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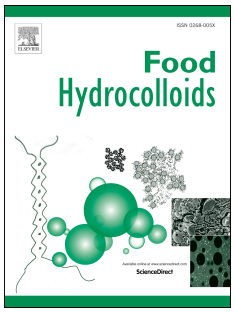

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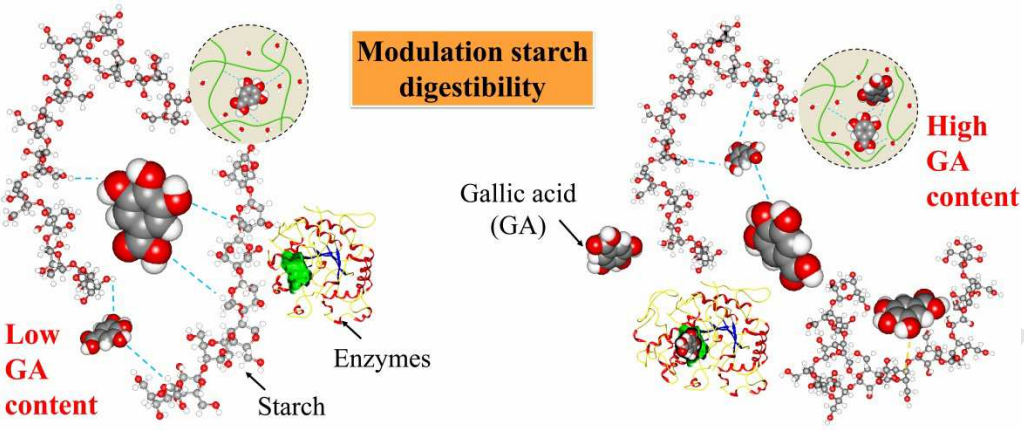

Modulating *in vitro* digestibility and predicted glycemic index of rice starch gels by complexation with gallic acid

Chengdeng Chi[1], Xiaoxi Li[1,*], Yiping Zhang[1], Ling Chen[1], Fengwei Xie[2,3], Lin Li[1], Guanghui Bai[4]

[1] Ministry of Education Engineering Research Center of Starch and Protein Processing, Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China

[2] Institute of Advanced Study, University of Warwick, Coventry CV4 7HS, United Kingdom

[3] International Institute for Nanocomposites Manufacturing (IINM), WMG, University of Warwick, Coventry CV4 7AL, United Kingdom

[4] Hebei Hengshui Laobaigan Liquor Group Co., Ltd., Hengshui, 053000, China

[*] **Correspondence:** Xiaoxi Li

Fax: +86 20 8711 3252

E-mail: xxlee@scut.edu.cn

Abstract: The starch digestibility strongly depends on the food composition and microstructure formed during food processing. To control rice starch digestion and glycemic response, rice starch was complexed with a dietary polyphenol. The interaction between starch and gallic acid (GA) and its influence on the *in vitro* digestibility of rice starch gel were investigated, which was correlated to the variation in GA fluorescence and the changes in starch pasting and gel properties. It was found that GA influenced the starch molecular rearrangement and aggregation and subsequently the multi-scale structures of rice starch gel. ATR-FTIR and SAXS results revealed that GA not only acted as a molecular chaperone to assist starch reassembly and to form starch ordered structures at a lower amount (4.54 and 11.24 mg/g starch), but also broke starch hydrogen bonding networks and reduced the ordered multi-scale structures of starch gel at a higher level (13.21 mg/g starch). After GA complexation, the resistant starch content in starch gel increased from 15.52% to 45.93% and thus decreased the predicted glycemic index (pGI). The synergistic effects of the reassembled ordered structures and the GA inhibitory activity against enzymes should be responsible for its nutritional changes. Thus, the digestibility and pGI of starch gel can be modulated through starch reassembly chaperoned with GA molecules.

Keywords: Rice starch; starch gel; digestibility; glycemic response; phenolic compound

1. Introduction

Rice (*Oryza sativa L.*) is the most important agricultural cereal in south Asia. It was estimated that rice was harvested at *ca.* 480 million metric tons (milled rice basis) every year (Muthayya, Sugimoto, Montgomery, & Maberly, 2014). In most developed countries, rice starch provides as much as 80% of daily calorific intake (Burrell, 2003). However, the glycemic response of rice starch foods is relatively high compared with other foods (Jenkins, Wolever, Jenkins, Josse, & Wong, 1984). Long-term consumption of high glycemic index (GI) food was regarded as a fundamental cause or contributor to a wide variety of pathological conditions such as obesity, Type II diabetes, and other metabolic complications (Brand-Miller, 2007; Brand-Miller, Dickinson, Barclay, & Celermajer, 2007).

To control the rice starch digestion and glycemic response, various methods such as heat-moisture treatment (Wang, Wang, Li, Chen, & Zhang, 2017) and the recrystallization of debranched starches (Kiatponglarp, Tongta, Rolland-Sabate, & Buleon, 2015) have been used. Moreover, the starch digestion behavior can be simply modified by complexation of starch with other food-derived ingredients, especially the polysaccharides (Chen et al., 2017), protein (Chi et al, 2018a), and phenolic compounds (Chi et al., 2018b; Koh, Wong, Loo, Kasapis, & Huang, 2010), which is regarded as a safe, eco-friendly, and cost-effective technique. In particular, phenolic compounds are well documented as inhibitors against starch digestive enzymes to reduce the starch digestibility (Miao, Jiang, Jiang, Zhang, & Li, 2015). However, phenolic compounds consist of one or multi-hydroxyl groups that can both covalently and non-covalently interact with starch to form starch-phenolic complexes (Bordenave, Hamaker, & Ferruzzi, 2014; Zhu, 2015), and in turn

57 influencing the starch structure and digestibility (Li, Pernell, & Ferruzzi, 2018). It has been indicated
58 that phenolic compounds may modulate starch digestibility via versatile modes such as decreasing
59 enzymes activity and/or increasing the starch ordering (Li et al., 2018; Zhu, 2015).

60 Recent research has shown that phenolic compounds could interact with starch especially
61 amylose to assemble into V-type inclusion complexes driven by hydrophobic interactions (Cohen,
62 Schwartz, Peri, & Shimoni, 2011). Li et al. (2018) found that, while caffeic acid and ferulic acid
63 could interact with amylose and amylopectin to form V-type complexes, gallic acid (GA) would
64 rather associate with starch to form non-inclusive starch-GA complexes. The hydrophobic
65 interactions and hydrogen bonds formed between starch and phenolics should be responsible for the
66 changes of starch conformation in those cases, respectively (Li et al., 2018). More interestingly, not
67 only V-type complexes but also non-inclusive starch-GA complexes are capable of inhibiting starch
68 hydrolysis, indicating that the modulation of starch digestion can be achieved by GA through
69 forming hydrogen bonding between starch and GA. Nevertheless, there is insufficient knowledge on
70 whether and how dietary phenolics such as GA can vary the hydrogen-bonding network and the
71 ordering of starch. In our previous research, we found that GA could alter the starch semi-crystalline
72 structure by interacting with starch in a non-covalent way, which decreases the starch digestibility
73 (Chi et al., 2017). However, phenolic compounds have been reported with the potential of inhibiting
74 starch retrogradation rather assisting starch reassembly (Wang, Li, Copeland, Niu, & Wang, 2015).
75 To tailor the starch structure and functionality including digestibility by phenolic compounds such as
76 GA, it is crucial to have a more complete understanding of the interactions between phenolic
77 compounds and starch and its effects on starch architectures and digestibility.

