Endoxylanases in Durum Wheat Semolina Processing: Solubilization of Arabinoxylans, Action of Endogenous Inhibitors, and Effects on Rheological Properties

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Endoxylanases seriously affect the rheological properties of durum wheat (*Triticum durum* Desf.) semolina spaghetti doughs prepared with, and as evaluated, by the farinograph. Under the experimental conditions, control doughs (34.9% moisture content) made from two semolinas (semA and semB) yielded a maximal consistency of 525 and 517 farinograph units (FU), with, respectively, 19.4 and 16.4% of the total level of arabinoxylans (TOT-AX) being water-extractable (WE-AX). When 75.4 Somogyi units/50 g of semolina of the endoxylanases from *Trichoderma viride* (XTV), rumen microorganisms (XRM), *Bacillus subtilis* (XBS), and *Aspergillus niger* (XAN) were used, the maximal consistencies at 34.9% moisture decreased for semA to 467, 436, 448, and 417 FU, respectively. This was accompanied by increased WE-AX contents of 60.8, 71.2, 70.7, and 73.0%, respectively. Similar results were observed for semB. By reducing the total water content of doughs, it was possible to recover the maximal consistency of the original doughs. Both the decrease in maximal consistency and the amount of water to be omitted were significantly related to the decrease in molecular weight (MW) of the WE-AX and the percentage of WE-AX solubilized as a result of the enzymic action. At the same time, it was clear that endogenous endoxylanase inhibitors were present in the durum wheat semolinas and that they inhibited the endoxylanases used to different degrees. Part of the differences in effects between the different endoxylanases (decrease in maximal consistency, amount of AX solubilized, MWs of the WE-AX, and amount of water that could be omitted) could be ascribed to the differences in inhibition of the endoxylanases by endogenous inhibitors.

Keywords: *Durum wheat (Triticum durum Desf.); spaghetti; arabinoxylan; endoxylanase; endoxylanase inhibitors; dough rheology*

INTRODUCTION

Semolina, the milling product of durum wheat (*Triticum durum* Desf.), is the product usually used for the production of spaghetti. Its arabinoxylans (AX) are either water-extractable (WE-AX) or water-unextractable (WU-AX). The contents of AX in durum wheat grains vary between 4.07 and 7.46% (dry basis) for total content of AX (TOT-AX) and between 0.37 and 0.56% (dry basis) for WE-AX (Bains and Irvine, 1965; Lempereur et al., 1997). In semolina, the contents reported vary between 0.58 and 3.02%, respectively (dry basis), for TOT-AX (Bains and Irvine, 1965; Lintas and D'Appolonia, 1973; Lempereur et al., 1997). For WE-AX, a level of 0.36% (dry basis) has been reported (Lintas and D'Appolonia, 1973). The percentage of WE-AX in spaghetti is higher than in semolina. This phenomenon has been ascribed to the action of endogenous endoxylanases (Neukom et al., 1962; Lintas and D'Appolonia, 1973).

AX consist of a backbone of 1,4-linked β-D-xylopyranosyl units partially substituted with α-1,2 and/or α-1,3 L-arabinofuranosyl side chains (Perlin, 1951). Ferulic acid can be esterified to the O-5 position of arabinose side chains of such AX (Joseleau et al., 1992). The ferulic acid on AX can give rise to stable gels as a result of oxidative gelation (Geissmann and Neukom, 1973). Durum AX contain a higher proportion of arabinose than do aestivum AX, indicating a more substituted structure (Medcalf and Gilles, 1968; Medcalf et al., 1968; Ciaccio et al., 1982; Roels et al., 1999). Roels et al. (1999) showed that semolina WE-AX have 63.1% unsubstituted, 11.8% monosubstituted, and 25.1% disubstituted xylose residues. Much earlier, it was reported that in durum WU-AX, 44.5% of the xylose residues are unsubstituted, 33.5% monosubstituted, and 22.0% disubstituted (Medcalf and Gilles, 1968).

When, in dough-making, part of semolina was replaced with an AX-rich fraction, the rheological properties of the resulting product were altered significantly (Bains and Irvine, 1965). When durum semolina WE-AX were interchanged with hard red spring wheat WE-AX, macaroni samples with a decreased firmness after cooking were obtained (Sheu et al., 1967). When spaghetti was produced with 1.0 or 2.0% added WE-AX, only small effects on the texture of the final product were noticed (Edwards et al., 1995). In bread-making, durum and regular wheat AX behaved differently (Tao and Pomeranz, 1967).

