

Tyrosine Cross-Links: Molecular Basis of Gluten Structure and Function

Katherine A. Tilley,^{*,†,‡} Rachel E. Benjamin,[†] Katherine E. Bagorogoza,[†] B. Moses Okot-Kotber,^{†,‡} Om Prakash,[§] and Haidoo Kwen^{†,‡}

Department of Grain Science and Industry, 201 Shellenberger Hall, Kansas State University, Manhattan, Kansas 66506; Food Science Graduate Program, 209 Call Hall, Kansas State University, Manhattan, Kansas 66506; and Department of Biochemistry, 209 Chemistry-Biochemistry Building, Kansas State University, Manhattan, Kansas 66506

The formation of the large protein structure known as “gluten” during dough-mixing and bread-making processes is extremely complex. It has been established that a specific subset of the proteins comprising gluten, the glutenin subunits, directly affects dough formation and breadmaking quality. Glutenin subunits have no definitive structural differences that can be directly correlated to their ability to form gluten and affect dough formation or breadmaking quality. Many protein structural studies, as well as mixing and baking studies, have postulated that disulfide bonds are present in the gluten structure and contribute to the process of dough formation through the process of disulfide-sulfhydryl exchange. Evidence presented here indicates that tyrosine bonds form in wheat doughs during the processes of mixing and baking, contributing to the structure of the gluten network. The relative contributions of tyrosine bonds and disulfide–sulfhydryl interchange are discussed.

Keywords: *Wheat; Triticum aestivum; gluten; glutenin; tyrosine; cross-link; dough; bread-making; quality*

INTRODUCTION

The ability of a given wheat (*Triticum aestivum*), or flour derived from that wheat, to form gluten determines its utilization quality. Many aspects of wheat breeding technology that is designed to improve bread-making quality, from the traditional plant crossing techniques to the more recent molecular breeding techniques, are focused upon the improvement of wheat's gluten-forming properties. Although numerous studies have been conducted, full comprehension of the molecular associations and covalent bond formation in dough during mixing has not been realized. A complete understanding of the molecular mechanisms by which gluten proteins interact and dough forms is crucial in the agriculture industry and represents a phenomenon of worldwide importance with vast economic impact. Wheat is a major commodity in world agriculture. The ability to understand ways in which gluten proteins can be modified and altered to fit various specifications represents a revolution in applications that range from the food to the nonfood industry.

The formation of the large quaternary protein structure known as “gluten” during dough-mixing and bread-making processes is extremely complex and not well characterized. It has been established that a specific subset of the proteins comprising the gluten complex, the glutenin subunits, directly affects dough formation and bread-making quality. Glutenin subunits that have

been identified in wheat varieties around the world have not been shown to exhibit any definitive structural differences that can be directly correlated to their ability to aggregate into the gluten complex and affect dough formation or bread-making quality (1–3).

A major barrier to understanding the quaternary structure of gluten has been its complexity. The aggregated glutenin polymers range in M_r from (~ 1 to 10) $\times 10^6$ and consist of three, four, or five high M_r glutenin subunits, together with an uncertain number of low M_r subunits (4). These polymers are stabilized by disulfide bonds, but little is currently known about which of the four to seven cysteine residues present in different subunits form interchain disulfide bonds and which, if any, form intrachain disulfide bonds. The potential for variation in glutenin polymer size and composition is extensive.

The individual mature high M_r glutenin subunit proteins have M_r values ranging from 65000 to 120000. These rod-shaped proteins consist of three domains: a repetitive central domain flanked by nonrepetitive N- and C-terminal domains. The central domain varies considerably in length (from about 440 to 680 residues), and this is largely responsible for the differences in the M_r values of the whole proteins. The N- and C-terminal domains consist of 81–104 residues and 42 residues, respectively. These domains contain most or all of the cysteine residues, although single cysteine residues are present in the repetitive domains of a few high M_r glutenin subunits (4).

The secondary structures of the high M_r glutenin subunits have been studied using predictive methods (6) and by spectroscopic analyses of subunit 1Bx20 purified from *Triticum durum* (7, 8) and of synthetic peptides corresponding to the central repetitive motifs

* Author to whom correspondence should be addressed [e-mail kat@wheat.ksu.edu; telephone (785) 532-4811; fax (785) 532-7010].

[†] Department of Grain Science and Industry.

[‡] Food Science Graduate Program.

