

## Enzymatic Solubilization of Arabinoxylans from Native, Extruded, and High-Shear-Treated Rye Bran by Different Endo-xylanases and Other Hydrolyzing Enzymes

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The overall objective of this research was to find a new way to valorize rye bran, by producing a gellifier from the enzymatic solubilization of arabinoxylans (AX). The effects of three pure endo-xylanases from *Aspergillus niger* (Xyl-1), *Talaromyces emersonii* (Xyl-2), and *Bacillus subtilis* (Xyl-3) and of Grindamyl S100 (GS100), a commercial enzyme preparation containing a Xyl-1 type endo-xylanase, were tested on rye bran to study the solubilization of water-unextractable arabinoxylans (WUAX). Eight different extrusion-treated rye brans were also used as substrates to find the best physical treatment to facilitate enzymatic arabinoxylan (AX) solubilization. Arabinoxylans were better solubilized from the bran extruded at high temperature using Xyl-3. This enzyme was then tested in combination with pure (1,4)- $\beta$ -D-arabinoxylan arabinofuranohydrolase (AXH) and endo- $\beta$ -D-glucanase or ferulic acid esterase (FAE), from *A. niger*. Only  $\beta$ -glucanase in combination with Xyl-3 improved the AX extraction, but it did not have a marked effect on the viscosity of the extracts. Xyl-3 was then tested on a high-shear-treated rye bran, and results were compared to those obtained with the high-temperature-extruded rye bran. The high-shear treatment did not improve the bran AX enzymatic solubilization. The combination of FAE with Xyl-1 or Xyl-3 did not improve the AX extraction from untreated and high-shear-treated rye bran. Finally, to study the gelation capacity of the enzymatically solubilized AX, the effect of the hydrogen peroxide/horseradish peroxidase ( $H_2O_2$ /POD) was tested on the Xyl-3 high-temperature-extruded bran extracts. Solubilized AX did not gel in the presence of the oxidizing system.

**KEYWORDS:** Arabinoxylans; arabinofuranohydrolase; bran; extrusion; ferulic acid esterase; gelation; glucanase; high shear; horseradish peroxidase; oxidative gelation; rye; solubilization; xylanase

### INTRODUCTION

Rye is a cereal raw material with interesting functional properties from technological, physiological, and nutritional points of view. This justifies the increasing consumption of this particular grain and the research in innovative products (1). The main components of rye grain [starch, dietary fiber (DF), and protein] are the same as in wheat, with the difference that rye proteins do not form a viscoelastic network. Nonstarch polysaccharides in rye constitute the major DF fraction and include components such as cellulose, mixed-linked  $\beta$ -glucans, arabinoxylans (AX), and xyloglucans. AX are the quantitatively most important DF components in rye. Both water-extractable ara-

binoxylans (WEAX) and water-unextractable arabinoxylans (WUAX) are present in higher concentrations in rye than in wheat. Bran, an important byproduct of the cereal industry, is rich in noncellulosic polysaccharides, with heteroxylans as the major component (2–8).

WUAX have a high water-binding capacity and, in addition, a capacity to swell (9–11). They have been reported to decrease crumb firming rate (12) and to depress loaf volume (13), thus having a negative impact on rye baked goods (14). In wheat breadmaking, WUAX are also detrimental, whereas WEAX and solubilized AX with medium to high molecular weights (MW) have a positive impact on loaf volume (15–17). WEAX is an important component for the baking quality of rye flour as it contributes to viscosity and air entrapment in the dough and to the volume and texture of breads. Rye WEAX have a higher degree of polymerization compared to wheat WEAX, thus producing more viscous solutions in water (14, 18–23). From

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this appears the importance of solubilizing AX from WUAX to increase the concentration of high molecular weight AX (HMWAX) in rye dough, which would increase quality in rye breadmaking.

Solubilization of AX can be obtained by using xylanolytic enzymes. There exist different types of endo-xylanases with different specificities, which can be tested to achieve a good rate of solubilization of HMWAX from WUAX, without excessive depolymerization of WEAX that leads to sticky doughs (15, 24–31). Thus, an endo-xylanase will perform well in breadmaking if it has a bias toward WUAX instead of WEAX.

In rye, the extractability of AX is lower in the outer layers of the kernel than in the starchy endosperm. As cell-wall material is very complex, several enzymes are frequently needed to ease the AX solubilization. From this has grown an interest in the study of different hydrolyzing enzymes, that is,  $\beta$ -glucanases, arabinofuranosidases, and ferulic acid esterases, to help the liberation of water-soluble HMWAX in the presence of endo-xylanases (12, 22, 24, 30, 32–41). AX extraction can also be achieved or facilitated by physical or chemical treatments (42–44). Bergmans et al. (43) observed that a pretreatment (autoclave treatment, alkaline peroxide, and chlorite delignification) of wheat bran was not effective in increasing the extract yield in the subsequent saturated barium hydroxide extraction of AX.

Besides the good properties conferred to dough by WEAX in their native form, they can be very interesting in their cross-linked form because of their gelling potential, in the presence of free radical oxidizing agents (45–49). By oxidative gelation, WEAX could then set a polysaccharide network in cereal dough where no viscoelastic protein network (gluten) is present. This network would increase water absorption and retention, decrease stickiness, and increase stability, gas retention, bread volume, and shelf life. To set this polysaccharide network to improve the quality of rye baked products, water-extractable HMWAX are needed. This justifies the importance of finding good combinations of hydrolyzing and oxidizing enzymes. Rye bran is rich in WUAX. Enzymatic release of HMWAX in combination with oxidizing enzymes could produce a gellifier, usable as an improver for the food and nonfood industries. Several applications could be then considered, that is, improvers for breadmaking, thickeners, gelling materials in both the food and pharmaceutical industries, constituents of biodegradable films, etc. This would constitute a new method of valorization for rye bran.

The aim of this work was to examine the enzymatic release of HMWAX from rye bran and different treated brans (extrusion and high shear), using different pure endo-xylanases: Xyl-1 from *Aspergillus niger*, Xyl-2 from *Talaromyces emersonii*, and Xyl-3 from *Bacillus subtilis*. Combinations of Xyl-3 with pure arabinofuranosidase (AXH), endo- $\beta$ -D-glucanase, or ferulic acid esterase (FAE) from *A. niger* were also tested. The effect of hydrogen peroxide/horseradish peroxidase ( $H_2O_2$ /POD) was also studied on the solubilized AX from bran previously treated with Xyl-3. The final objective was to find the best enzyme combination to valorize rye bran, using it as a source of HMWAX with gellifying properties.

