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Enzymatic Solubilization of Arabinoxylans from Isolated Rye
Pentosans and Rye Flour by Different *endo*-Xylanases and
Other Hydrolyzing Enzymes. Effect of a Fungal Laccase on the
Flour Extracts Oxidative GelationM. C. FIGUEROA-ESPINOZA,^{*,†,‡} C. POULSEN,[‡] J. BORCH SØE,[‡]
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Water-extractable (WEP) and water-unextractable (WUP) pentosans were isolated from a rye flour. The effect of a commercial enzyme preparation, Grindamyl S 100 (GS100), containing pentosanase activities, was investigated on WEP, WUP, a mix of WEP and WUP, and the rye flour, with the aim to monitor the solubilization and depolymerization of high molecular weight arabinoxylans and the effect on the viscosity of the reaction medium. The effects of other hydrolyzing enzymes were also tested. Three xylanases were used: xylanase 1 (Xyl-1) from *Aspergillus niger*, the main activity present in GS100; xylanase 2 (Xyl-2) from *Talaromyces emersonii*; and xylanase 3 (Xyl-3) from *Bacillus subtilis*. Xyl-3 was used in combination with Xyl-1, (1,4)- β -D-arabinoxylan arabinofuranohydrolase, *endo*- β -D-glucanase, or ferulate esterase from *A. niger*, but no synergism was observed. GS100 and xylanases increased the arabinoxylan solubilization, Xyl-3 and Xyl-1 being those that presented the best yields of extraction without extensive depolymerization of water-extractable arabinoxylans. Both xylanases were affected by an inhibitor in rye flour. Flour treated with hot ethanol was used to study the oxidative gelation of flour extracts treated with xylanases, in the presence of laccase from *Pycnoporus cinnabarinus*. Two doses of xylanases were tested (0.5 and 2.5 units). Only the flour extracts treated with 0.5 unit of Xyl-1 thickened.

KEYWORDS: Arabinoxylans; arabinofuranohydrolase; ferulate esterase; glucanase; laccase; oxidative gelation; rye; solubilization; xylanase; xylanase inhibitor

INTRODUCTION

Pentosans, the main nonstarch polysaccharide fraction of most cereal grains, are mainly composed of arabinoxylans (AX) that are partly extractable with water (water-extractable pentosans or WEP) (1). Water-extractable AX (WEAX; 30–40% in rye) are concentrated in the flour fraction (endosperm) and are coextracted with arabinogalactan-peptides. Together with various amounts of β -glucans, they form the water-extractable fraction. The water-unextractable pentosans (WUP) [rich in water-unextractable AX (WUAX)] are associated in the cell-wall fragments with other components, including cellulose, β -glucans, glycoproteins, and phenolic compounds, constituting the water-unextractable fraction. In comparison with wheat grain, rye grain has a higher AX content [6.5–12.2% dry basis (db)], with a large portion of the AX fraction being water-extractable (1.5–3% db). Rye flour contains ~5% db of total AX and ~2%

db of WEAX. In wheat flour, total AX occur in a range of 2–3% db, being 20–30% water-extractable (2–4).

The presence of WEAX in rye dough increases dough viscosity, bread volume, gas retention, shelf life, crumb texture, color, and taste. In contrast, WUAX swell during dough fermentation, increase crumb firming rate, and decrease loaf volume (4–6). Similar results were observed by Rouau et al. (7) and Courtin et al. (8) in wheat bread-making. The solubilization of AX from WUAX can be achieved by the use of *endo*-xylanases alone or in combination with other hydrolyzing enzymes such as arabinofuranosidases, β -glucanases, or ferulate esterases (FAE) (6, 9–22).

endo-(1,4)- β -D-Xylanases [EC 3.2.1.8, (1,4)- β -D-xylan xylanohydrolase] hydrolyze the 4-linked β -D-xylopyranosyl residue, cutting the internal linkages in AX and provoking a decrease in viscosity (23). Certain *endo*-xylanases preferentially release high molecular weight AX (HMWAX) from WUP and slowly degrade WEAX; others solubilize AX of low molecular weight and/or extensively degrade WEAX (24–27). Bread-making-improving xylanases should be able to solubilize large amounts of AX from WUP with limited depolymerization of AX in the

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water-extractable fraction of the dough (7, 10, 25, 26, 28–31). The amount of HMWAX that can be released from flour cell-wall material during a bread-making process is limited: adding too high a level of enzyme causes a degradation of WEAX and solubilized AX, resulting in a too soft and sticky dough (7, 10, 19, 25, 31). Grindamyl S 100 (GS100, *Aspergillus niger*), widely used as a dough and bread improver, is an example of a commercial enzyme preparation containing a specific *endo*-xylanase (7, 19, 20, 28).

The structure of AX and their linkages to other cell-wall components determine their water solubility and extractability by endogenous and exogenous enzymes. The extractability of pentosans from rye is lower in the outer layers of the kernel than in the starchy endosperm. This physical inaccessibility of the substrate may retard or hinder the reactions. Thus, both depolymerizing and substituent-cleaving enzymes are needed for the complete hydrolysis of heteroxylans. *endo*-Xylanases can act synergistically with (1,4)- β -D-arabinoxylan arabinohydrolase (AXH; EC 3.2.1.55, α -L-arabinofuranosidase) and *endo*- β -D-glucanase (EC 3.2.1.4; β -1,4-glucan glucanohydrolase). AXH render the xylan backbone less branched and more accessible to depolymerization by xylanases. β -Glucanases help to release AX by degrading the β -glucans linked to them (11, 12, 17, 19, 32–34). Synergy of *endo*-1,4- β -xylanases and ferulic acid esterases (EC 3.1.1.73) has also been reported (9, 12, 14, 15, 19, 21, 22). The substituent-cleaving enzymes could also affect the solubilization of xylans from the cell walls or the oxidative gelation that happens through the dimerization of their esterified ferulic acid in the presence of free radical generating oxidants (12, 35–39).

Enzymatic AX solubilization can be limited not only by the structure of WUP and the affinity of the xylanase for the WUP but also by the presence of inhibitors in the substrate as flour or bran. Proteinaceous *endo*-xylanase inhibitors were discovered in wheat by Debyser et al. (40). Rouau and Surget (41) confirmed the existence on wheat grain endosperm and bran of a water-extractable and thermolabile compound that inhibits exogenous hemicellulases. The presence of xylanase inhibitors in wheat was also reported by Sorensen and Poulsen (42) and McLauchlan et al. (43). Today it is accepted that there exist in wheat two families of proteinaceous inhibitor of xylanases, the TAXI family (*Triticum aestivum* xylanase inhibitor) and the XIP family (xylanase-inhibiting protein). There is no sequence homology between the two families, and they exhibit different specificities toward xylanases and different mechanisms of inhibition. Inhibitors in other cereal species were also reported (44, 45, 61).

The aim of this work was to find the best combination of enzymes to be used as a rye-bread-making improver. For this, we examined the enzymatic release of HMWAX from rye WUP, from a mix of WEP and WUP, and from rye flour, using GS100, three different xylanases alone or in combination with AXH, β -D-glucanase or FAE. The effect of GS100 and xylanases on the depolymerization of WEAX was also studied. The positive effect of HMWAX enzymatic release on rye bread-making would be due to not only their capacity to hold water and form viscous solutions but also their capacity to gel. Thus, laccase from *Pycnoporus cinnabarinus* was used to verify the oxidative gelation capacity of the flour WEAX released by xylanase.

MATERIALS AND METHODS

Flour. German rye flour type 947 with 93% extraction rate and ash content of 1.06% was provided by Danisco Cultor (Brabrand, Denmark). The composition of the flour is presented in **Table 1**.

Table 1. Composition of Rye Flour, WEP, and WUP in Percent Dry Matter

fraction	lipids	protein	sugars ^a	AX ^b	FA ^c	FA dimers ^d
flour	1.1	7.8	79.8	6.7	0.08	0.06
WEP1 ^e	nd ^f	6.1	77.4	65.0	0.15	0.00
WUP1 ^g	nd	30.2	48.2	28.2	0.20	0.35
WEP2 ^h	nd	13.6	87.9	62.9	0.03	0.00
WUP2 ⁱ	nd	15.2	55.7	32.7	0.36	0.34

^a Sum of arabinose, galactose, glucose, mannose, rhamnose, and xylose. ^b Sum of (arabinose + xylose) \times 0.88. ^c Ferulic acid = *cis*- + *trans*-ferulic acid. ^d Sum of ferulic acid dehydromers: 5-5' [(*E,E*)-4,4'-dihydroxy-5,5'-dimethoxy-3,3'-bicycinnamic acid], 8-5' [(*E,E*)-4,4'-dihydroxy-3,5'-dimethoxy- β ,3'-bicycinnamic acid], 8-5'-benzofuran [*trans*-5-[(*E*)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid], 8-8' [4,4'-dihydroxy-3,3'-dimethoxy- β , β '-bicycinnamic acid], and 8-O-4' [(*Z*)- β -[4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy]-4-hydroxy-3-methoxycinnamic acid] (60). ^e Water-extractable pentosans extracted from a flour not treated with Pronase. ^f Not determined. ^g Water-unextractable pentosans extracted from a flour not treated with Pronase. ^h Water-extractable pentosans extracted from a flour treated with Pronase. ⁱ Water-unextractable pentosans extracted from a flour treated with Pronase.

Flour treated with hot ethanol (Flour_{etOH}) was obtained by treating 34.135 g of rye flour with 110 mL of 80% ethanol (v/v) under reflux for 30 min. Centrifugation (5 min, 15000g, 4 °C) and washing of the pellet with absolute ethanol were repeated three times. The pellet was dried by solvent exchange (absolute ethanol, acetone, diethyl ether) on a glass filter (17–40 μ m).

Enzymes. Termamyl (α -amylase, *Bacillus licheniformis*, 120 KNU/g, Novo, Denmark), amyloglucosidase (glucamylase, *Aspergillus niger*, 75 units/mg, Merck, Darmstadt, Germany), and Pronase (*Streptomyces griseus*, 700 units, Boehringer, Mannheim, Germany) were used to purify WEP1, WEP2, WUP1, and WUP2 from rye flour. Danisco Cultor provided the following enzymes: Grindamyl S 100 (*A. niger*, 2.5 units/mg), Xyl-1 (*A. niger*, EC 3.2.1.8, MW 20000 Da, pI 4.07, optimal pH 3.5, 166.9 units/mL), Xyl-2 (*Talaromyces emersonii*, EC 3.2.1.8, MW 38000 Da, pI 4.59, optimal pH 3.0, 117.4 units/mL), Xyl-3 (*Bacillus subtilis*, EC 3.2.1.8, MW 20000 Da, pI 9.03, optimal pH 6.0, 142 units/mL), *endo*- β -glucanase (*A. niger*, EC 3.2.1.4, MW 24230 Da, pI 4.2, optimal pH 4.0, 120000 β -glucanase units [BGU]/mL), AXH (*A. niger*, EC 3.2.1.55, MW 332700 Da, pI 3.7, optimal pH 4–5) (46), and FAE (*A. niger*, EC 3.1.1.73, MW 30000 Da, pI 3–4, optimal pH 5 (substrate: ferulic acid methyl ester), 5.2 units/mL). Laccase (*P. cinnabarinus*, EC 1.10.3.2, 0.04 unit/ μ L (one unit of activity is the nanomoles of syringaldazine oxidized per second), optimal pH 5) was provided by the Unité de Biotechnologie des Champignons Filamenteux-INRA (Marseille, France) (38, 47).

