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The effect of sodium chloride on gluten network formation and rheology

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ABSTRACT

Gluten samples were obtained from two wheat flours with different levels of total protein in the presence or absence of sodium chloride (2% flour base). The dynamic oscillation rheology, large extensional deformation, confocal laser scanning microscopy (CLSM), transmission electron microscopy (TEM) and chemical analysis of disulfide bond linkages and the ratio of polymeric glutenins and monomeric gliadins were used to investigate the effect of salt on the structure and rheological properties of gluten. CLSM and TEM images showed that NaCl caused the gluten to form fibrous structure. The presence of NaCl increased non-covalent interactions and β-sheet structure, measured by FTIR, in gluten proteins. The gluten matrix formed with salt resulted in higher tan δ values corresponding to a less elastic network when measured using oscillatory rheometry. Large deformation extensional measurements showed that the maximum force to fracture were lower for the gluten samples prepared in the presence of NaCl. The results from this study indicate that changes in the solvent quality due to the presence of NaCl during dough mixing result in different molecular conformation and network structure of gluten proteins which contributed to the differences in the rheological properties.

1. Introduction

Wheat is a very important cereal crop for human food production. This reflects the unique ability of wheat flour to produce a viscoelastic dough when it is mixed with water. The principal characteristic that governs the wide usage of wheat flour is the viscoelasticity of the gluten network formed by its major protein fractions: the polymeric glutenins and the monomeric gliadins. The rheological properties of the gluten protein network, their impact on handling properties of the dough during processing and the resultant end-product quality, depend on a number of factors. These include the quantity and quality of the gluten proteins, their structure at the molecular level, conformational re-arrangements effected by the solvent environment upon hydration as well as physical strain induced by mechanical shear as mixing proceeds (Delcour et al., 2012).

The glutenin and gliadin components have different roles within the gluten matrix. Glutenin proteins consist of two subunit populations: high molecular weight glutenin subunits (HMW-GS) which have apparent molecular weights in the range of 80,000–120,000 Da and low molecular weight glutenin subunits (LMW-GS) with weights of 40,000–55,000 Da (Shewry et al., 2002). Gliadin proteins are monomeric and classified as ω-, α/β-, and γ-gliadins based on their mobility in electrophoretic gel systems and their amino acid sequences (Shewry et al., 2002). Based upon the particular characteristics of the glutenin and gliadin fractions, various models have been developed to describe the unique structure of the gluten network as well as its viscoelastic properties.

The HMW-GS and LMW-GS together form a disulfide cross-linked protein polymeric network thereby contributing to the strength and the elastic properties of the gluten and the dough (Shewry et al., 2002). Both intra- and inter-molecular disulfide bonds are involved contrasting with the structure of the gliadins, which have only intra-molecular disulfide linkages. Gliadins do not contribute to the protein matrix formation, but they interact with the glutenin structures via non-covalent bonds and thus affect the viscous properties of dough (Shewry et al., 2002). However, this structural model with the disulfide cross-links cannot fully explain the observed elasticity of gluten. The existence of some cross-linking may explain the resistance to extension but does not explain the elasticity. Gluten proteins contain a high level of glutamine residues which has a very high capacity to form both intra- and inter-molecular hydrogen bonds. This may contribute to elasticity through the formation of inter-molecular hydrogen bonds (Belton, 1999). In addition, like any other proteins, the structure of

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the gluten proteins and their interactions can also be affected by solvent environment upon hydration, including the presence of salt. This is because salt acts as a co-solute and influences the hydration of wheat proteins and starch under the particular moisture conditions during the manufacture of a diverse range of products including bread, breakfast cereal, pasta, and Asian noodles.

There have been a number of studies regarding the effect of salts on the rheological properties of wheat flour dough (Butow et al., 2002; He et al., 1992; Kinsella and Hale, 1984; Preston, 1989; Salovaara, 1982). Empirical rheology of dough systems has shown that salt generally increases dough development time, resistance to extension and dough extensibility. In addition, the studies show that the effects are determined by concentration as well as the type of salts from comparisons using various compounds belonging to the Hofmeister series. In contrast, results on the fundamental rheology of doughs have shown apparently contradictory effects of salt at different concentrations upon G' values (Larsson, 2002; Lynch et al., 2009; Salvador et al., 2006). Larsson (2002) found that the G' of dough increased as salt was added in increasing concentrations. Similar results were also reported recently by Beck et al. (2012). On the other hand, Lynch et al. (2009) found decreasing G' of the dough with increasing NaCl addition. These results indicate that the effect of salt on dough rheology is relatively complex and measurement of small deformation rheology may not provide adequate explanations. This may be due to the differences in protein concentration between flours and the possible contribution of starch components which are a larger fraction of the dough than the protein (McCann and Day, 2013).

