Konjac glucomannan-induced changes in thiol/disulphide exchange and gluten conformation upon dough mixing

Yun Zhou a, Dan Zhao a, Tim J. Foster b, Yixin Liu a, Yu Wang a, Satoru Nirasawa c, Eizo Tatsumi c, 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
c Japan International Research Center for Agricultural Sciences, Tsukuba 305-8686, Japan

1. Introduction

Wheat gluten is unique for conferring cohesive and viscoelastic properties to wheat dough, which is responsible for the ability to process wheat flour into a range of wheat products including bread, pasta and noodles. Gluten comprises monomeric gliadins, which interact mostly by non-covalent interaction and intrachain disulphide bonds, and polymeric glutenins consisting of high and low molecular weight subunits (HMW and LMW, respectively) stabilised mainly by hydrophobic interaction and interchain disulphide bonds (Köhler et al., 1993). Gliadins take the role to act as plasticizers for glutenins, and thus increase the viscosity of gluten network and decrease the high levels of elasticity conferred by glutenins. Water is necessary for gluten proteins to fully hydrate and plasticize during dough formation, therefore contributing to the supramolecular organisation of dough structure. Shear-triggered continuous exchange between sulfhydryl groups and disulphide bonds happened during mixing as well (Morel, Redl, & Guilbert, 2002). Presumably, the strain from mechanical stress of mixing is relieved by transformation of highly cross-linked chains to less branched systems. Different degrees of depolymerisation and re-polymerisation of wheat gluten taking place during the mechanical action of mixing are likely due to differences in susceptibility of disulphide bonds to cleavage (Lindsay & Skerritt, 1999; Weegels, Pijpekamp, Graveland, Hamer, & Schofield, 1996).

Dietary fibres have aroused particular attention in recent years as they are not enzymatically degraded within the human alimentary digestive tract and demonstrated to be effective in weight reduction, modification of intestinal microbial metabolism, and cholesterol reduction. An increase in consumption of dietary fibres was recommended in most European countries, where cereal products constitute the major source of dietary fibre (Wang, Rosell, & Barber, 2002). However, addition of dietary fibre changes dough rheological behaviour and impairs dough-handling properties to a large extent. The deleterious effects of dietary fibre on wheat dough formation have been attributed to a physical interfering effect and the water competition between fibres and gluten (Kohajdová et al., 2012). Ferrer et al, 2011 observed wheat gluten depolymerisation in the presence of lignin and demonstrated lignin impaired gluten cross-linking during mixing. The degree of the disruption of the continuity of the protein matrix increased with the amount of fibres increasing (Bock & Damodoran, 2013; Tudorica, Kuri, & Brennan, 2002).

Konjac glucomannan (KGM) is a water-soluble, non-ionic polysaccharide, extracted from the tubers of the Amorphophallus konjac plants. It is mainly composed of β-1,4-linked α-mannosyl and β-glucosyl residues at a molar ratio of 1:6:1 (Maeda, Shimahara,
& Sugiyama, 1980; Katsuraya et al., 2003). KGM has been used as dietary supplement for the management of metabolic disorders and also used for modifying characteristics of wheat products (Chua, Baldwin, Hocking, & Chan, 2010; Sim, Noor Aziah, & Cheng, 2011; Zhou et al., 2013). In our previous study, it was found that the substitution of low-protein wheat flour with more than 3% KGM can result in negative changes in noodle quality, including loss of continuous sheet-like structure in gluten network microstructure and deterioration in textural properties of noodles (Zhou et al., 2013), which might be related with deleterious effects of KGM on gluten network formation. Thousands of hydroxyl groups presented in the KGM structure allow more water interaction through hydrogen bonding according to Silva et al. (2013). The hydrophilic character of KGM was expected to modify water distribution when it was incorporated into the dough (Bock & Damodaran, 2013). Competitive water binding by KGM was supposed to be the major factor affecting dough mixing properties (Sim et al., 2011). If KGM causes redistribution of water in dough, it is reasonable to expect conformational changes in gluten as a consequence in a way related to the chemical structure of KGM.

