Carbohydrate Polymers 114 (2014) 357–364

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effect of a small amount of sodium carbonate on konjac glucomannan-induced changes in thermal behavior of wheat starch

Yun Zhou [a], Charles G. Winkworth-Smith [b], Yu Wang [a], Jianfen Liang [a], Tim J. Foster [b], Yongqiang Cheng [a,*]

[a] Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China
[b] Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 5 August 2014
Accepted 6 August 2014
Available online 23 August 2014

Keywords:
Konjac glucomannan
Na_2CO_3
Wheat starch
Thermal behavior
CLSM

ABSTRACT

The effects of konjac glucomannan (KGM) on thermal behavior of wheat starch have been studied in the presence of low concentrations of Na_2CO_3 (0.1–0.2 wt% of starch). Confocal laser scanning microscopy (CLSM) allows the visualization of the starch gelatinization process and granule remnants in starch pastes. Heating the starch dispersion in KGM–Na_2CO_3 solution significantly delays granule swelling and inhibits amylose leaching, whereas Na_2CO_3 alone, at the same concentration, has little effect. Na_2CO_3 assists KGM in producing the extremely high viscosity of starch paste, attributing to a less remarkable breakdown of viscosity in subsequent heating, and protecting starch granules against crystallite melting. The distinct partially networked film around the surface of starch granules is evident in the CLSM images. We propose that Na_2CO_3 could trigger the formation of complexes between KGM and starch polymers, which exerts a protective effect on granular structure and modifying gelatinization characteristics of the mixtures.

1. Introduction

Konjac glucomannan (KGM) is a neutral polysaccharide obtained from the tuber of *Amorphohallus* konjac C. Koch (Maeda, Shimahara, & Sugiyama, 1980). KGM is composed of β-1,4-linked glucose and mannose residues in the approximate ratio of 2:1 as the main chain with a small number of branches through β-1,3-mannosyl units (Katsuraya et al., 2003). The glucomannan backbone possesses between 5% and 10% acetyl substituted residues (Williams et al., 2000). Compared with other commercial gums, KGM forms an extremely high-viscosity solution with a pronounced shear thinning characteristic (Wang et al., 2012). KGM has several health benefits, such as modification of intestinal microbial metabolism and cholesterol reduction, making it quite popular in the development of functional foods (Chua, Baldwin, Hocking, & Chan, 2010; Sim, Noor Aziah, & Cheng, 2011).

Addition of KGM to starch is known to modify and control the pasting and rheological properties of starch (Charoenrein, Tatirat, Rengsutthi, & Thongngam, 2011; Xu et al., 2012; Yoshimura, Takaya, & Nishinari, 1996). With respect to KGM–starch interactions, a preliminary study showed that KGM might promote thermodynamic incompatibility (Funami et al., 2005). The behavior of KGM was similar to a physical barrier to prevent amylopectin chain association (Khanna & Tester, 2006).

Starch and hydrocolloids usually coexist with other ingredients in multi-component systems of foods, among which one such ingredient is alkali. It is known that alkali can dramatically alter the pasting properties of starch dispersions (Karim et al., 2008; Lai, Karim, Norziah, & Seow, 2004). Not only does pH affect the rheological behaviors of ionic hydrocolloids, acidic pH values, even those found in food products, will effect hydrolysis of starch, resulting in substantial losses in viscosity, and of the hydrocolloid, resulting in both average molecular weight decreases and structural changes (BeMiller, 2011). However, the concentration of alkali is critical in determining its effectiveness. Among the very few reports in the literature concerning the multiple-component system based on starch–hydrocolloid combinations, ionic-polysaccharides continue to be the subject of extensive research. It was suggested that the pasting temperatures of a mixed system of hsian-tsao leaf gum and starch shifted progressively to higher temperature with increasing concentrations of added salts (Lai & Chao, 2000). Wheat starch and hsian-tsao leaf gum formed junction zones whose formation was facilitated by the presence of appropriate amount of salts (Chao & Lai, 1999). The effects of xanthan addition on the

* Corresponding author. Tel.: +86 10 62737424; fax: +86 10 62737424.
E-mail address: chengyq@cau.edu.cn (Y. Cheng).

http://dx.doi.org/10.1016/j.carbpol.2014.08.033
0144-8617/

pasting and rheological properties of rice starch were more pronounced with salts added (Viturawong, Achayuthakan, & Suphantharika, 2008). It is reasonable to expect changes in thermal properties of starch as a consequence in a way related to alkali–non-ionic hydrocolloid or alkali–starch interaction.There are still gaps in our knowledge of the basic phenomena associated with heating (that is, pasting and gelatinization) in the presence of both non-ionic hydrocolloid and alkali. Our previous work demonstrates that Na_2CO_3 affects diffusion-controlled chain aggregation of KGM–starch systems, but does not significantly affect conformational ordering of amylose and amylopectin evident in short-term and long-term retrogradation (Zhou, Zhao, Winkworth-Smith, et al., 2014). In the present work, we has explored the role and potential usefulness of Na_2CO_3 in controlling rheology of starch–KGM systems by preparing mixtures of starch with KGM–Na_2CO_3 solution and characterizing their thermal behavior by differential scanning calorimeter (DSC), rapid viscosity analyzer (RVA), and laser diffraction particle size analyzer. The microstructure of starch granules during heating was observed by confocal laser scanning microscopy (CLSM) using direct staining. To investigate the action modes of KGM–Na_2CO_3 interaction upon heating starch granules, all the experiments were designed at cross levels of both KGM and Na_2CO_3.

2. Materials and methods

2.1. Materials

Wheat starch and fluorescein 5-isothiocyanate (FITC) was purchased from Sigma Chemical Co. (St. Louis, USA). Moisture and protein contents of the wheat starch were 11.5 g/100 g and ≤0.3 g/100 g, respectively. Amylose and amylopectin were 20.2 g/100 g and 75.1 g/100 g (dry basis), which were determined using the spectrophotometric method described by Jarvis and Walker (1993). Konjac flour HXZC was kindly provided by the Huaxianzi Konjac Product Co. (Hubei, China) and purified by alcohol precipitation. The contents of moisture, ash, crude protein, fat, glucomannan were 3.7 g/100 g, 0.8 g/100 g, 0.6 g/100 g, 0.1 g/100 g and 97.8 g/100 g (dry basis, w/w), respectively. Analytical grade of Na_2CO_3 (Beijing Chemical Reagent Co., Beijing, China) was used in all the experiments.