## MATERIALS AND METHODS

**Bran.** Two batches of rye bran were provided by Eider Mühle (Germany) obtained as byproducts from the extraction of a German rye flour type 947 with 93% extraction rate (50). The first batch, Br1, was used to obtain eight different extruded brans (BrT1–BrT8) (Table 1). The second batch, Br9, was received one year later and was used

**Table 1.** Extrusion Conditions To Obtain the Eight Different Extruded Brans

	extrusion conditions							
	BrT1	BrT2	BrT3	BrT4	BrT5	BrT6	BrT7	BrT8
product temp (°C)	98.9	104.4	112.3	126.6	127.6	142.4	123.8	89.3
screw (rpm)	300	300	300	300	300	300	300	300
feeder (rpm)	20	31	31	40	40	40	40	40
steam flow (kg/h)	6	6	6	4	6	5	5	5
shaft pressure (atm)	0	2	2	5	3	4	4	1
product pressure (atm)	4	6	6	10	7	9	9	5
throughput (kg/h)	14.3	17.2	16.6	18	18.3	17.2	22.6	25.8
energy (amp)	7	9–10	10	14	13	16	11	6

to obtain the high-shear-treated bran (BrT9). Treated and nontreated rye brans were milled in a Janke and Kunkel (IKA Labortechnik) grinder during 20 s at ambient temperature before utilization. Br1 and Br9 contained 24.5 and 21% (dm) of total AX, respectively. The Ara/Xyl ratios were 0.37 and 0.43 for Br1 and Br9, respectively.

**Extruded Bran.** The extrusion conditions of rye bran are presented in Table 1. After extrusion, the samples were dried in a heating cabinet at 35 °C and milled as described before.

**High-Shear-Treated Rye Bran.** Two kilograms of rye bran Br9 was mixed with 23.5 kg of water at 12 °C. The slurry was pumped through a Dispax-reactor DR3/6/6 (shear rate = 45600 s<sup>-1</sup>; throughput = 2500 L of H<sub>2</sub>O/h; back pressure = 1.5 bar; rotor speed = 8000 rpm; peripheral speed = 23 m/s; maximum internal discharge head at throughput = 0–2 bar) (IKA Works, Inc., Wilmington, NC). This reactor is a high-shear, three-stage dispersing machine for the production of microemulsions and very fine suspensions. This treatment was repeated seven times, and the final temperature was 31 °C. Finally, samples of BrT9 were freeze-dried.

**Enzymes.** Grindamyl S100 (*A. niger*, 2.5 units/mg) and the following pure enzymes were provided by Danisco Cultor (Brabrand, Denmark): endo-xylanase Xyl-1 [*A. niger*, EC 3.2.1.8., MW 20000 Da, pI 4.07, optimal pH 3.5, 166.9 units/mL, high affinity for HMW WUAX, with low affinity for low molecular weight (LMW) soluble AX]; endo-xylanase Xyl-2 (*T. emersonii*, EC 3.2.1.8., MW 38000 Da, pI 4.59, optimal pH 3.0, 117.4 units/mL, more exo-activity and more affinity for WEAX); endo-xylanase Xyl-3 (*B. subtilis*, EC 3.2.1.8., MW 20000 Da, pI 9.03, optimal pH 6.0, 142 units/mL, more affinity for WUAX than Xyl-1); endo- $\beta$ -glucanase [*A. niger*, EC 3.2.1.4, MW 24230 Da, pI 4.2, optimal pH 4.0, 120000  $\beta$ -glucanase units (BGU)/mL]; arabinofuranosidase AXH (*A. niger*, EC 3.2.1.55, MW 332700 Da, pI 3.7, optimal pH 4–5) (51); and ferulic acid esterase 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]. Horseradish peroxidase type I (POD, donor: hydrogen peroxide oxidoreductase EC 1.11.1.7, 96 PU/mg) was purchased from Sigma Chemical Co. (St. Louis, MO).

**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 (52) using as the enzyme standard a noncommercial enzyme preparation, TXU Standard 98/99, which contains 0.56 mg of Xyl-1/g.

**Endo- $\beta$ -glucanase Activity.** Enzyme (0.2 g) was weighed into a 100 mL volumetric flask and dissolved in the assay buffer (sodium acetate buffer 100 mM, 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  $\mu$ L) was added to 1.0 mL aliquots of the assay buffer, and the mixtures were equilibrated at 40 °C for 5 min. One  $\beta$ -glucosylase tablet (Megazyme, Megazyme International, Dublin, Ireland) was added to each test tube, which must not be stirred. After exactly 10 min, 10 mL of the stop solution [tris(hydroxymethyl)aminomethane 1% (w/v)] was added. The test tubes were stirred, and the solutions were filtered through a Whatman no. 1 filter paper. The absorbance at 590 nm of the standard ( $\beta$ -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 within 0.2–1.1 at 590 nm.

**Determination of FAE Activity by Using Methyl Ferulate (MeFA) as Substrate.** FAE activity was determined by measuring the formation of ferulic acid (FA) released from 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  $\mu$ L of FAE to 300  $\mu$ L of MeFA. The mixture was incubated at 45 °C for 10 min. The reaction was stopped by adding 50  $\mu$ 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 using a spectrophotometer, and lecture was made at 325 nm. A FA standard curve was then constructed by using various concentrations of the FA (1000–1500  $\mu$ M). One unit of enzyme activity is defined as the amount of enzyme that produces 1  $\mu$ mol of FA/min at 45 °C.

**Analysis of Alkali-Labile Phenolics.** Total FA (*cis*- + *trans*-FA) was determined as described in Figuerola-Espinoza et al. (50). The total contents of FA were 0.4% (dm) for Br1 and BrT6, 0.3% (dm) for Br9 and BrT9, and 0.08% (dm) for flour, respectively. These values represent 85.8, 88.8, and 61.4 nmol of FA/mg of AX, for Br1 and BrT6, for Br9 and BrT9, and for flour, respectively.

**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 (53), using an Alliance Instruments Evolution II autoanalyzer (Alliance Instruments, France). One hundred microliters of the filtered (2.7  $\mu$ m) reaction mixes were diluted before analysis.

**Gas-Liquid Chromatography (GLC).** The carbohydrate content was determined by GLC following the procedure of Blakeney et al. (54). Alditol acetates obtained after sulfuric acid hydrolysis (2 M H<sub>2</sub>SO<sub>4</sub>, 100 °C, 2 h) were injected on a DB 225 capillary column (J&W Scientific, Folsom, CA), using inositol as the internal standard.

**Size Exclusion Chromatography (SE-HPLC).** SE-HPLC was performed at 35 °C using a Waters (Millipore Co., Milford, MA) Ultrahydrogel 1000, 10  $\mu$ m, column (7.8  $\times$  300 mm), with a pullulan limit exclusion 10<sup>6</sup> Da, eluted with 0.1 M sodium acetate buffer, pH 5.0, at 0.6 mL/min. Twenty microliters of the filtered (2.7  $\mu$ 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 (2 mL of extract + 100  $\mu$ L of enzyme solution) 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 ( $\eta_{rel}$  = flow time of sample/flow time of solvent) and specific viscosity ( $\eta_{sp}$  =  $\eta_{rel}$  - 1) were obtained 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 (55)

$$[\eta]_{app} = 1/c \times [2(\eta_{sp} - 1_n(\eta_{rel}))]^{0.5} \times 1000$$

where *c* (expressed as mg/mL) represents the AX concentration (15).

