Enzymatic Oxidative Treatments of Wheat Bran Layers: Effects on Ferulic Acid Composition and Mechanical Properties

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Enzymatic treatments known to induce the gelation of feruloylated arabinoxylans solutions were applied to tissue strips isolated from peripheral layers of wheat grain to tentatively produce in situ arabinoxylan reticulation. The treatments by horseradish peroxidase (HRP) and manganese dependent peroxidase (MnP) induced a dimerization of ferulic acid (FA) in wheat bran with concomitant decrease of arabinoxylan solubility. Similar results were obtained, but to a lesser extent, by simple incubation of bran strips in water, suggesting the action of endogenous peroxidases. The fact that these treatments proved to be ineffective on the isolated aleurone layer and pericarp suggested that dimerization occurred mostly at the aleurone−pericarp interface. In addition, the MnP system generated a consumption of monomer and dimer of ferulic acid in the pericarp, perhaps due to their incorporation into lignin. Micro-mechanical tests using DMTA were performed on isolated tissue strips and showed that oxidation of wheat bran increased their mechanical strength (increase of stress and strain to rupture).

Keywords: *Wheat bran; ferulic acid; dehydrodimer; peroxidase; oxidative treatment; mechanical properties*

INTRODUCTION

Several functions are assigned to the hydroxycinnamic acids present in the cell walls of plants. In monocots, ferulic and *p*-coumaric acids are mostly esterified to α-L-arabinose units of cell wall arabinoxylans (AX) (*1, 2*). AX chains are cross-linked in the cell wall through diferulic bridges (*3*) which might control the cell wall extensibility and, consequently, the cell growth (*4*−*6*), as well as cell−cell adhesion (*7*). The content of dehydrodiferulic acids (DHD) present in cell walls seems to strongly influence the mechanical properties of tissues (*8, 9*). Parker and Waldron (*10*) mentioned the involvement of phenolic compounds on cooking behavior of chinese chesnut. Recently, DHD content of cell wall was studied in relation with thermal resistance of beets (*11, 12*).

Many studies have been carried out on oxidative AX gelation. Different enzymatic systems (peroxidase/H_2O_2, manganese peroxidase, and laccase) are able to provoke cross-linking of feruloylated AX chains in solution (*13*−*15*). In 1995, Grabber et al. (*16*) induced DHD formation in cell walls from maize cell suspensions using HRP/H_2O_2 treatment.

In the milling process of wheat grain, differences in elasticity and friability between endosperm and outer layers are used to favor the separation of flour and semolina from bran. After optimum grain conditioning, the endosperm should be friable while the bran should remain resistant against fragmentation (*17*). A high content of DHD in the cell walls of bran (aleurone layer and pericarp) could influence the toughness of these tissues and would constitute a favorable factor to the bran−endosperm separation during grinding. Recently, rheological tests were developed on hand-isolated tissues to measure the mechanical strength of wheat bran and to identify relevant mechanical parameters for assessing the milling behavior of wheat and the particle size of bran fraction (*18*).

The aim of this work was to study the modifications of bran mechanical properties after oxidative cross-linking of cell-wall AX. Enzymatic treatments (HRP/H_2O_2 and MnP) were applied to induce FA dimerization of aleurone layer and pericarp within the cell walls . The treated tissues were then subjected to rheological analyses in order to relate the content in dimers with the mechanical strength of the tissues.

MATERIALS AND METHODS

Wheat Samples. Durum wheat (*Triticum durum* Desf.) used in this study was cv. Ardente grown in 1999 (INRA, Melgueil, France). Bran fractions were obtained from a conventional milling process carried out in a semi-industrial semolina mill (150 kg/h).

Enzymes and Reagents. Manganese peroxidase (MnP) from *Phanerochaete chrysosporium* I-1512 (EC 1.11.1.13, Mn^{2+}: hydrogen-peroxide oxidoreductase) was supplied by Dr. M. Asther (Laboratoire de Biotechnologie des Champignons Filamenteux, INRA, Marseille, France). Feruloylated sugar-beet pectins (GENU beta pectin) from Hercules (Copenhagen Pectin A/S, Lille Skensved, Denmark) were supplied by Dr. M. C. Ralet-Renard (URPOI/INRA, Nantes, France). Peroxidase type I (HRP) from horseradish (EC 1.11.1.1.7, hydrogen-peroxide oxidoreductase), *trans*-ferulic acid (FA), TMCA, cysteine, H_2O_2, and $MnSO_4$ were purchased from Sigma Chemical Co (St. Louis, MO).

