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

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ABSTRACT

Effects of konjac glucomannan (KGM) on the changes in gluten upon dough mixing were investigated in this study. Wheat flour was blended with KGM and processed into dough. Farinographic analysis showed that KGM caused a significant increase in water absorption and dough development time to reach maximum consistency. Comparison of electrophoretic protein profile from control dough and KGM-dough revealed that protein fractions were similar in molecular size distribution, but the lability of glutenin aggregates slightly differed. Addition of KGM to gluten induced negative effects on exchange between sulfhydryl groups and disulphide bonds. Fourier transform-Raman spectroscopy indicated that secondary structure of gluten proteins was differentially modified related with water absorption of flours before dough formation. This study reveals that when KGM is added to the dough, conformational behaviours of gluten proteins are changed and the hydroxyl groups of KGM might be involved in the interaction by forming strong intermolecular hydrogen bonding system.

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 glutenin 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, 2011observed 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 & Damodaran, 2013; Tudorica, Kuri, & Brennan, 2002).

Konjac glucomannan (KGM) is a water-soluble, non-ionic polysaccharide, extracted from the tubers of the *Amorphaphallus* konjac plants. It is mainly composed of β-1,4-linked D-mannosyl and D-glucosyl residues at a molar ratio of 1.6:1 (Maeda, Shimahara,

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& 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 $\geqslant$ 95%) was supplied by Yuanli 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, Puppo, 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% v/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 9% 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_{free}) and disulphide groups (SS) were assayed as described elsewhere (Morel et al., 2002). Briefly, for the determination of accessible thiol (SH_{free}), 100 mg of ground freeze-dried dough was shaken for 10 min with 5.5 mL of Ellmann 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 (SHeq), 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 acetone, 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.