Polymerization Reactions of Wheat Gluten: The Pretzel Case[1]

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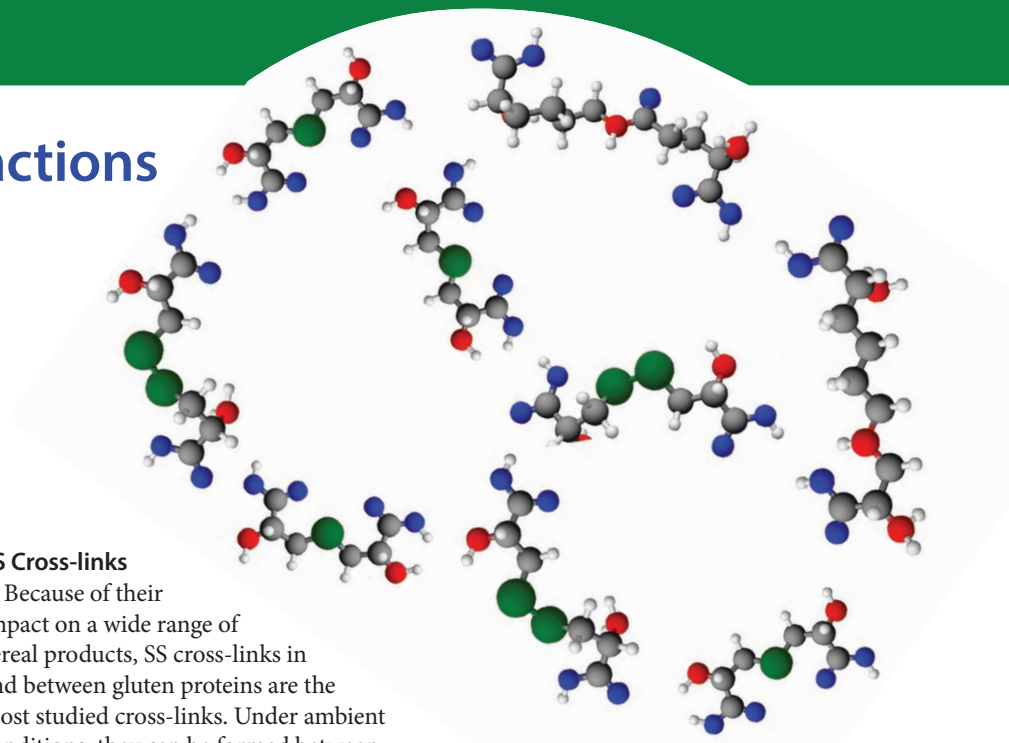

Network formation of wheat gluten proteins (containing monomeric gliadin and polymeric glutenin) is essential for many wheat-based food products, including breads, cakes, cookies, pasta noodles, pizza doughs, biscuits, doughnuts, croissants, and bagels. Gluten network formation impacts, for example, bread volume (29), cake collapse (57), cookie break strength (33), and pasta cooking losses (11). The mechanisms of polymerization depend on processing conditions. Disulfide (SS) cross-linking determines the end-product quality of many, if not all, wheat-based food products. In addition, non-SS cross-links can be formed under certain processing conditions, e.g., at acidic or basic pH levels, low moisture contents, or high temperatures.

This article provides an overview of potential cross-linking reactions in wheat-based food products (Fig. 1). The final section deals with the gluten network formed in hard pretzels. Because the production of these popular savory wheat-based snacks involves a heat and alkaline dip treatment followed by baking and drying to a final moisture content of 2%, it induces various types of cross-links in and between gluten proteins.