Preparation of Wheat Bran and Aleurone Layer Strips. Two strips were isolated by hand on each of the lateral faces of the grain. Germ, dorsal, and ventral parts of the grain were sanded with sandpaper to give them a plane shape. After 10 h of immersion in distilled water, the sample (in the form of a disk) was divided into two parts by incising the crease. Each

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part was soaked again in distilled water for 2 h and the endosperm was removed using a scalpel. After the two strips were rinsed, they were dried for 12 h between two plates to impose upon them a plane shape (final water content, 15% wet basis (W.b.)). The strips obtained were approximately 6 mm long and 2 mm wide.

Treatment of Bran and Tissues Strips. The HRP treatment was carried out on 80 mg of ground wheat bran at 20 °C in 4.5 mL of distilled water with HRP (6.4 U) and H_2O_2 (3.4×10^{-2} mM). The MnP treatment was performed on 80 mg of material (ground wheat bran, aleurone layer, and pericarp isolated by hand) in 4.5 mL of tartrate buffer (0.5 M, pH 5) with MnP (8 U), $MnSO_4$ (0.18 mM), and H_2O_2 (0.5 mM). The cysteine treatment was performed on 80 mg of material (wheat bran strips isolated by hand) in 4.5 mL of distilled water containing cysteine (35 mM).

Water-Extractable Arabinoxylans (WEAX) Determination. The WEAX concentration in the reaction mixtures was determined according to a semi-automated method (*19*), using an Evolution II auto-analyzer (Alliance Instruments, France). Reaction mixtures were diluted 3-fold in distilled water.

Analysis of Esterified Phenolic Acids. Wheat bran fraction, aleurone layer, and pericarp isolated by hand were ground (Freeze-mill 6570, Avantec, France) and freeze-dried before FA extraction. Ground samples (80 mg) were de-esterified for 2 h in the dark with 2 M NaOH (10 mL) at 35 °C. Internal standard, 2.5 trimethoxy-*trans*-cinnamic acid (TMCA), was added, and the solution was adjusted to pH 2 with 4 N HCl. Phenolic acids were extracted twice with diethyl ether (5 mL). The ether phase was evaporated at 30 °C in the presence of argon. The dried extract was dissolved in methanol/water (50:50, v/v), filtered (0.45-μm filter), and injected (20 μL) on RPHPLC using an Alltima (Alltech, Deerfield, IL) C_{18} 5-μm column (250 × 4.6 mm). UV detection was carried out using a 996 Water photodiode array detector (Waters, Milford, MA) at 320 nm. Linear gradient elution was performed by acetonitrile and sodium acetate buffer 0.05 M (pH 4.0) at 1 mL/min at 35 °C, from 15:85 to 35:65, respectively, in 30 min, from 35:65 to 60:40 in 0.5 min, from 60:40 to 15:85 in 4.5 min, and maintained at 15:85 for 5 min. Response factors of ferulic acid dehydrodimers determined by Saulnier et al. (*20*) were used.

Determination of Endogenous Phenol Oxidase Activity in the Bran Fraction. The endogenous phenol oxidase activity was measured in bran aqueous extract (a) and bran aqueous suspension (b).

(a) Bran Aqueous Extract. Bran fractions (2.15 g) were immersed in distilled water (21.5 mL) for 1 h at 20 °C under gentle agitation. After centrifugation (300*g*, 10 min), the supernatant was collected (4.5 mL) and filtered (0.1 mm). *Trans*-FA solution (1.5 mL, 1 mg/mL) was added, and the reaction mix was incubated at 20 °C for 1 or 24 h. The reaction was stopped by addition of 4 N HCl. The reaction mixture was adjusted to pH 2, and phenolic acids were quantified as described above.

(2) Bran Aqueous Suspension. Trans-FA solution (1.5 mL, 1 mg/mL) was added to the bran suspension (2.15 g in 21.5 mL), and the reaction mixture was incubated for 1 or 24 h at 20 °C under agitation. After centrifugation (300*g*, 10 min), the supernatant was filtered (0.1 mm) and the solution was adjusted to pH 2 with 4 N HCl. Phenolic acids were quantified as described above. A bran extract and bran suspension boiled for 30 min was used as control.

Oxidative Gelation. Because of the presence of endogenous xylanases in the water bran extract, gelation tests were performed on feruloylated sugar-beet pectins instead of on purified wheat AX. The reaction was carried out in water at room temperature (pH range 6−6.5). The mixture contained 0.5 mL of pectin solution (50 mg/mL) and 0.5 mL of water bran extract or bran suspension, and 10 μL of H_2O_2 (5 mM) was added to the pectin/bran suspension.

The gel formation was estimated by visual inspection of the reaction mixture and the system was considered gelled when it did not deform under its own weight upon turning the tube upside down.

