Wheat Gluten Functionality as a Quality Determinant in Cereal-Based Food Products

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Annu. Rev. Food Sci. Technol. 2012. 3:469–92

First published online as a Review in Advance on December 12, 2011

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi: 10.1146/annurev-food-022811-101303

1941-1413/12/0410-0469$20.00

Keywords

gliadin, glutenin, processing, cross-linking, bread, pasta

Abstract

The unique properties of wheat reside primarily in its gluten-forming storage proteins. Their intrinsic viscoelastic behavior is responsible for the characteristics of different wheat-based foods and for the use of wheat gluten proteins in different food products. Wheat-based food processing generally develops and sets the gluten protein network. Heat-induced gluten aggregation proceeds through cross-linking within and between its protein fractions. Prominent reactions include sulfhydryl (SH) oxidation and SH-disulfide (SS) interchange, which lead to SS cross-links. Other covalent bonds are also formed. Gluten functionality can be (bio-) chemically impacted. We focus on bread making, in which gluten proteins contribute to dough properties, bread loaf volume, and structure, and on pasta production, in which gluten proteins generate the desired cooking quality. Furthermore, it is speculated that the structure and texture of soft wheat products are also, at least to some degree, shaped by the heat-induced changes in the gluten protein fraction.

INTRODUCTION AND SCOPE

Protein: biochemical compound consisting of one or more polypeptides folded into a globular or fibrous form

Dough: paste made by mixing wheat flour with water (typically 600 g of water per kg flour)

Of all cereal grains, wheat and rice are the most important food grains. Wheat ranks fourth (2008 data) in total production of all commodities produced worldwide (FAOSTAT 2011). Most (>90%) of the wheat grown worldwide is from the hexaploid (three genomes, AABBDD) *Triticum aestivum* L. species often referred to as common or bread wheats. Genes on the D genome encode for proteins with a profound impact on dough rheology and hence bread-making characteristics of wheat flour. In the case of *Triticum durum* Desf. (durum wheat, tetraploid, A and B genomes), its coarse milling product, i.e., semolina (also referred to as middlings) is primarily used for pasta production (Wrigley 2009). Durum wheat has protein levels varying from 9% to 18% (Feillet 1988) and is the hardest of all wheat species. In North American terminology, the *Triticum aestivum* wheats are divided into soft and hard wheat cultivars, based on the force required to crush the kernels (Delcour & Hoseney 2010). Unlike flour from hard wheat (10% to 14% protein), which is mainly used for bread making, soft wheat (8% to 11% protein) has more than one major use. Soft wheats are, as their name implies, softer in nature and have been bred to have low protein content (Huebner et al. 1999). Cakes, cookies, crackers, and pretzels are important product categories made from soft wheat flour.

Wheat is unique because its flour can form dough that exhibits properties required for the production and the quality of a wide diversity of well-known food products. Starch [for a profound discussion of the role of starch in food processing, the interested reader is referred to Delcour et al. (2010)] is quantitatively the major constituent of wheat grain, but the unique properties of the kernel reside primarily in the gluten-forming storage proteins of its endosperm. Hydrated gluten proteins have unique viscoelastic properties (Veraverbeke & Delcour 2002). The most common use (and well-known effect) of gluten proteins is as a component of wheat flour used in bread making that determines the bread loaf quality. The quality of other wheat-based products, such as cookies, cakes and pretzels, also heavily relies on the functionality of gluten proteins. Likewise, semolina gluten is very important in the context of pasta making.

Other than the intrinsic quality of wheat, which resides in genetic as well as in environmental factors, processing of wheat flour is also important for gluten functionality and its contribution to final product quality. We focus on the physicochemical changes of gluten proteins in typical cereal-based food processes. Although such proteins play a decisive role in the production and/or quality attributes of most cereal-based products, the complex underlying mechanisms are not fully understood at present. This not only has to do with the difficulties encountered during gluten isolation or the natural, complex, and diverse structure of the gluten constituents, but also with the wide spectrum of chemical reactions occurring during wheat processing.

TRITICUM AESTIVUM AND *TRITICUM DURUM* GLUTEN PROTEINS

Classification

Cereal-seed proteins are generally classified based on their sequential extraction in a procedure originally developed by Osborne (1907). At present, many slightly modified versions of this procedure exist and are used to isolate the different wheat protein fractions. Alternatively, wheat proteins are classified into the nongluten-forming proteins on the one hand, which generally enclose approximately 15% to 20% of total wheat protein, and the proteins that form a viscoelastic network, the gluten, on the other hand. The nongluten-forming proteins correspond to the albumins (extractable in water) and globulins (extractable in salt solutions but not in water) in Osborne type fractionation procedures. The gluten proteins, conversely, have very low extractabilities in water or salt solutions and consist of gliadins (extractable in aqueous ethanol) and glutenins (unextractable

POLYMERIC POLYMERS?

Although proteins already consist of polypeptides, with amino acids being their building blocks, the terms monomeric and polymeric are used for gluten proteins to refer to their particular quaternary structure. Gliadins represent a heterogeneous mixture of single polypeptides, whereas glutenin proteins consist of more polypeptide chains (subunits) associated through interchain disulfide (SS) bonds. For glutenin, this SS bonding leads to proteins with enormous sizes, making them the largest protein molecules in nature (Wrigley 1996). This large size of glutenin is certainly important for its unique elastic properties because it provides structural continuity, a prerequisite for polymer elasticity.

in aqueous ethanol). In what follows, gluten and gluten proteins are used interchangeably. Factors contributing to the low water extractability of gluten proteins include a low content of amino acids with ionizable side chains and high contents of glutamine and the nonpolar amino acids proline and glycine. The gliadins are monomeric and have molecular weights (mol wt) between 30,000 and 60,000, whereas glutenin represents a mixture of polymers with mol wt ranging from approximately 80,000 to more than twenty million (Veraverbeke & Delcour 2002) (see sidebar Polymeric Polymers?). Both common and durum wheats contain roughly equal proportions of gliadins and glutenins (Feillet 1988, Wieser 2007). However, because *T. durum* species lack the D genome, they do not possess certain gliadins and glutenin subunits (GS) that common wheats have (Feillet 1988).

In durum wheat, low molecular weight (LMW) glutenins, referred to as durum sulfur-rich glutenin, can be found. This fraction corresponds to known α-amylase inhibitor subunits and is believed to bind to gluten proteins during dough formation (Kobrehel et al. 1991).

Cysteine residues occur in most gliadin and glutenin polypeptides. They either occur as free sulfhydryls (SH) (in glutenin but not in gliadin) or are involved in disulfide (SS) bonds either within the same polypeptide (intrachain SS bonds) or between different polypeptides (interchain SS bonds) (Shewry et al. 1986).