SS Cross-links

Because of their impact on a wide range of cereal products, SS cross-links in and between gluten proteins are the most studied cross-links. Under ambient conditions, they can be formed between glutenin proteins during dough mixing as a result of sulfhydryl (SH)-SS interchange or the presence or use of oxidants as a result of SH oxidation (15). In the case of SH-SS interchange reactions, a free thiolate (S^-) anion group carries out a nucleophilic attack on the sulfur atom of an SS bond (Fig. 2B). Because the pK_a value of cysteine is ≈8.5, this type of reaction is enhanced under alkaline conditions and inhibited under more acidic conditions (30,51). The oxidation of two SH groups leads to an additional SS bond (Fig. 2A). Even a few cross-links between large glutenin polymers can substantially enlarge the gluten network (32). Under ambient conditions, the intramolecular SS bonds in gliadins are not involved in SS cross-linking. When fully hydrated gluten is heated above 75°C, both gliadin and glutenin can be incorporated into the protein network (30,43,46). The reaction of gliadin with glutenin molecules follows first-order kinetics (27). Gluten is most susceptible to SS cross-linking at moisture contents exceeding 20% (54,55).

The importance of free SH groups for gluten polymerization was demonstrated by adding redox agents to gluten-in-water suspensions and monitoring viscosity and protein extractability losses in sodium dodecyl sulfate (SDS)-containing media during subsequent heating (26). Oxidants decreased protein extractability loss during heating, while low levels of reducing agents increased it. This suggests that oxidants hinder gluten polymerization during heating due to decreased levels of free SH groups, while reducing agents facilitate gluten polymerization by increasing levels of free SH groups. Similarly, the addition of redox agents has been shown to impact gliadin-glutenin cross-linking during baking of breads (29), cakes (57), and cookies (33) and during drying and cooking of pasta (10), which affects final product quality.

Cross-links Derived from Tyrosine

Tilley et al. (49) pointed out the potential importance of dityrosine and isodityrosine cross-links in wheat gluten. Such cross-links, in principle, can be formed by a radical mechanism involving laccase (EC 1.10.3.2) or peroxidase (EC 1.11.1.7). In the presence of molecular oxygen and the enzyme tyrosinase (EC 1.14.18.1), tyrosyl residues transform to *o*-quinone structures, which then can react nonenzymically with, for example, the ε-amino group of lysine or the SH group of cysteine (1,2,17).

However, the percentage of dimeric tyrosine is <0.1% of the tyrosine residues of wheat protein in common wheat dough (22). In addition, Peña et al. (34) showed that the number of cross-links between tyrosine residues in such dough is small and of much less importance to the structure of the gluten network than SS cross-links. Attempts to stimulate the formation of tyrosine-derived cross-links throughout the breadmaking process have failed. The use of peroxidase (48),

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tyrosinase (45), and laccase (25,45) in baking applications can improve dough handling, crumb structure, loaf volume, and/or resistance to staling, but the effects on dough and bread are primarily ascribed to SS cross-linking and cross-linking of ferulic acid residues of the arabinoxylan thionine fraction. In conclusion, in spite of several studies on tyrosine-derived cross-linking of wheat proteins, experimental evidence pointing to the importance of this type of cross-linking for wheat-based food products remains limited.

Cross-links Derived from Dehydro Amino Acids

Other possible cross-links result from the formation of dehydro amino acids. In most cases, an alkaline pH-favored elimination reaction in the β-position of the chiral carbon atom of cystine yields dehydroalanine and cysteine. The newly formed free SH group of cysteine can either oxidize or initiate SH-SS interchange reactions, resulting in SS cross-links; however, dehydroalanine residues can also react with cysteine or lysine to form the cross-links lanthionine or lysinoalanine, respectively (18). Figure 2A–E shows such reactions. In addition to cystine, cysteine, serine, and threonine also are consumed in β-elimination reactions, but at only ≈3–7% of the rate at which cystine is consumed (56).