Table 1. AX, FA, and DHD Contents of Different Histological Layers, and Dehydrodimers-to-Ferulic Acid Molar Ratio (DHD/FA), and Total Ferulic Acid to Total Arabinoxylan Ratio (FAt/AXt)

	endosperm[a]	aleurone layer	pericarp
AXt[b] (%DM)	1.6	46.0	42.0
FAt[c] (mg/g)	0.17	6.75 ± 0.20	12.88 ± 0.20
FA (mg/g)	0.15	5.00 ± 0.10	4.90 ± 0.10
DHD (mg/g)	0.02	1.65 ± 0.10	3.72 ± 0.10
FAt/AXt	0.11	0.14	0.29
DHD/FA	0.16	0.34	1.65

[a] (*22*)(Ardente variety). [b] (*23*). [c] FA + DHD.

Mechanical Property Measurements. Mechanical tests were performed using dynamic mechanical thermal analysis DMTA Mk III E (Rheometrics Inc., Piscataway, NJ). The strip moisture content was stabilized at about 17% W.b. (a moisture content generally used in wheat milling) by setting the relative humidity at 78%. Humidity control was achieved according to the principle of water vapor saturation at different temperatures (*21*). The furnace was flushed with air that was bubbled through water at 25.2 °C. The furnace temperature was set at 30 °C in order to established the desired humidity (%HR = P_{H_2O}(30 °C)/P_{H_2O}(25.2 °C) × 100 = 78). Sample equilibration was followed by a time sweep test at imposed strain (0.01%) (total time = 10 min; frequency = 1.59 Hz). The stability of elastic modulus (E) was used as an indicator of the sample equilibration.

Uniaxial tension tests were performed at a rate of 0.05 mm/s until disruption of the sample. Stress−strain curves were used to determine maximum tensile strain (ϵ_{max}), stress of rupture (σ_{max}), elastic modulus (E), and energy of rupture (W_{max}). Tension tests were performed on at least 10 bran strips. Tests in which failure did not occur in the center of the strip were discarded.

RESULTS AND DISCUSSION

Phenolic Acid Composition of Wheat Grain. In aleurone layer and pericarp, the major monomeric phenolic acid was *trans*-ferulic acid. *Cis*-ferulic acid, *p*-coumaric, and vanillic acids were present in low amounts. Five structural isomers of ferulic acid dehydrodimers (DHD) were detected (data not shown). 8-*o*-4′ and 5-8′ benzofuran DHD were mainly encountered, but the 5-5′, 8-5′, and 8-8′ forms were also detected. Although FA concentration was almost the same in the pericarp and the aleurone layer, DHD content was 4.8 times higher in pericarp (Table 1). Consequently, this variation was similar for the dehydrodimer-to-ferulic acid molar ratio (DHD/FA) which can be considered as an index of cross-linking. The degree of cross-linking of AX cell wall increased from the inner to the outer layers of the grain teguments which was in agreement with the involvement of DHD in grain resistance to insects and phytopathogens (*24*).

In addition, considering the distribution of AX concentration within the grain (*22*), a gradient was also observed for the feruloylation degree of AX as expressed by the ratio of ferulic acid/AX (FAt/Axt). However, the difference between aleurone layer and endosperm, which are both seed tissues, was lower than that between pericarp (fruit tissue) and other layers. Phenolic acid composition and AX concentration seem then to depend on the histological origin of tissues.

Enzymatic Oxidative Treatment of Wheat Bran. Changes in phenolic acid composition were followed for 72 h in samples treated with HRP/H_2O_2 and MnP (Figure 1). In both cases, an increase in DHD content and simultaneous decrease in FA content indicated FA

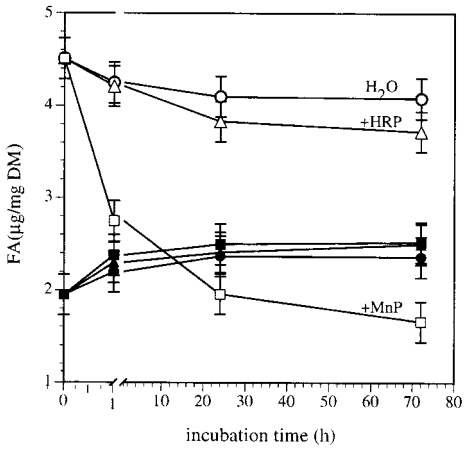

Figure 1. Evolution of phenolic acid content of wheat bran during immersion in water (FA (○); DHD (●)), during HRP treatment (FA(△); DHD(▲)), and during MnP treatment (FA-(□); DHD (■)).

dimerization. After a fast dimerization during the first hour of treatment, the reaction rate decreased gradually until stabilization of FA and DHD concentrations. After 24 h of treatment, the DHD content did not increase again indicating that the maximum possible dimerization was reached.

Increases in DHD content were roughly similar with the two enzymatic treatments (≈28%). The reaction yield was twice lower than that in a suspension of cell walls isolated from maize cell suspension (*16*) suggesting that molecular mobility of AX chains was more limited in bran layers.