Much is known about the role of rye (Casier et al., 1973; Kühn and Grosch, 1989; Delcour et al., 1991; Vanhamel et al., 1993) and wheat (Pence et al., 1950; Udy, 1957; Cawley, 1964; Casier et al., 1973; Michniewics et al., 1992; Biliaderis et al., 1995; Courtin and Delcour, 1998) AX in bread-making, as well as about the effect of endoxylanases (McCleary, 1986; ter Hase-

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borg and Himmelstein, 1988; Rouau et al., 1994; Krishnaray and Hoseney, 1994; Courtin et al., 1999). Courtin and Delcour (1998) found a difference in functionality between high molecular weight and low molecular weight WE-AX in bread-making. Also, endoxylanases have a beneficial effect on bread volume by converting the WU-AX into WE-AX (Rouau et al., 1994; Courtin et al., 1999). As reviewed by Courtin and Delcour (1998), the role of AX in bread-making can be related to their impact on viscosity, water distribution, oxidative gelation, and steric hindrance of gluten network formation. According to Jelaca and Hlynka (1971), AX can bind one-third of the total amount of water in a bread dough with WE-AX and WU-AX having different water binding capacities. In view of the above, we judged it to be possible to solubilize WU-AX to influence the characteristics of a pasta dough and the resulting product.

The purpose of this study was therefore to elucidate the effect of different dosages of a number of endoxylanases on spaghetti doughs, prepared in the farinograph. Changes in the WE-AX to WU-AX ratio were monitored, as were the gel permeation profiles of the purified AX. At the same time, we wanted to study to what extent the differences in endoxylanase action could be related to the presence of endoxylanase inhibitors in durum wheat (Debyser and Delcour, 1998). Detailed reports about such inhibitors in common wheat and durum have recently appeared (Debyser et al., 1997, 1999; Rouau and Surget, 1998; McLauchlan et al., 1999).

MATERIALS AND METHODS

Chemicals. All reagents were at least of analytical grade. Specialty chemicals were heat-stable α-amylase (Thermamyl 120 LS, Novo Nordisk, Bagsvaerd, Denmark) and amyloglucosidase (Boehringer Mannheim, Mannheim, Germany). For both enzymes, units were as defined by the supplier. β-D-Allose and clay (Montmorillonite K10) were obtained from Sigma Chemical Co. (St. Louis, MO) and Aldrich (Bornem, Belgium), respectively.

Semolinas. Durum wheat semolina A (semA) was from a blend of Greek and French durum wheats (Soubry, Roeselare, Belgium), and durum wheat semolina B (semB) was from the variety Avonlea (Canadian Grain Commission, Winnipeg, Canada). Samples were stored at 4 °C until used. Protein contents (N × 5.7) were determined according to a Kjeldahl procedure [AACC Method 46-11A (AACC, 1983)] to be 13.6 and 13.1% (dry basis), respectively. Ash (dry basis) contents (AACC Method 08-01) were 0.93% for semA and 0.81% for semB. Moisture contents (AACC Method 44-15A) were 14.0 and 14.1% for semA and semB, respectively.

Activity of the Endoxylanase Preparations. Enzyme solutions were prepared from the endoxylanases XTV (M1, from *Trichoderma viride*, Megazyme, Bray, Ireland; pH optimum 4.7), XRM (M6, from a rumen microorganism, Megazyme; pH optimum 6.0), XBS (from *Bacillus subtilis*, Puratos NV, Groot-Bijgaarden, Belgium; pH optimum 6.0), and XAN (from *Aspergillus niger*, Puratos, pH optimum 4.7). Endoxylanase solutions were prepared in deionized water (30 °C) just before addition to the farinograph. Their activities were determined according to a method by Somogyi (1952), with modifications as depicted in Megazyme product sheet 9/95. One unit is defined as the amount of enzyme that releases 1 μmol of xylose reducing sugar equiv/min at 40 °C from wheat AX (Megazyme) (1% w/v) in sodium phosphate (0.1 M, pH 6.0).