In this work, starch gel systems with different GA contents were studied, which allowed us to confirm the correlation between the changes in the starch gel structure and digestibility and the GA complexation amount. Based on the results, we obtained new knowledge of the interactions between starch and phenolics and explored the underlying mechanism regarding the effect of GA on the starch digestibility, which could be instrumental to the rational design of healthy starch-based foods containing phenolics.

2. Materials and Methods

2.1. Materials

Rice starch was purchased from Jinnong Biotechnology Co., Ltd. (Jiangxi, China). The moisture content was determined by a moisture analyzer (MA35, Sartorius Stedim Biotech GmbH, Germany). GA in this study was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pancreatin and Amyloglucosidase were purchased from Sigma-Aldrich LLC (Santa Clara, USA). A glucose oxidase/peroxidase (GOPOD) used to determine the glucose content was supplied by Megazyme International Ireland (Bray Business Park, Bray, Co. Wicklow, Ireland). Other reagents were of analytical grade.

2.2. Preparation of GA-complexed rice starch systems

Rice starch-GA complexes were prepared according to our previous method (Chi et al., 2017) with slight modification. Briefly, starch suspensions (10%, w/v) with 4% (GA/starch, w/w), 20% and 50% contents of GA (labeled as SGA-1, SGA-2 and SGA-3, respectively) were incubated at 37 °C. The slurries were homogenized by high-speed shearing (6000 rpm) bubbled with a slow stream of nitrogen for 0.5 h. Afterwards, starch-GA complex slurries were centrifuged and washed with

distilled water until no GA could be detected in the supernatants. The starch-GA complexes were air dried at 40 °C, smashed and sieved within 30 μm for further analysis.

2.3. Determination of GA content in the complexes

The GA content was estimated by the Folin-Ciocalteu procedure (Kaluza, McGrath, Roberts, & Schroeder, 1980). Briefly, 10 mg (dry basis) of the starch-GA complex was dissolved in 10 mL of dimethyl sulfoxide and then centrifuged for 10 min at 4000 rpm. 0.5 mL of the solution was removed and mixed with 1.5 mL of deionized water, which was then added with 1 mL of the Folin-Ciocalteu reagent and mixed homogeneously. Afterwards, 1 mL of 8% (w/w) sodium carbonate was added, and then mixed and kept in the dark at room temperature for 90 min. The absorbance of the solution was detected at 760 nm. GA was used to prepare the standard curve.

2.4. Preparation of starch gel system

The GA-starch complexes (1g, dry basis) were dispersed in 20 mL of acetate buffer (0.1 M, pH 5.2). The suspensions were cooked at 95 °C with continuous stirring for 30 min to make completely gelatinized starch pastes. Sufficient cooling at 4 °C for 24 h was carried out to prepare starch gels with varied structure. The starch gels were used for physical characterization and digestibility analysis. A native rice starch gel as a control was prepared in a way similar to starch-GA complexes but without GA. Starch gel systems prepared from rice starch, SGA-1, SGA-2 and SGA-3, respectively, were referred to RSG, RSG-1, RSG-2 and RSG-3, respectively.

2.5. *In vitro* digestibility

In vitro digestibility of starch-GA complexes and starch-GA gels were measured based on the *Englyst* method (Englyst & Cummings, 1985) with slight modification. 12 g of porcine pancreatin

120 (1.4×10^4 USP, Sigma Aldrich) was completely suspended in 80 mL of deionized water and followed
121 by centrifugation at 3000 g for 15 min to obtain working solution A. Afterwards, amyloglucosidase
122 (3.15 mL, 45 units) was mixed with 3.85 mL of deionized water to obtain working solution B. Then,
123 the fresh enzyme working solution was prepared by mixing 54 mL of solution A and 6 mL of
124 solution B.

125 The prepared starch gels were equilibrated at 37 °C for 10 min, and 5 mL of the enzyme
126 working solution and 5 glass balls were added. Then, the samples were incubated at 37 °C with
127 continuous stirring (190 rpm) in a water bath. 0.5 mL of the hydrolysate at different times (0, 10, 20,
128 30, 40, 50, 60, 80, 100, and 120 min) was removed and mixed with 20 mL of 66% ethanol to
129 inactivate the enzymes. The samples were centrifuged at 3000 g for 10 min and the hydrolyzed
130 glucose content was measured using a GOPOD reagent. The glucose content after 20-min and
131 120-min hydrolysis were labeled as G20 and G120, respectively, by which starch fractions were
132 classified as RDS, SDS and RS based on the hydrolysis rate using the following formulas:

$$\mathrm{RDS} = \mathrm{G20} \times 0.9/\mathrm{TS}$$

$$\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9/\mathrm{TS}$$

$$\mathrm{RS} = [\mathrm{TS} - \mathrm{RDS} - \mathrm{SDS}]/\mathrm{TS}$$

133 where TS means the total starch (TS) content of the complexes used for digestibility measurement.
134 Herein, TS equals 1 g.

135 **2.6. Hydrolysis kinetic and predicted glycemic index (pGI)**

136 To investigate the hydrolysis kinetics and pGI of starch gels, the first order kinetic model [$C = C_\infty(1 - e^{-kt})$] was applied (Goñi, Garcia-Alonso, & Saura-Calixto, 1997), where C, C_∞ and k

represent the percentage of starch hydrolyzed at time *t* (0, 10, 20, 30, 40, 50, 60, 80, 100 and 120 min), the maximum hydrolysis extent, and the kinetic constant, respectively.

The hydrolysis index (HI) was expressed as the ratio of the area under the hydrolysis curve (AUC) of the sample to the AUC of the fresh white bread (a standard reference for calculating pGI). Then, the pGI was obtained from HI according to the equation, pGI = 39.71 + 0.549HI (Goñi et al., 1997).

2.7. Fluorescence spectrum analysis

The interaction between GA and rice starch was evaluated by a steady-state fluorescence technique using an F4500 fluorescence spectrophotometer (Hitachi Co., Japan). The fluorescence determination was performed with a constant GA concentration of 10 μM and increasing concentrations (0-1.5 mg/mL) of rice starch (cooked in a boiling water bath for 10 min). The emission spectra were recorded from 280 to 410 nm with an excitation wavelength of 250 nm, and the excitation and emission slit widths used were both 5 nm.

2.8. Pasting analysis

The pasting properties of native rice starch and starch-GA complexes were evaluated by a Brabender Viscoamylograph (Brabender OHG, Germany). A 6% suspension (w/w) of the sample was stirred at a paddle speed of 210 rpm, heated from 30 to 95 °C at 7.5 °C/min, held at 95 °C for 15 min, then cooled from 95 °C to 50 °C at 7.5 °C /min, followed by holding at 50 °C for another 15 min.

