1 Effects of konjac glucomannan on heat-induced changes of wheat gluten
2 structure

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14 **ABSTRACT**

15 Effects of konjac glucomannan on the structure of wheat gluten were investigated at
16 variable temperatures in this study. Dynamic oscillatory rheology study showed that
17 konjac glucomannan conferred stiffness on gluten with a higher $\tan \delta$ data at 25 °C
18 and 55 °C, but this parameter was lower at 75 °C and 95 °C. Konjac glucomannan
19 decreased the content of thiol equivalent groups and increased the α -helix/ β -sheet
20 content ratio, respectively. The thicker layer of gluten protein with 5% konjac
21 glucomannan was observed by scanning electron microscopy. This study revealed that
22 konjac glucomannan could alter the conformations of gluten proteins upon heating via
23 non-covalent interactions and physical entanglements. It is likely that konjac
24 glucomannan promotes protein aggregation by strengthening hydrophobic interaction
25 at 25°C and 55 °C, and alleviates heat-induced denaturation by decreasing the
26 flexibility of polypeptide chain at higher 75 °C and 95 °C.

27 *Keywords:* Konjac glucomannan, Heat treatment, Gluten, Disulfide bond, Fourier
28 transform infrared spectroscopy (FTIR), Gluten secondary structure

29 Chemical compounds studied in the article

30 Glucomannan (PubChem CID: 24892726); Gliadin (PubChem CID: 17787981)

1. Introduction

Gluten is defined as the viscoelastic mass that remains when starch granules are removed from wheat dough. It confers some functional properties such as viscosity, elasticity and water absorption capacity to wheat products through formation of three-dimensional matrix. Gluten proteins can be divided mainly into glutenins (30-40 wt %) and gliadins (40-50 wt %) based on their solubility to aqueous alcohol. Glutenin is a kind of aggregated protein in which subunits are linked by interchain disulfide bonds. Glutenin varies in size, ranging from 500,000 to over 10 million. The structure of its subunit can be classified into repetitive domains and non-repetitive domains. The repetitive domain exists as a spiral structure which is organized by β -turns; the non-repetitive domain is mainly α -helical structure and comprises almost all cysteine residues. The spiral structure is found to be responsible for the viscoelasticity via intermolecular bonding of its glutamine residues. These findings led to the development of loop-train model, proposing that the state of hydrogen bonding could influence the secondary structure and consequently affect viscoelastic properties of dough: intrachain hydrogen bonds in β -sheet, which are associated with “train” structure, will break during processing, leading to the formation of turn-like structures referred to “loop” and an increase in protein flexibility (Anjum et al., 2007; Belton, 1998; Wellner et al., 2005). Moreover, β -turn has a low resistance to extension and can increase the ability of dough to trap gas bubbles (Bock & Damodaran, 2013). Gliadins are monomeric proteins with a molecular weight being around 28,000-55,000. Cysteine residues of S-rich α -type and γ -type gliadins locate at the highly conserved positions and are bonded as intrachain disulfide bonds (Shewry & Tatham, 1997). S-poor ω -type gliadin could not form an elastic polymer by themselves

55 because of their inability to form covalent cross-links (Tatham, Mifflin, & Shewry,
56 1985).

57 Considering the widespread application of thermo-processing like boiling, baking,
58 and air-drying in productions of gluten-based foods, structural changes of gluten upon
59 heating were investigated previously. Generally, the changes induced by heating
60 eventually lead to gluten protein aggregation. It was proposed that mainly glutenin
61 species participate in thermal-induced changes at the temperature between 55 °C and
62 75 °C. However, when heated to above 90 °C, gliadins begin cross-linking to
63 glutenins through the SH-SS exchange reaction (Lagrain, Thewissen, Brijs, &
64 Delcour, 2008). Although disulfide bridges are thought to be the primary
65 intermolecular interaction responsible for the formation of gluten networking during
66 heating, other non-covalent bonds also participate in this process. Hydrophobic
67 interaction could contribute to the stabilization of gluten structure during baking and
68 its strength increases with temperature (Wieser, 2007). Furthermore, eliminating
69 hydrogen bonding upon heating results in changes in rheological properties of gluten
70 protein (Lagrain, Brijs, Veraverbeke, & Delcour, 2005).

