Modification of Some Physicochemical Properties of Wheat Flour Pentosans by an Enzyme Complex Recommended for Baking[1]

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ABSTRACT

The effects of a fungal α-amylolytic complex containing side activities were studied on water-insoluble pentosans (WIP) and water soluble pentosans (WSP) purified from wheat flour. This enzymatic preparation is used in breadmaking for its capacity to improve dough and bread characteristics. The enzymatic complex brought about a partial solubilization of the WIP flour cell-wall fraction, causing up to a 50% weight loss under prolongated exposure. During hydrolysis, 75% of the insoluble arabinoxylans passed into solution. The insoluble residue, resistant to the enzymatic degradation, was enriched in glucans. The swelling power of the hydrolyzed residue was double that of the intact WIP, but its water-holding capacity remained unchanged. Therefore, the amount of water retained was reduced proportionally compared to the weight loss of the WIP fraction. On the other hand, the high viscosity of WSP was dramatically decreased by the action of the enzymatic complex. The drop in viscosity during hydrolysis on a mixture of WIP and WSP was limited by the contribution of the solubilized arabinoxylans. It is apparent from these data that the so-called side activities of the enzymatic complex were mainly endoxylanase and arabinofuranosidase.

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Pentosans are the major nonstarchy polysaccharides of wheat flours. They originate from the endosperm cell walls of wheat grains. They are composed mostly of arabinoxylans that are partly extractable with water in their native form. The mean structure of the molecules is a linear backbone of β-1,4 linked xyloses carrying single arabinofuranose residues on C-3 or on both C-2 and C-3. A few of the arabinose are esterified with ferulic acids. The insoluble pentosans are associated in the cell-wall fragments with other components, including cellulose, β-glucans, glycoproteins, and polyphenols. The soluble pentosans are composed of arabinoxylans often mixed with arabinogalactan-peptides in flour water extracts (Amado and Neukom 1985, Fincher and Stone 1986, Meuser and Suckow 1986).

Although they occur as minor components of the flour (2–3%), pentosans play an important role in dough rheology and bread quality because of their remarkable functional properties. They exhibit a high affinity for water and are partly responsible for elevated water absorption and viscosity of doughs (Bushuk 1966; Jelaca and Hlynka 1971, 1972; Shelton and D'Appolonia 1985, McCleary 1986). They are also able to form chemical gels by the oxidative coupling of their feruloyl groups (Geissmann and Neukom 1973, Hoseney and Faubion 1981, Izydorczyk et al 1990).

In the European Economic Community (EEC), several kinds of enzymes are used in the baking industry to regularize or improve the breadmaking ability of flours. Fungal α-amylasic preparations are the most widely used. Some of these preparations are composed prevalently of amylases designed to enhance the availability of fermentable sugars from the hydrolysis products of starch. Others are standardized enzymatic complexes containing side activities necessary for optimal results on dough and bread, especially in french bread processing. Pentosanases occur among the side activities, but the mechanisms of the improvement remains unknown.

In this work, we investigated the effect of a commercial α-amylolytic complex containing side activities on purified wheat flour pentosans to determine the modifications of their structure and properties caused by the enzymes.

MATERIALS AND METHODS

Flour

The flour was a commercial white bread flour (T550) obtained from the Centre de Recherche sur les Pulvérulents de Créteil (CRPC), Créteil, France. It corresponded to a composite of

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different varieties harvested at a number of locations in France. The protein and ash contents were 11.1% and 0.59%, respectively, expressed on a dry basis.

Enzymes

Grindamyl S100, a fungal α-amylolytic complex with side activities from *Aspergillus*, was kindly provided by Grindsted Products, Brabrand, Denmark. The recommended enzyme dosage for french bread baking is 100–200 mg per kilogram of flour. Other enzymes used for the purification of pentosans were: Termamyl 60L, a heat-stable α-amylase from *Bacillus licheniformis* (Novo Nordisk, Copenhagen, Denmark); amyloglucosidase (100 units per milligram) from *Aspergillus niger* (Merck, Darmstadt, Germany); and pronase from *Streptomyces griseus* (Boehringer, Mannheim, Germany).

Extraction and Purification of Pentosans

The different steps for extracting and purifying WIP and WSP are shown in Figure 1. Flour (100 g) was mixed with water (50 ml) then hand-kneaded 20 min and allowed to rest for 20 min at room temperature. The dough was washed under cold (4°C) deionized water, and the effluent was centrifuged at 15,000 × *g* for 20 min at 4°C.

The "tailings" were taken from the upper part of the pellets, dispersed in 100 ml of water, adjusted to pH 5 with HCl, and incubated in Termamyl (0.4 ml) at 96° C for 30 min under agitation. After a cooling period, amyloglucosidase (50 mg) was added, and the mixture was agitated for 3 hr more at 60°C to complete the starch removal. After the addition of phosphate buffer (5 ml, 1*M*, pH 7.5) containing 30 mg of pronase, the mixture was incubated for 4 hr at 40°C under constant agitation to degrade insoluble proteins. The slurry was then centrifuged (15,000 × *g* for 20 min at 20°C). The WIP pellets were extensively washed with distilled water and dried by solvent exchange (ethanol, acetone, ether).