[§] Department of Biochemistry.

found within the high M_r glutenin subunits (9). Secondary structure prediction indicated that the repetitive domains are rich in β -reverse turns, which are predicted to occur within individual motifs and to span the junctions of adjacent motifs. In contrast, the nonrepetitive N- and C-terminal domains are predicted to consist predominantly of α -helical structure (9).

Gluten forms a three-dimensional protein network during the dough-mixing process, but the cross-links that form have been attributed solely to disulfide bonds, which can occur only at the N and C termini of the glutenin proteins (4). Many protein structural studies, as well as mixing and baking studies, have postulated that disulfide bonds are present in the gluten structure and contribute to the general process of dough formation. However, disulfide bonds that are cleaved and reformed according to the disulfide interchange theory, and are therefore ultimately responsible for the quaternary structure of gluten, have never been definitively identified (10, 11).

Sullivan et al. first proposed that thiol groups and disulfide bonds were responsible for determining dough properties (10). Subsequently, the work of Frater et al. formed the foundation for the hypothesis that related strength and elasticity of dough to thiol groups, intermolecular disulfide bonds, and the rate at which they interchange (11). Disulfide bond interchange reactions in wheat dough were supported by studies conducted by Mauritzen and Stewart, and the concept became accepted theory in the study of dough rheology (5). Many variations on these fundamental experiments, which involved using oxidizing and reducing agents, have been conducted over the past 40 years, but it has yet to be proven conclusively that disulfide bond interchange occurs during dough mixing or plays a significant role in gluten structure formation. Consequently, the work of Frater et al. (11) remains a hypothesis that has not been proved or disproved.

Due to the lack of confirmatory data proving that disulfide bond interchange occurs during dough mixing, it appeared to be possible that the formation of non-disulfide covalent intermolecular bonds among the glutenin proteins might occur. Because of the primary and secondary structures of the glutenin proteins, the formation of tyrosine cross-links during dough mixing was investigated. Tyrosine cross-links, in the form of isodityrosine, are common in plant proteins and have been thoroughly studied in extensin proteins, which have amino acid contents and secondary structures that closely resemble those of the high M_r glutenin proteins (12, 13). Another form of tyrosine cross-link, dityrosine, has been found in animal proteins from resilin, a protein found in the cuticle of insects and arthropods, to elastin and collagen in vertebrates (14). Although it is more common in nature for only one type of tyrosine cross-link to be present in a given system, both dityrosine and isodityrosine, as well as the higher order structures trityrosine and pulcherosine, have been shown to be synthesized simultaneously in human phagocytes by a myeloperoxidase-hydrogen peroxide system via a tyrosyl radical-dependent pathway (15).

Individual high M_r glutenin subunits contain from 3 to 5% tyrosine within their structures (16). Tyrosine residues occur periodically throughout the length of the proteins and are most often found in repeats of pairs of tyrosine residues (YY). These pairs of tyrosine residues

occur ~ 11 – 22 times throughout the length of the spiral protein backbone, depending on the particular glutenin subunit.

The repeat sequences that occur throughout the central domains of all glutenin subunits have been studied extensively (4). However, the repeating double-tyrosine sequence has never been discussed as particularly significant in the structure except as part of the tyrosine-tyrosine-proline-threonine-serine (YYPTS) repeat, which is the predicted site for β -turns in the protein structure (9). Double-tyrosine residues occur within the structure of the glutenins in the YYPTS repeats, as well as variations of this amino acid sequence, within the protein structure.

MATERIALS AND METHODS

Wheat Flour. The wheat flour used was produced from the spring wheat variety Bronze Chief (Wheat Montana, Three Forks, MT) and contained 14.76% protein.

Mixing Procedure. Mixing was performed using AACC method 54-40A. Ten grams of flour was used at 58% water absorption. Dough samples were taken at various times during mixing, flash frozen, and immediately lyophilized. Fifty milligrams of the lyophilized material was placed in 6 N HCl/1% phenol and evacuated for hydrolysis.

Amino Acid Analysis. Amino acid composition analysis of wheat flour or lyophilized dough developed under various treatments was accomplished by hydrolysis in 6 N HCl containing 1% phenol under vacuum at 110 °C for 24 h. Phenol and HCl were completely evacuated under vacuum, and residual materials were reconstituted in double-distilled H₂O and filtered. Amino acids were separated by liquid chromatography using a reversed phase column (LUNA RP 5 μ m C18, 2; 250 \times 4.6 mm, Phenomenex) and stepwise gradient (3, 10, 40, 95, 95, and 3%) of acetonitrile containing 1% trifluoroacetic acid at 0, 35, 50, 60, 65, and 85 min, respectively, a flow rate of 1 mL/min, and a column temperature was 30 °C. The eluent was monitored simultaneously at 285 nm by an HP diode array detector and by an HP fluorescence detector set at 285 and 405 nm excitation and emission wavelengths, respectively.