2.2. Preparation of aqueous starch–KGM–Na_2CO_3 mixtures

To create starch–KGM–Na_2CO_3 mixtures, Na_2CO_3 solution was first diluted with distilled water from 0.1 g/ml Na_2CO_3 stock solution; then the required amount of purified konjac powder was slowly dissolved in Na_2CO_3 solution under magnetic stirring (Xinyijia Equipments, Jintan, China), heated to 80 °C for 5 min and cooled to room temperature; last, starch was dispersed in the KGM–Na_2CO_3 solutions with an overhead stirrer. RVA, DSC and particle size distribution studies were carried out after the mixtures were stirred at room temperature at 100 rpm for 5 min to achieve sufficient consistency. Starch dispersions were prepared at three concentrations, that is, 4% (w/w) for the median diameter and leached amylose determinations and CLSM, 8% (w/w) for the RVA, and 20% (w/w) for the DSC. All samples were prepared just before the measurements were performed. Each sample name was simplified: (1) WS; (2) WS + 0.1% Na_2CO_3; (3) WS + 0.2% Na_2CO_3; (4) WS + 5% KGM; (5) WS + 5% KGM + 0.1% Na_2CO_3; (6) WS + 5% KGM + 0.2% Na_2CO_3; (7) WS + 10% KGM; (8) WS + 10% KGM + 0.1% Na_2CO_3; (9) WS + 10% KGM + 0.2% Na_2CO_3, where percentage means weight dividing by that of wheat starch.

2.3. Particle size

The starch dispersions (4%, w/w) were heated onto a L-129A tube heater (Laiheng Lab-Equipments, Beijing, China), temperature ranging from 25 to 95 °C at 5 °C/min and then maintaining at 95 °C for 4 min. In order to ensure the uniform heating of starch dispersions, an overhead stirrer was used. A series of samples was taken for every 2 min from 45 °C till the end of heating. Samples were suspended into the loading cell of LS230 laser diffraction particle size analyzer (Beckman Coulter, CA, USA) to determine the particle size distribution. The flow rate of water was set at 60% and run for 90 s per test. All of the experiments were performed in triplicate.

2.4. Viscosity profiles

The viscosity profiles of starch pasting in an aqueous system were measured using the RVA (Newport Scientific, Sydney, Australia). 8% starch dispersions containing various concentrations of KGM (0–10 wt% of starch) and Na_2CO_3 (0–0.2 wt% of starch) were weighed into the RVA canisters to make up a total weight of 28 g and stirred manually using a plastic paddle for 20–30 s. The canisters were then placed into the instrument and treated using the following temperature profile: heated to 50 °C and held for 1 min, ramped to 95 °C at 12 °C/min, held at 95 °C for 3.5 min, cooled to 50 °C at 12 °C/min and held for 2 min at 50 °C. This method is known as the standard 1 profile of the RVA.

2.5. Leached amylose

To determine the amount of amylose leached during gelatinization, starch–KGM–Na_2CO_3 (4% starch, w/w) paste sampled immediately after standard 1 heating profile was subjected to the determination reported previously by Funami et al. (2005) with small modifications. The composite was centrifuged at 20,000 × *g* for 30 min. The volume of the supernatant of each sample was determined. 1 ml of the supernatant obtained was added to 0.33 M NaOH aqueous solution (6 ml), which was then heated at 95 °C for 30 min. The solution (0.1 ml) was mixed with 0.5% trichloroacetic acid (5 ml) to decrease pH of the mixture toward pH 5.5. The mixture was subsequently dyed with 0.01 N I_2–KI aqueous solution (0.05 ml). The resultant blue color was read at 620 nm after incubation at 25 °C for 30 min using a distilled deionized water as a reference. Reagent grade of potato amylose (Sigma Chemical Co., St. Louis, USA) was used as a standard substance. The amount of dissolved amylose in the supernatant was calculated from the concentration and the volume. Wheat starch as a dry ingredient was also subjected to this assay to investigate the original amylose content of wheat starch before gelatinization. Leached amylose was calculated as:

$$\text{Al}\ \ (\%) = \frac{A_d - A_o}{A_t} \times 100\%$$

where Al is leached amylose, A_d is dissolved amylose of starch paste, A_o is original amylose of wheat starch, and A_t is total amylose of wheat starch determined using the method described by Jarvis and Walker (1993). Data were presented as means ± SD of triplicate.

2.6. Determination of thermal properties

DSC analysis was conducted to determine the thermal properties of starch–KGM–Na_2CO_3 blends using DSC-60 calorimeter (Shimadzu, Tokyo, Japan). Starch slurries (20%, w/w) in KGM–Na_2CO_3 solution were prepared following the method described above. After hydration for 30 min at room temperature, approximate 20 mg of starch slurries were weighed into aluminum pans, hermetically sealed and equilibrated for 12 h. Then

temperature scans were performed from 20 to 90 °C at a controlled constant rate of 10 °C/min. An empty pan was used as the reference. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures as well as enthalpy (ΔH, expressed as J/g starch) were determined in triplicate.

2.7. Confocal laser scanning microscopy (CLSM)

The CLSM was used to visualize the gelatinization process of starch granules. Stock solution of fluorescein 5-isothiocyanate (FITC) was prepared by dissolving 0.2 g FITC in 100 ml distilled water. Starch suspensions (100 μL) were stained by mixing with 20 μL FITC stock solution. Starch pastes (4%, w/w) were prepared in aqueous solutions of KGM–Na_2CO_3 using the RVA apparatus following temperature profile: held at 25 °C for 1 min, ramped to the target temperatures at 2 °C/min, and held for 1 min. Samples were then cooled to room temperature and analyzed within 2 h, to avoid structural reordering as a result of retrogradation. An aliquot of the stained sample was deposited onto a glass slide and observed within 15 min. A Leica TCS SP5 confocal scanning laser microscope (CLSM) equipped with an inverted microscope was used in single photon mode with an Ar laser. The following Leica objective lenses were used: 20 × 0.4 NA/dry/HC PL FLUOTAR. The excitation wavelength was 488 nm and the emission maxima were within 500–525 nm. Digital image files were acquired in 512 × 512 pixel resolution.

2.8. Statistical analysis

The results were statistically analyzed using SPSS (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) was used to determine significant differences between the results and Duncan's test was used to separate the mean with a significance level of 0.05.