The dough solubles were boiled for 10 min then cooled at room temperature so that the denatured soluble proteins were decanted. The extract was centrifuged (15,000 × *g* for 20 min at 20°C), and the supernatant was adjusted to pH 5 with HCl before amyloglucosidase (50 mg) was added. The solution was allowed to react at 60°C for 3 hr. After it cooled to 40°C, it was mixed with pronase (30 mg) and phosphate buffer (5 ml, 1*M*, pH 7.5) and agitated for 4 hr at 40°C. The solution was boiled for 10 min using a microwave oven to inactivate the protease activity, then it was cooled and passed through filter paper. Thereafter, five volumes of 95% ethanol were added to the clear solution under agitation. The precipitated material was allowed to settle overnight at 4°C. The supernatant was partially removed by suction, and the WSP pellets were centrifuged (15,000 × *g* for 20 min at 20°C) and then dried by solvent exchange.

Enzymatic Hydrolysis

The purified pentosans were submitted to hydrolysis by Grindamyl S100 in liquid medium at 25°C. The enzyme-to-arabinoxylan ratio was kept at 0.011, which is in the range of the breadmaking process. The ionic strength of the solution was brought to 0.5*M* using acetate buffer (0.1*M*, pH 5) and NaCl (0.4*M*), as calculated for the ionic strength in the dough.

The hydrolysis of WIP samples (100 mg of sample containing 60 mg of arabinoxylan) was conducted in 10-ml glass cylinders after a vigorous initial agitation. After appropriate periods of time (15 and 30 min, or 1, 2, 4, 8, and 24 hr), the content of each cylinder was centrifuged. The pellets were extensively washed with distilled water and then freeze-dried. The supernatant was boiled for 5 min and analyzed or kept frozen.

The hydrolysis of WSP was made on a 0.2% solution (0.16% arabinoxylan solution to match the insoluble-to-soluble arabinoxylan ratio in flour). For the hydrolysis of total pentosans, a mixture of WIP and WSP was used so that the ratio of insoluble to soluble arabinoxylan was the same as in flour (60 mg of insoluble plus 16 mg of soluble arabinoxylans). The slurry was continuously agitated and aliquots (5 ml) were sampled at appropriate periods of time. The centrifuged pellets and supernatants were treated as indicated above.

Analyses

All analyses were performed in duplicate. Results are expressed on a moisture-free basis.

Polysaccharide content and monosaccharide composition. The arabinoxylan content was calculated as the sum of anhydroarabinose and anhydroxylose, which was determined by gas chromatography of alditol acetates after total acid hydrolysis of polysaccharides (Blakeney et al 1983). In some cases, polysaccharides were precipitated in 80% final aqueous ethanol concentration before carbohydrate determination with 5 volumes of 95% ethanol added to the solution. The mixture was allowed to stand at 4°C for 2 hr and then centrifuged for 10 min at 10,000 × *g*. The monosaccharide content was determined as above, except that the hydrolysis step was omitted.

Glycosidic linkages determination. The methylation of polysaccharides was performed according to Lomax et al (1983). The methylated polysaccharides were hydrolyzed by formic acid (100°C, 1 hr) and trifluoracetic acid (100°C, 3 hr). The partially methylated alditol acetates were reduced and acetylated by the procedure of Harris et al (1984) and analyzed by gas chromatography on DB225 and OV1 columns.

Swelling power and water-holding capacity. Swelling power was measured by the expansion of the insoluble material in an excess of aqueous medium in a glass cylinder according to the bed volume technique (Kuniak and Marchessault 1972). Results are expressed as milliliters per gram of dry sample (ml · g^{-1}). The water-holding capacity (WHC) was determined with distilled water by centrifugation as described by Bertin et al (1988); results are expressed as grams of water per gram of sample (g · g^{-1}).

Viscosity. Flow times of filtrated solutions (0.45 μm) were measured at 25 ± 0.1°C using an Ubbelhode capillary viscometer. Relative viscosities (n_{rel}) and specific viscosities ($n_{sp} = n_{rel} - 1$) were calculated using distilled water flow time. Reduced viscosities ($n_{red} = n_{sp}$ / arabinoxylan concentration) were plotted against the polymer concentration and extrapolated to zero concentration using the Huggins equation (Huggins 1942) to determine the intrinsic viscosities ([*n*]). The average viscometric molecular weight (M_v) was calculated from [*n*] according to Anger et al (1986): $[n] = 3.47 \cdot 10^{-3} M_v^{0.98}$.

Gel-permeation chromatography. Chromatographic analyses were performed at 35°C using a Superose 6 column (1.6 cm i.d. × 40 cm; Pharmacia XK 16-40) eluted with 0.2*M* NaCl at a flow rate of 0.6 ml · min^{-1}. The effluent was monitored with a refractive index detector (Shimadzu RID 6A).

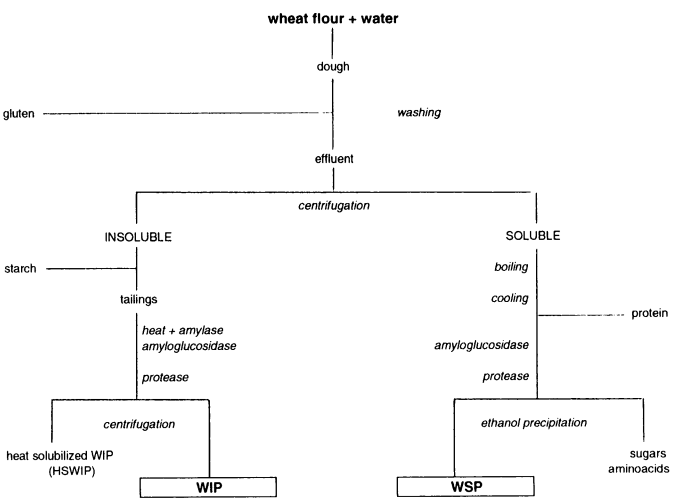

Fig. 1. Scheme of extraction and purification of the water-insoluble pentosans (WIP) and water-soluble pentosans (WSP).