To investigate the potential occurrence of such reactions in wheat-based products, gliadin, the monomeric fraction of gluten containing no free SH groups, was heated at pH 8.0 and 130°C (37). Because the treatment decreased gliadin extractability in SDS-containing media under reducing and nonreducing conditions, it was concluded that both SS and non-SS cross-links were formed between gliadins. In addition, decreased SS levels and the presence of dehydroalanine and SH groups in heated samples indicated cleavage of SS bonds by β-elimination reactions, while amino acid analysis revealed the irreversible non-SS cross-link lanthionine. In an additional study, gluten was heated at pH 13.0 and 70°C. This treatment induced not only lanthionine formation, but also lysinoalanine formation (37). The reaction of lysine with dehydroalanine requires reactive (i.e., nonprotonated) lysine residues. Therefore, at pH levels exceeding 10 (the pK_i value of lysine [7]), lysinoalanine formation is favored. Lanthionine formation is also pH dependent. However, because the pK_i value of cysteine is lower (≈8.4) (7), lanthionine is formed at lower pH levels than lysinoalanine. The kinetics of β-elimination of cystine and subsequent formation of lanthionine were studied by heating model systems containing gliadin at different pH levels (6.0, 8.0, and 11.0) and temperatures up to 120°C (28). Multiresponse modeling was used to simultaneously describe the levels of the reaction partners, intermediates, and products. The rate constant for the elimination reaction, which followed first-order kinetics, increased with temperature and pH. Lanthionine formation followed second-order reaction kinetics, and the corresponding reaction rate constant was less dependent on temperature and pH than that of the elimination reaction. The proposed kinetic model was used to estimate the reaction product concentrations in cereal-based foods. β-Elimination and subsequent lanthionine formation are probably less important during breadmaking but may well contribute to gluten network formation in the case of alkaline processing. For instance, gluten is subjected to heat and alkali treatment to prevent microbial attack on moist grain (18) and to increase the solubility of gluten proteins (4). In addition, lysinoalanine has been found in alkaline noodles (≈1.5 μmol/g of protein) (23) and pretzels (0.2–9 μmol/g of protein) (23,35,38,47).