2.9. Dynamic oscillatory measurements

To measure the viscoelastic property of starch-GA gels, a strain sweep test of the starch paste was first performed from 0.1 to 10% strains at 1 Hz and 25 °C to identify the linear viscoelastic

159 region. Then, dynamic oscillatory analysis was performed in the linear viscoelasticity range. Storage
160 modulus (G') and loss modulus (G'') were recorded at 25 °C in a 0.1-10 rad/s angular frequency
161 sweep. The edge of the gap was covered with silicon oil to minimize the water evaporation.

162 **2.10. Short-range ordered structure analysis**

163 The IR spectrum of starch has been shown to be sensitive to changes in short-range orders (J. J.
164 G. van Soest, D. De Wit, H. Tournois, & Vliegenthart, 1994). A Tensor 37 spectrometer (Bruker,
165 Germany) with an attenuated total reflectance (ATR) accessory was used to detect the molecular
166 structure of starch gels from 4000 to 400 cm^{-1}. Each spectrum was obtained at a resolution of 4 cm^{-1}
167 with 64 scans against the air as the background. Deconvoluted spectra over a range from 1200 cm^{-1}
168 to 800 cm^{-1} were used to investigate the short-range ordered structure of starch gels. Each gel system
169 was equilibrated at 37 °C for 10 min before determination.

170 **2.11. Small Angle X-ray Scattering**

171 Small-Angle X-ray Scattering (SAXS) measurements were performed on a SAXSess
172 small-angle X-ray scattering system (Anton-Paar, Austria). Samples were measured with a PW3830
173 X-ray generator (PANalytical) with the X-ray source of Cu Kα radiation (λ = 0.1542 nm). The
174 voltage was set at 40 kV and the current at 50 mA. Each starch gel was filled into a capillary of 1
175 mm diameter and 0.01 mm wall thickness, and the temperature kept at 25 °C for 10 min with the
176 X-ray exposure. The recorded data in an image plate was collected by the IP Reader software using a
177 PerkinElmer Storage Phosphor System. All collected data were normalized, the background and
178 smeared intensity were subtracted using SAXSquant 2D and SAXSquant 3.0 software was used to
179 further analyze the data (Chi et al., 2018b).

2.12. *In vitro* GA release from starch-GA gel system

The GA release behaviors of starch-GA gel systems during digestion were investigated by a dialysis method. Briefly, the starch-GA gel was transferred into a dialysis bag which was added with 5 mL of the enzyme working solution (Section 2.8). The dialysis bag was incubated in a triangular flask with 60 mL of acetate buffer (0.1 M, pH 5.2) with continuous stirring (190 rpm) in a water bath at 37 °C. After hydrolysis (5, 10, 15, 20, 30, 50, 70, 90 and 120 min), 1.0 mL of acetate buffer was removed and the absorbance of the solution was detected by the Folin-Ciocalteu procedure (Kaluza et al., 1980).

2.13. Statistical analysis

All tests were conducted at least in triplicate and the data analyzed using IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA). Analysis of variance (ANOVA) was followed by Tukey's HSD test to compare the treatments and the significance level was set at $p < 0.05$.

3. Results and Discussion

3.1. Phenolic compounds content

Phenolic compounds are an important type of phytochemicals in vegetables, fruits, and cereals. Native rice starch contained *ca*. 0.11 mg of phenolic compounds per gram of starch. The GA contents in the form of complexes were much higher and in the range of 4.54-13.21 mg/g starch. To be more specific, SGA-1 had 4.54 mg of GA per gram of rice starch, while SGA-2 and SGA-3 had 11.24 mg and 13.21 mg of GA per gram of rice starch, respectively. Based on a previous study (Réblová, 2012), GA has relatively high thermostability and thus short-time hydrothermal treatment would not influence the GA functionality. In this study, the infrared spectrogram of GA after cooking (cooked at

95 °C for 30 min) remained unchanged (**Fig. S1**), exhibiting short time of cooking would not influence the GA structures and functionality.

3.2. *In vitro* digestibility and predicted glycemic index of rice starch-GA complexes

Starch is one of the major components in cereal-based foods. The interactions between starch and other food ingredients tend to influence *in vivo* digestion of starch and its postprandial blood glucose response. As shown in **Table 1**, the different starch fractions (RDS, SDS and RS) were determined in native and GA-complexed rice starch gels.

After complexation with GA, the digestibility of rice starch gels was significantly decreased. RSG had the highest content of RDS (75.75 ± 1.23%, mean ± SD), followed by RSG-1 (69.34 ± 1.97%) > RSG-2 (64.78 ± 0.86%) > RSG-3 (47.91 ± 1.92%). These results indicate that starch digestibility can be mitigated after GA complexation. To predict the glycemic response, the *in vitro* kinetics of starch digestion was shown in **Fig. 1** and the pGI was calculated and presented in **Table 1**. The maximum hydrolysis extents (C_∞) of starch-GA gels, ranging between 59.25 and 91.85, were obviously lower than that of native starch gel (95.17). This observation was in agreement with the RDS changes and further demonstrates that the complexation with GA can reduce the starch digestibility. The kinetic constant, *k*, which reflects the rate of hydrolysis in the early stage, ranged between 0.062 and 0.080. The values of *k* followed the sequence of RSG > RSG-3 > RSG-1 > RSG-2. Interestingly, the trend of C_∞ and *k* were not fully consistent with each other. In other words, the *k* value of SGA-3 was higher than those of RSG-1 and RSG-2, but the former had a much lower C_∞ than the those of latter two. The initial stage of starch digestion is always determined by the starch hierarchical structure, while C_∞ is additionally governed by other factors such as the enzyme

222 activity. Hence, the changes in *k* and C_∞ for starch-GA complexes must result from the synergistic
223 effects of the starch gel structure and the GA inhibitory activity against α-amylase.

224 Based on the Goñi method (Goñi et al., 1997), pGI can be obtained from starch digestion curves.
225 From the data (Table 1), it can be observed that RSG had the highest pGI value (84.41). RSG-1
226 (81.98) and RSG-2 (79.38) displayed lower pGI than that of RSG and RSG-3 possessed the lowest
227 value (67.43). Although gel systems complexed with GA were rich in SDS and RS fractions, RSG-1
228 and RSG-2 were considered as high GI (GI > 75) foods. Notably, RSG-3, which had the highest SDS
229 and RS contents, was considered as an intermediate GI starch food. Hence, complexation with GA
230 can be considered as an alternative approach to modulate the digestibility and pGI of rice starch gels.