3. Results and discussion

3.1. Starch granule size

Size–distribution curve presented in percentage-size of all the starch suspensions were bimodal (data not shown), since in mature wheat, two distinct classes of granules occur that differ in size, i.e. large, lenticular (A-type) granules ranging between 15 and 40 μm and small, spherical (B-type) granules ranging between 1 and 10 μm. Median diameter was chosen to assess the granule size instead of average diameter, for comparing the granule size without bias in standard deviation generated from size difference in the original starch granules.

Median diameters of starch granules as a function of temperature are presented in Fig. 1. The median diameter of the native starch granules was 9.7 ± 0.4 μm on heating to the temperature of 45 °C (Fig. 1A). Then the median diameters sharply increased, which has been ascribed to the irreversible starch swelling, because of water uptake upon heating in water. It can be seen that after temperature holding at 95 °C for 4 min, the median diameters of starch granules decreased around 18 μm as a result of the granule disruption, mainly crystallite melting and starch components dissolving. Na_2CO_3 has been described previously to accelerate starch granule swelling and causing starch granules to swell to a greater extent before rupturing (Lai et al., 2004). The starch granule structure could also be weakened by some salts (Jyothi, Sasikiran, Sajeev, Revamma, & Moorthy, 2005). However, those conclusions are quite suspect at much lower Na_2CO_3 concentrations of 0.08‰ and 0.16‰ (w/w) (i.e. 0.1% and 0.2 wt% of starch), since the median diameters in Na_2CO_3–starch were insignificantly different to those of the wheat starch at all the sampling temperature ($p > 0.05$).

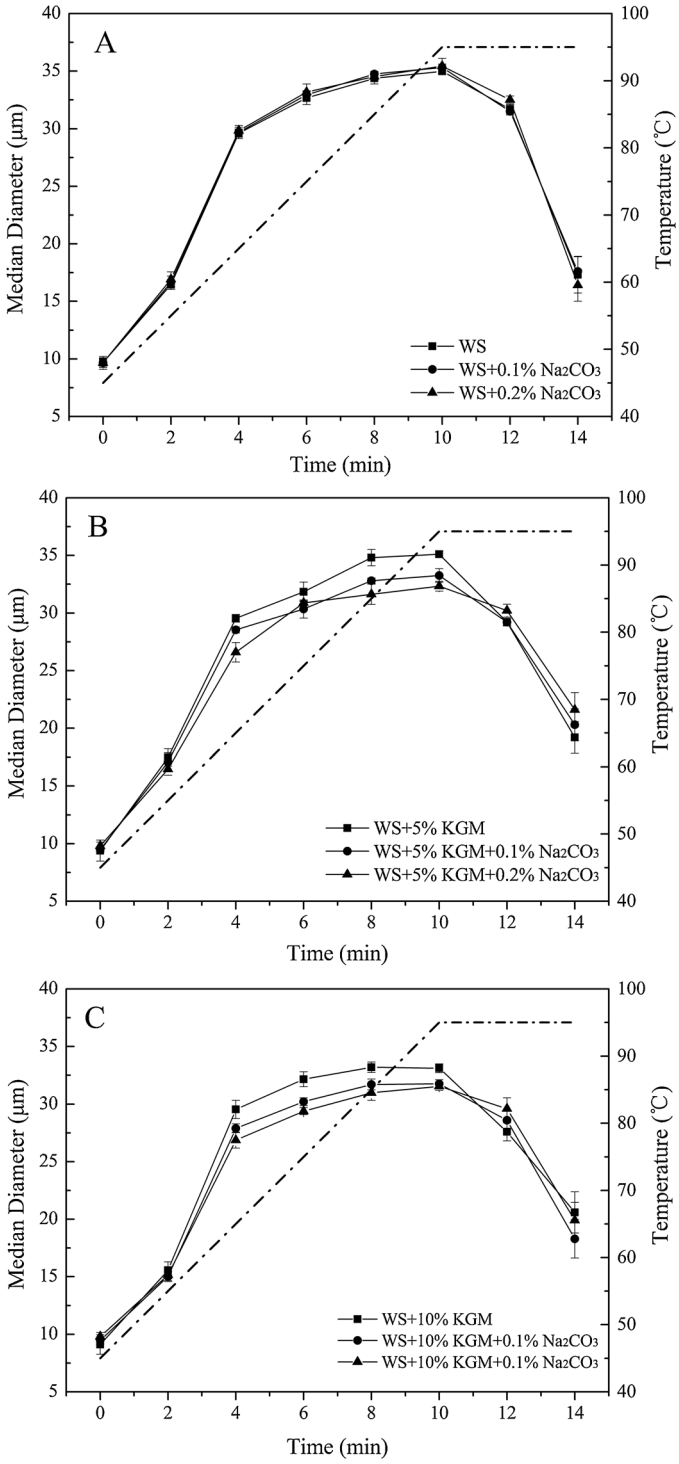

Fig. 1. Median diameter (solid line) of starch granules in WS–Na_2CO_3 (A), WS-5% KGM–Na_2CO_3 (B), WS-10% KGM–Na_2CO_3 (C) system during the pasting process. WS: wheat starch; KGM: konjac glucomannan.

Starch swelling was slightly inhibited by KGM, which can be indicated from the right-shifted curve shown in Fig. 1B and C. The maximum median diameter was significantly decreased at 95 °C in the presence of 0.4% KGM ($p \leq 0.05$), suggesting that KGM might take the dominating role in preventing starch swelling. With its strong water binding capacity decreasing the amount of free water in the starch matrix, hydrogen bonds could be redistributed between starch-to-water and intra- and interchain within starch molecules, resulting in an improvement in the interaction energy

between starch chains (Zhou, Zhao, Foster, et al., 2014; Kalichevsky, Jaroszkiewicz, & Blanshard, 1993). Moreover, the distance between starch chains might be changed when hydrocolloids with strong water-binding capacity are applied.

Starch in the presence of both KGM and Na_2CO_3 underwent very slow and restricted swelling before progressively reaching 95 °C. During holding at peak temperature of 95 °C, the median diameter decreased by 45.2%, 38.9% and 33.1% of WS + 5% KGM samples, compared to 50.5%, 50.1% and 50.8% of WS samples at the equal concentrations of Na_2CO_3, respectively, suggesting that Na_2CO_3 could function synergistically with KGM in determining the extent of starch swelling. The similar yet more pronounced tendency exhibited in WS + 10% KGM samples reflected that the effect of Na_2CO_3 was conditional and highly depended upon the KGM concentration.

