

Solubilization of Arabinoxylans from Isolated Water-Unextractable Pentosans and Wheat Flour Doughs by Cell-Wall-Degrading Enzymes¹

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ABSTRACT

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Water-unextractable pentosans (WUP) isolated from the flours of three wheat cultivars (Apollo, Soissons, Thésée) were treated with enzymes to solubilize the arabinoxylans. The water-unextractable arabinoxylans from the three cultivars had similar susceptibility to solubilization by enzymes: Grindamyl S 100 (GS100), a commercial preparation for baking, rich in pentosanase activities that originated from an *Aspergillus niger* culture; and three endoxylanases (E1, E2, E3), an arabinofuranosidase (Af), a β -glucanase (β G), and a ferulate esterase (FAE) purified from GS100. A cellulase (C) and a pure endoglucanase (eG) from *Trichoderma reesei* were also used. GS100 was able to solubilize high molecular weight arabin-

oxylans (HMWAX) from WUP that markedly enhance the viscosity of the reaction mixture supernatants. The endoxylanase E1 was responsible for this solubilizing activity of GS100, whereas E2 and E3 made only a very low contribution. Combining E1 with FAE led to a limited increase in the arabinoxylan-solubilizing effect. Also, enzymes hydrolyzing cellulose and β -glucans slightly improved the arabinoxylan solubilization from WUP when combined with GS100 or E1, but produced arabinoxylans of lower intrinsic viscosity. Similar effects of the enzymes were observed on arabinoxylan solubilization when applied to dough instead of isolated WUP.

Wheat flours contain 2–3% pentosans. These components originate from the endosperm cell-walls of wheat grains. They are mainly composed of arabinoxylans with a linear backbone of β -1,4 linked xyloses, half of which carry single arabinofuranose residues at O-3 or at both O-2 and O-3 positions. Some of the arabinoses are esterified with phenolic acids, mostly ferulic acid (Izydorczyk and Biliaderis 1995).

About one-third of the flour pentosans is extractable with water. Water-extractable pentosans (WEP), due to their high molecular weight arabinoxylans (HMWAX) form viscous solutions and increase dough viscosity (Rouau 1993). Although conflicting results have been published about the functional role of water-unextractable pentosans (WUP) and WEP in breadmaking (Izydorczyk and Biliaderis 1995), it appeared that the water-extractable arabinoxylans (WEAX) content of wheat flours was positively correlated to their breadmaking potential in a French-type breadmaking process, whereas total arabinoxylans (AXT) were detrimental to dough and bread quality (Rouau et al 1994).

Certain enzymes are able to solubilize arabinoxylans from WUP (Gruppen et al 1993). Grindamyl S 100 (GS100), a commercial preparation used as a dough and bread improver, can release HMWAX from flour cell-wall material, due to the presence of a specific endoxylanase (Rouau and Moreau 1993, Rouau 1993, Rouau et al 1994). The efficiency of the enzyme preparation as an improver was related to its ability to solubilize polysaccharides. However, the amount of HMWAX that can be released during a breadmaking process is limited because adding too high a level of enzyme causes a degradation of WEAX and solubilized arabinoxylans. In such a case, the effect of the depolymerization of arabinoxylans is clearly negative on dough characteristics (Rouau et al 1994). Generally, the detrimental effect occurs while amounts of arabinoxylans are still high in WUP and potentially available for solubilization.

The aim of this work was to examine and to optimize the enzymatic release of HMWAX from wheat flour WUP, isolated and in dough to obtain a good balance between a large arabinoxylan release and a high M_r of the solubilized molecules so that the func-

tional properties are preserved. The solubilization process was investigated using enzymes acting specifically on arabinoxylans and also other cell-wall-degrading enzymes (C, β G) that could help the arabinoxylan release by liberating them from associations with other cell-wall components such as cellulose and β -glucans. Indeed, Rouau and Moreau (1993) showed that cell-wall material resistant to degradation by GS100 was enriched in β -glucans. Also, the extraction of arabinoxylans from whole rye by a xylanase was favored by a combination with protease and β G (Harkonen et al 1995).

MATERIALS AND METHODS

Flours

Wheat flours from cultivars Apollo, Soissons, and Thésée were provided by Grands Moulins de Paris (Genevilliers, France). Ash contents were determined by incineration at 900°C as 0.48, 0.57, and 0.56%, respectively. Protein contents ($N \times 5.7$) were determined by a Kjeldahl procedure as 9.8, 10.0, and 10.2%, respectively. Arabinoxylan contents were calculated as the sum of arabinose and xylose determined by gas-liquid chromatography of alditol acetates obtained after acid hydrolysis of flour samples and flour-water extracts (Rouau 1993). Contents in AXT and WEAX were 1.7 and 0.46%, 1.6 and 0.37%, 2.0 and 0.56%, for Apollo, Soissons, and Thésée, respectively.

WEP and WUP

Pentosans fractions were obtained from the three flours using the procedure developed by Faurot et al (1995).