Arabinoxylans. Protein-free WEP and WUP were purified from rye flour using an adaptation of different methods (28, 48–50). Two hundred grams of rye flour was boiled for 30 min with 80% (v/v) ethanol under reflux, to reduce protein solubility through denaturation, to inactivate endogenous enzymes, and to remove ethanol-soluble substances such as low molecular weight sugars and peptides. Flour was then filtrated in a glass filter (17–40 μ m) and successively washed with 80, 95, and 100% (v/v) ethanol and then dried. Five hundred milliliters of ultrapure water (pH 5.0 adjusted with HCl) was added to the dry flour, and the suspension was homogenized by stirring. Termamyl (1.6 mL) was added, and the enzyme reaction was allowed to proceed for 30 min at 95 °C, under continuous mechanical stirring. After the mixture had cooled to 40 °C, 5 mL of amyloglucosidase solution (40 mg/mL in sodium acetate buffer, 0.1 M, pH 5.0) was added and incubated 2 h at 40 °C, under continuous mechanical stirring. After the pH had been adjusted to 7.5 by adding 50 mL of sodium phosphate buffer (0.1 M, pH 7.5), 5 mL of Pronase solution (12 mg/mL in sodium phosphate buffer) was added, and the solution was incubated for 2 h at 40 °C under stirring. Heating the solution at 95 °C for 30 min inactivated the Pronase. The solution was then cooled to room temperature and centrifuged for 20 min at 15300g and 20 °C. WEP were extracted from the supernatant and WUP from the pellet. The supernatant volume was measured, and ethanol was added to obtain a

final concentration of 80% (v/v). The solution was left at 25 °C during 1 h to precipitate AX and then filtrated in a glass filter (17–40 μ m) under vacuum. WEP were then successively washed with 65, 95, and 100% (v/v) ethanol and then acetone and finally dried with diethyl ether. The pellet containing the WUP was suspended in ultrapure water and then centrifuged for 20 min at 34500g and 20 °C. The pellet was suspended in absolute ethanol under mechanical agitation overnight at 20 °C. WUP were then successively washed with 95% (v/v) ethanol, absolute ethanol, and acetone and finally dried with diethyl ether.

Two batches of flour were used to obtain WEP1 and WUP1 (from a flour not treated with Pronase) and WEP2 and WUP2 (from a flour treated with Pronase).

Gas-Liquid Chromatography (GLC). The carbohydrate content was determined by GLC following the procedure of Blakeney et al. (51). Alditol acetates obtained after sulfuric acid hydrolysis (2 M H₂SO₄, 100 °C, 2 h) were injected on a DB-225 capillary column (J&W Scientific, Folsom, CA), using inositol as the internal standard.

Enzymatic Depolymerization and Solubilization of AX. Four milliliters of a WEP solution (containing 1.25 mg of AX/mL in 0.1 M sodium acetate buffer, pH 5.0) or amounts of WUP or flour (containing 20 or 40 mg of AX) were suspended in 4 mL of sodium acetate buffer and 1 mL of enzyme solution (GS100, xylanases, AXH, β -glucanase, or FAE) or 1 mL of buffer (for the blank) was added. Samples were agitated by rotation (40 rpm) in a room at 20 °C, for 25 min, 55 min, 1 h and 55 min, 2 h and 55 min, 3 h and 55 min, and 23 h and 55 min and then centrifuged for 1 min at 1400g and 25 °C. Supernatants were filtered (glass microfiber, ref 95320B-0025, K'lab, Fisher Scientific) in a glass tube and then boiled for 10 min after a total reaction time of 0.5, 1, 2, 3, 4, and 24 h. After cooling, they were filtered (2.7 μ m) and frozen. Resulting reaction mixes were analyzed by colorimetric determination of AX, capillary viscometry, and size exclusion high-performance liquid chromatography (SE-HPLC). For the study of the effect of GS100 or xylanases on a WEP–WUP mixture, 4 mL of the WEP solution (1.25 mg of AX/mL) was added of the amount of WUP containing 20 mg of AX and 1 mL of the enzyme solution. Blanks, in which the enzyme solution was replaced by buffer, were vortexed (30 s), centrifuged (1 min, 1400g, 25 °C), paper filtered, boiled (10 min), cooled, filtered (2.7 μ m), and frozen. Different doses of GS100 (0, 0.5, 1, 1.5, 2, and 4 units) were tested on two different amounts of WUP (containing 40 and 20 mg of AX). Then, the effect of 0.5 unit of GS100 was tested on WEP, WUP, WEP–WUP, and flour, using different reaction times. The effect of 0.5 unit of xylanases 1, 2, and 3 was studied on WEP1, WUP1, WEP1–WUP1, and flour. AXH (0.2 and 1 μ g), β -glucanase (1000 and 5000 BGU), and FAE (0.026 and 0.26 units) were also tested on flour containing 20 mg of AX. These enzymes were also tested in combination with 2.5 units of Xyl-3.

For assays with flour treated with hot ethanol, a sample of 2.05 g of flour_{etOH} was treated with 6 mL of xylanase (containing 0.5 or 2.5 units) and 2 mL of 0.1 M sodium buffer, pH 5.0, for 15 min in a rotary shaker (40 rpm, 20 °C). The sample was centrifuged (10 min, 22000g, 4 °C), and the supernatant was boiled for 10 min and then centrifuged (10 min, 600g, 20 °C), cooled, and frozen. WEAX were determined colorimetrically.

The percentage of AX solubilization and specific (η_{sp}) and apparent intrinsic ($[\eta]_{app}$) viscosities of reaction mixes were calculated as follows: % solubilized AX = [(AX in solution – initially soluble AX)/(total AX – initially soluble AX)] \times 100; % of initial η_{sp} = (final η_{sp} in the reaction mix/initial η_{sp} in the blank) \times 100.

WEAX Determination. AX present in supernatants and released by enzymes in the reaction mixes were determined according to the semiautomated colorimetric method of Rouau and Surget (52), using an Alliance Instruments Evolution II autoanalyzer. One hundred microliters of the filtered (2.7 μ m) reaction mixes was diluted before analysis.

Analysis of Alkali-Labile Phenolics. Supernatant reaction mixes (0.5 mL), to which was added 0.5 mL of 4 N NaOH, were incubated under argon during 1 h at 20 °C in the dark. Five micrograms of 3,4,5-trimethoxy-*trans*-cinnamic acid (TMCA; Sigma Chemical Co., St. Louis, MO) was added as internal standard, and the pH was adjusted to 2.0 \pm 0.02 with HCl. Phenolic acids were extracted twice with 2 mL of ether. The ether phase was transferred to an amber test tube and

evaporated at 30 °C under argon. Methanol (350 μ L) was added, and samples were filtered (0.45 μ m) and then injected (20 μ L) in reversed phase (RP) HPLC, using an Alltima (Alltech, Deerfield, IL) C18, 5 μ m, column (250 \times 4.6 mm). Detection was by UV absorbance at 320 nm. Gradient elution was performed using acetonitrile/sodium acetate buffer, 0.05 M, pH 4.0, at 1 mL/min at 35 °C, in linear gradients of 15/85 during the first 3 min, then from 15/85 to 35/65 in 27 min, from 35/65 to 60/40 in 0.5 min, from 60/40 to 15/85 in 4.5 min, and finally maintained at 15/85 for 5 min. A Waters 996 photodiode array detector (Millipore Co., Milford, MA) was used to record the FA and its dimers spectra. The solvents were of HPLC grade, and the mobile phase was sparged with helium. Dimer identification and quantification were carried out as described in Figueroa-Espinoza and Rouau (38).

Size Exclusion Chromatography (SE-HPLC). SE-HPLC was performed at 35 °C using a Waters (Millipore Co.) Ultrahydrogel 1000, 10 μ m, column (7.8 \times 300 mm), with a pullulan limit exclusion 10⁶ Da, eluted with 0.1 M sodium acetate buffer, pH 5.0, at 0.6 mL/min. Twenty microliters of the filtered (2.7 μ m) reaction mix was injected. The eluent was monitored with a Waters 410 differential refractometer.

Capillary Viscometry. Flow times of 2 mL of the reaction mixes were measured at 25 °C using an AVS 400 (Schott Geräte, Hofheim/Ts, Germany) capillary viscometer, equipped with an Oswald capillary tube 518 23 Ic (water flow time = 29.1 s). Relative viscosity (η_{rel} = flow time of sample/flow time of solvent) and specific viscosity (η_{sp} = η_{rel} – 1), were calculated using 0.1 M sodium acetate buffer, pH 5.0, as the solvent. An apparent intrinsic viscosity ($[\eta]_{app}$, mL/g) was calculated using the Morris equation (53): $[\eta]_{app} = 1/c \times [2(\eta_{sp} - 1, n(\eta_{rel}))]^{0.5} \times 1000$, where c (expressed as mg/mL) represented the AX concentration, assuming that only AX contributed to the viscous properties of the reaction supernatants (7).

endo-Xylanase Activity. *endo*-Xylanase activities of GS100 (2.5 units/mg) and of xylanases Xyl-1, Xyl-2, and Xyl-3 were determined according to an adaptation of the method of McCleary (54) using as the enzyme standard a noncommercial enzyme preparation (TXU Standard 98/99), which contains 0.56 mg of Xyl-1/g.

endo- β -Glucanase Activity. Enzyme (0.2 g) was weighed into a 100 mL volumetric flask and dissolved in the assay buffer (100 mM sodium acetate buffer, pH 5.0). The solution was stirred for 15 min, the volume was adjusted with the assay buffer, and the sample was filtered through Whatman GF/A. If necessary, the sample was diluted with the assay buffer. The enzyme solution (50, 75, or 100 μ L) was added to 1.0 mL aliquots of the assay buffer, and the mixtures were equilibrated at 40 °C for 5 min. One β -glucanase tablet (Megazyme, Megazyme Int.) was added to each test tube, which must not be stirred. After exactly 10 min, 10 mL of the stop solution [1% tris(hydroxymethyl)aminomethane (w/v)] was added. The contents of the test tubes were stirred and the solutions filtered through a Whatman no. 1 filter paper. The absorbance at 590 nm of the standard (β -glucanase from *A. niger* with an activity of 100 BGU/g) and of the test samples was measured against a blank sample (without enzyme). The concentrations of the standard and the sample enzymes were adjusted to have an optical density (OD) within 0.2–1.1 at 590 nm.

