

Effects of Laccase and Ferulic Acid on Wheat Flour Doughs¹

E. Labat,² M. H. Morel,² and X. Rouau^{2,3}

ABSTRACT

Cereal Chem. 77(6):823–828

The effects of a laccase from the fungus *Pycnoporus cinnabarinus* on the mixing of a wheat flour dough with or without added ferulic acid (FA) were studied. Laccase reduced dough time-to-peak and accelerated dough breakdown in comparison with the control. Its effect was enhanced with FA. The water extractability of arabinoxylans (AX) increased during mixing of a dough free of added laccase, especially with exogenous FA. At the same time, the extractability of FA decreased during mixing. Added FA may have competed with endogenous AX feruloyl esters, inhibiting partly oxidative gelation. Laccase decreased AX extractability by chain

cross-linking through oxidative dimerization of feruloyl esters. FA and, moreover, FA plus laccase, increased the oxidation of sulfhydryl (SH) groups. FA and, even more, FA in combination with laccase, increased the rate of protein depolymerization during mixing. FA and the products of FA laccase oxidation participated in a redox reaction involving SH groups. A coupling reaction involving enzymatically generated feruloyl radicals and thiol radicals generated through the mechanical breakdown of inter-chain disulfide bonds might explain these results.

The viscoelastic properties of wheat flour dough depend primarily on the dough's protein constituents (MacRitchie 1992), and prolamins are recognized as the most important functional proteins (Weegels et al 1996). The glutenins provide elasticity, whereas gliadin provides viscosity and extensibility in a dough system (Ciaffi et al 1996). Arabinoxylans (AX), despite their low content in wheat flour, are also important in determining dough-handling properties and bread quality (Delcour et al 1991, Michniewicz et al 1991, Biliaderis et al 1995). Due to high water-binding capacity, AX play a regulatory role with respect to the water economy in bread-making. With feruloyl adducts, pentosans are subject to oxidative gelation (Geissmann and Neukom 1973, Hoseney and Faubion 1981).

Among other oxidative systems, peroxidases and laccases have been used successfully as gelling agents of AX solutions (Izydorczyk et al 1990, Moore et al 1990, Figueroa-Espinoza and Rouau 1998). These enzymes also have been applied to doughs (Si 1994a,b; van Oort et al 1995; Hilhorst et al 1999). Peroxidases need hydrogen peroxide to oxidize a wide range of substrates, including phenolic and thiol compounds (RSH). The generation of hydrogen peroxide, however, may represent a limiting factor to the use of peroxidases as a dough-oxidizing agent. Laccase, a *p*-diphenol-oxygen oxidoreductase (EC 1.10.3.2), is a copper-containing enzyme that catalyzes the oxidation of a wide variety of phenolic substrates. In oxygen, it induces the catalytic oxidation of phenols to free radicals. *Pycnoporus cinnabarinus* laccase oxidizes ferulic acid (FA) into a phenoxy radical that reacts nonenzymatically to produce dehydrodimers and polymers of FA. In feruloylated AX, laccase catalyzes gelation by dimerization of feruloyl esters (Figueroa-Espinoza and Rouau 1998). According to Vinkx et al (1991) and Figueroa-Espinoza and Rouau (1998), adding RSH to an AX solution delayed oxidative gel formation. No direct coupling of RSH to phenoxy radicals through an addition reaction was evidenced. It was proposed that FA oxidized by laccase into phenoxy radicals was regenerated by an oxidoreduction reaction involving the conversion of RSH into disulfides (RSSR). Moreover, the use of laccases from *Myceliophthora thermophila* and *Trametes hirsuta* in a wheat flour dough resulted in a partial oxidation of some gluten amino acids, especially cysteine (S. A. Virtanen et al, unpublished). Because FA is the main phenolic compound of wheat flours occurring as free, soluble-bound, and insoluble-bound forms as feruloylated AX (Sosulski et al 1982), the use of laccase as oxidizing agent in doughs appears interesting.

In this work, we have studied the effects of the laccase from *P. cinnabarinus* in with and without added free FA on macromolecules involved in dough mixing properties. The flour content in free FA is very low; therefore, this compound was added in some experiments to magnify the effects of laccase. The extent of pentosan gelation was assessed by measuring changes in AX extractability and FA contents during mixing. The oxidative effects of laccase on the gluten network were evaluated by measuring the dough sulfhydryl (SH) content and following the changes in size distribution of proteins during mixing.

MATERIALS AND METHODS

The flour used (cv. Scipion) contained 8.4% protein (14% wb; $N \times 5.7$ determined by the Dumas method) and 0.6% ash (Approved Methods, AACC 2000). All reagents (highest grade available) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). A laccase preparation (solution in 35% glycerol) was obtained from a culture supernatant of *P. cinnabarinus* MIC11 (supplied by M. Asther, Laboratoire de Biotechnologie des Champignons Filamenteux [INRA, Marseille, France]). Laccase activity (0.03 nkat/ μ L) was measured with syringaldazine as substrate (Figueroa-Espinoza et al 1998). One nkat corresponds to the oxidation of 1 nmol of syringaldazine/sec under the experimental conditions.