The objective of the present work was to determine the underlying mechanisms responsible for properties of doughs incorporated with KGM. To investigate the protein–KGM interaction upon gluten network formation, we monitored the time-varying changes in thiol and disulphide groups during dough mixing. Protein subunits of gluten from developed doughs were analysed by SDS–PAGE. Fourier transform-Raman was employed to investigate the molecular conformation of gluten proteins. The results allowed us to make correlations between molecular interaction among KGM and gluten in order to understand macroscopic properties of KGM dough.

2. Materials and methods

2.1. Materials

Commercial wheat flour ('Great value' brand) was purchased from De Jiafu Flour Mills (Guangdong, China). Analysis (moisture, ash, protein and wet gluten) of wheat flour was performed according to approved methods of AACC International, 2000 (methods 44–15, 08–01, 46–13 and 38–12, respectively) in triplicate and the results were expressed as average. The contents of moisture, ash, crude protein and wet gluten were 11.95 g/100 g, 0.69 g/100 g, 8.02 g/100 g, and 19.58 g/100 g (dry basis, w/w), respectively. Konjac glucomannan (KGM ≥ 95%) was supplied by Yulian Biotechnology Co., Ltd. (Hubei, China).

2.2. Farinographic assay

Flour and KGM blends were thoroughly pre-mixed. The farinographic assays were conducted following the AACC standard method (2000) in the Brabender equipment (Duisburg, Germany). KGM substitution levels used were 1%, 2%, 3%, 4% and 5% of the mixed blends. Water absorption (WA, percentage of water to yield dough consistency of 500 BU), dough development time (DDT, time to reach maximum consistency), dough stability (DS, time dough remains at a consistency of 500 BU) and degree of softening index (DSI, difference in BU between the peak time point and 12 min after peak time is reached) were recorded. The results were expressed as average ± standard deviation.

2.3. Dough preparation

Flours were thoroughly dry-blended with KGM. Flour–KGM blends of 200 g were mixed in a small scale kneader (Kenwood, UK) with planetary kneading action over a 30 s interval at the mini speed setting for 8 min. Adapted water amounts were determined based on each farinograph water absorption value accounting for the higher water absorption induced by incorporating KGM. For SDS–PAGE analysis and thiol/disulphide contents determination, doughs prepared were immediately frozen at −80 °C. Frozen samples were lyophilised, grounded, and sieved to pass through a 250 μm mesh screen. Dried powders were put into hermetic plastic bags and kept in a drier.

2.4. SDS–PAGE

Protein fractions were extracted from freeze-dried dough in a sequential extraction described by Linlaud, Ferrer, Poppe, and Ferrero (2011) with some modifications. Gliadins were extracted from freeze-dried dough (0.5 g) with 1.5 mL of 50% (v/v) 1-propanol (Solution A), heated at 65 °C for 30 min with constant shaking, and centrifuged for 10 min at 8900×g. Supernatants were collected and kept at 4 °C. For glutenin extraction, the precipitate was washed three times with Solution A, disregarding the supernatants. 50% (v/v) 1-propanol containing 0.08 M Tris–HCl buffer (pH 8.0) (Solution B) containing dithiothreitol (DTT) [1% (w/v), mixed immediately before use] was added (0.4 mL) to the former residue, incubated for 30 min at 65 °C. 0.4 mL of solution B containing 4-vinylpyridine (1.4% w/v) was added and vortexed thoroughly, followed by incubation at 65 °C for 15 min and centrifugation at 8900×g for 5 min. Supernatants were transferred to another microtube and used for glutenin characterisation by SDS–PAGE.