In addition, although the formation of 8-*o*-4′ and 5-5′ linkages were more favored in maize cell suspension, five DHD forms were generated in equal proportions in wheat bran (data not shown). The enzymatic systems (MnP and HRP/H_2O_2) induced the oxidation of the FA phenolic function to a phenoxyl radical, which forms DHD through nonenzymatic reaction (*25*). Considering the limited FA mobility in bran cell walls, the type of linkage, and thus, the DHD form, may be imposed by the spatial orientation of the two involved FA.

In addition, FA consumption was very different for the two enzymatic treatments. FA content decreased from 4.2 µg/mg in the control to 3.8 and 1.5 µg/mg in the presence of HRP and MnP, respectively. During MnP treatment, DHD formation did not compensate fully the decrease in FA, suggesting that DHD were not the only products formed, or that, alternatively, DHD can be further oxidized or radicals could bind to other cell-wall components (proteins, lignin, etc.). In both cases, the products formed would not be detected by the method used. This difference in FA consumption can be explained by the mode of action of the enzymatic systems. Whereas HRP acts directly in contact with the substrate (FA), MnP is mediated by a Mn^{3+} ion, which forms an oxidizing chelate with an organic acid, freely diffusible through the cell-wall structure (*26*). This mechanism allowed oxidation of substrates not directly accessible to the enzyme. The difference in FA consumption suggests that the polysaccharidic network of the cell wall is too compact to allow the free diffusion of enzyme such as HRP (molecular weight ≈40 kDa). HRP action could then be limited to the surface of the bran particles. Furthermore, MnP seems to act deeply in cell wall tissues with effects other than those of unique FA dimerization.

FA dimerization was also observed in a control sample immersed in water. In this case, an increase of the DHD/FA ratio (15%) suggested the possible presence of endogenous peroxidases and/or phenoloxidases.

Detection of Ferulate Oxidase Activity in Bran Fractions. Measurements of ferulate oxidase activities were carried out on a bran suspension and an extract of bran (Figure 2, a and b). The results confirmed the presence of ferulate oxidase activities in wheat bran. Even though FA oxidation did not occur with the boiled bran extract, the large consumption of free *trans*-FA in the reaction mix demonstrated the ability of bran endogenous enzymes to oxidize FA. In parallel, a low synthesis of DHD was observed in bran suspension, with 8-8′ and 5-8′ benzofuran DHD being the major products formed, and a low formation of 8-*o*-4 DHD. These three major forms of DHD were also produced during incubation of WEAX with HRP/H_2O_2 (*27*). FA decrease and DHD formation were not proportional, suggesting that FA oxidation led to the formation of polymers or other products.

This oxidation could be catalyzed by either a phenol oxidase (PO) or a peroxidase (POD). It is interesting to notice that no addition of H_2O_2 was necessary, suggesting that FA oxidation was catalyzed by PO using O_2 as cosubstrate. Alternatively, if POD was responsible for FA oxidation, either H_2O_2 was available in the wheat bran or POD exhibited oxidase activity (*28*). The fact that the FA consumption was 3-fold higher with the bran suspension than with the bran extract indicated that the extraction of the enzyme was not complete, and it may also suggest that ferulate oxidation was achieved by a peroxidase. Actually, it has been reported that PO occurs as a soluble form in the cell cytoplasm, whereas POD may be wall-bound (*29*). Moreover, H_2O_2 addition was necessary to induce the feruloylated pectin gelation in bran extract, confirming that ferulate dimerization was probably catalyzed by POD. On the other hand, pectin gelation occurred without H_2O_2 addition in the presence of bran suspension. This observation indicated that H_2O_2 was probably produced and released by bran cells during a simple bran immersion in distilled water.

In the mature wheat grain, PODs are more particularly concentrated in the bran layers (*30*). Several isoforms were identified, and their immunolocalization in durum wheat showed that each isoform appeared to be tissue specific (*31*). PODs were localized in the cell wall of external layers of kernel, except in aleurone layer, where enzymes were found inside the cell. It is likely that a low amount of H_2O_2 was released by aleurone cells when wheat bran was immersed in water. H_2O_2 is formed when NADH, produced by wall-bound malate deshydrogenase, is oxidized by peroxidase (*32*). The low H_2O_2 concentration in bran extract may allow free *trans*-FA oxidation in solution but would be insufficient for feruloylated pectin gelation by extract endogenous POD.

Results obtained during the oxidizing treatment of wheat bran showed that maximum ferulate dimerization was not naturally reached in the mature grain. During grain development, the activity of these endogenous enzymes or the H_2O_2 production could be the limiting factors for FA dimerization or incorporation of FA and DHD into lignin. Isolation of these enzymes from different peripheral layers would allow understanding of the cell wall development.