3.2. Rapid viscosity analysis (RVA)

RVA characteristics of each sample are given in Table 1. The onset of viscosity increase (i.e., pasting temperature) was shifted to a lower temperature by the addition of KGM. The remarkable increase in the viscosity of the starch paste in the whole process of RVA testing was also found by the addition of KGM, which agreed with previous reports (Charoenrein et al., 2011; Funami et al., 2005). Viscosity enhancement in starch–hydrocolloid mixtures was generally thought to arise from modification in granule swelling and resultant granule deformability during gelatinization, as well as starch–hydrocolloid interactions in the continuous phase. The increase in the effective concentration of starch in the continuous phase as a result of mutual exclusion could lead to detection of pasting at a lower temperature and changes in viscosity of a heated starch suspension, based on thermodynamic incompatibility between starch and polysaccharide (Alloncle & Doublier, 1991; Funami et al., 2005). The physicochemical nature of KGM determines that it can hydrate and take up water quickly. Upon heating, a quickly formed semisolid network, mainly composed of highly hygroscopic KGM might form a viscous water layer at the surface of starches to produce high viscosity (Zhou et al., 2013).

However, it is interesting that 0.1% and 0.2% Na_2CO_3 had nearly no effect on the initiation of paste formation, but contribute to a more pronounced increase in peak viscosity at the same KGM concentration. It was hypothesized that anions could promote gelatinization by the disruption of hydrogen bonding between the starch chains on the basis of electrical double layer theory (Oosten, 1983). According to this theory, an electrical double layer of cations surrounding the starch exists which excludes the anions, and hence the anions cannot penetrate and cause gelatinization.

Rather than at the onset of gelatinization, Na_2CO_3 might be more involved in pasting after the crystalline structure is disordered resulting in the dissociation between amylose and amylopectin. Based on the solute-specific nature of Na_2CO_3, electrostatic interactions between the ions and the starch polymers of weak acid character might promote the solubilization of starch components. As the increase of Na_2CO_3 concentration proceeds, Na_2CO_3 might play another consequential role by transforming the polymer into a less charged species, facilitating the stability of entanglement and paste of higher viscosity. However, deacetylation of KGM could be initiated by alkali after a certain induction period when KGM was included (Gao & Nishinari, 2004). The removal of acetyl groups resulted in reducing the solubility and expansion of the molecular chain in solution (Luo, He, & Lin, 2013), making it difficult to explain the enormously increased peak viscosity by merely a binary interaction between Na_2CO_3 and KGM.

Partial fragmentation of the swollen granules takes place during continuous heating, thus reducing friction between starch granules, which is the primary cause of viscosity breakdown. Breakdown for each starch–KGM system was significantly larger than that for the control. Starch granules were hypothesized to be less resistant to thermal treatment and mechanical shearing due to addition of polysaccharide, suggesting the increased fragmentation could account for the larger breakdown (Lee, Baek, Cha, Park, & Lim, 2002). Funami et al. (2005) argued that dissociations between starch and polysaccharide through the structural shrinkage of these polymers when temperature decreased should be responsible for increase in the breakdown rather than the polysaccharides induced changes of starch granule resistance. When small amount of Na_2CO_3 was included in the system, the breakdown was slightly decreased in the presence of KGM. This might be partially explained by the results of particle size, which indicated that Na_2CO_3 could contribute to larger fraction in the presence of KGM. However, the WS + 5% KGM sample exhibited both larger medium diameter and higher breakdown, indicating that other factors influencing viscosity breakdown except for the particle size variation and polymeric dissociation.

Setback is the difference of viscosity between the trough viscosity and the final viscosity at 50 °C. There was a significant increase in setback and final viscosity with increasing KGM concentration, suggesting an increased tendency to aggregate in the continuous phase. Na_2CO_3 could aggravate the degree of setback by addition of KGM, which was concentration-dependent (Table 1). According to our previous study (Zhou, Zhao, Foster, et al., 2014; Zhou, Zhao, Winkworth-Smith, et al., 2014), Na_2CO_3 at 0.2 wt% of starch allowed a greater gap of swollen granules between each other with KGM involved in fast gelling. Such a highly ordered network with certain rigidity developed could coat the starch granules, increasing the volume and thereby abrasions between the granules to form gels with significantly increased final viscosity.

3.3. Leached amylose

Starch molecules leach easily from swollen granules at a temperature slightly above the gelatinization temperature. Leached materials are mainly composed of amylose, which present in close proximity to amylopectin and concentrated more at the periphery (Li, Blanco, & Jane, 2007). The determination here should be regarded as “apparent amylose content”, which involves not only amylose but also amylopectin with long-chain segments that can behave in a similar manner to amylose during gelatinization.

In fact, some alkalis have been reported to be capable of hydrolyzing starch into shorter chain molecules at a concentration as low as 0.001 M in the aqueous phase (Lai et al., 2004). However, leached amylose content was not strongly affected by only incorporating Na_2CO_3 (Fig. 2).

The amount of leached amylose for each starch–KGM system was significantly lower than that for the control (Fig. 2). For the starch at a fixed concentration of KGM, the higher the Na_2CO_3 concentration, the lower the amount of leached amylose. In this study, the significantly decreased leached amylose amount of KGM samples as compared with pure starch paste was probably due to the increase in viscosity of the continuous phase exerting larger forces, which effected the diffusion of amylose from the starch granules, in accordance with an earlier report (Sudhakar, Singhal, & Kulkarni, 1995).

There is generally a positive relation between swelling and the amount of soluble solids leached outside the granules during heating. Significant changes in particle diameter of the starch granules indicate that incorporating Na_2CO_3 into the KGM–starch system inhibited swelling and prevented melting of crystalline structures composed mainly of the amylopectin fraction. Amylose could hardly leach from crystalline structure without starch granules disruption. It might be related with the surface control by adsorbing onto and coating around the starch granules to form a film covering the starch granules with certain rigidity, thus

Table 1
Gelatinization properties of wheat starch alone (WS) and in the presence of konjac glucomannan (KGM) or Na_2CO_3 obtained from rapid viscosity analysis (RVA) profiles.[a]