Polypeptide: linear polymer chain of amino acids linked by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues

Quaternary structure: the arrangement of folded or coiling polypeptides in a multi-subunit complex

SH-SS interchange: reaction in which a sulfhydryl (SH) group carries out a nucleophilic attack on a sulfur atom of a disulfide (SS) group

Reverse or β-turns: structural motif in which the polypeptide chain reverses direction over the span of a few amino acids

Gliadin Structures

Three structurally distinct groups of gliadins, i.e., α-, γ-, and ω-types, can be distinguished. Cysteine residues in α- (six cysteine residues) and γ- (eight cysteine residues) gliadins are located at highly conserved positions and are all involved in intrachain SS bonds, preventing them from participating in the quaternary structure of glutenin as well as from becoming involved in SH-SS interchange reactions (see below) at ambient conditions. In contrast, ω-gliadins lack cysteine residues. The primary structure of gliadins consists of several domains of variable sizes (**Figure 1*a***). The short N terminal domain consists of 5 to 14 amino acid residues. The central repetitive domain contains up to 100 residues organized in repeat sequences of one or two motifs mainly made up of glutamine, proline, and hydrophobic amino acids (phenylalanine or tyrosine). Finally, the C-terminal nonrepetitive domain is a succession of polyglutamine and unique lysine and arginine-rich sequences that include all the sulfur-containing amino acids (Müller & Wieser 1997). The secondary protein structure of α- and γ-gliadins comprises reverse or β-turns in the repetitive domain and α-helices in the nonrepetitive domains, giving these an overall compact globular structure. The ω-gliadins also contain β-turns but only contain low levels of α-helices and β-sheets (Tatham & Shewry 1985). However, it was later stated that, although α-gliadins have a compact and less regular structure, γ-gliadins rather form an extended-spiral tertiary structure

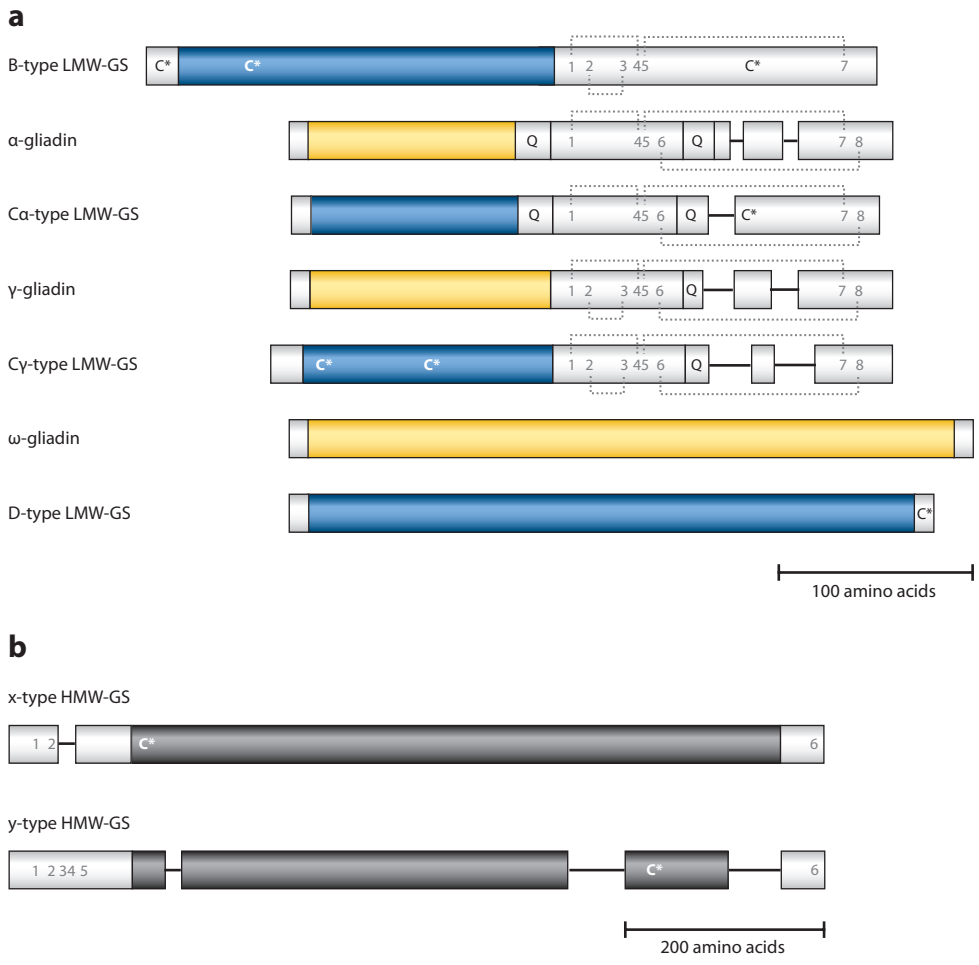

Figure 1

(*a*) Structures of α-, γ-, and ω-gliadin (*yellow*) and B, C, and D low molecular weight–glutenin subunits (LMW-GS; *blue*). (*b*) Structure of x- and y-type high molecular weight (HMW)-GS. Repetitive domains are colored and nonrepetitive domains are white. Positions of the conserved cysteine residues are indicated with Arabic numerals. Additional unconserved cysteine residues are indicated with C*. Intramolecular disulfide bonds are indicated by the gray dotted lines. Q represents a polyglutamine sequence. Similar polypeptide regions are aligned based on their amino acid sequences.

(Tatham et al. 1990). The ω-gliadins presumably adopt a stiff coil rather than a compact structure (Shewry et al. 2009).

Glutenin Structure

Glutenin is largely insoluble in most common solvents because of its large size. However, its subunit building blocks (GS) have solubilities comparable to those of gliadins. The GS can be obtained by treating glutenin with an SS reducing agent. The drastic extraction conditions needed

to isolate glutenin (subunits) hamper the study of the native structure of the polymeric protein. High molecular weight glutenin subunits (HMW-GS, mol wt 70,000 to 90,000) and low molecular weight glutenin subunits (LMW-GS, mol wt 30,000 to 45,000) are distinguished.

Elasticity: physical property that makes a material return to its original shape after removal of the stress that made it deform

Although primary and secondary structures of LMW-GS are similar to those of α- and γ-gliadins (**Figure 1*a***), they differ in one very important characteristic. Indeed, apart from intrachain SS bonds, LMW-GS also contain interchain SS bonds by which they are incorporated in glutenin polymers.

The LMW-GS are classified in B, C, and D types, based on their mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Alternatively, three subgroups of typical LMW-GS can be recognized, i.e., LMW-s, LMW-m, and LMW-i types, according to their first amino acid residue (serine, methionine, or isoleucine, respectively). LMW-s and LMW-m are the predominant sequence types in the B-LMW-GS group of bread and durum wheats. They are inter alia related to pasta quality (see below).

Two types of HMW-GS, a higher mol wt x-type (mol wt 83,000 to 88,000) and a lower mol wt y-type (mol wt 67,000 to 74,000), exist. Bread and durum wheats, in theory, can contain six and four different HMW-GS, respectively. However, because of silencing of some of their genes, most common and durum wheat cultivars have only three to five and one to three HMW-GS, respectively. Although HMW-GS are minor components in terms of quantity, they are major determinants of gluten elasticity and functionality and hence are key factors in the process of bread making (see below) (Gianibelli et al. 2001).

Both x- and y-type HMW-GS have a typical three-domain structure consisting of relatively small N- and C-terminal domains flanking a major central domain (**Figure 1*b***). Whereas the C-terminal domain has a constant size (42 amino acid residues) and the N-terminal domain only slightly varies in length (approximately 80 to 100 amino acid residues), the central domain is of a much more variable length (between 600 and 850 amino acid residues). All cysteine residues are located near the ends of the HMW-GS (**Figure 1*b***). The secondary structure of the N- and C-terminal domains is predominantly organized as an α-helix. In contrast, the central domain consists of repetitive sequences rich in glutamine, proline, and glycine. These are supposed to produce a series of overlapping reverse turns that form a β-spiral super secondary structure. It is generally believed that HMW-GS adopt a rigid rod-like conformation due to the specific structure of their central repetitive domain (Shewry et al. 1992).