Farinography. Farinograms were recorded according to the method of Irvine et al. (1961). Semolina (50 g, 14.0% moisture basis) was mixed with deionized water (34.9% total water content) in a 50 g stainless steel farinograph bowl connected to a DO-Corder E DCE 330 (Brabender, Duisburg, Germany) operating system.

To test the effect of the endoxylanases, 5.0 mL of the deionized water was replaced by 5.0 mL of enzyme solution. The range of activities of the endoxylanases used was between 0 and 75.4 units/50 g of semolina. By reducing the water added to the dough by 2.0, 3.0, and 4.5%, it was determined which enzyme level could give the same consistency as the control doughs.

Inhibition of Endoxylanases by Durum Wheat Endogenous Endoxylanase Inhibitors. Semolina was extracted (w/v 1:5, 20 min, 20 °C) with a sodium phosphate buffer (0.1 M, pH 6.0). The suspension was centrifuged (4500*g*, 15 min, 20 °C). Half of the supernatant was boiled for 15 min to inactivate the endogenous endoxylanase inhibitors. Both the boiled and the unboiled supernatants were centrifuged (12000*g*, 10 min).

Enzyme solutions (0.2 unit/ mL, 0.5 mL) were added to 0.5 mL of boiled or unboiled supernatant at room temperature. After 30 min, the enzymatic activity was measured, according to the AZCL-xylan procedure described by Cleemput et al. (1995), at 40 °C for 10 min. The activity was measured as the difference in absorbance at 590 nm between the sample and controls (containing buffer instead of semolina extract). Residual activity, in the presence of an active endogenous inhibitor, was then expressed as the ratio (in percent) between the activity of the sample with unboiled extract and the one with boiled extract.

Inactivation of Enzyme-Treated Semolina Doughs. Exactly 25 min (starting with 20 min of dough-mixing in the farinograph) after addition of the endoxylanases, the farinograph doughs (which were divided in pieces of ~1 cm^3) were boiled in ethanol (under reflux) for 2 h. After cooling, the ethanol was removed by vacuum rotary evaporation (45 °C), and the material was air-dried. The material was crushed with a mortar and pestle until it passed a 250 μm sieve. It is hereafter referred to as inactivated material.

Purification of Nonstarch Polysaccharides. Inactivated material (80.0 g) was extracted with 10 mM $HgCl_2$ in deionized water (w/v 1:5, 15 min, 4 °C), taking all safety precautions into account. This additional inactivation step eliminated the action of even small residual endoxylanase activities (Coughlan, 1992). The suspension was centrifuged (8000*g*, 15 min, 4 °C), and the supernatant was boiled for 10 min. Samples were dialyzed (24 h, 4 °C) to remove $HgCl_2$. Following a Thermamyl (3000 units, 30 min, 90 °C) treatment and a centrifugation step (3000*g*, 15 min, 15 °C), the supernatant was clay-treated, as described by Courtin and Delcour (1998), to adsorb residual proteins. The clay and the adsorbed material were removed by centrifugation (8000*g*, 40 min, 15 °C). Samples were then treated with amyloglucosidase at pH 4.5 (50 units, 12 h, 60 °C) and centrifuged (8000*g*, 40 min, 15 °C), and the supernatant was boiled (10 min). After a final centrifugation step (8000*g*, 40 min, 15 °C) to remove the denatured proteins, the supernatant was dialyzed (48 h, 4 °C) and freeze-dried to obtain the nonstarch polysaccharide material.

Because enzymatically degraded AX have a precipitation behavior in ethanol solutions similar to that of arabinogalactan-peptide (AGP) (Courtin et al., 1998), no further separation between AX and AGP was performed.

Determination of Carbohydrate Content. For the determination of the water-extractable carbohydrates in inactivated doughs, 2.0 g of inactivated material was extracted with 20 mL of 10 mM $HgCl_2$ (15 min, 4 °C). After centrifugation (3000*g*, 15 min, 4 °C), the supernatant (2.5 mL) was hydrolyzed (60 min, 110 °C) with 2.5 mL of 4.0 M trifluoroacetic acid (TFA). Both the extraction and the hydrolysis and derivatization were done in duplicate.