Determination of FAE Activity Using Methyl Ferulate as Substrate. FAE activity was determined by measuring the formation of FA released from methyl ferulate [MeFA] (Danisco Cultor). MeFA was dissolved in very small amounts of ethanol before 100 mM sodium acetate buffer, pH 5.0, was added to a final concentration of 2 mg/mL. The solution was kept at 45 °C in a water bath. The reaction was initiated by the addition of 50 μ L of FAE to 300 μ L of MeFA. The mixture was incubated at 45 °C for 10 min. The reaction was stopped by adding 50 μ L of enzyme/substrate complex to 2.5 mL of stop reagent (10 mM glycine buffer, pH 10, adjusted with NaOH). The blank contained ultrapure water instead of enzyme solution. The formation of FA was measured at 325 nm using a spectrophotometer. A FA standard curve was then constructed by using various concentrations of the FA (1000–1500 μ M). One unit of enzyme activity is defined as the amount of enzyme that produces 1 μ mol of FA per minute at 45 °C.

Arabinose Determination. Arabinose was detected by HPLC on a Polyspher CHCA column (Merck, Darmstadt, Germany) on 5 μ L of filtered (0.45 μ m) samples. Water was used as the mobile phase at a

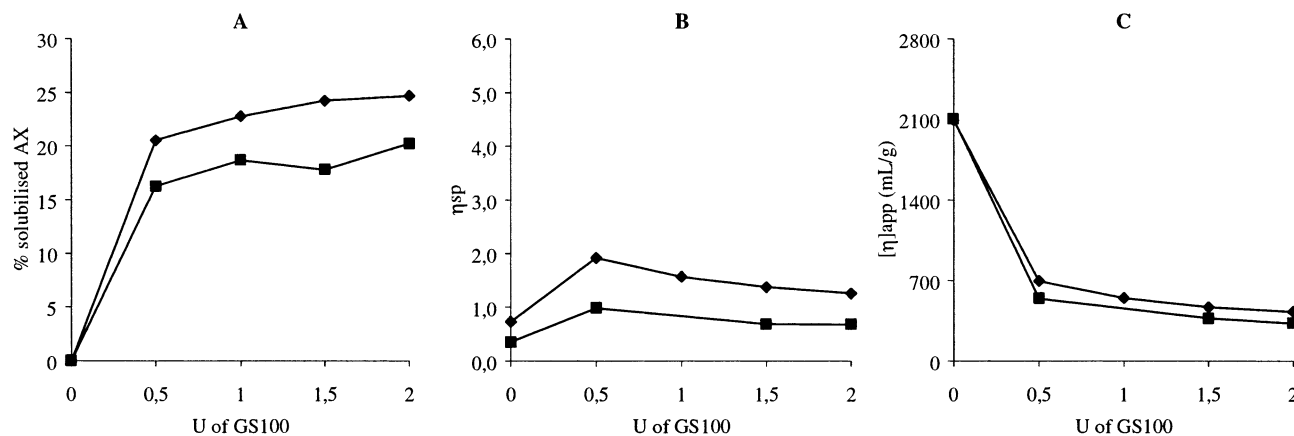


Figure 1. Effect of different doses of GS100 on WUP1 (◆) and WUP2 (■) containing 40 mg of AX, after 5 h of reaction at 20 °C. Results are expressed as (A) percentage of AX solubilization from WUP, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

flow rate of 0.5 mL/min and 85 °C. Arabinose content was quantified by refractometry using a standard curve.

General. Dry matter was determined by measuring the loss of weight of samples (2–5 g) after 1.5 h at 130 °C. The coefficients of variation for the viscometry, the arabinoxylan, and the ferulic acid determination procedures were 3, 3, and 4%, respectively. Results are expressed as mean values of replicates.

RESULTS AND DISCUSSION

Characterization of the Substrates. Compositions of rye flour, WEP, and WUP are presented in Table 1.

Effect of GS100 on WUP and Rye Flour. Preliminary Experiments. In a first experiment, GS100 was added to suspensions of WUP containing 40 mg of AX. The total reaction time was fixed at 5 h at 20 °C, including 4 h of incubation plus 1 h of centrifugation, according to the method used by Petit-Benvegnen et al. (19). Results are shown in Figure 1. AX solubilization from WUP1 and WUP2 increased with the enzyme addition level. With 0.5 unit of GS100, the solubilization extent increased rapidly and reached 20.5% for WUP1 and 16.2% for WUP2 and then slowed to reach an ascending plateau up to a dose of 2 units, with maximum solubilizations of 24.7 and 20.3% for WUP1 and WUP2, respectively. Solubilization of AX in rye WUP is more difficult than in wheat WUP. Petit-Benvegnen et al. (19) observed a solubilization of ~50% of AX from wheat WUP after the same reaction time for 2 units of GS100.

The η_{sp} of the reaction mixture increased due to the release of AX and passed through a maximum with 0.5 unit of GS100; then it decreased with the enzyme dose (Figure 1B). As observed by Petit-Benvegnen et al. (19), the blank (without enzyme) presented a η_{sp} superior to zero, which is explained by the release of some AX by mechanical action during agitation (0.29 mg of AX/mL for WUP1 and 0.15 mg of AX/mL for WUP2). As explained before (cf. Materials and Methods), the percentage of AX solubilization includes only the AX enzymatically solubilized. The $[\eta]_{app}$ of solubilized AX decreased with the addition of GS100, until it was reduced by ca. 74% (547 mL/g) and 66% (453 mL/g) for a dose of 1 unit of GS100 and by ca. 80% (429 mL/g) and 85% (327 mL/g) for a dose of 2 units of GS100, for WUP1 and WUP2, respectively (Figure 1C). Working on wheat WUP, Petit-Benvegnen et al. (19) observed that $[\eta]_{app}$ reduced by ~64% (300 mL/g) for a dose of 1 unit of GS100 and by ~75% (200 mL/g) for a dose of 2 units of GS100. In conclusion, AX solubilized from rye material are of higher molecular weight than wheat AX (55) but extraction is more difficult.

From Figure 1C, it can be observed that AX released from WUP1 and WUP2 presented similar molecular sizes as indicated by $[\eta]_{app}$. Surprisingly, in WUP1 (without proteolysis), AX were more easily enzymatically extractable than in WUP2. Nilsson et al. (56) investigated how protease treatment could influence the extractability and separability of AX from water unextractable material from rye bran. They observed that more disubstituted xylose residues were eluted in the fractions pretreated with protease. It is likely GS100 solubilizes easily AX with a low content in disubstituted xyloses.

In a second experiment, different doses of GS100 (0.5, 2, and 4 units) were tested with 24 h incubation time on WUP and flour containing 20 mg of AX (Figure 2). Four units of GS100 provoked solubilization rates of 35, 29, and 27% for WUP1, WUP2, and flour, respectively (Figure 2A). Maximum values of η_{sp} were reached when 0.5 unit of GS100 was added (Figure 2B). Solubilization was accompanied by a degradation of WEAX and thus a decrease in $[\eta]_{app}$ (Figure 2C). This effect was more important on WUP than on flour. In general, 0.5 unit of GS100 was the dose exhibiting the best ratio between solubilization of AX and high η_{sp} . In the case of flour, no further solubilization was observed at doses of GS100 > 0.5 unit, whereas moderate increases were obtained on WUP preparations.

From these results, a dose of 0.5 unit of GS100 or xylanases was chosen for further experiments on WEP and on WUP, WEP–WUP, and flour, containing 20 mg of AX, with incubations of 0, 0.5, 1, 2, 4, and 24 h at 20 °C. In the case of xylanases, only WEP1 and WUP1 were tested.

Effect of GS100 and Xylanases on WEP. For a similar AX concentration (1 mg/mL of reaction mix), results of $[\eta]_{app}$ showed that WEP2 contained chains of higher molecular weight than WEP1. Initial $[\eta]_{app}$ values for WEP1 (1900 mL/g) were inferior to those from WEP2 (2600 mL/g). GS100 provoked a similar extensive degradation on both WEP1 (Figure 3) and WEP2 (not shown). After 30 min of reaction, $[\eta]_{app}$ decreased by 34% (WEP1) and 44% (WEP2), and after 24 h of reaction, $[\eta]_{app}$ decreased by 80 and 83%, respectively.

The three xylanases provoked important degradation on WEP1, Xyl-2 being the most and Xyl-1 the least active (Figure 3). In all cases, there was an important decrease of $[\eta]_{app}$ during the first 30 min of reaction, with viscosity losses of 55, 75, and 58%, for xylanases 1, 2, and 3, respectively. In all cases, degradation was more important than with GS100. Xyl-1 is the *endo*-xylanase present in GS100. It is possible that the side activities in GS100 limit the degradative action of *endo*-xylanase.

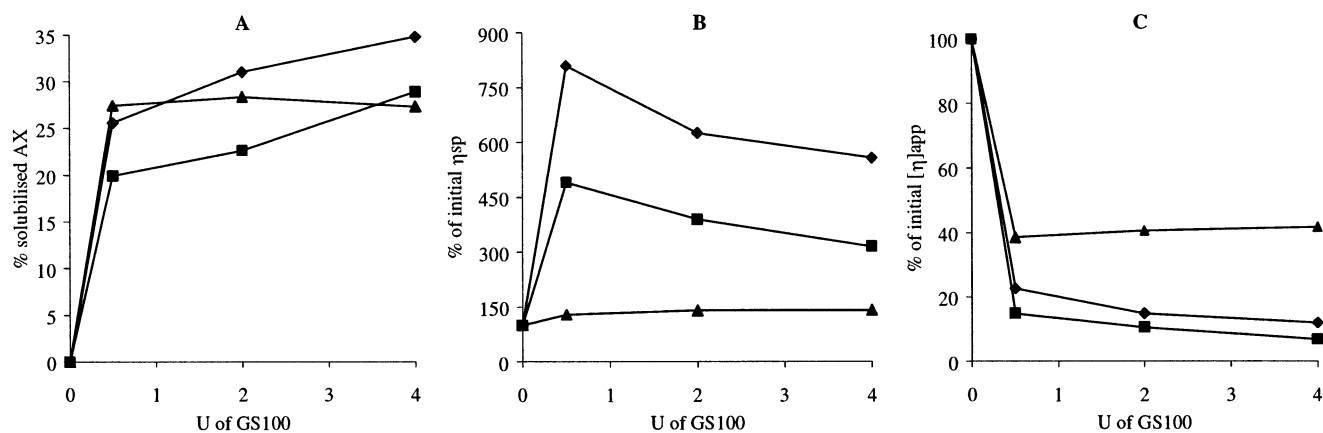


Figure 2. Effect of different doses of GS100 after 24 h of incubation at 20 °C on the solubilization of AX from WUP1 (◆), WUP2 (■), and flour (▲), containing 20 mg of arabinoxylans. Results are expressed as (A) percentage of AX solubilization from WUP, (B) percentage of initial specific viscosity (η_{sp}), and (C) percentage of initial apparent intrinsic viscosity ($[\eta]_{app}$).