Doughs were mixed in a 10-g mixograph (National Manufacturing Co., Lincoln, NE) equipped with Mixsmart software at 60% absorption (14% wb). FA, previously dissolved in hot water, was added to the flour at 340 ppm. Laccase was added at a level of 30 nkat. Different doughs were made for different mixing times. They were immediately frozen in liquid nitrogen, then freeze-dried, and ground in a laboratory mill (IKA, Janke & Kunkel, Staufen, Germany) to pass a 0.5-mm sieve.

Extraction of protein and separation by size exclusion (SE) HPLC were as described by Dachkevitch and Autran (1989), with modifications. Proteins were extracted from 160 mg of flour or ground freeze-dried dough, with 20 mL of 0.1M sodium phosphate buffer (pH 6.9) containing 1% (w/v) SDS (buffer A) for 80 min at 60°C. After centrifugation (30 min, 39,000 $\times g$, 20°C), the pellet was suspended in buffer A (5 mL) and sonicated (180 sec, 3.5W power output) (Vibracell, Bioblock Scientific, Illkirch, France), and centrifuged as above, yielding a second supernatant consisting of SDS-unextractable proteins known as glutenin macropolymers (Weegels et al 1996). The supernatants were injected (20 μ L) onto a size-exclusion column TSK G 4000-SW (Merck, Chelles, France) (7.5 mm \times 30 cm) with a TSK 3000-SW (Merck) guard column (7.5 mm \times 7.5 cm) using a HPLC (Waters, St Quentin en Yvelines, France) system comprising a model 600 pump, a 715 automatic sampler, and a model 486 UV detector at 214 nm. Pump control and data acquisition were directed with Millennium 32

¹ Presented in part at the AACC 84th Annual Meeting, Seattle, WA, November 1999.

² Unité de Technologie des Céréales et des Agropolymères, ENSAM/INRA, 2 Place Viala, 34060 Montpellier Cedex 01, France.

³ Corresponding author. E-mail: rouau@ensam.inra.fr

version 3.0 software. The column was eluted with 0.1M sodium phosphate buffer (pH 6.9) containing 0.1% (w/v) SDS (0.7 mL/min, at room temperature). Apparent molecular weights (M_r) were estimated by calibrating the column with protein standards according to Dachkevitch and Autran (1989).

SH groups were determined as described by Chan and Wasserman (1993) using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid [DTNB]), which reacts with SH to produce 1 mol of NTB²⁻ ($\epsilon = 13,600 M^{-1} cm^{-1}$ at 412 nm) per mol of SH. A solution (50% isopropanol-2, 80 mM Tris-HCl, pH 8.8, 10 mM EDTA, DNTB at 0.2 mg/mL) was added (1.2 mL) to 100 mg of flour or ground freeze-dried dough. Agitation (Maxi-Mix III, Bioblock Scientific) for 20 min at room temperature was followed by centrifugation (15,000 $\times g$, 20°C, 15 min). Absorbance at 412 nm was read using a spectrophotometer (Ultrospec 2000 UV/visible, Amersham Pharmacia Biotech, St Quentin en Yvelines, France).

Water extractable AX (WEAX) were extracted from 1.0 g of flour or ground freeze-dried dough and dispersed in 4.0 mL of water at 4°C for 15 min. Total AX and WEAX concentrations were determined semiautomatically, as described by Rouau and Surget (1994), using an auto-analyzer (Evolution II, Alliance Instruments, Cergy-Pontoise, France). WEAX are expressed as % total AX.

Flow times of WEAX solutions were measured at 25°C using a capillary viscometer (AVS 400, Schott Geräte, Hofheim, Germany) equipped with an Oswald capillary tube. Relative viscosities (η_{rel} = flow time of sample/flow time of solvent) and specific viscosities ($\eta_{sp} = \eta_{rel} - 1$) were calculated using water flow time (78 sec). An apparent intrinsic viscosity ($[\eta]_{app} = 1/c \times [2(\eta_{sp} - \ln [\eta_{rel}])]^{0.5} \times 1,000$) was evaluated using the Morris equation (Morris 1984), where c represented the AX concentration, assuming that only AX contributed to the viscous properties of the dough extracts (Rouau et al 1994).