Because glutenin polymers have mol wt reaching over twenty million (Gianibelli et al. 2001) and their extraction does not prove to be straightforward, it has been difficult to determine their precise native quaternary structure and mol wt distribution. In the absence of sound experimental approaches and hence evidence, several hypotheses on their structure have been put forward. In a model proposed by Graveland et al. (1985), the backbone of the molecule is only made up by HMW-GS, whereas LMW-GS are present as lateral attachments, with SS bonds strengthening the structure. The experimental findings available to date partly support this model. At least dimers of HMW-GS must be considered in the models. They are widespread in partly reduced glutenin preparations (Gianibelli et al. 2001). Apparently, HMW-GS and LMW-GS polymerize separately in vivo, both forming linear polymers (Wieser et al. 2006), and a cross-link between a HMW-GS and a LMW-GS has been identified (Keck et al. 1995). Based on Graveland's model and stepwise reduction of the glutenin macropolymer and subsequent immunocytochemical studies of the localization of GS, branched models of the glutenin macropolymer have been proposed (Lindsay & Skerritt 1998, 1999) in which HMW-GS occur as chains forming a network to which the LMW-GS-containing polymers are attached (**Figure 2*a***). The glutenin backbone contains HMW-GS chains and has branches of LMW-GS, which is in agreement with the hypothesis of

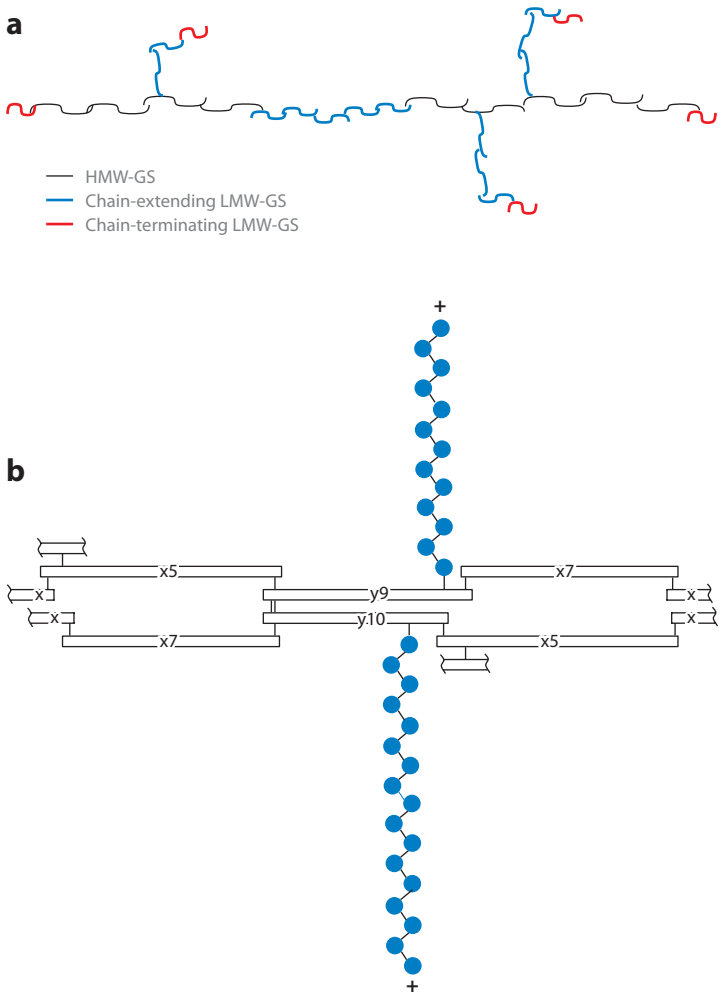

Figure 2

Models for the structure of the glutenin macropolymer. (*a*) Branched model of Lindsay & Skerritt (1999) composed of a backbone of high molecular weight (HMW)- and low molecular weight (LMW)-glutenin subunits (GS) with linear branches containing only LMW-GS. (*b*) Model unit of Wieser et al. (2006) for the interchain SS structures of LMW-GS (•) and HMW-GS (□) of gluten polymers.

Graveland, but also a model with a backbone and/or branches containing both LMW-GS and HMW-GS has been put forward (Lindsay & Skerritt 1999). The branched models can be further refined by taking into account the ratio between the weight proportions of HMW-GS and LMW-GS (≈1:2) and the mol wt of subunits. As such, a basis molecular unit of glutenin polymers might consist of six HMW-GS and approximately 30 LMW-GS linked through interchain SS bonds (Wieser et al. 2006) (**Figure 2*b***). The mol wt of this unit totals about 1.5 million. The largest of the glutenin molecules might include more than ten of these units (Wieser et al. 2006).

Although intermolecular SS bonding is a major factor defining polymer stability, hydrogen bonding between adjacent HMW-GS and between HMW-GS and other proteins is also important in this regard (Belton et al. 1995, Humphris et al. 2000).

Hydrogen bond: interaction of a hydrogen atom with an electronegative atom, such as nitrogen or oxygen, coming from another chemical group

PHYSICAL AND CHEMICAL CHANGES OF GLUTEN PROTEINS DURING PROCESSING

Mechanical energy input and heating are essential treatments during production of cereal-based foods. Therefore, gluten development, as a result of hydration and shear (mixing), and cross-linking, as a result of heat (baking), have been intensively studied.

Gluten or dough development: mixing gluten or flour particles with water into a viscoelastic mass

Glass transition: reversible transition in amorphous materials from a hard and relatively brittle state into a molten or rubber-like state

Viscosity: measure of the resistance or thickness of a fluid that is being deformed by either shear or tensile stress

Physical Changes in Wheat Processing Chains

Gluten and its subfractions, gliadin and glutenin, exhibit a glass transition (Hoseney et al. 1986), i.e., a transition from a glassy to a rubber-like state. The glass transition occurs at a certain temperature, the glass transition temperature (T_g). Literature has reported on a broad range of T_g values for dry gliadin (from 120°C to 170°C), glutenin (from 140°C to 180°C), and gluten (from 130°C to 190°C) (Ferrari & Johari 1997, Kokini et al. 1994, Micard & Guilbert 2000, Noel et al. 1995, Pouplin et al. 1999, Toufeili et al. 2002). In certain moisture content ranges, T_g generally decreases when moisture content increases. To be able to interact or react, gluten polymers need to be in their rubber-like state and thus above their T_g (Levine & Slade 1990, Slade et al. 1989). Dry gluten (moisture contents below 20%) at room temperature occurs in its glassy state and thus below its T_g (Toufeili et al. 2002, Weegels et al. 1994b). At higher moisture contents, however, gluten appears leathery and when fully hydrated (moisture contents above 35%) (Almutawah et al. 2007), it forms a rubbery, viscoelastic mass. Indeed, mixing or extrusion of flour or semolina with the plasticizer water causes gluten proteins to interact. In wheat flour dough, the proteins are cohesive and exhibit a moderate elastic recovery (Kasarda 1989).

The origin of gluten's viscoelasticity has been studied at different length scales. On a molecular scale, it is believed that glutenin is the main wheat flour constituent for dough cohesiveness and elasticity. Its molecular size and the entanglement of different glutenin molecules provide continuity to dough. Its elasticity most likely depends on reversible stretching of an energetically more favorably folded glutenin conformation. Although the β-spiral structure may confer intrinsic elasticity to HMW-GS (Tatham et al. 1985) (**Figure 3*a***), its precise contribution to the polymer elastomeric properties is not yet understood.

On a molecular scale, glutenin elasticity has been suggested to be mediated by noncovalent interactions (mainly hydrogen bonds) between and within individual glutenin chains. In this context, a loop and train model (Belton 1999) considers protein-protein interactions (trains) between HMW-GS through interchain hydrogen bonding of glutamine residues, and hydrogen bonds between water and glutamine that result in formation of regions in which interchain interactions are broken (loops) [**Figure 3*b***, (1)]. Logically, upon higher gluten protein hydration, more loop regions are formed. Low extension of dough stretches the loops first and then unzips the train regions [**Figure 3*b***, (2)]. Under further extension, the resistance to deformation increases because of protein-protein interactions in the extended stiff chains [**Figure 3*b***, (3)]. In addition, rearrangement of SS bonds under extension can result in an apparent decrease in stiffness along the direction of extension (Shewry et al. 2000). Continued input of energy into the dough can eventually cause breakdown of the network or overmixing with a concomitant loss of dough elasticity.