Mass Spectral Analysis. Mass spectral analysis was carried out on a high-resolution matrix-assisted laser desorption/ionization, Fourier transform (HiResMALDI-FT) mass spectrometer (Ion Spec Corp., Irvine, CA). Sample was injected in a solution in methanol/water (1:1, v/v) using 2,5-dihydroxybenzoic acid (DHB) as a matrix.

NMR Analysis. High-resolution one-dimensional ¹H NMR experiments were performed with a 11.75 T Varian Unity plus spectrometer (Varian, Palo Alto, CA), operating at 499.96 MHz for 1 h with a 5 mm triple-resonance inverse detection probe. NMR data sets were acquired for ~ 0.1 mg of sample dissolved in 0.7 mL of deuterated methanol at 25 °C and were processed and analyzed using Varian software 6.1b on a Silicon Graphics Indigo² XZ workstation. The tetramethylsilane (TMS) chemical shift was used as reference for ¹H resonance assignment.

Baking Procedure. One hundred gram pup loaves were prepared using a 90 min fermentation according to AACC method 10-10B. One hundred milligram samples were taken at mixing peak (5 min), first punch (25 min), second punch (77 min), and pan (90 min) and during baking at 10 and 27 min. Dough/bread samples were immediately flash frozen and lyophilized. Freeze-dried samples were ground with a mortar and pestle, and 50 mg of each sample was hydrolyzed for amino acid analysis.

RESULTS AND DISCUSSION

In the present study, dough was isolated at various points during the mixing process and HPLC amino acid analysis was performed to identify the presence of tyrosine cross-links. Dityrosine and isodityrosine standards eluted at 18.5 and 24.5 min, respectively, under

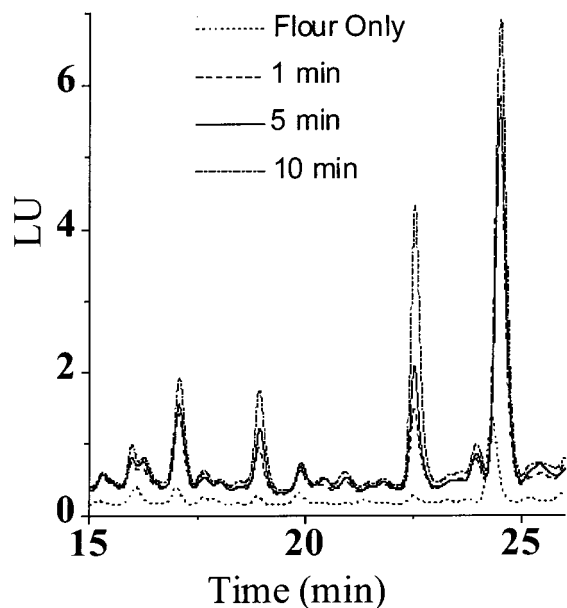


Figure 1. Direct fluorescence detection during HPLC analysis of amino acids hydrolyzed from flour and dough made from that flour mixed for 1, 5, and 10 min. Four significant peaks at 17, 18.5, 22.5, and 24.5 min increased during mixing. Dityrosine and isodityrosine standards eluted at approximately 18.5 and 24.5 min, respectively. Each of these peaks represented cross-links that were not present or were present in very low levels in the flour and form upon mixing the flour in water over time. The peaks eluting at 22.5 and 24.5 min also increased notably when 1% free tyrosine was added into the mixograph analyses (data not shown).

these analytical conditions. Peaks at 17, 18.5, 22.5, and 24.5 min increased as dough-mixing time increased (Figure 1), indicating that the gluten structure being developed was coincident with the formation of at least four species that resist hydrolysis under these analytical conditions. The peaks at 22.5 and 24.5 min continued to increase during the baking process (data not shown) with the greatest increase occurring in the 22.5 min peak. The peak eluting at 22.5 min was collected, further purified by HPLC using simultaneous diode array and fluorescent detection, and analyzed by mass spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The results indicated a cross-link structural unit having a mass of 361, the mass of both dityrosine and isodityrosine (12). The ^1H NMR spectrum of the isolated cross-link structural unit showed resonances from three aromatic protons as follows: a singlet at δ 7.26 and two doublets at δ 7.05 and 6.91. Integration of the peaks was approximately equal to 1:1:1. The NMR spectrum described for the isolated cross-link structural unit is consistent with the NMR spectrum taken from the standard dityrosine (14). Thus, the tyrosine cross-link structure eluting at 22.5 min most closely resembles dityrosine. The cross-link structural unit is being further characterized by ^{13}C and 2D NMR spectroscopy. Figure 2 shows the structures of dityrosine and isodityrosine. Figure 3 shows a proposed model of two glutenin peptides linked together by two intermolecular tyrosine cross-links.