Enzymes

Grindamyl S 100 (GS100) is a commercial enzyme preparation derived from the fermentation of a selected strain of *Aspergillus niger*. GS100 is a complex preparation that contains different kinds of activity, including xylanase, Af, β G, and FAE. Some enzymes were purified to homogeneity from GS100: three endoxylanases (E1, E2, E3), an arabinofuranosidase (Af), FAE, and β G. C, partly purified, was derived from a crude fermentation of *Trichoderma reesei*. GS100, E1, E2, E3, Af, FAE, β G and C were provided by Danisco Ingredients (Brabrand, Denmark). Enzymes were purified by desalting on a Sephadex G25 SF (Pharmacia, Uppsala, Sweden) column (50 \times 200 mm, distilled water), followed by ionic exchange chromatography on a Q-Sepharose (Pharmacia) column (25 \times 100 mm, buffer A: 20 mM piperazine buffer (pH 5.5), buffer B: A + 1M NaCl, gradient: 0–100% B), hydrophobic interaction chromatography on a Phenyl-Sepharose (Pharmacia) column (16 \times 100 mm, buffer A: 50 mM phosphate buffer (pH 6.0) + 1.5M (NH₄)₂SO₄, buffer B:

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50 mM phosphate buffer (pH 6.0), gradient: 0–100% B), and size-exclusion chromatography on a Superdex 75 (Pharmacia) column (50 × 600 mm, buffer: 0.2M phosphate buffer (pH 7.0) + 0.2M NaCl). The homogeneity of the purified enzymes was assessed by SDS-PAGE and iso-electric focusing (IEF) using precast gels (Novex, San Diego, CA). E1, E2, E3, βG, and FAE presented a single band after silver staining performed according to manufacturer's instructions. E1, E2, Af, FAE, βG, and C were standardized on a weight basis using starch or lactose as filler with GS100 as reference, so that a given mass of one of the pure enzyme preparations contained the same activity as the same mass of GS100. The substrate used to follow the purification and standardize the endoxylanases E1, E2, and E3 was a dyed-xylan (Azo Wheat Arabinoxylan, Megazyme, Australia). An endo-1,4-glucanase (eG) from *Trichoderma reesei*, obtained as a pure protein was kindly provided by Massiot (1992).

Characterization of WUP and WEP

The carbohydrate content of WUP and WEP was analyzed by gas-liquid chromatography of alditol acetates obtained after sulfuric acid hydrolysis (2M H₂SO₄, 2 hr) on a DB 225 capillary column (J&W Scientific) according to the procedure of Blakeney et al (1983). Inositol was used as an internal standard. Arabinoxylans present in reaction supernatants and released by enzymes were also determined according to a semiautomated colorimetric method (Rouau and Surget 1994). Ferulic and dehydrodiferulic acids were determined by RP-HPLC after saponification according to the procedure described by Figueroa-Espinoza and Rouau (1998).

Viscometry

Viscometric determinations used flow times of solutions measured at 25°C with an Ubbelohde capillary viscometer. Relative viscosity (η_{rel} = flow time of sample/flow time of solvent) and specific viscosity ($\eta_{sp} = \eta_{rel} - 1$) were calculated using Na-acetate buffer 0.1M (pH 5.0) as a solvent. An apparent intrinsic viscosity ($[\eta]_{app}$, mL/g) was evaluated using the Morris equation (Morris 1984), where c (expressed as mg/mL) represented the arabinoxylan concentration: $[\eta]_{app} = 1/c \times [2[\eta_{sp} - I_n(\eta_{rel})]]^{0.5} \times 1,000$ assuming that only arabinoxylans contributed to the viscous properties of the reaction supernatants and dough extracts (Rouau et al 1994).

Statistical Analysis

The precision of the methods were determined by multifactor analysis of variance (Stat-ITCF computer package, ITCF, Paris France) using large sets of replicate determinations (20) on two different samples. The coefficients of variation for the determinations of carbohydrate contents, extents of solubilization, specific and apparent intrinsic viscosities, and ferulic acid measurement were 2.5, 3, 3, 3.1, and 4%, respectively. Samples were analyzed in duplicate and results are expressed as mean values, on a dry basis.

Enzyme Treatments of Pentosans

Amounts of WUP containing 40 mg of arabinoxylan were weighed in 10-mL centrifuge tubes and suspended in 5 mL of Na-acetate buffer 0.1M (pH 5.0) without (control) or with enzymes and agitated (40 rpm) on a rotary shaker at 25°C for 4 hr. Tubes were then centrifuged 1 hr at 15,000 × g , 25°C. Supernatants were recovered and boiled for 10 min. After cooling, they were centrifuged for 10 min at 15,000 × g and filtered through 2.7- μ m pore size filters (Millipore). The arabinoxylan content of the supernatants was determined colorimetrically (Rouau and Surget 1994). The percentage of WUP arabinoxylan solubilization was calculated as: (amount of arabinoxylan in solution/amount of arabinoxylan initially present in WUP) × 100. The viscosity of the supernatant was determined.

Solutions of WEP containing 20 mg of arabinoxylan, in 4 mL of Na-acetate buffer 0.1M (pH 5.0) were prepared by overnight agitation (40 rpm) on a rotary shaker at 4°C, followed by centrifugation at 15,000 × g for 10 min. The final arabinoxylan content was determined colorimetrically (Rouau and Surget 1994). Volumes of solutions corresponding to 15 mg of soluble arabinoxylan were adjusted to 4 mL with the same buffer. Na-acetate buffer 0.1M (pH 5.0) (1 mL) containing enzymes was added. After 4 hr of rotative agitation at 25°C, samples were boiled for 10 min to stop the reaction and centrifuged for 10 min at 15,000 × g . Supernatants were filtered through 2.7- μ m filters and the viscosity was determined.