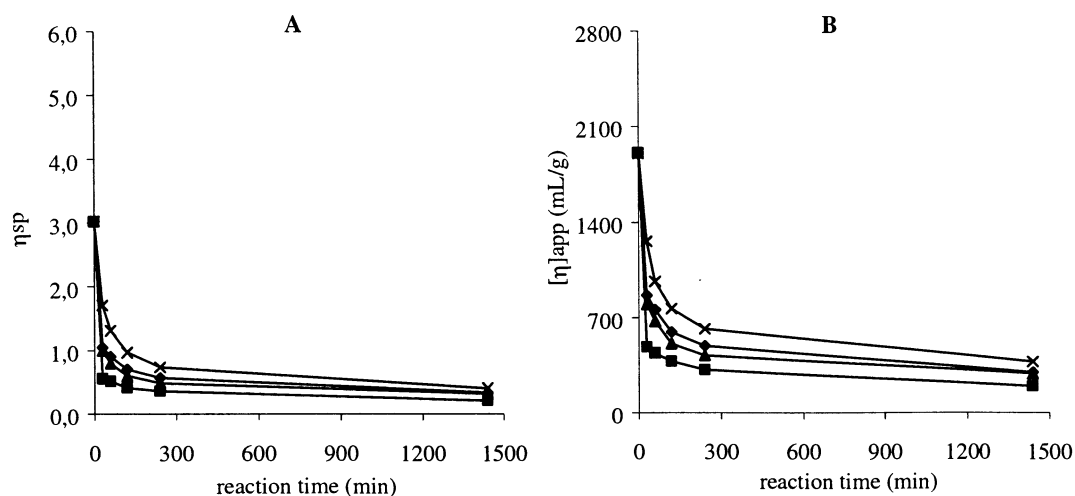


Figure 3. Effect of 0.5 unit of GS100 (×), Xyl-1 (◆), Xyl-2 (■), and Xyl-3 (▲) on WEP1 solutions (1 mg of AX/mL) at different reaction times at 20 °C. Results are expressed as (A) specific viscosity (η_{sp}) and (B) apparent intrinsic viscosity ($[\eta]_{app}$).

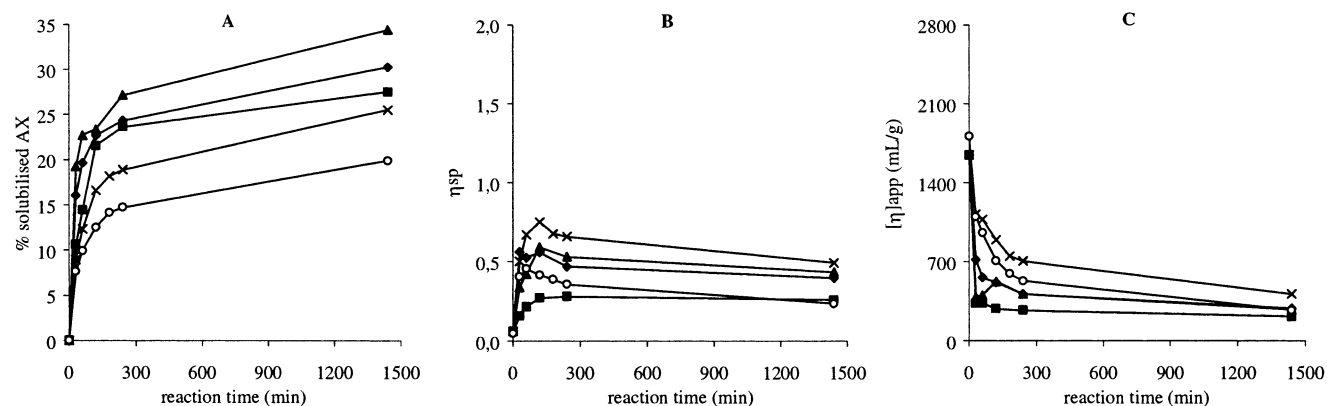


Figure 4. Effect of 0.5 unit of GS100 (×), Xyl-1 (◆), Xyl-2 (■), and Xyl-3 (▲) on WUP1 containing 20 mg of AX at different reaction times at 20 °C. (○) represents the effect of GS100 on WUP2. Results are expressed as (A) percentage of AX solubilization from WUP, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

Effect of GS100 and Xylanases on WUP. When WUP preparations containing 20 mg of AX were exposed to a dose of 0.5 unit of GS100, the AX solubilization reached maxima of 25.5% for WUP1 and 19.9% for WUP2 (Figure 4A). As observed before, AX from WUP1 were more susceptible to enzymatic solubilization. Maximum values of η_{sp} were observed at 120 min for WUP1 and at 60 min for WUP2 (Figure 4B). $[\eta]_{app}$ decreased with reaction time, and after 24

h of treatment, it was reduced by 75% for WUP1 and by 85% for WUP2. Therefore, solubilized AX were degraded by GS100.

In the WUP1 blank (without enzyme) analyzed after 24 h of stirring, 4.6% of AX were solubilized and exhibited values of 2011 mL/g for $[\eta]_{app}$ and 0.51 for η_{sp} , whereas the corresponding values observed in the WUP1 sample treated for 30 min with GS100 were 8.9%, 1120 mL/g, and 0.50, respectively. Thus,

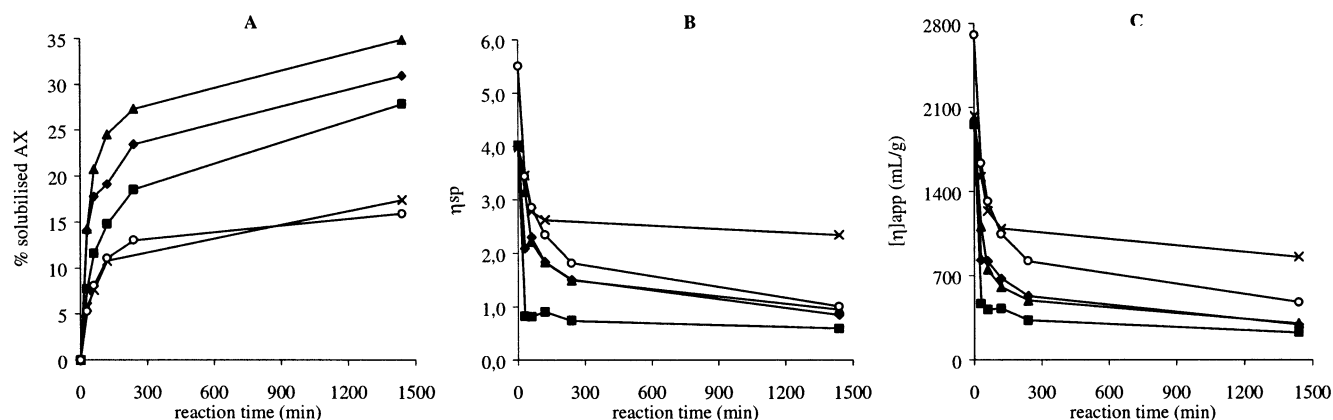


Figure 5. Effect of 0.5 unit of GS100 (×), Xyl-1 (◆), Xyl-2 (■), and Xyl-3 (▲) on WEP-WUP1 solutions at different reaction times at 20 °C. (○) represents the effect of GS100 on WEP-WUP2. Results are expressed as (A) percentage of AX solubilization, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

the same η_{sp} was obtained in both samples but with very different AX concentrations and apparent molecular weights. The xylanolytic enzyme is necessary to extract important amounts of AX from WUP: 5.5-fold more AX were solubilized from WUP1 after a 24 h GS100 treatment than from the corresponding blank.

The SE-HPLC profiles (not shown) confirmed the results from viscosity measurements, with information on the AX molecular weight distribution and solubility of the filtered samples. The addition of GS100 provoked an increase in the amount of soluble material, with new shoulders on the elution pattern corresponding to large- and medium-sized AX. After 120 min of reaction, an important increase in the low molecular weight AX was observed, with a concomitant decrease of the large- and medium-sized AX.

The profiles of solubilization of the three xylanases were similar until 4 h (Figure 4A), and then Xyl-3 presented the highest percent of AX solubilization (34.4%) and Xyl-2 the lowest (27.5%) after 24 h of reaction. In all cases, xylanases solubilized more AX than GS100 (25.5%).

When the viscosity results were analyzed, xylanases 1 and 3 liberated chains of higher molecular weight than Xyl-2. The maximum values of viscosity reached with xylanases 1 and 3 were similar (0.6) (Figure 4B). Xyl-2 liberated AX of low molecular weight (Figure 4C). These results were confirmed by SE-HPLC (not shown). It is likely that xylanases 1 and 3 first solubilized AX of higher molecular weight than Xyl-2, and then degradation took place in solution. Depolymerization of solubilized AX was more important with Xyl-2. AX released by GS100 were of higher molecular weight than those released by pure xylanases. This confirms that GS100 degraded less soluble AX than pure xylanases.

Effect of GS100 and Xylanases on WEP-WUP. The maximum values of solubilization after 24 h of reaction in the presence of 0.5 unit of GS100 reached 17.4 and 15.9% for WEP-WUP1 and WEP-WUP2, respectively (Figure 5A). This rate of solubilization was lower than in the case of WUP preparations alone (Figure 4A). Comparison of the effects observed separately on WEP (Figure 3) and WUP (Figure 4) with the effect on the mixture WEP-WUP (Figure 5) shows that solubilization and WEP depolymerization were more pronounced in the separate experiments than in WEP-WUP. For a given level of GS100, the presence of WEP in the mixture WEP-WUP limited the extraction of AX from WUP, and the presence of WUP in the mixture WEP-WUP limited the degradation of the soluble AX.

Solubilization profiles of AX were similar in the presence of the three xylanases, xylanases 3 and 1 being the ones that provoked the highest values (35 and 31%, respectively) (Figure 5A). Xyl-2 was the enzyme that degraded the most soluble AX, and xylanases 1 and 3 presented very similar viscosity profiles (Figure 5B,C). Solubilization and degradation were less important with GS100.

Effect of GS100 and Xylanases on Rye Flour. Treating rye flour with 0.5 unit of GS100 provoked an AX solubilization profile similar to those obtained with Xyl-1, Xyl-2, and Xyl-3. Approximately 27% of AX were solubilized after 24 h of reaction (Figure 6A). Maximum η_{sp} and $[\eta]_{app}$ were observed after 60 min of reaction (Figure 6B,C); thereafter, AX were degraded in the solution. Viscosity profiles from flour experiments were similar to those observed with WUP (Figure 4) but with higher values, but not to those observed with the WEP-WUP model (Figure 5), as expected. In the WEP-WUP mixture, HMWAX from WEP were already present and were readily degraded by the enzyme. Solubilization of AX from WUP and degradation of HMWAX and solubilized AX were simultaneous. AX from WEP presented a higher molecular weight than those extracted from flour. It is likely that some non-covalent interactions were established between AX during the purification of WEP. The contribution of these HMWAX to viscosity in WEP-WUP (Figure 5B) was therefore more important than that of the AX enzymatically extracted from rye flour (Figure 6B).