Treatments	Pasting temperature (°C)	Peak viscosity	Breakdown	Setback	Final viscosity
WS	79.6 ± 1.3 a	1770 ± 18.2 a	418 ± 9.7 a	618 ± 7.1 a	1970 ± 16.2 a
WS + 0.1% Na_2CO_3	79.1 ± 1.9 a	1832 ± 8.4 b	420 ± 4.4 a	617 ± 3.8 a	2029 ± 7.9 b
WS + 0.2% Na_2CO_3	78.4 ± 0.6 a	1894 ± 20.6 c	436 ± 12.6 a	621 ± 5.0 a	2079 ± 32.1 c
WS + 5% KGM	70.2 ± 0.5 b	3311 ± 50.2 d	700 ± 20.5 c	756 ± 5.9 b	3367 ± 31.8 d
WS + 5% KGM + 0.1% Na_2CO_3	66.0 ± 1.1 c	3445 ± 11.2 e	692 ± 8.3 c	768 ± 3.3 c	3521 ± 18.4 e
WS + 5% KGM + 0.2% Na_2CO_3	66.1 ± 0.6 c	3601 ± 41.8 f	639 ± 3.4 b	824 ± 9.8 d	3786 ± 17.2 f
WS + 10% KGM	65.8 ± 1.0 cd	4795 ± 23.3 g	1009 ± 10.6 f	933 ± 7.3 e	4719 ± 9.3 g
WS + 10% KGM + 0.1% Na_2CO_3	65.9 ± 1.3 cd	4898 ± 31.9 h	973 ± 17.9 e	919 ± 14.9 e	4844 ± 15.5 h
WS + 10% KGM + 0.2% Na_2CO_3	64.8 ± 0.2 d	4993 ± 10.6 i	931 ± 10.1 d	1034 ± 7.5 f	5096 ± 8.9 i

[a] Results are presented as mean values ± standard deviation in triplicate. Values in the same column with different letters (a–f) indicate significantly different ($p \leq 0.05$) between each parameter tested.

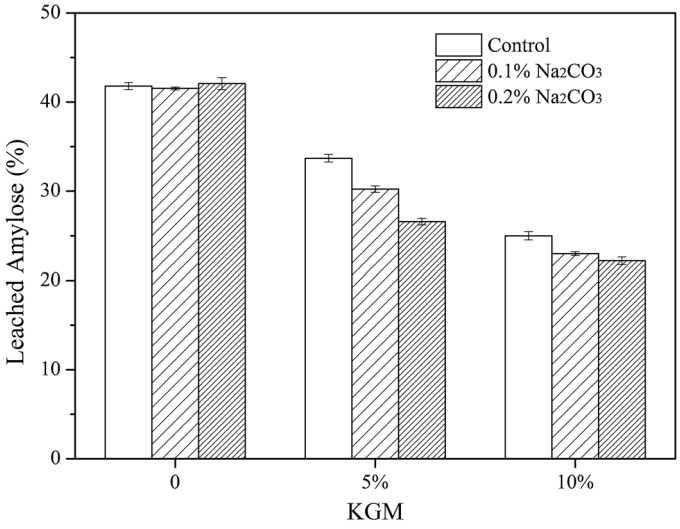

Fig. 2. The amount of amylose leached from the starch granules during gelatinization. Each datum is presented as mean ± standard deviation of triplicate.

prevent leaching of some starch components from the granules and depressing deformability of the granules. However, assuming the leaking has been inhibited to a certain extent by the film, the available amount of amylose would be questionable considering the proportion of amylose being centrifuged to the supernatant.

3.4. Differential scanning calorimetry (DSC)

DSC was applied to monitor the temperatures and enthalpies of starch thermal transitions in the presence of KGM across a concentration range of Na_2CO_3, from 0 to 0.2 wt% of wheat starch. The results of DSC transition temperatures (T_o, T_p and T_c) standing for the onset, peak and final gelatinization temperature, and gelatinization enthalpy (ΔH) are shown in Table 2. The transition is endothermic if ΔH is negative.

For Na_2CO_3 alone, gelatinization temperatures were similar to those of the pure starch suspensions. Both the peak (T_p) and final (T_c) gelatinization temperature shifted to higher temperature due to the KGM addition in the suspensions. However, the onset gelatinization temperature (T_o), was slightly changed with all the samples. This result was in good agreement with previous studies reported by Zhou, Wang, Zhang, Du, & Zhou, 2008. Xu et al. (2012) observed that T_o and T_p of wheat starches were minimally effected by the addition of increasing amounts of KGM, but T_c shifted to higher values with the suspension moisture over 60% (moisture not specified). The contradictions might be attributed to structure diversity with the botanical source of the starch. The DSC transition temperatures primarily reflected loss of double helical order as to the structural origin (Biliaderis, 1982). This infers that the forces responsible for the structural stability of starch granules are largely at the double helical level, which might be very stable in a given particular starch. Thereby, immobilization of water molecules is one of the most important reasons in explaining polysaccharides-induced DSC transition temperatures shifts.

T_p and T_c became significantly higher for starch suspensions when Na_2CO_3 was incorporated along with KGM. One possible explanation lied in the partial loss of energy reaching to starch granule and protection of the integrity to some extent, when starch granules are subjected to heating gradually. Moreover, the DSC transition temperatures were not in accordance with the pasting temperature from RVA (Table 1) with a remarkable advance in viscosity increase presented in KGM–Na_2CO_3 starches. It confirmed that once energy required for irreversible swelling of starch granules was reached, the initial viscosity was dependent on media viscosity (Christianson, Hodge, Osborne, & Detroy, 1981) and the combined network of KGM–Na_2CO_3 did contribute to the media viscosity.

A significant ($P < 0.05$) decrease in gelatinization enthalpy (ΔH) was observed. The gelatinization enthalpy (ΔH) of granular starch represents net thermodynamic quantities of different events: granule swelling and crystallite melting (endothermic); hydration and chain reorder (exothermic) (Slade & Levine, 1994). The swelling of amorphous regions of the granule initiates a force which then destabilizes the crystallites (BeMiller & Whistler, 2009). The restrictive effects of KGM–Na_2CO_3 on swelling and crystalline melting could decrease the extent of conformational disordering of starch chains on gelatinization of wheat starch, as inferred by the reduced enthalpy values observed in the presence of KGM–Na_2CO_3. Direct ion–macromolecule interactions as well as interactions with water molecules are involved in the hydration of the macro-molecule, which might also affect the exothermic endotherm (Zhang & Cremer, 2006).

3.5. Confocal laser scanning microscopy (CLSM)

A series of changes in starch granular structure with or without KGM and Na_2CO_3 upon heating were visualized by CLSM. Fig. 3 shows samples which were taken just before starch gelatinization at 55 °C (A_1–D_1), during rapid swelling at 75 °C (A_2–D_2) and after starch granule disruption at 95 °C (A_3–D_3).

