



Review

Microstructure of hydrated gluten network

Vassilis Kontogiorgos*

Department of Chemical and Biological Sciences, University of Huddersfield, Queensgate, HD1 3DH, UK

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ABSTRACT

The present work reviews the microstructure of hydrated gluten network and its protein fractions i.e., gliadin and glutenin. We summarize microstructural imaging of the last forty years as observed by various techniques such as atomic force or electron microscopy. Furthermore, rheological and calorimetric modeling that provides additional information on the microstructural features of the network is also discussed. Combining most of the available evidence it is suggested that the building blocks of gluten network are *gluten-sheets* with embedded aggregates. The sheets are arranged in parallel fashion to give rise to a nanoporous ultrastructure.

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Contents

1. Introduction	2582
2. Microstructure of gluten protein fractions	2583
2.1. Microstructure of gliadins	2583
2.2. Microstructure of glutenins	2583
3. Microstructure of gluten network	2583
3.1. Microscopical observations	2583
3.2. Microstructural modeling	2584
4. Concluding remarks	2584
References	2585

1. Introduction

Wheat is one of the most important arable crops because of its great financial significance. Wheat grain is a staple food in most of the world and the special rheological properties of gluten allows wheat flour to be processed into a multitude of food as well as non-food products (Lagrain, Goderis, Brijs, & Delcour, 2010; Reddy & Yang, 2007).

Protein fractions of gluten are traditionally classified according to their solubility in alcohol or dilute acid solutions that is, gliadins or glutenins, respectively. Gliadins consist of a heterogeneous mixture of monomeric proteins while glutenins are composed of several subunits cross-linked via interchain disulfide bridges (Veraverbeke, 2002; Wieser, 2007). Interactions among these proteins define the mechan-

ical properties of gluten network and consequently its industrial performance. Modifying the strength and extend of protein-protein interactions by incorporation of oxidizing, reducing or other agents allows controlling the mechanical response of this material. Although molecular understanding of the structure of individual proteins of gluten provides useful information on the state of interactions it cannot account for the mechanical, ultrastructural and other physical properties of the three-dimensional network that is formed. Because gluten network cannot be crystallized (unlike individual proteins) to elucidate its structure with established techniques such as X-ray scattering, other methods need to be employed. For that reason, several imaging techniques, rheological and calorimetric modeling have been used in an effort to understand, predict and control the mechanical properties of this system.

The aim of the present work is to revisit the rather scattered microstructural work on gluten and its components, summarize our current understanding and suggest a model on the structural hierarchy of the microstructure of this material.

* Tel.: +44 1484 472488; fax: +44 1484 472182.

E-mail address: v.kontogiorgos@hud.ac.uk.

2. Microstructure of gluten protein fractions

2.1. Microstructure of gliadins

Gliadins are traditionally classified into three distinct groups according to their electrophoretic mobility that is, α -, γ - and ω -gliadins. Reassessment of gliadin classification based on amino acid sequence analysis distinguishes gliadins into sulfur rich (α - and γ -) or poor (ω -) prolamins (Shewry & Tatham, 1990; Shewry, Tatham, Forde, Kreis, & Mifflin, 1986). Gliadins are monomeric proteins that they interact mostly by non-covalent interactions forming transient binding partners and their role in gluten network is to act as plasticizers and reduce its elasticity. Distribution of gliadins varies depending on wheat cultivar as well as growing conditions. Generally, α - and γ -gliadins are the major components whereas ω -gliadins are found in lower amounts (Wieser & Kieffer, 2001).