Using the procedure above, WUP containing 40 mg of insoluble arabinoxylan was added to the WEP solution to obtain the same ratio of water-extractable to unextractable arabinoxylan as in flour. The reaction volume was made up to 5 mL using Na-acetate

TABLE I

Composition of Water-Extractable Pentosan (WEP) and Water-Unextractable Pentosans (WUP) Obtained from Three Wheat Cultivar Flours^{a,b}

	Apollo		Soissons		Thésée	
	WEP	WUP	WEP	WUP	WEP	WUP
Arabinoxylan ^c	38.8	23.4	34.0	32.2	41.9	29.7
Ferulic acid ^d	1.8	8.0	2.6	9.5	2.3	10.2
Glucose ^c	3.2	...	10.4	...	5.0	...
Cellulose, β-glucan ^c	...	11.8	...	4.4	...	4.8
Starch ^c	...	43.7	...	34.3	...	46.8
Protein ^c	30.9	13.7	28.8	11.9	31.0	7.1

^a Prepared according to Faurot et al (1995).

^b Mean values of duplicates.

^c Expressed as % of WEP or WUP (db).

^d Expressed as mg/g of arabinoxylan contained in WEP or WUP.

TABLE II

Effects of Different Concentrations of Grindamyl S 100 on the Arabinoxylan Solubilization of Water-Unextractable Pentosans (WUP) from Three Wheat Flours^a

	Apollo WUP			Soissons WUP			Thésée WUP		
	0.35	1.8	3.5	0.35	1.8	3.5	0.35	1.8	3.5
Arabinoxylan solubilization ^b	18.0	42.3	53.2	16.5	43.2	51.6	21.0	42.6	51.0
Specific viscosity	1.1	1.2	0.9	0.9	1.5	1.1	1.1	1.3	0.9
Apparent intrinsic viscosity ^c	505	245	160	475	290	190	445	255	160

^a Mean values of duplicates. Grindamyl S 100 to arabinoxylan ratio × 10⁻³.

^b Expressed as % db of arabinoxylan content of WUP.

^c Expressed as mL/g of arabinoxylan in solution.

buffer 0.1M (pH 5.0). The percentage of solubilization was calculated taking into account the initially soluble arabinoxylan.

Enzymatic Solubilization of Arabinoxylans in Doughs

Reconstituted or partially reconstituted flours were made up with gluten (Vital Gluten, Roquette, Lestrem, France), WUP, WEP, and starch (Wheat Starch, Roquette, Lestrem, France). The level of gluten was kept at 10% (db) of the reconstitution. WUP and WEP were added to yield arabinoxylan concentrations of 1.44% water-unextractable and 0.56% water-extractable arabinoxylan in the reconstituted flour, equal to the arabinoxylan concentration in the Thésée flour. The mixture was completed by starch. For example, Thésée flour was reconstituted with 0.86 g (db) of gluten, 0.42 g of WUP, and 0.11 g of WEP, and was made up to 8.6 g with starch. Doughs were formed at 25°C in a thermostated 10-g mixograph (National Mfg. Co., Lincoln, NE). The level of dough hydration was 61.5% (for a 14% moisture content of the flour) corresponding to the usual value for breadmaking with Thésée flour. After mixing for 9 min, doughs were allowed to rest for 50 min at the same temperature. The doughs were then immediately frozen and freeze-dried. The dried doughs were ground using a water-cooled laboratory grinder (IKA-Werk A10, Janke and Kunkel, Staufen, Germany) and sieved through a 0.5-mm screen. The resulting powder was suspended in distilled water (1:4 ratio of solid to liquid) at 4°C in centrifuge tubes and agitated for 15 min at 40 rpm. Tubes were then centrifuged for 15 min at 15,000 × g, 4°C. After boiling for 10 min, supernatants were centrifuged for 5 min at 15,000 × g, 20°C, and filtered through a 2.7-µm filter. The percentage of WUP arabinoxylan solubilization was: [(extractable arabinoxylans – initially soluble arabinoxylan)/arabinoxylans initially present in WUP] × 100. The viscosity of supernatants was determined as described above.

RESULTS AND DISCUSSION

Solubilization of Arabinoxylan from Pentosans

The compositions of the WUP and WEP from the three flours are reported in Table I. GS100 was applied to suspensions of WUP, isolated from Thésée flour, with an enzyme to arabinoxylan ratio (w/w) of 0 to 3.5 × 10⁻³. The percentage of arabinoxylan solubilization, specific and apparent intrinsic viscosities of supernatants as a function of enzyme dose are shown in Fig. 1. The treatment by GS100 brought about a solubilization of arabinoxylan that increased with enzyme addition level. Up to a 2.1 × 10⁻³ ratio, the solubilization extent increased rapidly (from 0 to 48%). Beyond this ratio, the solubilization reached a pseudo-plateau where ≈50% of arabinoxylans were released.

The specific viscosity of the supernatant increased first due to the release of arabinoxylans and passed through a maximum for a 0.7 × 10⁻³ ratio of GS100 to arabinoxylan, then it decreased with

increasing enzyme addition. The apparent intrinsic viscosity of solubilized arabinoxylan was high for a low solubilization extent (ratio 0.14 × 10⁻³) then decreased with increasing enzyme concentration until it was reduced by half for a 1.4 × 10⁻³ ratio and by 70% for a 3.5 × 10⁻³ ratio.