When oxidizing agents [ascorbic acid, azodicarbonamide (ADA), and potassium bromate (KBrO_3)], commonly used in the baking industry (17, 18), were added to bread doughs and baked, an increase in the peak at 22.5 min during processing was observed (Figure 4). The formation of this cross-link structure was the only one

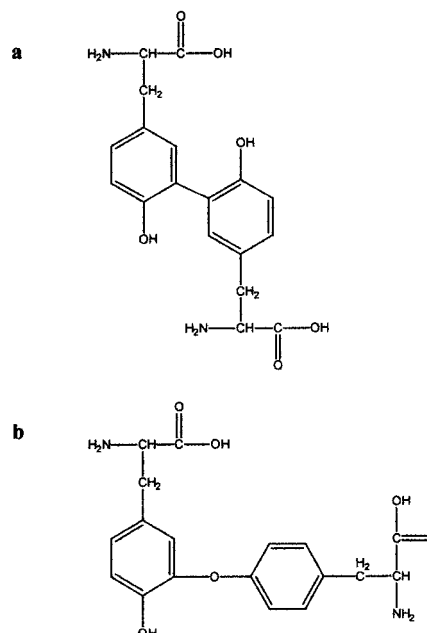


Figure 2. Structures of (a) dityrosine and (b) isodityrosine.

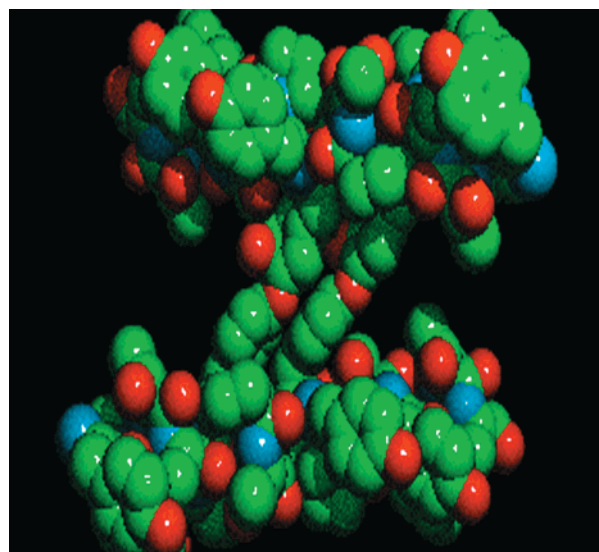


Figure 3. Model showing two glutenin peptides with the sequence YYPTSQQGYPTSQQGYPTS linked together through double-tyrosine cross-links.

facilitated by the addition of oxidizing agents, with potassium bromate having a singular affect.

Potassium bromate has been used in the baking industry for many years and is the best known dough conditioner available. However, there has been increased concern over its use due to toxicological studies implicating it as a possible carcinogen (17). Because the precise mechanism of action of potassium bromate was unknown, attempts to develop nontoxic agents with similar effects have been impeded. All of these oxidants have been reported to act by oxidizing gluten protein thiol groups, thus causing formation of disulfide bonds between proteins, although there was no direct evidence of the mechanisms of action of these agents (18).

A number of studies have shown that agents such as cysteine and glutathione affect dough by altering the properties of the elastic gluten network. For years, it has been hypothesized that those reducing agents affected dough solely by cleaving disulfide bonds, thereby