Gliadin fraction has been visualized using scanning probe microscopy revealing that proteins are present as single molecules (McMaster, Miles, Kasarda, Shewry, & Tatham, 1999; Thomson, Miles, Tatham, & Shewry, 1992). α - and γ -gliadins revealed compact architecture as opposed to ω -gliadins that showed variable structures that depend on the degree of hydration (Tatham, Thomson, McMaster, Humphris, Miles & Shewry, 1999). A-gliadins, a special group of α -gliadins, are monomeric at low pH and ionic strengths but at higher values the proteins self-assemble to form a fibrillar network stabilized by physical interactions (McMaster, Miles, Kasarda, et al., 1999). Fibril formation of α -gliadins that dissociate at low ionic strength and pH and reform at high has been also observed (Kasarda, Bernardin, & Thomas, 1967) indicating that fiber formation may be the preferred ultrastructure of this particular fraction. However, mixing of α - and ω -gliadins results in phase-separated bicontinuous morphology depending on the composition of the mixtures and the conditions of mixing (McMaster, Miles, Wannerberger, Eliasson, Shewry & Tatham, 1999). Differences in the modes of interactions between α - and ω -gliadins may play key role in determining network formation and mechanical properties of gluten (Paananen, Tappura, Tatham, Fido, Shewry, Miles et al., 2006). These observations show that the complexity in the composition of gliadin fraction results in various structures depending on the specific set of circumstances leading up to visualization. Although visualizing individual proteins gives valuable information, the direct extrapolation from single protein solutions to interacting mixtures of proteins should be regarded with trepidation. This is because all different sample preparation and imaging techniques show strong evidence that gliadins interact at molecular level with each other *via* physical forces mostly hydrogen and ionic interactions. Furthermore, immunocytochemical localization of gluten proteins revealed that gliadins are scattered throughout the network filling the space around glutenin polymers (Lindsay & Skerrett, 2000). Consequently, in hydrated gluten network the extent and strength of interactions between gliadins as well as gliadins and glutenins will greatly influence the structuring and the mechanical properties of the network. In a more recent study, the gliadin fraction has been visualized by transmission electron microscopy using ambient and subzero temperature-embedding protocols to obtain information of the morphology of gliadin assemblies over a range of temperatures. Gliadin structures appeared to be continuous without evidence of a fibrous protein assembly in all specimens even at high magnifications (Jiang, Kontogiorgos, Kasapis, & Goff, 2008) something that has been also observed in other gliadin preparations (Shomer, Lookhart, Vasiliver, & Bean, 1998). It appears that there is a critical transition in the morphology of assembled gliadins as we pass from the molecular to a higher level of structure. It is not known at the moment what are the specific conditions that influence the transition from fibrillar assemblies to continuous sheet-like structures. However, identification of this mechanism and control of this transition could lead to engineering new material with novel physical properties.

2.2. Microstructure of glutenins

Glutenin fraction consists of high and low molecular weight subunits (HMW, LMW, respectively) cross-linked by disulfide bonds. HMW subunits in particular have been associated with bread making performance of flours (Payne, 1987; Wieser, 2007) and viscoelastic properties of the matrix (Popineau, Cornec, Lefebvre, & Marchylo, 1994). Therefore, understanding the microstructure of this fraction is of paramount importance in relation to its functionality.

Early studies on glutenin microstructure using scanning tunneling microscopy revealed sheets of aligned protein molecules with a spiral structure (Miles, Carr, McMaster, l'Anson, Belton, Morris et al., 1991). Further studies showed that proteins form fibrils at the atomic level that possibly align side by side probably due to hydrogen bond formation (Humphris, McMaster, Miles, Gilbert, Shewry & Tatham, 2000; Mackintosh, Meade, Healy, Sutton, Larsen, Squires et al., 2009; McIntire, Lew, Adalsteins, Blechl, Anderson & Kasarda, 2005). However, it is stressed that interactions of proteins with the surface of the mica and sample deposition protocols may contribute to fibril formation. Nevertheless, fibril formation appears to be the morphology of preference for both gliadin and glutenin preparations as opposed to spherical or aggregated structures when visualized independently. Another school of thought argues based on confocal microscopy imaging findings that glutenin macropolymer (GMP) forms spherical particles (Don, Lichtendonk, Plijter, & Hamer, 2003; Peighambaroust, van der Goot, Hamer, & Boom, 2005). This microstructural detail of GMP isolates apparently does not persist in more complex mixtures of glutenins. Distribution of HMW subunits appears to be continuous forming the backbone of gluten network whereas the LMW subunits show discrete topology with clustered structures scattered in gluten matrix (Lindsay & Skerrett, 2000). Individual fibers or particles cannot be visualized as proteins interact to form a compound structure. Continuous morphology of glutenin isolates has been also observed using transmission electron microscopy (Jiang et al., 2008; Shomer et al., 1998) also revealing sheet-like structures similar to those of gliadin. It is important to note the remarkable change of microstructure when proteins interact at high concentrations. This is important considering that both glutenin and gliadin are required in high concentrations in order to form the three-dimensional structure that occurs in industrial applications. Therefore, the sheet like structure seems to be the morphology of preference when gluten protein fractions interact with each other.