For the determination of total carbohydrate content of inactivated materials, 50 mg was hydrolyzed (120 min, 110 °C) with 5.0 mL of TFA (2.0 M). After cooling, the hydrolysate was centrifuged (3000*g*, 15 min). All analyses were done at least in duplicate.

Alditol acetates were prepared according to the method of Englyst and Cummings (1984) and were separated on a Supelco SP-2380 (Bellefonte, PA) column (30 m, 0.32 mm i.d., 0.2 μm film thickness) in a Chrompack 9011 chromatograph

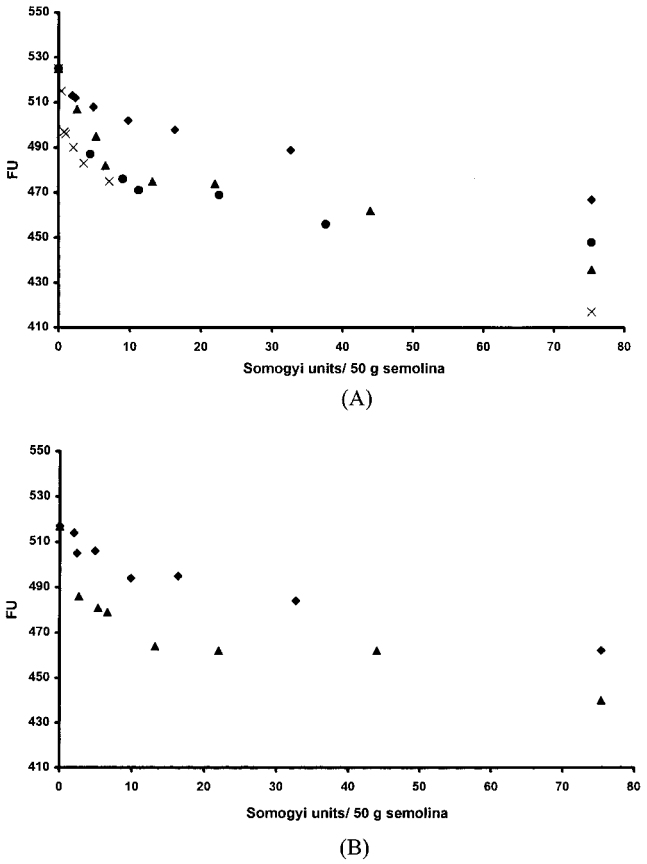

Figure 1. Maximal farinograph consistencies (FU) at 34.9% total moisture content for semolina doughs prepared from semA (A) and semB (B), as a function of dosages (Somogyi units/50 g of semolina) of different endoxylanases (XBS, ●; XAN, ×; XTV, ◆; and XRM, ▲).

(Middelburg, The Netherlands) equipped with a flame ionization detector. The carrier gas was He. Separation was at 225 °C, with injection and detection temperatures of 275 °C and β-D-allose as internal standard (1.0 mL added, with a concentration of 1.0 mg/mL).

Gel Permeation Chromatography (GPC). Nonstarch polysaccharide material (6.0 mg) was solubilized in 0.3% NaCl (3.0 mL) and centrifuged (10000*g*, 10 min). The solution was filtered (0.45 μm) and separated on a Shodex B-804 HQ (Showa Denko K.K., Tokyo, Japan) GPC column (300 × 8 mm) by elution with 0.3% NaCl (0.5 mL/min). The eluate was monitored using a refractive index detector (VDS Optilab, Berlin, Germany). Molecular weight markers were Shodex standard P-82 pullulan standards (Showa Denko K.K.) with molecular weights (MW) of 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, and 0.59×10^4. Comparison of elution times of pullulan standards and nonstarch polysaccharides (AGP and WE-AX) made approximate calculations of MW of the latter possible.

RESULTS AND DISCUSSION

Effects of Added Endoxylanases on Dough Rheology. The maximal consistencies of the control doughs (34.9% moisture content) prepared with semA and semB were 525 and 517 FU, respectively. The addition of endoxylanases decreased the maximal consistency (FU) of semolina doughs significantly (Figure 1). Despite the low moisture content of these semolina doughs, the endoxylanases needed only limited times to be effective because maximal consistency was already reached a few minutes after the addition of the enzymes. No significant impact on other farinograph characteristics was noticed. The decrease of maximal consistency was, for the four enzymes used, dose-dependent (Figure 1). However, large differences were noted between the curves obtained for the different enzymes. When 75 Somogyi units of endoxylanase/50 g of semolina was used for semA, doughs with maximal consistencies of 467, 436, 448, and 417 FU were obtained with XTV, XRM, XBS, and XAN, respectively. Comparable values were obtained for semB (maximal consistencies of 462 and 440 FU for XTV and XRM, respectively).