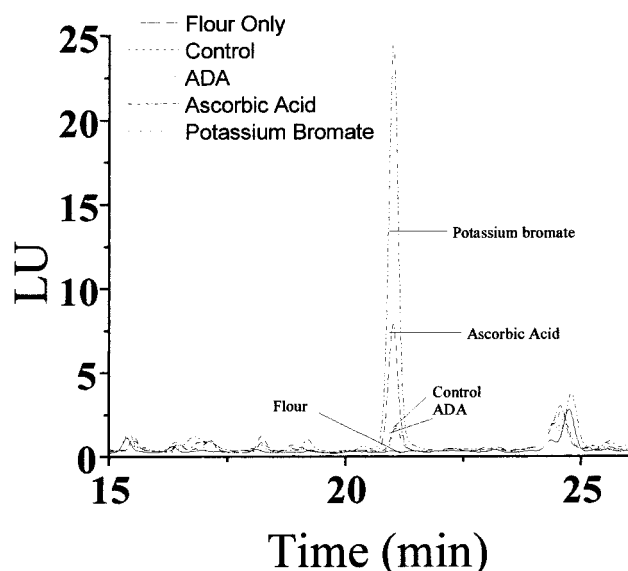


Figure 4. Direct fluorescence detection during HPLC analysis of amino acids hydrolyzed from flour, fully baked dough made from that flour, and fully baked doughs made from that flour and either 100 ppm of ascorbic acid, 45 ppm of ADA, or 30 ppm of KBrO_3 . The tyrosine cross-link that eluted at 22.5 min increased during each stage of bread-making and was enhanced by the addition of oxidizing agents. Most notably, the dough containing 30 ppm of KBrO_3 contained a level of this tyrosine cross-link that was ~ 10 times greater than the amount found in the baked control dough.

destroying the gluten network (10, 19, 20). Cysteine and glutathione are capable of not only cleaving disulfide bonds but also inhibiting tyrosine bond formation via their free radical scavenging activities (21). Koh et al. found decreased intermolecular cross-linking of large protein aggregates in flour dough when cysteine was added in an extrusion system (22). However, it appeared that thiol–disulfide interchange played little or no role in the observed phenomenon. Thiol–disulfide interchange is a slow reaction, requiring tens of minutes of mixing of hydrated doughs before mixed disulfides accumulate (20, 22). Koh et al. (22) reasoned that the thiol–disulfide reaction was inhibited in their extrusion studies due to the low water content and very short (~ 2 min) reaction time and that the decrease in cross-links among the gluten proteins was due to the inhibition of other, non-disulfide, bonds among gluten proteins.

To test the hypothesis that free radical scavengers inhibit the formation of tyrosine bonds during dough formation, cysteine and glutathione were added to flour doughs during mixograph analyses and the doughs were subsequently analyzed for tyrosine bond content. With the addition of 0.0064% (w/v) cysteine or 0.016% (w/v) glutathione, formation of the tyrosine bond that elutes at 22.5 min was eliminated in the dough (data not shown).

In an attempt to show that the gluten proteins could be cross-linked via tyrosine bond formation at specific tyrosine-containing amino acid sequences commonly found in the repeat sections of the glutenin proteins, several peptides representing modifications of the repetitive sequences commonly found in the high M_r glutenin subunits were synthesized. These sequences included QQGYPTS, YYPTS, YY, and free tyrosine (Y). The three peptides (0.5 mM) and the free tyrosine (0.5 mM) were exposed to various experimental conditions based on the work by Michon et al. (23). Tyrosine cross-

links between two adjacent tyrosine residues are not conformationally possible (24), so the possibility of a cross-link forming between the adjacent tyrosine residues within the peptides was not a concern. The tyrosine cross-link that elutes at 22.5 min was monitored at 285 nm, and the results are shown in Figure 5.

No cross-link formation was detected in any of the samples incubated in water only. The synthetic peptides were incubated in the water-soluble extract of flour because cereal chemists have reported that the water-soluble extract of flour contains some “unknown factor” that causes dough to reach its mixing peak more rapidly and subsequently lose its elasticity (25). The tyrosine cross-link that eluted at 22.5 min was detected when the peptides or free tyrosine was incubated in the water-soluble extract of flour for either 150 min or 24 h. It is postulated that an enzyme or secondary product was present in the water soluble extract of flour that was capable of catalyzing the synthesis of tyrosine cross-links. Preliminary studies have shown that the “unknown factor” that causes dough to reach its mixing peak more rapidly and subsequently lose its elasticity (25) is sensitive to protease treatment (Figure 6). When the water-soluble extract of flour was incubated with bovine pancreas protease (2 mg/mL) (Sigma Chemical Co., St. Louis, MO) for 24 h at 37 °C, it was no longer capable of causing the formation of tyrosine cross-links. These initial studies indicate the presence of an enzyme or enzymes in the water-soluble extract that are responsible for the formation of tyrosine cross-links.