Explanations of the effect of salt on the dough rheological properties have generally been proposed on the basis of effects on the gluten proteins. When salt is present in low concentrations, it shields the charges of the gluten molecules, thereby reducing electrostatic repulsion between proteins, allowing them to associate and produce a stronger dough (Kinsella and Hale, 1984; Miller and Hoseney, 2008). At higher concentrations, salt interacts more with solvent molecules and its effect on gluten proteins is primarily determined by the type of ions present. The somewhat contradictory results on the effect of salt on dough rheology reported by various research groups are probably due to the nature of small deformation rheology which measure both protein network characteristics as well as the packing of starch granules. As the protein is a smaller proportion, the rheological properties of dough explained on the basis of the effect of salt on gluten structure may also be influenced by starch/starch and starch/protein interactions (McCann and Day, 2013).

Up until now, most of the studies have been focussed on the effects of salt on the rheological properties of wheat flour dough. However, the effects of salts on the gluten protein network at the molecular level and resulting rheological properties have not been adequately investigated. Our hypothesis is that firstly, salt affects the formation of the gluten network during the initial hydration of the gluten proteins when wheat flour is mixed with water and then the rheological properties as a result of the gluten network formed in the presence of salt. Thus, this study aimed to examine the rheological properties of the gluten in relation to the protein network formed with and without salt. Both small and large deformation rheology were applied. Structural characterisation at different length scales were carried out using microscopic techniques, FTIR and biochemical analyses.

2. Materials and methods

2.1. Wheat flour samples

Two commercial wheat flours (FSB and Redbase) were kindly provided by Allied Mills (Kensington, Victoria, Australia). The protein contents of the flours were 13.2 and 10.4% for FSB and Redbase, respectively, determined by AACC method 46-30 (AACC International, 2000). The moisture contents were 12.8 and 12.9% for FSB and Redbase, respectively, measured by AACC method 44-15a (AACC International, 2000). Chemicals used were of analytical grade and NaCl was purchased from Chem-Supply (Sydney, Australia).

2.2. Gluten washing

Preparation of water-washed gluten (WW) and salt-washed gluten (SW) was carried out according to the method previously described by Day et al. (2009). Briefly, flour (300 g) was mixed with water (180 mL) with or without 2% NaCl (flour base) in a Hobart mixer at setting 1 (63 rpm) for 2 min followed by setting 2 (111 rpm) for a further 2.5 min to form a dough. The dough was then rested for 30 min in either water or 2% NaCl solution, then washed 3 times by hand in 5 L water or salt solution (150 g/5 L). The wet gluten was then freeze-dried for 72–96 h. The freeze-dried gluten was ground to a powder using a coffee grinder and sieved through a 250 μm sieve. The gluten samples were prepared in two batches for each flour. The moisture and protein content of dried gluten powders were determined according to AACC methods (AACC International, 2000).

Fresh wet gluten samples were also obtained by mixing flour (4 g) with 2.4 mL water or 2% salt solution for 4.5 min, using a Microdough lab (Perten Instruments, NSW, Australia). The gluten sample was then washed in either water for WW gluten or salt solution for SW gluten. A proportion of the fresh wet gluten (approx. 1.5 g) was used for the rheological measurement. The remainder of the wet gluten was used for the determination of protein and moisture contents.

2.3. Rheological measurements

2.3.1. Dynamic rheological measurements

Freeze dried gluten (0.5 g) was rehydrated with 0.75 mL water or salt solution using a mortar and spatula to obtain a rehydrated gluten dough containing approximately 67% w/w water content. The gluten dough was then wrapped in plastic film and allowed to rest for 1 h. Dynamic oscillation measurements were performed on a controlled stress-strain rheometer (Paar Physica MCR 300, Messtechnik GmbH, Stuttgart, Germany), using parallel-plate geometry. Top (25 mm) and bottom plates were both serrated to prevent the sample from slipping during measurement. The gluten dough was placed between the plates and the upper plate was lowered to a fixed gap of 2 mm and allowed to rest for 10 min following loading. A purpose-built compartment with a water-saturated filter paper was used to minimize dehydration of the gluten sample during measurement (Day et al., 2009). Oscillation measurements were performed at strain values within the range of 0.01–1000% at a constant frequency of 1 Hz at 25 °C. Once the strain sweep curves were obtained, the linear viscoelastic region was determined. Further, the G', G'', and tan delta values were measured within the strain of 0.05–10%. Measurements were carried out in duplicate for each gluten preparation. Similar rheological measurements were performed on the fresh wet gluten from duplicate preparations from each flour.

2.3.2. Uniaxial extensional rheology

Uniaxial extensional rheology was carried out using a Universal Testing Machine (Instron 5546, Instron, UK) with a 100 N load cell using the modified method described by McCann and Day (2013). The gluten sample (1.0 g) was rehydrated with 1.5 mL water and rested for 30 min in plastic wrap before the measurement. The wet