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Xylanase, β-Glucanase, and Other Side Enzymatic Activities Have Greater Effects on the Viscosity of Several Feedstuffs than Xylanase and β-Glucanase Used Alone or in Combination

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This study was carried out to evaluate the effects of a pure xylanase, a pure β-glucanase, a mix of the two pure enzymes, and a commercial enzyme preparation (Quatrazyme HP, Nutri-Tomen Les Ulis, France) on the viscosity exhibited by water-soluble nonstarch polysaccharides of several feedstuffs (Rialto wheat, Sidéral wheat, Isengrain wheat, triticale, rye, barley, oats, corn, wheat bran, rice bran, wheat screenings, soybean meal, rapeseed meal, sunflower meal, and peas). The viscosity depended on the feedstuffs and varieties of the same feedstuff. There was a correlation ($R^2 = 0.86$) between viscosity of cereals and their arabinoxylan and β-glucan contents. The correlation was greater ($R^2 = 0.99$) when the type of cereal was taken into account. The addition of pure xylanase significantly decreased the viscosity of all feedstuffs except sunflower meal ($P \leq 0.05$). However, pure β-glucanase was unable significantly to decrease the viscosity of Isengrain wheat, corn, rice bran, wheat screenings, soybean meal, and sunflower meal. There was a greater decrease in viscosity with the combination of xylanase and β-glucanase than with addition of xylanase or β-glucanase alone. This synergistic action of xylanase and β-glucanase was observed only in Rialto wheat, Sidéral wheat, triticale, rye, barley, oats, and peas. Finally, the commercial enzyme preparation produced a greater reduction ($P \leq 0.05$) in viscosity for all feedstuffs compared to xylanase or β-glucanase used alone or in combination. The greater effectiveness of the commercial enzyme preparation was due to the presence of side enzymatic activities (arabinofuranosidase, xylosidase, glucosidase, galactosidase, cellulase, and polygalacturonase).

KEYWORDS: Xylanase; β-glucanase; side enzymatic activities; viscosity; feedstuffs

INTRODUCTION

The endosperm cell wall of cereal contains complex sugars known as nonstarch polysaccharides (NSP). Arabinoxylans are the major NSP of wheat, triticale (a cross between wheat and rye), and rye grains (*1*). They consist of a linear β-(1→4) linked xylose backbone to which α-L-arabinofuranose units are attached as side-chain residues either on position O_3 or on positions O_2 and O_3 (*2*). The L-arabinose to D-xylose ratio of wheat arabinoxylans varies between 0.51 and 0.61 (*3*). Mixed-linked β-glucans are nonstarch polysaccharides present at high levels in oats and barley (*4*, *5*). They are linear polymers of glucose residues linked through β-(1→3) and β-(1→4) linkages. They are usually considered as chains of β-(1→3) linked cellotriosyl and cellotetraosyl units arranged randomly (*6*). In noncereal feedstuffs such as soybean, rapeseed, and sunflower meals, pectins are another important NSP. Pectin is characterized by a linear backbone of (1→4) linked α-D-galacturonic acid units (*7*). Finally, pea seeds are characterized by a high level of crude protein and α-galactosides (*8*) compared to other feedstuffs. The NSP of pea seeds are mainly pectins and xylans (*9*).

In general, NSP have antinutritional effects (especially in chicks) related to their viscous properties, which interfere with digestion and absorption of nutrients (*10*). The antinutritive effects of NSP can be attenuated by adding xylanase to wheat-based diet (*11*) and rye-based diet (*12*) or β-glucanase to barley-based diet (*13*).

However, due to the heterogeneity of the composition and structure of NSP feedstuffs (*14*, *15*), xylanase, which hydrolyzes the β-1,4 linkages between the xylose units forming the backbone in an endo mode of action (*16*), or β-glucanase alone is insufficient to reduce the antinutritional effects of NSP significantly, especially in diets containing more than one cereal, that is, wheat and barley. A wide range of exogenous enzymes is therefore required for the biodegradation of these polysaccharides (*17*). Synergy between endo-xylanase and certain side enzymatic activities has previously been demonstrated (*18*). De Vries et al. (*3*) recently studied in detail the synergy between

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side enzymatic activities involved in the degradation of water-insoluble pentosan from wheat flour. However, they did not investigate the role of β-glucanase or the effectiveness of these enzymes on the water-soluble NSP of feedstuffs. Furthermore, Salobir et al. (*19*) has reported that the combination of xylanase and β-glucanase is essential to reduce the intestinal viscosity of broilers significantly and to maximize the beneficial effect of the addition of xylanase and β-glucanase. We therefore decided to investigate whether the combination of xylanase and β-glucanase is sufficient to hydrolyze water-soluble NSP and to reduce their antinutritional effects to a maximum. The second issue addressed was to identify the side enzymatic activities required to enhance and complete the actions of xylanase and β-glucanase in water-soluble NSP hydrolysis.