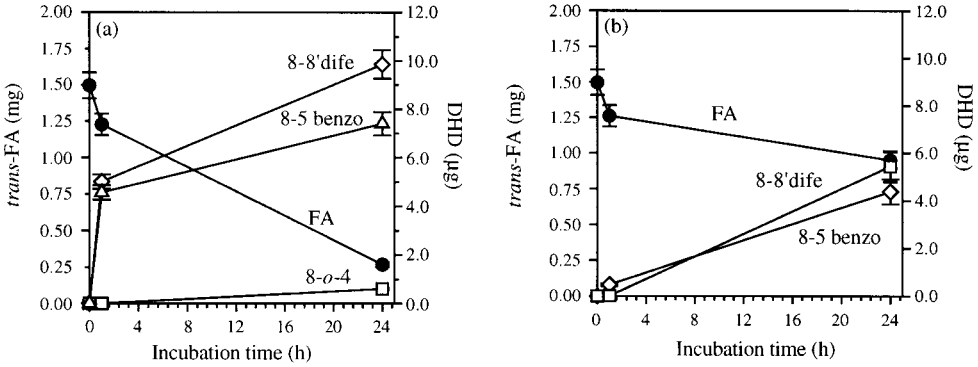

Figure 2. Evolution of *trans*-FA solution incubated with bran suspension (a) and with bran extract (b).

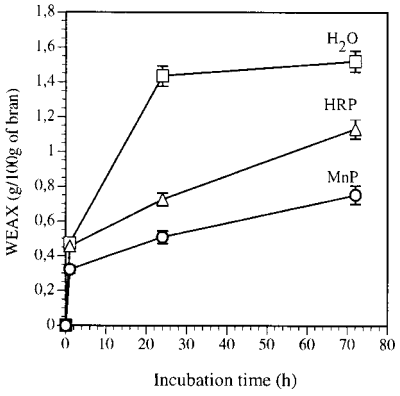

Figure 3. AX solubilization in treated (H_2O, HRP, and MnP) wheat bran.

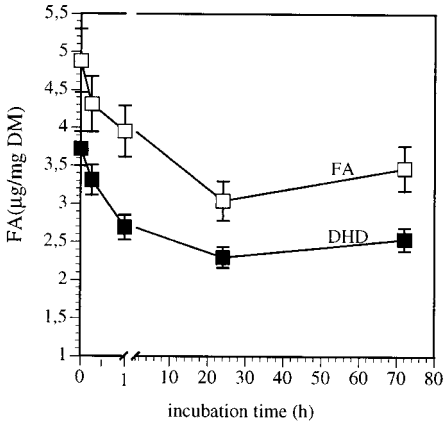

Figure 4. Evolution of phenolic acid content of isolated pericarp layer during MnP treatment.

AX Solubilization in Wheat Bran. FA dimerization data were confirmed by AX solubilization patterns presented in Figure 3. In a control sample immersed in water, a rapid AX solubilization during the first 24 h was observed. Beyond 24 h of incubation, the maximum extent of solubilization (1.52% of DM) was reached.

The AX solubilization curves obtained with samples treated by MnP and HRP showed lower solubilization rates. After 72 h of incubation, the maximum extent of solubilization was not reached. The increase of DHD content observed in the presence of HRP and MnP corresponded to a decrease in AX solubility. These results thus showed that a low increase of DHD content induced significant reduction in AX solubilization extent.

Effect of MnP Treatment on Phenolic Acid Composition of Aleurone Layer and Pericarp. The MnP treatment did not induce any modification of FA composition of isolated aleurone layer. Neither the DHD nor the FA contents were affected despite the diffusibility of the oxidizing chelate. These observations suggest that the FA present in the aleurone layer cell wall was protected by the thick polymer deposition or was not favorably located to undergo dimerization.

On the other hand, MnP quickly reduced FA and DHD content in pericarp (Figure 4). In contrast to the results obtained on bran fractions, no FA dimerization was measured which suggested that FA units involved in the dimerization reaction were located neither in aleurone nor in pericarp layers. It is possible that cross-linking occurred at the aleurone−pericarp interface. In the oxidized pericarp, FA and DHD could bind other cell-wall components such as lignin. Such linkages have been identified in wheat straw and internodes highly concentrated in lignin (*33*, *34*). It has been established that FA and DHD were able to form cold-alkali stable bonds (ether bonds) with the lignin units (i.e., coniferyl and sinapyl alcohols). These linkages led to a copolymerization between AX and lignin which might contribute to the rigidity of the cell wall (*4*, *35*).

Table 2. Effect of MnP, HRP, and Cysteine Treatments on Mechanical Properties of Wheat Bran Strips[a]

	E^b (10^8 Pa)	$\sigma_{max}{}^c$ (10^7 Pa)	$\epsilon_{max}{}^d$ (%)	W^e (J/mm^3)
control	3.20[1]	2.87[1]	12.39[1]	5.37[1]
cysteine (24 h)	6.61[2]	3.29[1]	4.95[2]	1.85[2]
H_2O (24 h)	7.27[2]	4.50[2]	5.95[2]	3.12[2]
MnP (24 h)	6.68[2]	4.89[2]	7.67[3]	4.77[1]

[a] Superscripts 1, 2, and 3 indicate homogeneous groups (ANOVA multiple range tests (n = 5) significant at the 0.01 level of probability). [b] Elastic modulus (E). [c] Maximum stress of rupture (σ_{max}). [d] Maximum tensile strain (ϵ_{max}). [e] Rupture energy (W_{max}).