The synthetic peptides were incubated with potassium bromate to determine if it was possible for these peptides to form tyrosine cross-links in aqueous potassium bromate at standard baking temperature and time with no additional flour components present (Figure 5). Peptides were also incubated in aqueous potassium bromate that included 5% cysteine (w/v), 500 ppm of glutathione, and 500 ppm of butylated hydroxytoluene (BHT), respectively, as potential free radical scavengers.

The tyrosine cross-link that eluted at 22.5 min formed *in vitro* in the presence of aqueous potassium bromate when the peptides were held either at 37 °C for 24 h or at 215 °C for 25 min. However, the tyrosine cross-link formed in greater amounts when the peptides were incubated at 215 °C for 25 min.

When the individual peptides or free tyrosine were incubated for 150 min or 24 h in water-soluble extract of flour, intermolecular tyrosine cross-links formed. However, the greatest amount of tyrosine cross-link formed when the peptides were incubated for either 150 min or 24 h in water-soluble extract of flour and 30 ppm of KBrO_3 and then held at 215 °C for 25 min (Figure 5). This most closely resembles the conditions that would exist during the baking process. Subsequent studies have shown that glutenin subunits, purified from wheat kernels, form these same tyrosine cross-links under similar conditions.

When the free radical scavengers cysteine, glutathione, and BHT were added to the system, the level of tyrosine cross-links decreased dramatically, indicating that a free radical mechanism was responsible for the cross-link formation under those conditions (data not shown). For many years, the scientific literature has taught that cysteine and glutathione were acting upon disulfide bonds in dough, thereby causing breakdown in the gluten structure. These data indicate that these agents act as free radical scavengers preventing tyrosine cross-link formation.