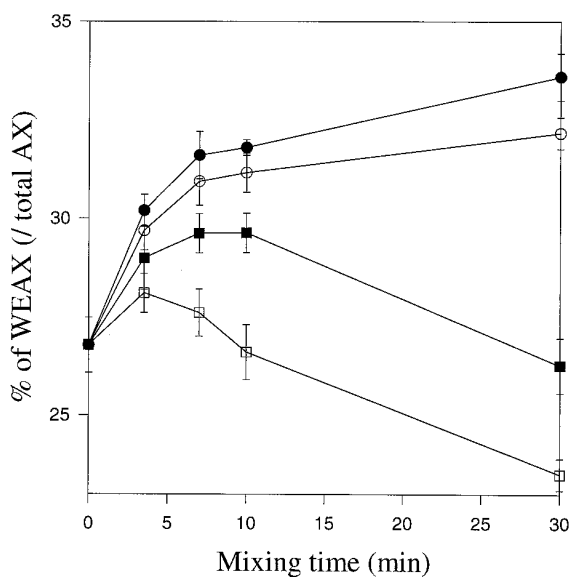


Fig. 1. Changes in % of water-extractable arabinoxylan (WEAX) during mixing. Control (○), laccase (□), ferulic acid (FA) (●), and laccase + FA (■) doughs.

The extraction of free phenolic compounds was based on the method described by Krygier et al (1982) using acetone-methanol-water (7:7:6, v/v) as extraction solvent. Ester-linked FA and FA dehydrotimers were determined as described by Figueroa-Espinoza and Rouau (1998).

The coefficients of variation for AX, $[\eta]_{app}$, SH group, FA, and protein size determination procedures were 3.0, 3.0, 3.0, 6.0, and 3.3%, respectively. Results are expressed as mean values of at least duplicate analyses.

RESULTS AND DISCUSSION

Mixograph Study

Adding laccase to standard flour dough shortened dough development time, increased consistency at peak, and decreased dough tolerance (Table I). Added FA provoked similar effects. The combined addition of laccase and FA was very effective in accelerating dough formation and breakdown. Indeed, time-to-peak decreased by 25% in comparison with control dough. The torque for time-to-peak increased, and then a significant breakdown occurred, as shown by a loss of 30 Nm after 10 min, whereas only 12 Nm was lost for control dough. Laccase (with or without FA) affected dough overmixing in a manner similar to that of the SH-blocking reagent N-ethylmaleimide (Schroeder and Hosoney 1978) or fast-acting oxidants (Weak et al 1977).

Evolution of Water Extractability and Viscosity of AX During Mixing

Mixing increased water extractability of AX (Fig. 1). A rapid increase in WEAX content was observed during the first 7 min of mixing (26.7% of total AX at the initial, up to 30.9%). Then, only minor changes occurred, and the WEAX content finally reached 32.1% when mixing duration was extended to 30 min. Increase in water extractability of AX during mixing might result from a partial breakdown of water-unextractable AX chains due to mixing shear

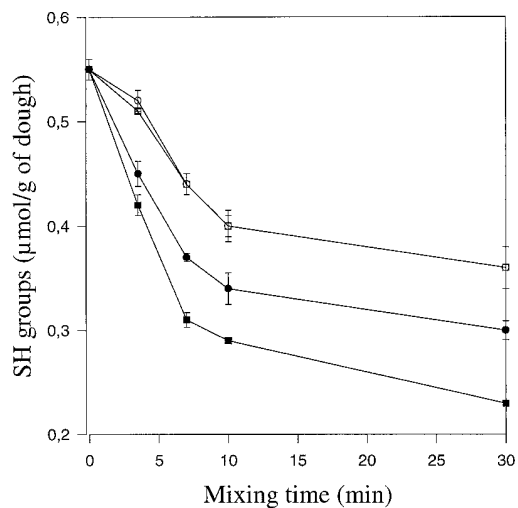


Fig. 2. Sulfhydryl (SH groups) content (% of flour value) of control (○), laccase (□), ferulic acid (FA) (●), and laccase + FA (■) doughs.

TABLE I
Mixograph Data for Doughs Prepared With or Without Laccase or Ferulic Acid (FA)^a

	Time-to-Peak (min)	Torque (% of scale)			
		Time-to-Peak	10 min	20 min	30 min
Control	4.2 ± 0.1	73.2 ± 1.9	61.3 ± 1.5	58.0 ± 1.2	49.2 ± 1.7
Laccase	4.0 ± 0.1	76.8 ± 1.6	59.2 ± 1.9	50.4 ± 2.0	47.8 ± 2.2
FA	3.6 ± 0.1	77.3 ± 1.5	58.1 ± 1.7	50.2 ± 2.1	46.5 ± 2.3
Laccase + FA	3.1 ± 0.1	82.3 ± 2.5	53.1 ± 2.0	49.7 ± 2.1	46.6 ± 2.3

^a Mean ± standard deviation.

stress or to the activation of endogenous xylanases (Cleemput et al 1993, Rouau 1993).