231 **3.3. Molecular interaction between rice starch and GA**

232 **3.3.1. GA fluorescence variation after complexation with rice starch**

233 To confirm the interactions between GA and rice starch, the intrinsic fluorescence emission
234 spectra of GA as affected by increasing concentrations of rice starch were determined, as displayed
235 in **Fig. 2**. It was shown that GA was excited at 250 nm, it had an emission fluorescence peak at
236 around 330 nm, which can be assigned to the π-system of the benzene ring. Based on the variation in
237 GA fluorescence intensity, the interactions between GA and other molecules can be detected. Notably,
238 the fluorescence intensity of GA was slightly enhanced when the content of available starch was
239 increased from 0.1 to 1.5 mg/mL, indicating non-covalent interactions were formed between GA and
240 starch. A similar phenomenon has been observed for phenolic compounds (e.g., curcumin) when
241 other polysaccharides (e.g., ι-carrageenan and soy soluble polysaccharides) were mixed (Chen, Ou,
242 Chen, & Tang, 2017; Yang, Wu, Li, Zhou, & Wang, 2013). It is indicated that phenolic compounds

243 could interact with polysaccharides via non-covalent molecular interactions.

244 **3.3.2. Changes of rice starch pasting properties after complexation with GA**

245 The changes in starch pasting properties can be used to reveal starch structural changes and the
246 interactions between starch and other substances. According to **Fig. 3** and **Table 2**, the paste
247 viscosity of starch-GA complexes was remarkably different from that of native rice starch paste. The
248 whole paste viscosities of SGA-1 and SGA-2 were greatly increased, while SGA-3 displayed a
249 significant reduction in the overall paste viscosity comparing to that of native rice starch paste. This
250 was the first time that starch complexed with phenolic compounds presented such pasting behavior.

251 After complexation with GA, SGA-1 and SGA-2 showed a higher peak viscosity (η_{pk}) while
252 SGA-3 had lower η_{pk} compared with that of native rice starch. Regarding these changes, it is
253 considered that suitable amounts of GA interacted with starch and promoted the formation of starch
254 ordered structures, which exhibited elevated resistance to hydrothermal treatment. However, excess
255 GA also tends to break the network of hydrogen bonding and in turn decreasing the starch
256 thermostability and η_{pk}. Once the swollen granules were disrupted, amylose molecules were leached
257 and the viscosity was decreased. All of the GA-treated starches showed higher thermostability (lower
258 η_{bd} in **Table 2**) than that of native rice starch, verifying the interaction between GA and starch.
259 During the cooling cycle, the viscosity of all the starch pastes increased rapidly, resulting in the
260 formation of starch gels at lower temperatures. SGA-1 and SGA-2 showed higher η_{sb} (i.e, higher
261 reassociation or rearrangement behavior), which could be due to the interaction of GA with starch
262 and subsequently assisted the reassembly of starch in forming a gel network. Interestingly, the gel of
263 SGA-3 had high stability with η_{rb} of 0.5 BU, which was much lower than that of SGA-1 (50.6 BU)

and SGA-3 (49.7 BU). This indicates a high level of GA would reduce starch reassociation at low temperatures. Based on these investigations, a feasible way to improve starch gel characteristics could be achieved by simply complexing starch with GA.

3.3.3. Changes of rice starch gel network after GA complexation

As shown in **Fig. 4**, with the increased oscillation frequency, the storage modulus (*G′*) and loss modulus (*G″*) of starch gel systems with or without GA complexation were gradually increased. Moreover, as the oscillation frequency increased from 0 to 10 rad/s, *G′* was always greater than *G″*, indicating the elastic modulus of rice starch gels were greater than its viscous modulus. When rice starch was treated with GA, *G′* and *G″* of starch-GA gel systems except SGA-3 were increased. This observation could also reflect the interaction between GA and starch. The assumption goes that suitable amounts of GA acted as a molecular chaperone to assist the reassembly of starch and the formation of a starch network or a starch-GA-starch architecture, which improved the strength of starch gel. However, a higher amount of GA in the gel system contributed to a reduction in *G′*, indicating a decreased gel strength. Polyphenols have been considered a classical antiager for starch retrogradation (Wu, Chen, Li, & Li, 2009). Herein, the reduced starch reassociation induced by GA molecules should be responsible for the reduced *G′* of RSG-3.

3.4. Short-range ordered molecular structure of starch-GA complex gels

As can be seen from **Fig. S1**, the GA in RSG-3 did not show any absorption peak over FTIR spectra comparing to RSG, indicating that GA within starch gel systems was undetectable by FTIR due to its limited amount. Hence, the ratio of absorbance (1047/1022 cm^{-1}) in starch FTIR spectra can be used to determine starch short-range orders (J. J. G. van Soest et al., 1994). Although starch

285 short-range molecular orders were severely disrupted after cooking, starch rearranged during cooling,
286 resulting in its elevated degree of ordering. As shown in **Table 2**, the ratio of absorbance at
287 1047/1022 cm^{-1} for RSG was 0.25 and increased to the range of 0.48-0.57 when starch gels were
288 complexed with GA at 4.54 to 11.24 mg/g starch, indicating the formation of a more ordered starch
289 gel network. RSG-3, which complexed with an even higher amount of GA, showed reduced
290 short-range orders (0.23) compared to RSG (0.25). This observation may result from the reduction of
291 starch rearrangement induced by excessive GA. Therefore, controlling the complexation content of
292 GA in starch gel systems would be an alternative approach to change the ordered structure of rice
293 starch gels.

294 **3.5. Aggregation structures of starch-GA complex gels**

295 SAXS has been extensively applied to analyze starch aggregated structures (Blazek & Gilbert,
296 2011; Fan et al., 2014). As presented in **Fig. 5**, the characteristic peak for the starch lamellar structure
297 at *ca.* 0.65 nm^{-1} was disappeared for all the starch samples due to the complete disruption of
298 multi-scale structures of starch granules after hydrothermal treatment. According to the literature, the
299 SAXS intensity at low-*q* region was correlated with the difference in the electron density between
300 ordered and amorphous regions (Chi et al., 2017; Suzuki, Chiba, & Yano, 1997). It can be seen that
301 the intensity of RSG-1 and RSG-2 at low-*q* region was higher than that of RSG, indicating RSG-1
302 and RSG-2 had a larger difference in the electron density between ordered and amorphous regions.
303 This could be attributed to the reduced amounts of molecular orders in amorphous regions, and/or the
304 enhancement of starch molecular aggregates within the ordered regions. Bearing the elevated setback
305 viscosity (**Fig. 3**) and storage modulus (**Fig. 4**) for RSG-1 and RSG-2, the SAXS changes must result

306 from the enhancement of gel ordered structures when a suitable content of GA was complexed.
307 However, the intensity of RSG-3 in the small-angle region was remarkably lower than that of RSG.
308 It could be explained that the ordering of RSG-3 was decreased when a higher amount of GA was
309 complexed. In addition, the intensity of all the samples decreased progressively from the low-q
310 region to the higher scattering region, with the extents of decrease following the sequence of RSG-1 >
311 RSG-2 > RSG > RSG-3. It is indicated that RSG-1 had the largest fractal dimension with the most
312 compact network architecture, while RSG-3 possessed the loosest structure with a smaller fractal
313 dimension (Suzuki et al., 1997). These observations further confirmed that a suitable content of GA
314 would promote the formation of ordered starch aggregates, while a high content of GA would inhibit
315 starch rearrangement after hydrothermal treatment.