Gliadins weaken the interactions between glutenin chains and essentially have a plasticizing effect on the gluten structure. Furthermore, they impart viscosity on dough. In practice, the ratio of monomeric gliadin to polymeric glutenin determines the balance between dough viscosity and elasticity (Veraverbeke & Delcour 2002). Overall, there is a scientific consensus that the gluten network has rubberlike properties and on a molecular level, consists of flexible chains between junction points. Such junction points have been suggested to be entanglements, trains, and/or SS bonds with a typical mesh size of 20 nm (Ng & McKinley 2008).

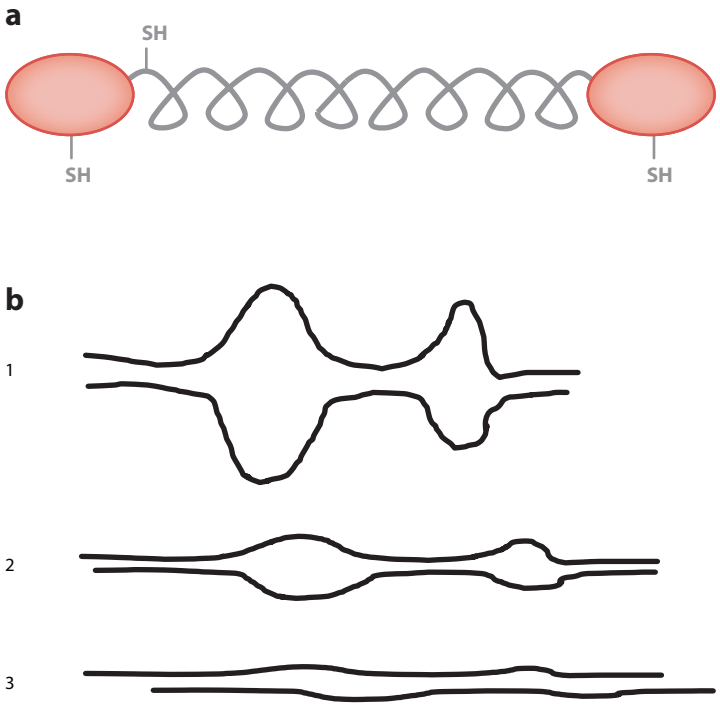

Figure 3

(*a*) Structural features of the high molecular weight-glutenin subunits (HMW-GS) which may determine the elasticity of gluten and dough (Shewry et al. 2000). (*b*) Loop and train behavior of HMW-GS as proposed by Belton (1999). With high levels of hydration, the system contains many loops, but sufficient bonds (trains) exist that maintain interchain contacts. (1) The equilibrium configuration. (2) Small extension, only the loops are deformed. (3) Large extension, loops are flattened and the interchain hydrogen bonds are broken so that the chains slip over each other.

The structural organization of the protein polymers at larger length scales is still under debate. On a mesoscopic scale, the gluten network seems to be organized into sheets with no visible network structures (Bache & Donald 1998). Don and coworkers (2003) presented a view on the structural organization of gluten that maintains that glutenin is a colloidal particle system with particle sizes ranging from 5 to 30 μm based on noncovalent reactions. Upon shear, the particles form a macroscopic network or gel responsible for the viscoelastic properties. Glutenin can then further interact with gliadin and other wheat components, impacting dough rheological properties (van Vliet 2007). The particulate model of glutenin has been heavily debated (Belton 2005, Belton & Dobraszczyk 2006, MacRitchie 2007), and no consensus has been reached on the exact nature of glutenin structure and interactions.

Mesoscopic scale: length scale intermediate between that of a molecule (>nm) and of materials (<μm)

Temperature clearly affects the overall mobility and hydrogen bonds in wet gluten (Lefebvre et al. 2000). In addition, temperatures exceeding 40°C can induce structural changes. Guerrieri and coworkers (1996) observed that at approximately 45°C, gluten hydrophobicity increases, indicating conformational changes that expose hydrophobic groups of gliadin and glutenin. Above 50°C, cross-links are formed. To observe gluten protein cross-linking, the decrease in protein extractability in solvents containing denaturing agents, such as SDS and/or urea, is often used (Domenek et al. 2003). Glutenin is more sensitive to extractability loss during heating than gliadin (Schofield et al. 1983). Because of the cross-linking reactions upon heating, the elastic modulus of

wet gluten increases (Attenburrow et al. 1990, Kokini et al. 1994). Upon heating, gliadins also react with glutenin polymers and drastically decrease glutenin polymer mobility (Redl et al. 1999). The reaction kinetics of wet gluten extractability loss can be described by a simple first-order reaction with an activation energy of 172 to 183 kJ mol^{-1} (Pence et al. 1953, Pommet et al. 2004).

Reaction kinetics: the study of rates and order of chemical processes

First-order reaction: reaction that depends on the concentration of only one reactant

Enzyme: protein that increases the rate of (catalyzes) a chemical reaction

Chemical Changes in Wheat Processing Chains

Covalent SS bonds in glutenin are critical for gluten functionality at ambient conditions, even if the prevalence of SH-containing amino acids in the gluten protein fraction is reasonably small. During dough mixing, SH-SS interchange occurs between glutenin proteins. Under such conditions, the stability of the intramolecular SS bonds in gliadins prevents them from becoming involved in SH-SS interchange reactions. Breaking and remaking SS bonds in glutenin under extension then leads to a network with polymers aligned along the direction of extension (see above) (Shewry et al. 2000). However, continued input of energy can physically destroy this network. In the above SH-SS interchange reactions, SH-containing LMW compounds, which already naturally occur in flour, can lower the average mol wt of the glutenin polymers. Another way by which SS bonds in glutenin can be impacted at ambient conditions is through the presence or use of oxidizing agents. Reactions involving such agents are common in wheat processing, inter alia owing to the occurence of certain wheat endogenous enzymes (Joye et al. 2009a). Covalent dityrosine bonds play little if any role in the structure and properties of wheat gluten in mixed dough (Hanft & Koehler 2005).

Heating readily breaks hydrogen bonds and thus initially decreases the degree to which glutenin and/or gliadin interact in gluten (Guerrieri et al. 1996, Lagrain et al. 2005). SS bonds play a key role in further gluten polymerization. Glutenin polymerization involves oxidation of SH groups and thus leads to additional SS bonds (Weegels et al. 1994a,b) (**Figure 4*a***). There is, however, also evidence for the occurrence of SH-SS interchange reactions under these conditions (Lagrain et al. 2005, Schofield et al. 1983). Already a small increase in the level of SS bonds can exert a large structural effect. Only a few cross-links between large glutenin polymers are needed to substantially enlarge the gluten network (Pareyt et al. 2010b). Moreover, formation of a single SS bond between, on the one hand, protein originally extractable in a given solvent and on the other hand, unextractable protein suffices to ensure loss of extractability of the first (Domenek et al. 2002).

Heating to at least 90°C leads to SS-linked aggregates between mainly α- and γ-gliadins and glutenin. The incorporation of gliadins into the glutenin structure at higher temperatures results from SH-SS interchange reactions (Lagrain et al. 2008b, Schofield et al. 1983, Singh & MacRitchie 2004). Such interchange reaction readily occurs in (other) proteins at higher temperatures (Volkin & Klibanov 1987). When occurring, a free SH group of glutenin carries out a nucleophilic attack on the sulfur atom of a gliadin SS bond (**Figure 4*b***). With the pK_a value of cysteine being approximately 8.5, this type of reaction is enhanced in alkaline conditions but inhibited in more acidic conditions (Lagrain et al. 2008b, Volkin & Klibanov 1987). The reaction of gliadin with the large glutenin molecules obeys a first-order rate law (Lagrain et al. 2008a).