Xyl-3 and Xyl-1 presented the highest values of η_{sp} followed by GS100 and Xyl-2. In flour, GS100 provoked an effect similar to that of Xyl-1. When applied to pure substrates, GS100 solubilized more AX than from flour but degraded less soluble AX.

Oxidative Gelation of Flour Extracts Treated with Xylanases. Flour was thermally treated with 80% ethanol (v/v) (flour_{etOH}) to inactivate the endogenous xylanases to better study oxidative gelation of flour WEAX after treatment with Xyl-1, Xyl-2, and Xyl-3. Washing of flour with 80% ethanol (v/v) and absolute ethanol eliminates also the free phenolic compounds susceptible to inhibit AX gelation (38, 57). The AX content of the flour extracts is reported in Table 2. In the sample of flour_{etOH} treated with Xyl-3 + laccase, 100 μ L of the 2 mL of buffer was substituted with laccase to verify if the addition of an oxidant during AX solubilization could limit the AX depolymerization.

Two milliliters of the extracts listed in Table 2 was added to 100 μ L of laccase, and 2 mL of this reaction mix was analyzed

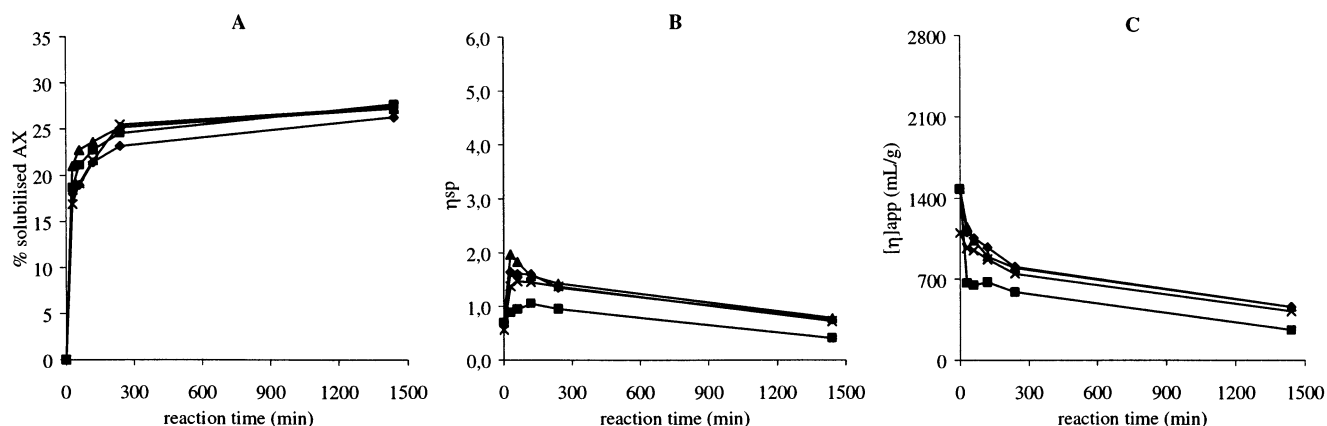


Figure 6. Effect of 0.5 unit of GS100 (×), Xyl-1 (◆), Xyl-2 (■), and Xyl-3 (▲) at different reaction times on flour containing 20 mg of AX at 20 °C. Results are expressed in terms of (A) percentage of AX solubilization, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

Table 2. Arabinoxylan Solubilized from Flour_{etOH} after 15 min of Treatment with Different Xylanases

sample	% WEAX ^a	flow time (s)
flour _{etOH} ^b blank ^c	0.21	377.640
flour _{etOH} + Xyl-1 (0.5 unit)	0.34	56.266
flour _{etOH} + Xyl-1 (2.5 units)	0.42	24.410
flour _{etOH} + Xyl-2 (0.5 unit)	0.29	15.693
flour _{etOH} + Xyl-2 (2.5 units)	0.35	12.036
flour _{etOH} + Xyl-3 (0.5 unit)	0.37	28.266
flour _{etOH} + Xyl-3 (2.5 units)	0.43	14.636
flour _{etOH} + Xyl-3 (2.5 units) + laccase	0.44	15.006

^a Water-extractable arabinoxylans. ^b Flour thermally treated with 80% ethanol (v/v). ^c Without addition of xylanase.

Table 3. Content in Ferulic Acid and Ferulic Acid Dehydrodimers of Flour_{etOH} Extracts Treated with Laccase at 25 °C for 1 h

sample	nmol of FA ^a /mL	nmol of dimers/mL	nmol of FA/initial mg of WEAX ^b	nmol of dimers/initial mg of WEAX
flour _{etOH} ^d blank ^e	36.4	0.8	17.8	0.4
flour _{etOH} diluted 2-fold	1.7	1.6	1.7	1.6
flour _{etOH} + Xyl-1 (0.5 unit)	6.2	7.2	1.8	2.1
flour _{etOH} + Xyl-1 (2.5 units)	5.1	10.4	1.2	2.5
flour _{etOH} + Xyl-2 (0.5 unit)	5.3	5.3	1.9	1.9
flour _{etOH} + Xyl-2 (2.5 units)	5.0	6.7	1.4	1.9
flour _{etOH} + Xyl-3 (0.5 unit)	4.5	9.0	1.2	2.5
flour _{etOH} + Xyl-3 (2.5 unit)	5.8	13	1.3	3.0
flour _{etOH} + Xyl-3 (2.5 units) + laccase	5.2	11.8	1.2	2.7

^a Ferulic acid = *cis*- + *trans*- ferulic acid. ^b Water-extractable arabinoxylans. ^c Sum of ferulic acid dehydrodimers: 5-5', 8-5', 8-5'-benzofuran, 8-8', and 8-O-4' (60). ^d Flour thermally treated with 80% ethanol (v/v). ^e Without addition of laccase.

by capillary viscometry at 25 °C (results not shown). The control flour_{etOH} extract containing 0.21% of AX was diluted twice before the addition of laccase, to avoid the rapid formation of a gel. For the flour_{etOH} treated with xylanases, no dilution was made. After 1 h of reaction with laccase in the viscometer, FA and FA dehydrodimers were determined on the flour_{etOH} extracts (Table 3).

Among the xylanase-treated samples, only the flour_{etOH} treated with 0.5 unit of Xyl-1 thickened. It is likely that at this dose, degradation was not very extensive and the extracted AX (0.34% w/v) had a sufficient molecular weight to gel. This was confirmed by measuring the flow times of the initial extracts

(Table 2). For the three xylanases dosed at 2.5 units, none of the extracts thickened. Thus, the size or the structure of the WEAX was not suitable to form a network, even if the initial content in AX was ~0.4% (w/v). According to Figueroa-Espinoza and Rouau (38), a wheat WEAX solution containing >0.2% (w/v) of AX forms a gel rapidly. The samples treated with Xyl-1 presented the highest values in flow time for similar contents in WEAX (Table 2). Thus, Xyl-1 was the enzyme that less depolymerized the AX. In both cases, AX extraction and flow time, Xyl-2 was the least efficient of the three enzymes.

These results were confirmed by SE-HPLC (not shown). Extracts obtained with 0.5 unit of Xyl-1 contained AX of large and medium molecular size. AX extracted with Xyl-2 and Xyl-3 at both doses were more degraded.

From results in Table 3, the balance between the content in FA or dimers and the initial content in WEAX of all samples shows that the consumption of FA is proportional in all samples. The addition of laccase to the flour_{etOH} extracts provoked FA to decrease by a mean value of 91% and FA dimers were formed, which would provoke a thickening in all samples. In a previous work (38), it was demonstrated that a 0.2% (w/v) wheat WEAX solution forms a gel in the presence of equivalent doses of laccase, with a decrease of 79% of FA. Rye WEAX from flour_{etOH} presented 17.8 nmol of FA/mg of WEAX. A purified wheat WEAX contained a mean value of 11.4 nmol of FA/mg of WEAX according to Figueroa-Espinoza and Rouau (38) and Figueroa-Espinoza et al. (47). In the case of rye, it is likely that the lack of thickening is attributed to the previous treatment with xylanases that provokes an extensive depolymerization of WEAX.

The presence of laccase during the AX solubilization with Xyl-3 did not limit depolymerization (Table 2) and did not improve gelation (not shown). Similar concentrations of AX were solubilized with Xyl-3 with and without laccase (Table 2), and SE-HPLC profiles were similar (not shown). FA consumption and dimer production were also similar (Table 3).

It is likely that due to the heterogeneity of rye AX, xylanases solubilized large fragments of AX poor in FA and short fragments rich in FA were liberated. FA esterified to short fragments could form polymers and precipitate and thus be nondetectable in RP-HPLC. The large fragments of AX would increase the viscosity of the extract but would not form a gel upon addition of laccase. This could explain both the low level of dimers and the absence of thickening. It would be interesting to fractionate by molecular weight the xylanase flour_{etOH} WEAX and to dose FA in each fraction to validate this hypothesis.

Table 4. Effect of Xylanase Xyl-3 at Different Doses on the Solubilization of AX from Rye Flour

dose of Xyl-3 (units)	reaction time (min)	% solubilized AX ^a	% η_{sp} ^b	% $[\eta]_{app}$ ^c (mL/g)
0	0	0.0	100	100
	60	16.6	299	88
	240	19.0	268	74
	1440	22.2	181	48
0.5	0	0.0	100	100
	60	23.6	224	61
	240	25.2	204	54
	1440	27.2	111	31
2.5	0	0.0	100	100
	60	31.8	350	63
	240	36.0	277	47
	1440	39.7	183	31
5	0	0.0	100	100
	60	35.1	296	51
	240	38.0	259	43
	1440	41.4	178	30
10	0	0.0	100	100
	60	35.8	252	44
	240	36.9	230	40
	1440	45.1	165	26
20	0	0.0	100	100
	60	39.1	191	33
	240	39.8	199	34
	1440	48.5	98	16

^a Arabinoxylans. ^b Specific viscosity. ^c Apparent intrinsic viscosity.

These results suggest that to solubilize gellable AX, the best enzyme would be Xyl-1 at low concentrations and short reaction time. It has to be considered that the FA content and the AX molecular size are important parameters in view of cross-linking AX in rye products, so the obtained materials could have highly viscous properties but no gelling potential.