In the present study we investigated the effects of pure xylanase and β-glucanase used alone or in combination and those of a commercial enzyme preparation on the viscosity of the water-soluble NSP of several feedstuffs commonly used in poultry feeding.

MATERIALS AND METHODS

Feedstuffs. Experiments were performed on the 15 most often used feedstuffs in poultry nutrition. Wheat (Isengrain), barley (Scarlett), and triticale (Trimaran) harvested in 1998 were supplied by ITCF (Paris, France). Wheat (Rialto), wheat (Sidéral), and corn were harvested in 1999 at INRA (Tours, France). Oats and rye harvested in 1999 were supplied by Agralys (Bonneval, France). Soybean meal, rapeseed meal, sunflower meal, peas, and wheat screenings were supplied by Agralys (Bonneval, France). Wheat and rice brans were supplied by Grands Moulins (Semblancay, France).

Enzyme Preparations. Four enzyme preparations were used in the current study. The first two preparations were xylanase (210 IU/mg of product, Megazyme) and β-glucanase (118 IU/mg of product, Megazyme), respectively. The third enzyme preparation was a mix of the latter two enzymes at 1/5 of xylanase and 4/5 of β-glucanase, respectively. The fourth was a commercial enzyme preparation (Quatrazyme HP, Nutri-Tomen Les Ulis, France) containing xylanase (28 IU/mg of product) and β-glucanase (140 IU/mg of product) as enzymes stated by the manufacturer. All of these enzyme preparations were used at the same level of activity as xylanase (560 IU/kg of feedstuff) and β-glucanase (2800 IU/kg of feedstuff).

Analyses and Measurements. Water, ash, crude fat, and crude protein of feedstuffs were determined according to the Official European Methods (*20*), and starch was determined according to the method of Carré et al. (*21*). Water-insoluble cell wall content (cellulose, water insoluble hemicellulose, water-insoluble pectic matter, lignin, and proteins) was determined according to an AFNOR method (*22*). Feedstuffs were milled through a 0.5-mm sieve, and then an aliquot (3.5 g) was homogenized with 30 mL of acetate buffer (0.2 mol/L of sodium acetate; pH 4.5) and incubated for 1 h at room temperature (20 °C). Feedstuff suspensions were centrifuged at 1000*g* for 15 min, and the supernatant was isolated for viscosity measurements. The viscosity of each sample was measured according to Carré et al.'s (*23*) method using a Rheomat 115 A Contraves Rheoanalyzer (Société Lamy, Caluire, France) as viscometer. Viscosity was expressed as real applied viscosity [RAV = Ln(η_r) per g^{-1}·mL, where η_r represents relative viscosity, calculated as the ratio of the viscosity of feedstuffs in the buffer to the viscosity of the buffer]. The potential applied viscosity [PAV = Ln(η_r) per g^{-1}·mL] was analyzed as the real applied viscosity except that the samples were first treated with hot ethanol (80%) to inactivate endogenous enzymes and remove low molecular weight sugars. The four enzyme preparations were added to supernatants of feedstuffs treated with hot ethanol and prepared as described previously. The mixture was then incubated for 1 h at 39 °C. The reaction was stopped by heating the mixture in a boiling water bath for 10 min. The viscosity of the mixture was determined at room temperature after cooling (20 °C) using the same method and viscometer as described above. Aliquots of this solution were used for measurement of soluble arabinoxylans and β-glucans. Soluble sugars (arabinose and xylose) were determined in feedstuffs using the method described by Harris et al. (*24*). Arabinoxylans were calculated as arabinose plus xylose. Soluble β-glucan contents in feedstuffs were analyzed according to the method of McCleary and Codd (*25*).