**Enzymatic Solubilization and Depolymerization of AX.** Amounts of bran or processed bran (containing 20 mg of AX) suspended in 4 mL of 0.1 M sodium acetate buffer, pH 5.0, were added to 1 mL of enzyme solution (xylanases, AXH,  $\beta$ -glucanase, or FAE) or 1 mL of buffer (for the control). 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 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, or 24 h. After cooling, they were filtered (2.7  $\mu$ m) and frozen. Resulting reaction supernatants were analyzed by colorimetric determination of AX, capillary viscometry, and SE-HPLC. In blanks, the enzyme solution was replaced by buffer; the blanks were vortexed (30 s), centrifuged (1 min, 1400g, 25 °C), paper filtered, boiled (10 min), cooled, filtered (2.7  $\mu$ m), and frozen. The effect of GS100 and xylanases Xyl-1,

**Table 2.** Effect of Different Doses of GS100 on the Solubilization of AX from Rye Br1 after 24 h of Reaction at 20 °C

dose of GS100 <sup>a</sup> (units)	% of solubilized AX <sup>b,c</sup>	% of initial $\eta_{sp}$ <sup>b,d</sup>	% of initial [ $\eta$ ] <sub>app</sub> <sup>b,e</sup>
0.0	0.2	93.5	69.9
0.5	5.0	92.7	12.4
2.0	5.2	91.5	11.9
4.0	5.1	84.4	11.1

<sup>a</sup> Grindamyl S100. <sup>b</sup> Results obtained from duplicates; the coefficient of variation was 3%. Calculation was made by taking the initial values in the sample without enzyme at 0 min. <sup>c</sup> Arabinoxylans. <sup>d</sup> Specific viscosity. <sup>e</sup> Apparent intrinsic viscosity.

Xyl-2, and/or Xyl-3 was studied on the untreated bran, extruded bran, and/or high-shear-treated bran. AXH (0.2 and 1  $\mu$ g),  $\beta$ -glucanase (1000 and 5000 BGU), and FAE (0.026, 0.26, and 2.6 units) were also tested. The latter enzymes were also tested in combination with 2.5 units of Xyl-3.

The percentages of AX solubilization and specific ( $\eta_{sp}$ ) and apparent intrinsic ( $[\eta]_{app}$ ) viscosities of reaction mixes, were calculated as follows:

$$\% \text{ solubilized AX} = \frac{[(\text{AX in solution} - \text{initially soluble AX}) / (\text{total AX} - \text{initially soluble AX})] \times 100$$

$$\% \text{ of initial } \eta_{sp} \text{ or } [\eta]_{app} = \frac{(\text{final } \eta_{sp} \text{ or } [\eta]_{app} \text{ in the reaction mix} / \text{initial } \eta_{sp} \text{ or } [\eta]_{app} \text{ in the blank}) \times 100$$

**Oxidative Gelation Tests.** Rye flour (4.10 g; German rye flour type 947 with 93% extraction and ash content of 1.06%, Danisco Cultor) (50) and 1.05 g of BrT6 were added to 8 mL of 0.1 M sodium acetate buffer, pH 5.0, and treated with 12 mL of a Xyl-3 solution (2.5 units/mL of buffer), during 1 and 24 h, respectively, in a rotary shaker (40 rpm, 20 °C). The samples were centrifuged (5 min, 22000g, 4 °C), and the supernatants were boiled for 10 min and then centrifuged (10 min, 22000g, 20 °C), frozen, freeze-dried, and redissolved in water to obtain solutions of 6.25 and 3.12 mg of AX/mL, pH 6.6, for flour and 32.88 mg of AX/mL, pH 6.2, for BrT6. Two milliliter solutions added to 50  $\mu$ L of 0.1 M acetate buffer, pH 5.0, 25  $\mu$ L of H<sub>2</sub>O<sub>2</sub> (2.09 mM), and 25  $\mu$ L of horseradish POD (7.1 PU/mL) were tested by capillary viscometry.

**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

**Effect of GS100 on Bran.** The effect of 0.5, 2, and 4 units of GS100 was studied on the solubilization of AX from rye bran Br1, after 24 h of reaction at 20 °C (Table 2). GS100 allowed a maximum solubilization of 5%, similar for the three doses. Increasing doses of GS100 provoked a degradation of the solubilized AX as evidenced by viscosity values. After 24 h of reaction, the blank without enzyme contained 6-fold less WEAX (0.04 mg of AX/mL) than the assays with GS100 (0.24 mg of AX/mL), but  $[\eta]_{app}$  was ~6-fold higher (1330 mL/g). More pronounced solubilization and less pronounced degradation rates were observed when the effect of GS100 was tested on rye flour or endosperm water-unextractable AX, as previously reported (50). Stronger linkages of AX to other cell wall components and more complex AX structure in rye bran would contribute to the inaccessibility of the enzyme to the substrate and would explain the lower extractability rate of AX from the rye kernel outer layers (6, 12). As no further solubilization was observed at doses above 0.5 unit, and to limit AX degradation, this dose was chosen for the rest of the experiments.

**Table 3.** Effect of 0.5 Unit of GS100 and Xylanases Xyl-1, Xyl-2, and Xyl-3 on the Solubilization of Arabinoxylans from Rye Bran Br1 at 20 °C

enzyme	reaction time (min)	% solubilized AX <sup>a,b</sup>	% of initial $\eta_{sp}$ <sup>a,c</sup>	% of initial $[\eta]_{app}$ <sup>a,d</sup>
blank <sup>e</sup>	0	0.0	100.0	100.0
	60	0.5	161.6	91.9
	240	1.7	126.8	39.8
	1440	1.7	101.4	32.0
GS100 <sup>f</sup>	0	0.0	100.0	100.0
	60	2.4	174.9	42.1
	240	3.0	137.9	27.9
	1440	5.0	92.7	12.4
Xyl-1	0	0.0	100.0	100.00
	60	3.1	182.4	35.8
	240	3.4	146.9	26.9
	1440	5.1	111.0	14.7
Xyl-2	0	0.0	100.0	100.0
	60	3.0	116.3	23.8
	240	4.3	110.6	17.0
	1440	6.7	69.5	7.2
Xyl-3	0	0.0	100.0	100.0
	60	4.6	175.3	25.0
	240	5.1	169.9	22.2
	1440	7.7	139.6	12.7

<sup>a</sup> Results obtained from duplicates; the coefficient of variation was 3%. Calculation was made by taking the initial values in the sample without enzyme at 0 min.