When flour was pretreated (4 h at 130 °C in an oven or 30 min with boiling 80% ethanol v/v), Xyl-1 solubilized less AX than Xyl-3, but WEAX presented a higher molecular weight as probed by a higher viscosity and SE-HPLC profiles (results not shown). With non-pretreated flour, SE-HPLC profiles were similar for both xylanases. AX extraction was slightly superior with Xyl-3 compared to that with Xyl-1, and viscosity was higher after 1 h of reaction with Xyl-3. This suggests that the endogenous enzymes of the flour had more affinity for AX solubilized by Xyl-1 than by Xyl-3. This would mean that Xyl-1 solubilized less AX than Xyl-3 but of higher molecular weight. This would explain the thickening of only flour_{etOH} treated with 0.5 unit of Xyl-1.

Effect of Xyl-3 Combined with AXH, β -Glucanase, or FAE on Rye Flour. *Preliminary Experiments.* Different doses of Xyl-3 (0.5, 2.5, 5, 10, and 20 units) were tested on rye flour to study the evolution of AX solubilization and to find the best concentration to use in combination with other enzymes (Table 4). The solubilization of AX increased with the dose of Xyl-3. Maximum η_{sp} values were obtained after 1 h of reaction with 2.5 units of Xyl-3, followed by the doses 0.5, blank, 5, 10, and 20 units. Above 2.5 units, the important solubilization of AX was accompanied by a depolymerization. Thus, 2.5 units of Xyl-3 was chosen to solubilize amounts of AX, without excessive depolymerization, compared to the higher dosages. One hour of reaction was chosen to test the effect of Xyl-3 combined with other enzymes.

Surprisingly, a tendency to reach the same rates of solubili-

zation after long reaction times (independently of the dose) was not observed (Table 4), probably due to an inhibitory effect (40–45).

Combination of Xyl-3 with AXH. The effect of AXH in combination with Xyl-3 is presented in Figure 7. AX extraction profiles of samples of flour with only AXH (control) differ from those of the blank (without enzyme). After 1 and 4 h of reaction, more AX were solubilized from the controls, but they presented inferior $[\eta]_{app}$ values. The control η_{sp} values after 1 and 4 h of reaction were not significantly inferior to those from the blank (Figure 7B), and the SE-HPLC profiles (not shown) were all similar. It is likely that more AX of an inferior molecular weight were extracted when AXH was added. Some non-covalent interactions between the less substituted chains could explain the viscosity values after 24 h of reaction. Arabinose side groups could favor chain entanglement of AX with other polymers. This could explain in part the increases in extractability in controls and in assays (samples with AXH and Xyl-3) for which the effect of AXH was less pronounced. In controls, the reduction in the amount of extractable AX with increasing AXH dosage was probably due to the reduced solubility of the less substituted polymer after arabinose removal. As a consequence of the lower concentration of soluble AX, the viscosity of the solution decreased. It is also possible that AXH rendered AX more accessible to flour endogenous xylanases, which could explain the increases in extraction in controls and also the decreases in viscosity. The addition of 0.2 and 1.0 μ g of AXH to samples containing 2.5 units of Xyl-3 slightly improved the solubilization of AX from rye flour but did not change either the viscosity (Figure 7) or the SE-HPLC profiles (not shown). Arabinose was quantified by HPLC of supernatants of flour treated with AXH with and without Xyl-3 (results not shown). More arabinose was extracted when the two enzymes acted together. Thus, AXH linearized AX and improved their extraction by Xyl-3.

Combination of Xyl-3 and β -Glucanase. The effects of 1000 and 5000 BGU of β -glucanase on flour AX extraction were tested in the absence and presence of 2.5 units of Xyl-3 (Figure 8). Solubilization of AX was similar on the one hand in the blank (flour without enzymes) and in the 1000 BGU β -glucanase control and, on the other hand, in the Xyl-3 control and the sample Xyl-3 + 1000 BGU of β -glucanase. The addition of 5000 BGU of β -glucanase (with and without xylanase) provoked a decrease in the extraction of AX after 4 h of reaction and resulted in the lowest viscosities. Rye β -glucan also contributed to viscosity; thus, increasing doses of β -glucanase contributed to the decrease of viscosity. In conclusion, the addition of β -glucanase did not improve the viscosity of the extracts.

It is possible that at low concentrations or short reaction times, β -glucanase could improve AX extraction. After 1 h of reaction with 5000 BGU of β -glucanase, and both 1 and 4 h of reaction with 1000 BGU of β -glucanase, AX extraction increased, providing more substrate to xylanases (both endogenous and Xyl-3). This hypothesis is supported by results of SE-HPLC (not shown): in the presence of 1000 BGU of β -glucanase, there was more material extracted after 1 h, and then depolymerization occurred more rapidly than in the blank. With 5000 BGU of β -glucanase the same phenomenon was accelerated. When xylanase was present, the phenomenon was also accelerated.

Combination of Xyl-3, AXH, and β -Glucanase. In rye flour treated with the three enzymes together, Xyl-3 (2.5 units), AXH (0.1 or 0.2 μ g), and β -glucanase (500 or 1000 BGU), the extractions of AX were similar until 4 h of reaction (Figure 9). After 24 h of reaction, the amount of extractable AX decreased.

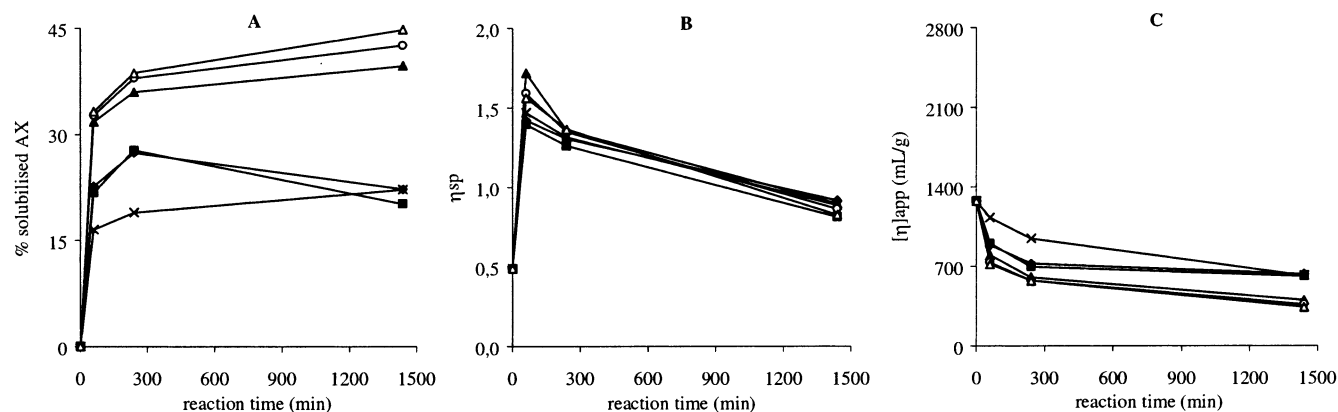


Figure 7. Effect of the combination of 2.5 units of Xyl-3 with different concentrations of (1,4)- β -D-arabinoxylan arabinofuranohydrolase [AXH] on the solubilization of AX from rye flour containing 20 mg of AX at different reaction times at 20 °C: blank (x); 0.2 μ g of AXH (◆); 1 μ g of AXH (■); control Xyl-3 (▲); Xyl-3 + 0.2 μ g of AXH (○); Xyl-3 + 1 μ g of AXH (△). Results are expressed in terms of (A) percentage of AX solubilization, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

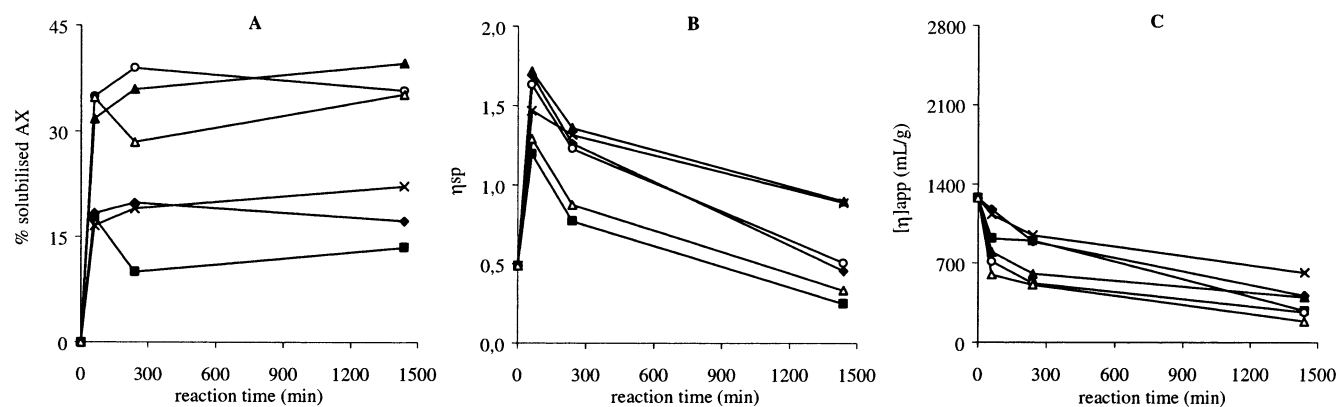


Figure 8. Effect of the combination of 2.5 units of Xyl-3 with different concentrations of β -glucanase on the solubilization of AX from rye flour containing 20 mg of AX at different reaction times at 20 °C: blank (x); 1000 BGU (◆); 5000 BGU (■); control Xyl-3 (▲); Xyl-3 + 1000 BGU of β -glucanase (○); Xyl-3 + 5000 BGU of β -glucanase (△). Results are expressed in terms of (A) percentage of AX solubilization, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

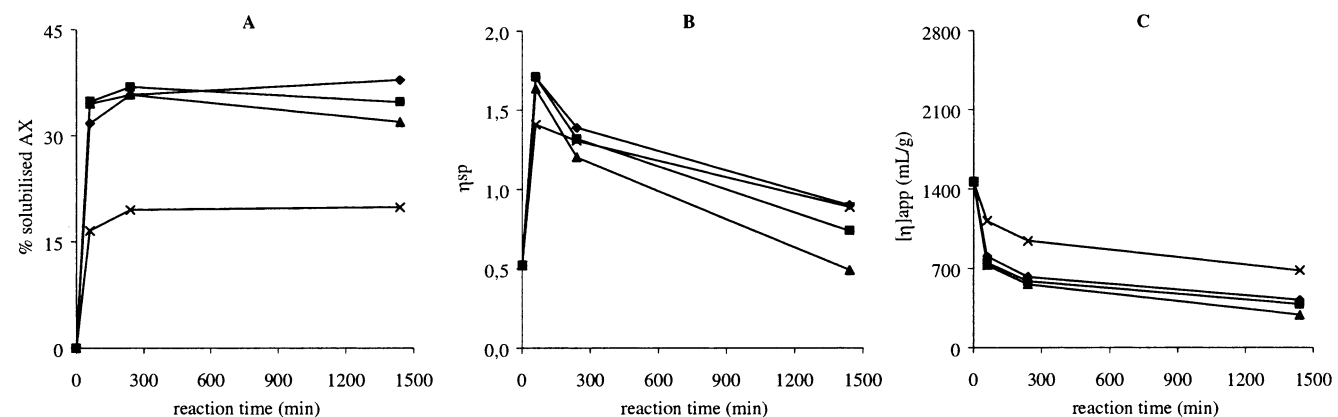


Figure 9. Effect of the combination of 2.5 units of Xyl-3 with different concentrations of (1,4)- β -D-arabinoxylan arabinofuranohydrolase [AXH] and β -glucanase on the solubilization of AX from rye flour containing 20 mg of AX at different reaction times at 20 °C: blank (x); control Xyl-3 (◆); Xyl-3 + 0.1 μ g of AXH + 500 BGU of β -glucanase (■); Xyl-3 + 0.2 μ g of AXH + 1000 BGU of β -glucanase (▲). Results are expressed in terms of (A) percentage of AX solubilization, (B) specific viscosity (η_{sp}), and (C) apparent intrinsic viscosity ($[\eta]_{app}$, mL/g).