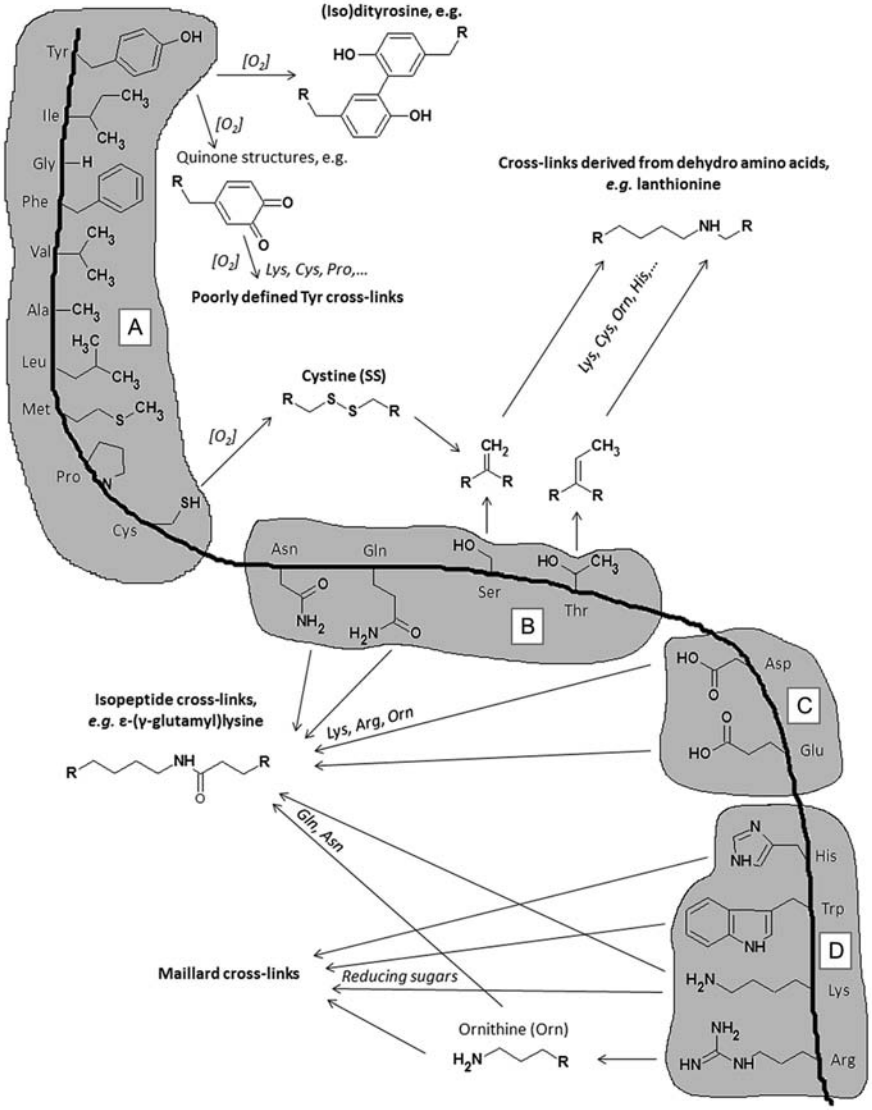

Fig. 1. Potential cross-linking of nonpolar (**A**), polar (**B**), acidic (**C**), and basic (**D**) amino acids. R indicates peptide chain.

Isopeptide Cross-links

The reactive ε-amino group of lysine can be involved in isopeptide bond formation. This reaction is catalyzed by transglutaminase (EC 2.3.2.13) but also occurs nonenzymically at high temperatures and neutral pH and under dry conditions. Isopeptide bonds cannot be identified by conventional amino acid

analysis because the technique involves acid hydrolysis of all peptide bonds and, thus, also of isopeptide bonds. In nongluten proteins, they have been identified after enzymic hydrolysis (42). For this approach, exhaustive proteolytic digestion of the samples is crucial because incomplete hydrolysis results in underestimation of isopeptide levels (42).

A method for complete enzymic hydrolysis of gluten proteins, to the best of our knowledge, has not been reported. However, numerous studies have demonstrated the cross-linking potential of transglutaminase and its impact on the texture, rheological properties, and water-holding capacity of gluten gels (53), wheat doughs (3,40), and pasta (41). Transglutaminase-induced cross-links mainly involve high molecular weight glutenin subunits (HMW-GS) and α-gliadins (5,6). To investigate heat-induced formation of isopeptide cross-links in wheat-based products, dry wheat gluten was heated at 130°C (39). This treatment decreased gluten extractability in SDS-containing media, even after reduction of SS bonds, which indicated that both SS and non-SS cross-links are responsible for the loss of extractability. Lysinoalanine and lanthionine were not present in the heated samples, but levels of available amino groups were reduced. All of these observations support the hypothesis that isopeptide cross-links are formed in and between gluten proteins during heating under dry conditions.

In the next step, the cysteine and cystine residues of purified HMW-GS were alkylated with iodoacetamide. Thermal treatment of these samples under dry conditions also resulted in loss of extractability, which demonstrated that cross-linking does not depend on the availability of cysteine or cystine.

In a final step, isopeptide cross-links were detected by liquid chromatography-tandem mass spectrometry between heated lysine- and glutamine-containing model peptides that naturally occur in HMW-GS. Isopeptide cross-linked dipeptides were detected after 60 min of heating at 130°C, and their level increased with heating time. It is uncertain whether isopeptide bond formation occurs at lower temperatures as well. The identification of isopeptide cross-links after thermal treatment of complete gluten proteins, or even a flour sample, remains a challenge. Nevertheless, all available studies point to the possibility that isopeptide cross-links may be relevant for pasta making, industrial isolation of wheat gluten, thermomolding of wheat gluten through dry processing, and drying of cereal-based food products to very low moisture contents. Methods that allow accurate quantification of isopeptide bonds in wheat gluten would be helpful to further evaluate the importance of isopeptide bonds for wheat-based applications.