Although most of the gluten cross-linking during heat treatment occurs through formation of additional SS bonds, cross-links between other amino acids may also occur. Tilley and coworkers (2001) reported an increase in dityrosine bond levels as a result of bread baking. Also, at pH ≥ 6 and high temperatures, β-elimination of cystine forms dehydroalanine (DHA) and free SH groups in gluten proteins (**Figure 4*c***) (Rombouts et al. 2010). Both the formed DHA residues and free SH groups can participate in forming new cross-links. For example, DHA residues can react with cysteine to form the DHA cross-link lanthionine (LAN) (**Figure 4*d***). At pH ≥ 10,

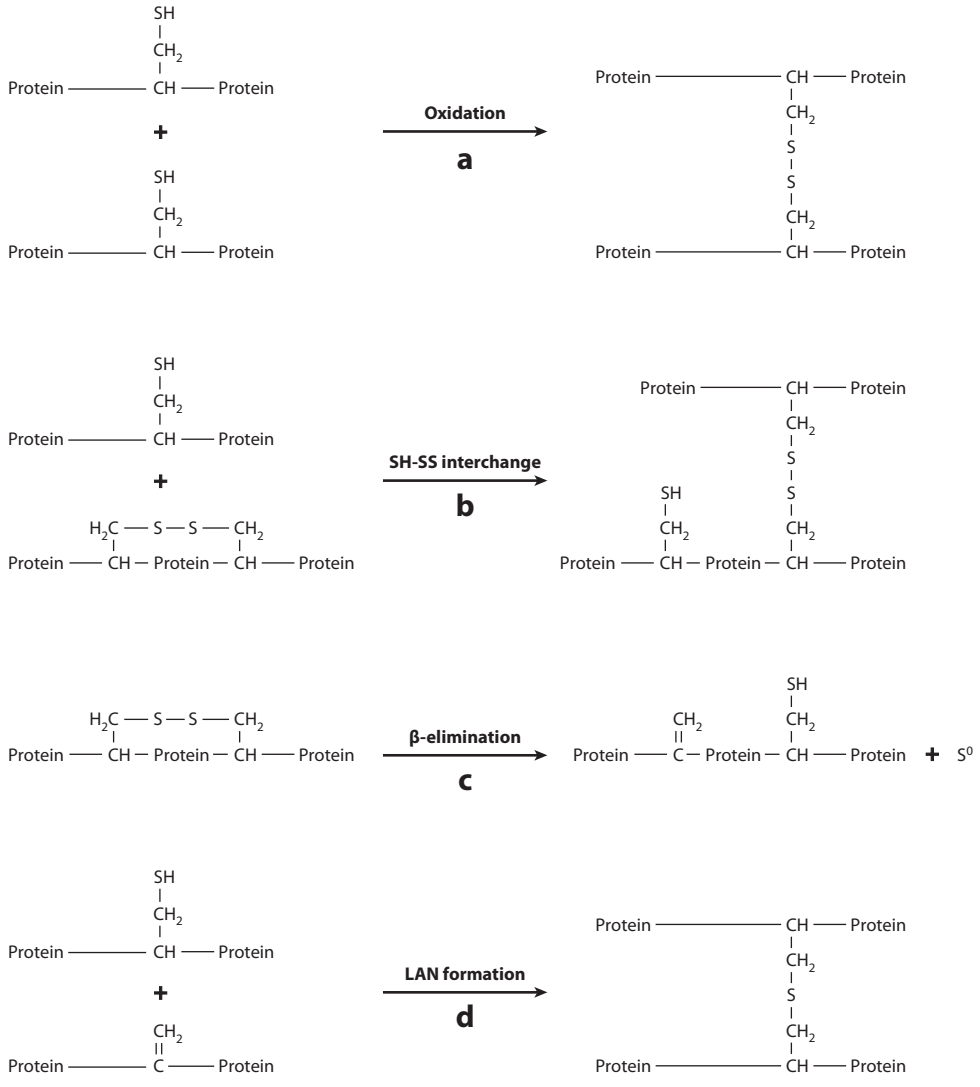

Figure 4

Schematic representation of reactions involving cyst(e)ine residues adapted from Rombouts et al. (2010). (*a*) Oxidation of cysteine residues with a free sulfhydryl (SH) to an intermolecular disulfide (SS) bond. (*b*) SH-SS interchange reaction transforming an intramolecular into an intermolecular SS bond. (*c*) Cleavage of an intramolecular SS bond by a β-elimination reaction leading to dehydroalanine (DHA) and cysteine residues. (*d*) Formation of a lanthionine (LAN) residue by the reaction between DHA and cysteine.

lysinoalanine (LAL), a cross-link between DHA and lysine, can also be formed in gluten (Haraguchi et al. 1980, Rombouts et al. 2010). The newly generated free SH groups can also participate in oxidation or SH-SS interchange reactions leading to additional intermolecular SS bonds in gluten proteins (Lagrain et al. 2011). Furthermore, heating gluten proteins in a dry state at neutral pH may lead to isopeptide bonds between the ε-amino groups of lysine residues and the β- or γ-carboxamide groups of asparagine and glutamine residues, respectively, as shown for gluten peptides and proteins (Rombouts et al. 2011b).

Impacting Gluten Functionality

Chemical additives. Given the crucial role of SS bonds for the elastic properties of gluten, additives impacting the SH functionality in proteins are widely used to optimize gluten rheology during food processing. As outlined above, the levels and types of SS groups can be manipulated by the pH of the reaction environment and by adding oxidizing or reducing agents. Redox agents are frequently used by the milling and bread-making industry to optimize gluten quality in view of the final application. The overall effect of reducing agents, such as cysteine (CSH) or glutathione (GSH), which also naturally occur in wheat flour (see above), is that they reduce the average mol wt of glutenin proteins. As a result, they increase gluten extensibility. Reducing agents react with glutenin proteins through SH-SS interchange reactions freeing one protein unit and leaving a SS bond between protein and reagent. The latter can react with another CSH or GSH, whereby the protein SH group is set free and the oxidized form of the LMW thiol is formed (Wieser 2003). Hence, reducing agents generally cause a loss of glutenin structure prior to heat treatment.

During heat treatment, however, a network structure is formed (Attenburrow et al. 1990, Lagrain et al. 2006) because of the free SH groups initiating cross-linking reactions at high temperatures. After all, free SH groups are obligatory intermediates in interchanges with SS groups present in, for example, gliadins (Lagrain et al. 2006, Lagrain et al. 2008b), and at elevated temperatures, the gliadins also become involved in SH-SS interchange reactions.

Oxidizing agents, such as potassium bromate, potassium iodate, azodicarbonamide, dehydroascorbic acid, and peroxides, oxidize free SH groups to SS moieties. These oxidizing agents generally increase the number of cross-links between proteins, minimize SH-SS interchange reactions and increase gluten's elastic properties. Use of oxidants leads to a more heat stable protein structure as a result of the removal of free SH groups (Hayta & Schofield 2004, Nagao et al. 1981, Veraverbeke et al. 1999).

Enzymes. Peptidases [enzyme commission number (EC) 3.4] catalyze the hydrolysis of the amide (peptide) bond between two amino acids, reducing the mol wt of a protein. Some such enzymes have been used for many years in bakery applications to modify dough stiffness, to improve flavor profiles, and to tenderize product texture (Stauffer 1987).

Transglutaminase (EC 2.3.2.13) catalyzes the formation of isopeptide bonds (see above). The enzyme can be used as a dough improver and because of its excellent protein cross-linking capacity, it also finds application in production of gluten-free bakery products. In regular bread making using wheat flour, it increases gluten elasticity and affects bread volume (Bauer et al. 2003).

Other enzymes promoting formation of covalent bonds between gluten proteins can serve as clean label alternatives to chemical bread improvers. As they are generally inactivated after heat treatment, they are also well accepted by consumers. Such enzymes include glucose oxidase, hexose oxidase, lipoxygenase, and laccase. An overview of these gluten cross-linking enzymes and their functionality in wheat-based foods can be found in Joye et al. (2009b).