RESULTS AND DISCUSSION

Extraction, Purification, and Characterization of Pentosans from Wheat Flour

Carbohydrate content of flour. The carbohydrate content of the flour after total acid hydrolysis is shown in Table I. The large amount of glucose was derived from starch. The major components of the nonstarchy polysaccharides were arabinose and xylose, yielding 1.7% of the flour. About 30% of arabinose and xylose were water soluble, which was nearly all precipitated by 80% aqueous ethanol. Therefore, all arabinose and xylose occur in their polymeric forms, mostly as arabinoxylans.

Mannose and galactose were also found in the flour. Quite surprisingly, although it is generally assumed to be part of the hemicellulosic glucomannans of the cell wall (Fincher and Stone 1986) and normally insoluble in cold water, mannose was contained almost totally in the water-soluble fraction. Galactose is usually related to the arabinogalactan-peptides found in flour solubles (Fincher and Stone 1974). However, our results show that 60% of the galactose remained associated with the insoluble fraction. Part of these galactose residues may belong to galactolipids found in the gluten (Bushuk 1986).

Extraction and purification of pentosans. The pentosan extraction scheme (Fig. 1) resulted in WIP and WSP fractions. Insoluble arabinoxylans were distributed between WIP, the heat-solubilized fraction after hot amylolysis, and gluten. WIP contained 80% of the total insoluble arabinoxylans, 13% were recovered from the heat-solubilized fraction after hot amylolysis, and 7% remained associated with gluten. Gluten nonstarchy polysaccharides accounted for 0.13% of the flour dry matter. This value is higher than the 0.07% reported by Meuser and Suckow (1986) and the 0.04% reported by Gruppen et al (1989). This nonstarchy polysaccharide fraction was especially rich in galactose (21% of the total galactose content of the flour). Whether it belongs to galactolipids or polysaccharides is not known.

On the other hand, only minute amounts of soluble arabinoxylans were associated or coprecipitated with heat-denatured proteins; most of them (98% of the total extracted arabinoxylan) were found in the WSP fraction.

The sum of arabinoxylan contents of WIP and WSP was more than 85% of the total extracted arabinose and xylose. The overall yield was 77% of the arabinoxylan content of the flour, with a similar arabinose-xylose ratio (0.73 vs. 0.74). The proportion of soluble-insoluble arabinoxylans was the same between WSP and WIP as that determined by direct dosage on the flour. Therefore, WIP and WSP can be considered as representative of the original flour pentosans.

Composition of WIP and WSP model substrates. The carbohydrate content of the WIP and WSP fractions is shown in Table II. WIP contained nearly 69% of polysaccharides. These polysaccharides were mainly composed of arabinose, xylose, and glucose. The glucose yield was three times higher when the sample was first mixed with 72% sulphuric acid than it was before the hot dilute acid hydrolysis. This suggests that amorphous β-glucans represented only one third of the glucose residues of WIP, the remainder was resistant cellulose. However, the prehydrolysis procedure was not used for further analyses because it caused important losses in pentoses, especially xylose (up to 20% lost).

The arabinoxylans of WIP accounted for 88% of the recovered

TABLE I
Carbohydrate Content of Flour Samples[a]

	Polysaccharides[b]			
Anhydrosugars	Total	Solubles	Solubles[c]	Insolubles[d]
Arabinose	0.73 (0.01)	0.24 (0.00)	0.23 (0.01)	0.48 (0.02)
Xylose	0.98 (0.01)	0.26 (0.01)	0.24 (0.00)	0.72 (0.01)
Mannose	0.29 (0.01)	0.27 (0.00)	0.02 (0.00)	0.02 (0.01)
Galactose	0.43 (0.03)	0.17 (0.01)	0.15 (0.01)	0.26 (0.04)
Glucose	78.6 (2.10)	2.26 (0.03)	0.52 (0.02)	76.30 (2.10)
Total carbohydrates	81.0 (2.2)	3.21 (0.05)	1.17 (0.04)	77.80 (2.20)
Pentosans (ara + xyl)	1.70 (0.02)	0.50 (0.01)	0.47 (0.01)	1.20 (0.03
Ara/xyl ratio	0.74	0.92	0.96	0.67

[a] Values in parentheses are standard deviations of duplicates.
[b] Expressed as g/100 g of flour (dm).
[c] 80% ethanol-precipitable.
[d] Calculated by subtracting solubles from total polysaccharides.