For electrophoresis runs, supernatants of 1 mg/mL gliadin and 3 mg/mL glutenin were mixed separately with running buffer solution (1:1) composed of 0.4% (w/v) SDS, 12% (w/v) glycerol, 50 M Tris–HCl buffer (pH 6.8), 2% (v/v) mercaptoethanol and 0.01% (w/v) Bromophenol Blue. Suspensions were heated in a boiling water bath for 1 min and allowed to cool, followed by centrifuge at 3950×g for 10 min. Supernatant of 10 μL was for loading into 12% and 10% poly acrylamide gels. SDS–PAGE analysis was carried out on Mini-PROTEAN Tetra horizontal electrophoresis system (Bio-Rad Laboratories, USA). Electrophoresis was run at a constant voltage (200 V) for about 0.5 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 colouring solution containing 5% acetic acid (v/v), 45% methanol (v/v), and 0.25% Coomassie Blue R (w/v). The same solution without the colourant was used for discolouring the gels overnight.

2.5. Accessible thiol and disulphide contents determination

Doughs mixing for 2, 4, 6, 8, 10 and 12 min were processed as previously described in dough preparation for the measurements. Contents in accessible thiol (SH_{eq}) and disulphide groups (SS) were assayed as described elsewhere (Morel et al., 2002). Briefly, for the determination of accessible thiol (SH_{eq}), 100 mg of ground freeze-dried dough was shaken for 10 min with 5.5 mL of Ellman reagent [propan-2-ol, 250 mM Tris–HCl buffer (pH 8.5) and 4 g/L 5,5-dithiobis-2-nitrobenzoic acid in ethanol (5/5/1, v/v/v)]. After centrifugation (6 min, 15,800×g), absorbency of the nitro-thiobenzoate anion (NTB-) was measured at 412 nm (ε = 13,600 M^{−1} cm^{−1}). For measurement of the total thiol equivalent groups (SH_{eq}), 30 mg of sample was exhaustively reduced with dithioerythritol (40 mM DTE in 80 mM Tris/HCl, pH 8.5, 1.3 mL) for 2 h at 60 °C, before adding 3 mL of glacial (−18 °C) acetic acid (100 mM) in aceton, to precipitate and stop the reaction. Samples were centrifuged at 959×g for 4 min at 4 °C. The precipitates were suspended in 300 μL of acetic acid (100 mM), before being precipitated again with 3 mL of acetone acid (see above) and centrifuged.
2.6. Fourier transform (FT)-Raman spectroscopy

Considering moisture might influence dough formation, dough preparations were conducted under 55% and 81% water absorptions. Gluten samples were prepared by an automatic gluten washing apparatus Glutomatic 2200 (Perten, Sweden) according to approved methods of AACC (2000) 38-12A, immediately frozen at −80 °C and lyophilised. Raman spectra of dried gluten samples were collected as previously described by (Ferrer et al., 2011) with small modifications.

Raman spectroscopy was performed with a Nicolet Nexus 670 FT-IR system with Raman attachment (Thermofisher, USA). The excitation wavelength was 1064 nm from a Nd:YAG laser with a laser power of 370 mW. Each spectra was vector normalised in the whole range from 100 to 3700 cm⁻¹. Average of 256 scans at 4 cm⁻¹ resolution in a reflective mode were collected from each sample. The collected spectra were baseline-corrected and analysed using OMNIC software provided with the Nicolet 670.

2.7. Statistical analysis

The results were statistically analysed 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. Farinographic behaviour of KGM-flour mixture

Table 1 shows results of farinographic assays registered in the farinogram. A great increment in water absorption was produced by the addition of KGM. The highest absorption was found with the substitution of 5% KGM. A similar effect of KGM on water absorption at a much lower addition level of KGM was observed (Sim et al., 2011). Much higher amount of water is required to reach 500 FU in the presence of KGM, even though the water uptake was affected by swelling of gluten network, suggesting that KGM might take the dominating role in water absorption due to its strong water binding capacity. The great number of hydroxyl groups in the KGM structure increases the monolayer moisture capacity and leads to a more solid water combination (Yan et al., 2012). The change in the distribution of water might prevent gluten hydration and apparently decrease dough consistency. Dough development time (DDT) reflecting the resistance of the dough against the blades was also strongly affected by incorporating KGM. DDT was increased by 426.7% at a substitution level of 5% KGM, indicating that KGM impeded typical gluten matrix formation subject to shear stress action. Dough stability (DS) and degree of softening index (DSI) give an indication of dough strength. In the case of KGM doughs, DS was also increased in the substitution level from 1% to 4%, showing an enhanced resistance to successive mixing and an improved capacity to sustain shear stress of KGM doughs, while DSI was much higher than the control indicating that KGM consequently weakened the wheat doughs.