The solubility loss was stronger with 0.1 μ g of AXH and 500 BGU of β -glucanase.

Extract viscosity followed the same evolution, yielding lower values after 24 h of reaction and with higher doses of AXH and β -glucanase. Decreases in viscosity are explained as a combination of the degradation of β -glucan and AX. The linearization of AX by AXH could favor the depolymerization

by xylanase. The results showed additional effects of both enzymes and no synergism with Xyl-3.

Combination of Xyl-1 and Xyl-3 with FAE. Solubilizations of AX were similar between blank and controls with 0.026 and 0.26 units of FAE (Table 5). The addition of FAE at both levels did not improve the solubilization of AX in the presence of either Xyl-1 or Xyl-3. The addition of FAE did not improve

Table 5. Effect of FAE^a at Different Doses on the Solubilization of AX from Rye Flour in Combination with 0.5 Unit of Xylanases 1 and 3

sample	reaction time (min)	% solubilized AX ^b	% of initial η_{sp} ^c	% of initial $[\eta]_{app}$ ^d
blank	0	0.0	100	100
	60	26.3	222	20
	240	29.6	211	17
	1440	32.7	138	11
FAE (0.026 unit)	0	0.0	100	100
	60	24.8	227	21
	240	29.9	215	17
	1440	33.9	139	11
FAE (0.26 unit)	0	0.0	100	100
	60	26.0	221	20
	240	32.3	216	16
	1440	34.1	138	11
Xyl-1	0	0.0	100	100
	60	39.7	265	16
	240	40.1	228	14
	1440	40.5	132	9
Xyl-3	0	0.0	100	100
	60	42.1	264	15
	240	40.5	202	12
	1440	46.7	141	8
FAE (0.026 unit) + Xyl-1	0	0.0	100	100
	60	40.0	276	16
	240	39.0	228	14
	1440	35.6	137	10
FAE (0.26 unit) + Xyl-1	0	0.0	100	100
	60	40.6	273	16
	240	38.3	232	15
	1440	39.3	143	10
FAE (0.026 unit) + Xyl-3	0	0.0	100	100
	60	42.4	257	14
	240	40.3	222	14
	1440	38.9	143	10
FAE (0.26 unit) + Xyl-3	0	0.0	100	100
	60	43.4	254	14
	240	39.4	224	14
	1440	41.8	145	9

^a Ferulic acid esterase. ^b Arabinoxylans. ^c Specific viscosity. ^d Apparent intrinsic viscosity.

the viscosity of the extracts either. SE-HPLC profiles of flour treated with FAE with and without xylanases were all similar (not shown). The released FA was measured for all the samples, but values were very low.

Petit-Benvegnen et al. (19) observed a synergism between a xylanase and a FAE, both from *A. niger*, when applied to wheat dough. A 38% AX solubilization was observed when the enzymes were used together, whereas in separate experiments, they observed 12 and 10%, respectively. Thus, FAE increased the extraction of HMWAX from wheat WUP. In the case of rye pentosans, no synergism was observed.

CONCLUSIONS

On testing the effects of GS100 and xylanases on rye WEP, WUP, and WEP–WUP preparations, it was observed that the AX solubilization pattern was similar to that with rye flour. It is apparent that WUP is a better model than WEP–WUP to study the AX solubilization and depolymerization by these enzymes in rye flour. AX from WEP presented a higher molecular weight than flour WEAX, probably due to non-covalent interactions established during WEP purification.

Differences in η_{sp} in the extracts may be attributed to differences in initially available WEAX.

AX extracted from rye by GS100 and xylanases presented a higher average molecular weight than those from wheat (19). In the case of GS100, for equivalent enzyme and WUAX contents, rye AX were more resistant to solubilization than wheat AX: the rate of AX solubilization from wheat WUP was on average 2-fold higher than that from rye WUP. This may be due to the presence of bran in rye flour and to fine structural differences between rye and wheat AX (1, 6), in relation with enzyme specificity, or to differences in the endosperm cell-wall structure with consequences on accessibility. Solubilization and degradation were less important when GS100 was used in model solutions than when pure xylanases were. Solubilization rates from rye flour were similar with the four enzymes, and Xyl-1 and Xyl-3 degraded less the soluble AX than GS100 and Xyl-2. In all cases, Xyl-2 presented an important depolymerizing activity.

AXH and β -glucanase in combination with Xyl-3 did not improve either the extraction of AX or the viscosity of the flour extracts. The insolubilization of AX in the presence of AXH was explained as the result of the non-covalent interactions between less substituted chains. According to Kormelink et al. (59), the removal of arabinose by AXH causes intermolecular aggregation and increases the insolubility of AX. AXH and β -glucanase together with Xyl-3 exhibited no synergism for AX extraction. With the aim to obtain enzymatic extracts of high viscosity, β -glucans should be preserved when AX are extracted because of their contribution to the viscosity of the extracts.

The effect of a FAE from *A. niger* was studied in combination with Xyl-1 and Xyl-3 on rye flour. No synergism was observed when FAE and xylanases were combined. It is likely that xylanase activity is not affected by the presence of FA on rye AX.

It was demonstrated that AX solubilized from rye flour by xylanases could not easily gel in the presence of a fungal laccase. It is likely that dimerization of ferulic acid esterified to WEAX was not enough to form a gel, because of an important WEAX degradation. From the action of the three xylanases (Xyl-1 from *A. niger*, Xyl-2 from *T. emersonii*, and Xyl-3 from *B. subtilis*), only the AX extracted during 15 min with 0.5 unit of Xyl-1 thickened, but no gel was formed after 1 h of reaction with laccase. Xyl-1 solubilized AX of higher molecular weight than Xyl-3 in a rye flour treated with 80% ethanol (v/v).

LITERATURE CITED

- (1) Izydorczyk, M. S.; Biliaderis, C. G. Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydr. Polym.* **1995**, *28*, 33–48.
- (2) Fincher, G. B.; Stone, B. A. Cell walls and their components in cereal grain technology. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; AACC: St. Paul, MN, 1986; Vol. 8, pp 207–295.
- (3) Meuser, F.; Suckow, P. Non-starch polysaccharides. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frazier, P. J., Galliard, T., Eds.; The Royal Society of Chemistry: London, U.K., 1986; pp 42–61.
- (4) Vinkx, C. J. A.; Delcour, J. A. Rye (*Secale cereale* L.) arabinoxylans: a critical review. *J. Cereal Sci.* **1996**, *24*, 1–14.
- (5) Delcour, J. A.; Vanhamel, S.; Hosney, R. C. Physicochemical and functional properties of rye nonstarch polysaccharides. II. Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chem.* **1991**, *68*, 72–76.