316 To detect the inhomogeneous distribution of starch aggregates in a finite size region, the
317 effective structure factor $S(q)$ was calculated from SAXS profiles. It is defined as (Svergun & Koch,
318 2003):

$$S(q) = \frac{c_0 I_j(q)}{c_j I_0(q)}$$

319 Where c_0 and c_j, are the concentration of rice starch (they are equal in this work), $I_j(q)$ and $I_0(q)$ are
320 the intensity of starch-GA gel and that of rice starch gel, respectively. The value of $S(q)$ close to 1
321 suggests the system is homogenously distributed, less than 1 indicates a depletion region at given
322 size range, and larger than 1 shows the enrichment of starch gel in a given separation distance (Shi et
323 al., 2017). As shown in **Fig. 6**, the $S(q)$ values of RSG-1 and RSG-2 were always larger than 1 in the
324 whole q range. This result clearly indicates that the presence of GA would induce the aggregation of
325 starch at the entire SAXS detectable region. For RSG-3, $S(q)$ value was smaller than 1 when $q < 1.15$

326 nm^{-1}, indicating a high level of GA inhibited starch reassembly and thus led to a smaller size
327 (calculated by Woolf-Bragg's equation: $d=2\pi/q$) of aggregation structure which was detected at a q
328 range of 1.15-1.5 nm^{-1}. All the $S(q)$ vs. q profiles had fluctuations in the large q range that
329 corresponds to the smaller size of aggregates. These fluctuations should be contributed by the partial
330 inhomogeneous distribution of starch aggregates.

331 **3.6. *In vitro* GA release behaviors**

332 GA showed critical α-amylase inhibition activity (Chi et al., 2017) and its release kinetics from
333 starch-GA gel systems during digestion may influence the starch digestibility. To reveal the GA
334 release behavior of starch-GA gel, the dialysis method combined with the starch *in vitro* digestion
335 procedure were carried out. As shown in **Fig. 7**, the cumulative released GA drastically increased in
336 the initial stage, then significantly reduced and finally reached an equilibrium state as the digestion
337 time was prolonged. Notably, RSG-3 released the highest GA content and reached an equilibrium
338 state within a short time, which was the same as that for RSG-1. RSG-2 released an intermediate GA
339 content but reached a plateau for a longer time compared to those for RSG-1 and RSG-3. If RSG-3
340 had a more ordered structure of starch gel than RSG-1 and RSG-2, it should have reached a plateau
341 of GA release content after a longer time because the ordered starch assembly suppressed the access
342 to enzymes. However, this assumption cannot be verified by the GA release profiles. Therefore, the
343 degree of structural ordering of RSG-3 should be lower than those of RSG-1 and RSG-2, and the
344 release behavior of GA should have resulted from the synergistic effects of the ordered architecture
345 and the GA inhibitory activity against enzymes.

346 **3.7. Mechanism of structure and digestibility changes of rice starch gels**

347 The interaction between GA and starch has been validated by the elevated GA fluorescence
348 intensity and the changes in starch pasting and gel properties when rice starch was complexed with
349 GA. As presented in **Fig. 8**, GA migrated into the interior of starch granules and interacted with
350 starch. The elevated viscosity for the overall pasting curve (**Fig. 3**) suggested the interactions among
351 starch were improved when starch was complexed with GA at low levels (<11.24 mg/g starch). We
352 postulate that suitable amounts of GA acted as a "molecular chaperone" and assisted starch
353 reassembly, which increased the starch swelling capacity and the peak viscosity η_{pk}. Once the
354 swelled starch granules were disrupted with the leaching of amylose, the breakdown viscosity of
355 starch paste was shown. However, GA interacted with starch and reduced the breakdown viscosity
356 η_{bd} (**Table 2** and **Fig. 3**). During the cooling cycle, starch was favorably reassociated with the
357 assistance of suitable amounts of GA but was inhibited when the amounts of complexed GA was
358 higher (>13.21 mg/g starch) (**Table 2** and **Fig. 3**). Therefore, the multi-scale structures of starch gel
359 including short-range molecular orders (**Table 2**) and aggregation structures (**Fig. 4**, **Fig. 5** and **Fig. 6**)
360 were reinforced by complexation with low (suitable) amounts of GA, but were decreased when
361 higher levels of GA were complexed. GA consists of three hydroxyl groups and one carboxyl group
362 as functional groups, which could favor GA to interact with starch. On the other hand, the steric
363 hindrance of phenolic benzene of GA and the interaction between GA and starch tend to inhibit the
364 aggregation of starch and starch-GA reassembly, thereby decreasing the ordering of starch gel. GA
365 acted as a "double-edged sword" for modulating starch rheological properties as shown in **Fig. 9**.

366 Phenolic compounds have been proposed to decrease starch digestibility in two modes: (i)

suppressing the activity of enzymes and inhibiting the access of starch to enzymes and (ii) improving the starch structural features that slow or prevent the amylase action (Chi et al., 2017). As to gel-like starch foods complexed with phenolic compounds, the multi-scale structures of starch gel determine the food textural quality and digestibility and the glycemic response. Based on the results discussed above, it can be concluded that starch gels were reassembled with higher contents of short-range molecular orders and aggregated structures when GA was complexed at a suitable content. These ordered structures may reduce the accessibility of enzymes to starch. The evidence from the GA release behaviors during starch digestion verified this assumption (**Fig. 7**). RSG-3 released a higher GA content and thus may show higher inhibitory activity against α-amylase during the digestion than those of RSG-1 and RSG-2, which, in turn, decreased the RSG-3 hydrolysis and the GA release rate. However, RSG-3 reached a GA release plateau not after a longer time compared with RSG-1 and RSG-2, indicating a lower gel strength of RSG-3 also affected the gel digestion behavior. Compared with RSG-1 and RSG-3, RSG-2 released an intermediated GA content during the initial stage but it took a longer time to reach a plateau over the GA release profile (**Fig. 7**). Regarding this observation, it is considered that RSG-2 had greater amounts of ordered structures than RSG-3 and showed a stronger inhibitory effect against α-amylase than RSG-1. Therefore, the synergetic effects of the ordered multi-scale structures of starch gel and the GA inhibitory activity against the enzymes determine the starch digestibility in starch-GA gel systems. To be more specific, the digestibility/pGI of RSG-3 was mainly controlled by the GA inhibitory activity against α-amylase, and the digestion behaviors of RSG-1 and RSG-2 were caused by the synergistic effects. Ordered structures suppressed enzymes from attacking the gel network, while GA released from the starch-GA systems

had potent inhibitory activity against α-amylase and thus retarded the binding of starch to enzymes.

In our diet menus, cereals had phenolic compounds ranging 0.5 mg/g to 4.0 mg/g (Angelino et al., 2017) and vegetables had phenolic contents between 8.9 mg/g to 71.7 mg/g (Ismail, Marjan, & Foong, 2004). It is indicated that the digestibility of cereals would be determined by the synergetic effects of the ordering degree of multi-scale structures of starch gel and the inhibitory activity of phenolic compounds during cooking, while the inhibitory activity against α-amylase could be the rate-determining factor for starch foods incorporated with vegetables.

