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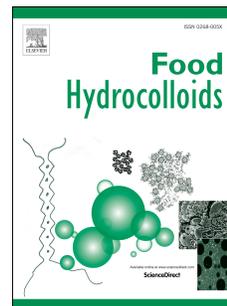
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Lamellar structure change of waxy corn starch during gelatinization by time-resolved synchrotron SAXS

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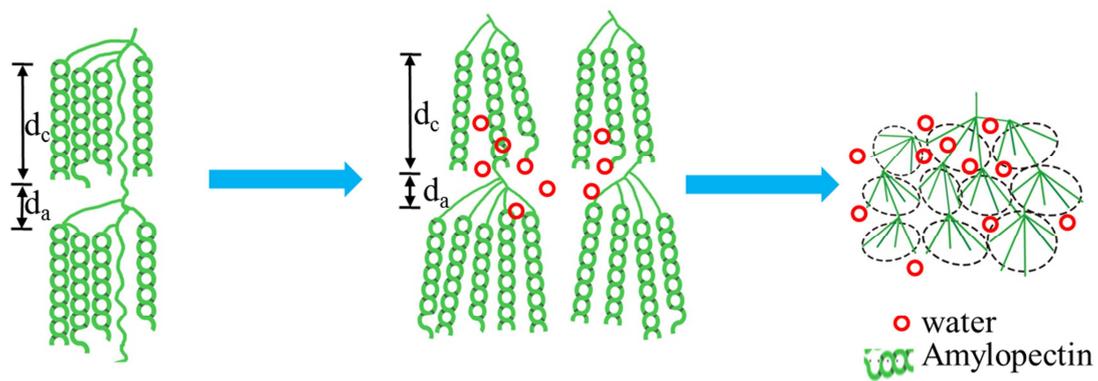
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ACCEPTED MANUSCRIPT

1 Lamellar Structure Change of Waxy Corn Starch during Gelatinization by
2 Time-resolved Synchrotron SAXS

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33 **Abstract:** *In situ* experiment of synchrotron small- and wide-angle X-ray scattering
34 (SAXS/WAXS) was used to study the lamellar structure change of starch during
35 gelatinization. Waxy corn starch was used as a model material to exclude the effect of
36 amylose. The thicknesses of crystalline (d_c), amorphous (d_a) regions of the lamella
37 and the long period distance (d_{ac}) were obtained based on a 1D linear correlation
38 function. The SAXS and WAXS results reveal the multi-stage of gelatinization. Firstly,
39 a preferable increase in the thickness of crystalline lamellae occurs because of the
40 water penetration into the crystalline region. Then, the thickness of amorphous
41 lamellae has a significant increase while that of crystalline lamellae decreases. Next,
42 the thickness of amorphous lamellae starts to decrease probably due to the
43 out-phasing of starch molecules from the lamellae. Finally, the thickness of
44 amorphous lamellae decreases rapidly, with the formation of fractal gel on a larger
45 scale (than that of the lamellae), which gradually decreases as the temperature further
46 increases and is related to the concentration of starch molecular chains. This work
47 system reveals the gelatinization mechanism of waxy corn starch and would be useful
48 in starch amorphous materials processing.

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51 *Keywords:* waxy starch, lamellar structure, gelatinization, SAXS, synchrotron

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54 1. Introduction

55 Starch is the main component of food and provides an essential energy for humans.
56 Recently, starch has attracted much attention as a renewable polymer resource for
57 eco-friendly uses due to its advantages of biodegradability and low costs (Yu, Dean, &
58 Li, 2006). Starch granules are always heated in water before used, and this results in
59 an order-disorder phase transition, termed “gelatinization”. Gelatinization is one of
60 the most significant processing methods in industry and food application of starch,
61 which determines the proper conversion of starch in the processing of food and
62 emerging biodegradable starch-based materials (Liu, et al., 2011).

63 The structure of native starch granules is a unit entirety and organized in different
64 length scales, *i.e.*, whole granule (μm), growth rings ($\sim 0.1 \mu\text{m}$), lamellar structure
65 (8-11 nm) and molecular scale ($\sim 0.1\text{nm}$) (Pérez & Bertoft, 2010; Tran, et al., 2011). It
66 is widely recognised that the native starch granule is composed of alternating
67 amorphous and semi-crystalline growth rings. The semi-crystalline growth ring
68 consists of the repeats of alternating amorphous and crystalline lamellae. The
69 amorphous lamellae are related to branch points of the amylopectin side chains, and
70 the crystalline lamellae are formed by the short-chain fractions of amylopectin
71 arranged as double helices and packed in small crystallites, respectively (Witt, Douth,
72 Gilbert, & Gilbert, 2012). Also, linear amylose molecules and probably less ordered
73 amylopectin are present in an amorphous state within each native granule (Fan, et al.,
74 2013; Pérez, et al., 2010)

75 In our previous papers (Chen, Yu, Kealy, Chen, & Li, 2007; Chen, et al., 2011),
76 the changes of granule and growth rings of starch during gelatinization have been
77 studied by light microscopy and confocal light scanning microscopy (CLSM).
78 However, there had been no non-destructive and efficient methods to observe the
79 lamellar structure of starch until the use of SAXS. SAXS measures the variations in
80 electron density distributions of amorphous and crystalline lamellae in granule starch
81 (Blazek & Gilbert, 2011). Although the lab SAXS is widely used in starch lamellar

82 structure characterization (Zhang, Chen, Zhao, & Li, 2013), the lab SAXS is still
83 rarely used for *in-situ* experiments due to its lower light brightness.