<sup>b</sup> Arabinoxylans. <sup>c</sup> Specific viscosity. <sup>d</sup> Apparent intrinsic viscosity. <sup>e</sup> Without enzyme.

<sup>f</sup> Grindamyl S100.

**Effect of Xylanases on Bran.** As shown in **Table 3**, after 24 h of reaction using 0.5 unit of enzymes, the best rates of AX solubilization from Br1 were obtained with Xyl-3 ( $7.7 \pm 0.25\%$ ), followed by Xyl-2 ( $6.7 \pm 0.20\%$ ), Xyl-1 ( $5.1 \pm 0.15\%$ ), and GS100 ( $5.0 \pm 0.15\%$ ). As expected, the effects of GS100 (containing a Xyl-1 type endo-xylanase) and Xyl-1 were similar. Compared to the blank (without enzyme), where a basal AX solubilization occurred, the rates of AX solubilization were 3-, 4-, and 5-fold higher when GS100 or Xyl-1, Xyl-2, and Xyl-3, respectively, were added. After 24 h of reaction, only  $1.7 \pm 0.05\%$  of AX was solubilized in the blank. In all cases, the enzymatically solubilized AX were more degraded than those from the blank. Xyl-2 was the enzyme that degraded the most solubilized AX, as previously observed with rye flour AX (50). After 24 h of reaction, Xyl-2-solubilized AX presented half values of  $\eta_{sp}$  and  $[\eta]_{app}$  compared to other enzymes.

**Effect of Xylanases on Extruded Brans (BrT1–BrT8).** After 24 h of reaction, the AX solubilization rates were 3–5- and 4–17-fold higher in the Br1 and extruded brans, respectively, than in the blank Br1 (**Tables 3 and 4**). In general, extrusion increased the enzymatic solubilization rates (**Table 4**). Maximums of  $22 \pm 0.66$  to  $27.4 \pm 0.82\%$  of solubilization were obtained with Xyl-3 after 24 h of reaction with the eight different BrT. Maximum values for Xyl-1 ( $9.8 \pm 0.30$  to  $10.9 \pm 0.33\%$ ) and Xyl-2 ( $6.6 \pm 0.20$  to  $10.3 \pm 0.31\%$ ) were in all cases lower than those of Xyl-3. As observed for Br1, the lowest values of AX solubilization from BrT were observed with Xyl-2. The highest AX solubilizations by Xyl-3 were obtained with BrT6 ( $27.4 \pm 0.82\%$ ), BrT4 ( $26.2 \pm 0.79\%$ ), BrT5 ( $25.9 \pm 0.78\%$ ), and BrT1 ( $25.9 \pm 0.78\%$ ). It was decided to choose BrT6, the bran treated with the highest extrusion energy and temperature (**Table 1**). According to Bergmans et al. (43), increasing the temperature of extraction improves the AX solubilization yield from wheat bran by barium hydroxide.

All supernatants from the enzymatically treated BrT were analyzed by SE-HPLC (not shown). BrT5, BrT6, and BrT7

**Table 4.** Effect of 0.5 Unit of Xylanases Xyl-1, Xyl-2, and Xyl-3 on the Solubilization of Arabinoxylans from Untreated and Extruded Rye Bran at 20 °C

enzyme	reaction time (min)	% of solubilized AX <sup>a</sup>									
		Br1	BrT1	BrT2	BrT3	BrT4	BrT5	BrT6	BrT7	BrT8	
Xyl-1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	60	3.1	5.5	5.0	5.2	5.6	5.1	5.4	5.1	5.3	
	240	3.4	8.4	7.3	7.6	7.7	7.8	7.8	7.4	7.5	
	1440	5.1	9.8	10.8	10.5	10.6	10.9	10.8	10.9	9.9	
Xyl-2	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	60	3.0	4.5	3.3	3.1	3.4	2.9	3.3	2.7	4.1	
	240	4.3	5.1	5.1	5.0	4.9	4.8	4.7	6.0	6.0	
	1440	6.7	10.3	8.1	7.4	7.2	7.0	6.9	6.6	8.9	
Xyl-3	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	60	4.6	6.3	5.8	6.0	6.4	6.1	6.2	6.0	6.0	
	240	5.1	9.5	9.3	9.7	9.6	9.7	9.7	9.1	9.1	
	1440	7.7	25.9	25.2	25.6	26.2	25.9	27.4	25.4	22.0	

<sup>a</sup> Results obtained from duplicates; the coefficient of variation was 3%. Calculation was made by taking the initial values in the sample without enzyme at 0 min.

contained high ( $MW \sim 85 \times 10^4$  using a pullulan calibration standard) and medium ( $MW \sim 38 \times 10^4$ ) MW AX after a 24 h treatment with any of the three xylanases. LMWAX ( $MW < 5 \times 10^4$ ) were extracted from BrT1 with the three xylanases. Low and medium MW AX were extracted from BrT2, BrT3, BrT4, and BrT8 with the three xylanases. Xyl-3 extracted AX of medium and low MW from brans BrT1, BrT2, BrT3, and BrT8. BrT4, BrT5, BrT6, and BrT7 contained larger sized AX than the other BrT, BrT6 presenting the highest MW AX.

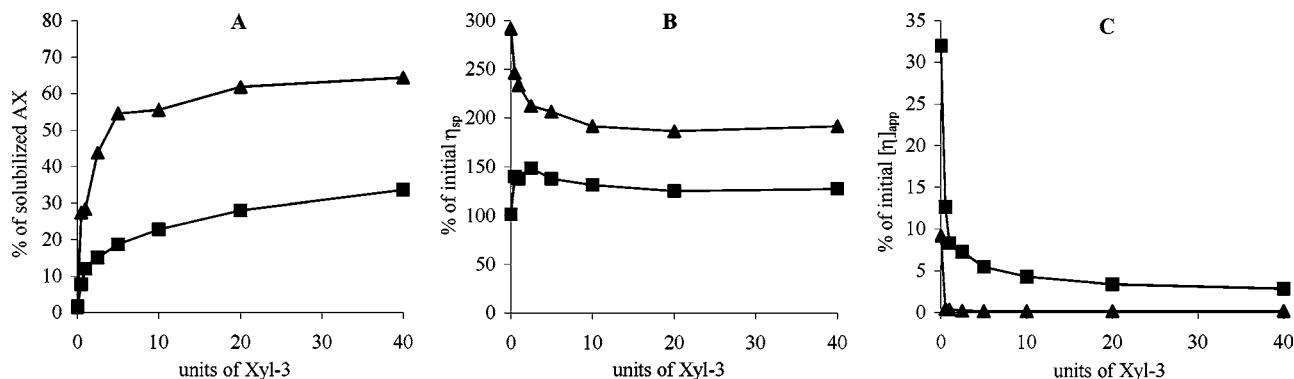
These results confirm that a thermomechanical treatment of bran can improve the enzymatic solubilization of AX and that Xyl-3 was the more efficient among the tested enzymes to release AX from both Br1 and BrT. BrT6 was the extruded bran that presented the largest AX solubilization rates with a high apparent molecular size of solubilized AX.

