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Water-Extractable and Water-Unextractable Arabinoxylans Affect Gluten Agglomeration Behavior during Wheat Flour Gluten−Starch Separation

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Water-extractable arabinoxylan (WE-AX) of variable molecular weight (MW) and water-unextractable arabinoxylan (WU-AX) were added to wheat flour to study their effect on gluten agglomeration in a dough and batter gluten−starch separation process with recovery of gluten from the batter with a set of vibrating sieves (400, 250, and 125 μm). Low MW WE-AX had almost no impact on the distribution of the gluten on the different sieves. High MW WE-AX decreased yields of the largest (400 μm sieve) gluten aggregates, more than their medium MW counterparts, indicating the importance of AX MW for their effect on gluten interactions. Correlations between the total level of gluten protein recovered on the three sieves and the batter extract viscosity as well as between the proportion of gluten protein recovered on the 400 μm sieve to that on the three sieves and the batter extract viscosity pointed to the importance of viscosity as an indicator for gluten agglomeration, as did the fact that another viscosity increasing plant polysaccharide (guar gum) also negatively influenced gluten agglomeration. However, the obtained data cannot rule out that AX and guar gum also exert steric effects on gluten agglomeration. WU-AX, present as discrete cell wall fragments, had a negative impact on the level of large gluten aggregates. Taken together, the results show that both native WE-AX and WU-AX detrimentally impact gluten agglomeration.

KEYWORDS: Arabinoxylan; gluten agglomeration; gluten−starch separation

INTRODUCTION

The gluten agglomeration behavior is of great importance for the efficiency of industrial wheat flour gluten−starch separation processes and the end-use quality of the resulting vital gluten. It is strongly dictated by the levels and physicochemical properties of the different wheat flour constituents, that is, proteins, starch, arabinoxylans (AX), lipids, and other minor components. In addition, interactions between these constituents can influence the flour processing properties.

AX are nonstarch polysaccharides present in cell walls of different cereals. They consist of a backbone of β-1,4-linked D-xylopyranosyl-units substituted with α-L-arabinofuranosyl-units at the C(O)-2 and/or C(O)-3 positions. Some arabinose residues are substituted with ferulic acid. Approximately one-third of the 1.5−2.5% AX in wheat flour is water-extractable (WE-AX), while the remainder is water-unextractable (WU-AX) (*1*). The difference in extractability between these two AX populations can be explained by differences in chemical and/or physical interactions between AX and other cell wall constituents. WU-AX are strongly embedded in the cell wall network by interactions with other AX through ferulic acid-based cross-linking or with proteins, lignin, cellulose, β-glucans, and glucomannans in the cell wall, while WE-AX are only loosely bound at the cell wall surface (*2*, *3*). As a consequence, both AX populations have different physicochemical and functional properties. WE-AX have a high viscosity forming potential (*4*, *5*) and can be oxidatively cross-linked through ester-linked ferulic acids (*6*−*8*). WU-AX have a high water holding capacity (*9*). These properties strongly dictate the functional effects of AX during biotechnological processes.

Xylanases are endo-acting enzymes that solubilize WU-AX and/or degrade WE-AX and solubilized AX (S-AX) and thus change their structure and physicochemical and functional properties. They profoundly impact gluten agglomeration behavior during gluten−starch separation (*10*−*12*) and indirectly provided insight into the role of different AX populations in gluten interactions. A xylanase with preference for WE-AX degradation decreased batter viscosities and improved gluten agglomeration, as indicated by an increased yield of large gluten aggregates. In contrast, a xylanase with preference for WU-AX solubilization increased batter viscosities and had a detrimental impact on gluten yield. However, complete hydrolysis

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of the total AX population improved gluten agglomeration, even to a larger extent than degradation of WE-AX alone (*11*, *12*).

The evidence available to date suggesting that both endogenous WU-AX and WE-AX have a negative impact on gluten protein agglomeration properties during gluten−starch separation is hence largely based on deducing their roles from the impact of xylanolytic enzymes in the process. From a theoretical point of view, it is not to be excluded that hydrolysis products of different molecular weight (MW) generated by the enzymes have a positive impact on gluten agglomeration. However, studies in which natural high MW WE-AX and WU-AX were added to a gluten−starch mixture (*13*) or to wheat flour (*14*−*16*) revealed the detrimental impact of both AX populations on gluten agglomeration and suggest that the effect of xylanases in gluten−starch separation can probably be explained by the hydrolysis of these deleterious polysaccharides rather than generation of beneficial fragments.