Addition of FA further increased the effect of mixing on AX extractability. At the time-to-peak, 30.2% of total AX was WEAX in dough with added FA, versus 29.7% in control dough. This difference in extractability further increased during mixing because the WEAX content reached 33.6% after 30 min of mixing versus 32.1% for control dough. Moore et al (1990) and Vinkx et al (1991) reported that FA, vanillic acid, and cysteine inhibited the oxidative gelation of WEAX treated by hydrogen peroxide-horseradish peroxidase. According to those authors, added free FA competed with FA esterified to AX chains, partly preventing an oxidative cross-linking of feruloylated AX naturally occurring in doughs.

Laccase impeded the increase in WEAX content at the beginning of mixing (28% at peak time). Then, WEAX content dropped sharply and, after 30 min of mixing, was lowered to 23.5%. Laccase from *P. cinnabarinus* induced gelation of isolated WEAX solutions (Figuroa-Espinoza and Rouau 1998). It is likely that a similar mechanism occurred during dough mixing, resulting in a large decrease in AX water extractability.

Addition of FA delayed the effect of laccase and more WEAX was obtained for all samples compared with dough with laccase. It is likely that added FA competed with FA esterified on AX chains, thereby preventing excessive polymerization of AX chains.

The viscosity of a flour-water extract is mainly due to extractable polysaccharides (Udy 1956), especially WEAX (Rouau 1993). The calculated $[\eta]_{app}$, based on the concentration of WEAX, are shown Table II. The $[\eta]_{app}$ increased slightly after the time-to-peak for the control dough, and then decreased slowly, suggesting a balanced effect of both oxidative cross-linking and depolymerization of AX chains by endogenous xylanases and shear stress. The $[\eta]_{app}$ decreased slightly with FA, whereas with laccase $[\eta]_{app}$ diminished immediately after the beginning of mixing because the high M_r AX were rendered water-unextractable by cross-linking, the highest M_r chains were generally first cross-linked (Ciacco and D'Appolonia 1982, Izydorczyk et al 1991). This was previously described in solution (Figuroa-Espinoza and Rouau 1998) and occurs in dough. When FA and laccase were added in combination, the $[\eta]_{app}$ increased after the peak, then decreased as in the laccase-treated dough, for the last 20 min. The gelation of feruloylated AX probably occurred but was delayed by the competition with added FA, perhaps until this substrate was entirely con-

sumed (first 10 min). After consumption of FA, the cross-linking of high M_r WEAX occurred.

Vemulapalli and Hosney (1998) and Miller and Hosney (1999) suggested that oxidative gelation of water-extractable pentosans caused by added glucose oxidase could be responsible of a drying effect on the dough. From our results, however, it appears that the amount of AX cross-linked by *P. cinnabarinus* laccase did not markedly affect dough mixing curve.

Quantification of SH Groups

In the control dough, SH groups oxidized rapidly (-27% of the initial SH group content) during the first 10 min of mixing and then more slowly (-7.5%) in the next 20 min (Fig. 2). Laccase did not affect SH oxidation. This result confirms SH groups did not react with laccase (Figuroa-Espinoza et al 1998). The extent of SH oxidation increased with FA (-38%), and even more when FA and laccase were combined (-47%), when compared with control dough, an increase in the rate of SH oxidation was observed. These results are in contrast with the findings of Figuroa-Espinoza et al (1998) that FA needed laccase to oxidize SH compounds in model solution. In flour dough, SH oxidation could result from the same coupled reactions, in which FA radicals generated by endogenous flour enzymes (i.e., phenol oxidase) (Honold and Stahmann 1968, Hatcher and Kruger 1993) would be involved. The SH oxidation was accelerated by the laccase from *P. cinnabarinus* only with added FA; it is likely that laccase-catalyzed SH oxidation needs sufficient amounts of mobile FA (or phenolic compounds). FA esterified to the water-unextractable AX appeared ineffective in this respect.

TABLE II
Apparent Intrinsic Viscosity of Water Extracts from Dough
With or Without Laccase Ferulic Acid (FA) Mixed for Different Times^a

Time (min)	Control	Laccase	FA	Laccase + FA
0	725 ± 20	725 ± 20	725 ± 20	725 ± 20
3.5	698 ± 19	703 ± 19	686 ± 22	792 ± 18
7	745 ± 22	684 ± 21	688 ± 19	751 ± 19
10	740 ± 20	610 ± 23	687 ± 23	738 ± 22
30	689 ± 25	551 ± 19	673 ± 21	528 ± 20

^a Viscosity = $[\eta]_{app}$ expressed as arabinoxylan in solution in mL/g; mean ± standard deviation.