Mechanical Properties. Traction tests were carried out until rupture of bran strips (Table 2). The influence of each treatment was determined from rupture characteristics (elastic modulus (E), maximum stress of rupture (σ_{max}), maximum tensile strain (ϵ_{max}), and rupture energy (W_{max})). Control samples corresponded to bran strips isolated by hand that had not been subjected to any treatment. The other strips were treated during 24 h in distilled water, in the presence of MnP or presence of cysteine used to inhibit FA dimerization.

The elastic modulus (E), which expresses the stiffness of the material, corresponds to the slope of the linear part of curves. Samples treated in aqueous suspensions (H_2O, MnP, or cysteine) presented E values twice higher than that of the control sample, showing that aqueous treatment had a significant influence on tissue rigidity.

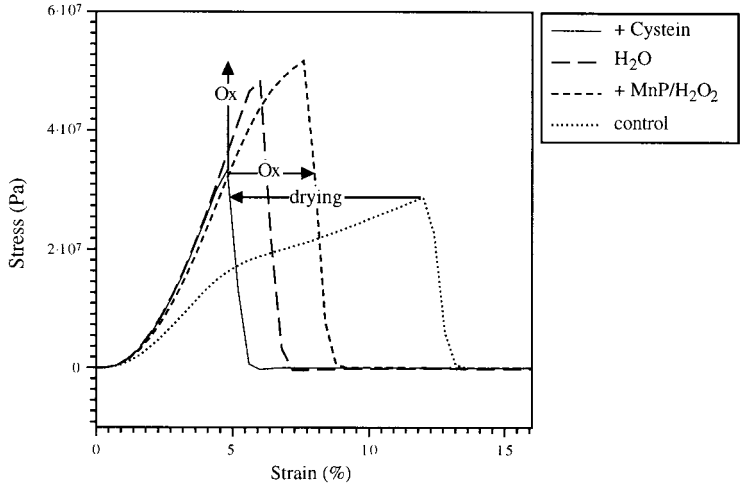

Figure 5. Effect of FA oxidation and tissue drying on the mechanical properties of bran layers.

During strip drying, cell-wall components, such as polysaccharides and proteins, could undergo aggregations affecting tissue elasticity (increase in elastic modulus and decrease in maximum tensile strain (ϵ_{max})).

The stress of rupture (σ_{max}) corresponds to the maximum strength exerted per unit of volume. Measurements of this parameter allowed determination of the contribution of an oxidizing treatment on the mechanical resistance of wheat bran. The sample treated by cysteine showed a rate of dimerization and a σ_{max} value similar to those of the control sample. Samples treated with water and MnP, whose extents of dimerization were similar, presented σ_{max} values 1.5 times higher than those of the control. However, it is difficult to assign this strong increase to FA dimerization only. Other reactions could occur during the oxidizing treatment. For example, disulfide bridges between two cysteines and dityrosine formations are reactions catalyzed by MnP (*36*, *37*). Such protein cross-linking in the aleurone cell could contribute to the increase of tensile strength. However, simultaneous increases in DHD/FA ratio and stress of rupture (σ_{max}) tend to confirm the involvement of DHD in the mechanical strength of bran. However, the strips treated by MnP presented a higher extensibility than that of a sample immersed in water. In the event of cross-linking between arabinoxylans and lignin, it would be surprising that MnP treatment could induce an increase of the tissue extensibility and not of its stiffening.

Considering energy of rupture measurements (W_{max}), added effects of water and MnP treatments did not modify significantly the *W* values of bran despite very different values of stress and strain at rupture. Drying and oxidation of samples generates modifications of distinct mechanical properties. Their respective actions can be shown schematically as in Figure 5.

From a technological point of view, oxidative pretreatment of grain might decrease the size of bran particles and could favor semolina contamination by fine bran particles during milling under constant conditions. On the other hand, stiffening of brans could have a positive impact on alternative technology, e.g., dehulling or bran micronization.

CONCLUSION

Enzymatic treatments carried out on the external part of wheat grain resulted in FA dimerization in situ. MnP and HRP treatments, as well as simple water immersion, induced AX reticulation in bran cell-wall. The extents of DHD formation were 20% in water and 28% in the presence of HRP and MnP. FA oxidation after water treatment could be assigned to wheat bran endogenous peroxidases or phenol oxidases.