84 Compared with lab-bench SAXS instruments, synchrotron SAXS may offer much
85 higher spectral brilliance, small source size and high beam flux (Koch, 2006).
86 Therefore, synchrotron SAXS is very effective to study the *in-situ* (real time) lamellar
87 structure change during gelatinization. Vermeulen et al. (Vermeulen, et al., 2006a,
88 2006b) have studied the gelatinization behavior of rice starch and potato starch with
89 bound or limited water by *in-situ* SAXS experiments. It was found that the water
90 content plays a major role in gelatinization and the change of lamellar and crystalline
91 structures during gelatinization. Waigh et al. (Waigh, Gidley, Komanshek, & Donald,
92 2000) also studied the starch structure change during gelatinization by *in-situ* SAXS
93 and found two different processes for the A-type and B-type starches. However, the
94 SAXS analysis in their study did not investigate the changes in the amorphous and
95 crystalline layers. Yang et al. (Yang, et al., 2016) have used synchrotron SAXS
96 coupled with diamond anvil cell (DAC) to study the effect of high hydrostatic
97 pressure on starch gelatinization and used the correlation function to reveal the change
98 in thickness of the crystalline and amorphous layers during this process.

99 In this study, synchrotron SAXS and WAXS were used to *in-situ* study the
100 lamellar structure of waxy corn starches during gelatinization. Waxy starches were
101 selected as a model material since there is nearly no amylose starch in its lamellar
102 structure and waxy starch shows a clear peak corresponding to the lamellar phase. The
103 correlation function was used to analyze the *in-situ* synchrotron SAXS results of waxy
104 starch in excess water. Those studies would help to probe the changes in waxy starch
105 amorphous and crystalline layers during gelatinization.

106 **2. Material and Method**

107 **2.1 Sample and sample prepared**

108 Waxy corn starch with the amylose/amylopectin ratio of 0/100 was obtained
109 from Lihua Starch Industry Co., Ltd. (Qinhuangdao, China). The amylose content was

110 determined by the method of concanavalin A while the moisture content (MC) (about
111 10%) of each sample was determined using a moisture analyzer (MA35, Sartorius
112 Stedim Biotech GmbH, Germany). Distilled water was added to the starch to obtain a
113 starch: water ratio of 1:3 (w/v) in a glass vial and equilibrated 24 h for SAXS/WAXS
114 tests.

115 **2.2 Differential scanning calorimetry**

116 The gelatinization behavior of starch was determined by a differential scanning
117 calorimeter (200 F3, Netzsch, Germany) equipped with a thermal analysis data station.
118 Exactly 3 mg of starch were weighed into an aluminum sample pan. Distilled water
119 was added to the starch in the DSC pans with a pipette to obtain a starch: water ratio
120 of 1:3 (w/v) in the DSC pans. When water was added, care was taken to ensure that
121 the starch granules were completely immersed in the water by gentle shaking. The
122 pans were sealed, and the sealed pans were allowed to stand overnight at room
123 temperature before DSC analysis. An empty pan was used as a reference. The pans
124 were heated from 20 to 120 °C at a scanning rate of 10 °C/min. The analysis was
125 undertaken in triplicate. The software of Netzsch Proteus Thermal Analysis Version
126 6.1.0 was used to analyze the DSC traces.

127 **2.3 Small and wide Angle X-ray scattering (SAXS/WAXS)**

128 Synchrotron time-resolved small and wide-angle X-ray scattering (SAXS/WAXS)
129 measurements were carried out at BL16B1 beamline at Shanghai Synchrotron
130 Radiation Facility (SSRF), China. We loaded the suspension (0.70 mL) into
131 2-mm-thick sample cells, of which the front and back windows were both covered
132 with Kapton tape. Two-dimensional (2D) Mar165 were used to collect the 2D SAXS
133 and WAXS patterns. The wavelength of the incident X-ray was 1.24 Å for both SAXS
134 and WAXS, and the sample to detector distance (SDD) was 1940 mm for SAXS and
135 115 mm for WAXS measurements. A beef tendon specimen and Cerium oxide (CeO₂)
136 were used as standard materials for the calibration of the scattering vector of SAXS
137 and WAXS, respectively. The air and water scattering were subtracted from the

138 original SAXS and WAXS data. A Linkman (STC200) hot stage was used to control
 139 sample temperatures, which was calibrated by a temperature calibrator (Fluker 724)
 140 with a K type thermocouple (Omega) before use. The temperature rose from 35 to
 141 85 °C, at a speed of 2 °C/min, with a holding time of 1 min. Data were collected at
 142 each degree rise and were measured for 60 seconds. 2D SAXS and WAXS patterns
 143 were recorded by a Mar165 charge-coupled device (CCD) detector. By measuring
 144 sample adsorption using ionization chambers in front and back of the sample cell, we
 145 performed data correction, calibrated the SAXS data from the background scattering,
 146 and normalized the data on the primary beam intensity. Background subtraction
 147 follows the equation: $I_s(\theta) = I_t(\theta) - \frac{I_t}{I_b} \frac{T_t}{T_b} I_b(\theta)$. $I_t(\theta)$, $I_b(\theta)$ and $I_s(\theta)$ represent the
 148 distribution of scattering intensity of samples held in cells, sample cells and pure
 149 samples respectively. I_t and I_b represent the values of samples held in cells and sample
 150 cells, read from the ionization chambers in front of sample cell. T_t and T_b represent the
 151 transmissivity of samples held in cells and sample cells.