The complex protein interactions described above culminate in the formation of gluten network with mechanical properties distinct from the network structures of its constituent proteins. Imaging techniques and modeling that have been used to describe the microstructure of hydrated gluten network are discussed in the following section.

3. Microstructure of gluten network

3.1. Microscopical observations

Investigation of hydrated gluten using various microscopical techniques may reveal the microstructure of the three-dimensional network. In such a complex polymeric mixture transmission and scanning electron techniques play more important roles than single molecule techniques, as the molecular packing of proteins will give rise to a larger structure. The structure that results from combination of various proteins has distinct mechanical properties from its constituent fractions, which may be reflected in the microstructure of the material. Identification and modeling of microstructural characteristics of gluten network may be an important step towards improving the industrial performance of flours.

Early microstructural studies of gluten network using electron microscopy opened the debate on the nature of the network at the various microstructural scales i.e., fibrillar or membrane-like (Bechtel,

Pomeranz, & de Francisco, 1982; Bernardin & Kasarda, 1973; Crozet, Godon, Petit, & Guilbot, 1974; Cumming & Tung, 1976; Fretzdorff, Bechtel, & Pomeranz, 1982; Orth, Dronzek, & Bushuk, 1973a, 1973b). Although the terminology of these studies seems nowadays to be confusing, as concepts from synthetic polymer science had not been introduced at that time, they provide valuable information on the microstructure of gluten network. A fibrous network would imply that fibers would have high degree of molecular orientation and linked by intramolecular bonds such as in collagen or cellulose (Gupta & Kothari, 1997). We now know that to produce fibers from gluten proteins requires chemical modification and special processing conditions (Athamneh & Barone, 2009; Reddy & Yang, 2007; Van Der Goot, Peighambardoust, Akkermans, & Van Oosten-Manski, 2008). Although some fiber-like structures may appear in micrographs of this early work they should be attributed mostly to shear forces that have been exerted to the samples during sample preparation prior to imaging. Furthermore, various sample preparation techniques result in changes in the microstructure and care should be taken when we try to interpret electron microscopy imaging (Freeman, Shelton, Bjerke, & Skierkowski, 1991). Microstructure of gluten has been revisited a decade or so later identifying that the interacting and interpenetrating proteins form a continuous structure that has been described as “gluten sheet” (Amend & Belitz, 1989, 1990a, 1990b; Amend, Belitz, & Kurthen, 1990; Amend, Belitz, Moss, & Resmini, 1991; Bernardin & Kasarda, 1973; Hermansson & Larsson, 1986; Jiang et al., 2008). In this model, gluten network is formed by cross-linked protein sheets that are layered one over the other forming a three dimensional structure. Upon application of shear forces on gluten sheets (i.e., stretching) perforation may occur which may eventually lead to the disruption of the network and possible appearance of fiber-like structures. The idea of a fibrous network has been challenged by the use of techniques that require minimum sample preparation such as environmental scanning electron microscopy (ESEM), cryo-SEM or confocal laser microscopy (CLSM). These methodologies allow observing gluten structure at its most native state. Using ESEM it was not possible to observe fibrillar structures at mesoscopic scale giving further support to the sheet-like arrangement of the network (Bache & Donald, 1998). This view is also in agreement with more recent cryo-SEM studies of gluten network where the structure appears to be homogeneous indicating the absence of a fibrous network at micron scale (Bonet, Rosell, Caballero, Gomez, Perez-Munuera & Lluch, 2006; Kontogiorgos & Goff, 2006). Furthermore, CLSM imaging of various bread dough preparations revealed a continuous gluten network that surrounds starch granules (Baier-Schenk, Handschin, von Schonau, Bittermann, Bachi & Conde-Petit, 2005; Peighambardoust, 2006; Peighambardoust, Dadpour, & Dokouhaki, 2010).