The sugars released after the action of pure xylanase and commercial enzyme preparation (560 IU of xylanase/kg of feedstuff) on pure soluble arabinoxylan solution (4 mg/mL, Megazyme) were determined using high-performance anion exchange chromatography (HPAEC) performed on a Dionex system with pulsed amperometric detection. The Carbopac PA1 column was eluted at a flow rate of 1 mL/min as follows: 0−45 min isocratic with 0.1 M NaOH; 46−60 min linear gradient from 0 to 0.25 M sodium acetate in 0.075 M NaOH; 61−70 min linear gradient from 0.25 to 0.3 M sodium acetate in 0.07 M NaOH; 71−75 min linear gradient from 0.3 to 1 M sodium acetate; 76−105 min linear gradient from 0 to 0.1 M NaOH. Monomers (arabinose and xylose, from Sigma, St. Louis, MO) and oligomers (xylobiose to xylohexaose from Megazyme) were used as standards.

The molecular weights of sugars released after the action of pure xylanase and commercial enzyme preparation (560 IU of xylanase/kg of feedstuff) at 5, 15, and 30 min and 1 h of incubation (39 °C) were measured using high-performance size exclusion chromatography (HPSEC) performed on two columns per series (Shodex OH-Pack SB-804 and HQ-Pack SB-805 HQ, exclusion limits of 1×10^6 and 4×10^6 for pullulan, respectively) at room temperature and eluted at 0.7 mL/min with 0.05 M sodium nitrate and 0.02% (w/v) sodium azide; column effluent was monitored with an on-line multiangle laser light-scattering detector (Mini Dawn, Wyatt Technology Corp.), a viscometer (Viscotek T50A, Viscotek), and a differential refractometer (Erma 7512). Average molecular weights and intrinsic viscosities were calculated using Astra 1.4 (Wyatt Technology Corp.) and TriSec (Viscotek) software, respectively. A refractive index increment of $dn/dc = 0.146$ g/mL was used for all calculations.

Arabinofuranosidase, xylosidase, glucosidase, and galactosidase activities were measured in xylanase, β-glucanase, and commercial enzyme preparations using *p*-nitrophenyl α-L-arabinofuranoside, *o*-nitrophenyl β-D-xylopyranoside, *p*-nitrophenyl α-L-glucofuranoside, and *p*-nitrophenyl α-L-galactofuranoside, respectively. Enzyme preparations (0.1 mL) were incubated at 40 °C with solutions of substrates (0.1 mL) in which the substrate concentrations were 0.004 M in 0.05 M sodium acetate buffer, pH 5. The reaction was stopped by adding 1 M sodium carbonate (0.6 mL). Release of *p*-nitrophenol and *o*-nitrophenol was measured by absorbance at 400 and 410 nm, respectively. Activity was calculated using molar extinction coefficients (M^{-1} cm^{-1}) of 18350 and 6514, respectively. Before cellulase and polygalacturonase activities were measured, the commercial enzyme preparation was dialyzed in sodium acetate buffer (0.1 M, pH 4.5) for 4 h. Cellulase activity was determined in the presence of two substrates [insoluble (Avicel, Fluka) and soluble (carboxymethyl cellulose, Sigma)]. Polygalacturonase activity was determined using polygalacturonic acid as substrate. Cellulase and polygalacturonase activities were measured according to the method of Nelson (*26*).

Statistical Analyses. All of the measurements were performed in quintuplicate. The level of statistical significance was preset at $P \leq 0.05$. Data were statistically analyzed for treatment effect by the General Linear Models procedures of SAS software (*27*). Multiple-regression analysis was performed using the REG procedure of SAS to determine the correlation between the viscosity level and arabinoxylan and β-glucan contents and type of cereal (Rialto wheat, Sidéral wheat, Isengrain wheat, triticale, rye, barley, oats, and corn). Mean differences were determined using the Newman−Keuls test for multiple mean comparisons.