Maillard Cross-links

High temperatures, low moisture contents, and alkaline conditions promote a number of consecutive reactions classified under the term "Maillard reaction," which among other things involves the ε-NH_2 group of lys and reducing sugars (8). Maillard products impact flavor, aroma, color, and nutritional characteristics (31,50). For instance, Maillard reactions play a key role in the development of bread crust aroma, color, and flavor. As early as 1910, the occurrence of maltol and isomaltol, which act as natural bread flavorants, was reported. Gradually, the list of Maillard compounds found in dough, oven vapors, and bread has grown to include more than 70, including alcohols, aldehydes, ketones, carboxylic acids, and esters (13). Without Maillard reactions, bread, as well as cakes and pastries, would be pale (16). Many key odorants resulting from Maillard reactions have been reported in breads (wheat and rye), toasted breads, puff pastries made with different fats, fragrant and nonfragant rices, sweet corn, popcorn, and tortillas (21). Furthermore, flavor compounds that result from reactions between lipids and Maillard reaction products are formed during extrusion cooking of wheat flour (9).

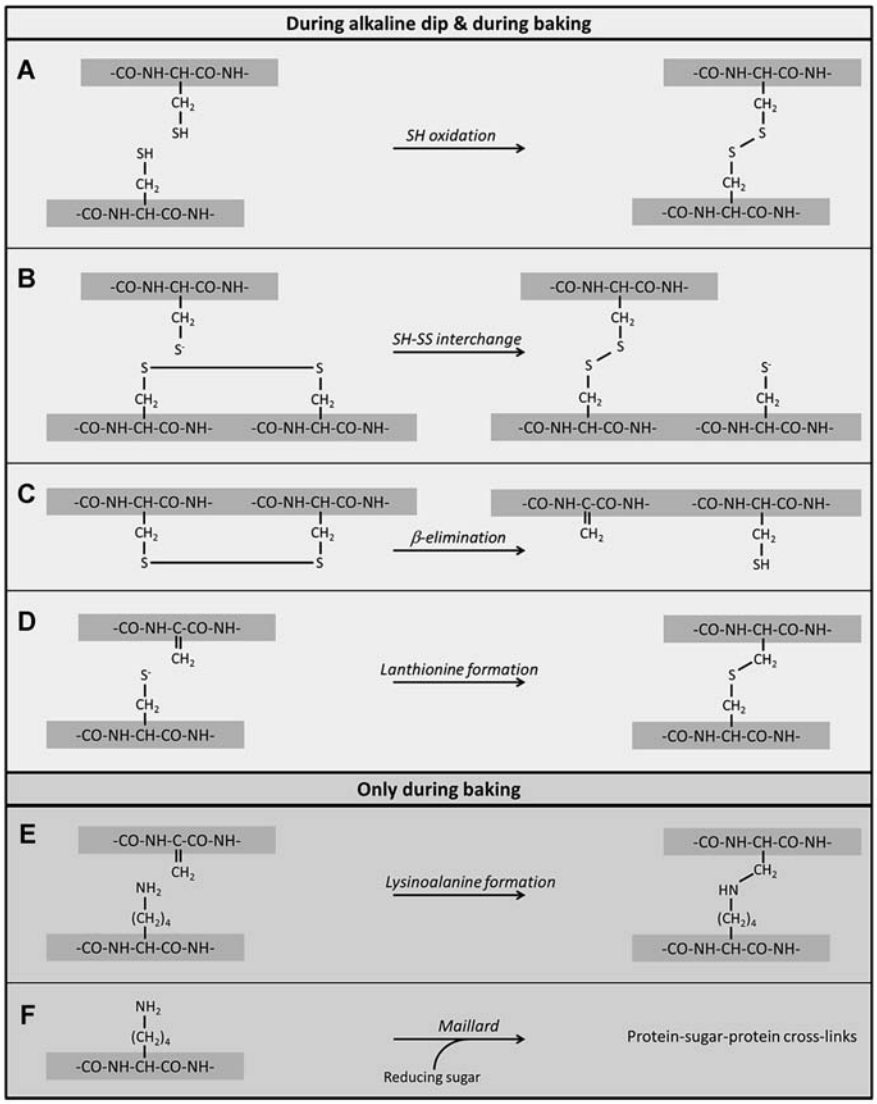

Fig. 2. Overview of reactions during pretzel production that result in protein cross-links: **A,** sulfhydryl (SH) oxidation; **B,** SH-disulfide (SS) interchange; **C,** β-elimination; **D,** lanthionine formation; **E,** lysinoalanine formation; and **F,** Maillard reactions. Reproduced from Rombouts et al. (38) with permission from Springer Science+Business Media.