Summarizing the structural studies using a multitude of imaging techniques, specimens and sample preparation methods it appears that the structural model that is based on layered sheets is the most probable arrangement of gluten network upon hydration. Microscopy is usually limited by microscope resolution something that is particularly important when trying to visualize specimens at nano scales. Furthermore, presence of artifacts especially when there is a demanding sample preparation protocol may mislead the interpretation of imaging. For this reason, it is essential to combine information from modeling using other techniques in order to seek for further evidence to support the interpretation of microscopy.

3.2. Microstructural modeling

The microstructural intricacies of gluten network result from the complexity of its composition and interactions among the constituent proteins. It is unlikely, however, that imaging alone will provide conclusive results of the ultrastructure of gluten network as it usually gives information on two dimensions with exception of CLSM that

investigates the third dimension of the specimen. Additional techniques based on different fundamental physical principles than those of imaging are required to improve our understanding of the network.

An in-depth rheological modeling of gluten in retardation and oscillatory modes suggested that gluten forms a colloidal network in which particles are aggregated by hydrogen and hydrophobic bonds (Lefebvre, Pruska-Kedzior, Kedzior, & Lavenant, 2003; Lefebvre & van Vliet, 2003). Confocal laser imaging of glutenin macropolymer dispersions where discrete particles were observed somewhat reinforces this model (Don et al., 2003; Peighambardoust et al., 2005). As it has already been discussed, microscopical imaging of protein preparations at various length scales and with a large number of techniques fails to provide further evidence of existence of discrete particles. Even when imaging provides some evidence of particles (Lindsay & Skerritt, 2000) these are embedded in a continuous protein phase. It has not been demonstrated as yet by means of microscopy the existence of a particulate network such as those that are observed in, for example, acid induced milk protein gels. It is possible, however, that incomplete protein hydration may allow the presence particles or aggregates that are embedded in a continuous gluten matrix, which may affect the overall mechanical response of the network. This is, however, related to the details of protein hydration rather than to the mechanism of network formation.

Thermal events of hydrated gluten at subzero temperatures have been recently investigated to create a model that could be tested calorimetrically (Kontogiorgos & Goff, 2006; Kontogiorgos, Goff, & Kasapis, 2007). This model was based on first thermodynamic principles outlined by Defay and Prigogine for the phase behavior of liquids confined in porous materials (Defay & Prigogine, 1966). In this model, gluten network was considered as a porous matrix where after hydration pores are filled with water (confined water) and are surrounded by ice (bulk ice) (Fig. 1). Modeling of the thermal transitions using empirical porosimetric models in combination with microstructural investigations gave strong evidence that gluten forms a nano-porous structure giving further support to the sheet-like arrangement of gluten building blocks. Thermal events were attributed to the presence of a porous network that forms nanocapillaries with an estimated pore diameter in the range of 5 nm. Building on the previous model of sheet arrangement we could argue that gluten sheets are separated from each other by about 5 nm. The thermal traces observed for gluten were also observed in flour–water mixtures (Kontogiorgos, Goff, & Kasapis, 2008) suggesting that the nanoporous structure persists in the presence of starch granules as well as other minor flour components (arabinoxylans, lipids, etc.).

4. Concluding remarks

Revisiting research findings of the last forty years on the microstructure of hydrated gluten network showed a remarkable improvement in our capacity to understand this intricate biological system. Progression of the technical aspects of imaging, resolution of microscopes and sample preparation techniques has enabled us to observe more microstructural and topological features of the network. Furthermore, introduction of fundamental concepts from polymer and material sciences boosted our capability to describe soft matter pertaining to food systems.