RESULTS

The three wheat varieties (**Table 1**) had comparable levels of crude fat and ash. However, Isengrain wheat had the lowest insoluble cell wall content, and Rialto wheat showed the highest value of insoluble cell wall content. High, medium, and low viscosity (RAV and PAV) characterized three wheat varieties, that is, Rialto, Sidéral, and Isengrain. Rye contained the lowest

Table 1. Nutritional Characteristics of Feedstuffs (Grams per Kilogram of Dry Matter)

feedstuff	ash	CP	starch	CF	cell wall[a]	RAV[b] (mL/g of DM)	PAV[c] (mL/g of DM)
Rialto what	18.1	124	740	22.5	123.4	2.79	4.79
Sidéral wheat	17.6	121	700	21.2	114.5	2.27	3.24
Isengrain wheat	14.7	111	718	20.1	102.8	1.63	2.07
triticale	20.4	110	702	17.7	104.2	2.55	3.34
rye	18.8	87	747	15.5	146.3	18.78	24.13
barley	20.0	103	662	27.4	141.2	4.81	9.98
oats	31.4	113	565	55.2	310.6	4.23	8.83
corn	12.9	97	728	48.2	96.1	0.34	0.39
wheat bran	53.3	177	324	37.4	405.9	2.00	3.36
rice bran	82.3	139	382	187.4	193.6	0.47	0.83
wheat screenings	89.3	156	582	26.8	194.8	0.78	1.51
soybean meal	72.4	506	102	16.8	211.1	1.83	2.04
rapeseed meal	76.8	366	91	24.0	409.2	1.36	1.51
sunflower meal	67.5	311	84	9.2	519.7	1.03	0.87
peas	28.9	234	551	9.0	148.1	1.64	2.22

[a] Cell wall, water insoluble cell wall. [b] RAV (Incubation at room temperature for 1 h). [c] PAV after hot ethanol treatment (incubation at room temperature for 1 h).

Table 2. Contents in Water-Extractable Arabinoxylan and β-Glucan and Viscosity Level in Feedstuffs[a]

feedstuff	viscosity[b] (mL/g of DM)	arabinoxylans (g/kg of DM)	β-glucans (g/kg of DM)
Rialto wheat	4.73d	8.0b	2.4d
Sidéral wheat	3.21e	5.6c	1.2f
Isengrain wheat	2.03g	3.9e	0.6h
triticale	3.38e	4.8d	1.8e
rye	24.08a	14.4a	7.6c
barley	9.91b	3.3f	24.3b
oats	8.78c	1.3h	43.5a
corn	0.33j	0.3k	0.5h
wheat bran	3.31e	5.0d	0.7g
rice bran	0.79i	0.6j	0.8g
wheat screenings	1.47h	3.5f	0.5h
soybean meal	2.00g	1.1i	0.6h
rapeseed meal	1.48h	2.7g	0.5h
sunflower meal	0.83i	1.3h	1.8e
peas	2.20f	0.7j	1.1f

[a] Means (five measurements per treatment) in the same column with different letters are significantly different ($P \leq 0.05$). [b] Viscosity, sample treated with hot ethanol and incubated at 39 °C for 1 h.

Table 3. Regression of Viscosity Level on Contents of Water-Extractable Arabinoxylan and β-Glucan and Type of Cereal When Using the Multiple Regression Procedure of SAS ($R^2 = 0.99$)[a]

variable	DF	parameter estimate	standard error	probability
intercept	1	21.55	0.98	0.0001
Rialto wheat	1	–18.18	0.51	0.0001
Sidéral wheat	1	–19.28	0.67	0.0001
Isengrain wheat	1	–20.17	0.77	0.0001
triticale	1	–19.00	0.68	0.0001
corn	1	–21.28	0.96	0.0001
barley	1	–12.85	2.30	0.0001
oats	1	–14.19	4.34	0.0027
arabinoxylans	1	0.16	0.08	0.0472
β-glucans	1	0.03	0.10	0.7913

[a] The equation of the model is $\text{viscosity}_i = 21.55 + F_i + 0.16[\text{arabinoxylans}] + 0.03[\beta\text{-glucans}] + \epsilon$ (where viscosity_i represents the viscosity of the cereal i, F_i represents the parameter estimate of the cereal i, and ϵ was the residual error).

crude protein and fat contents and the highest starch and viscosity (RAV or PAV). Triticale, which is obtained from a cross between wheat and rye, had nutritional characteristics similar to those of wheat. Triticale had lower crude protein and fat content than wheat. Barley and oats had similar crude protein contents. They were the two most viscous feedstuffs after rye. Corn had the lowest insoluble cell wall content and the lowest viscosity level. Wheat bran had a higher level of insoluble cell water than rice bran. The latter contained the highest crude fat content. The highest level of ash characterized wheat screenings. Soybean had the highest level of crude protein. Moreover, when compared with rapeseed meal and sunflower meal, soybean meal had the lowest cell wall content. Sunflower meal contained the highest cell wall content and the lowest starch content. However, rapeseed meal was characterized by nutritional values midway between those of soybean meal and sunflower meal. Finally, peas had the lowest crude fat content. For all of these feedstuffs (**Table 1**) PAV was higher than RAV.