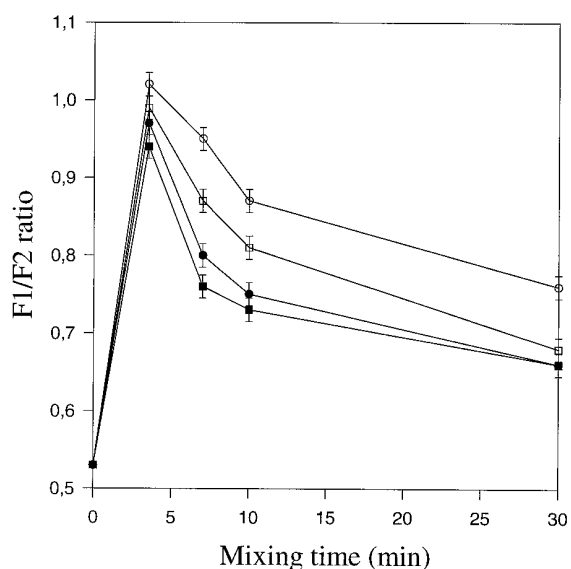


Fig 3. Alteration of size distribution of protein polymers during mixing, as assessed by F1/F2 ratio. Control (○), laccase (□), ferulic acid (FA) (●), and laccase + FA (■) doughs. F1/F2 ratio (F1 = $65 \times 10^4 < M_r < 10^6$ and F2 = $15 \times 10^4 < M_r < 65 \times 10^4$).

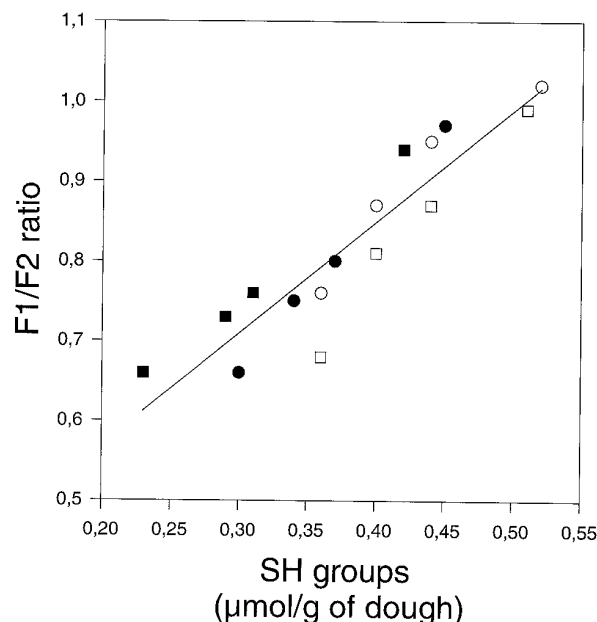


Fig. 4. Sulphydryl (SH) content and F1/F2 ratio. Control (○), laccase (□), ferulic acid (FA) (●), and laccase + FA (■) doughs; $r^2 = 0.85$.

Quantification of FA

Natural content of free FA in flour is very low (0.007 $\mu\text{mol/g}$, db) (Table III). In the control dough, it remained practically constant during mixing and was almost all consumed with laccase. The decrease in added free FA during mixing was much accelerated by laccase. The decrease in FA was probably due to oxidation leading to undetectable compounds (i.e., self-polymerization or addition products).

According to Figueroa-Espinoza et al (1998), the laccase from *P. cinnabarinus* can oxidize FA and feruloyl esters into phenoxyl radicals that are the three most important mesomeric forms (semi-quinones). Cysteine and other SH-containing compounds reduce the semiquinones into the original FA with formation of disulfide compounds. According to those authors, these coupled reactions continue until all the SH groups are consumed. Thereafter, the dimerization of FA by coupling of two FA-phenoxyl radicals could take place.

During the mixing of the control dough, the amount of FA esterified to AX decreased slightly and the level of FA dehydromers increased (Table III). This corresponds to a slight cross-linking of feruloylated AX. An excess of free FA inhibited the dimerization process. With added laccase, both FA and FA dehydromers disappeared, suggesting that a further oxidation of the dehydromers could occur.

Change in Size Distribution of Protein Polymers

The SE-HPLC elution profiles of proteins gave five peaks (F1 to F5) with M_r from 600×10^3 to 1×10^6 for F1, from 150×10^3 to 600×10^6 for F2, from 20×10^3 to 150×10^3 for F3 and F4 (corresponding to gliadins) (Singh et al 1990), and $<20 \times 10^3$ for F5. The second extract (protein extracted by sonication) consisted