152 2.4 SAXS analysis

153 The normalized 1D correlation function $\gamma_1(r)$ is defined as

$$154 \quad \gamma_1(r) = \int_0^{\infty} I(q)q^2 \cos(qr) dq / Q$$

155 where $I(q)$ is scattering intensity, q is scattering vector defined as $q = 4\pi \sin\theta / \lambda$ (2θ
 156 is the scattering angle) and r is the direction along the lamellar stack.

157 The scattering invariant, Q , is defined as

$$158 \quad Q = \int_0^{\infty} I(q)q^2 dq$$

159 Because of the finite q range of experimental SAXS data, extrapolation of the 1D
 160 SAXS data to both the low and high q ranges are necessary for the integration of the
 161 intensity, $I(q)$. Extrapolation to low q was performed using an intensity profile based
 162 on Guinier's law, and the extension of the intensity to large q values can be
 163 accomplished using the Porod-Ru land model (Yang, Liang, & Han, 2015; Yang,
 164 Liang, Luo, Zhao, & Han, 2012). The parasitic scattering and thermal fluctuation were
 165 corrected using a normalized 1D correlation function.

166 **3. Result and Discussion**

167 **3.1 Thermal behavior by DSC**

168 DSC is a quick and efficient method to test gelatinization behavior of starch. Corn
169 starch with different amylose content has been studied before using DSC (Chen, et al.,
170 2007). From Fig.1, it can be seen that the waxy starch exhibited a significant
171 gelatinization endotherm at about 71°C, which has been well accepted as the
172 representation of the gelatinization of amylopectin. The onset, peak and end
173 temperature of waxy starch used in this experiment are 60, 71 and 83°C, respectively.
174 According to previous studies (Liu, Yu, Xie, & Chen, 2006; Liu, et al., 2013), DSC
175 with a stainless steel pan could be used to study the phase transition during
176 gelatinization.

177 **3.2 SAXS curves analysis**

178 The SAXS one-dimensional (1D) scattering intensity distributions for waxy
179 starch at different temperatures are shown in Fig. 2A and Fig. 2B. It is clearly seen
180 that there is one typical scattering peak around the q value of $0.6-0.7\text{nm}^{-1}$ in each
181 SAXS curves below the temperature of 70.7°C, indicating a 9-10 nm semi-crystalline
182 structure according to Bragg's law $D=2\pi/q$. No typical scattering peak was observed
183 above 72.9°C. In fact, the scattering peak indicated a long period (also known the
184 lamellar repeat distance, or Bragg spacing) in granule starches (Blazek, et al., 2011).
185 The position of SAXS peak is reciprocally related to the average total thickness of the
186 crystalline and amorphous regions in lamellar arrangements (Waigh, Perry, Riekell,
187 Gidley, & Donald, 1998). Moreover, the intensity depends on the amount of the
188 ordered semi-crystalline structures and/or on the differences in electron density
189 between crystalline and amorphous lamellae on the amorphous background (Yuryev,
190 et al., 2004).

191 From Fig.2A and Fig.2B, it could be clearly seen that the peak intensity
192 decreased with increasing temperature. This reduction means that the destruction to
193 crystalline lamellae may lead to a reduction in the electron density contrast between
194 crystalline and amorphous lamellae. However, there are several rises in peak intensity,

195 as the temperature went from 61.8°C to 66.3°C. Meanwhile, the peak becomes
196 broadening with increasing temperature. In fact, the peak width depends on the
197 regularity of the lamellar arrangements within the starch granule (Blazek, et al., 2011;
198 Yang, et al., 2016).

199 To further analyze the SAXS curves, Lorentz correction was used, and the
200 selected temperature could be found in Fig.3. The peak intensity shows similar trends
201 as in Fig.2. Scattering invariant (Q) is proportional to the electron density difference
202 between the crystalline and amorphous phases, and the volume fractions of the two
203 phases are based on a two-phase model. From Fig.2, it could be seen that Q increased
204 firstly and then decreased. Correspondingly, the contrast of electron density was
205 firstly enhanced, which should be due to the water uptake and swelling in the
206 amorphous parts and/or the leaching of amylose from the amorphous parts. The
207 decrease in peak intensity suggests a gradually decreasing electron density contrast
208 between amorphous and crystalline lamella. However, a slight increase of the peak
209 between 61.8 °C and 66.3 °C is observed, which is a new phenomenon and needs to
210 be studied in the future.

211 3.3 SAXS analysis with correlation function

212 The 1D correlation function can provide the structure parameters of lamellar
213 structures of polymers (Chen, et al., 2016; Yang, et al., 2012). Recently, the
214 correlation function is widely used in the analysis of starch aggregation structure and
215 provides basic structure parameters such as the thickness of crystalline (d_c),
216 amorphous (d_a) region of the lamella and long period distance ($d_{ac} = d_a + d_c$) (Chen, et
217 al., 2016; Fan, et al., 2013; Yang, et al., 2016). In this method, the long period
218 distance (d_{ac}) is the value of x at the second maximum of $\gamma_1(x)$, d_a is representing the
219 solution of linear regression in the auto correlation triangle (LRAT) at $y =$ value of the
220 flat minimum of $\gamma(x)$. Hence, the average thickness of the crystalline lamellae d_c ,
221 equals ($d_{ac} - d_a$) (Goderis, Reynaers, Koch, & Mathot, 1999).