The starch granules swelled slightly when heated to 55 °C and did not initiate the onset of viscosity increase yet, which was supported by the RVA results. Starch granules of A_1–D_1 were comparable in size and shape with those of another study reported previously (van de Velde, van Riel, & Tromp, 2002). The granules of wheat starch in A_1 were ranging between 5 and 55 μm, a little larger than those in B_1–D_1.

Expansion of starch granules proceeded in all directions when heated to 75 °C. The fluorescence concentrated at the periphery of the starch granules suggesting that the fluorescein isothiocyanate (FITC) dissolved in water was adsorbed into the granules during

Table 2
Thermal properties of wheat starch alone (WS) and in the presence of konjac glucomannan (KGM) or Na_2CO_3 obtained from the differential scanning calorimetry (DSC) thermograms.[a]

Treatments	Thermal properties			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
WS	57.3 ± 0.2 a	63.4 ± 0.4 a	70.0 ± 0.5 a	−9.3 ± 0.1 a
WS + 0.1% Na_2CO_3	57.4 ± 0.1 a	63.3 ± 0.6 a	69.9 ± 0.3 a	−9.2 ± 0.1 ab
WS + 0.2% Na_2CO_3	57.4 ± 0.3 a	63.2 ± 0.1 a	69.7 ± 0.1 a	−9.0 ± 0.5 ab
WS + 5% KGM	57.3 ± 0.4 a	64.5 ± 0.4 b	71.4 ± 0.2 b	−8.9 ± 0.1 b
WS + 5% KGM + 0.1% Na_2CO_3	58.0 ± 0.5 ab	65.0 ± 0.3 bc	71.6 ± 0.3 bc	−8.1 ± 0.3 c
WS + 5% KGM + 0.2% Na_2CO_3	57.8 ± 0.3 ab	65.5 ± 0.4 c	72.2 ± 0.5 c	−7.5 ± 0.2 d
WS + 10% KGM	57.8 ± 0.2 ab	65.1 ± 0.4 bc	72.4 ± 0.2 c	−7.5 ± 0.3 d
WS + 10% KGM + 0.1% Na_2CO_3	58.0 ± 0.1 b	65.3 ± 0.2 c	72.6 ± 0.3 c	−7.1 ± 0.3 de
WS + 10% KGM + 0.2% Na_2CO_3	58.1 ± 0.1 b	65.6 ± 0.1 c	73.2 ± 0.4 d	−6.9 ± 0.2 e

[a] Results are presented as mean values ± standard deviation in triplicate. Values in the same column with different letters (a–e) indicate significantly different ($p \leq 0.05$) between each parameter tested.

swelling (Savary, Handschin, Conde-Petit, Cayot, & Doublier, 2008). Apparently, starch in A_2 and B_2 swelled to a greater extent. The starch granules which were smaller in size were observed in samples heated in the KGM-added system with no distinction between the morphology of the granules (C_2 and D_2). However, close inspection of D_2 shows that fibrous network in the dark space surrounding starch granules (indicated by arrow in D_2). The FITC accumulates in hydrophobic parts of samples, for its affinity is largely equivalent to hydrophobicity. Consequently, the polymeric network will be highlighted in a way which is dominated by the kinetics and thermodynamics of the probe molecules (Hans Tromp, van de Velde, van Riel, & Paques, 2001). Since KGM cannot be distinguished from starch components which is quantitatively larger than KGM (Zhou, Zhao, Foster, et al., 2014; Zhou, Zhao, Winkworth-Smith, et al., 2014), it was not concluded but speculated that KGM might be associated or at least in close relevance with what was formed surrounding starch granules.

Starch granules were damaged and finally disrupted at 95 °C. Starch components diffused outside the granules, forming a continuous and glutinous network around the surface of the swollen granules in all the samples as indicated by the smearing of A_3–D_3. Na_2CO_3 seemed to promote the complete disruption of highly swelled starch granules and the solubility of starch components to form non-separated (homogeneous) starch paste (B_3). It was suggested that the interactions of Na^+ cation and starch could presumably raise the temperature for the initial water assisted melting of the least stable crystallites (Day, Fayet, & Homer, 2013). Conversely, the CO_3^{2-} anion was a gelatinizing agent, promoting starch gelatinization by rupturing hydrogen bonds between starch molecules, even though it was less strong than the OH^- (Lai et al., 2004). As a result, the swollen starch granules ruptured more easily with alkaline hydrolysis of amylose.

Starch granules in KGM solution were stabilized in morphology (C_3 and D_3), probably because they restrict starch granule swelling, which is in good accordance with what was observed in the particle size determination. Opening of pores in the surface of the granule would allow some polysaccharides to penetrate inside swollen starch granules (Savary et al., 2008). However, macromolecules of