Two doses of GS100 appeared particularly interesting: at a 0.35 × 10⁻³ ratio, the solubilization extent was low (20%), the apparent intrinsic viscosity of solubilized arabinoxylan (445 mL/g) was high and similar to WEP arabinoxylan. At a ratio of 1.8 × 10⁻³, the solubilization was twofold higher (40%). Although a high specific viscosity was observed due to significant liberation of arabinoxylan, the apparent intrinsic viscosity was approximately twofold lower (255 mL/g).

Pentosan Comparison

WEP and WUP were extracted from the three wheat cultivars of varying technological potential. Three doses of GS100 (0.35 × 10⁻³, 1.8 × 10⁻³ and also a high dose of 3.5 × 10⁻³ ratio) were applied to the three different WUP. Similar effects in terms of extent of arabinoxylan solubilization and apparent intrinsic viscosity of the products were observed (Table II). For the lowest dose only, differences were probably due to small variations in structural features that appreciably modify the initial rate of solubilization. However, due to the general similarity in WUP behavior of the three cultivars, only Thésée was selected for further studies.

A mixture of WEP and WUP with a ratio of water-extractable to water-unextractable arabinoxylans similar to that of Thésée flour was treated at a 1.8 × 10⁻³ ratio. WEP were strongly degraded. The viscosity of the solution was reduced by 76% (Table III). However, when the enzyme was applied to a mixture of WEP and WUP, the fall in viscosity was only 30%. Rouau and Moreau (1993) observed a similar effect on pentosans extracted from a commercial flour. The degradation of isolated WEP was more pronounced than in a mixture of WEP + WUP for a given amount of enzyme. The enzymatic solubilization of WUP arabinoxylan was only slightly reduced when they were in the presence of WEP. Therefore, suspensions of WUP in buffer were chosen as a model system, allowing a simplification in the analysis of enzyme performances in terms of solubilization and viscosity measurements.

Enzyme Performance on Arabinoxylan

Table IV reports the effects on WUP of enzymes acting only on arabinoxylans. The rate of WUP arabinoxylan solubilization obtained with the endoxylanase E1 at ratios of 0.35 × 10⁻³ and 1.8 × 10⁻³ was similar to the rate observed with these doses of GS100. This result confirms that this endoxylanase is responsible for the effect of GS100 on pentosans (Rouau 1993, Rouau et al 1994). With a 1.8 × 10⁻³ ratio of E1, the specific viscosity of the reaction supernatant was slightly lower than with a same amount of GS100. At a ratio of 0.35 × 10⁻³, 1.8 × 10⁻³, 3.5 × 10⁻³, and even 35 × 10⁻³,

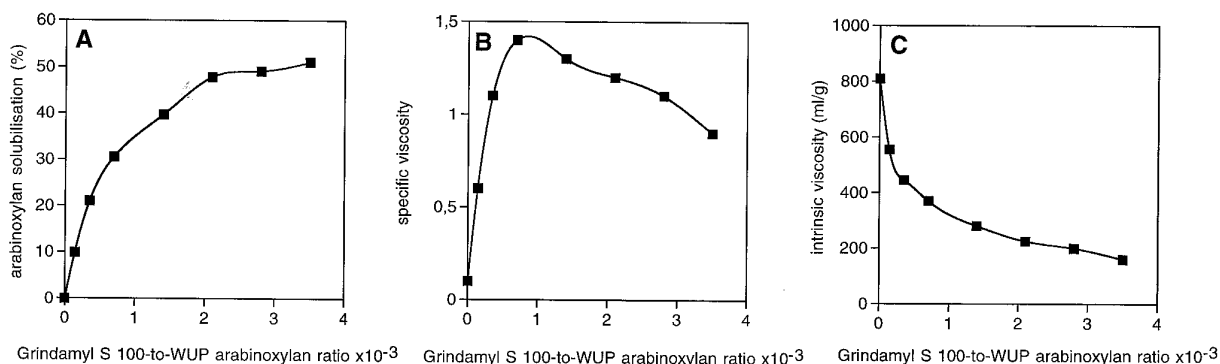


Fig. 1. Effects of increasing doses of Grindamyl S 100 (expressed as enzyme-to-arabinoxylan ratio) applied to a water-unextractable pentosan (WUP) suspension on the percentage of arabinoxylan solubilization from WUP (A), on the specific viscosity of the supernatant (B), and on the apparent intrinsic viscosity of the solubilized arabinoxylans (C).

the endoxylanase E2 was totally inefficient for arabinoxylan solubilization. However, at a ratio of 35×10^{-3} , E2 was able to solubilize 10% of the glucose content of WUP. This cellulolytic activity could be due to incomplete specificity of the xylanase or to a minor contamination by a glucanase. The endoxylanase E3 was provided without activity equivalent with GS100. An arbitrary dose solubilized 18% of WUP arabinoxylan but yielded a viscosity of supernatant fourfold lower than GS100 for a similar solubilization. E3 was able only to release arabinoxylans of low molecular weight from WUP or it degraded rapidly HMWAX when they passed into solution.

E1, E2, and E3 were purified and standardized using a dyed-xylan assay. It is likely that their action toward wheat WUP arabinoxylan differed due to differences in structural features when compared to AzoWheat Arabinoxylan. Kormelink et al (1991) purified two endoxylanases from *Aspergillus awamori*, Endo I and Endo III, which required different specific sites on the xylan backbone, in terms of length and degree of substitution, for binding their substrate.