TABLE II
Carbohydrate Composition (% dm) of Water-Insoluble Pentosans (WIP) and Water-Soluble Pentosans (WSP)[a]

	WIP[b]	WIP[c]	WSP[c]
Arabinose	23.0 (0.3)	24.4 (0.2)	28.3 (0.3)
Xylose	28.4 (0.2)	36.2 (0.4)	51.5 (0.4)
Mannose	2.2 (0.1)	1.3 (0.1)	1.0 (0.1)
Galactose	0.6 (0.0)	0.9 (0.0)	1.9 (0.1)
Glucose	17.2 (0.2)	6.1 (0.1)	0.4 (0.0)
Total anhydrosugar	71.5 (0.8)	68.8 (0.8)	83.1 (0.9)
Arabinoxylan	51.4 (0.5)	60.6 (0.6)	79.8 (0.7)
Ara/xyl	0.81	0.67	0.55

[a] Values in parentheses are standard deviations of duplicates.
[b] Analysis includes a 72% sulphuric acid prehydrolysis step.
[c] Used as model substrates.

TABLE III
Partially Methylated Alditol Acetates from Arabinoxylans of Initial and Hydrolyzed Water-Insoluble Pentosans (WIP) and Water-Soluble Pentosans (WSP) (% of recovered sugars)[a]

	WIP						
	Residue			Supernatant[b]		WSP	
Methylated Sugars	Unhydrolyzed	1 hr	24 hr	1 hr	24 hr	Unhydrolyzed	24 hr
2.3.5. Me Ara[c]	81.5 (1.0)	54.7 (0.6)	58.0 (0.2)	81.0 (0.8)	72.8 (1.1)	85.0 (0.7)	87.4 (1.1)
2.5. Me Ara	4.0 (0.8)	12.1 (0.2)	14.8 (0.3)	...	...	...	...
3.5. Me Ara	6.6 (0.4)	11.0 (0.5)	10.0 (0.4)	9.7 (0.1)	17.1 (0.6)	6.3 (0.4)	5.5 (0.2)
2.3. Me Ara	5.1 (0.3)	12.4 (1.0)	13.5 (0.9)	9.3 (0.3)	10.1 (1.0)	8.7 (0.4)	7.1 (0.5)
5. Me Ara	2.8 (0.3)	9.8 (0.7)	3.7 (0.3)	...	...	...	...
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2.3.4 Me Xyl	2.7 (0.1)	5.0 (0.2)	5.6 (0.2)	3.1 (0.2)	3.5 (0.1)	1.1 (0.3)	1.8 (0.1)
2.3. Me Xyl	52.0 (1.2)	41.9 (0.8)	32.5 (1.1)	51.3 (1.6)	50.2 (0.6)	51.2 (0.9)	53.4 (0.9)
2. Me Xyl	20.6 (0.7)	28.5 (1.0)	30.7 (0.5)	13.9 (0.3)	9.9 (0.5)	18.0 (0.4)	15.3 (1.1)
Xyl	24.7 (1.0)	24.6 (2.1)	31.2 (0.8)	31.7 (1.9)	36.4 (1.6)	29.7 (1.0)	29.4 (1.3)
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Values in parentheses are standard deviations of duplicates.
[b] Analyses on 80% ethanol-precipitated polysaccharides.
[c] 2.3.5 Me Ara denotes 1.5 di-*O*-acetyl-2.3.5-tri-*O*-methylarabinitol.

polysaccharides. The arabinose-xylose ratio was 0.67. The glycosidic linkages were determined by methylation analysis (Table III). Arabinose occured mostly as terminal units, but some residues were branched through O-2, O-3, and O-5. Arabinoxylans with extended side chains of arabinose have been already described (Hoffmann et al 1991). They may be purely endospermic arabinoxylans or fragments of complex, highly branched xylans from the outer tissues of the wheat grain that are contaminating the flour. Xylose was 1-4 linked, and half of the residues were unsubstituted. The others carried substituents (arabinose) at O-3 or at both O-2 and O-3 positions.

WSP was composed of arabinose and xylose (80%), representing 96% of the recovered soluble carbohydrates. The carbohydrate moiety could thus be considered as a pure arabinoxylan with an arabinose-xylose ratio of 0.55, which is more linear than that of the WIP arabinoxylan (0.67). This ratio was also lower than that obtained directly on flour solubles (0.92). The loss of arabinogalactan-peptide, occurring during the preparation of WSP (heat treatment and protease treatment), probably induced some degradative changes of arabinogalactan-peptides that were no longer precipitated by ethanolic solutions. Arabinogalactan-peptides are soluble in rather concentrated ethanol solutions: 70% (Neukom 1976) or even 80% (v/v) aqueous ethanol solutions (Amado and Neukom 1985). The glycosidic linkages of WSP arabinoxylans (Table III) appeared similar to those of WIP, with less terminal xylose and more doubly branched xylose. These results are consistent with previously published data on pentosan structure (Perlin 1951, Montgomery and Smith 1955, Renard et al 1990, Hoffmann et al 1991).

Enzymatic Hydrolysis of WIP with Grindamyl S100

The enzymatic treatment brought about a partial solubilization of WIP (Fig. 2). Approximately 45% of WIP weight loss was obtained after 1 hr. The rate of solubilization slowed down, and a maximum of 53% was reached at 24 hr. Therefore, 47% of WIP was not degraded by the enzymatic complex, even after prolonged exposure.