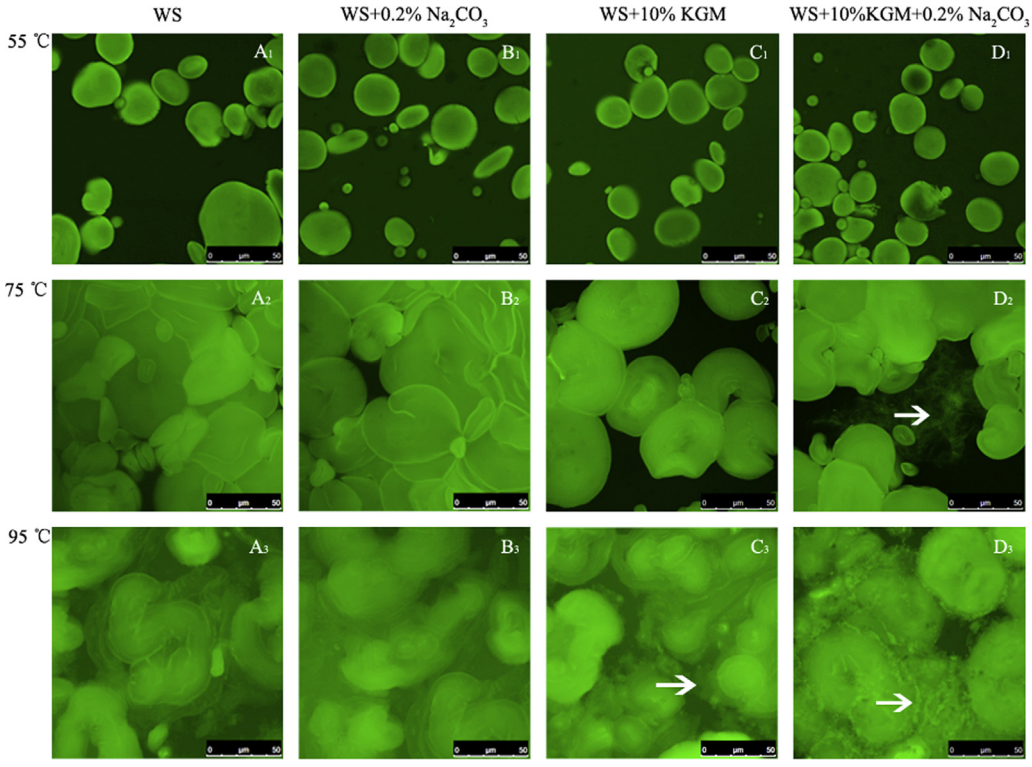

Fig. 3. CLSM images of the Na_2CO_3–KGM–starch composite systems. WS (A_1–A_3), WS + 0.2% Na_2CO_3 (B_1–B_3), WS + 10% KGM (C_1–C_3) and WS + 10% KGM + 0.2% Na_2CO_3 (D_1–D_3) were heated to 55 °C, 75 °C and 95 °C. WS: wheat starch; KGM: konjac glucomannan.

KGM were not able to penetrate the granule (Christianson & Bagley, 1983). It is more likely that the majority of KGM was adsorbed on the surface of starch granules, since a higher concentration of cloud-like extensions of starch granules were evident in the periphery as indicated by arrow in C_3.

D_3 shows the presence of KGM and Na_2CO_3 reinforced the starch granules so that their swollen size and structure were retained in integrity at 95 °C. A certain amount of brighter complexes indicated by arrow in D_3 appeared to be situated on the cloud-like extensions shown in C_3, and were unevenly distributed. Na_2CO_3 was likely to be critical in developing such discontinuous highlighted complexes by the growth of polymeric network of C_3 in addition to increasing the resistance of the granules to disintegration and decreasing the extent of swelling in the presence of KGM. This finding corresponds to a previous work investigating Na_2CO_3–KGM starch gels by Zhou, Zhao, Foster, et al. (2014); Zhou, Zhao, Winkworth-Smith, et al. (2014), which showed that those highlighted complexes remained in the periphery along with swollen starch granules shrinking after being stored for 24 h at room temperature. This led to a hypothesis regarding the electrostatic interactions between the ions and the polymer chains. Since the hydroxyl group of the linear amylose dissociate more readily than water, there is a higher local concentration of hydrogen ions around the chains than in the bulk of the solvent. As a result of the behavior, hydrogen ions diffuse away from the amylose to remove this imbalance, leaving the chains with a net negative charge. These Na^+ cations may neutralize a negative charge density concentrated in starch and molecular association, which might occur when KGM simultaneously, binds to the surface of adjacent starch granules with starch polymers leaking out. This gave rise to the possibility that KGM stabilized the granular shape with a joint effect of Na_2CO_3, making the shear forces and heating energy exerted on granules much smaller than those encountered in starch dispersions of the equal KGM concentration.

4. Conclusion

The present work attempts to clarify the effect of a small amount of Na_2CO_3 on konjac glucomannan (KGM)-induced changes in wheat starch thermal properties, which are important for predicting and controlling the structure and texture of processed formulated starch-based products. In summary, the main conclusions are as follows:

- Na_2CO_3 is considered to magnify the effects of KGM on inhibiting starch swelling and protecting starch granule crystals against melting even at low concentrations.
- The extremely high viscosity of the starch paste was mainly contributed by KGM. Na_2CO_3 enhances the effectiveness of KGM in this respect. However, the breakdown in subsequent heating was less remarkable in the presence of Na_2CO_3, compared with the KGM sample.
- Na_2CO_3 triggers the formation of the complexes between KGM and amylose. These complexes are adsorbed onto or around the starch granules to form a film covering the starch granules and amyloses.
- The reaction aforementioned takes place once the starch components begin to exude. The molecular mechanism for the role of Na_2CO_3 in the binding of KGM and starch polymers awaits further elucidation.

Acknowledgements

This work was supported by the grants from National Science Foundation of China (31371717), Specialized Research Fund for the Doctoral Program of Higher Education (20130008110016) and National Key-Technologies R&D Project (2012BAD37B05) during the 12th 5-year Plan of the People's Republic of China.