71 To improve overall quality of wheat dough, many water-soluble polysaccharides
72 coming with different chemical structures and diverse functional properties such as
73 gelling, thickening, foaming, emulsifying, as well as water-retention and textural
74 enhancing properties are applied. Konjac glucomannan (KGM) as one of these
75 popular polysaccharides becomes increasingly recognized due to its healthy benefit
76 (Chua, Baldwin, Hocking, & Chan, 2010) and unique rheological and gelling
77 properties. KGM, extracted from *Amorphophallus konjac* tubers, is a water-soluble
78 and neutral polysaccharide with a molecular weight around 480,000 g/mol (Nishinari,

Williams, & Phillips, 1992). It is mainly composed of D-glucosyl and D-mannosyl residues which are linked by β -1, 4-linkage in an approximate ratio of 1:1.6 (Maeda, Shimahara, & Sugiyama, 1980; Nishinari, 2000). The main chain of KGM has branches through β -1, 3-mannosyl units or acetyl groups at the C-6 position. The degree of water solubility is influenced by the presence of the acetyl units (Davé & McCarthy, 1997; Katsuraya et al., 2003). Preliminary studies show its effects on sensory properties of food products. The replacement of low-protein wheat flour with KGM (5.0%) can improve the overall quality of noodles except several sensory properties, like hardness and stickiness (Zhou et al., 2013). Some attempts have been made to explain the mechanism how KGM affects dough system. The studies conducted by Sim, Aziah, & Cheng (Sim, Aziah, & Cheng, 2011) and Silva et al. (Silva, Birkenhake, Scholten, Sagis, & Van der Linden, 2013) revealed that KGM has stronger water binding capacity than gluten, thus enabling it to influence the water distribution and protein structure through hydrogen bonding. At molecular level, the addition of KGM might induce the formation of entanglement knots with enough spacing in the dough system, leading to a greater molecular slippage than chain elongation when stretching doughs. This could increase the extensibility of dough (Sim et al., 2011). Zhou et al. (Zhou et al., 2014) found that KGM could increase the content of accessible thiol (SH_{free}) in dough during mixing. This might be attributed to radical scavenging effects of active hydroxyl groups on D-glucose and D-mannose of KGM, which interferes the disulfide exchange reactions. They also claimed that KGM might not form covalent bonds with gluten proteins, whereas it functions by impairing hydrogen bonding system of hydrated gluten hydration via its hydroxyl groups.

Most of the experiments about KGM-gluten interaction were performed extensively in doughs, which consist of both starch and gluten. This makes it hard to specify the

interaction among dough components or interactions involving KGM. In addition, studies of KGM-gluten interactions focusing on thermo-processing are still required in order to understand how KGM, as a commercial food colloid, affects wheat products. To simplify problem, present work directly used wheat gluten which is the most important structural component in dough to determine the effects of KGM on heat-induced changes. This study was designed to look into the rheological properties and microstructure of KGM-gluten system. Heat-induced changes of secondary structure of gluten were investigated by Fourier transform infrared spectroscopy (FTIR). Thiol content analysis and SDS-PAGE were performed to give a clue on tertiary structure.

2. Material and method

2.1 Materials

Gluten of wheat was purchased from Sigma Chemical Co. (St. Louis, USA). Analysis (moisture, crude protein) of gluten from wheat was performed according to approved methods of AACC International, 2000 (Methods 44-19, 46-13) in triplicate and the results were expressed as average. The moisture and protein presented 6.34 and 74.96 % of dry mass of gluten, respectively. Konjac glucomannan (KGM $\geq 95\%$) was supplied by Yuanli Biotechnology Co., Ltd. (Hubei, China). It was purified by alcohol precipitation before being lyophilized, grounded to a fine powder and sieved. The contents of moisture, ash, crude protein, fat and glucomannan were 1.7%, 0.8%, 0.6%, 0.1% and 96.8% (dry basis, w/w), respectively.

2.2 Preparation of mixture of gluten and KGM

Gluten was mixed with different portions of KGM: gluten (control), gluten with

1.0% KGM, 2.5% KGM, and gluten with 5.0% KGM. Take 1.0 g of mixtures and add excess distilled water (5.0 mL) which is used to ensure that the water content will not be a limiting factor (Bárcenas, De La O-Keller, & Rosell, 2009). Each sample was heated at 55 °C, 75 °C and 95 °C for 10 minutes, respectively. Samples were cooled in the water bath (4 °C) immediately after heating. Controls were also kept in water for 10 minutes at room temperature (25 °C) and then cooled. All samples were freeze-dried, ground and sieved (0.125 mm) to obtain a powder with uniform particle size.