2.3. SDS-PAGE

To understand the role of KGM on wheat protein fractions of dough, SDS-PAGE was carried out which allows a rough correlation between the physicochemical properties of proteins, as inferred from their aggregation state and their behaviour during dough mixing. Fig. 1A shows the electrophoretic profiles for the gliadins extracted from control dough and dough prepared in the presence of 5% KGM. No high molecular mass proteins (>97 kDa) was extracted from doughs with or without KGM. This result of the present study was not in accordance with Linlaud et al. (2011), who indicated aggregation could be promoted by other hydrocolloids including xanthan gum and highly methoxylated pectin through non-cova lent interactions. This difference might be due to the anionic groups like pyruvyl residues and carboxyl groups present in the primary structure of the polysaccharides are involved in the interaction, bringing the overall net charge to zero and thus promoting aggregation in the dough by incorporating negative charges into the complex; however, KGM with no anionic groups in the giving dough system could not exert such effect. Within the range of Mr. values, from 30 to 45 kDa, a defined group of bands was observed in all samples, corresponding to α-, β-, and γ-gliadins (Linlaud et al., 2011). Below 20 kDa, several intense bands corresponding to fast moving (possibly albumin-like) components were observed. No differences in the profile of gliadins and band intensity were detected among samples, indicating that wheat protein fractions were unaffected including KGM polysaccharide in the gluten network.

The profile of glutenins extracted with the reducing agent (DDT) is shown in Fig. 1B. In the KGM substitution samples, a depolymerisation effect can be observed since lesser proportion of aggregates (>120 kDa) was detected. It can be concluded that the presence of KGM during the gluten network formation can hinder glutenin aggregation. Suggesting KGM might promote the formation of a weak gluten network probably due to the rupture of intermolecular cross-linking that stabilised the polymeric glutenin polymers. As the extent of protein aggregation in the form of polymeric glutenins is highly correlated with mechanical properties of dough (Weegels et al., 1996). It could be hypothesised that this effect of KGM reflects distinct farinographic dough stability (DS) of KGM doughs. No difference in the molecular size distribution in glutenin profile was evidenced. This may indicate that KGM is impossible to change the polymeric structure resulted from selectively interacting with gluten proteins through covalent bonds. However, we could not rule out the possibility that KGM could interact with other gluten proteins in alcohol insoluble complex.

3.3. Changes in thiol and disulphide groups during dough mixing

Fig 2 shows the effect of KGM on the contents of accessible thiol (SHfree) and disulphide bonds (SS) during dough mixing performed under different mixing time. The replacement of wheat flour with KGM tended to increase the concentration of SHfree at both 2.5% and 5.0% KGM level (Fig 2A). A strong increment in the gluten free thiol content during mixing was also observed in native gluten (control), similar to the results achieved by Morel et al. (2002).
indicating shear stress action could not be excluded for releasing buried thiol groups in native gluten, probably by increasing the catalysis of thiol/disulphide exchange (Auvergne et al., 2008). In 2.5% KGM–gluten, the increase in $S_{\text{free}}$ was obviously slower than that of the control. The tendency is different from that of the control with increasing KGM supplement to 5%. The content of $S_{\text{free}}$ decreased by 15.1% during the mixing from 2 to 12 min, suggesting the mixing incorporated with KGM might not only comply with the catalytic mechanism. Radical and nucleophilic mechanisms have been proposed to play important role in interchain disulphide bonds induced gluten aggregation (Auvergne et al., 2008). Active hydroxyl groups on $\alpha$-glucose and $\alpha$-mannose in KGM molecules reacting through a radical scavenging effect were probably able to partly prevent the accumulation of radical species formed on protein during disulphide exchange to alleviate protein molecule aggregation during dough mixing.