Analysis of the resistant fraction. The analysis of the insoluble resistant fraction shows a decrease in pentoses and a relative increase in glucose content (Fig. 3). Arabinose and xylose were 60% of WIP but only 34% of the residue after 1 hr of hydrolysis, whereas glucose was 6 and 12%, respectively. The release of arabinose and xylose was parallel, confirming their occurrence as arabinoxylans. Within 1 hr of hydrolysis, the solubilization of WIP arabinoxylans was already close to 70% (Fig. 2). After 24 hr of enzymatic treatment, 76% of the arabinoxylans passed into solution, but one fifth still was not hydrolyzed by Grindamyl S100. The arabinose-xylose ratio in the insoluble fraction was modified from 0.6 to 0.8 in 24 hr. The enzyme preferentially liberated the more linear zones of arabinoxylan; among a mixture of structurally different arabinoxylan polymers, some were not degraded.

This is confirmed by the analysis of the glycosidic linkages of WIP remnants (Table III). The percentage of unsubstituted, 1-4 linked xylose (linear zones) and terminal arabinose were relatively reduced on behalf of more substituted forms. The resistant part of WIP consisted of complex arabinoxylans and a nondegradable glucan, possibly cellulose. From a technological point of view, the disappearance of an important part of the insoluble fiber fraction may have a positive influence on dough and bread characteristics. Indeed, WIP could behave like non-endospermic cell-wall fragments that interrupt the gluten film (Gan et al 1989). Therefore, their partial disappearance by enzymatic degradation should improve the homogeneity of the dough.

Analysis of the solubilized fraction. The 80% ethanol precipitation allowed the recovery of soluble polysaccharides from the reaction mixture (Table IV). The amount of precipitable arabinoxylan was about half of the total released arabinoxylan. This remained almost constant, which means that the enzyme solubilized some high molecular weight arabinoxylans that cannot be hydrolyzed further or, at least, not beyond the limit of alcohol precipitation. The methylation analysis of these polysaccharides (Table III) indicated that their structure was not very different from the original WIP arabinoxylans; they were only slightly modified along the hydrolysis course, with an enrichment in doubly substituted xylose and a concomitant decrease in mono-substituted xylose. Perhaps, arabinofuranosidasic activity present

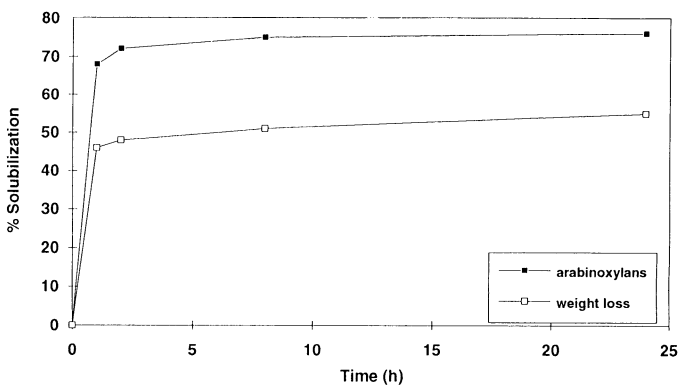

Fig. 2. Weight loss and arabinoxylan release from water-insoluble pentosans (WIP) during hydrolysis. Each point corresponds to a mean of duplicates with maximum standard deviation < 2.0 for all series.

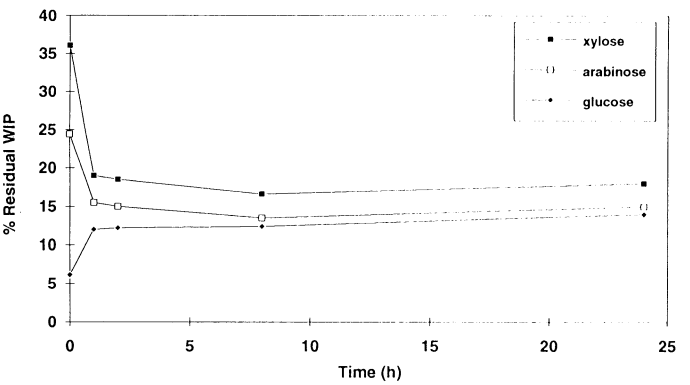

Fig. 3. Composition of the residues of water-insoluble pentosans (WIP) after hydrolysis. Each point corresponds to a mean of duplicates with maximum standard deviation < 1.0 for all series.

TABLE IV
Evolution of the Hydrolysis Products of Water-Insoluble Pentosans (WIP) and Water-Soluble Pentosans (WSP)[a]

	WIP, %		WSP, %		
Hydrolysis, hr	WIP Arabinoxylan	Total Flour[b] Arabinoxylan	WSP Arabinoxylan	Total Flour[b] Arabinoxylan	(WIP + WSP) Arabinoxylan, %
0.5	33.0 (0.7)	24.6 (0.7)	78.2 (1.7)	20.0 (0.8)	48.6 (0.9)
1	35.5 (0.4)	26.4 (0.6)	73.9 (1.2)	18.9 (0.6)	51.8 (1.7)
2	31.3 (0.4)	23.3 (0.5)	68.7 (0.6)	17.6 (0.4)	51.4 (1.1)
8	33.0 (0.5)	24.6 (0.7)	61.8 (1.8)	15.8 (0.7)	49.5 (0.7)
24	33.8 (0.2)	25.2 (0.3)	41.2 (0.8)	10.5 (0.5)	33.7 (0.5)

[a]Values in parentheses are standard deviations of duplicates for 80% ethanol-precipitable arabinoxylans.
[b]Calculated from the relative contribution of WIP and WSP in (WIP + WSP).

in Grindamyl S100 was able to split arabinose from the O-3 position of xylose. This was not the case for double substitution, perhaps because of steric hindrance. At the end of hydrolysis, the (ethanol-precipitable) arabinoxylans in solution were much more linear than the resistant, insoluble ones.