2.3. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of different gluten samples were recorded using an iS50 FT-IR spectrometer (Thermo Nicolet Inc, Waltham, MA, USA) equipped with a horizontal multi-reflectance diamond accessory. Each sample was carried in triplicate. Spectra were collected in the 400–4000 cm^{-1} infrared spectral range at room temperature. Each spectrum was an average of 64 scans at a 4 cm^{-1} resolution. A background spectrum of the empty trough sampling plate was collected before each sample was run. The overlap of individual peaks in the amide I region (1600 cm^{-1} to 1700 cm^{-1}) was resolved with the Fourier self-deconvolution (FSD) and the curve fitting. The quantity of secondary structure of protein, presented as a percentage of the sum of the areas measured in the Amide I region of gluten was estimated via Omnic software (version 8.0, Thermo Nicolet Inc, Waltham, MA, USA) (Bradley & Nishikida, 2005).

2.4 Accessible thiol and disulfide contents determination

Samples were processed as previously described in 2.2 for the measurements. The contents of accessible thiol (SH_{free}) and thiol equivalent groups (SH_{eq}) were assessed as described by Zhou et al. (Zhou et al., 2014). 80 mg of ground freeze-dried sample

was used to determinate the content of accessible thiol (SH_{free}). 20 mg of the sample was used to measure the total thiol equivalent groups (SH_{eq}). Determinations were carried out in triplicate. Results were expressed in μmol per gram of protein.

2.5 SDS-PAGE

Protein fractions were extracted from freeze-dried samples referring to the method described by Zhou et al. (Zhou et al., 2014) with some modifications. Gliadin was extracted from freeze-dried samples (50 mg). The mixture of the sample and solution A was centrifuged at $4000\times g$ for 4 min. Then the precipitate was used for glutenin extraction.

Gliadin and glutenin extracts at same concentration were mixed separately with running buffer solution (1:1) which was composed of 0.4% (w/v) SDS, 36% (w/v) glycerol, 50 mM Tris-HCl buffer (pH 6.8), 2% (v/v) mercaptoethanol and 0.01% (w/v) bromophenol blue. The mixtures were heated in a boiling water bath and allowed to cool, which was followed by centrifugation. 10 μL supernatant was loaded into 12% polyacrylamide gels. SDS-PAGE analysis was carried out on Mini-PROTEAN Tetra vertical electrophoresis system (Bio-Rad Laboratories, USA). Electrophoresis was run at a constant voltage (75 V) for about 2.5 h until the tracking bromophenol blue dye reached 1 cm above the end of the gel. Gels were stained for 30 min by an aqueous coloring solution. The same solution without the colorant was used for discoloring the gels overnight.

2.6 Dynamic oscillatory tests

Dynamic rheological measurements of gluten with KGM were determined using an AR1500ex rheometer (TA instruments, New Castle, DE, USA) under strain control

mode as described by Han et al., 2013 (Han et al., 2013). The measuring system included a cone and plate (plate diameter 20mm, gap 1mm) to eliminate slippage during a test. 1.0 g of gluten was mixed with 5.0 mL distilled water, and centrifuged at 2000×g for 10min; The supernatant was discarded. KGM was mixed with the gluten (0%, 2.5%, 5.0% (w/w) per gram of gluten) before adding the distilled water. After centrifugation, samples consisting of hydrated gluten had the to the optimum water binding capacity. Gluten dough was placed between the plates within 1 h after hydration and the test started after 15 min resting so that residual stresses could relax. The rim of the sample was coated with silicon oil in order to prevent evaporation during the measurements. Strain sweeps at 1 Hz frequency at 25 °C, 55 °C, 75 °C and 95 °C. A strain of 3×10^{-3} was selected within the linear viscoelastic region. A frequency sweep from 0.1 to 10 Hz was performed at constant strain. Frequency sweep tests were performed from 0.1 to 10 Hz at 25 °C, then go through programmed heating up to 55 °C, 75 °C and 95 °C at a heating rate of 5 °C/min. Elastic modulus G' and viscous modulus G'' were determined as a function of frequency at 25 °C, 55 °C, 75 °C and 95 °C.