GLUTEN FUNCTIONALITY IN FOOD PRODUCTS: FROM FARM TO FORK

Wheat processing generally includes hydration and shearing steps, in which wheat flour is converted to viscoelastic dough, and a heating step, during which the gluten protein structure sets. In the case of bread making, heating sets the loaf structure during baking, while both drying during dry pasta production and subsequent boiling prior to consumption generate the desired pasta

quality. Furthermore, the structure and texture of soft wheat products are also shaped, at least to some extent, by the heat-induced changes in the gluten fraction of the wheat flour.

Milling

T. aestivum wheat cultivars differ in the force needed to mill them, leading to different types of flour with different end-use specificity (Delcour & Hoseney 2010). Wheat roller milling separates, as efficiently as possible, the anatomical parts of the grain (endosperm, germ, and bran) and reduces their size. Prior to the actual milling and to facilitate it, some water is added to wheat kernels in a process known as tempering. Even after tempering, kernels differ in the force required to crush them and in the level of starch damaged during milling. Hard wheat kernels break mainly at the interface between endosperm cell walls and the cell contents. Soft wheat kernels are more easily reduced to flour and break mainly along the starch-protein interface. The mechanism dictating endosperm hardness still remains unclear, but all evidence points to an involvement of an interaction between gluten and starch granules. In hard wheat kernels, the adhesion between the starch granules and the protein matrix is stronger than in soft wheat kernels. This origin of endosperm hardness is genetically controlled (Giroux & Morris 1998). The main difference can be assigned to the presence of the two proteins puroindoline a and b in soft wheats, and the absence or sequence polymorphism of one of those proteins in hard wheats. The presence of both puroindolines has been suggested to weaken the interaction between starch and gluten, resulting in softer wheat kernel texture (Morris 2002).

Durum wheat lacks puroindolines and most likely as a result thereof, it is so hard that it is difficult to reduce it to flour fineness without creating substantial starch damage. Hence, durum wheat is generally tempered to higher moisture levels than common wheat and milled into semolina, i.e., its purified middlings.

Bread Making

In bread making, flour, water (typically 60 g per 100 g of flour), salt, yeast, and often also nonessential ingredients, such as fat and sugar, are mixed into viscoelastic dough, which is fermented and baked (Goesaert et al. 2005). During bread making, complex (bio-)chemical and physical transformations occur and proteins are generally recognized to be very important for bread-making quality. The importance of gluten proteins for bread quality is in part genetically determined as illustrated by the fact that quality scores have been assigned to HMW-GS (Payne et al. 1987).

Mixing disrupts the discrete masses of gluten proteins in wheat flour, hydrates them and the other flour constituents, and transfers elastic energy to dough (Belton 2005). The gluten proteins are transformed into a continuous cohesive gluten network surrounding the starch granules and give dough its viscoelastic properties (Singh & MacRitchie 2001). In optimally kneaded or fully developed dough, protein films or sheets are the predominant structural element (Amend et al. 1991, Bache & Donald 1998). Mixing increases the extractability of the gluten proteins (Veraverbeke et al. 1997, Weegels et al. 1997). Whether this is due to (a combination of) depolymerization (Tanaka & Bushuk 1973a,b, Weegels et al. 1997), conformational rearrangement (Eckert et al. 1993), and /or better dissolution by changed effective surface properties and/or area (Belton 2005) is still subject of debate.

The gluten network gives dough the capacity to hold carbon dioxide gas produced during fermentation and hence is essential for the liquid foam structure of dough (Campbell 2003). An optimal ratio of dough viscosity and elasticity is required for quality bread making. Insufficiently elastic dough as well as dough that is too stiff lead to low bread loaf volume, as they cannot

efficiently retain the carbon dioxide produced or impede the expansion of gas cells (Kasarda 1989), respectively.

During fermentation, carbon dioxide is produced, and the dough rheology changes. Yeast has an oxidizing effect on gluten proteins (Liao et al. 1998). As a result, the dough feels drier and stiffer. In any case, during fermentation, gluten proteins become less extractable (Hamer & Lichtendonk 1987, Veraverbeke et al. 1999). One hypothesis is that glutenin, after being partly depolymerized during mixing, repolymerizes during dough rest (Weegels et al. 1996). Another possibility is that the protein structure, extended during mixing, relaxes and decreases its surface area exposed to the extracting solvent (Eckert et al. 1993).

Strain hardening: strengthening of gluten under large deformation

During baking, dough undergoes irreversible and very manifest structural transformations to form a light, porous product. At the temperatures and moisture contents typical for bread baking, starch gelatinization occurs at approximately 65°C (Delcour et al. 2010). Starch gelatinization withdraws water from the gluten proteins making the air cell walls stiffer. Furthermore, gluten undergoes strain hardening due to gas cell expansion (Delcour & Hoseney 2010). In addition, the increased temperature during baking promotes the formation of protein cross-links as evidenced by a decrease of the level of extractable protein with time of baking (Lagrain et al. 2007, Singh 2005, Veraverbeke et al. 1999, Wieser 1998). SH are involved in the main reactions occurring during bread baking (Lagrain et al. 2007, Singh 2005). Firstly, glutenins polymerize through oxidation of free SH and SH-SS interchange reactions (Lagrain et al. 2008a, Veraverbeke et al. 1999). When bread is further baked, free SH groups initiate gliadin-glutenin cross-linking, resulting in a large protein network (Lagrain et al. 2007). The increase of gluten strength because of loss of plasticizer, strain hardening, and polymerization contributes to the sharp increase in tensile stress that occurs in dough on heating, leading to the rupture of gas cells and to the loss of gas retention (Gan et al. 1995). As a result of the above, the continuous gluten network contributes to the structure and initial texture of bread crumb.

Pasta Production

Durum wheat is the preferred raw material for producing pasta. The levels of a given durum wheat cultivar's LMW-GS are an important genetically controlled quality attribute (D'Ovidio & Masci 2004). In particular, good quality is positively correlated with high levels of LMW-m and -s type GS in glutenin and negatively correlated with levels of α- and γ-gliadins (D'Ovidio & Masci 2004). However, it has been suggested that the quality of the raw material is not very critical when pasta is dried at high temperature (HT) and a controlled relative humidity (Cubadda et al. 2007, Zweifel et al. 2003).

The minimum formula for pasta is semolina and water to obtain crumbly dough with a moisture content of about 31%, which is substantially lower than that in bread dough. The added water plasticizes wheat proteins and allows shaping of pasta by extrusion or sheeting. In the subsequent drying steps, the product is stabilized by transforming it from the rubbery to the glassy state (Zweifel et al. 2003). During shaping, drying, and boiling the protein phase determines pasta properties by forming a structured network (Fardet et al. 1998, Zweifel et al. 2003).

Fresh pasta is viscoelastic and soft, whereas dry pasta is elastic and rigid (Cuq et al. 2003). Pasta drying is a delicate process with the risk of serious quality defects, such as undesired color, surface cracks, and fissures. Generally, the outer surface of the pasta strand is dried rapidly in approximately 30 min. The rapid drying period is followed by a longer and more moderate drying period (hours) at a relatively constant temperature of approximately 80°C to 85°C in humid air to decrease the moisture gradients in the pasta product. In a final step, the pasta is dried to a moisture content of 12% (Delcour & Hoseney 2010).

Although during the pasta drying process, starch maintains it granular identity (Delcour et al. 2010), proteins cross-link at temperatures exceeding 60°C. When the temperature is raised above 80°C, glutenin and gliadin extractabilities are diminished (Favier et al. 1996, Zweifel et al. 2003). In line with the observations during bread baking, glutenins polymerize first in drying pasta, inter alia because of oxidation of their free SH groups (Lamacchia et al. 2007) and part of the monomeric gliadins cross-links with the unextractable glutenin fraction. The thermal loss of extractability of gluten proteins proceeds according to a first-order reaction (Favier et al. 1996). The protein polymerization is not affected by the decrease in moisture levels during HT (>60°C) drying (Zweifel et al. 2003) because the proteins remain above T_g during drying (Cuq et al. 2003). The polymerization of gliadin and glutenin during HT drying promotes the formation of a dense and continuous protein network that at least partly encapsulates the starch granules (Bruneel et al. 2010, Zweifel et al. 2003). The extent of protein polymerization after drying is critical for final pasta cooking quality.