222 The normalized 1D correlation function can be seen in Fig. 4, where we
223 assigned the larger layer thickness to the amorphous and crystalline thickness. Fig.5

224 shows the temperature function of d_c , d_a , and d_{ac} and the Bragg lamellar repeat
225 distance, D ($D=2\pi/q$). From Fig.5, it could be seen that the long period distance (d_{ac})
226 from the correlation function have a proper fitting with D from Bragg's law. A
227 significant decrease in D , d_{ac} and d_c , as well as an increase in d_a above 70 °C, could be
228 clearly observed. However, when the temperature is below 70 °C, D and d_{ac} remain
229 almost identical at approx. 8.5 nm. It is noteworthy that d_c rises slightly with the
230 temperature increasing from 50 to 55 °C, before it decreases to around 6.4 nm. A
231 slight increase in d_c could be seen along with the temperature rising from 65 to 70 °C.
232 An opposite trend is observed in d_a , which is as expected since d_{ac} did not change
233 greatly. The side-chain model (Waigh, et al., 2000) could explain the increase in the
234 thickness of crystalline lamellae, implying that the amylopectin branching points
235 could be compressed by the double helices of the amylopectin side chains because of
236 the plasticization of the spacers.

237 3.4 WAXS analysis

238 The time-resolved wide-angle X-ray diffractogram is shown in Fig.6. A typical
239 A-type crystalline structure can be observed with peaks near 15°, 17°, 18° and 23°.
240 WAXS is always used to study the longer range scale structure of starch crystallites,
241 which mainly consists of monoclinic and/or hexagonal crystal units (Zhang, et al.,
242 2015). From Fig.6, the peak intensity gradually becomes weaker as the temperature
243 increases, indicating the reduction in crystallinity and increase of amorphous zones.
244 Peaks are almost invisible at 72.7 °C, showing the absence of crystalline structure.
245 This is consistent with results from SAXS and DSC, which results from
246 gelatinization.

247 3.5 Starch gel structure analysis

248 The fractal dimension indicates the compactness of a system (Beaucage, 1996)
249 and has been used to describe the self-similar structure of gel structure (Tamon &
250 Ishizaka, 1998). In the low- q region the curves comply with a simple power law
251 equation (Zhu, Li, Chen, & Li, 2012), $I(q) \sim q^{-\alpha}$, where the exponent α gives insight
252 into the surface/mass fractal structure. Moreover, the mass fractal dimension

253 ($0 < \alpha < 3$) is used to indicate the compactness, whereas the surface fractal dimension
254 ($3 < \alpha < 4$) is regarded as an indicator of the degree of smoothness of the scattering
255 objects.

256 After gelatinization, starch becomes a gel. From Fig.7, it could be seen that the
257 exponent α decreased from 2.70 to 1.21 (within the q of 0.1 to 0.2 nm^{-1} , the
258 corresponding size of 31.4 nm to 62.8 nm) with the increasing temperature from 75
259 to 85°C, which means the starch gel is a mass fractal structure. Moreover, α till
260 decreases when the temperature keeps at 85 °C for 1 min. These results suggest that
261 the scattering objects of gelatinized waxy starch were more compact with the
262 increasing temperature. Since the SAXS measurements were performed *in-situ*, the
263 measured change in the starch gel fractal structure could depend on the concentration
264 of amylopectin, and a high concentration may lead to a mass fraction. This
265 phenomenon will be studied by rheology and *in-situ* SAXS in future.

266 3.6 Gelatinization mechanism from SAXS/WAXS

267 Generally, the well-accepted conception of “gelatinization” means destroying the
268 crystalline structure in the starch granule (Liao, et al., 2014; Xie, et al., 2006), which
269 is an irreversible multi-stage process including granule swelling, native crystalline
270 melting, loss of birefringence and starch solubilization (Sullivan & Johnson, 1964).
271 SAXS and WAXS would provide the information of lamellar and crystallinity
272 structure change during gelatinization.

273 Starch suspensions with a higher concentration were used in this study as a model
274 system to reveal the gelatinization mechanism. The simply lamellar structure changes
275 during heating for waxy starch could be found in Fig.8. The results of d_a , d_c and d_{ac}
276 from SAXS by a correlation function would further clarify the changes of the starch
277 lamellar structure. (A) First, water is slowly and reversibly taken up in the
278 crystallinity lamellar with the increasing temperature although the water is already
279 equilibrated in starch (Chen, et al., 2007; Liu, et al., 2011). At this stage, the size of
280 amorphous lamellar does not change, but the size of crystallinity lamellar has a slight
281 increase. (B) From 55 to 60 °C (T_o , onset temperature), the size of amorphous
282 lamellar has a modest increase. This phenomenon is also observed in the

283 gelatinization caused by ultra-high hydrostatic pressure (Yang, et al., 2016). However,
284 the reduction of the size of crystallinity lamellar is unexpected. (C) After the onset
285 temperature, the amorphous lamellar starched to decrease probably due to the
286 out-phasing of starch molecules from them (Zhang, et al., 2015). Meanwhile, the
287 SAXS intensity has a clear increase. (D) From T_p , the decrease of d_c could attribute to
288 the disrupted crystalline layer, and all amylopectin double helices are dissociated to
289 form a gel.

290 4. Conclusion

291 Gelatinization is essential for industry and food application of starch. The present
292 study investigated the lamellar structure of starch during gelatinization. *In situ*
293 synchrotron SAXS and WAXS are used in this work. Waxy starches were selected as
294 model materials since there is no amylose starch in its lamellar structure and waxy
295 starch shows a clear peak corresponding to the lamellar phase. The correlation
296 function was used to analyze the in situ synchrotron SAXS results of waxy starch in
297 excess water.

298 During gelatinization, WAXS intensity decreases gradually with the increasing
299 temperature, and the temperature for the disappearance of the WAXS peak is
300 consistent to that of the DSC and SAXS lamellar peaks. The thickness of crystalline
301 (d_c), amorphous (d_a) regions of the lamellae and the long-period distance (d_{ac}) were
302 obtained from a 1D linear correlation function. The average thicknesses of amorphous
303 layers and crystalline layers show different change trends with the increasing
304 temperature.