2.7. Scanning electron microscopy (SEM)

The processed gluten samples were cut transversally into cubes, immediately frozen at -80 °C and lyophilized. Freeze-dried samples were mounted on a silver specimen holder and coated with gold for 60 s. The microstructure of cross-section was observed by a scanning electron microscope (HITACHI, Japan) at a voltage of 15.0 kV.

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 compare the means with a significance difference at the level of 0.05.

3. Results and discussion

3.1 Changes in secondary structure of gluten

The FTIR spectra of different samples (shown in Supplementary Fig. S1) at amide I spectral region ($1600\text{-}1700\text{ cm}^{-1}$), which is mainly caused by the C=O stretch vibrations of the peptide linkages, were used to estimate the secondary structure of protein because of its high sensitivity and strong signal. Each type of secondary structure gives rise to a somewhat different C=O stretching frequency due to unique molecular geometry and hydrogen bonding pattern. Because of the extensive overlap of the broad component bands, the Fourier self-deconvolution (FSD) and curve fitting were carried out to resolve the individual band component corresponding to specific secondary structure. The estimates of α -helix, β -sheet, β -turn and random coil, contents were determined from the relative band areas in the spectra in Fig. 1. The relative amount of secondary structures of wheat gluten followed the order β -sheet > β -turn > random coil > α -helix and confirmed that gluten predominantly forms β -sheet structure in developed gluten dough, which is in consistence with studies (Bock & Damodaran, 2013; Marti, Bock, Pagani, Ismail, & Seetharaman, 2016). The secondary structure of the control changed as the heating temperature was increased from 25 °C to 95 °C. The β -turn contents increased from about 26.0% to 27.6% and

the random coil content increased from 20.5% to 22.0%, while contents of β -sheet and α -helix decreased accordingly. It was supposed that temperature influenced content of β -turn and β -sheet by impairing hydrogen bonding at higher temperature. The β -turn structure is an open reverse turn, requiring fewer hydrogen bonds than the multi-hydrogen bonded β -sheet structure (Tatham et al., 1985). The conversion of β -sheet to β -turn as temperature increased might be attributed to those hydrogen bonds being weakened and even cleaved. As α -helix of the gluten was uncoiled gradually upon heating, gluten developed into lower-energy structure stabilized by conformations such as random coils and β -turn, which are in the absence of specific interactions, but could account for much of the energy barrier to protein refolding. The α -helix/ β -sheet content ratio (shown in Supplementary Fig. S2) was also calculated as an indication of structural flexibility of protein (Wang et al., 2016), which estimated that gluten dominated a more flexible structure with temperature increased.

However, with KGM added, effects of heating were offset and even reversed at a concentration of 5.0%, indicating KGM functioned oppositely in terms of gluten conformation compared with increasing temperature. The α -helix content increased with KGM content increasing at the temperature ranging from 55 °C to 95 °C. The α -helical conformation, which was thought to be related to protein folding, was possible to be adopted by gluten interacted with KGM and contribute to a more compact gluten structure (Linlaud, Ferrer, Puppo, & Ferrero, 2011). The β -sheet content was decreased by KGM at room temperature or moderate heating temperature of 55 °C, but increased when heating to 95 °C. The content of β -turn and random coil were decreased by KGM when gluten was subjected to continues heating to 75 °C and even higher, while β -turn and random coil content of control remained increasing.

According to the “loop and train” model, the transformation between β -sheet and β -turn implies that the equilibrium between “loops” and “trains” sections could be modified by KGM (Belton, 1998). The different effects of KGM on conformations observed between moderate heating temperature (55 °C) and high heating temperature (75 and 95 °C) were possibly due to the fact that both the structure of KGM and the interaction between KGM and gluten are affected by heating. At moderate heating temperature (55 °C), 5.0% KGM might strengthen the hydrophobic interaction of repeat regions of high molecular weight subunits of glutenin, leading to more rupture of β -sheet at these regions. At higher temperature, hydrogen bonding within KGM got weaker. Heating also induced more random motion of segments and increased the internal entropy driven motion of KGM. All these heat-induced changes maintained KGM in extended form and exposed more hydroxyl groups and therefore enhancing physical entanglement effect (Case & Hamann, 1994; Karim et al., 2005). Hydrated KGM could quickly form a highly entangled network surrounding gluten, slowing down heat exchange and making protein retain more β -sheet structure. Furthermore, the α -helix/ β -sheet content ratio (shown in Supplementary Fig. S2) elevated as KGM content was increased to 5.0% at all temperatures, indicating a limitation of flexibility of protein. This supports the hypothesis that KGM could alleviate the denaturation of gluten proteins.