In the light of this new information, we would like to propose the structural hierarchy of gluten that at the moment is the best compromise among other arrangements and it takes into consideration most microscopical observations and experimental findings with other techniques (Fig. 1). Gluten network structure could be divided into four structural levels. At molecular level individual gluten strands interact with various physical or covalent forces (Fig. 1, atomic scale). At some critical point that depends on the conditions (e.g., ratio of protein fractions, hydration, pH etc.) there is a transition in the

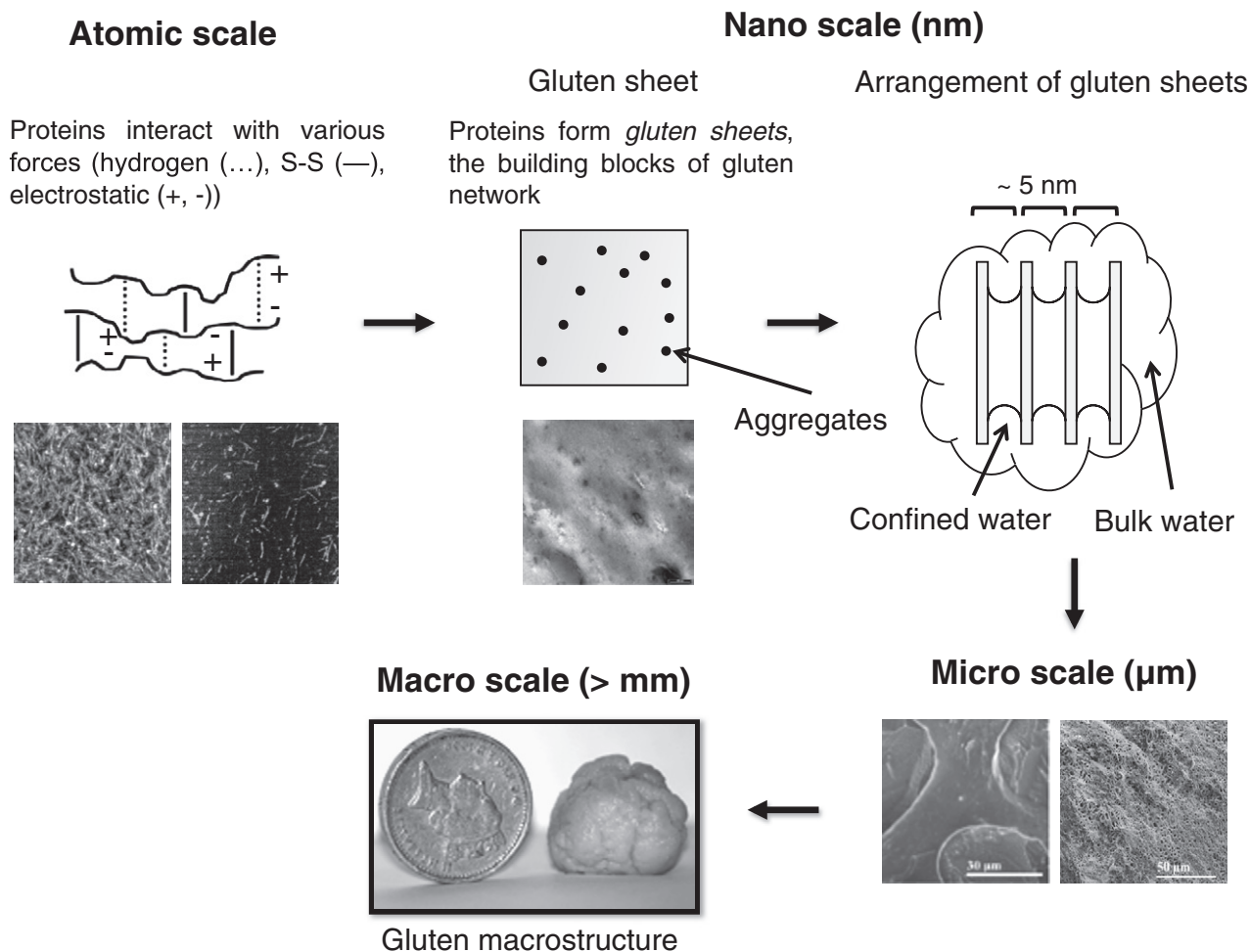


Fig. 1. Structural hierarchy of hydrated gluten network. At the atomic scale, gluten proteins interact with each other to form gluten sheets. Gluten sheets may include protein aggregates that influence the rheology of the material. At nanoscale, gluten sheets are arranged in a manner so as to form nanocapillaries that are filled with water. Nanopores are surrounded by bulk water. Consequent packing of the nanoporous structure leads to the three dimensional network. (Schematic adopted from (Kontogiorgos & Kasapis, 2010). All images are reproduced by permission. Clockwise: McMaster, Miles, Kasarda, et al., 1999; McMaster, Miles, Wannerberger, et al., 1999; McIntire et al., 2005; Jiang et al., 2008; Kontogiorgos & Goff, 2006, respectively).