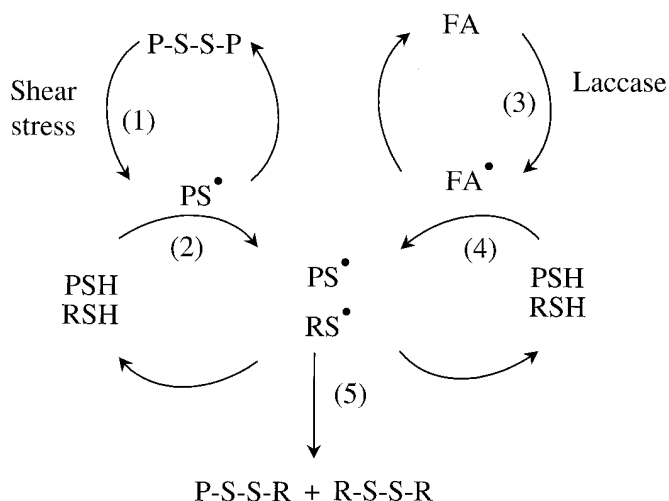


Fig. 5. Proposed reactions for action of laccase and ferulic acid (FA). PSSP = oxidized protein, PSH = reduced protein, PS^{\bullet} = thiol radical on protein, RSSR = oxidized low M_r compound, RSH = reduced low M_r compound, and RS^{\bullet} low M_r thiol radical.

of SDS-unextractable proteins known as glutenin macropolymers (Weegels et al 1996).

In flour, SDS-extractable proteins accounted for 84% of total protein. SDS-unextractable proteins were rapidly brought into solution at the beginning of mixing and accounted for $<2\%$ of protein at time-to-peak. Increase in protein extractability during mixing has already been reported (Tanaka and Bushuk 1973, Graveland et al 1980, Weegels et al 1996). The amount of protein monomers (F3, F4, F5) did not change during mixing. All changes after peak time concerned F1 and F2 contributions. F2 increased at the expense of F1. Therefore, the ratio of F1 to F2 of SDS-extractable proteins was used as an indicator of the changes in the size distribution of protein polymers during mixing.

The F1/F2 ratio increased dramatically up to time-to-peak compared with flour (Fig. 3). This rise coincided with the depolymerization of SDS-unextractable polymers, which were preferentially recovered into F1 of SDS-extractable polymers. Control dough showed the highest F1/F2 ratio at time-to-peak, indicating that the SDS-unextractable polymers were degraded to a lesser extent. After time-to-peak, the F1/F2 ratio decreased, indicating a depolymerization of the larger SDS-extractable aggregates eluted in F1. The depolymerization rate increased slightly when laccase was added to dough and even more when FA and FA + laccase were added.

There was a strong relationship (Fig. 4) between the F1/F2 ratio and the SH content during mixing, suggesting that the glutenin depolymerization was closely related to SH oxidation. The unique linear relationship with any adduct considered ($r^2 = 0.85$), suggesting that FA and laccase interacted with SH compounds, leading to dough breakdown.

A polemic about the dough breakdown phenomenon has existed a long time. Different mechanisms have been proposed to explain the depolymerization of the dough proteins. It has been suggested that the size of protein aggregates decreases by physical disruption of the aggregates or by chemical breakdown of noncovalent or covalent bonds. Shear stress would disrupt disulfide bonds, leading to the creation of thiol radicals. Schroeder and Hoseney (1978) proposed that FA could react with thiol radicals created during the mixing process. The mechanisms proposed to explain our results are shown in Fig. 5. According to a number of studies (Weak et al 1977, Schroeder and Hoseney 1978, Sidhu et al 1980, Danno and Hoseney 1982), shear stress would break protein interchain disulfide bonds (PSSP) forming thiol radicals (PS^{\bullet}). These radicals could be transferred to a smaller thiol compound (RSH) to form a labile species RS^{\bullet} . On the other hand, FA is oxidized into a highly reactive phenoxyl radical (FA^{\bullet}) by added laccase. FA^{\bullet} can propagate onto RSH to form another RS^{\bullet} . The rate of this reaction is likely to increase with an excess of FA^{\bullet} . Consequently, most of RSH present in flour would be rapidly oxidized into RS^{\bullet} , which would rapidly react with another thiol radical (RS^{\bullet} or PS^{\bullet}). According to Grosch and Wieser (1999), RSH compounds like cysteine or glutathione were linked to the cysteine residues of glutenin subunits after dough mixing. The addition of RS^{\bullet} and PS^{\bullet} radicals would lead to PSSR and RSSR. These coupled reactions, accelerated with FA, led to a partial blocking of SH groups in gluten, which were rendered unavailable for further protein-protein cross-linking. Therefore,

TABLE III
Free Ferulic Acid (FA) and Alkali-Labile FA (AL-FA) and Dehydromer Contents of Doughs^a

Time (min)	Control			Laccase			FA			Laccase + FA		
	Free FA	AL-FA	Dimer	Free FA	AL-FA	Dimer	Free FA	AL-FA	Dimer	Free FA	AL-FA	Dimer
0	0.007	0.51	0.12	0.006	0.51	0.12	1.87	0.52	0.10	1.73	0.51	0.15
3.5	0.008	0.49	0.12	0.006	0.50	0.12	1.79	0.52	0.11	1.43	0.59	0.17
7	0.007	0.50	0.15	0.002	0.41	0.10	1.67	0.51	0.13	0.55	0.51	0.16
10	0.006	0.49	0.19	0.001	0.32	0.05	1.67	0.48	0.11	0.13	0.48	0.22
30	0.006	0.46	0.17	0.001	0.36	0.08	1.49	0.48	0.11	0.01	0.37	0.13