4. Conclusion

The *in vitro* enzymatic digestibility and the pGI of rice starch gels complexed with GA were evaluated, and the mechanisms involved in the changes in structure and digestibility of rice starch gels were revealed. The non-covalent interaction between GA and starch was validated. Lower amounts of GA (< 11.24 mg/g starch) acted as a molecular chaperone to assist the reassembly of starch and thus enhanced the ordering of short-range molecular structure and aggregates, but higher levels of GA (>13.21 mg/g starch) weakened the starch gel network and decreased its structural ordering. Complexation with GA increased the total contents of SDS and RS fractions and reduced the pGI value. Based on the results of the gel structures, GA release behavior, and starch digestibility, we conclude that starch complexation with GA would alter the multi-scale structures of gel-like starch foods, and, in turn, modulating the starch digestibility or pGI by the synergistic effects of the physical barriers from ordered structures and the GA inhibitory activity against enzymes. Selecting a suitable mode of GA complexation will open a pathway to control the structures and digestion behaviors of starch gels.

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502

503 **Table 1** Digestibility, model parameters, calculated hydrolysis indices and predicted glycemic

504 indices (pGI) of native and GA-treated starch gel system.

	RDS (%)	SDS (%)	RS (%)	C_∞	*k*	HI	pGI
RSG	75.75±1.23^{a}	12.40±1.37^{b}	12.21±0.75^{d}	95.17^{a}	0.080^{a}	108.57^{a}	84.41^{a}
RSG-1	69.34±1.97^{b}	15.14±0.78^{a}	15.52±1.46^{c}	91.85^{b}	0.071^{b}	102.66^{b}	81.98^{b}
RSG-2	64.78±0.86^{c}	15.61±1.89^{a}	19.61±1.72^{b}	88.54^{c}	0.062^{c}	96.35^{c}	79.38^{c}
RSG-3	47.91±1.92^{d}	7.06±2.33^{c}	45.93±0.99^{a}	59.25^{e}	0.078^{a}	67.32^{e}	67.43^{d}

505 ^{a,b,c,d} Values within column with different superscript letters are significantly different ($p < 0.05$).

506 RSG-1, RSG-2, and RSG-3 indicated the rice starch gel (RSG) complexed with 4.54, 11.24 and 13.21 mg GA per gram starch,

507 respectively. RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

508

509 **Table 2** Pasting characteristics and short-range orders of native and GA complexed rice starch gels.

Sample	T_p (°C)	η_{pk} (BU)	η_{bd} (BU)	η_{sb} (BU)	η_{rb} (BU)	1047/1022 $cm^{-1\#}$
Rice starch	64.8^{b}	237.0^{c}	151.8^{a}	208.6^{c}	9.7^{b}	$0.25^{c\#}$
SGA-1	66.3^{a}	279.2^{a}	113.6^{c}	257.5^{a}	50.6^{a}	0.48^{b}
SGA-2	65.7^{a}	270.5^{b}	107.9^{d}	249.0^{b}	49.7^{a}	0.57^{a}
SGA-3	65.8^{a}	165.4^{d}	119.5^{b}	62.7^{d}	0.50^{c}	0.23^{d}

510 T_p, pasting temperature; η_{pk}, peak viscosity; η_{bd}, breakdown viscosity (the difference between peak viscosity and the viscosity at the

511 start of cooling); η_{sb}, setback viscosity (the difference between the viscosities at the end of cooling and at the start of cooling); η_{rb},

512 retrogradation viscosity (the difference between viscosity at the end of cooling and final viscosity).

513 ^{a,b,c} Values within column with different superscript letters are significantly different ($p < 0.05$).

514 $^{\#}$ Values of the ratio was related to the short-range orders of starch gel systems prepared from rice starch, SGA-1, SGA-2 and SGA-3.

515 RSG-1, RSG-2, and RSG-3 indicate the rice starch gel (RSG) complexed with 4.54, 11.24 and 13.21 mg of GA per gram of starch,

516 respectively.

517

518

519 **Figure captions**

520 **Fig. 1.** Typical digestion curves and fit curves for native rice and GA-treated-starch gels. RSG-1,
521 RSG-2, and RSG-3 indicate the RSG complexed with 4.54, 11.24 and 13.21 mg of GA per gram of
522 starch, respectively.

523 **Fig. 2.** Fluorescence emission spectra of GA in the presence of increasing concentrations (0-1.5
524 mg/mL) of cooked rice starch.

525 **Fig. 3.** Pasting profiles of native and GA complexed rice starch. SGA-1, SGA-2, and SGA-3 indicate
526 the starch granules complexed with 4.54, 11.24 and 13.21 mg of GA per gram of starch, respectively.

527 **Fig. 4.** Storage modulus (G') and loss modulus (G'') of native rice and GA-treated-starch gels as a
528 function of oscillation frequency. RSG-1, RSG-2, and RSG-3 indicate the RSG complexed with 4.54,
529 11.24 and 13.21 mg of GA per gram of starch, respectively.

530 **Fig. 5.** $I \sim \log q$ SAXS patterns ($q < 1.5$ nm^{-1}) of native and GA-treated-rice starch gel. RSG-1,
531 RSG-2, and RSG-3 indicate the RSG complexed with 4.54, 11.24 and 13.21 mg of GA per gram of
532 starch, respectively.

533 **Fig. 6.** Structure factor ($S(q)$) plot of starch-GA gel systems. RSG-1, RSG-2, and RSG-3 indicate the
534 RSG complexed with 4.54, 11.24 and 13.21 mg of GA per gram of starch, respectively.

535 **Fig. 7.** *In vitro* GA release content at different time intervals during gel digestion. RSG-1, RSG-2,
536 and RSG-3 indicate the RSG complexed with 4.54, 11.24 and 13.21 mg of GA per gram of starch,
537 respectively.

538 **Fig. 8.** Schematic presentation for the starch-GA complexes during cooking and cooling.