The Af, purified from GS100, solubilized <2% of WUP arabinoxylan at doses equivalent to a ratio of 0.35×10^{-3} , 1.8×10^{-3} , and even up to 3.5×10^{-3} . This enzyme was combined with the three endoxylanases in separate experiments. Af did not modify the arabinoxylan solubilization by E1 and E3. The site of action of these endoxylanases on arabinoxylan chains perhaps was not affected by the presence of single arabinoses at O-3 position, because Rouau and Moreau (1993) have shown that this Af released only arabinose at O-3 position of xylose in arabinoxylans. Alternatively, the action of the Af could be hindered in heavily substituted regions corresponding to sites of hydrolysis of the endoxylanases. However, the combination of the Af with E2 yielded a 12% arabinoxylan solubilization. There was a synergy between E2 and Af.

The FAE was not efficient for solubilizing WUP arabinoxylan at a dose equivalent to a ratio of 0.35×10^{-3} of GS100. A dose of 35×10^{-3} solubilized <2%. Ferulic acid (3.6 ng/100 mg of WUP) was released during the reaction time, compared to only 0.27 ng/100 mg of WUP for the control. A 10-fold increase in the amount of FAE solubilized 14% of WUP arabinoxylan. But at this dose, traces of contaminating activities could be responsible for this solubilization. FAE was interesting because of its ability to de-esterify feruloyated arabinoxylans. Results reported in the literature have shown the difficulty of releasing ferulic acids from polysaccharides. A feruloyl esterase partly purified from *Streptomyces commune* released nearly no ferulic acid from arabinoxylan without combination with a xylanase (Tenkanen et al 1991). The efficiency of hydrolysis of a combination of FAE and xylanase could mainly be explained by increased accessibility of the enzyme to the substrate (Ralet et al 1994).

The combination of FAE and E1 solubilized 29% of WUP arabinoxylan (22% solubilization for E1). The elimination of some of the ferulic acids probably improved the accessibility and binding of E1 on the arabinoxylans. In these experiments, FAE also released traces of dehydrodiferulic acids. This could explain the liberation of some arabinoxylans that are bound to cell-walls by diferulic acid bridges.

TABLE III
Extent of Specific Viscosity, Apparent Intrinsic Viscosity, and Arabinoxylan Solubilization in Thésée Wheat Flour Treated with Grindamyl S 100^a

	WEP		WEP + WUP	
	Control	Treated	Control	Treated
Specific viscosity	3.7	0.9	5.3	3.7
Apparent intrinsic viscosity ^b	620	200	650	320
Arabinoxylan solubilization ^c	0	38.2

^a Mean values of duplicates. Water-extractable pentosans (WEP) and water-unextractable pentosans (WUP) suspension supernatants. Enzyme to arabinoxylan ratio: 1.8×10^{-3} .

^b Expressed as mL/g of arabinoxylan in the solution.

^c Percentage of insoluble arabinoxylan rendered water-extractable.

Enzyme Performance on Other Polysaccharides

Results are reported in Table IV on the effects of glucanases and cellulase on the WUP arabinoxylans. β G solubilized no WUP arabinoxylan at a ratio of 0.35×10^{-3} . With a 1.8×10^{-3} ratio, only 3% of arabinoxylan was released. A ratio of 7×10^{-3} allowed the solubilization of 7% of WUP arabinoxylan that was not degraded very much (apparent intrinsic viscosity of 365 mL/g compared to 445 mL/g with GS100). Up to 32% of arabinoxylan could be solubilized with an equivalent ratio of 70×10^{-3} of β G, but the apparent intrinsic viscosity was lowered to 135 mL/g. The solubilization was close to that obtained with a ratio of 1.8×10^{-3} of GS100 (43%), but the arabinoxylan molecular weight was twofold smaller. With very high doses of β G, minor contaminating activities could interfere with the major glucanase activity. For Harkonen et al (1995), treating rye meal with a β G did not affect the molecular weight of WEP arabinoxylans. The extraction of pentosans was also less efficient with β G than with xylanase. Mulder et al (1991) tested a commercial β G (Grindstedt GV) that was not able to solubilize arabinoxylan from wheat aleurone cell-wall.

WUP arabinoxylan (9%) was solubilized by C at a dose equivalent to a ratio of 0.35×10^{-3} . This corresponded to half of the solubilization extent obtained with GS100, but the apparent intrinsic viscosity of liberated arabinoxylans was fourfold lower. Higher amounts of C solubilized more arabinoxylan but of reduced size (apparent intrinsic viscosity 75 mL/g). This enzyme was only partly purified and contained at least also xylanase activity. The xylanase-cellulase activity ratio was 0.1:1 (Danisco Ingredients, *personal communication*).