305 Overall, the multiple stages of gelatinization could be concluded: firstly, a
306 preferable increase in the thickness of the crystalline lamellae because of the water
307 penetration into crystalline regions; then, the thickness of amorphous lamellae has a
308 significant increase while that of crystalline lamellae decreases; next, the amorphous
309 lamellae start to decrease probably due to the out-phasing of starch molecules from
310 them; at last, the thickness of amorphous lamellae decreases rapidly with the
311 formation of fractal gel on a larger scale (than that of the lamellae) which gradually

312 decreases as the temperature increases further and is related to concentration of starch
313 molecular chains. This work reveals the gelatinization mechanism of waxy corn starch
314 and would be useful in starch amorphous materials processing.

315

316 Conflict of interest

317 The authors declare that there is no conflict of interests regarding the publication of
318 this paper.

319

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422 **Figures**

423 Fig.1 DSC thermograms of native waxy cornstarch in abundant water.

424 Fig.2 *In-situ* synchrotron SAXS patterns of waxy corn starch at different temperatures

425 (A) 40-61.8°C; (B) 61.8-84°C.

426 Fig.3 Lorentz-corrected 1D SAXS profiles of waxy corn starch under selected

427 temperature

428 Fig.4 Normalized 1D correlation function of waxy corn starch

429 Fig.5 Changes in Bragg lamellar repeat distance (D), long period (d_{ac}), thickness of

430 amorphous layer (d_a) and thickness of crystalline layer (d_c) as a function of

431 temperature for waxy corn starch

432 Fig.6 *In-situ* synchrotron WAXS patterns of waxy corn starch at different

433 temperatures

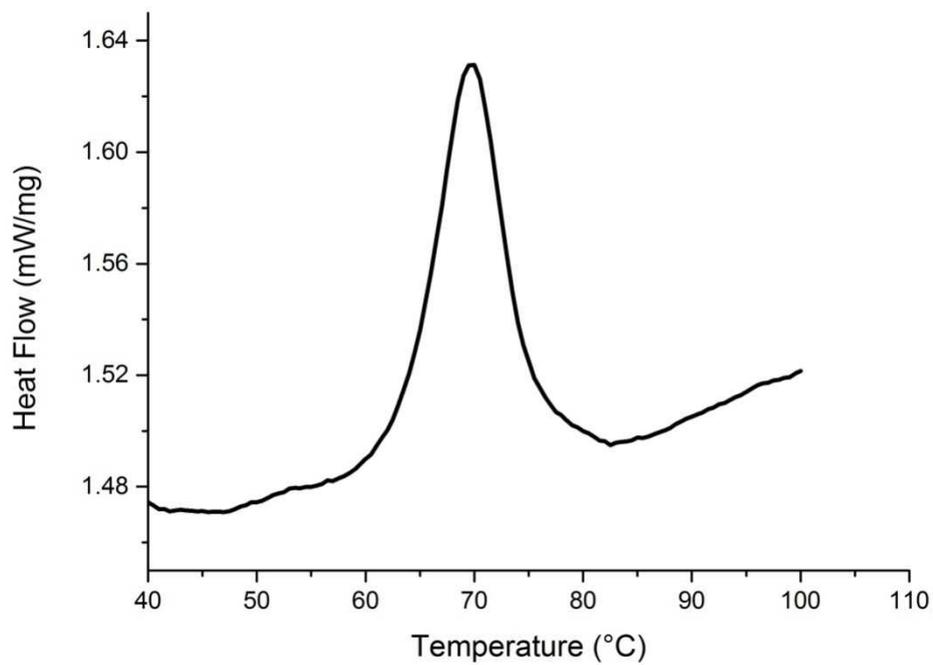
434 Fig.7 SAXS patterns (log-log) of waxy corn starches. The black scattering dot lines

435 show the relationship $I \sim q^\alpha$ at selected temperature (the last 85 °C means this

436 temperature was kept for 1 min.)

437 Fig. 8 Schematic representation of the changes of waxy starch lamellar structure

438 during heating.



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Fig.1 DSC thermograms of native waxy cornstarch in abundant water.