The viscosity depended on the feedstuffs and varieties of the same feedstuff (**Table 2**). There was a correlation between the level of viscosity of the feedstuff and the water-extractable arabinoxylan and β-glucan contents. Rye, wheat (Rialto, Sidéral, and Isengrain), triticale, wheat screenings, soybean meal, and rapeseed meal contained more water-extractable arabinoxylans than water-extractable β-glucans, whereas the contrary was observed for barley, oats, corn, rice bran, sunflower meal, and peas (**Table 2**).

The viscosity of cereals (wheats, triticale, rye, barley, oats, and corn) was well correlated ($R^2 = 0.86$) with the water-extractable arabinoxylan and β-glucan contents. The correlation was greater (**Table 3**) when the type of cereal was taken into account ($R^2 = 0.99$). In this case, the probability of the β-glucan factor was not significant because the viscosity of barley and oats was mainly due to their high β-glucan contents.

The addition of pure xylanase significantly decreased ($P \leq 0.05$) the viscosity of all feedstuffs excepted sunflower meal (**Table 4**). However, pure β-glucanase was unable to significantly decrease the viscosity of Isengrain wheat, corn, rice bran, wheat screenings, soybean meal, and sunflower meal. Viscosity was decreased more significantly by the combination of xylanase and β-glucanase than by the addition of xylanase or β-glucanase alone. This synergistic action of xylanase and β-glucanase was observed only in Rialto wheat, Sidéral wheat, triticale, rye, barley, oats, and peas. Moreover, the combination of xylanase and β-glucanase increased the viscosity of Isengrain wheat, corn, wheat bran, rice bran, wheat screenings, soybean meal, rapeseed meal, and sunflower meal compared to xylanase alone. Furthermore, when compared with xylanase or β-glucanase used alone or in combination, the commercial enzyme preparation produced a noteworthy decrease ($P \leq 0.05$) in the viscosity of all feedstuffs (**Table 4**).

The results in **Table 5** show that the "pure" xylanase preparation was contaminated by cellulase activity. However,

Table 4. Effect of Xylanase and β-Glucanase Alone or in Combination and a Commercial Enzyme Preparation Addition on the Viscosity of Feedstuffs[a]

	viscosity (mL/g of DM)				
MP	control	[XYL]	[GLU]	[XYL] + [GLU]	QHP
Rialto wheat	4.73a	0.46c	4.42b	0.33d	0.26e
Sidéral wheat	3.21a	0.29c	2.91b	0.20d	0.09e
Isengrain wheat	2.03a	0.28c	1.95a	0.50b	0.19c
triticale	3.38a	0.38c	3.34b	0.19d	0.19d
rye	24.08a	2.92c	19.38b	2.76d	1.56e
barley	9.91a	3.80b	1.62c	0.50d	0.19e
oats	8.78a	4.76b	0.15c	0.06d	0.02e
corn	0.33a	0.28b	0.34a	0.31a	0.02c
wheat bran	3.31a	0.58d	3.02b	0.86c	0.29e
rice bran	0.79a	0.44c	0.78a	0.56b	0.37d
wheat screenings	1.47a	0.38c	1.45a	0.89b	0.07d
soybean meal	2.00a	1.80c	2.05a	1.94b	0.48d
rapeseed meal	1.48a	0.77d	1.22b	1.08c	0.59e
sunflower meal	0.83a	0.75a	0.80a	0.78a	0.55b
peas	2.20a	1.73c	1.89b	0.99d	0.29e

[a] XYL, xylanase (Megazyme); GLU, β-glucanase (Megazyme); XYL + GLU, mixture of xylanase and β-glucanase; QHP, commercial enzyme preparation (Quatrazyme HP, Nutri-Tomen). Means (five measurements per treatment) in the same row with different letters are significantly different ($P \leq 0.05$).