In conclusion, BrT6 and Xyl-3 were chosen for further experiments using different concentrations of xylanase and combinations with other enzymes.

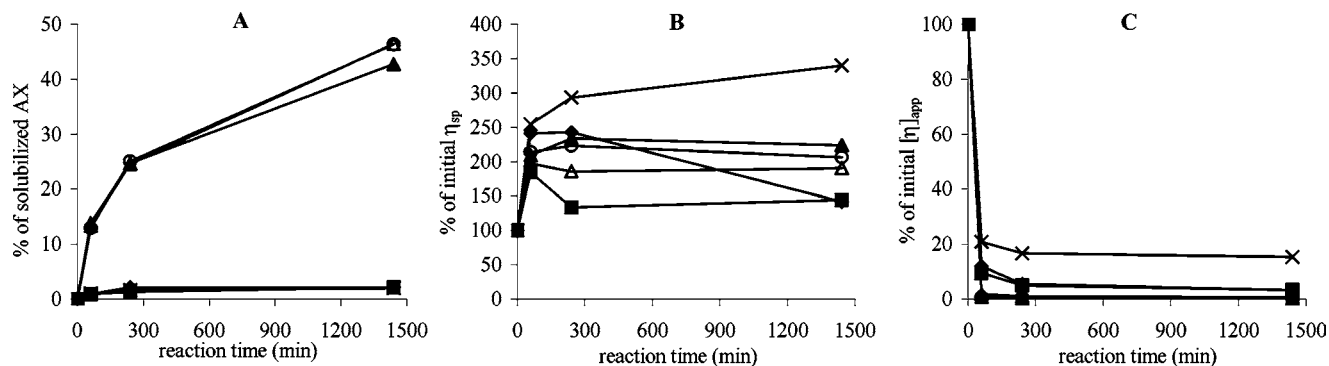
**Effect of Different Concentrations of Xyl-3 on Br1 and BrT6.** The effect of different concentrations of enzyme was studied after 1, 4, and 24 h of reaction at 20 °C. Only results after 24 h of reaction are presented (**Figure 1**). In both, Br1 and BrT6, increasing concentrations of Xyl-3 provoked higher rates of solubilization (**Figure 1A**). For each dose of enzyme, AX solubilization (**Figure 1A**) and  $\eta_{sp}$  (**Figure 1B**) were between 2- and 4-fold more important in BrT6 than in Br1. Solubilization of AX was accompanied by a depolymerization, which increased with the dose of enzyme (**Figure 1C**) and was more important with BrT6. From these results, it is observed that high doses of Xyl-3 extracted LMWAX and/or depolymerized the WEAX.

With 2.5 units of Xyl-3, intermediate values of solubilization and viscosity were obtained (**Figure 1**). Increasing doses of Xyl-3 allowed increasing solubilizations, but increased also AX depolymerization. With 2.5 units an appreciable solubilization of AX was obtained from Br1 and BrT6, without great losses in viscosity. For the rest of this work, this dose was chosen for enzyme combinations and oxidative gelation studies.

**Oxidative Gelation of BrT6 and Rye Flour Extracts Treated with Xyl-3.** No increase in viscosity was observed when  $H_2O_2/POD$  was added to the BrT6 extract rich in AX (32.9 mg of AX/mL) (results not shown). The  $H_2O_2/POD$  doses were then increased 4- and 8-fold to test the effect of the oxidizing system on gelation, but no thickening was observed.



**Figure 1.** Effect of different doses of Xyl-3 on arabinoxylans from (■) untreated bran (Br1) and (▲) extruded bran (BrT6), after 24 h of reaction at 20 °C. Results are expressed as percentages of (A) arabinoxylan solubilization from 20 mg of bran arabinoxylan, (B) initial specific viscosity, and (C) initial apparent intrinsic viscosity. The coefficient of variation was 3%.



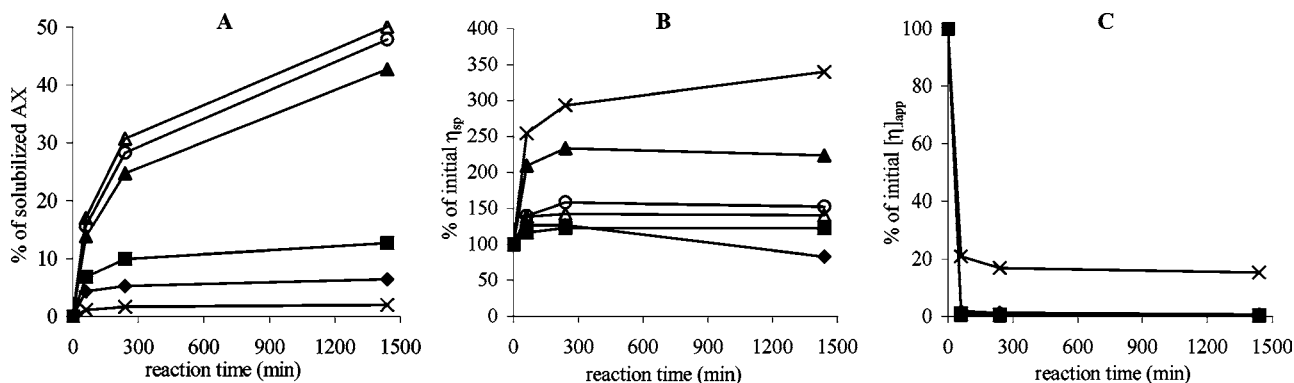
**Figure 2.** Effect of the combination of 2.5 units of Xyl-3 with different concentrations of (1,4)- $\beta$ -D-arabinoxylan arabinofuranohydrolase (AXH) on the solubilization of AX from extruded rye bran (BrT6) at different reaction times: blank (x); 0.2  $\mu$ g of AXH (◆); 1  $\mu$ g of AXH (■); control Xyl-3 (▲); Xyl-3 + 0.2  $\mu$ g of AXH (○); Xyl-3 + 1  $\mu$ g of AXH (△). Results are expressed as percentages of (A) arabinoxylan solubilization, (B) initial specific viscosity, and (C) initial apparent intrinsic viscosity. The coefficient of variation was 3%.