Monomeric arabinose and xylose were also found in the hydrolysis solution (Table V). The sugars were released regularly during the hydrolysis. After 24 hr, they represented, in combination, 5.8% of WIP arabinoxylans (4% of the original weight of WIP). Hence, 8% of the total arabinose content of WIP was monomeric in solution. Arabinose monomers were liberated earlier and in larger amounts than those of xylose. Thus, Grindamyl S100 also exhibits arabinofuranosidasic and weak xylosidasic activities.

Enzymatic Hydrolysis of WSP with Grindamyl S100

The amount of ethanol-precipitable arabinoxylans (80%) decreased continuously between 0 and 24 hr (Table IV), but the rate of hydrolysis slowed after 2 hr. Within 24 hr of contact with the enzyme, nearly 60% of WSP polysaccharides were no longer precipitable. Practically no structural differences appeared during methylation analysis between initial and hydrolyzed WSP arabinoxylans (Table III).

Hydrolysis of a Mixture of WIP and WSP

WSP and WIP were blended so that soluble and insoluble arabinoxylans were in the same proportion as in original flour (0.42:1). After enzyme addition, the reaction mixture was continuously stirred for homogeneous sampling. The analysis of the soluble part (Table IV) shows that the amount of ethanol-precipitable pentosans increased in the first stage of the experiment and maximized after 1 hr. The precipitable arabinoxylans accounted for less than 20% of the original (WIP + WSP) arabinoxylan (8% of the cumulative weight of WIP and WSP) at the beginning of the reaction, but they were almost 50% after 30 min. Thereafter, these arabinoxylans were progressively split by the enzyme. Between 1 hr and 24 hr, the loss in precipitable arabinoxylans was 35%.

Arabinose was first released as the free monomer, whereas xylose is measurable only after 8 hr (Table V). The cumulative amount of monomers accounted for 14.3% of the mixed arabinoxylans at 24 hr (4 and 3%, respectively, of the mixed weight for arabinose and xylose). We confirmed, therefore, that the arabinofuranosidic activity is more important than xylosidasic activity in Grindamyl S100.

TABLE V
Evolution of Hydrolysis Products of Water-Insoluble Pentosans (WIP) and Water-Soluble Pentosans (WSP)[a]

	WIP, %[b]		(WIP + WSP), %[c]	
Hydrolysis, hr	Arabinose	Xylose	Arabinose	Xylose
0.5	0.9 (0.1)	Tr[d]	2.9 (0.1)	Tr
1	1.1 (0.0)	Tr	2.9 (0.1)	Tr
2	1.2 (0.1)	Tr	3.1 (0.1)	Tr
8	2.0 (0.2)	0.8 (0.0)	5.7 (0.2)	1.3 (0.2)
24	3.5 (0.1)	2.3 (0.1)	8.6 (0.3)	5.7 (0.1)

[a] Values in parentheses are standard deviations of duplicates for arabinose and xylose released as monomers.
[b] % of WIP arabinoxylan.
[c] % of (WIP+WSP) arabinoxylan.
[d] Traces.

TABLE VI
Evolution of the Swelling Power and Water-Holding Capacity of Untreated and Enzyme-Treated Water-Insoluble Pentosans (WIP)[a]

	Swelling Power[b]			Water-Holding Capacity[c]		
		Enzyme-Treated WIP			Enzyme-Treated WIP	
Reaction Time, hr	Control	Specific	Actual[d]	Control	Specific	Actual[e]
1	90 (2.5)	190 (3.5)	115 (4.5)	9.4 (0.2)	9.2 (0.1)	5.2 (0.2)
2	nd[f]	210 (5.0)	105 (7.0)	nd	9.8 (0.2)	5.0 (0.3)
8	nd	155 (4.5)	75 (5.3)	nd	10.2 (0.2)	5.4 (0.5)
24	65 (2.0)	150 (2.0)	70 (3.5)	10.0 (0.2)	10.6 (0.3)	5.0 (0.5)

[a] Values in parentheses are standard deviations of duplicates.
[b] Expressed as milliliters per gram of dry sample.
[c] Expressed as grams of water per gram of sample.
[d] Calculated from the specific swelling power and the percentage of WIP weight loss.
[e] Calculated from the specific water-holding capacity.
[f] Not determined.

Modifications of Physical Properties by Enzymatic Action

Hydration properties of WIP. Data related to the swelling power (SP) and the WHC are reported in Table VI. The specific SP and specific WHC are values obtained per gram of final residue; actual SP and actual WHC are the real values obtained starting from 1 g of WIP. In other words, the specific values were affected by the extent of solubilization.

SP is a measurement of the capacity of expansion of the cell-wall network in a liquid medium. The original SP of WIP control was important (90 ml · g^{-1} vs. 11 ml · g^{-1} for sugar beet fibers) (Bertin et al 1988). The value was then decreased slowly until it was 65 ml · g^{-1} within 24 hr. The specific SP of enzyme-treated WIP was at a maximum after 2 hr (210 ml · g^{-1}), and it remained twice as high as the control SP throughout the experiment. The breakdown of many internal bonds allowed the remnants of the cell-wall fragments to expand more in the medium. Similar results were observed on cell-wall material from different sources treated by chemicals (Bertin et al 1988). However, when too many linkages are broken, for instance, after a long exposure to enzyme action, a partial collapse of the polysaccharidic network diminishes the SP. When the solubilization of WIP is taken into account (which could really occur in the dough), the actual SP of the cell-wall fraction remains identical to that of the control throughout the process.