Table 5. Side Enzymatic Activities in Xylanase, β-Glucanase, and Commercial Enzyme Preparations (International Units per Milligram of Product)

enzyme	XYL[a]	GLU[b]	QHP[c]
arabinofuranosidase			28
xylosidase			13
glucosidase			642
galactosidase			2464
cellulase (IS:[d] Avicel)			0.02
cellulase (SS:[e] CMC)	98		801
polygalacturonase			85340

[a] Xylanase (Megazyme). [b] Glucanase (Megazyme). [c] Quatrazyme HP (Nutri-Tomen). [d] Insoluble substrate. [e] Soluble substrate.

Table 6. Products Released during the Incubation of Xylanase or the Commercial Enzyme Preparation with Pure Wheat Arabinoxylans

product released (μg/mL)	SAX (control)	SAX + XYL[a]	SAX + QHP[b]
arabinose	0	0	19
xylose	0	11	171
xylobiose	0	70	171
xylotriose	0	53	115
xylotetraose	0	0	13

[a] Xylanase (Megazyme). [b] QHP, Quatrazyme HP (Nutri-Tomen).

no side enzymatic activities were detected in the pure β-glucanase preparation. Besides the two main xylanase and β-glucanase activities claimed by the manufacturer, the commercial enzyme preparation also contained several polysaccharide-degrading activities such as arabinofuranosidase, xylosidase, galactosidase, glucosidase, cellulase, and polygalacturonase.

The products released during the incubation of pure xylanase or a commercial enzyme preparation with pure soluble arabinoxylan solution were analyzed by HPAEC. The use of standards (arabinose, xylose, and xylo-oligosaccharides up to a degree of polymerization of 6) made it possible to identify and quantify several peaks. Pure xylanase released xylose, xylobiose, and xylotriose (**Table 6**). The commercial enzyme preparation (Quatrazyme HP) released not only new products [arabinose (1.28% of total arabinose) and xylotetraose] but also more xylose (7 vs 0.46% of total xylose), xylobiose (171 vs 70 μg/mL), and xylotriose (115 vs 53 μg/mL) than pure xylanase (**Table 6**). However, some peaks were not identified. For example, the three peaks that were eluted at 29.792, 30.183, and 30.683 min, respectively (**Figure 1**) were released after the action of pure xylanase and disappeared after the action of the commercial enzyme preparation. The disappearance of these peaks was probably due to their hydrolysis by arabinofuranosidase and xylosidase, which were detected in the commercial enzyme preparation (**Table 5**).

The average molecular weight (MW) of arabinoxylans after incubation with pure xylanase or the commercial enzyme preparation was determined using HPSEC (**Table 7**). The MW of arabinoxylans decreased rapidly (5 min of incubation) after the action of xylanase or the commercial enzyme preparation. However, the degradation of arabinoxylans was more pronounced in the case of the commercial enzyme. A similar pattern was observed for intrinsic viscosity (**Table 7**).

DISCUSSION

The nutritional characteristics of feedstuffs were similar to those reported by Larbier and Leclercq (*28*), Gonçalvez et al. (*29*), and Carré et al. (*30*). The PAV values of feedstuffs reported in the present study were generally in agreement with those obtained by Carré et al. (*23*). Moreover, the viscosity depended on feedstuffs. This was well demonstrated with wheat varieties (Rialto, Sidéral, and Isengrain), which exhibited different levels of viscosity and different water-extractable arabinoxylan and β-glucan contents. Similar results were obtained by Saulnier et al. (*31*), who reported that the amount of water-soluble arabinoxylans was largely dependent on the variety of wheat. Izydorczyk et al. (*2*) reported that β-glucans were significantly different in different types of barley. These variations in total arabinoxylan and β-glucan content between wheat and barley varieties could be explained by genetic differences rather than differences in size and/or amount of endosperm in the cereal kernel (*2*, *31*).

The water-soluble arabinoxylan contents of wheat and barley indicated in this study were in agreement with those reported by Austin et al. (*32*) and Debyser et al. (*33*). There were also high amounts of water-soluble arabinoxylan molecules in rye (*34*).