With the aim to compare to a flour extract rich in Xyl-3-solubilized AX, two flour extracts were tested containing 6.2 mg of AX/mL and 3.1 mg of AX/mL, using the same doses of  $H_2O_2/POD$ . As observed before with laccase (50), no thickening was obtained. After 30 min of reaction with  $H_2O_2/POD$ , total FA on Xyl-3 flour extracts decreased by 35.9 and 89.6%, and FA dimers increased by 32.6 and 59.6% of the initial FA dimer content in the blank (Xyl-3 flour extract without  $H_2O_2/POD$ ), for 1- and 4-fold the  $H_2O_2/POD$  dose, respectively. The Xyl-3 flour extract contained 13.9 and 0.9 nmol of FA and FA dimers/mg of AX, respectively. According to Vinkx et al. (19), a pentosan-protein solution obtained from whole rye, and containing 2 mg of AX/mL and 4.8 nmol of FA/mg of rye AX, could gel when  $H_2O_2/POD$  was added. It is likely that the extracted AX were strongly degraded and therefore unable to form gels by FA dimerization.

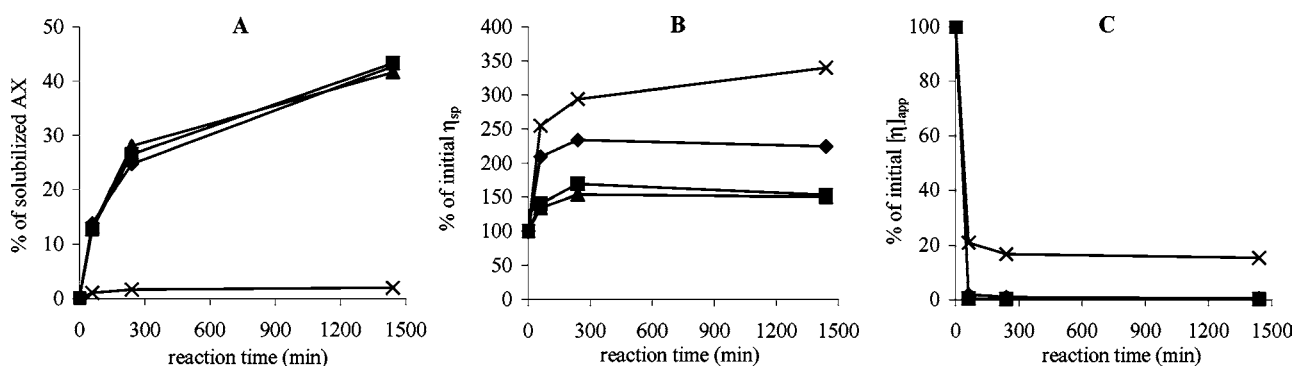
**Effect of Xyl-3 Combined with AXH,  $\beta$ -Glucanase, or FAE on BrT6. Combination of Xyl-3 and AXH.** Solubilizations of AX in the blank and in samples with added AXH (0.2 and 1  $\mu$ g) were similar (Figure 2A). In the presence of Xyl-3, the solubilization was similar (~25%) to that in the control (BrT6 + Xyl-3) until 4 h of reaction. Then values were slightly superior in samples with added AXH ( $46.4 \pm 1.4\%$ ) compared to those without AXH ( $42.7 \pm 1.3\%$ ). Luonteri et al. (39) observed that in combination with xylanase, a low  $\alpha$ -arabinosidase dosage increased the AX solubilized compared to the xylanase-treated rye bran. Samples with AXH presented lower viscosities than in the blank. After 24 h, samples with both enzymes presented ~2-fold the initial  $\eta_{sp}$  against 3.4-fold for

the blank (Figure 2B). In both cases, a decrease in  $\eta_{sp}$  was observed after 1 h of reaction with 1  $\mu$ g of AXH and after 4 h of reaction with 0.2  $\mu$ g of AXH. According to Andrewartha et al. (56), Luonteri et al. (39), and Schooneveld-Bergmans et al. (57), the rodlike shape of AX becoming less asymmetric provokes a decrease in  $\eta_{sp}$ . More recently, Dervilly et al. (58) and Dervilly-Pinel et al. (59) demonstrated that the less arabinose substituted AX present lower hydrodynamic volumes and a lower intrinsic viscosity. SE-HPLC profiles of blank and samples with AXH were similar (not shown) and also in the case of the reference and the samples with both enzymes Xyl-3 and AXH. In conclusion, AXH did not exhibit a synergistic effect with Xyl-3. Kormelink et al. (32) observed that AXH was strongly synergistic with endo-(1,4)- $\beta$ -xylanase for the degradation of AX in wheat. The synergistic effect reported by these authors was evaluated by measuring the amount of released reducing sugars.

**Combination of Xyl-3 and  $\beta$ -Glucanase.** Solubilization of AX was improved by the addition of  $\beta$ -glucanase in both samples with and without Xyl-3. AX were rendered more extractable with the higher doses of  $\beta$ -glucanase (Figure 3A). After 24 h of reaction, solubilization rates were  $2.0 \pm 0.1$ ,  $6.4 \pm 0.2$ , and  $12.7 \pm 0.4\%$  for the blank and samples with 1000 BGU and 5000 BGU of  $\beta$ -glucanase, respectively, and  $42.7 \pm 1.3$ ,  $47.9 \pm 1.4$ , and  $50.1 \pm 1.5\%$  for samples treated with Xyl-3, Xyl-3/1000 BGU, and Xyl-3/5000 BGU, respectively. In the presence of  $\beta$ -glucanase, viscosity values were lower than in the blank and control (Figure 3B,C). It is likely that the hydrolysis of the  $\beta$ -glucan associated with AX in bran liberated



**Figure 3.** Effect of the combination of 2.5 units of Xyl-3 with different concentrations of  $\beta$ -glucanase on the solubilization of AX from rye extruded bran (BrT6) at different reaction times: blank ( $\times$ ); 1000 BGU ( $\blacklozenge$ ); 5000 BGU ( $\blacksquare$ ); control Xyl-3 ( $\blacktriangle$ ); Xyl-3 + 1000 BGU  $\beta$ -glucanase ( $\circ$ ); Xyl-3 + 5000 BGU  $\beta$ -glucanase ( $\triangle$ ). Results are expressed as percentages of (A) arabinoxylan solubilization, (B) initial specific viscosity, and (C) initial apparent intrinsic viscosity. The coefficient of variation was 3%.



**Figure 4.** Effect of the combination of 2.5 units of Xyl-3 with different concentrations of (1,4)- $\beta$ -D-arabinoxylan arabinofuranohydrolase (AXH) and  $\beta$ -glucanase on the solubilization of AX from rye extruded bran (BrT6) at different reaction times: blank ( $\times$ ); control Xyl-3 ( $\blacklozenge$ ); Xyl-3 + 0.2  $\mu$ g of AXH + 1000 BGU  $\beta$ -glucanase ( $\blacksquare$ ); Xyl-3 + 0.4  $\mu$ g of AXH + 2000 BGU  $\beta$ -glucanase ( $\blacktriangle$ ). Results are expressed as percentages of (A) arabinoxylan solubilization, (B) initial specific viscosity, and (C) initial apparent intrinsic viscosity. The coefficient of variation was 3%.

more AX in assays without xylanase, making AX more accessible to this enzyme. To obtain enzymatic extracts with high viscosity, it would be better to conserve polymerized  $\beta$ -glucan, which also participated in the extract viscosity (14, 35).