Table 1. Nonstarch Polysaccharide Compositions (Percent, Dry Basis) of semA and semB Doughs Treated with Endoxylanases and Subsequently Inactivated[a]

	semA				semB			
	AX[b]	AG[c]	A/X[d]	A/Xs[e]	AX	AG	A/X	A/Xs
total hydrolysate	2.22	0.37	0.83		1.83	0.29	0.62	
water extract								
control	0.43	0.37	0.52		0.30	0.29	0.44	
control + XTV								
1.9 units	0.53	0.34	0.53	0.50	0.40	0.28	0.45	0.43
4.9 units	0.70	0.37	0.53	0.52	0.53	0.30	0.48	0.56
32.7 units	1.16	0.35	0.57	0.58	1.02	0.28	0.54	0.56
75.4 units	1.35	0.37	0.57	0.60	1.13	0.28	0.54	0.56
control + XRM								
2.6 units	1.12	0.36	0.56	0.57	1.05	0.30	0.53	0.57
6.6 units	1.27	0.38	0.58	0.62	1.16	0.30	0.53	0.56
44.0 units	1.54	0.37	0.58	0.60				
75.4 units	1.58	0.37	0.57	0.59	1.40	0.29	0.54	0.57
control + XBS								
4.6 units	1.14	0.38	0.54	0.56				
11.3 units	1.44	0.38	0.56	0.58				
75.4 units	1.57	0.36	0.55	0.56				
control + XAN								
0.5 unit	0.75	0.35	0.57	0.58				
1.1 units	1.06	0.37	0.58	0.62				
7.2 units	1.39	0.34	0.60	0.62				
75.4 units	1.62	0.37	0.57	0.59				
CV[f] (%)	6	9	7	6	8	9	6	5

[a] Endoxylanase units are those added per 50 g of semolina. The endoxylanases used originated from *Trichoderma viride* (XTV), rumen microorganisms (XRM), *Bacillus subtilis* (XBS), and *Aspergillus niger* (XAN), respectively. [b] AX = [% Xyl + (% Ara − (A/G) × % Gal)] × 0.88 with A/G = substitution degree of the AGP; i.e., for semAa, 0.68, and for semB, 0.66. [c] AG = [% Gal × 0.9 + % Gal × (A/G) × 0.88]. [d] A/X = [% Ara − (% Gal × (A/G))]/% Xyl. [e] A/Xs = (% Ara − % Ara control)/(% Xyl − % Xyl control). [f] CV, maximal coefficient of variation.

Nonstarch Polysaccharide Composition of Enzyme-Treated Doughs. In Table 1, the nonstarch polysaccharide levels and structural characteristics for the inactivated dough samples are shown. The maximal percentage of AX solubilized [(% WE-AX in endoxylanase-treated samples − % WE-AX in control)/% AX, calculated from the values in Table 1] for 75.4 Somogyi units/50 g of semolina of XAN was 53.6%, compared to an initial WE-AX percentage of 19.4% for the nontreated semA dough. The percentage of WE-AX in nontreated semB was 16.4%. By treating semB with 75.4 Somogyi units/50 g of semolina of endoxylanase XRM, 60.1% AX were solubilized. In an analogous experiment on bread wheat flour doughs treated with endoxylanases, Petit-Benvegnen et al. (1998) obtained a maximum AX solubilization of 41.8%, whereas in the untreated sample 28.0% of the AX were WE-AX.