3.2 Changes in thiol and disulfide groups during heating

Fig. 2 shows the effects of KGM on the content of accessible thiol (SH_{free}) and total equivalent thiol (SH_{eq}) in gluten upon heating at different temperatures. Fig. 2A shows the SH_{free} in almost all samples declined as the heating temperature increased from 25 °C to 95 °C and the downward trend became more evident as heating temperature

was over 55 °C. It seemed like that various KGM substitutions exerted no pronounced effect on SH_{free} except that at 55 °C the downward trend was mitigated by the presence of 5.0% KGM. Morel, Redl, & Guilbert (Morel, Redl, & Guilbert, 2002) suggested that free sulfhydryl groups might not be changeable below 60 °C. However, a slight decrease occurred as heating temperature arising from 25 °C to 55 °C in regard to our results. Protein heating at 55 °C was partially denatured with hydrophobic groups exposed, keeping more thiol groups inaccessible in the outer part. 5.0% KGM addition could slow the heat-induced denaturation of gluten and subsequent aggregation in water (Zhang, Li, Wang, Xue, & Xue, 2016). The further drop in the amount of SH_{free} at 75 °C and 95 °C was assumed to be a result of disulfide bonding with free sulfhydryl groups and dynamic disulfide/sulfhydryl exchange reaction (Lagrain et al., 2005).

As shown in Fig. 2B, the SH_{eq} content of gluten which was heated from 25 °C to 55 °C remained stable before a continuous increase observed as heating directly to 95 °C. In general, the KGM added to gluten played a role in reducing SH_{eq} content. It is interesting to find that the reductive effect was significantly enhanced by 5.0% KGM at 55 °C. From 25 °C to 55 °C, there is no pronounced change in the content of SH_{eq} of gluten with less than 5.0% KGM added, which could be ascribed to the seemingly opposite effects of hydrophobic interaction and partial denaturation on protein structure. To be specific, increased intensity of hydrophobic interaction as heating at moderate temperature could contribute to protein aggregation which might depress the exposure of disulfide bonds to reducing agents like dithioerythritol (DTE) (Guerrieri, Alberti, Lavelli, & Cerletti, 1996). In contrast, the denaturation process unfolded proteins and made the protein structure irregular and less compact. Similar hypothesis, the denaturation of gluten proteins involving hydrophobic interaction at

50~60 °C is followed by thiol-disulfide exchange reaction as heating temperature increases, was brought out by Micard, Morel, Bonicel, & Guilbert (Micard, Morel, Bonicel, & Guilbert, 2001) and Morel et al. (Morel et al., 2002). The role of KGM played on affecting content of SH_{eq} might be identified as a filler in the interactive gel matrix postponing the protein denaturation by inhibiting heat transition consequently maintaining the original folding of gluten (Marti et al., 2016; Zhang et al., 2016).

However, there was no obvious difference between 1.0% KGM, 2.5% KGM and 5.0% KGM at 95 °C. The subsequent increase in the content of SH_{eq} occurring at 75 °C and 95 °C might be caused by weaker hydrophobic interaction at higher temperatures, which promoted disaggregation and consequently made disulfide bonds more susceptible to DTE (Cecil & Wake, 1962). There is another assumption that disulfide/sulfhydryl interchange reactions could be facilitated through temperature-dependent unfolding of protein (Lagrain et al., 2005; Schofield et al., 1983; Wieser, 1998). These newly formed bonds were more sensitive to reducing agents (Morel et al., 2002; Wagner, Morel, Bonicel, & Cuq, 2011). At the same time, the interchange of disulfide bonds was remarkably suppressed within a viscous gluten evident in 5.0% KGM gluten, of which molecular chain was inflexible and hardly move close to a certain distance enabling disulfide bonding taking place, thus causing the sudden decrease of the SH_{eq} content (Karim et al., 2005).