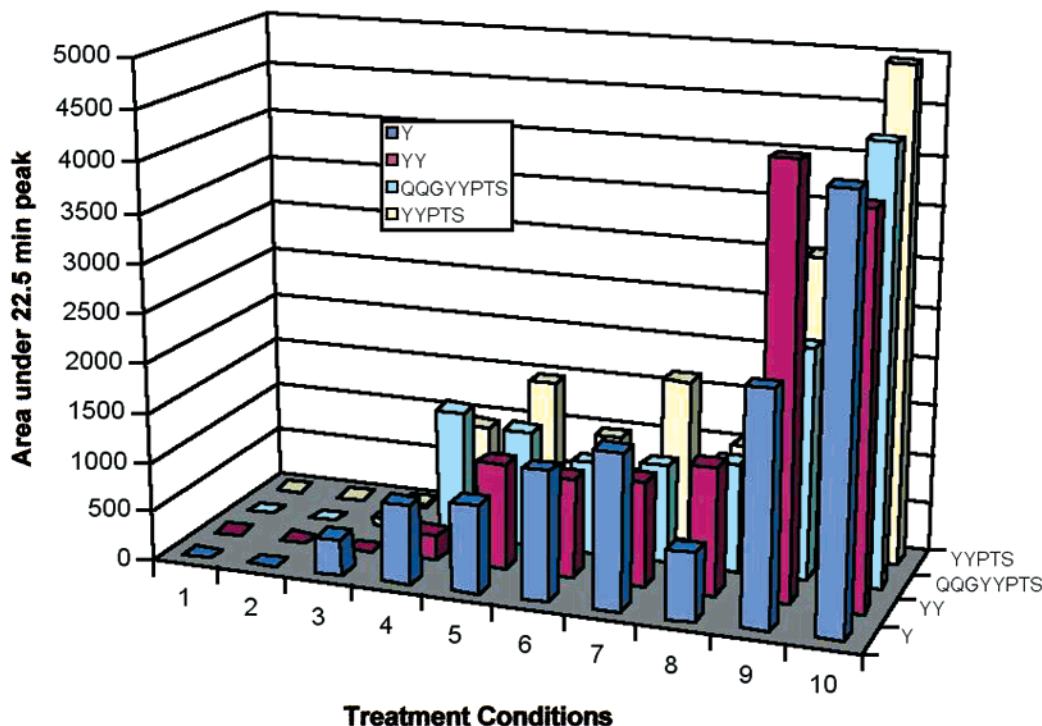


Figure 5. Graph showing the amount of tyrosine cross-link (eluting at 22.5 min) that is produced from exposure of three peptides (YY, YYPTS, and QGGYYPTS) (0.5 mM) and free tyrosine (Y) (0.5 mM) to the following experimental treatment conditions: 1 = peptide incubated in water at 37 °C for 24 h; 2 = peptide incubated in water at 215 °C for 25 min; 3 = peptide incubated in 30 ppm of aqueous KBrO₃ at 37 °C for 24 h; 4 = peptide incubated in 30 ppm of aqueous KBrO₃ at 215 °C for 25 min; 5 = peptide incubated in water-soluble extract of flour at 37 °C for 150 min; 6 = peptide incubated in water-soluble extract of flour at 37 °C for 24 h; 7 = peptide incubated in water-soluble extract of flour with 30 ppm of aqueous KBrO₃ at 37 °C for 150 min; 8 = peptide incubated in water-soluble extract of flour with 30 ppm of aqueous KBrO₃ at 37 °C for 24 h; 9 = peptide incubated in water-soluble extract of flour with 30 ppm of aqueous KBrO₃ at 37 °C for 150 min and then held at 215 °C for 25 min; 10 = peptide incubated in water-soluble extract of flour with 30 ppm of aqueous KBrO₃ at 37 °C for 24 h and then held at 215 °C for 25 min.

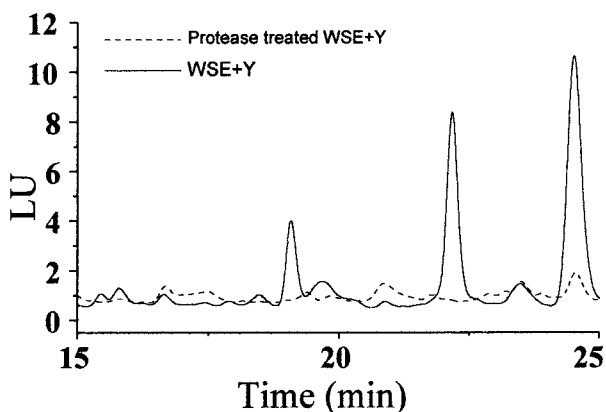


Figure 6. Direct fluorescence detection during HPLC analysis of tyrosine cross-links (eluting at approximately 18, 22.5, and 24.5 min, respectively) that are produced from exposure of free tyrosine (Y) to water-soluble extract of flour (WSE) and WSE that has been treated with protease prior to incubation with free tyrosine. WSE is capable of producing tyrosine cross-links when incubated with free tyrosine. However, exposure to protease renders the WSE incapable of synthesizing tyrosine cross-links from free tyrosine.

Since the work of Mauritzen and Stewart (5) in 1963, disulfide-sulfhydryl exchange has been erroneously assumed to be the only molecular mechanism by which gluten is developed and dough formation occurs. We have now shown that previously unidentified tyrosine cross-links occur during dough formation and bread-making processes. These tyrosine cross-links are affected by experimental conditions utilized in research designed to support the theory of disulfide-sulfhydryl

exchange. Thus, conclusions from previous investigations require re-evaluation because the formation of tyrosine cross-links in dough was unknown until the present study.

ACKNOWLEDGMENT

We gratefully acknowledge S. Anderson and S. C. Fry for dityrosine and isodityrosine standards, respectively. G. Radke is recognized for his assistance with amino acid analyses, and Y. X. Gong is acknowledged for her excellent technical assistance with NMR spectroscopy. Coauthor contributions are as follows: R.E.B. and K.E.B. contributed technical assistance and performed HPLC analyses; B.M.O.-K. performed initial amino acid analyses, O.P. performed mass spectral analyses and initial NMR analyses, and H.K. performed definitive NMR analyses and provided critical interpretation of data. We most gratefully acknowledge the editorial contributions of R. W. Trewyn, G. P. Rabold, and B. J. Donnelly.

LITERATURE CITED

- (1) Payne, P. I.; Corfield, K. G.; Blackman, J. A. Identification of high-molecular-weight subunit of glutenin whose presence correlates with breadmaking quality in wheats of related pedigree. *Theor. Appl. Genet.* **1979**, *55*, 153–159.
- (2) Payne, P. I.; Corfield, K. G.; Holt, L. M.; Blackman, J. A. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.* **1981**, *32*, 51–60.