TABLE IV
Effect of Enzyme Concentrations on Arabinoxylan Solubilization in Flour and Apparent Intrinsic Viscosity of Liberated Arabinoxylans^a

Enzymes ^b	Ratio ^c	Arabinoxylan Solubilization ^d	Apparent Intrinsic Viscosity ^e
GS100	0.35×10^{-3}	21	445
	1.8×10^{-3}	43	255
E1	0.35×10^{-3}	22	455
	1.8×10^{-3}	46	210
E2	0.35×10^{-3}	0	nd ^f
	1.8×10^{-3}	0	nd
	3.5×10^{-3}	1	nd
	35×10^{-3}	1	nd
E3	d1 ^g	4	190
	d10 ^h	18	150
E2 + Af	$35 \times 10^{-3} + 3.5 \times 10^{-3}$	12	160
E3 + Af	$0.7 \times 10^{-3} + 3.5 \times 10^{-3}$	20	145
FAE	0.35×10^{-3}	0	nd
	35×10^{-3}	2	nd
	350×10^{-3}	14	510
E1 + FAE	$0.35 \times 10^{-3} + 350 \times 10^{-3}$	29	385
β G	0.35×10^{-3}	0	nd
	1.8×10^{-3}	3	nd
	7×10^{-3}	7	365
	70×10^{-3}	32	135
C	0.35×10^{-3}	9	120
	0.8×10^{-3}	22	75
eG	d1	4	370
	d10	30	170
E1 + β G	$0.35 \times 10^{-3} + 7 \times 10^{-3}$	27	405
E1 + C	$0.35 \times 10^{-3} + 0.35 \times 10^{-3}$	25	330
E1 + eG	$0.35 \times 10^{-3} + d1$	26	400

^a Mean values of duplicate samples of water-unextractable pentosans (WUP) obtained from Thésée flour.

^b Grindamyl S 100 (GS100) and enzymes purified from GS100; three endoxylanases (E1, E2, E3); arabinofuranosidase (Af); ferulic acid esterase (FAE); β -glucanase (β G). Prepared from *Trichoderma reesei*: cellulase (C); endo-glucanase (eG)

^c Doses equivalent to Grindamyl S 100.

^d Arabinoxylan content of WUP (% db).

^e Expressed as mL/g of arabinoxylan.

^f Not determined.

^g Arbitrary dose 1.

^h Arbitrary dose 10-fold.

The enzyme preparation can solubilize arabinoxylans by degradation of cellulose to which they were linked or by its xylanase activity. In general, polysaccharides from *Trichoderma* (like C) exhibit an higher hydrolytic power than does *Aspergillus*.

The eG used at an arbitrary dose (dose 1) allowed the solubilization of 4% of WUP arabinoxylan with a high apparent intrinsic viscosity (370 mL/g). However, part of the specific viscosity of the supernatant can be due to liberated β -glucan fragments. A 10-fold dose solubilized 30% of arabinoxylan but with a 2.5-fold size reduction of liberated molecules. This enzyme was purified to homogeneity (Massiot 1992) and was completely devoid of xylanase activity. It hydrolyzed specifically β 1-4 linkages between glucose residues of cellulose or β -glucans. Therefore, eG released arabinoxylan fragments that were closely associated with cell-wall β -glucans. Accordingly, Kulp (1968) and Mulder et al (1991) explained the liberation of arabinoxylan by cellulases by their association with cellulose in the cell wall. When the enzyme-substrate ratio increased, the molecular weight of liberated arabinoxylans decreased.

The C, eG, and β G, at a dose yielding 5–10% of WUP arabinoxylan solubilization, were added to endoxylanase E1 in separate experiments at a dose equivalent to a ratio of 0.35×10^{-3} of GS100. The solubilization extent was increased from 22 to 25–27%, depending on the enzymes. The apparent intrinsic viscosity of liberated arabinoxylans was ≈ 400 mL/g for β G and eG and slightly less for C (330 mL/g). The hydrolysis of β -glucans and cellulose clearly improved the release of arabinoxylans. However, the apparent intrinsic viscosity of products from C was lower because of the xylanase activity remaining in this enzyme preparation.

Harkonen et al (1995) showed also that a crude culture filtrate, a commercial enzyme preparation (both containing xylanases and cellulases) and a combination of xylanase and β G, brought about an increase in the release of arabinoxylan from rye meal when compared to a single xylanase. Molecules released by the crude culture filtrate were extensively hydrolyzed, although small amounts of solubilized polysaccharides were present in xylanase digest.

Native and Reconstituted Flour Doughs

A previous study (El-Hayek 1991) showed that 20-fold more GS100 was needed in dough as compared to isolated WUP to yield a similar extent of arabinoxylan solubilization. For example, a ratio of 0.4×10^{-3} applied to WUP was equivalent in solubilizing effect to a ratio of 8×10^{-3} in a commercial flour dough. This ratio corresponds to an addition level of 100 mg of GS100/kg of flour (100 ppm), which is in the range of recommended addition rates for breadmaking of a normal wheat flour. Therefore, the 1.8×10^{-3} ratio used also in the model system corresponded to a high level of application in technology (500 mg/kg). However, Rouau et al (1994) showed that bread loaf volumes can increase more, even above the recommended dosage, although dough characteristics deteriorated.

The results in Fig. 2 concern the behavior of arabinoxylans in a flour-buffer system following various doses of GS100. Comparison of Figs. 1 and 2 pointed out the similarities of effect when doses are corrected for the factor 20 of correspondence between isolated WUP system and dough system. However, in dough, a very high enzyme dose (1,000 mg/kg) liberated arabinoxylans of unexpectedly high apparent intrinsic viscosity.

An investigation on the effects of the enzymes in doughs used mixtures of flour-buffer and fully or partially reconstituted flours (starch + gluten + WUP or + WEP).

