24) decrease. While just the opposite as the ratios of WRS:WA continue to increase (1:1.0–1:1.2). The relative crystallinity gradually decreases since increased degree of branching and more short branch chains (DP $\leq$ 12) restrain chain entanglement not to develop the order structure as the ratios of WRS:WA (1:0.2–1:0.8) increase. On the contrary, they increase because higher degree of branching and more symmetric middle chain branches with similar chain length (DP 13–24) may be more prone to entangle each other, resulting in the formation of the ordered structure. With increasing ratios of WRS:WA from 1:0.2 to 1:0.8, the solubility and Tan δ increase, whereas the rate of starch retrogradation, the gelatinization temperatures and enthalpy values, as well as G′ and G″ decrease. However, with increasing ratios of WRS:WA from 1:1.0 to 1:1.2, just the opposite. Overall, different amylose ratios in WRS:WA mixtures have a significant influence on the catalytic properties of sequential GA and BE, which contributes to the regulation of the physicochemical properties of waxy rice starch-based products.

CRediT authorship contribution statement

Li Guo: Conceptualization, Methodology, Funding acquisition, Writing - original draft. **Yu Zhu:** Investigation, Formal analysis. **Jiahao Li:** Investigation. **Yifan Gui:** Investigation. **Haiteng Tao:** Investigation. **Feixue Zou:** Investigation. **Pengfei Liu:** Investigation. **Srinivas Janaswamy:** Investigation. **Bo Cui:** Conceptualization, Funding acquisition.

Declaration of competing interest

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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