The sensitivity of rheological tests carried out on bran strips isolated by hand allowed determination of the contribution of oxidizable bran compounds to the mechanical properties. The increase in rupture stress (σ_{max}) observed after oxidizing treatments confirmed the role of DHD in bran mechanical strength. However, this effect was accompanied with a decrease of tissues extensibility due to their drying after aqueous immersion. This points out the importance of the hydric history of the sample in the study of mechanical properties of the tissues. The enzymatic oxidizing treatment could still constitute an interesting approach to modifying bran strength which is a relevant factor in the technological processing of wheat.

ABBREVIATIONS USED

AX, arabinoxylan; FA, ferulic acid, 4-hydroxy-3-methoxycinnamic acid; DHD, dehydrodimer; 5-8′, (E−E)-4-4′ dihydroxy 3,5′ dimethoxy-β-3′ bicinammic acid; 5-8′ benzo, *trans*-5-((E)-2carboxyvinil)-2-(4-hydroxy-3-methoxyphenyl)-7-2,3-dihydrobenzofuran-3-carboxylic acid; 8-*o*-4, (Z)-β-(4((E)-2 carboxyvinyl)-2-methoxyphenoxy)-4-hydroxy-3-methoxycinnamic acid; 5-5′, (E,E)-4-4′ dihydroxy 5,5′ dimethoxy 3′-bicinammic acid; TMCA, 3,4,5-trimethoxy-*trans*-cinnamic acid.

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LITERATURE CITED

(1) Smith, M. M.; Hartley, R. D. Occurrence and nature of ferulic acid substitution of cell-wall polysaccharides in graminaceous plants. *Carbohydr. Res.* **1983**, *118*, 65−80.

(2) Kato, Y.; Nevins, D. J. Isolation and identification of *o*-(5-*o*-feruloyl-α-L-arabinofuranosyl)−(1′3)-*O*-β-D-xylopyranosyl-(1′4)-D-xylopyranose as component of zea shoot cell walls. *Carbohydr. Res.* **1985**, *137*, 139−150.