Purified AGP of semolina had arabinose to galactose (A/G) ratios of 0.68 (semA) and 0.66 (semB) (results not shown). These A/G values were used to calculate the level of arabinose originating from AX. In general, the WE-AX from the endoxylanase-treated doughs had a higher A/X than did the corresponding WE-AX from the untreated doughs (Table 1). Fragments released by the enzyme had a higher average A/X ratio than did the WE-AX population. From the A/X of the total hydrolysates (Table 1), it is obvious, however, that of the total

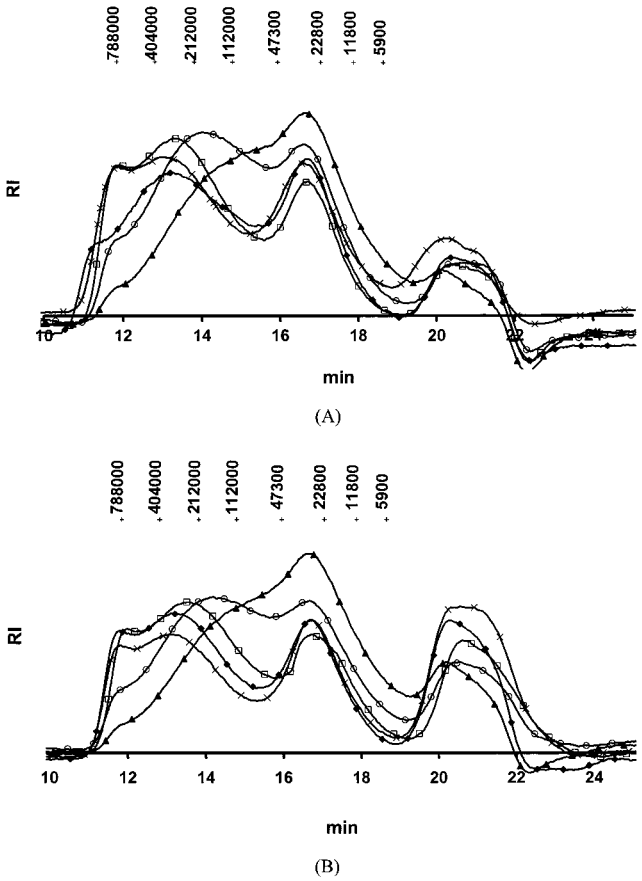

Figure 2. GPC profiles of AX-AGP material, purified from semolina doughs of semA (A) and semB (B) treated with XTV (control, ×; 1.9 units, ◆; 4.9 units, □; 32.7 units, ○; 75.4 units, ▲) (units are expressed as Somogyi units per 50 g of semolina).

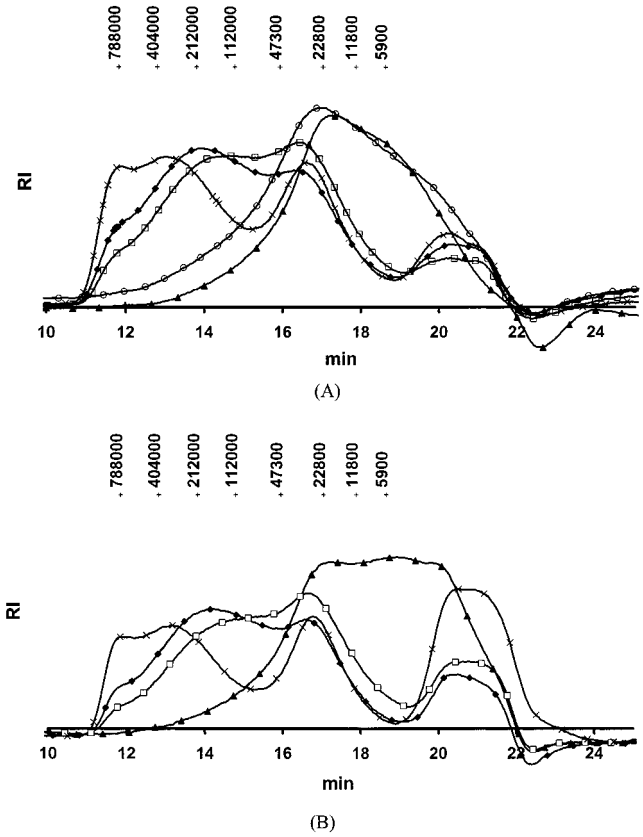

Figure 3. GPC profiles of AX-AGP material, purified from semolina doughs of semA (A) and semB (B) treated with XRM (control, ×; 2.6 units, ◆; 6.6 units, □; 44.0 units, ○; 75.4 units, ▲) (units are expressed as Somogyi units per 50 g of semolina).