Compared with free thiol groups (Fig 2A), much more disulphide bonds (SS) are presented in gluten (Fig 2B) suggesting intermolecular disulphide bonds probably came from intramolecular and intermolecular disulphide bond exchanges, catalysed by the presence of a small amount of thiol groups. Fig 2B shows significant differences between KGM samples and the control in disulphide bonds cleavage and bonding reflected in disulphide bond contents. Lower degree of aggregation made disulphides more susceptible to exposure to reducing agent (Cecil & Wake, 1962). Additionally, the interchain disulphide bonds are more readily cleaved than intrachain bonds which are likely to be buried into the core of the native molecule, being protected from chemical reduction (Morel et al., 2002). The disappearance of glutenin aggregates ($>$120 kDa) in SDS–PAGE electrophoresis (Fig 1) led us to suppose disulphide bond concentration might be even lower despite of the protein depolymerisation in the presence of KGM. In KGM samples, disulphide bonds (SS) content peaked at 4 min, while in the control SS content decreased constantly. Farinographic behaviour based largely on cohesive elastic character of hydrated wheat glutenin could be probably affected by the reversible increment in SS bonds which could produce a viscous structured supramolecular cross-linking. Fig 2B shows excessive mixing led to sharp declines in SS concentration suggesting a negative effect of shear stress action on accessibility of disulphide bonds. From Fig2B, during the mixing of 10 min (begin sampling from 2 to 12 min), the control decreased by 17.6% in disulphide bonds, while 2.5% KGM and 5% KGM samples decreased by 11.2% and 7.6%, respectively. When KGM was mixed with gluten, shear stress induced accessibility reduction of disulphide bonds was weakened to a certain extent, suggesting special arrangements of disulphides might be changed. Therefore, there is possibility that KGM might be involved in the protein tertiary structure alternation to account for dissimilar thiol/disulphide exchange.

3.4. Effect of KGM on protein structure

3.4.1. Backbone conformation

Generally, proteins with a high proportion of $\alpha$-helix show an amide I band at 1650–1658 cm$^{-1}$ and amides III band at 1260–1300 cm$^{-1}$; proteins with predominantly high $\beta$-sheet contents

**Fig. 1.** Effect of KGM on the contents of accessible thiol ($S_{\text{free}}$) and disulphide bonds (SS) during dough mixing.

**Fig. 2.** SDS–PAGE pattern of protein from wheat dough: (A) gliadins and (B) glutenins. Lanes: S, Molecular weight standard; (1) Control dough; (2) 1% KGM dough; (3) 2% KGM dough; (4) 3% KGM dough; (5) 4% KGM dough; (6) 5% KGM dough.
show an amide I band centred at 1665–1680 cm\(^{-1}\) and an intense amides III band at 1225–1235 cm\(^{-1}\); proteins containing high contents of random coil structures have an amide I band close to 1660–1665 cm\(^{-1}\) and an amide III transition near 1250 cm\(^{-1}\); the region between 1625 and 1600 cm\(^{-1}\) has been attributed to intermolecular β-sheet in prolamins (Beattie, Bell, Farmer, Moss, & Patterson, 2004; Wellner et al., 2005; Wong, Phillips, & Ma, 2007).

Fig. 3A shows the spectra obtained from samples that were subjected to different KGM additions by application of two water absorption level. Secondary structure of gluten has been described previously as vast majority of proteins are in the α-helical conformation (Wong et al., 2007) and union of hydrocolloids to gluten would lead to conformational changes to a certain extent without changing the dominating role of α-helix (Linlaud et al., 2011). However, these conclusions are quite suspected because the major protein peaks showed KGM and water dependent changes in the shape or in the position of the bands (Fig 3A).