(3) Ralph, J.; Quideau, S.; Grabber, J. H.; Hatfield, R. D. Identification and synthesis of new ferulic dehydrodimers present in grass cell walls. *J. Chem. Soc. Perkin Trans. 1* **1994**, 3485−3498.
(4) Fry, S. C. Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth. *Planta* **1979**, *146*, 343−351.
(5) Fry, S. C. Feruloylated pectins from the primary cell wall: their structure and possible functions. *Planta* **1983**, *157*, 111−123.
(6) Tan, K. S.; Hoson, T.; Masuda, Y.; Kamisaka, S. Correlation between cell wall extensibility and the content of diferulic and ferulic acids in cell walls of *Oryza sativa* coleoptiles grain under water and in air. *Plant Cell. Physiol.* **1991**, *33*, 103−108.
(7) Fry, S. C. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annu. Rev. Plant Physiol.* **1986**, *37*, 165−186.
(8) Kamisaka, S.; Takeda, S.; Takahashi, K.; Shibata, K. Diferulic and ferulic acid in the cell wall of Avena coleoptiles. Their relationships to the mechanical properties of the cell wall. *Physiol. Plant.* **1990**, *78*, 1−7.
(9) Parvez, M. M.; Wakabayashi, K.; Hoson, T.; Kamisaka, S. Change in cellular osmotic potential and mechanical properties of cell walls during light induced inhibition of cell elongation in maize coleoptiles. *Physiol. Plant.* **1996**, *96*, 179−185.
(10) Parker, M. L.; Waldron, K. W. Texture of chinese water chesnut: involvement of cell wall phenolics. *J. Sci. Food Agric.* **1995**, *68*, 337−346.
(11) Waldron, K. W.; Ng, A.; Parker, M. L.; Parr, A. J. Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. *J. Sci. Food Agric.* **1997**, *74*, 221−228.
(12) Ng, A.; Harvey, A. J.; Parker, M. L.; Smith, A. C.; Waldron, K. W. Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (*Beta vulgaris*). *J. Agric. Food Chem.* **1998**, *46*, 3365−3370.
(13) Crowe, N. L.; Rasper, V. F. The ability of chlorine and chlorine-related oxidants to induce oxidative gelation in wheat flour pentosans. *J. Cereal Sci.* **1988**, *7*, 283−294.
(14) Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.* **1990**, *11*, 153−169.
(15) Figueroa-Espinoza, M. C.; Morel, M. H.; Surget, A.; Rouau, X. Oxidative cross-linking arabinoxylans by manganese peroxidase. Comparison with laccase and horseradish peroxidase. Effect of cysteine and tyrosine on gelation. *J. Sci. Food Agric.* **1999**, *79*, 460−463.
(16) Grabber, J. H.; Hatfield, R. D.; Ralph, J.; Zon, J.; Amrhein, N. Ferulate cross-linking in cell walls isolated from maize cell suspensions. *Phytochemistry* **1995**, *40*, 1077−1082.
(17) Butcher, J.; Stenvert, N. L. Conditioning studies on Australian wheat. I. The effect of conditioning on milling behaviour. *J. Sci. Food Agric.* **1973**, *24*, 1055−1066.
(18) Mabille, F.; Gril, J.; Abecassis, J. Mechanical properties of wheat bran. *Cereal Chem.* **2001**, *78*, 231−235.
(19) Rouau, X.; Surget, A. A rapid semi-automated method for the determination of total and water extractable pentosans in wheat flours. *Carbohydr. Polym.* **1994**, *24*, 123−132.
(20) Saulnier, L.; Crépeau, M. J.; Lahaye, M.; Thibault, J. F.; Garcia-Conesa, M. T.; Kroon, P. A.; Williamson, G. Isolation and structural determination of two 5,5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydr. Res.* **1999**, *320*, 82−92.
(21) Redl, A.; Vergnes, B.; Guilbert, S. Equivalence of temperature and hydratation on the viscoelastic behaviour of wheat gluten networks. *XIIIth International Congress on Rheology*: Cambridge, UK, 2000; Vol. 4, pp 344−346.
(22) Lempereur, I.; Rouau, X.; Abecassis, J. Genetic and agronomic variation in araninoxylan and ferulic acid contents of durum wheat (*Triticum durum* L.) grain and its milling fractions. *J. Cereal Sci.* **1997**, *25*, 103−110.
(23) Feillet, P. *Le grain de blé, composition et utilisation*. INRA: Paris, France, 2000.
(24) Arnason, J. T.; Gale, J.; Conilh de Beyssac, B.; Sen, A.; Miller, S. S.; Philogene, B. J. R.; Lambert, J. D. H.; Fulcher, R. G.; Serratos, A.; Mihlm, J. Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (horn) and *Sitophilus zeamais* (motch). *J. Stored Prod. Res.* **1992**, *28*, 119−126.
(25) Brown, B. R. Biochemical aspects of oxidative coupling of phenol. In *Oxidative Coupling of Phenols, Vol. 1.*; Taylor, W. I., Batlersby, A. R., Eds.; Dekker: New York, 1967; pp 167−201.
(26) Paice, M. G.; Reid, I. D.; Bourbonnais, R.; Archibald, F. S.; Jurasek, L. Manganese peroxidase, produced by *Trametes versicolor* during pulp bleaching, demethylates and delignifies kraft pulp. *Appl. Environ. Microbiol.* **1993**, *59*, 260−265.
(27) Figueroa-Espinoza, M. C.; Rouau, X. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase. Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chem.* **1998**, *75*, 259−265.
(28) Grambow, H. J.; Langenbeck-Schwich, B. The relationship between oxidase activity, peroxidase activity, hydrogen peroxide, and phenolic compounds in the degradation of indole-3-acetic acid in vitro. *Planta* **1983**, *157*, 131−137.
(29) Bolwell, G. P. Synthesis of cell wall components: aspects of control. *Phytochemistry* **1988**, *27*, 1235−1253.
(30) Honold, G. R.; Stahmann, M. A. The oxidation−reduction enzymes of wheat. IV. Qualitative and quantitative investigations of the oxidase. *Cereal Chem.* **1968**, *45*, 99−106.
(31) Fraignier, M. P.; Michaux-Ferrière, N.; Kobrehel, K. Distribution of peroxidases in durum wheat (*Triticum durum*). *Cereal Chem.* **2000**, *77*, 11−17.
(32) Cochrane, M. P. Observations on the germ aleurone of barley. Phenol oxidase and peroxidase activity. *Ann. Bot.* **1994**, *73*, 121−128.
(33) Scalbert, A.; Monties, B.; Lallemand, J. Y.; Guittet, E.; Rolando, C. Ether linkage between phenolic acids and lignin fractions from wheat straw. *Phytochemistry* **1985**, *24*, 1359−1362.
(34) Iiyama, K.; Lam, T. B. T.; Stone, B. A. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry* **1990**, *29*, 733−737.
(35) Fry, S. C. Phenolic components of the primary cell wall. *Biochem. J.* **1982**, *203*, 493−504.
(36) Wariishi, H.; Valli, K.; Renganathan, V.; Gold, M. H. Thiol-mediated oxidation of nonphenolic lignin model componds by manganese peroxidase of *Phanerochaete chrysosporium*. *J. Biol. Chem.* **1989**, *264*, 1415−1419.
(37) Michon, T.; Wang, W.; Ferrason, E.; Gueguen, J. Wheat prolamine cross-linking through dityrosine formation catalyzed by peroxidases: improvement in the modification of a poorly accessible substrate by "indirect" catalysis. *Biotechnol. Bioeng.* **1999**, *63*, 449−458.

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