3.3 SDS-PAGE

No difference among the profiles of glutenin and gliadin with varying KGM substitutions was detected (shown in Supplementary Fig. S3). But darker bands near 35-40 kDa and 14 kDa corresponding to cysteine-containing gliadins were observed at lower temperatures. It is possibly that the extraction rate for these specific gliadins

drastically decreased because the single gliadin types were differently influenced and aggregated to be ethanol-insoluble fraction due to disulfide crosslinking (Kieffer, Schurer, Köhler, & Wieser, 2007). It can, therefore, be concluded that intrachain disulfide bonds of α - and γ -gliadins are involved in heat-induced reactions by conversion into interchain bonds allowing the formation of ethanol insoluble aggregates (Kieffer et al., 2007). As for glutenins, the intensity of bands from 100 to 120 kDa became weaker as heating temperature increased, but the intensity of those around 14 kDa got stronger. It is hypothesized that original subunits of high-molecular weight glutenins subject to high temperature were degraded to smaller subunits because of the cleavage of disulfide bonds.

By comparison with data collected by SDS-PAGE and other analytical methods, there seems to be a non-negligible contradiction about if KGM could influence gluten cross-linking and aggregation through the cleavage and rearrangement of disulfide bonds. We interpreted that extraction of glutenins and gliadins did influence protein profile and effects of KGM had to be coupled with SDS, a strong anionic detergent, once SDS-PAGE was performed, making KGM less important on manipulating non-covalent bonds and disulfide bonds (Kieffer et al., 2007).

3.4 Dynamic oscillatory tests

The dynamic storage modulus (G') can be used to determine the energy recovered per cycle of deformation, in order to describe the solid or elastic character of a material. The loss modulus (G'') is an estimate of the energy dissipated as heat per cycle of deformation, taken as an indicator of the viscous response of the material. Two parameters obtained, intercept and slope, were present in Table 1. The intercept values correspond to the magnitude of the elastic modulus whereas the slope values

provide information about the frequency dependence (Hayta & Schofield, 2005). Loss tangent ($\tan \delta = G''/G'$) is a factor that measures damping. Gluten dough of higher $\tan \delta$ shows a more liquid-like behavior while gluten dough of lower $\tan \delta$ can maintain its elastic character to a greater extent.

The mechanical spectra of each sample holding at 25 °C (A) and heating at 55 °C, 75 °C and 95 °C (B-D) were presented in Fig. 3. All samples showed that the G' was higher than G'' , indicating that both control gluten and KGM-gluten exhibited solid-like behaviors. The mechanical spectra were evaluated with the linear variation fitting the logarithmic plot of G' versus frequency (Table 1). The slope gives information about the frequency dependence while intercept values correspond to the magnitude of the elastic modulus (Hayta & Schofield, 2005). Theoretically, the slope value of a completely cross-linked network is zero, with a higher value of slope indicating a lower fraction of cross-linked components (Kokini, Cocero, Madeka, & Graaf, 1994). In our study, as temperature increases, the slope values of all gluten dough moved downward, indicating an elevated extent of molecular cross-linking ascribed to protein aggregation (Hayta & Schofield, 2005). The value of slope increased when 2.5% and 5.0% KGM presented at 55 °C, but decreased when the gluten dough was heated at 75 °C or higher temperature.

With increasing temperature, $\tan \delta$ of both control and KGM-gluten dough decreased (Fig. 4), implying that heating process generally strengthened the gluten system. A substantial increase of $\tan \delta$ with KGM added occurred at 25 °C and 55 °C, which is in accordance with a previous study by Kokelaar, Van Vliet, & Prins (Kokelaar, Van Vliet, & Prins, 1996). At 25 °C and 55 °C temperature, KGM is less likely to form a gel network nor connect to protein via covalent bonds. It is more possible that the KGM exists as independent polymer chains in the system, increasing

the overall viscosity of mixture compared to pure gluten. When heating at 75 °C and 95 °C, hydrogen bonds got weaker. This might cause a partial disruption of original structure of polysaccharide, leading to a more extended form KGM. The extended KGM could interact with protein molecules and reduce the flexibility of protein molecules. More sliding friction between polymer chains could reduce energy dissipation, storing more energy in the strains and making them more elastically.