When GS100 at 100 mg/kg (Table V) was applied to the flour, $\approx 13\%$ of WUP arabinoxylan was solubilized, the specific viscosity of the dough extract was increased by comparison to the control, and the liberated arabinoxylans did not decrease the global apparent intrinsic viscosity of extractable pentosans. For the fully reconstituted flour (SGUE), the arabinoxylan solubilization was much higher; half passed into solution, fourfold more than in native flour. WUP may have been modified during extraction from flour leading to a greater susceptibility to enzymatic hydrolysis or, alternatively, endogenous xylanase inhibitors from flour could modified GS100 activity (Rouau and Surget, *in press*). A similar extent of arabinoxylan solubilization was obtained with a partially reconstituted

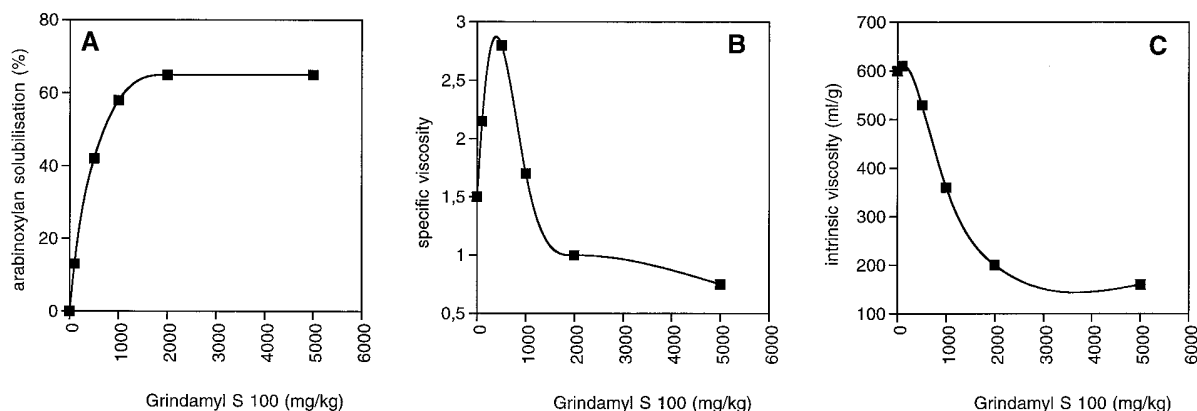


Fig. 2. Effects of increasing doses of Grindamyl S 100 (expressed as mg of enzyme per kg of flour) applied to a flour-buffer dough on the percentage of arabinoxylan solubilization (A), on the specific viscosity of the supernatant (B), and on the apparent intrinsic viscosity of arabinoxylans in solution (C).

TABLE V
Effect of Enzyme on Arabinoxylan Applied to Native and Reconstituted Thésée Flour^a

	Native Flour		SGUE ^b		SGU ^c		SGE ^d	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Arabinoxylan solubilization ^e	0	12.6	0	51.4	0	51.9
Specific viscosity	1.55	2.11	1.0	2.1	0.2	1.08	0.7	0.43
Apparent intrinsic viscosity ^f	600	610	435	380	...	375	400	250

^a Mean values of duplicates. Grindamyl S 100 applied at 100 mg/kg with an enzyme to arabinoxylan ratio of 7×10^{-3} .

^b SGUE = starch (7.21 g) + gluten (0.86 g) + water-unextractable pentosan (0.42 g) + water-extractable pentosan (0.11 g).

^c SGU = starch (7.32 g) + gluten (0.86 g) + water-unextractable pentosan (0.42 g).

^d SGE = starch (7.63 g) + gluten (0.86 g) + water-extractable pentosan (0.11 g).

^e Expressed as % of water-unextractable arabinoxylan.

^f Expressed as mL/g of arabinoxylan in solution.

TABLE VI
Effect of Enzyme Concentrations on Arabinoxylan Solubilization
in Dough and Apparent Intrinsic Viscosity
of Liberated Arabinoxylans^a

Enzymes ^b	Doses ^c	Arabinoxylan Solubilization ^d	Apparent Intrinsic Viscosity ^e
GS100	100	13	610
	500	43	530
E1	100	12	600
	500	58	300
FAE	5000	10	600
E1 + FAE	100 + 5,000	38	500
C	50	15	420
	250	28	300
E1 + C	100 + 50	20	450

^a Mean values of duplicate samples.

^b Grindamyl S 100 (GS100) and enzymes purified from GS100: endo-xylanase (E1); and ferulic acid esterase (FAE). Prepared from *Trichoderma reesei*: cellulase (C).

^c Equivalent doses of GS100 expressed as mg of enzyme per kg of flour.

^d Expressed as % db of water-unextractable arabinoxylan

^e Expressed as mL/g of arabinoxylan.

flour containing only WUP (SGU). It confirmed that the increase in specific viscosity was actually due to the presence of HMWAX (viscosity multiplied by a factor 5.5). The decrease in viscosity for a partial reconstitution with only WEP (SGE) reflected the hydrolysis of soluble arabinoxylans when no WUP can bind GS100. These results have been confirmed by the use of the endoxylanase E1 in dough systems.

Dough Arabinoxylans with Purified Enzymes

The effects of three enzymes (E1, C and FAE) are reported in Table VI. E1 alone gave results similar to those obtained in the model system, with a greater susceptibility to high doses. FAE exhibited the same effects as the model system. A combination of E1 and FAE allowed a 38% solubilization, while separated they gave 12 and 9%, respectively. The result of this synergism was especially interesting because the apparent intrinsic viscosity of released arabinoxylans was high. However, the loss in ferulic acid esters appreciably degraded the potential oxidative gelling power.