population with a high average A/X, the enzymes released the less substituted fragments as evidenced by the lower A/X ratio of the solubilized AX (A/Xs, Table 1). A WU-AX fraction then remained with a high degree of arabinose substitution. This may also help to explain why, to our knowledge, a complete solubilization of AX in wheat flour products has never been achieved with the use of endoxylanases. The highly substituted regions are indeed more resistant to enzymatic breakdown (Gruppen, 1992; Cleemput et al., 1997). WU-AX, which remained insoluble after 24 h of incubation, had an A/X ratio of 0.92 (Gruppen, 1992).

A significant negative correlation was found for all of the endoxylanases between the percentage of AX solubilized and the resulting maximal dough consistency. The R^2 values were 0.97 for XTV ($n = 5$, confidence level of 99%), 0.82 for XRM ($n = 5$, confidence level of 95%), 0.96 for XBS ($n = 4$, confidence level of 95%), and 0.86 for XAN ($n = 5$, confidence level of 95%) when doughs were made from semA. This negative correlation was confirmed for semB, with R^2 values of 0.94 ($n = 5$, confidence level of 99%) for XTV and 0.86 ($n = 4$, confidence level of 90%) for XRM.

MW Profiles of the Nonstarch Polysaccharides. The AGP in *Triticum aestivum* L. flour have apparent MW between 22000 and 25000 (Loosveld et al., 1998). For semA, a MW peak appeared at ~28000 (see control in Figure 2). This peak probably (results not shown) stems from the AGP in the semA. A corresponding peak was found at ~27000 for semB (Figure 3). With low dosages of endoxylanases, these peaks could still be recognized (Figures 2 and 3). With higher dosages, the MW profile of the AX overlaps with that of the AGP peak. It is equally clear that, for some reasons, the dialysis step, intended to remove the lower MW material, was not totally successful. Analogous profiles were obtained using XBS and XAN for semA (results not shown).

Thus, for the endoxylanases used, more AX were solubilized with higher enzyme dosages (Table 1), and the elution profile of the isolated AX shifted toward lower MW (Figures 2 and 3). It can be concluded that solubilized AX and the WE-AX initially present in the untreated dough were further cleaved. When 75.4 Somogyi units/50 g of semolina (semA) of each endoxylanase was used, the apparent MW of the AX purified from the doughs were approximately 11000, 18000, 27000, and 17000 for XBS, XAN, XTV, and XRM, respectively, whereas the original WE-AX of semA had an apparent MW of ~366000. For semB, the WE-AX were broken down from MW ~325000 to ~28000 and ~18000 for 75.4 Somogyi units/50 g of semolina XTV and XRM, respectively.

A positive correlation was found between the maximal consistency (FU) and the apparent MW of the WE-AX. The R^2 values (semA) were 0.89 for XBS ($n = 4$, at a confidence level of 90%), 0.85 for XRM ($n = 5$, at a confidence level of 95%), 0.78 for XAN ($n = 5$, at a confidence level of 99%), and 0.98 for XTV ($n = 5$, at a confidence level of 99.9%). For semB, R^2 was 1.00 for XTV ($n = 5$, at a confidence level of 99.9%). No significant correlation was found for XRM when semB was used.

It was thus demonstrated that, when endoxylanases were used, the decrease in maximal consistency (FU) goes hand in hand with the amount of AX solubilized,

Table 2. Endoxylanase Dosage[a] Necessary To Obtain the Same Consistency as That of the Control Doughs (semA, 525 FU; semB, 517 FU), by Omitting 2, 3, and 4.5% of the Water Added to the Semolina Doughs

water omitted (%)	semA				semB	
	XTV	XRM	XBS	XAN	XTV	XRM
2.0	4.0	0.5	2.0	1.0	4.0	3.0
3.0	7.5	2.0	3.5	2.0	7.5	6.0
4.5	27.5	12.5	10.0	7.5	22.5	12.5

[a] Units are expressed as Somogyi units used per 50 g of semolina; origin of the endoxylanases is as in Table 1.

on the one hand, and with their average apparent MW, on the other hand. As both factors change together, it is unclear to what extent these two factors or their combined impact is important. In any case, it is clear that high molecular weight arabinoxylans have a higher viscosity-forming potential than do their lower molecular weight counterparts and that such viscosity will contribute to the resistance measurement in the farinograph. Furthermore, other factors not studied here may play a role, such as breakdown of the WU-AX without solubilization and decrease of the amount WU-AX, as mentioned and studied by Courtin et al. (1999). Also, the oligosacharides and monosacharides enzymically released may have a function, for example, as plasticizing agent.