**Combination of Xyl-3, AXH, and  $\beta$ -Glucanase.** The combination of AXH and  $\beta$ -glucanase presented no marked improvement either in the rate of extraction of AX from BrT6 or in the viscosity of the extracts (Figure 4). SE-HPLC profiles (not shown) demonstrated that LMWAX were liberated with increasing doses of AXH and  $\beta$ -glucanase. The combination of AXH and  $\beta$ -glucanase did not present any synergistic effect with Xyl-3.

**Combination of Xyl-1 or Xyl-3 with FAE.** No improvements in AX rates of extraction from BrT6 or viscosity profiles were observed when FAE was combined at different doses with 2.5 units of either Xyl-1 or Xyl-3 (not shown). After 24 h of reaction, 35  $\pm$  1.1 and 42  $\pm$  1.3% of AX solubilization and viscosities 1.6- and 2-fold of the initial  $\eta_{sp}$  were observed in the control and the assays of FAE with Xyl-1 or Xyl-3, respectively. In contrast, when only FAE was added to BrT6, an increase of the extractability of AX and of their viscosity profiles was observed. At 24 h of reaction, the increases in WEAX were 2.7  $\pm$  0.1, 3.7  $\pm$  0.1, and 4.8  $\pm$  0.1% for the blank BrT6 and the samples BrT6-FAE (0.026 unit) and BrT6-FAE (0.26 unit), respectively. Specific viscosity increased 3.2, 3.8, and 4-fold, respectively. In this case, the addition of FAE helped to solubilize HMWAX. These results were confirmed

by SE-HPLC (not shown). More HMWAX were extracted after 24 h when doses of FAE were increased. No profile differences were observed between samples of BrT6 with xylanases and FAE at different doses. It is likely that xylanases masked the effects of FAE. When FAE was added alone, it liberated some HMWAX linked to the cell walls through FA.

Released FA was quantified in the BrT6 samples treated for 0, 1, and 4 h at 20 °C with FAE and xylanases (Table 5). It was observed that FA was liberated in a higher amount when FAE was combined with xylanases, as reported by Williamson et al. (40). After 4 h of reaction, 0.95  $\pm$  0.04 nmol/mL of FA was liberated with FAE (0.026 unit), and 1.94  $\pm$  0.08 and 5.82  $\pm$  0.23 nmol/mL were liberated for Xyl-1 and Xyl-3 combined with FAE (0.026 unit), respectively. Differences between the two xylanases could be explained by the higher solubilizing effect of Xyl-3. According to Bartolomé et al. (36), the source of the xylanase can influence the action of *A. niger* FAE on bran. These authors explained that the synergistic interaction is directly related to the rate of the hydrolysis of bran and to the ability of the xylanase to release fragments more suitable for attack by the esterase. In our case, the hydrolysis of WUAX bran by xylanases was not enhanced by the presence of FAE, thus no reciprocal cooperation between these enzymes was observed as reported by Bartolomé et al. (36) and Williamson et al. (40, 41). It is likely that FA liberated from the controls was oxidized after 4 h of the reaction with both xylanases, but further experiments should be done to confirm this hypothesis.

**Table 5.** Content of Free Ferulic Acid (FA) in Samples of Extruded Bran (BrT6), Untreated Bran (Br9), and High-Shear-Treated Bran (BrT9) after Different Reaction Times at 20 °C with 2.5 Units of Xyl-1 or Xyl-3 Combined with Different Doses of Ferulic Acid Esterase (FAE)

sample	nmol of FA/mL <sup>a</sup>				
	BrT6			Br9	BrT9
	0 h	1 h	4 h	4 h	4 h
blank (without enzyme)	0.09	0.09	0.10	0.05	0.04
+ FAE (0.026 unit)	0.09	0.58	0.95	0.00	0.05
+ FAE (0.26 unit)	0.09	1.04	1.13	0.00	0.06
+ FAE (2.6 units)	nd <sup>b</sup>	nd	nd	0.00	0.08
+ Xyl-1	0.09	0.22	0.05	0.00	0.06
+ Xyl-3	0.09	0.25	0.06	0.00	0.06
+ FAE (0.026 unit) + Xyl-1	0.09	1.64	1.94	0.00	0.05
+ FAE (0.26 unit) + Xyl-1	0.09	2.51	2.69	0.00	0.09
+ FAE (2.6 units) + Xyl-1	nd	nd	nd	0.00	0.10
+ FAE (0.026 unit) + Xyl-3	0.09	2.37	5.82	0.00	0.15
+ FAE (0.26 unit) + Xyl-3	0.09	3.71	7.79	0.00	0.49
+ FAE (2.6 units) + Xyl-3	nd	nd	nd	0.00	0.99

<sup>a</sup> Results obtained from duplicates; the coefficient of variation was 4%. <sup>b</sup> Not determined.

**Table 6.** Effect of Different Doses of Xylanases Xyl-1 and Xyl-3 on Untreated (Br9) and High-Shear-Treated (BrT9) Brans after 24 h of Reaction at 20 °C

sample	dose of enzyme (units)	% of solubilized AX <sup>a,b</sup>	% of initial $\eta_{sp}$ <sup>a,c</sup>	% of initial $[\eta]_{app}$ <sup>a,d</sup>
Br9 + Xyl-1	0.0	2.7	148.2	48.9
	0.5	10.2	186.7	21.3
	2.5	13.2	174.3	15.8
	5.0	15.2	157.1	12.6
	10.0	17.4	127.7	9.1
	20.0	23.9	105.6	5.6
BrT9 + Xyl-1	0.0	0.7	78.4	69.3
	0.5	5.4	86.6	39.8
	2.5	10.2	81.6	25.5
	5.0	12.3	76.5	21.0
	10.0	15.7	70.3	16.1
	20.0	19.3	51.4	10.1
Br9 + Xyl-3	0.0	2.7	148.2	48.9
	0.5	13.5	217.3	19.1
	2.5	18.6	167.8	11.1
	5.0	21.9	148.3	8.5
	10.0	26.7	130.0	6.2
	20.0	31.6	114.5	4.7
BrT9 + Xyl-3	0.0	0.7	78.4	69.3
	0.5	12.6	97.7	26.1
	2.5	18.6	87.7	17.5
	5.0	22.7	78.3	13.3
	10.0	27.8	69.7	10.0
	20.0	29.1	53.4	7.4
	40.0	36.8	44.9	5.1

<sup>a</sup> Results obtained from duplicates; the coefficient of variation was 3%. Calculation was made by taking the initial values in the sample without enzyme at 0 min.