In some cases, Maillard reactions cross-link two protein chains by bridging two amino acid side chains via a reducing sugar (8,19). Identified Maillard-derived protein cross-links in foods include histidino-threosidine (12), pentosidine, bispyrraline, and bisarg (8). In bread baking, for example, the addition of glutaraldehyde alters the crumb strength and texture of bread through Maillard cross-linking of wheat proteins (20).

Cross-links in Pretzels

Hard pretzels, often in the shape of a knot or a stick, owe their unique taste and hard shiny surface to alkaline dipping prior to baking, typically for 30–45 sec in 1.0% (wt/vol) sodium hydroxide at 80–90°C (14,44,52). This treatment causes gelatinization of starch granules at the dough surface, dissociates amylose-lipid complexes, and induces Maillard and caramelization reactions (58). The alkaline dip is followed by a rapid initial bake at high temperature and a slow drying step at a lower temperature (14,44,52).

Under standard pretzel-making conditions, reducible (SS) as well as nonreducible (non-SS) cross-links are formed during the alkaline dip and baking (38). This was concluded based on the decrease in protein extractability in the SDS-containing media of pretzel dough both under nonreducing and reducing conditions. To identify non-SS cross-links formed during pretzel production, potential precursors and cross-links were quantified in pretzel dough before and after the alkaline dip and in the end product (38). Figure 2 presents an overview of reactions that contribute to the protein network in hard pretzels. The oxidation of free SH groups (Fig. 2A) and SH-SS interchange reactions (Fig. 2B) can induce SS cross-links, which play a key role in the gluten network in pretzels. Another important reaction that results in protein cross-links is β-elimination of cystine (Fig. 2C). It not only yields SH groups, which can be involved in SS cross-linking, it also yields dehydroalanine, which can be involved in nonreducible dehydro amino acid-derived cross-linking. One such cross-link, lanthionine (Fig. 2D), has been detected in pretzel dough after dipping (7 μmol/g of protein) and in the end product (50 μmol/g of protein), while another, lysinoalanine (Fig. 2E), has only been detected in the end product (9 μmol/g of protein) (38). In addition, disproportionate lysine losses during baking, together with decreasing reducing sugar levels and increasing redness, suggested the occurrence of Maillard reactions in this process step (38). Several Maillard end products, including the Maillard cross-link pentosidine, were identified in baked pretzels (Fig. 2F). However, the magnitude of the impact of Maillard reactions on the color, flavor, aroma, and nutritional quality of a food product does not necessarily imply a high cross-linking potential. In fact, levels of Maillard-derived cross-links in pretzels (0.03–0.06 μmol pentosidine/g of protein) (24) are much smaller than those of dehydroalanine-derived cross-links. No indications of isopeptide cross-linking during pretzel production were found (36). It is possible that isopeptide bond formation during pretzel production is limited by competing Maillard reactions.

A distinction should be made between intra- and intermolecular cross-links. Based on the observed increasing molecular weights of nonreduced and reduced proteins during pretzel production, at least some SS and non-SS bonds, respectively, are assumed to form intermolecular links. With regard to non-SS cross-links, the highest levels were found for dehydroalanine-derived cross-links (36).

Higher dipping temperature, longer dipping, and higher sodium hydroxide concentration all led to increased protein extractability losses and redness and higher levels of lysinoalanine and lanthionine in the end product (38). However, no indications of Maillard-derived cross-links or lysinoalanine were found in any pretzel dough immediately after dipping, even when severe dipping conditions were used. In comparison to an alkaline dip, a dip in water (45 sec at 95°C) limits SS cross-linking and even prevents non-SS cross-linking, which illustrates the impact of alkaline conditions (38). As a result, the protein network in bagels, which are boiled in water prior to baking, is probably less strong than that in pretzels.

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