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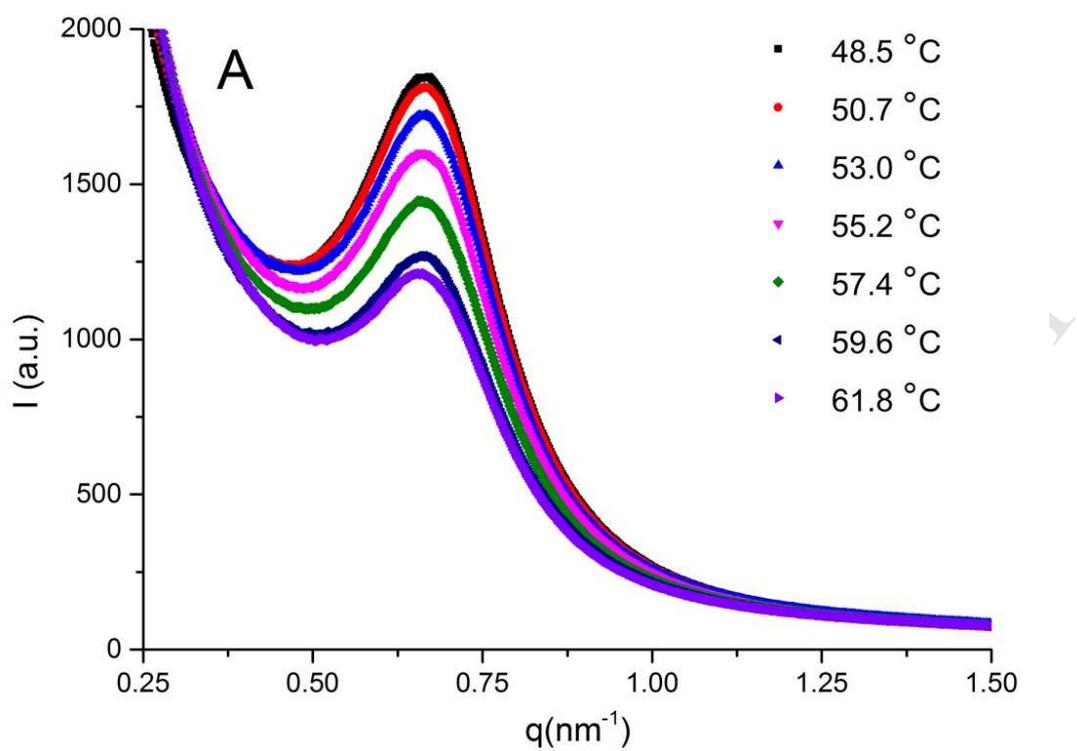
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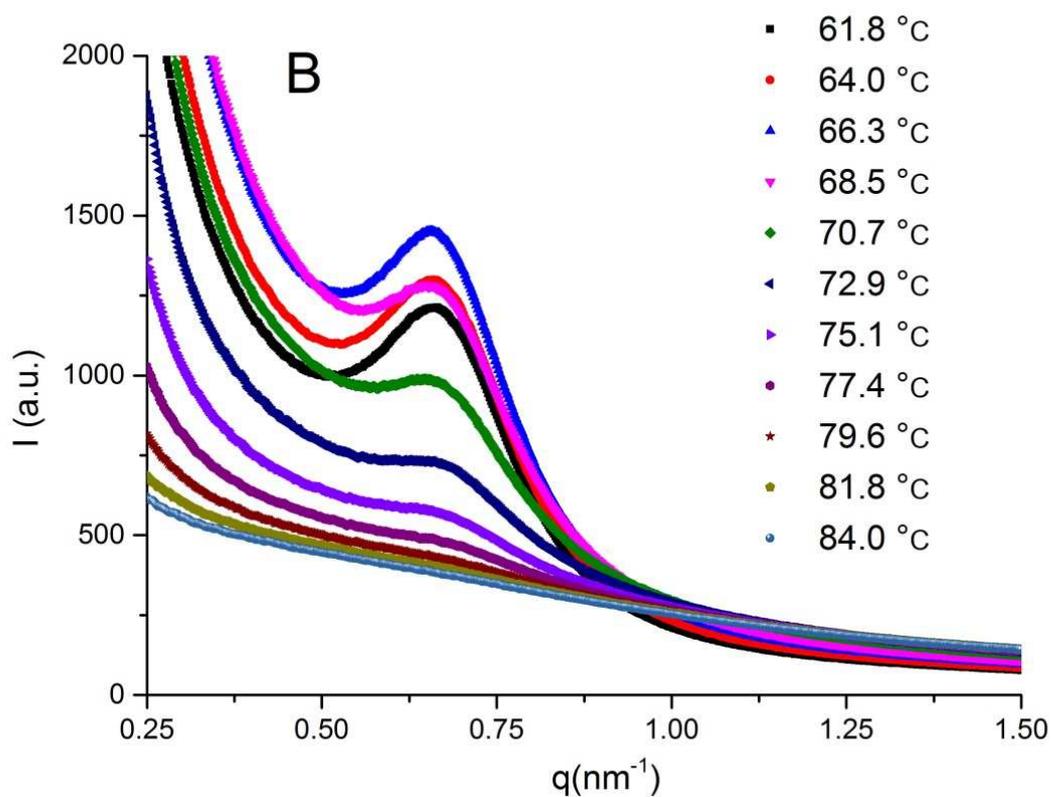
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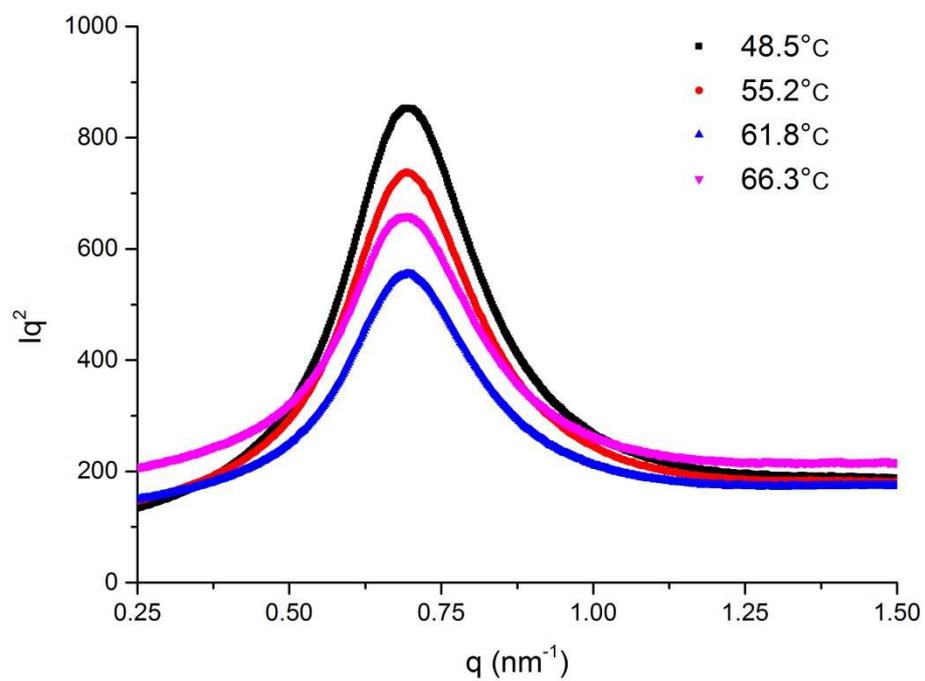


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(A) 40-61.8°C; (B) 61.8-84°C.