539

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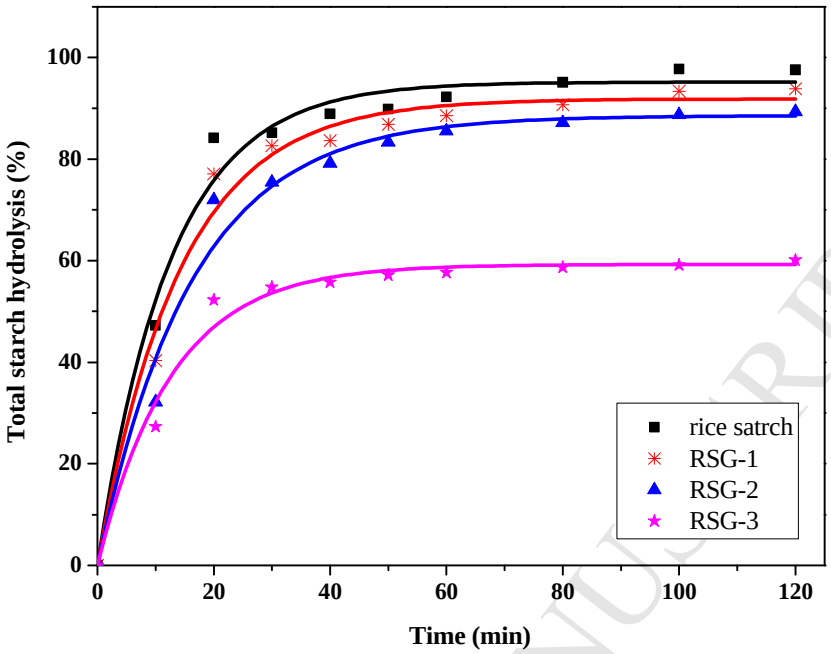

541

542 **Fig. 1**

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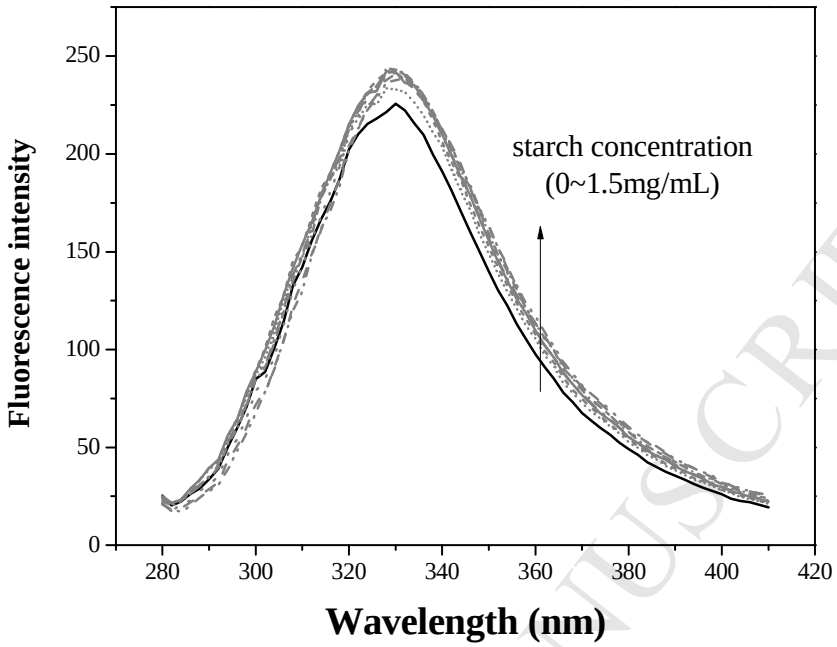

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546 **Fig. 2**

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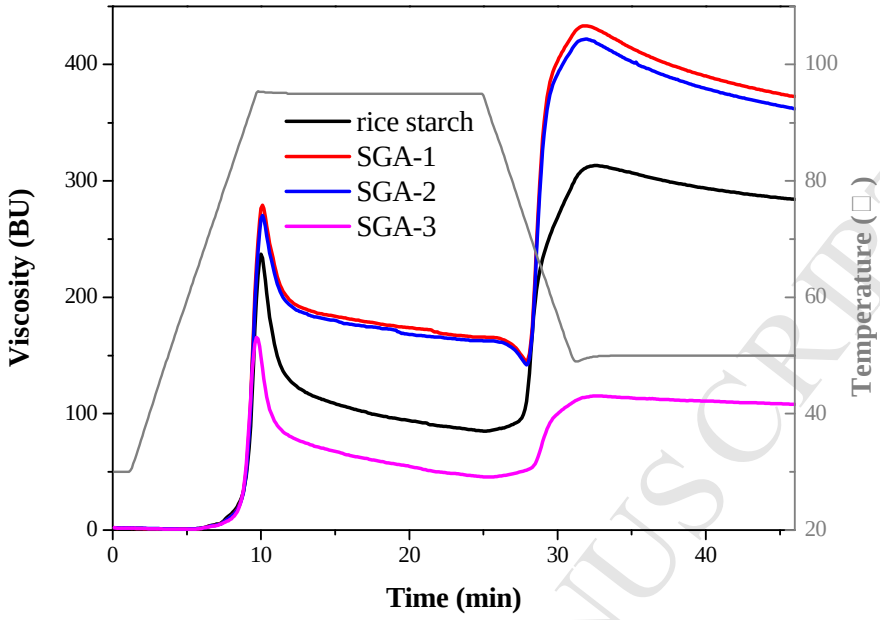

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550 **Fig. 3**

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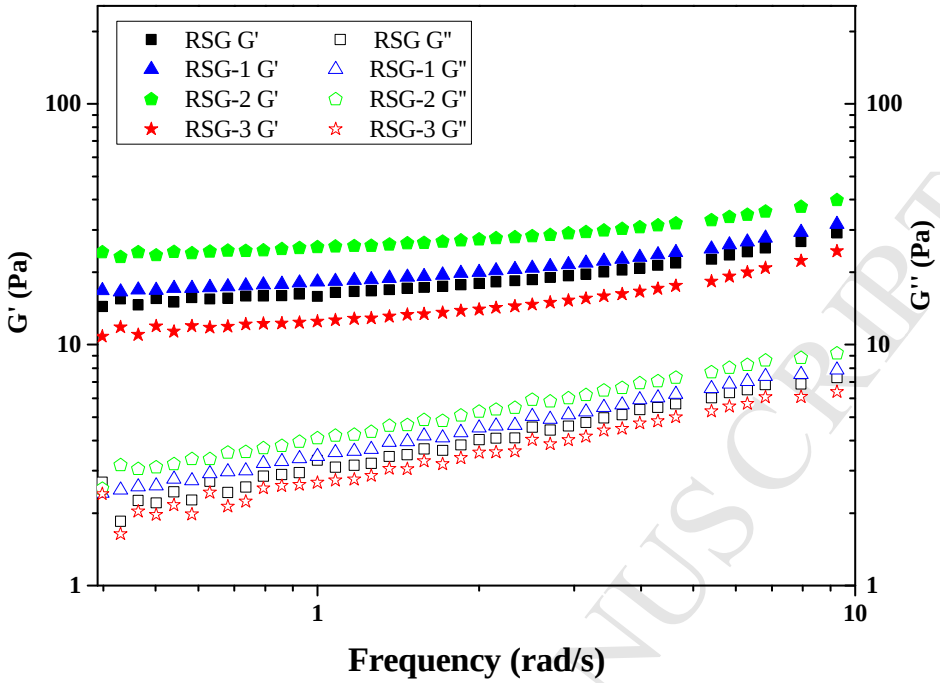