<sup>b</sup> Arabinoxylans. <sup>c</sup> Specific viscosity. <sup>d</sup> Apparent intrinsic viscosity.

**High-Shear-Treated Bran (BrT9).** Effect of Different Concentrations of Xyl-1 or Xyl-3 on Br9 and BrT9. Experiments were made with both untreated (Br9) and high-shear-treated (BrT9) brans. The effect of the enzymes on AX solubilization,  $\eta_{sp}$  and  $[\eta]_{app}$ , was followed at 0, 1, 4, and 24 h. Only results obtained after 24 h of reaction are shown in **Table 6**. The highest rates of solubilization of AX from Br9 and BrT9 were obtained with Xyl-3 for all doses. Better results of AX solubilization

and  $\eta_{sp}$  were observed with Br9 than with BrT9. With 0.5 unit of enzyme, AX presented higher viscosity values with Xyl-3 than with Xyl-1. The highest values of  $\eta_{sp}$  were observed when 0.5 unit of Xyl-1 or Xyl-3 was added. For both xylanases, when 2.5 units was added, samples presented an intermediate AX solubilization and still a high viscosity. For both xylanases, depolymerization of WEAX increased with the dose of enzyme. WEAX degradation was higher with Xyl-3 at doses above 2.5 units, which explains the lower value in  $[\eta]_{app}$ . Comparison of  $\eta_{sp}$  profiles of Br9 or BrT9 with Xyl-1 and Xyl-3 revealed similar results. High doses of enzyme provoked an extensive depolymerization of WEAX as observed by SE-HPLC (not shown).

In the blanks at 0 min, the reaction mixes of Br9 and BrT9 contained 0.05 and 0.18 mg of AX/mL, respectively. It is likely that the high shear treatment provoked the solubilization of some AX on BrT9. After 24 h, more AX were extracted from the blank Br9 ( $2.7 \pm 0.08\%$ ) than from BrT9 ( $0.7 \pm 0.02\%$ ), which would indicate that more endogenous enzymes are present in Br9.

Shiiba et al. (44) already observed that a steam–pressure treatment of wheat bran could increase the extractability of AX in combination with a cellulase from *Trichoderma viride*. They demonstrated that one treatment only, either steam–pressure or maceration with cellulase, did not provide large amounts of cell-wall hydrolysate, but both treatments were necessary to extract AX. In this work, in general, the high shear treatment did not improve AX extractability in the presence of xylanases as expected, and the viscosity of extracts was lower than those from Br9. This was the converse for the bran used in the first part of the work (bran, BrT6). The maximum values of AX extraction from Br9 and BrT9 did not reach the same limit value after extended reaction time with different doses of enzyme. This suggests the presence of xylanase inhibitory compounds in rye bran. The presence or inhibitors in cereals has been reported by Rouau and Surget (60), McLaughlan et al. (61), and Goesart et al. (62).

When the two bran batches were compared, the extraction of AX reached a pseudo-plateau in the Br1 blank but increased continuously with Br9. For a maximum of  $1.7 \pm 0.05\%$  of AX solubilization from Br1, a maximum of  $2.7 \pm 0.08\%$  was observed from Br9, after 24 h of reaction. The maximum increases in viscosity were observed after 1 h of reaction (1.6- and 2.8-fold the initial  $\eta_{sp}$  for Br1 and Br9, respectively). After 24 h of reaction, viscosity values were 1- and 1.5-fold the initial  $\eta_{sp}$  for Br1 and Br9, respectively. The total AX content of Br1 (24.5% dm) was higher than that of Br9 (21% dm). In the blanks, the percentage of AX solubilization and the  $\eta_{sp}$  values were more important and higher  $[\eta]_{app}$  values were found in Br9 than in Br1. This suggests more activity of endogenous endoxylanases in Br9.

In contrast with BrT9, BrT6 did not contain WEAX. However, only  $0.7 \pm 0.02\%$  of the initial AX were solubilized after 24 h from the BrT9 blank, versus  $2.0 \pm 0.06\%$  from the BrT6 blank. The MW of WEAX from BrT9 was inferior to that of BrT6. Thus, the high shear treatment provoked a physical degradation of BrT9 AX. In the presence of 2.5 units of Xyl-3, the extraction rates were 2-fold lower from BrT9 than from BrT6. A maximum of  $43.7 \pm 1.3\%$  was attained for BrT6 at 24 h, versus  $18.6 \pm 0.6\%$  for BrT9.

In conclusion, the extrusion treatment was more efficient than the high shear treatment to extract AX in the presence of Xyl-3.

*Effect of Different Concentrations of FAE Combined with Xyl-1 and Xyl-3 on Br9 and BrT9.* No improvements in AX solubilization or viscosity profiles were observed when FAE was added at different doses to Br9 with or without xylanases (not shown). Xyl-3 solubilized more AX ( $20 \pm 0.6\%$ ) than Xyl-1 ( $13 \pm 0.4\%$ ) with or without FAE. No differences appeared either in the SE-HPLC profiles (not shown). As observed for Br9, FAE did not improve the extraction of AX from BrT9 with or without xylanases. Without xylanases, no improvements in the solubilization were observed as in BrT6. Rates of AX extraction were better with Xyl-3 as observed before. SE-HPLC profiles were similar between the blank and the samples with FAE without xylanase and between the reference and the samples with FAE and xylanases (not shown).

Free FA was quantified in Br9 and BrT9. No free FA was found in Br9. In contrast, results obtained with BrT9 were similar to those from BrT6. More FA was released when FAE was combined with xylanases. More synergism was observed with Xyl-3 (Table 5). It is likely that FAE had more affinity for AX liberated by Xyl-3 than for AX liberated with Xyl-1. More FA was liberated from BrT6, but also more AX. AX ester-linked FA should be measured in the extracts to verify if AX extracted with Xyl-3 had higher FA contents than those solubilized by Xyl-1.

**Conclusions.** Three different xylanases (Xyl-1, Xyl-2, and Xyl-3) were tested on untreated rye bran and on different extruded and high-shear-treated brans. The difficulty of extracting AX from bran using enzymatic methods was demonstrated, as was the importance of complementing the enzymatic extraction with a physical pretreatment. The highest extrusion energy and temperature conditions gave the best Xyl-3 AX solubilization rates. The high shear treatment did not improve the AX extraction as expected. The AX FA content and their molecular size are important parameters in view of cross-linking AX in rye products. An excess of Xyl-3 provoked a hydrolysis of AX to short molecules, which cannot gel upon treatment with an oxidizing agent.

$\beta$ -Glucanase in combination with Xyl-3 improved the extraction of AX, but it did not have a marked effect on the viscosity of the extracts from BrT6. AXH in combination with Xyl-3 improved the AX extraction only after 24 h of reaction. No synergism was observed when FAE and xylanases were combined. Only an improvement of the rates of solubilization and on the molecular size of AX was observed when FAE was added alone to BrT6.

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