Restoring Maximal Consistency (FU). By reducing the amount of added water by 2.0, 3.0, and 4.5% in the doughs and treating them with a certain dosage of endoxylanase, the maximal consistency was restored (Table 2). Large differences were observed in the dosages of the different endoxylanases needed to restore the original 525 FU for semA and 517 FU for semB.

A positive correlation was found between the level of AX solubilized and the amount of water that could be omitted. R^2 values for semA were 0.98 for XTV ($n = 5$, at a confidence level of 99%), 1.00 for XRM ($n = 3$, at a confidence level of 99%), 0.90 for XBS ($n = 4$, at a confidence level of 90%), and 0.94 for XAN ($n = 4$, at a confidence level of 95%). The R^2 for semB when XTV was used was 0.97 ($n = 5$, at a confidence level of 99%). No significant correlation was found for XRM with semB. Significant correlations were also found between the apparent MW of the WE-AX and the amount of water to be omitted. The R^2 values were 0.97 for XTV ($n = 5$, at a confidence level of 99%), 0.99 for XRM ($n = 3$, at a confidence level of 90%), 0.83 for XBS ($n = 4$, at a confidence level of 90%), and 0.98 for XAN ($n = 4$, at a confidence level of 95%) using semA and 0.99 for XTV ($n = 5$, at a confidence level of 99.9%) and 1.00 for XRM ($n = 3$, at a confidence level of 95%) using semB.

Inhibition of Endoxylanase by Durum Wheat Endogenous Endoxylanase Inhibitors. Under the experimental conditions, endoxylanase XRM retained 95% of its activity, whereas XTV, XBS, and XAN retained 3, 30, and 56% of their activities, respectively, when in contact with semA extracts. For semB, the retained activities for XTV, XRM, XBS, and XAN were 2, 100, 32, and 54%, respectively. The data therefore confirmed that inhibitors are present in durum wheat. The observation that the endogenous endoxylanase inhibitors do not influence the activity of all endoxylanases to the same extent and were inactivated by a boiling step is in accordance with the findings of Debyser et al. (1999) for common wheat endogenous endoxylanase inhibitors. The above explains, in part, the observations made for the different endoxylanases concerning the decrease in maximal consistency (Figure 1), the degree of solubilization of AX (Table 1), the reduction of the apparent MW of the WE-AX (Figures 2 and 3), and the amount of endoxylanase needed to restore the original consistency of the untreated samples, when a certain level of the water was omitted (Table 2). The inhibitory effect was most important with the lower dosages of enzymes.

Conclusions. Endoxylanases seriously affect the rheological properties of durum semolina pasta doughs prepared in the farinograph. Through omission of a certain amount of water (2.0, 3.0, or 4.5%) and addition of a certain level of endoxylanase, the decrease of the maximal dough consistency could be restored. The maximal consistency depends on both the amount and the MW of the WE-AX. To what extent both factors and other factors (e.g., decrease in the amount of WU-AX and cleavage of the WU-AX without solubilization) are important is not known. These two factors also affected the amount of water that could be omitted to restore the original consistency of semolina doughs, when no endoxylanases were added. It remains to be clarified whether endoxylanases such as those used in the present work can have an impact on the processability of semolina (extrusion, drying) and/or on the quality of the final product. Our efforts will continue in this direction.

ABBREVIATIONS USED

AX, arabinoxylans; TOT-AX, total level of arabinoxylans; WE-AX, water-extractable arabinoxylans; WU-AX, water-unextractable arabinoxylans; AGP, arabinogalactan-peptides; A/G, arabinose-to-galactose ratio; A/X, arabinose-to-xylose ratio; AZCL-xylan, azurine cross-linked xylan; FU, farinograph units; GPC, gel permeation chromatography; MW, molecular weight; TFA, trifluoroacetic acid.

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