^a Free FA (cis + trans) and AL-FA (cis + trans); dehydromer contents ($\mu\text{mol/g}$ of dm); time = mixing time. Standard deviation $<6.0\%$.

the depolymerization of glutenin polymers associated with mixing was not compensated by reformation of disulfide bonds between protein chains. Limited addition of thiol groups on free FA remains possible, however, as an SH-blocking mechanism because the decrease in free FA was not compensated by FA dehydrodimer formation. If these additions involved cysteinyl residues of gluten proteins, the consequences on dough properties would be similar to those expected in SH-blocking by low M_r thiol compounds.

Phenol oxidases are endogenous in wheat flours (Honold and Stahmann 1968, Hatcher and Kruger 1993) but in limited amounts compared with the laccase added in this study. However, in a standard mixing process, such endogenous enzymes could contribute partly to dough properties by a mechanism similar to that described here for laccase.

CONCLUSIONS

The effects of a fungal laccase have been studied on wheat flour components during dough mixing with or without added FA. Laccase accelerated dough formation and dough breakdown and the effects on mixing properties were enhanced with added FA. Laccase used alone decreased AX water extractability. When used in combination with added FA, laccase increased the oxidation of SH groups and the rate of protein depolymerization during mixing.

The major substrate of laccase in a flour is FA, which occurs naturally in low amounts as free form and low M_r conjugates but mainly as AX esters. The oxidation of any form of FA by laccase results in the production of phenoxyl radicals, which further react nonenzymatically and can have different fates in the dough.

Adjacent AX-linked feruloyl radicals may come into contact. Their dimerization provoked AX cross-linking. In our experiments, however, the amount of cross-linked AX had little influence on dough mixing curves. Due to high mobility, free FA is quickly oxidized by laccase. Added FA limited AX oxidative cross-linking in the dough by competing with AX feruloyl esters. With SH groups, a displacement reaction occurs from phenoxyl radicals to SH groups, leading to the formation of thiol radicals. Their dimerization produced disulfide bonds that can involve proteins or low M_r thiols. Added FA amplified the effects of laccase on dough properties, probably by favoring the production of mobile thiol radicals that can block the reformation of protein interchain disulfide bonds in favor of disulfide between proteins and low M_r thiols. Also, limited addition reactions of thiol radicals on FA cannot be excluded.

LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 08-01. The Association: St. Paul, MN.

Ciacco, F., and D'Appolonia, B. 1982. Characterization of pentosans from different wheat flour classes and their gelling capacity. *Cereal Chem.* 59:96-99.

Ciaffi, M., Tozzi, L., and Lafiandra, D. 1996. Relationship between flour protein composition determined by size-exclusion high-performance liquid chromatography and dough rheological parameters. *Cereal Chem.* 73:346-351.

Chan, K.-Y., and Wasserman, B. P. 1993. Direct colorimetric assay of free thiol groups and disulfide bonds in suspensions of solubilized and particulate cereal proteins. *Cereal Chem.* 70:22-26.

Cleemput, G., Roels, S. P., van Oort, M., Grobet, P. J., and Delcour, J. A. 1993. Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chem.* 70:324-329.

Dachkevitch, T., and Autran, J. C. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. *Cereal Chem.* 66:448-456.

Danno, G., and Hosney, R. C. 1982. Effect of dough mixing and rheologically active compounds on relative viscosity of wheat proteins. *Cereal Chem.* 59:196-198.

Delcour, J. A., Vanhamel, S., and Hosney, R. C. 1991. Physicochemical and functional properties of rye nonstrach polysaccharides. II. Impact

of a fraction containing water-soluble pentosans and proteins on gluten-strach loaf volumes. *Cereal Chem.* 68:72-76.

Figuroa-Espinoza, M.-C., and Rouau, X. 1998. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase. Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chem.* 75:259-265.

Figuroa-Espinoza, M.-C., Morel, M.-H., and Rouau, X. 1998. Effect of lysine, tyrosine, cysteine and glutathione on the oxidative cross-linking of feruloylated arabinoxylans by a fungal laccase. *J. Agric. Food Chem.* 46:2583-2589.

Geissmann, T., and Neukom, H. 1973. On the composition of the water-soluble wheat flour pentosans and their oxidative gelation. *Lebensm. Wiss. Technol.* 6:59-62.

Graveland, A., Bosveld, P., Lichtendonk, W. J., and Moonen, J. H. E. 1980. Superoxide involvement in the reduction of disulphide bonds of wheat gel proteins. *Biochem. Biophys. Res. Commun.* 93:1189-1195.

Grosh, W., and Wieser, H. 1999. Redox reactions in wheat dough as affected by ascorbic acid. *J. Cereal Sci.* 29:1-16.