In the case of the lower water absorption level, native gluten (0% KGM) centres at 1656 cm\(^{-1}\) with a broad shoulder in the region of 1660–1680 cm\(^{-1}\) contributed to random coil and β-sheet. Peaks at 1608 cm\(^{-1}\) and 1625 cm\(^{-1}\) were observed of much less intensity. KGM addition cause a sharp decrease in the intensity over the range 1650–1658 cm\(^{-1}\) where the contribution of α-helix is dominant and an increase of the intensity around 1670 cm\(^{-1}\) where the contribution of β-sheet is dominant. Addition of larger amount of KGM to the dough probably forced gluten to adopt β-sheet at the same water absorption level, suggesting the extent of this conversion in the presence of KGM is likely to be inversely related to the moisture content of the dough by associating with the strong water binding capacity of KGM.

A more pronounced shoulder around 1618 cm\(^{-1}\) was observed in Fig 3A, showing an increase in intermolecular β-sheet. The formation of intermolecular β-sheet structures in gluten may be attributed to the behaviour of HMW which associates to form fibrils and sheets as previously shown by Fourier transform infrared (FT-IR) studies (Pézolet, Bonenfant, Dousseau, & Popineau, 1992). The formation of intermolecular β-sheet as evidenced from Fig 3A is consistent with the predictions of the loop and train model (Belton, 1999). This model postulates as water is added, there will be an increase in the number of water–protein hydrogen bonds formed, in balance with the residues decreased the involvement in interchain hydrogen bonds indicated in the decreasing amount of intermolecular β-sheet (Belton, 1999).

The huge number of amino acid residues ensured that it was unlikely that all the water–protein hydrogen bond be broken simultaneously. However, KGM addition decreased water availability due to the physicochemical nature of KGM which hydrates quickly. Thus, the long chains of HWM subunits tended to hydrogen bond to each other to form intermolecular β-sheet, which was expected to be more ordered and compact, and might inhibit the interaction in microenvironment. In such a model, the KGM combination with gluten proteins would result in a modification of their spatial configuration, making impossible their further cross-linking through disulphide bonds, as the resulting chain folding did not permit great accessibility of sulphhydrils to disulphides.

The differences observed between samples achieved from 55% and 81% water absorption were essentially dissimilar in the conversion of α-helix into β-sheet and random structures found in 55% water absorption. Gluten at higher water absorption of 81% cause a transition to a more chaotic conformation characterised by shift of peaks at 1600–1625 cm\(^{-1}\), implying the structure of gluten was looser, allowing for the better hydration of protein molecules.

3.4.2. disulphide stretch

The disulphide S–S stretch is visible in the region 500–550 cm\(^{-1}\), which give an approach to study structural change for disulphide band. Depending on the different conformations of the C–S–S–C atoms, the 500–550 cm\(^{-1}\) have been assigned to gauche-gauche-gauche-gauche conformation (510 cm\(^{-1}\)), gauche–gauche–trans (525 cm\(^{-1}\)) and trans–gauche–trans (540 cm\(^{-1}\)), respectively (Gniadecka, Nielsen, Christensen, & Wolf, 1998).

In the spectra of control, the S–S band was prominent and was located at around 510 cm\(^{-1}\) (Table 2), indicating that these bonds are primarily in the gauche–gauche–gauche–gauche conformation; however, a shoulder at 540 cm\(^{-1}\) indicated that some proportion of S–S bonds are in the trans–gauche–trans conformation (spectra not shown). The position of the S–S band around 540 cm\(^{-1}\) in 2.5% KGM gluten under 55% and 68% suggests a less stable conformation, trans–gauche–trans, of protein in 2.5% KGM gluten. Increasing water absorption to 81%, peak position shifts to around 510 cm\(^{-1}\), which implies trans–gauche–trans can be transformed to a more stable conformation under high extent of hydration. According to a survey of X-ray evidence, the great majority of cystine linkages in proteins are in a gauche–gauche–gauche S–S bonds correlate directly with the resistance of the protein to denaturation (Aoki et al., 1982). Similarly, the comparably high stability of native gluten in presence of denaturation factors could be mainly mediated by gauche–gauche–gauche conformation. In 5% KGM gluten, the S–S band was found at 526 cm\(^{-1}\), 541 cm\(^{-1}\) and 512 cm\(^{-1}\), corresponding to gauche–gauche–trans, trans–gauche–trans and gauche–gauche–gauche conformation. Three distinct conformational states of the C–S–S–C atoms could be explained by the disordered polypeptide chain displacement seen in abnormal protein folding and subunits aggregation which contribute directly to the unstable structure of gluten.