3.5 Morphology

SEM images of gluten with or without KGM subjected to variable temperatures are shown in Fig. 5. Gluten remained homogeneous yet discontinuous at 25 °C (Fig.5A) and started to show a sign of hollow network at 55 °C (Fig. 5C). In comparison with control, 5.0% KGM gluten went through severe fragmentation at 25 °C, which might be ascribed to the influence of KGM on disulfide bonds that could be cleaved and rearranged by thiol/disulfide exchange reactions in delaying protein cross-linking.

The network frame was less compact with the mesh size of the network increased compared to that at 55 °C, possibly because heating induced an intensified hydrophobicity of the glutenin surface. The layer thickness of samples with KGM increased with many large interconnected holes being formed, indicating a high accessibility of water to the amorphous regions of gluten network. KGM molecules could cause a change in conformation that resulted in the improvement of the water-holding capacity of samples and large pores resulted from ice crystal formation. This result showed that the hydrogen bonding was involved in the changes of system (Wen, Cao, Yin, Wang, & Zhao, 2009). Irregular lamellar domains were observed in Fig. 5G and Fig. 5H, since the degree of disulfide crosslinking strongly increased at the highest temperature.

4. Conclusion

The present study attempts to reveal the effect of KGM on heat-induced changes in gluten protein at different levels. The addition of KGM affects protein aggregation depending on heating temperature and concentration. Although KGM seems show an enhancing effect on thermal denaturation of gluten at moderate heating temperature (55 °C), it attenuates heat-induced aggregation at higher heating temperature (above 75°C). These contrary effects are possibly due to different driving forces of protein denaturation and different KGM status at various temperatures. The hydrophobic interaction of gluten peaks at around 55 °C and mainly contributes to the aggregation of protein molecules. However, as heating temperature increases, the rupture of hydrogen bonding and exchange reaction of disulfide bonding seems to be more pronounced. At moderating heating temperature, KGM could influence the denaturation of gluten by altering the viscosity of system and/or compressing the hydrophobic core of proteins via its hydrophilic groups. At higher heating temperature, heat-induced extended KGM chains might limit the flexibility of the protein molecules more efficiently, protecting original protein structure. Also, KGM could possibly interfere the disulfide exchange reaction with its hydroxyl groups, preventing the formation of irregular protein structures. It should be noted that this study has primarily provided circumstantial evidence for KGM affecting via hydrophobic interaction and hydrogen bonding upon heating. More efforts in the future may be needed to put on measuring changes in amount and strength hydrophobic interactions and hydrogen bonds formed with KGM at different heating temperature.

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539 **Figure captions**

540 **Fig. 1** Effect of KGM on secondary structure content of gluten: A (α -helix), B (β -
541 sheet), C (β -turn), D (random coil).

542 **Fig. 2** Effect of KGM on the thiol content upon heating: A (accessible thiol (SH_{free})),
543 B (total thiol equivalent (SH_{eq})).

544 **Fig. 3** Mechanical spectrum for gluten-KGM dough heating at different temperatures:
545 A (25 °C), B (55 °C), C (75 °C), D (95 °C).

546 **Fig. 4** Curves of $\tan \delta$ for gluten-KGM dough heating to different temperatures.

547 **Fig. 5** Scanning electron micrographs of the heated gluten with 0% and 5.0% KGM.

548 **Supplementary Fig. S1** FTIR amide I band of different gluten samples at different
549 temperature: A (25 °C), B (55 °C), C (75 °C), D (95 °C).

550 **Supplementary Fig. S2** Effect of KGM on α -helix/ β -sheet content ratio.

551 **Supplementary Fig. S3** SDS–PAGE pattern of protein from gluten: A (25 °C), B
552 (55 °C), C (75 °C), D (95 °C). Lanes: S, Molecular weight standard; (1) control
553 gluten; (2) 1.0 % KGM gluten; (3) 2.5% KGM gluten; (4) 5.0% KGM gluten.

1 **Table 1**

2 Slope value^a obtained from the regression lines of elastic modulus versus frequency
3 for gluten dough in the presence of varying concentrations of KGM.

KGM level (%)	25 °C		55 °C		75 °C		95 °C	
	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
0.0	3.63	0.2515	3.38	0.1954	3.35	0.1647	3.37	0.1287
2.5	3.61	0.2654	3.34	0.2014	3.35	0.1470	3.43	0.1387
5.0	3.53	0.2737	3.28	0.2033	3.31	0.1384	3.34	0.1142

4

5 ^aSlope of log G' vs. log frequency.