morphology from isolated fibrils to a continuous phase with sheet-like morphology. The resulting sheets (secondary structural level) could be considered as the building blocks or fundamental units of gluten network. At that length scale presence of particles or aggregates may be observed that could be an additional feature to the microstructural details of the network. The presence and extend of such aggregates may be responsible for significantly influencing the mechanical properties of the network. Fibrils that have been observed at times with various techniques are attributed to stretching and rupturing of the sheets during sample preparation and should not be considered building blocks of the network. At this level, organization of gluten sheets side-by-side culminates in the formation of a nanoporous ultrastructure (Fig. 1, nanoscale). Extensive arrangement of the sheets in a disordered manner concludes to the microscale (third structural level) where an additional feature can be observed (Fig. 1, microscale); large regions, where aqueous phase can be entrapped, disrupt the continuity of the nanoporous formations. This is where “bulk” water is located as opposed to “confined” water that interacts strongly with gluten matrix and is found within the nanopores. Eventually, arrangement of microstructural elements leads up to the fourth structural level showing various morphologies as well as mechanical properties (Fig. 1, macroscale). Ultimately, considering hydrated gluten network with a nanoporous structure may open new opportunities in designing novel materials beyond food applications with improved functionality.

References

- Amend, T., & Belitz, H. D. (1989). Microscopical studies of water/flour systems. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 189, 103–109.
- Amend, T., & Belitz, H. D. (1990a). The formation of dough and gluten – A study by scanning electron-microscopy. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 190, 401–409.
- Amend, T., & Belitz, H. D. (1990b). Gluten formation studied by the transmission electron microscope. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 191, 184–193.
- Amend, T., Belitz, H. D., & Kurthen, C. (1990). Electron microscopic studies on protein films from wheat and other sources at the air/water interface. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 190, 217–222.
- Amend, T., Belitz, H. D., Moss, R., & Resmini, P. (1991). Microstructural studies of gluten and a hypothesis on dough formation. *Food Structure*, 10, 277–288.
- Athamneh, A. I., & Barone, J. R. (2009). Enzyme-mediated self-assembly of highly ordered structures from disordered proteins. *Smart Materials and Structures*, 18, 104024.
- Bache, I. C., & Donald, A. M. (1998). The structure of the gluten network in dough: A study using environmental scanning electron microscopy. *Journal of Cereal Science*, 28, 127–133.
- Baier-Schenk, A., Handschin, S., von Schonau, M., Bittermann, A. G., Bachi, T., & Conde-Petit, B. (2005). In situ observation of the freezing process in wheat dough by confocal laser scanning microscopy (CLSM): Formation of ice and changes in the gluten network. *Journal of Cereal Science*, 42, 255–260.
- Bechtel, D. B., Pomeranz, Y., & de Francisco, A. (1982). Breadmaking studied by light and transmission electron microscopy. *Cereal Chemistry*, 55, 392–401.
- Bernardin, J. E., & Kasarda, D. D. (1973). The microstructure of wheat protein fibrils. *Cereal Chemistry*, 50, 735–744.
- Bonet, A., Rosell, C. M., Caballero, P. A., Gomez, M., Perez-Munuera, I., & Lluch, M. A. (2006). Glucose oxidase effect on dough rheology and bread quality: A study from macroscopic to molecular level. *Food Chemistry*, 99, 408–415.