The WHC of WIP was nearly 9.4 g · g^{-1}, which is higher than those generally found in literature for similar material (Jelaca and Hlynka 1971, Meuser and Suckow 1986). However, these WHC values were lower than those of pectin-rich fibers like sugar beet (26–29 g · g^{-1}) (Schaller 1978, Schweizer and Wursch 1979, Bertin et al 1988), carrot (23 g · g^{-1}) (Selvendran 1985), or lettuce (24–25 g · g^{-1}) (Holloway and Greig 1984). They were higher than the WHC of bran (2.4–7.3 g · g^{-1}) (McConnell et al 1974). The specific WHC of the residues of WIP after enzyme treatment was similar to the untreated control, and it was constant over the experiment time. Because of insoluble nonstarchy polysaccharides, the actual WHC was 50% lower (5 g · g^{-1}) in the presence of the enzymatic complex because of the solubilization of half the WIP dry matter. As a consequence, the percentage of water that should be associated with insoluble pentosans in a dough, as obtained by calculation, is decreased from 22% initially to 11% after 1 hr of hydrolysis. This water might become available for other dough components.

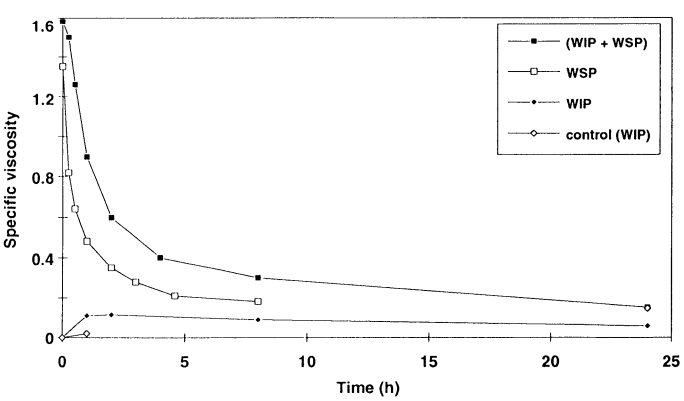

Fig. 4. Viscosity of hydrolysis supernatants of water-insoluble pentosans (WIP), water-soluble pentosans (WSP), and a mixture of WIP and WSP. Each point corresponds to a mean of duplicates with maximum standard deviation < 0.05 for all series.

Solution properties. The flow properties of the pentosan solutions and the reaction-mixture supernatants were investigated by capillary viscometry and gel-permeation chromatography. The specific viscosity of WIP supernatant increased during the first hour of reaction and remained constant for another hour (Fig. 4). It was then five to six times higher than the viscosity of the control sample (WIP with no enzyme). However, the specific viscosity of WIP was much lower than that of the WSP (see below). After 2 hr, the viscosity slightly decreased until it was approximately half of its value at 1 hr, due to the fractionation of liberated arabinoxylans. The refractometric detection of the chromatographic profiles showed very broad peaks at the total volume of columns. This was mainly due to the salts added in the reaction mixtures. Figure 5 shows some high molecular weight material was apparent in WIP solubles after 1 hr of hydrolysis. Some of these molecules were later reduced in size, but a portion (approximately one half) was still eluted at the void volume, even at 24 hr, which confirms solubility and viscosity results. Some arabinoxylans released from WIP by Grindamyl S100 are resistant to extensive hydrolysis with this enzymatic complex.

WSP exhibited an intrinsic viscosity of 210 ml · g^{-1} (Huggins coefficient = 0.51). This value is in agreement with other data published on soluble wheat flour pentosans (Girhammar et al 1985, Renard et al 1990). According to Anger et al (1986), the calculated viscometric average molecular weight of WSP is 75,700. The WSP solution was over 12 times more viscous than the WIP solubles were at their highest viscosity (1 hr) (Fig. 4). The WSP viscosity decreased rapidly with time when exposed to the enzyme. The fall was important in the earlier steps of hydrolysis: 40% at 15 min, 50% at 30 min, corresponding to hydrolysis of less than 1%. This demonstrates the major "endo" mode of action of the enzymatic complex. The residual viscosity after 24 hr was only 12% of the original value (11% hydrolysis). The elution profile of WSP solutions on Superose 6 is shown in Figure 5. Before enzyme addition, most of the material was eluted at the void volume. As the reaction progressed, the peak was broadened and displaced towards the total volume (i.e., lower molecular weights). However, some resistant material of high molecular weight remained eluted at the void volume, even after 8 hr of hydrolysis.

When WIP and WSP were blended together in water, the viscosity of the supernatant was higher than that for the WSP solution, probably because of some spontaneous (nonenzymatic) solubilization of WIP under agitation. The evolution of viscosity when the mixture was in contact with the enzymatic complex is shown in Figure 4. In this case, there was also a rapid reduction of viscosity, but it was less than that of WSP. The difference was especially marked in the first stages of the experiment. For example, after 15 min of reaction, the WSP viscosity decreased by 40%; it was only 10% in the presence of WIP. The gap is still appreciable up to 2 hr, but it reduces as the hydrolysis continues. Therefore, the release of arabinoxylans from WIP restricted the drop in viscosity when WSP arabinoxylans were hydrolyzed by the enzyme.