- (6) Härkönen, H.; Laurikainen, T.; Autio, K.; Tenkanen, M.; Poutanen, K. Cell wall degrading enzymes in baking. *Biotechnol. Food Res.* **1996**, *163*, 37–44.
- (7) Rouau, X.; El Hayek, M. L.; Moreau, D. Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties. *J. Cereal Sci.* **1994**, *19*, 259–272.
- (8) Courtin, C. M.; Gelders, G. G.; Delcour, J. A. Use of two endoxylanases with different substrate selectivity for understanding arabinoxylan functionality in wheat flour breadmaking. *Cereal Chem.* **2001**, *78*, 564–571.
- (9) Faulds, C. B.; Williamson, G. Ferulic acid release from plant polysaccharides by specific esterases. In *Xylans and Xylanases, Progress in Biotechnology*; Visser, J., Beldman, G., Kusters-van Someren, M. A., Voragen, A. G. J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; Vol. 7, pp 419–422, 576.
- (10) Gruppen, H.; Kormelink, F. J. M.; Voragen, A. G. J. Enzymic degradation of water-unextractable cell wall material and arabinoxylans from wheat flour. *J. Cereal Sci.* **1993**, *18*, 129–143.
- (11) Kormelink, F. J. M.; Voragen, A. G. J. Combined action of xylan-degrading and accessory enzymes on different ([glucuronol]-arabino)xylans. In *Xylans and Xylanases, Progress in Biotechnology*; Visser, J., Beldman, G., Kusters-van Someren, M. A., Voragen, A. G. J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; Vol. 7, 415–418, 576.
- (12) Poutanen, K.; Härkönen, H.; Parkkonen, T.; Autio, K.; Suortti, T.; Kantelinen, A.; Viikari, L. The use of xylanolytic enzymes in processing of cereals. In *Developments in Food Engineering*; Proceedings of the International Congress on Engineered Food, 6th Meeting, 1993; Yano, T., Matsuno, R., Nakamura, K., Eds.; Blackie: Glasgow, U.K., 1994; Vol. Pt. 1, pp 72–74.
- (13) Bartolomé, B.; Faulds, C. B.; Tuohy, M.; Hazlewood, G. P.; Gilbert, H. J.; Williamson, G. Influence of different xylanases on the activity of ferulic acid esterase on wheat bran. *Biotechnol. Appl. Biochem.* **1995**, *22*, 65–73.
- (14) Bartolomé, B.; Faulds, C. B.; Williamson, G. Enzymic release of ferulic acid from barley spent grain. *J. Cereal Sci.* **1997**, *25*, 285–288.
- (15) Bartolomé, B.; Faulds, C. B.; Kroon, P. A.; Waldron, K.; Gilbert, J.; Hazlewood, G.; Williamson, G. An *Aspergillus niger* esterase (ferulic acid esterase III) and a recombinant *Pseudomonas fluorescens* subsp. *cellulosa* esterase (XylD) release a 5-5' ferulic dehydrodimer (diferulic acid) from barley and wheat cell walls. *Appl. Environ. Microbiol.* **1997**, *63*, 208–212.
- (16) Kühn, M. C.; Grosch, W. Influence of the enzymatic modification of the nonstarchy polysaccharide fractions on the baking properties of reconstituted rye flour. *J. Food Sci.* **1998**, *53*, 889–895.
- (17) Luonteri, E.; Härkönen, H.; Suortti, T.; Stenholm, K.; Poutanen, K.; Tenkanen, M. *Trichoderma* and *Aspergillus* as sources of cell wall degrading enzymes for cereal processing. In *Proceedings of the 1st European Symposium on Enzymes and Grain Processing*. The Netherlands, Dec 2–4, 1996; Angelino, S. A. G. F., Hamer, R. J., van Hartingsveldt, W., Heidekamp, F., van der Lugt, J. P., Eds.; T.N.O. Nutrition and Food Research Institute: Zeist, 1997; pp 142–151, 267.
- (18) Martínez-Anaya, M.; Jiménez, T. Functionality of enzymes that hydrolyse starch and non-starch polysaccharide in breadmaking. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 209–214.
- (19) Petit-Benvegnen, M.-D.; Saulnier, L.; Rouau, X. Solubilization of arabinoxylans from isolated water-unextractable pentosans and wheat flour doughs by cell-wall-degrading enzymes. *Cereal Chem.* **1998**, *75*, 551–556.
- (20) Rouau, X. Investigations into the effects of an enzyme preparation for baking on wheat flour dough pentosans. *J. Cereal Sci.* **1993**, *18*, 145–157.
- (21) Williamson, G.; Faulds, C. B.; Kroon, P. A. Specificity of ferulic acid (feruloyl) esterases. *Biochem. Soc. Trans.* **1998**, *26*, 205–209.
- (22) Williamson, G.; Kroon, P. A.; Faulds, C. B. Hairy plant polysaccharides: a close shave with microbial esterases. *Microbiology* **1998**, *144*, 2011–2023.
- (23) Wood, T. M.; Castanares, A.; Smith, D. C.; McCrae, S. I.; Brown, J. A. Xylan-degrading enzyme systems of the fungi *Aspergillus awamori* and *Penicillium pinophilum*: studies on the isolation of over-producing mutant strains and aspects of the enzymology. In *Xylans and Xylanases, Progress in Biotechnology*; Visser, J., Beldman, G., Kusters-van Someren, M. A., Voragen, A. G. J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; Vol. 7, pp 187–202, 576.
- (24) Reilly, P. J. Xylanases: structure and function. In *Trends in the Biology of Fermentation for Fuels and Chemicals*; Holander, A., Ed.; Basic Life Sciences, Plenum Press: New York, 1981; pp 111–129.
- (25) Rouau, X. Les hémicelluloses en panification. *Ind. Céréales* **1996**, Jan-Fév-Mars, 13–19.
- (26) Courtin, C. M.; Roelants, A.; Delcour, J. A. Fractionation-reconstitution experiments provide insight into the role of endoxylanases in bread-making. *J. Agric. Food Chem.* **1999**, *47*, 1870–1877.
- (27) Courtin, C. M.; Delcour, J. A. Relative activity of endoxylanases towards water-extractable and water-unextractable arabinoxylan. *J. Cereal Sci.* **2001**, *33*, 301–312.
- (28) Rouau, X.; Moreau, D. Modification of some physicochemical properties of wheat flour pentosans by an enzyme complex recommended for baking. *Cereal Chem.* **1993**, *70*, 626–632.
- (29) Brümmer, J. M. Influence of rye flour treatment with pentosanases (International rye symposium: technology and products). *VTT Symp.* **1995**, No. 161, 155–159.
- (30) Mutsaers, J. H. G. M. The application of enzymes in baking. In *Proceedings of the 1st European Symposium on Enzymes and Grain Processing*, The Netherlands, Dec 2–4, 1996; Angelino, S. A. G. F., Hamer, R. J., van Hartingsveldt, W., Heidekamp, F., van der Lugt, J. P., Eds.; T.N.O. Nutrition and Food Research Institute: Zeist, 1997; pp 160–163, 267.
- (31) Si, J. Q. Synergistic effect of enzymes for breadbaking (feature). *Cereal Foods World* **1997**, *42*, 802–807.
- (32) Kormelink, F. J. M.; Searle-Van Leeuwen, M. J. F.; Wood, T. M.; Voragen, A. G. J. Purification and characterization of a (1,4)- β -D-arabinoxylan arabinofuranohydrolase from *Aspergillus awamori*. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 753–758.
- (33) Kormelink, F. J. M.; Gruppen, H.; Voragen, A. G. J. Mode of action of (1,4)- β -D-arabinoxylan arabinofuranohydrolase (AXH) and α -L-arabinofuranosidases on alkali-extractable wheat-flour arabinoxylan. *Carbohydr. Res.* **1993**, *249*, 345–353.
- (34) Autio, K.; Härkönen, H.; Parkkonen, T.; Frigard, T.; Poutanen, K.; Siika-aho, M.; Aman, P. Effects of purified endo- β -xylanase and endo- β -glucanase on the structural and baking characteristics of rye doughs. *Lebensm.-Wiss. Technol.* **1996**, *29*, 18–27.
- (35) Geissmann, T.; Neukom, H. On the composition of the water-soluble wheat flour pentosans and their oxidative gelation. *Lebensm.-Wiss. Technol.* **1973**, *6*, 59–62.
- (36) Hosney, R. C.; Faubion, J. M. A mechanism for the oxidative gelation of wheat flour water-soluble pentosans. *Cereal Chem.* **1981**, *58*, 421–424.
- (37) Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Oxidative gelation of water-soluble pentosans from wheat. *J. Cereal Sci.* **1990**, *11*, 153–169.
- (38) 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.
- (39) Figueroa-Espinoza, M. C.; Morel, M. H.; Surget, A.; Rouau, X. Oxidative cross-linking of wheat 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.

- (40) Debyser, A.; Derdelinckx, G.; Delcour, J. A. Arabinoxylan solubilisation and inhibition of the barley malt xylanolytic system by wheat during mashing wheat wholemeal adjunct; evidence for a new class of enzyme inhibitors in wheat. *J. Am. Soc. Brew. Chem.* **1997**, *55*, 153–156.
- (41) Rouau, X.; Surget, A. Evidence for the presence of a pentosanase inhibitor in wheat flours. *J. Cereal Sci.* **1998**, *28*, 63–70.
- (42) Sorensen, J. F.; Poulsen, C. H. Endogenous xylanase inhibitor from wheat flour: its characteristics and influence on xylanases used in bakery applications. In *Meeting Abstract*, 1999 AACC Annual Meeting, Oct 31–Nov 3, Seattle, WA; AACC: St. Paul, MN, 1999.
- (43) McLauchlan, W. R.; García-Conesa, M. T.; Williamson, G.; Roza, M.; Ravestien, P. A novel class of protein from wheat which inhibits xylanases. *Biochem. J.* **1999**, *338*, 441–446.
- (44) McLauchlan, W. R.; Flatman, R. H.; Sancho, A. I.; Kakuta, J.; Faulds, C. B.; Elliot, G. O.; Kroon, P. A.; Furniss, C. S.; Juge, N.; Ravestien, P.; Williamson, G. Xylanase inhibitors from cereals: implications for baking, brewing and plant technology. In *2nd European Symposium on Enzymes in Grain Processing*, Dec 8–10, 1999; Simoinen, T., Tenkanen, M., Eds.; Technical Research Centre of Finland: Helsinki, Finland, 2000; pp 55–61.
- (45) Goesaert, H.; Debyser, W.; Gebruers, K.; Proost, P.; Van Damme, J.; Delcour, J. A. Purification and partial characterization of an endoxylanase inhibitor from barley. *Cereal Chem.* **2001**, *78*, 453–457.
- (46) Mampusta, S.; Rasmussen, P.; Baruch, A. *Aspergillus* arabinofuranosidase. Int. Patent IPN WO96/29416, 1996.
- (47) Figueroa-Espinoza, M. C.; Morel, M. H.; Rouau, X. Effect of lysine, tyrosine, cysteine and glutathione on the oxidative cross-linking of feruloylated arabinoxylans by a fungal laccase. *J. Agric. Food Chem.* **1998**, *46*, 2583–2589.
- (48) Åman, P.; Nilsson, M.; Andersson, R. Chemical composition of rye grain (International rye symposium: technology and products). *VTT Symp.* **1995**, No. 161, 33–38.
- (49) Bengtsson, S.; Åman, P. Isolation and chemical characterization of water-soluble arabinoxylans in rye grain. *Carbohydr. Polym.* **1990**, *12*, 267–277.
- (50) Girhammar, U.; Nair, B. M. Isolation, separation and characterization of water soluble non-starch polysaccharides from wheat and rye. *Food Hydrocolloids* **1992**, *6*, 285–299.
- (51) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.
- (52) 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.
- (53) Morris, E. R. *Rheology of Hydrocolloids in Gums and Stabilisers for the Food Industry*; Phillips, G. O., Wedlock, D. J., Williams, P. A., Eds.; Pergamon Press: Oxford, U.K., 1984.
- (54) McCleary, B. V. Measurement of *endo*-1,4- β -D-xylanase. In *Xylans and Xylanases*; Visser, J., Beldman, G., Kusters-van Someren, M. A., Voragen, A. G. J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; pp 161–170.
- (55) Girhammar, U.; Nair, B. M. Certain physical properties of water soluble non-starch polysaccharides from wheat, rye, triticale, barley and oats. *Food Hydrocolloids* **1992**, *6*, 329–343.
- (56) Nilsson, M.; Andersson, R.; Åman, P. Arabinoxylan fractionation on DEAE-cellulose chromatography influenced by protease re-treatment. *Carbohydr. Polym.* **1999**, *39*, 321–326.
- (57) Figueroa-Espinoza, M. C.; Rouau, X. Effect of cysteinyl caffeic acid, caffeic acid and L-Dopa on the oxidative cross-linking of feruloylated arabinoxylans by a fungal laccase. *J. Agric. Food Chem.* **1999**, *47*, 497–503.
- (58) Debyser, W.; Peumans, W. J.; Van Damme, E. J. M.; Delcour, J. A. *Triticum aestivum* xylanase inhibitor (TAXI), a new class of enzyme inhibitor affecting breadmaking performance. *J. Cereal Sci.* **1999**, *30*, 39–43.
- (59) Kormelink, F. J. M.; Searle-Van Leeuwen, M. J. F.; Wood, T. M.; Voragen, A. G. J. (1,4)- β -D-Arabinoxylan arabinofuranohydrolase: a novel enzyme in the bioconversion of arabinoxylan. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 231–232.
- (60) Ralph, J.; Quideau, S.; Grabber, J. H.; Hatfield, R. D. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3485–3498.
- (61) Gebruers, K.; Debyser, W.; Goesaert, H.; Proost, P.; Van Damme, J.; Delcour, J. A. *Triticum aestivum* L. endoxylanase inhibitor consists of two inhibitors, TAXI I and TAXI II, with different specificities. *Biochem. J.* **2001**, *353*, 239–244.

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