During boiling, starch granules absorb water, swell, and gelatinize (Delcour et al. 2010), and protein extractabilities rapidly decrease as boiling proceeds, indicating further protein polymerization (Bruneel et al. 2010). This makes pasta a mixed polymer system with starch and protein as main structuring agents. Eating quality depends on two main parameters: viscoelastic behavior (particularly firmness after cooking) and surface condition (extent of disintegration). These determine product stickiness and degree of smoothness. Quality pasta tolerates moderate overcooking and has minimal cooking losses leading to a product with a smooth surface that has a certain firmness and resilience (Sissons et al. 2005). Cooking losses and surface stickiness are caused by excessive starch swelling. The latter can be prevented by the protein structure. Hence, pasta cooking quality largely depends on how the protein network withstands the starch swelling during cooking (Delcour et al. 2000a,b). In this view, a delicate competition during pasta cooking between (further) protein polymerization into a continuous network and starch swelling exists. An optimally cross-linked protein network traps starch particles in the network, restricts starch swelling and subsequent leaching, and promotes firmness in cooked pasta (Resmini & Pagani 1983, Vansteelandt & Delcour 1998). With insufficient cross-linking during cooking, the protein forms discrete masses lacking a continuous framework and pasta shows cooking losses, softness, and usually stickiness (Pagani et al. 1986, Resmini & Pagani 1983). However, with excessive protein cross-linking during harsh drying, the proteins lack resilience to cope with starch swelling during cooking, which is also deleterious for cooking quality (Bruneel et al. 2010).

From the above, it follows that the drying process all in all should be directed at an optimal rather than a maximal degree of protein cross-linking.

Manufacture of Soft Wheat Products

The following discussion is limited to the role of gluten proteins in cake and cookie products as well as hard pretzels.

Cake products. There are many types of cake, as follows from their recipes and/or production modes. In general, cake recipes contain flour, sugar, egg, and mostly fat. High ratio cakes contain more sugar than flour in their recipe, whereas pound cakes are produced from equal portions of sugar, egg, fat, and flour (Delcour & Hoseney 2010). During cake recipe mixing, full development of gluten into a continuous viscoelastic structure does not occur (Willhoft 1973), and cake ingredients form a viscous batter instead of viscoelastic dough. In cake recipes, gluten is diluted with eggs, fat, and sugar and is therefore less concentrated than in bread dough. Owing to lower viscosity than in, for example, bread dough, less friction and thus less energy is exerted on the

gluten during mixing (Cauvain & Young 2006). In cake batter, gluten serves as a water binder increasing viscosity (Donelson & Wilson 1960a,b). Cake batter needs to be sufficiently viscous to restrict migration, coalescence, and hence loss of gas cells (Wilderjans et al. 2008). During baking, individual bubble expansion must dominate over coalescence and disproportionation to make cake rise. With starch and eggs, cake batter contains two gel forming systems that, during baking, combine to form the structure of the cell walls. Final structure setting of cake occurs when starch gelatinizes and egg protein coagulates (Guy & Pithawala 1981). These events tremendously increase the viscosity of the batter, giving it a solid appearance and causing the end of oven rise. Temperature gradients in cake baking result in earlier structure setting in the bottom of the cake than in the top and center regions (Wilderjans et al. 2010a). During baking, interactions and reactions of both egg and gluten proteins are important for cake structural properties (Wilderjans et al. 2008). A major decrease in protein extractability during baking is observed, which indicates egg and gluten protein cross-linking. This decrease is more pronounced when the cake recipe contains more gluten (Wilderjans et al. 2008). Egg proteins are generally the largest protein fraction and contain seven times more free SH than gluten, making them highly susceptible to SS cross-linking when they are denatured. This results in a protein network in which SH-SS exchange reactions between gluten can be interrupted by interactions between egg and gluten protein (Kiosseoglou & Paraskevopoulou 2006). When cake is removed from the oven, it cools and the gases contract or condense. The structural strength of the crumb then determines whether collapse occurs (Guy & Pithawala 1981). The protein network reinforces the cake cellular structure and prevents its collapse (Wilderjans et al. 2010b, Wilderjans et al. 2008). If so, this results in high-quality cake with a high volume, low density, and a fine, relatively uniform crumb structure. Cake moisture contents typically vary between 18% and 28%, and are lower than those of bread but higher than those of cookies.

Cookie products. The term cookies refers to baked products generally containing flour, sugar, fat, and only low water levels (Pareyt & Delcour 2008). Two types of cookies are generally distinguished based on the type of dough they are made of, i.e., hard or short dough (Wade 1988). Hard dough resembles bread dough in that a gluten network is formed during mixing, and that it contains relatively high water (up to 30% on as-is basis) and low sugar and fat levels (up to 14% and 12%, respectively). Short dough contains no viscoelastic gluten network. It typically contains high sugar (up to 30%) and fat (up to 25%) levels, but low water levels (about 15%). Cookies are preferably made from soft wheat flour. European wheat flour used for cookie making generally is inferior to that used in North America in that the former yields smaller cookies. This is because European cookie flour is generally obtained from wheat that is less soft than the soft wheats available in North America. The flour then has higher damaged starch levels and stronger gluten. Still, within the available flour types, those with the lowest water absorption properties (low protein and damaged starch levels) are preferred.

We here discuss the role of gluten proteins in short-dough cookie making, with a focus on sugar-snap cookies. Short-dough mixing is generally a multistep process in which fat (either shortening or margarine) is first mixed together with the water and sugar to obtain a cream. To this, flour is added with minimal mixing (Pareyt & Delcour 2008). This type of mixing, together with high sugar, high fat, and low moisture levels, yields nonextensible and nonelastic dough with minimal if any gluten development (Baltsavias et al. 1999, Gaines 1990). During mixing, gluten proteins bind water and affect dough viscosity, but neither expand nor develop into a continuous network, probably because, under the conditions of the system, they are below their T_g and hence immobile (Doescher et al. 1987, Miller et al. 1996). Thus, at room temperature, the flour particles in dough remain intact and discontinuous, and dough cohesiveness is mainly determined by a

continuous sugar-syrup phase and when high fat levels are present, a continuous fat phase (Pareyt et al. 2010a).

Maillard reaction: chemical reaction between an amino acid and a reducing sugar resulting in nonenzymatic browning similar to caramelization

During short-dough cookie baking, the high sucrose and low water levels prevent starch gelatinization and changes in gluten proteins determine cookie quality. Short-dough cookies generally spread or flow at a constant rate during baking. This is partly caused by chemical leavening but also by gravitational flow due to the heat-induced decrease in apparent viscosity of the dough (Miller et al. 1997), which itself is determined by sugar dissolution, fat melting, and the competition for the available water (Hoseney & Rogers 1994). The cookie flows until the viscosity suddenly increases (Abboud et al. 1985). This sudden viscosity increase has been attributed to the flour proteins (Gaines 1990, Pareyt et al. 2009, Pareyt et al. 2010c). In the oven, the gluten proteins undergo an apparent glass transition (Doescher et al. 1987, Miller et al. 1996) and the dough matrix remains plasticized during baking (Chevallier et al. 2002). Under such conditions, the proteins are mobile and cross-link to a network consisting of glutenin and gliadin (Pareyt et al. 2008, Pareyt et al. 2010c). The viscosity of the now continuous gluten network suffices to offset the gravity force that causes the dough to flow (Doescher et al. 1987, Miller et al. 1996).