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16 Fig.3 Lorentz-corrected 1D SAXS profiles of waxy corn starch under selected
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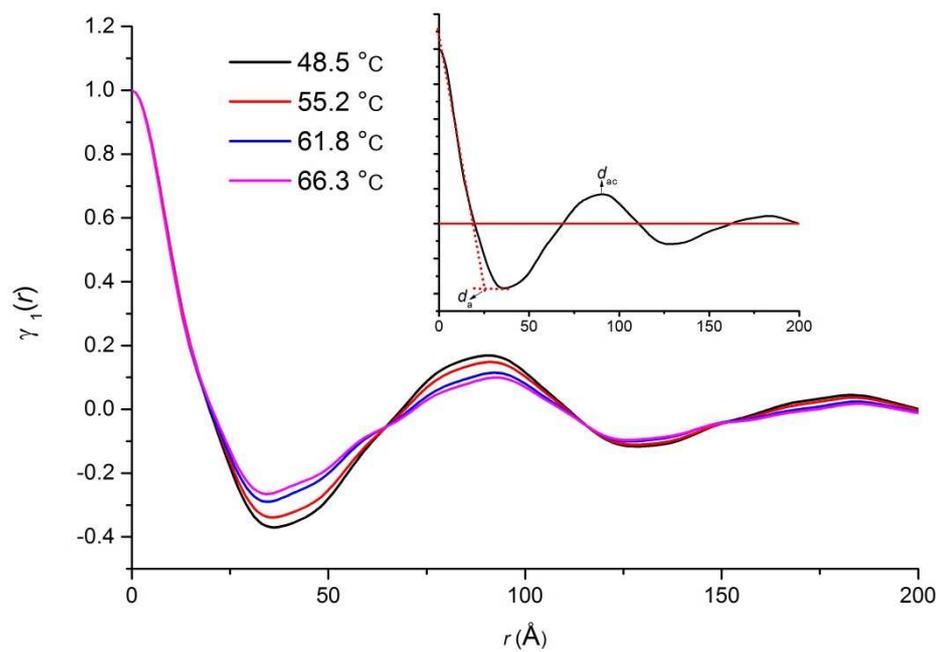
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Fig.4 Normalized 1D correlation function of waxy corn starch