Hatcher, D. W., and Kruger, J. E. 1993. Distribution of polyphenol oxidase in flour millstreams of Canadian common wheat classes milled to three extraction rates. *Cereal Chem.* 70:51-55.

Hilhorst, R., Dunnewind, B., Orsel, R., Stegeman, P., van Vliet, T., Gruppen, H., and Schols, H. A. 1999. Baking performance, rheology, and chemical composition of wheat dough and gluten affected by xylanase and oxidative enzymes. *Food Chem. Toxicol.* 64:808-813.

Honold, G. R., and Stahmann, M. A. 1968. The oxidation-reduction enzymes of wheat. IV. Qualitative and quantitative investigations of the oxidases. *Cereal Chem.* 45:99-108.

Hosney, R. C., and Faubion, J. M. 1981. A mechanism for the oxidative gelation of wheat flour water-soluble pentosans. *Cereal Chem.* 58:421-424.

Izydorczyk, M. S., Biliaderis, C. G., and Bushuk, W. 1990. Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.* 11:153-169.

Izydorczyk, M. S., Biliaderis, C. G., and Bushuk, W. 1991. Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chem.* 68:145-150.

Krygier, K., Sosulski, F., and Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. I. Extraction and purification procedure. *J. Agric. Food Chem.* 30:330-334.

MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr. Res.* 36:1-87.

Michniewicz, J., Biliaderis, C. G., and Bushuk, W. 1991. Effect of added pentosans on some physical and technological characteristics of dough and gluten. *Cereal Chem.* 68:252-258.

Miller, K. A., and Hosney, R. C. 1999. Effect of oxidation on the dynamic rheological properties of wheat flour-water doughs. *Cereal Chem.* 76:100-104.

Moore, A. M., Martinez-Munoz, I., and Hosney, R. C. 1990. Factors affecting the oxidative gelation of wheat water-solubles. *Cereal Chem.* 67:81-84.

Morris, E. R. 1984. Rheology of hydrocolloids in gums and stabilisers for the food industry. G. O. Phillips, D. J. Wedlock, and P. A. Williams, eds. Pergamon Press: Oxford.

Rouau, X. 1993. Investigations into the effects of an enzyme preparation for baking on wheat flour dough pentosans. *J. Cereal Sci.* 18:145-157.

Rouau, X., and Surget, A. 1994. A rapid semi-automated method for the determination of total and water-extractable pentosans in wheat flours. *Carbohydr. Polym.* 24:123-132.

Rouau, X., El-Hayek, M.-L., and Moreau, D. 1994. Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties. *J. Cereal Sci.* 19:259-272.

Schroeder, L. F., and Hosney, R. C. 1978. Mixograph studies. II. Effect of activated double bond compounds on dough-mixing properties. *Cereal Chem.* 55:348-360.

Si, J. 1994a. Use of laccase in baking. International patent 94/28728.

Si, J. 1994b. Use of peroxidase in baking. International patent 94/28729.

Sidhu, J. S., Nordin, P., and Hosney, R. C. 1980. Mixograph studies. III. Reaction of fumaric acid with gluten proteins during dough mixing. *Cereal Chem.* 57:159-163.

Singh, N. K., Donovan, G. R., Batey, I. L., MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem.* 67:150-161.

Sosulki, F., Krzysztof, K., and Hogge, L. 1982. Free, esterified, and insoluble-

- bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* 30:337-340.
- Tanaka, K., and Bushuk, W. 1973. Changes in flour proteins during dough-mixing. I. Solubility results. *Cereal Chem.* 50:590-596.
- Udy, D. C. 1956. The intrinsic viscosities of the water-soluble components of wheat flour. *Cereal Chem.* 33:67-74.
- van Oort, M., Hennink, H., and Moonen, H. 1995. Peroxidases in bread making. Pages 195-203 in: 1st European Symposium on Enzymes and Grain Processing (ESEGP-1). S. A. G. F. Angelino, R. J. Hamer, W. W. van Hartingsveldt, F. Heidekamp, and J. P. van der Lugt, eds. TNO Nutrition and Food Research Institute: Zeist, The Netherlands.
- Vemulapalli, V., and Hosney, R. C. 1998. Glucose oxidase effects on gluten and water solubles. *Cereal Chem.* 75:859-862.
- Vinkx, C. J. A., Van Nieuwenhove, C. G., and Delcour, J. A. 1991. Physicochemical and functional properties of the rye nonstarch polysaccharides. III. Oxidative gelation of a fraction containing water-soluble pentosans and proteins. *Cereal Chem.* 68:617-622.
- Weak, E. D., Hosney, R. C., Seib, P. A., and Biag, M. 1977. Mixograph studies. I. Effect of certain compounds on mixing properties. *Cereal Chem.* 54:794-802.
- Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-17.

[Received December 6, 1999. Accepted August 7, 2000.]