The cross-linking of gluten in cookie baking is affected by the initial sugar level. High sucrose concentrations in the formula not only delay the onset of gluten cross-linking but also decrease the rate of the cross-linking reactions. Thus, higher sugar concentrations lead to cookies with a larger diameter (Pareyt et al. 2009, Slade & Levine 1995). The extent of gluten cross-linking also affects cookie height because gluten cross-linking prevents structural collapse at the end of baking.

During cooling, the cookie structure is formed when the amorphous sugar syrup becomes glassy below its T_g. As such, a composite matrix made of a protein network with lipids and sugars embedding intact or little damaged starch granules is obtained (Chevallier et al. 2000, Chevallier et al. 2002).

Pretzels. Hard pretzels are popular savory wheat-based snacks, often with the shape of a knot or a stick and a hard shiny outer face. For pretzel making, wheat flour, water, shortening, salt, and a leavening agent are developed into a rather dry and tough dough that has approximately two-thirds of the water level used for bread dough (Delcour & Hoseney 2010). The dough is further shaped by extrusion at relatively low pressure. However, the unique step during pretzel making is the dough surface treatment with hot alkaline solution (1.0% NaOH about 90°C). Baking is divided into a rapid initial bake at high temperature, followed by a slow drying process at lower temperature (Delcour & Hoseney 2010, Seetharaman et al. 2004). The particular taste and hard shiny surface of pretzels result from the alkaline dipping prior to baking. This treatment gelatinizes starch granules at the dough surface and induces Maillard and caramelization reactions (Yao et al. 2006) that give pretzels their specific look. Moreover, the strong alkaline conditions induce and enhance protein cross-linking after dipping and certainly after baking, leading to both SS and non-SS bonds (Rombouts et al. 2011a). Indeed, the presence of LAN (Rombouts et al. 2011), LAL (Sternberg et al. 1975), and the Maillard cross-link pentosidine (Henle et al. 1997) have been observed in pretzels. However, to the best of our knowledge, no systematic study on the relative importance of SS bonds and non-SS bonds for pretzel texture is available.

CONCLUSIONS, RESEARCH NEEDS, AND PERSPECTIVES

In spite of the large number of excellent studies dedicated to the topics, important knowledge gaps remain concerning the (native) structure and functionality of gluten proteins. Because glutenins have mol wt ranging up to tens of millions, it has been difficult to isolate them and to determine both

their precise structure and mol wt distribution. Until now, only hypothetical structures of wheat glutenin have been proposed. Nevertheless, understanding the native cross-linking mechanism is of great value to control end-use quality and processing properties. One of the priorities should be to understand the nature, changes and dynamics of the SS structures from the synthesis of proteins in the growing plant to the final processed products (Wieser 2007). Additionally, the organization of gluten proteins in a developed gluten network structure, the importance of hydrogen bonds, and their rheological implications should be further elucidated. A better understanding of the relations between the exceptional structure and unique functionalities of gluten proteins would certainly lift gluten processing research from the current empirical approach to a more rational implementation of specific process principles.

Understanding gluten functionality is also important in view of the search for gluten alternatives. Both an increased awareness and diagnosis of gluten intolerance or celiac disease and the concomitant need to replace gluten proteins in certain food products would benefit from more basic insights. The lack of knowledge on the relation between the structural organization and unique functionality of gluten proteins, however, makes the search for satisfying alternatives for their properties a real challenge.

Gluten network formation during processing has now mainly been attributed to formation of intermolecular SS bonds by oxidation of SH groups of cysteine residues and/or SH-SS interchange reactions. Such reactions lead to further glutenin cross-linking at moderate heating and at higher temperatures also involve gliadin. Furthermore, the level and type of SS bonds can also be modified by the addition of redox agents or enzymes. Although of major importance, not all phenomena during thermal processing of gluten can be fully explained by reactions involving SS bonds. Especially gluten reactions at higher pH are of particular importance because they clearly enhance the thermal cross-linking of wheat gluten and lead to unreducible covalent cross-links such as LAN, LAL, and in the presence of reducing sugars, Maillard cross-links. These amino acid modifications, which can affect protein's nutritional properties (Friedman 1999), can also contribute to the gluten network structure in end products. However, further research is necessary to identify all possible unreducible bonds, to understand and control the chemistry behind their formation, and to determine their technological and nutritional importance.

Overall, challenges include generating knowledge on the characteristics of gluten proteins, on chemical reactions during processing and their impact on gluten polymer structure and physical performance, and how we can control and manipulate them. Hence, input from food chemistry and biochemistry, but also from polymer physical-chemistry and nutrition science, is required to design and deliver convenience products of high nutritional quality.

SUMMARY POINTS

1. Gluten is the rubbery, viscoelastic mass that is formed when the gluten-forming proteins, i.e., the major storage proteins of both bread wheat (*Triciticum aestivum* L.) and durum wheat (*Triticum durum* Desf.), are mixed with water. Gluten-forming proteins consist of gliadins and glutenins.
2. Gliadins are extractable in aqueous ethanol and have mol wt ranging from 30,000 to 60,000. The cysteine residues, when present, are all involved in intrachain SS bonds.

3. Glutenins are large polymers with mol wt up to twenty million made up of GS linked together through SS. Within GS, HMW (70,000 to 90,000) and LMW (30,000 to 45,000) GS are distinguished. GS differ from gliadins in that, at room temperature already, they also form interchain SS bonds. The exact glutenin structure is not known, but most models suppose a large linear polymer with branches.
4. Glutenin is responsible for gluten elasticity but its exact origin is still under debate. Gliadin contributes viscosity to the network. Upon heating, glutenin and gliadin cross-link through oxidation of free SH groups and SH-SS interchange reactions, leading to a very large protein network. The formation of SS bonds can also be manipulated by addition of redox agents.
5. In bread making, gluten gives dough its viscoelastic properties by forming a network, which during fermentation and baking is responsible for dough's gas-retaining properties. Upon baking, the gluten proteins undergo irreversible changes as they cross-link and contribute to final bread crumb structure.
6. Fresh pasta is made from durum semolina and water resulting in crumbly dough. After, extrusion pasta is mostly dried. During this drying step the gluten fraction undergoes comparable chemical changes, as in bread baking. The formed network largely affects pasta surface quality, cooking losses, and eating properties.
7. To prepare cake, a batter is made in which gluten serves as a water binder. As such, gluten increases viscosity, which is needed to retain gas cells in the batter. Along with gluten, egg proteins are important for final cake structure. Baking induces a network built up from egg and gluten proteins, providing the cake cell walls with strength against structural collapse.
8. Cookie types containing high sugar and fat levels and low water levels show no gluten development during dough mixing because the protein remains glassy. Gluten in such short dough mainly impacts dough viscosity through its water-binding properties. During heating, gluten proteins become mobile, react, and form a network that stops dough spread and increases resistance to collapse, determining cookie diameter and height.

FUTURE ISSUES

1. What is the exact molecular structure of glutenin?
2. How do gluten proteins interact in a developed gluten network, and how do these mesoscale properties determine the rheological properties of the network?
3. How does the structure of gluten proteins change during the different processing steps of food products?
4. How does the final gluten structure contribute to product quality?
5. How can the unique functionality of gluten proteins be replaced in food products for people with gluten intolerance?
6. What is the occurrence and relative importance of chemical bonds other than SS bonds in the structure of processed gluten proteins?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

B. Lagrain, I.J. Joye, and B. Pareyt wish to acknowledge the Research Foundation—Flanders (FWO, Brussels, Belgium); E. Wilderjans, the Leuven R&D (K.U. Leuven, Leuven, Belgium); and K. Brijs, the Industrial Research Fund (IOF, K.U. Leuven) for their positions as postdoctoral fellows.

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