Besides water-soluble arabinoxylans, β-glucans also affected the physicochemical properties of feedstuffs (*5*). Barley β-glucan contents reported by Beer et al. (*4*) and Saulnier et al. (*35*) were different from those found in the present study. This could be due to many factors such as barley variety, year, and location of cultivation (*31*, *35*). According to the present study, barley contained fewer β-glucans than oats. Moreover, Beer et al. (*4*) reported that not only is the difference between oats and barley limited to the amount of β-glucans but the β-glucan molecular weight in the oat cultivars was also significantly higher than in the barley cultivars.

The viscosity exhibited by wheat bran water extracts was lower in the present study than that of triticale water extracts, although they had similar water-soluble arabinoxylan content and wheat bran contained less β-glucan.

The addition of pure xylanase failed to reduce the viscosity of sunflower meal. The viscosity of sunflower meal water extracts was therefore not created by arabinoxylan but by another nonstarch polysaccharide. May (*7*) reported that pectin is the main constituent of NSP in soybean, rapeseed, and sunflower meals. The viscosity of wheat bran was dramatically reduced by the addition of pure xylanase. It could be suggested that

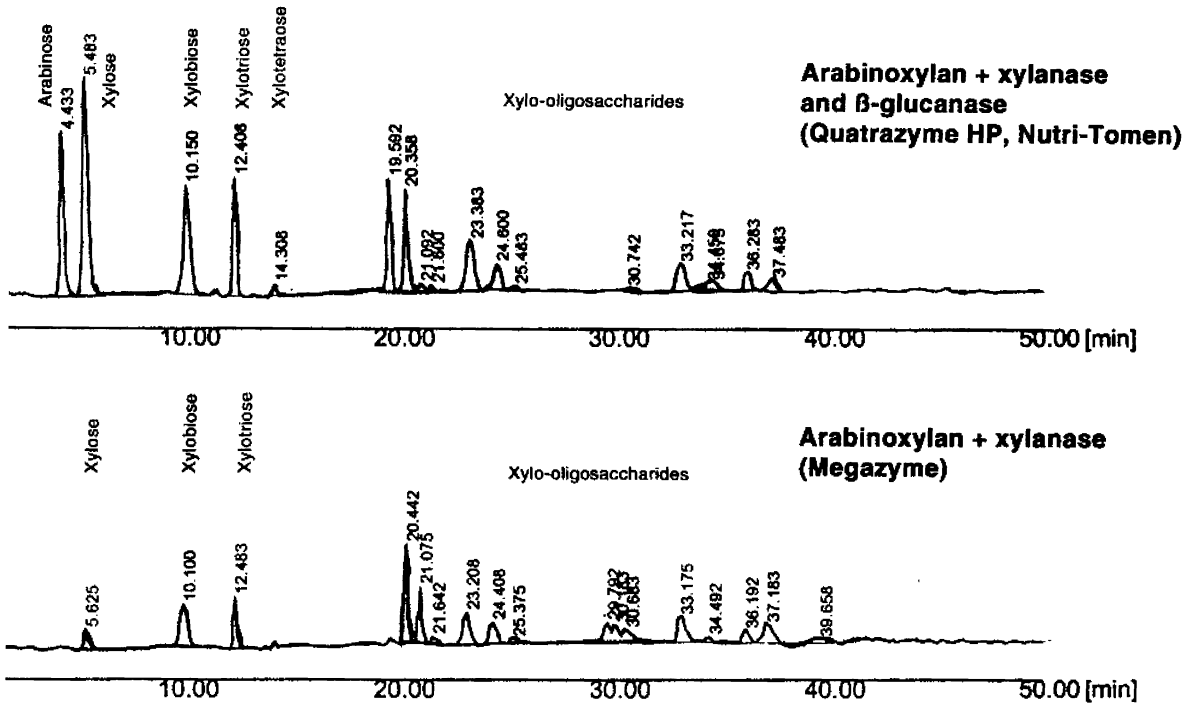

Figure 1. Product released after arabinoxylan hydrolysis by xylanase either alone or in combination with β-glucanase using HPAEC.