Combined with results of SH/SS exchange during dough mixing, it is conceived that KGM affects disulphide bonds in both content

![Fig. 3. FT-Raman amide I bands of gluten samples: (A) 55% water absorption and (B) 81% water absorption.](image-url)
and conformation and these effects are related with hydration level to a large extent. Compared with native gluten, not only is the total number of crosslinks mediated by disulphide bands reduced but conformation is also less stable.

3.4.3. Side chain vibrations

Microenvironment of tyrosine is reflected by the ratio of the tyrosyl doublet around 850 and 830 cm\(^{-1}\) (\(I_{850/830}\)), a good indicator of the hydrogen bonding of the phenolic hydroxyl group (Herrero, 2008). The tyrosyl doublet ratio has been proposed for determining if the tyrosine residue is exposed or buried (Herrero, 2008).

As KGM added to doughs of lower water absorption at 55%, there was a decrease of the \(I_{850/830}\) values in comparison with the control gluten thought \(I_{850/830}\) values are higher than 1 (Fig. 4). Tyrosine side chains were exposed on the surface of protein under low moisture condition and with KGM addition the extent of exposure was alleviated. Tyrosine is one of medium polar amino acids, which are not promoted the formation of a strong intermolecular hydrogen bonding system. Involvement of phenolic hydroxyl group in the system could explain that tyrosine is subject to be exposed in a significantly decreased extent under KGM hydrogen bonding conditions. These results echo a previous discovery that significant alterations in polarity around tyrosine residues was decreased and the hydrophobicity was increased as evidenced in a slight increase in the case of 2.5% KGM–gluten.

4. Conclusion

The present work attempts to clarify the KGM–gluten interaction responsible for farinographic properties of doughs incorporated with KGM. Addition of KGM affects conformational behaviour of gluten depending on water absorption of flours during dough formation, but does not affect molecular size distribution of wheat protein fractions. In native gluten, majority of proteins were in the \(\alpha\)-helical conformation and disulphide bonds are in the gauche–gauche–gauche (g–g–g) conformation. However, \(\beta\)-sheet became a major secondary structure at the expense of \(\alpha\)-helix in gluten and disordered conformation of disulphide bonds upon KGM treatment were obtained in incomplete hydrated state as demonstrated by FT-Raman. Indeed, determination of thiol and disulphide groups during dough mixing indicated KGM molecules are probably able to partly affect the accumulation of free thiol during disulphide exchange and alleviate disulphide bonds cleavage under continuous shear stress action. In general, KGM could not promote covalent bound units formation from gluten proteins, but the hydroxyl groups of KGM are involved in the interaction with gluten by forming strong intermolecular hydrogen bonding system which lead to flexible gluten conformation and therefore, result in distinct rheological behaviours of doughs.

Acknowledgements

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References


Table 2

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<tr>
<th>KGM (%)</th>
<th>Water absorption</th>
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<tr>
<td></td>
<td>55%</td>
</tr>
<tr>
<td>Control</td>
<td>509 (506–511)</td>
</tr>
<tr>
<td>2.5%</td>
<td>542 (540–545)</td>
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<tr>
<td>5.0%</td>
<td>526 (524–528)</td>
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Peak position, mean with 95% confidence intervals (cm\(^{-1}\)).

Fig. 4. FT-Raman normalised intensity of intensity ratio of tyrosine bands (\(I_{850/830}\)) of gluten samples. Bars on columns indicate standard deviation. Different letters superscripted on the columns indicate significant difference (P < 0.05).