- (3) Payne, P. I. Genetics of wheat storage proteins and the effect of allelic variation of bread-making quality. *Annu. Rev. Plant Physiol.* **1987**, *38*, 141–153.
- (4) Shewry, P. R.; Halford, N. G.; Tatham, A. S. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* **1992**, *15*, 105–120.
- (5) Mauritzen, C. M.; Stewart, P. R. Disulphide-sulphydryl exchange in dough. *Nature* **1963**, *197*, 48–49.
- (6) Tatham, A. S.; Shewry, P. R.; Mifflin, B. J. Wheat glutenin elasticity: a similar mechanism to elastin? *FEBS Lett.* **1984**, *177*, 205–208.
- (7) Field, J. M.; Tatham, A. S.; Shewry, P. R. The structure of a high M_r subunit of durum wheat. *Biochem. J.* **1987**, *247*, 215–221.
- (8) Miles, M. J.; Carr, H. J.; McMaster, T.; Anson, K. J.; Belton, P. S.; Morris, V. J.; Field, J. M.; Shewry, P. R.; Tatham, A. S. Scanning tunneling microscopy of a wheat gluten protein reveals details of an unusual supersecondary structure. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 68–71.
- (9) Tatham, A. S.; Drake, A. F.; Shewry, P. R. Conformational studies of synthetic peptides corresponding to the repetitive regions of the high molecular weight (HMW) subunits of glutenin. *J. Cereal Sci.* **1990**, *11*, 189–200.
- (10) Sullivan, B.; Howe, M.; Schmalz, F. D.; Astleford, G. R. The action of oxidizing and reducing agents on flour. *Cereal Chem.* **1940**, *17*, 507–528.
- (11) Frater, R.; Hird, J. F. R.; Moss, H. J.; Yates, J. R. A role for thiol and disulphide groups in determining the rheological properties of dough made from wheaten flour. *Nature* **1960**, *186*, 451–454.
- (12) Brady, J. D.; Sadler, I. H.; Fry, S. C. Di-isodityrosine, a novel tetrameric derivative of tyrosine in plant cell wall proteins: a new potential cross-link. *Biochem. J.* **1996**, *315*, 323–327.
- (13) Wilson, L. G.; Fry, S. C. Extensin—a major cell wall glycoprotein. *Plant Cell Environ.* **1986**, *9*, 239–260.
- (14) Malencik, D. A.; Sprouse, J. F.; Swanson, C. A.; Anderson, S. Dityrosine: Preparation, isolation and analysis. *Anal. Biochem.* **1996**, *242*, 202–213.
- (15) Jacobs, S.; Cistola, D. P.; Hsu, F. F.; Muzaffar, S.; Mueller, D. M.; Hazen, S. L.; Heinecke, J. W. Human phagocytes employ the myeloperoxidase-hydrogen peroxide system to synthesize dityrosine, trityrosine, pulcherosine and isodityrosine by a tyrosyl radical-dependent pathway. *J. Biol. Chem.* **1996**, *271*, 19950–19956.
- (16) Shewry, P. R.; Halford, N. G.; Tatham, A. S. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. *Oxford Surv. Plant Mol. Cell Biol.* **1989**, *6*, 163–219.
- (17) DuPuis, B. The chemistry and toxicology of potassium bromate. *Cereal Foods World* **1997**, *42*, 171–183.
- (18) Allen, W. G. Alternative oxidants as dough conditioners. *Cereal Foods World* **1999**, *44*, 642–649.
- (19) Kilborn, R. H.; Tipples, K. H. Factors affecting mechanical dough development. IV. *Cereal Chem.* **1973**, *50*, 70–86.
- (20) Moss, R. Bread microstructure as affected by cysteine, potassium bromate and ascorbic acid. *Cereal Foods World* **1975**, *20*, 289–292.
- (21) Holler, T. P.; Hopkins, P. B. A qualitative fluorescence-based assay for tyrosyl radical scavenging activity: Ovothiol A is an efficient scavenger. *Anal. Biochem.* **1989**, *180*, 326–330.
- (22) Koh, B. K.; Karwe, M. V.; Schaich, K. M. Effects of cysteine on free radical production and protein modification in extruded wheat flour. *Cereal Chem.* **1996**, *73*, 115–122.
- (23) Michon, T.; Chenu, M.; Kellershon, N.; Desmadril, M.; Gueguen, J. Horseradish peroxidase oxidation of tyrosine-containing peptides and their subsequent polymerization: a kinetic study. *J. Biochem.* **1997**, *36*, 8504–8513.
- (24) Epstein, L.; Lamport, D. An intramolecular linkage involving isodityrosine in extensin. *Phytochemistry* **1984**, *23*, 1241–1246.
- (25) Schroeder, L. F.; Hosney, R. C. Mixograph studies II. Effect of activated double bond compounds on dough mixing properties. *Cereal Chem.* **1978**, *55*, 348–360.

Received for review January 26, 2001. Revised manuscript received March 22, 2001. Accepted March 27, 2001. This work was supported by the Kansas Agricultural Experiment Station, Kansas State University. Contribution No. 01-388-J.

JF010113H