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554 **Fig. 4**

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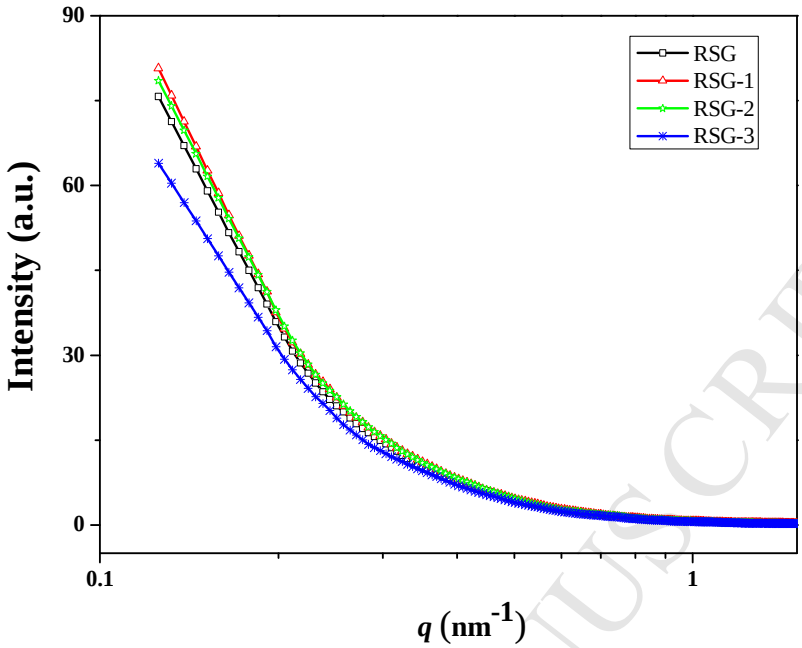

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558 **Fig. 5**

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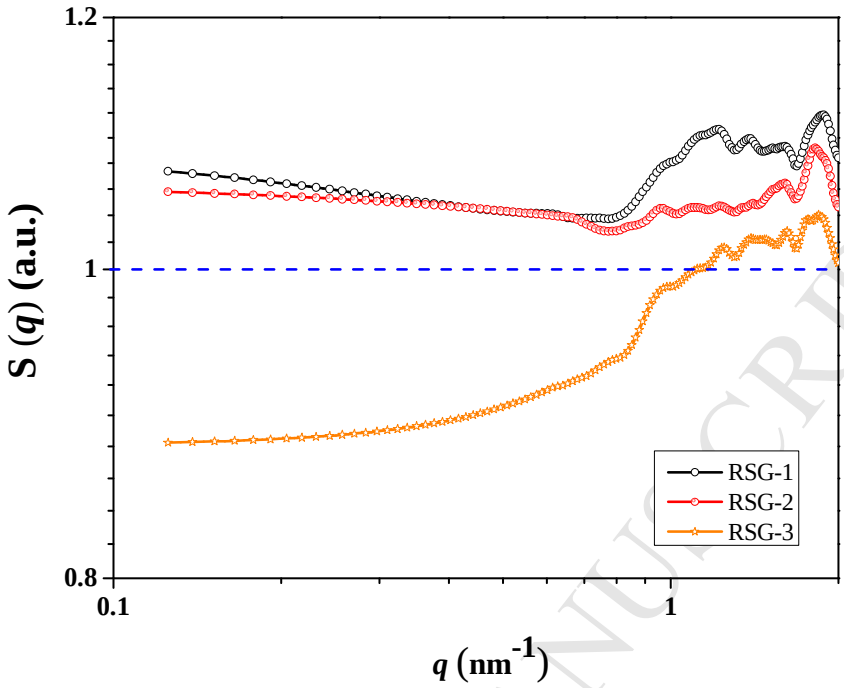

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562 **Fig. 6**

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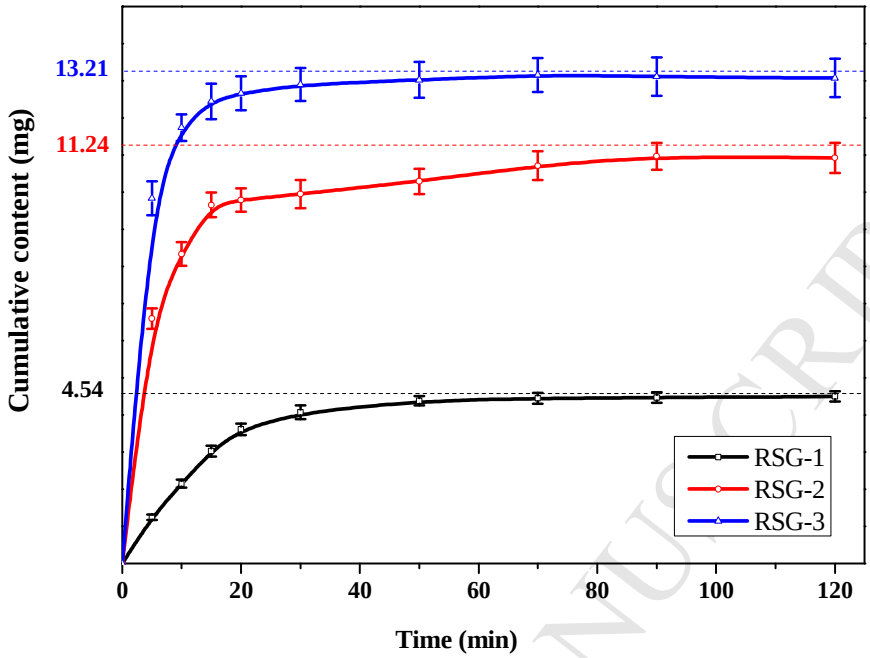

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566 **Fig. 7**

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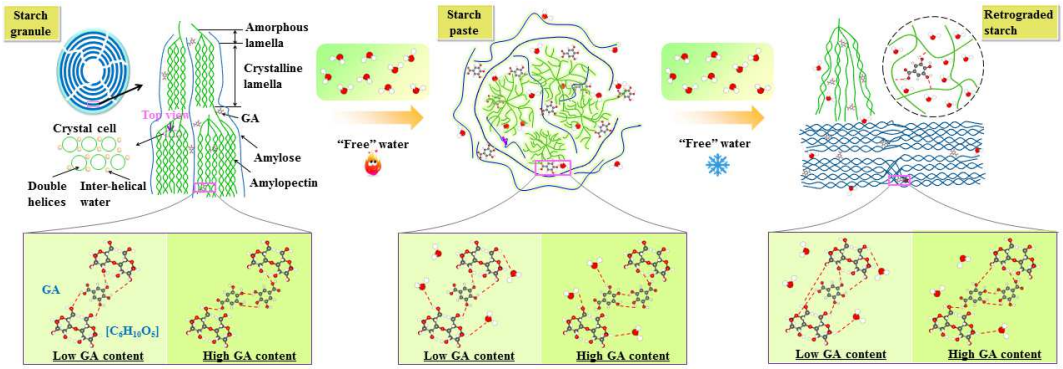

569

570 **Fig. 8**

571

- Digestibility of starch gels were decreased by complexation with gallic acid (GA).
- The non-covalent interactions between rice starch gels and GA were validated.
- Suitable amounts of GA assist starch assembly otherwise retard starch arrangement
- Starch digestion reduced by starch ordered structures and GA inhibition on enzymes.