Before enzyme addition, the chromatographic profile of (WIP + WSP) solubles on the Superose 6 column was composed of three peaks: 1) mainly WSP with some WIP materials, 2) material solubilized from WIP by agitating in water, and 3) salts and small molecules (Fig. 5). After 1 hr of enzymatic action, the amount of large molecules (void volume) strongly diminished in favor of molecules of various molecular weights, including some large ones. After 24 hr of reaction time, solubilization and viscosity were stabilized, and the bulk of molecules became smaller, but it is apparent that some resistant large and medium-sized molecules remained that were responsible for the residual viscosity of the supernatant.

CONCLUSION

The enzymatic complex used in this study contained several different activities, including α-amylases and pentosanases that were able to react with flour components. The main action on purified pentosans consisted of the solubilization and hydrolysis of arabinoxylans with a consecutive diminution of WHC and viscosity. The major enzymatic activities involved were endoxylanase and arabinofuranosidase. We can speculate from our data that the solubilization and hydrolysis of pentosans, especially insoluble cell-wall fragments, might possibly be enhanced by completing the complex with cellulase (or β-glucanase) to degrade the resistant glucan in WIP and with arabinofuranosidase to provide more binding sites for endoxylanase by splitting arabinosyl substituents off the xylan chains.

α-Amylolytic complexes containing side activities are of widespread use in the breadmaking industry because they are generally known to improve both dough rheology (machinability) and bread volume and are much better than pure α-amylases. Such complexes must be added to flours at an optimum dosage for the best results. Beyond this dosage, dough quality is dramatically altered.

Our results suggest that the mechanisms by which Grindamyl S100 improves flour doughs concern some modifications of physical properties of pentosans and their interaction with water. This dealt with the enzyme action on rather pure substrates in excess water, which corresponds to optimal conditions for hydrolases expression. Obviously, the action in the dough where substrates may be partly protected by other components, or where there is a lower availability of water, may be somewhat different. For these reasons, it is supposed that the extent of pentosans

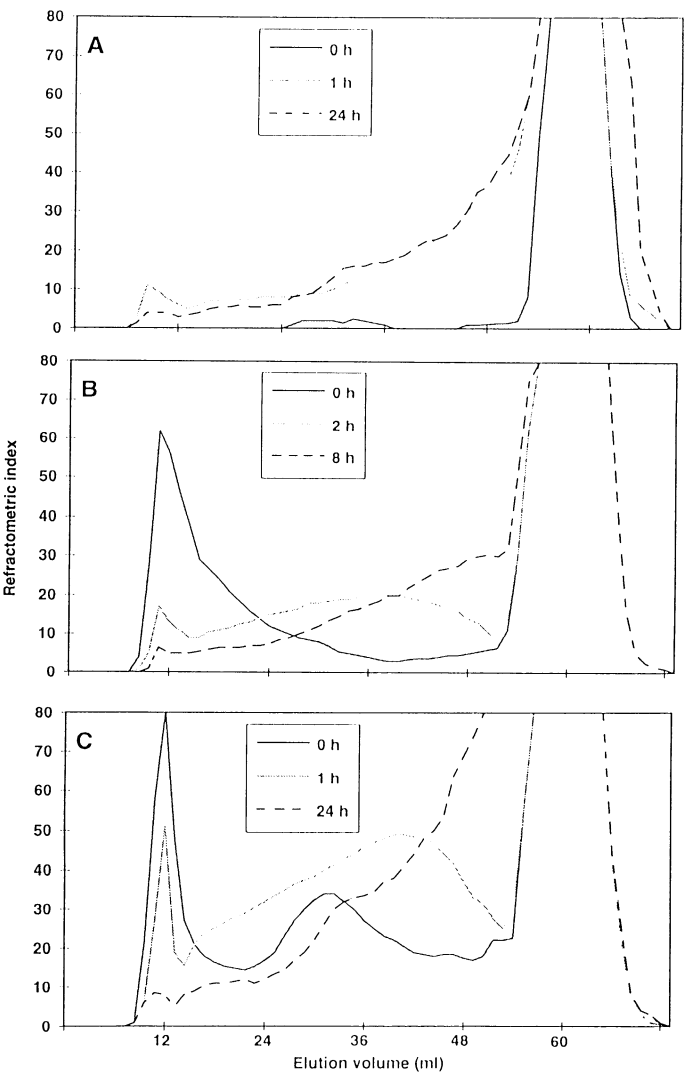

Fig. 5. Gel-permeation chromatography profiles of hydrolyzed supernatants. **A,** water-insoluble pentosans (WIP); **B,** water-soluble pentosans (WSP); **C,** a mixture of WIP and WSP. Column eluted with 0.2*M* sodium chloride aqueous solution at a flow rate of 0.6 ml/min. Effluent was monitored using a refractive index detector.

modifications in dough should be in the range observed for the short-time rather than extended exposure of this model. However, the amount of insoluble pentosans and their water retention decreases during the kneading when the enzymatic complex is added to the flour. The degradation of dough rheology observed in cases of enzyme overdosage could be ascribed to an extensive hydrolysis of WSP because of their importance to good dough rheology (McCleary et al 1986, Meuser and Suckow 1986).

Modifying pentosans in doughs in response to various enzyme dosages is currently under investigation.

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