At a first dose, C gave a 15% WUP arabinoxylan solubilization, twofold more than in the corresponding model (7%). A corresponding factor of 20 between model and dough was not suitable for this enzyme (El-Hayek 1991). When E1 and C were combined as in the model system, the solubilization increased compared to that of E1 alone. The M_r of arabinoxylans was degraded but remained high enough to yield interesting potential functional properties.

CONCLUSION

The WUP from three wheat cultivars of varying technological potential exhibited no important differences in terms of xylanase susceptibility and properties of liberated arabinoxylans. After screening seven enzymes at several doses, alone and in combination, on isolated WUP and dough systems, it appeared that the best enzymatic system to solubilize HMWAX was the endoxylanase type. The major part of the endoxylanase activity of GS100 (E1) exhibited an appropriate specificity toward flour insoluble pentosans so that HMWAX can be released. This was not the case with other xylanases in this preparation (E2 and E3). The fine mode of action of E1 remains to be studied. The FAE could improve the arabinoxylan solubilization, but the loss of ferulic acids should limit their use for oxidative cross-linking. Enzymes hydrolyzing cellulose and β -glucans allowed an improvement of pentosan solubilization by endoxylanases, but slightly degraded the size of liberated arabinoxylans. The solubilized feruloylated arabinoxylans (except those resulting from a treatment by FAE), as expected from their high M_r , are

suitable for an oxidative cross-linking. Together with the flour soluble arabinoxylans, they can participate in the setting of a polysaccharide network in enzyme-treated doughs that can improve the properties and quality of breads.

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LITERATURE CITED

- Blakeney, A. B., Harris, P. J., Henry, R. J., and Stone, B. A. 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* 113:291-299.
- El-Hayek, M.-L. 1991. Les pentosanes de la farine de blé: Acquisition de propriétés fonctionnelles dans les pâtes boulangères par modification enzymatique. Mémoire d'ingénieur de l'Institut National Agronomique Paris-Grignon: Paris.
- Faurot, A. L., Saulnier, L., Berot, S., Popineau, Y., Petit, M. D., Rouau, X., and Thibault, J. F. 1995. Large scale isolation of water-soluble and water-insoluble pentosans from wheat flour. *Lebensm. Wiss. Technol.* 28:436-441.
- Figueroa-Espinoza, M.-C., and Rouau, X. 1998. Oxidative cross-linking of pentosans by a fungal laccase and horseradish peroxidase: Mechanism of linkage between feruloylated arabinoxylans. *Cereal Chem.* 75:259-265.
- Gruppen, H., Kormelink, F. J. M., and Voragen, A. G. J. 1993. Enzymic degradation of wheat flour arabinoxylans. *J. Cereal Sci.* 18:129-143.
- Harkonen, H., Lehtinen, P., Suortti, T., Parkkonen, T., Siika-Aho, M., and Poutanen, K. 1995. The effects of a xylanase and a β -glucanase from *Trichoderma reesei* on the non-starch polysaccharides of whole meal rye slurry. *J. Cereal Sci.* 21:173-183.
- Izydorczyk, M., and Biliaderis, C. G. 1995. Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr. Polym.* 28:33-48.
- Kormelink, F. J. M., Searle-Van Leeuwen, M. J. F., Wood, T., and Voragen, A. G. J. 1991. (1,4)- β -D Arabinoxylan arabinofuranohydrolase: A novel enzyme in the bioconversion of arabinoxylans. *Appl. Microbiol. Biotechnol.* 35:231-232.
- Kulp, K. 1968. Enzymolysis of pentosans of wheat flour. *Cereal Chem.* 45:339-350.
- Massiot, P. 1992. Rapid purification procedure and characterisation of two 1,4- β -D-glucanases from *Trichoderma reesei*. *Lebensm. Wiss. Technol.* 25:120-125.
- Morris, E. R. 1984. Rheology of hydrocolloids in gums and stabilisers for the food industry. G. O. Phillips, D. J. Wedlock, and P. A. Williams, eds. Pergamon Press: Oxford.
- Mulder, M. M., Hotten, P. M., Lomax, J. A., and Chesson, A. 1991. Digestion of wheat aleurone by commercial polysaccharidases. *Anim. Food Sci. Technol.* 32:185-191.
- Ralet, M. C., Faulds, C. B., Williamson, G., and Thibault, J. F. 1994. Degradation of feruloylated oligosaccharides from sugar-beet pulp and wheat bran by ferulic acid esterases from *Aspergillus niger*. *Carbohydr. Res.* 263:257-269.
- Rouau, X. 1993. Investigations into the effect of an enzyme preparation for baking on wheat flour dough pentosans. *J. Cereal Sci.* 18:145-157.
- Rouau, X., and Moreau, D. 1993. Modification of some physicochemical properties of wheat flour pentosans by an enzyme complex recommended for baking. *Cereal Chem.* 70:626-632.
- Rouau, X., El Hayek, M. L., and Moreau, D. 1994. Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties. *J. Cereal Sci.* 19:259-272.
- Rouau, X., and Surget, A. 1994. A rapid semi-automated method for the determination of total and water-extractable pentosans in wheat flours. *Carbohydr. Polym.* 24:123-132.
- Rouau, X., and Surget, A. *In press*. Evidence for the presence of pentosanase inhibitor in wheat flours. *J. Cereal Sci.*
- Tenkanen, M., Schuseil, J., Puls, J., and Poutanen, K. 1991. Production, purification and characterization of an esterase liberating phenolic acids from lignocelluloses. *J. Biotechnol.* 18:69-84.

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