References

Alloncle, M., & Doublier, J. L. (1991). Viscoelastic properties of maize starch/hydrocolloid pastes and gels. *Food Hydrocolloids*, *5*(5), 455–467.
BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers*, *86*(2), 386–423.
BeMiller, J. N., & Whistler, R. L. (2009). *Starch: chemistry and technology* (3rd ed.). New York: Academic Press.
Biliaderis, C. G. (1982). Physical characteristics, enzymic digestibility and structure of chemically modified smooth pea and waxy maize starches. *Journal of Agricultural and Food Chemistry*, *30*(5), 925–930.
Chao, S. J., & Lai, L. S. (1999). Effects of salt on the gelling behavior of hsian-tsao (Mesona procumbens Hemsl) leaf gum as studied using rapid-visco analyzer. *Food Science (Taipei)*, *26*(2), 228–239.
Charoenrein, S., Tatirat, O., Rengsutthi, K., & Thongngam, M. (2011). Effect of konjac glucomannan on syneresis, textural properties and the microstructure of frozen rice starch gels. *Carbohydrate Polymers*, *83*(1), 291–296.
Christianson, D. D., & Bagley, E. B. (1983). Apparent viscosities of dispersions of swollen cornstarch granules. *Cereal Chemistry*, *60*(2), 116–121.
Christianson, D. D., Hodge, J. E., Osborne, D., & Detroy, R. W. (1981). Gelatinization of wheat starch as modified by xanthan gum, guar gum, and cellulose gum. *Cereal Chemistry*, 58.
Chua, M., Baldwin, T. C., Hocking, T. J., & Chan, K. (2010). Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex N.E.Br. *Journal of Ethnopharmacology*, *128*(2), 268–278.
Day, L., Fayet, C., & Homer, S. (2013). Effect of NaCl on the thermal behaviour of wheat starch in excess and limited water. *Carbohydrate Polymers*, *94*(1), 31–37.
Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005). Effects of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. *Food Hydrocolloids*, *19*(1), 1–13.
Gao, S., & Nishinari, K. (2004). Effect of degree of acetylation on gelation of konjac glucomannan. *Biomacromolecules*, *5*(1), 175–185.
Hans Tromp, R., van de Velde, F., van Riel, J., & Paques, M. (2001). Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. *Food Research International*, *34*(10), 931–938.
Jarvis, C. E., & Walker, J. R. (1993). Simultaneous, rapid, spectrophotometric determination of total starch, amylose and amylopectin. *Journal of the Science of Food and Agriculture*, *63*(1), 53–57.
Jyothi, A. N., Sasikiran, K., Sajeev, M. S., Revamma, R., & Moorthy, S. N. (2005). Gelatinisation properties of cassava starch in the presence of salts, acids and oxidising agents. *Starch-Stärke*, *57*(11), 547–555.
Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1993). A study of the glass transition of amylopectin—sugar mixtures. *Polymer*, *34*(2), 346–358.
Karim, A. A., Nadiha, M. Z., Chen, F. K., Phuah, Y. P., Chui, Y. M., & Fazilah, A. (2008). Pasting and retrogradation properties of alkali-treated sago (*Metroxylon sagu*) starch. *Food Hydrocolloids*, *22*(6), 1044–1053.
Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Sato, T., & Matsuzaki, K. (2003). Constitution of konjac glucomannan: chemical analysis and 13C NMR spectroscopy. *Carbohydrate Polymers*, *53*(2), 183–189.
Khanna, S., & Tester, R. (2006). Influence of purified konjac glucomannan on the gelatinisation and retrogradation properties of maize and potato starches. *Food Hydrocolloids*, *20*(5), 567–576.
Lai, L. S., & Chao, S. J. (2000). A DSC study on the gel–sol transition of a starch and hsian-tsao leaf gum mixed system. *Journal of Agricultural and Food Chemistry*, *48*(8), 3267–3274.
Lai, L. S., Karim, A. A., Norziah, M. H., & Seow, C. C. (2004). Effects of Na_2CO_3 and NaOH on pasting properties of selected native cereal starches. *Food Chemistry and Toxicology*, *69*, 249–256.
Lee, M. H., Baek, M. H., Cha, D. S., Park, H. J., & Lim, S. T. (2002). Freeze–thaw stabilization of sweet potato starch gel by polysaccharide gums. *Food Hydrocolloids*, *16*(4), 345–352.
Li, L., Blanco, M., & Jane, J. L. (2007). Physicochemical properties of endosperm and pericarp starches during maize development. *Carbohydrate polymers*, *67*(4), 630–639.
Luo, X., He, P., & Lin, X. (2013). The mechanism of sodium hydroxide solution promoting the gelation of konjac glucomannan (KGM). *Food Hydrocolloids*, *30*(1), 92–99.
Maeda, M., Shimahara, H., & Sugiyama, N. (1980). Detailed examination of the branched structure of Konjac glucomannan [pasta made from starch of devil's tongue, Amorphophalus konjac]. *Agricultural and Biological Chemistry*, *44*(2), 245–252.
Oosten, B. J. (1983). Explanations for Phenomena Arising from Starch-Electrolytes Interactions. *Starch-Stärke*, *35*(5), 166–169.
Savary, G., Handschin, S., Conde-Petit, B., Cayot, N., & Doublier, J.-L. (2008). Structure of polysaccharide–starch composite gels by rheology and confocal laser scanning microscopy: Effect of the composition and of the preparation procedure. *Food Hydrocolloids*, *22*(4), 520–530.
Sim, S. Y., Noor Aziah, A. A., & Cheng, L. H. (2011). Characteristics of wheat dough and Chinese steamed bread added with sodium alginates or konjac glucomannan. *Food Hydrocolloids*, *25*(5), 951–957.

Slade, L., & Levine, H. (1994). Water and the glass transition—dependence of the glass transition on composition and chemical structure: special implications for flour functionality in cookie baking. *Journal of food engineering*, *22*(1), 143–188.
Sudhakar, V., Singhal, R. S., & Kulkarni, P. R. (1995). Studies on starch–hydrocolloid interactions: Effect of salts. *Food Chemistry*, *53*(4), 405–408.
van de Velde, F., van Riel, J., & Tromp, R. H. (2002). Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *Journal of the Science of Food and Agriculture*, *82*(13), 1528–1536.
Viturawong, Y., Achayuthakan, P., & Suphantharika, M. (2008). Gelatinization and rheological properties of rice starch/xanthan mixtures: Effects of molecular weight of xanthan and different salts. *Food Chemistry*, *111*(1), 106–114.
Wang, C., Xu, M., Lv, W.-P., Qiu, P., Gong, Y.-Y., & Li, D.-S. (2012). Study on rheological behavior of konjac glucomannan. *Physics Procedia*, *33*, 25–30.
Williams, M. A. K., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. *Biomacromolecules*, *1*(3), 440–450.
Xu, Z., Zhong, F., Li, Y., Shoemaker, C. F., Yokoyama, W. H., & Xia, W. (2012). Effect of polysaccharides on the gelatinization properties of cornstarch dispersions. *Journal of Agricultural and Food Chemistry*, *60*(2), 658–664.
Yoshimura, M., Takaya, T., & Nishinari, K. (1996). Effects of konjac glucomannan on the gelatinisation and retrogradation of corn starch as determined by rheology and differential scanning calorimetry. *Journal of Agricultural and Food Chemistry*, *44*(10), 2970–2976.
Zhang, Y., & Cremer, P. S. (2006). Interactions between macromolecules and ions: the Hofmeister series. *Current opinion in chemical biology*, *10*(6), 658–663.
Zhou, Y., Cao, H., Hou, M., Nirasawa, S., Tatsumi, E., Foster, T. J., et al. (2013). Effect of konjac glucomannan on physical and sensory properties of noodles made from low-protein wheat flour. *Food Research International*, *51*(2), 879–885.
Zhou, Y., Wang, D., Zhang, L., Du, X., & Zhou, X. (2008). Effect of polysaccharides on gelatinization and retrogradation of wheat starch. *Food Hydrocolloids*, *22*(4), 505–512.
Zhou, Y., Zhao, D., Foster, T. J., Liu, Y., Wang, Y., Nirasawa, S., et al. (2014). Konjac glucomannan-induced changes in thiol/disulphide exchange and gluten conformation upon dough mixing. *Food Chemistry*, *143*, 163–169.
Zhou, Y., Zhao, D., Winkworth-Smith, C. G., Foster, T. J., Nirasawa, S., Tatsumi, E., et al. (2014). Effect of a small amount of sodium carbonate on konjac glucomannan-induced changes in wheat starch gel. *Carbohydrate Polymers*, http://dx.doi.org/10.1016/j.carbpol.2014.02.087