- Crozet, N., Godon, B., Petit, L., & Guilbot, A. (1974). Submicroscopic structure of wheat flour and gluten lipoprotein components. *Cereal Chemistry*, 51, 288–299.
- Cumming, D. B., & Tung, M. A. (1976). The ultrastructure of commercial wheat gluten. *Canadian Institute of Food Science and Technology Journal*, 8, 67–73.
- Defay, R., & Prigogine, I. (1966). *Surface tension and adsorption*. London: Longmans.
- Don, C., Lichtendonk, W., Plijter, J. J., & Hamer, R. J. (2003). Glutenin macropolymer: A gel formed by glutenin particles. *Journal of Cereal Science*, 37, 1–7.
- Freeman, T. P., Shelton, D. R., Bjerke, J. M., & Skierkowski, K. (1991). The ultrastructure of wheat gluten: Variations related to sample preparation. *Cereal Chemistry*, 68, 492–498.
- Fretzdorff, B., Bechtel, D. B., & Pomeranz, Y. (1982). Freeze-fracture ultrastructure of wheat flour ingredients, dough, and bread. *Cereal Chemistry*, 59, 113–120.
- Gupta, V. B., & Kothari, V. K. (1997). *Manufactured fibre technology* (1st ed.). London: Chapman & Hall.
- Hermansson, A. M., & Larsson, K. (1986). The structure of gluten gels. *Food Microstructure*, 5, 233–239.
- Humphris, A. D. L., McMaster, T. J., Miles, M. J., Gilbert, S. M., Shewry, P. R., & Tatham, A. S. (2000). Atomic force microscopy (AFM) study of interactions of HMW subunits of wheat glutenin. *Cereal Chemistry*, 77, 107–110.
- Jiang, B., Kontogiorgos, V., Kasapis, S., & Goff, H. D. (2008). Rheological investigation and molecular architecture of highly hydrated gluten networks at subzero temperatures. *Journal of Food Engineering*, 89, 42–48.
- Kasarda, D. D., Bernardin, J. E., & Thomas, R. S. (1967). Reversible aggregation of α -gliadin to fibrils. *Science*, 155, 203–205.
- Kontogiorgos, V., & Goff, H. D. (2006). Calorimetric and microstructural investigation of frozen hydrated gluten. *Food Biophysics*, 1, 202–215.
- Kontogiorgos, V., Goff, H. D., & Kasapis, S. (2007). Effect of aging and ice structuring proteins on the morphology of frozen hydrated gluten networks. *Biomacromolecules*, 8, 1293–1299.
- Kontogiorgos, V., Goff, H. D., & Kasapis, S. (2008). Effect of aging and ice structuring protein on the physical properties of flour–water mixtures. *Food Hydrocolloids*, 22, 1135–1147.
- Kontogiorgos, V., & Kasapis, S. (2010). Temperature dependence of relaxation spectra for highly hydrated gluten networks. *Journal of Cereal Science*, 52, 100–105.
- Lagrain, B., Goderis, B., Brijs, K., & Delcour, J. A. (2010). Molecular basis of processing wheat gluten toward biobased materials. *Biomacromolecules*, 11, 533–541.
- Lefebvre, J., Pruska-Kedzior, A., Kedzior, Z., & Lavenant, L. (2003). A phenomenological analysis of wheat gluten viscoelastic response in retardation and in dynamic experiments over a large time scale. *Journal of Cereal Science*, 38, 257–267.
- Lefebvre, J., & van Vliet, T. (2003). Physico-chemical aspects of gluten proteins. In W. Y. Aalbersberg & R. J. Hamer (Eds.), *Industrial proteins in perspective* (pp. 94–102). Amsterdam: Elsevier.
- Lindsay, M. P., & Skerritt, J. H. (2000). Immunocytochemical localization of gluten proteins uncovers structural organization of glutenin macropolymer. *Cereal Chemistry*, 77, 360–369.
- Mackintosh, S. H., Meade, S. J., Healy, J. P., Sutton, K. H., Larsen, N. G., Squires, A. M., et al. (2009). Wheat glutenin proteins assemble into a nanostructure with unusual structural features. *Journal of Cereal Science*, 49, 157–162.
- McIntire, M. T., Lew, L. J. E., Adalsteins, E. A., Blechl, A., Anderson, D. O., & Kasarda, D. D. (2005). Atomic force microscopy of a hybrid high-molecular weight glutenin subunit from a transgenic hexaploid wheat. *Biopolymers*, 78, 53–61.