Table 7. Intrinsic Viscosity and Average Molecular Weight of Water-Soluble Arabinoxylan as a Function of Incubation Time with Pure Xylanase or Commercial Enzyme Preparation[a]

treatment	MW (kDa)	intrinsic viscosity[b] (mL/g)
SAX (control)	260a	450a
SAX + XYL[c] (5 min)	96b	53b
SAX + QHP[d] (5 min)	73d	49c
SAX + XYL (15 min)	95b	46d
SAX + QHP (15 min)	62e	43e
SAX + XYL (30 min)	80c	45ed
SAX + QHP (30 min)	50f	34f

[a] Means (five measurements per treatment) in the same column with different letters are significantly different ($P \leq 0.05$). [b] Intrinsic viscosity $= -111.5 + 2.096 \times \text{MW} + \epsilon$, $R^2 = 0.96$, where MW is the molecular weight of the arabinoxylans after its incubation with the pure xylanase and the commercial enzyme preparation and ϵ is the residual error. [c] Xylanase (Megazyme). [d] Quatrazyme HP (Nutri-Tomen).

water-soluble arabinoxylans created the viscosity of wheat bran, as reported by Cui et al. (*36*). In general, pure xylanase was more effective in cereals than in their byproducts (wheat and rice bran) or in soybean meal, rapeseed meal, sunflower meal, and peas. The efficacy of pure xylanase was noteworthy in cereals rich in water-soluble arabinoxylans, that is, Rialto wheat and rye (*37*). The addition of pure β-glucanase was less effective than that of xylanase except for barley and oats, in relation to their high β-glucan content. The commercial enzyme preparation was more effective in reducing the viscosity of feedstuffs than the combination of xylanase and β-glucanase. The high efficacy of the commercial enzyme preparation was probably due to the presence of several side enzymatic activities, which enhance and complete the xylanase and β-glucanase action. Kormelink and Voragen (*38*) reported that rice bran arabinoxylan could be partly degraded by the combined action of endo-(1→4)-β-xylanase and arabinofuranosidase and that the extent of hydrolysis of rice bran with endo-(1→4)-β-xylanase alone was low due to its high degree of branching. We have shown that the commercial enzyme preparation was able to liberate arabinose from the side chain of arabinoxylans, due to the presence of an arabinofuranosidase that released arabinose directly from polymeric arabinoxylans and therefore enhanced the action of xylanase, because this enzyme prefers unsubstituted regions of xylan as a substrate (*3*, *38*). The release of xylose by the pure xylanase and the commercial enzyme preparation indicated that internal xylose−xylose bonds were hydrolyzed by xylosidase. This enzyme is involved in the degradation of oligosaccharides derived from the arabinoxylan hydrolyzed by xylanase (*1*). However, the amount of xylose released was greater in the case of the commercial enzyme preparation than in the case of pure xylanase. This may be due both to the presence of a high amount of xylosidase in the commercial enzyme preparation and to the presence of arabinofuranosidase and xylanase.

The essential role of side enzymatic activities in NSP hydrolysis was further sustained by molecular and intrinsic viscosity measurement of arabinoxylans degraded by pure xylanase or the commercial enzyme preparation. The molecular weight and the intrinsic viscosity of arabinoxylan were lower in the case of the commercial enzyme preparation than in pure xylanase.

In conclusion, viscosity depended not only on the concentrations of water-extractable β-glucans and arabinoxylans present in feedstuffs but also on the variety of cereals. The commercial enzyme preparation containing xylanase, β-glucanase, and side enzymatic activities was more effective than xylanase and β-glucanase used alone or in combination. Moreover, the results presented in this paper demonstrate that complex synergistic actions exist between all enzymes involved in the hydrolysis of soluble nonstarch polysaccharides. Finally, further studies are needed to evaluate the appropriate ratios of these enzymatic activities that can be used for different applications.

ABBREVIATIONS USED

[GLU], pure β-glucanase (Megazyme); **[**QHP**]**, Quatrazyme HP (Nutri-Tomen); **[**XYL**]** + **[**GLU**]**, pure xylanase and β-glucanase (Megazyme); **[**XYL**]**, pure xylanase (Megazyme);

CF, crude fat; CP, crude protein; DF, degree of freedom; DM, dry matter; HPAEC, high-performance anion exchange chromatography; HPSEC, high-performance size exclusion chromatography; IS, insoluble substrate; MW, molecular weight; NSP, nonstarch polysaccharides; PAV, potential applied viscosity; RAV, real applied viscosity; SAS, SAS Institute (*27*); SAX, soluble arabinoxylans; SS, soluble substrate.

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