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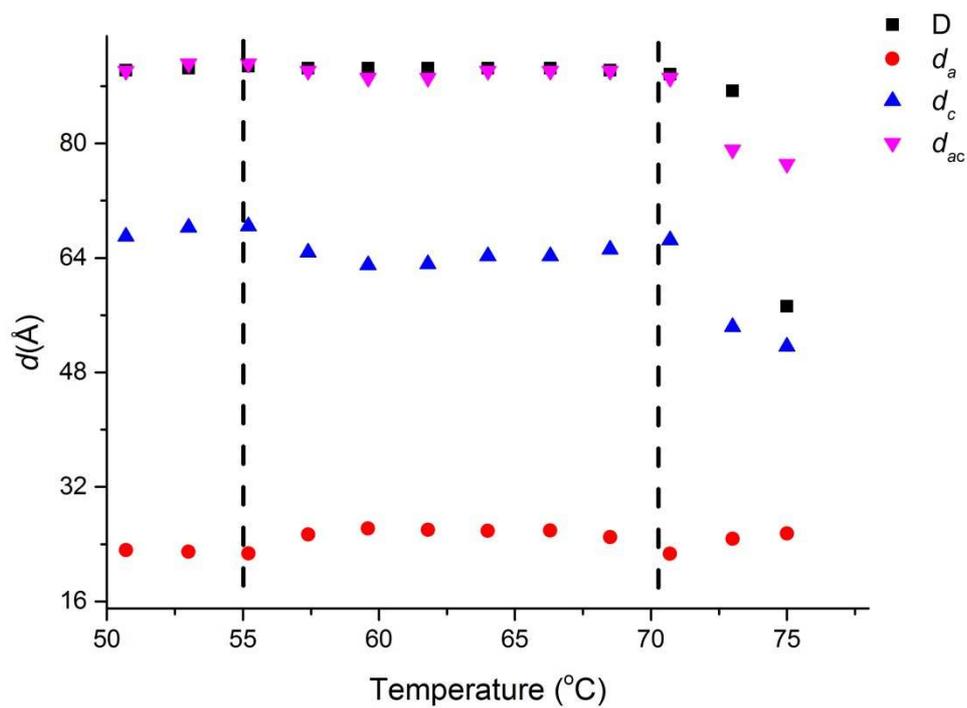
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41 Fig.5 Changes in Bragg lamellar repeat distance (D), long period (d_{ac}), thickness of
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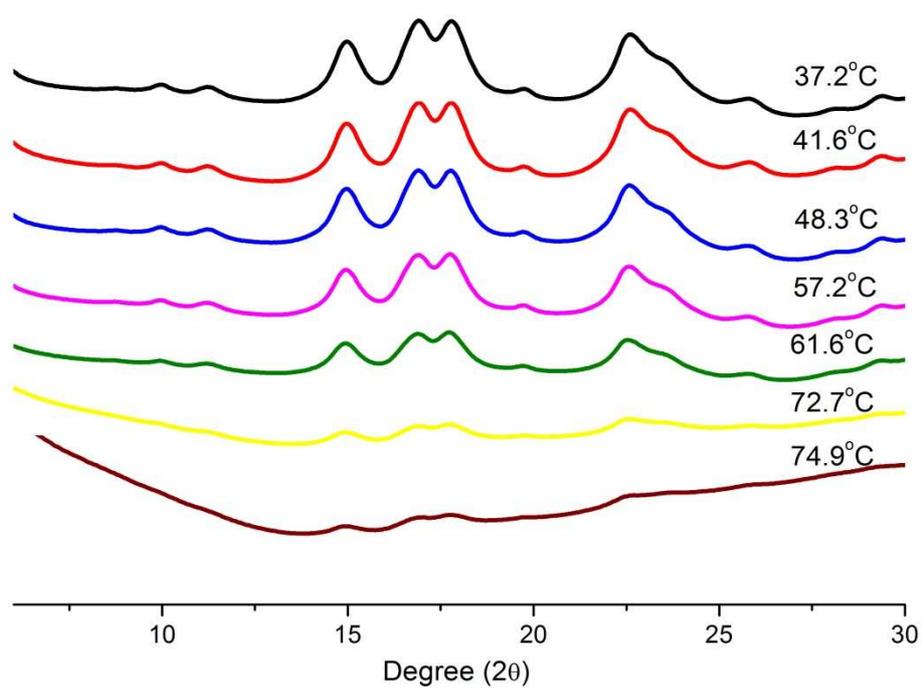
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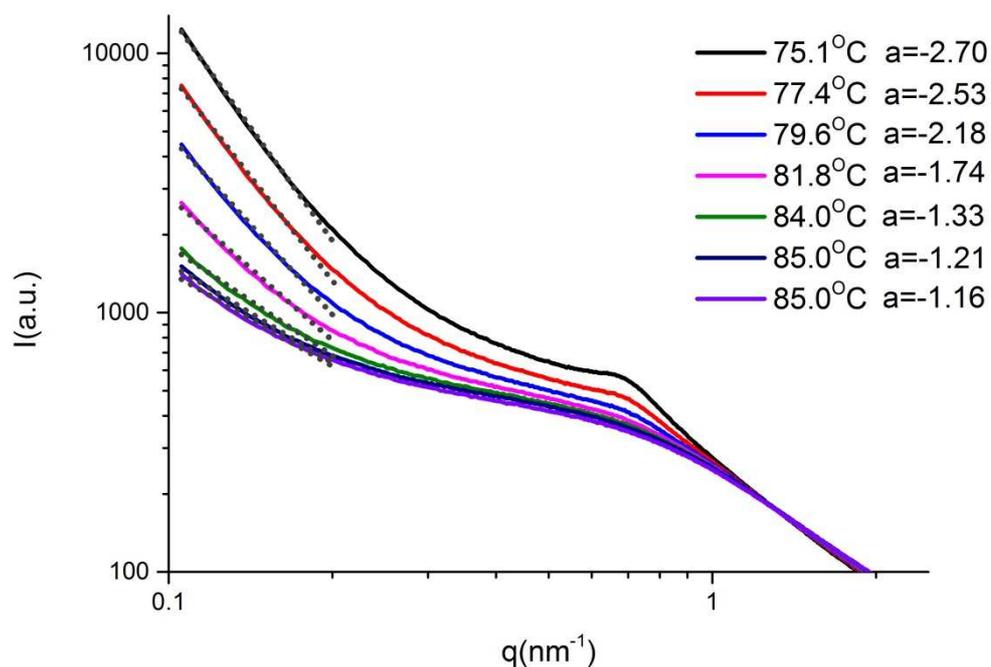
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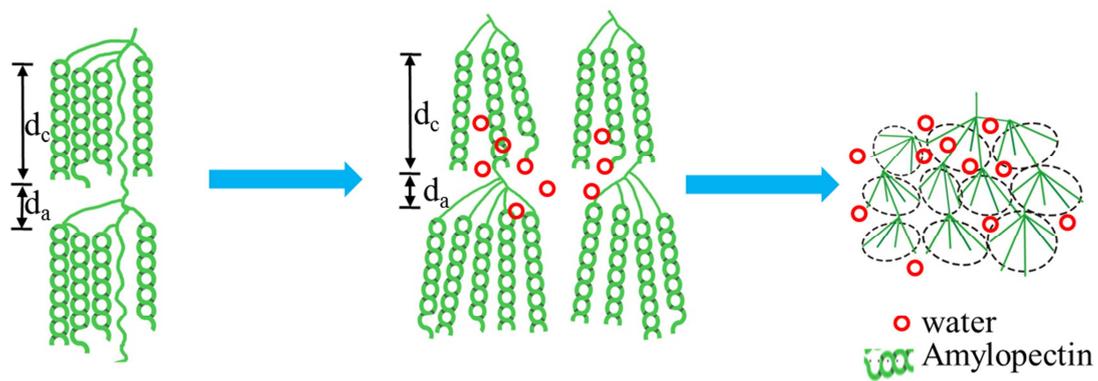
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81 Fig. 8 Schematic representation for waxy starch lamellar structure changes during
82 heating.

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Highlights

1. In situ SAXS/WAXS is used to study the lamellar structure change during gelatinization for waxy corn starch.
2. The multi-stage of gelatinization of starch is observed
3. The lamellar structure change for starch is a function of temperature.
4. Starch gel shows a mass fractal structure.