- McMaster, T. J., Miles, M. J., Kasarda, D. D., Shewry, P. R., & Tatham, A. S. (1999). Atomic force microscopy of α -gliadin fibrils and in situ degradation. *Journal of Cereal Science*, 31, 281–286.
- McMaster, T. J., Miles, M. J., Wannerberger, L., Eliasson, A. C., Shewry, P. R., & Tatham, A. S. (1999). Identification of microphases in mixed α - and ω -gliadin protein films investigated by atomic force microscopy. *Journal of Agricultural and Food Chemistry*, 47, 5093–5099.
- Miles, M. J., Carr, H. J., McMaster, T. C., l'Anson, K. J., Belton, P. S., Morris, V. J., et al. (1991). Scanning tunneling microscopy of a wheat seed storage protein reveals details of an unusual supersecondary structure. *Proceedings of the National Academy of Sciences of the United States of America*, 88, 68–71.
- Orth, R. A., Dronzek, B. L., & Bushuk, W. (1973a). Scanning electron microscopy of bread wheat proteins fractionated by gel filtration. *Cereal Chemistry*, 50, 696–701.
- Orth, R. A., Dronzek, B. L., & Bushuk, W. (1973b). Studies of glutenin. IV. Microscopic structure and its relations to bread making quality. *Cereal Chemistry*, 50, 688–695.
- Paananen, A., Tappura, K., Tatham, A. S., Fido, R. J., Shewry, P. R., Miles, J. M., et al. (2006). Nanomechanical force measurements of gliadin protein interactions. *Biopolymers*, 83, 658–667.
- Payne, P. I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology*, 38, 141–153.
- Peighambari, S. H. (2006). Microstructure formation and rheological behaviour of dough under simple shear flow. *Journal of Cereal Science*, 43, 183–197.
- Peighambari, S. H., Dadpour, M. R., & Dokouhaki, M. (2010). Application of epifluorescence light microscopy (EFLM) to study the microstructure of wheat dough: A comparison with confocal scanning laser microscopy (CSLM) technique. *Journal of Cereal Science*, 51, 21–27.
- Peighambari, S. H., van der Goot, A. J., Hamer, J. R., & Boom, R. M. (2005). Effect of simple shear on the physical properties of glutenin macro polymer (GMP). *Journal of Cereal Science*, 42, 59–68.
- Popineau, Y., Cornec, M., Lefebvre, J., & Marchylo, B. (1994). Influence of high Mr glutenin subunits on glutenin polymers and rheological properties of glutes and gluten subfractions of near-isogenic lines of wheat sicco. *Journal of Cereal Science*, 19, 231–241.
- Reddy, N., & Yang, Y. (2007). Novel protein fibers from wheat gluten. *Biomacromolecules*, 8, 638–643.
- Shewry, P. R., & Tatham, A. S. (1990). The prolamin storage proteins of cereal seeds: Structure and evolution. *The Biochemical Journal*, 267, 1–12.
- Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Mifflin, B. J. (1986). The classification and nomenclature of wheat proteins – A reassessment. *Journal of Cereal Science*, 4, 97–106.
- Shomer, I., Lookhart, G. L., Vasiliver, R., & Bean, S. (1998). Ultrastructure of consecutively extracted and flocculated gliadins and glutenins. *Journal of Cereal Science*, 27, 27–36.
- Tatham, A. S., Thomson, N. H., McMaster, T. J., Humphris, A. D. L., Miles, M. J., & Shewry, P. R. (1999). Scanning probe microscopy studies of cereal seed storage protein structures. *Scanning*, 21, 293–298.
- Thomson, N. H., Miles, J. M., Tatham, A. S., & Shewry, P. R. (1992). Molecular images of cereal proteins by STM. *Ultramicroscopy*, 42–44, 1204–1213.
- Van Der Goot, A. J., Peighambari, S. H., Akkermans, C., & Van Oosten-Manski, J. M. (2008). Creating novel structures in food materials: The role of well-defined shear flow. *Food Biophysics*, 3, 120–125.
- Veraverbeke, W. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition*, 42, 179.
- Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24, 115–119.
- Wieser, H., & Kieffer, R. (2001). Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. *Journal of Cereal Science*, 34, 19–27.