The objective of the present work was to study the direct impact of WE-AX of different MW and WU-AX on gluten agglomeration behavior during a dough and batter gluten−starch separation. To this end, such AX were added to wheat flour and the mixtures were fractionated according to a dough and batter procedure with recovery of gluten on a system of vibrating sieves with decreasing pore sizes. Besides the gluten protein recovery, the viscosity of the batter extracts and the gluten composition were studied. To further elucidate whether the impact of WE-AX on gluten interactions can be attributed to their viscosity increasing effect rather than to a specific interaction, the impact of another viscosity increasing plant polysaccharide, that is, guar gum, during the gluten−starch separation process was studied as well.

EXPERIMENTAL PROCEDURES

Materials. *Chemicals.* All chemicals and reagents were at least of analytical grade unless specified otherwise and were purchased from Sigma-Aldrich (Bornem, Belgium).

Flour. Wheat (variety Zohra, Aveve, Landen, Belgium, conditioned to 16% moisture) was milled on a Bühler MLU-202 laboratory mill (Uzwill, Switzerland) according to AACC method 26-31 (*17*). The resulting flour (yield 74%) had ash (AACC method 08-01) (*17*) and protein contents (*18*) of, respectively, 0.57% and 9.77% [dry matter (dm) basis] and contained 0.39% WE-AX and 1.59% WU-AX (dm) when analyzed as described further. The Farinograph absorption of the flour was 58.5% (14% moisture base, AACC method 54-21) (*17*). It was used in the dough and batter procedure described below.

WE-AX. WE-AX of high MW (HMW-WE-AX) and WE-AX of medium MW (MMW-WE-AX) were supplied by Leuven Bioproducts (Leuven, Belgium). WE-AX of low MW (LMW-WE-AX) were isolated from wheat pentosan concentrate (WPC, Pfeifer & Langen, Dormagen, Germany), a byproduct of the industrial gluten−starch separation process, as described by Courtin and Delcour (*19*). WPC, a mixture of mainly AX, proteins, and arabinogalactan-peptide (AGP), was thus treated with silica to remove proteins. In a next step, LMW-WE-AX were precipitated by addition of ethanol (95%) and recovered.

WU-AX. Wheat flour (variety Petrus, Clovis Matton, Avelgem, Belgium) was fractionated according to MacRitchie (*20*). Batches (250 g) were mixed with 160 mL of water and kneaded manually to a dough. Starch was washed from the gluten matrix by five subsequent washings with 500 mL of water. The resulting starch suspension was centrifuged (5000*g*, 10 min, 15 °C). The darker top layer or squeegee fraction (SQF), which contained insoluble cell wall material, starch, and proteins, was manually removed from the prime starch layer, suspended in water, and centrifuged as above to remove residual prime starch. This fractionation procedure was repeated several times to obtain a large quantity of SQF. WU-AX in this SQF were enriched by enzymic removal of starch and proteins. To that end, the SQF (400 g) was suspended in water (1:10 w/v), and the suspension was heated to 75 °C. Thermamyl 120L (16.7 mL, thermostable α-amylase, Novozymes, Bagsvaerd, Denmark) was added. The suspension was heated further to 90 °C and incubated for 30 min at this temperature. After centrifugation (10 000*g*, 15 min, 15 °C), the residue was suspended in sodium acetate buffer (25 mM, pH 5), and Neutrase 0.8L proteinase (13.4 mL, Novozymes) was added. The suspension was incubated for 24 h at 50 °C. It was finally boiled for 15 min to inactivate the enzymes and centrifuged (10 000*g*, 15 min, 15 °C). The residue was washed two times with water. It was then air-dried following several washings with ethanol and ground to pass a 250 μm sieve.

Guar Gum. Guar gum consists of a backbone of β-1,4-linked D-mannopyranosyl-units substituted with α-D-galactopyranosyl-units at the 6-positions. This galactomannan forms highly viscous solutions in water.

High-Performance Size Exclusion Chromatography. The MW profiles of the different WE-AX populations were determined with high performance size exclusion chromatography (HPSEC). Samples were separated on a Shodex SB-806 HQ HPSEC column with a MW range of 100 to 20×10^6 (Showa Denko K.K., Tokyo, Japan). Elution was with 0.3% NaCl (0.5 mL/min at 30 °C) on a Kontron 325 pump system (Kontron, Milan, Italy) and monitoring with a refractive index detector (VSD Optilab, Berlin, Germany). MW markers were glucose and Shodex standard P-82 pullulans with MW of 788×10^3, 404×10^3, 212×10^3, 112×10^3, 47.3×10^3, 22.8×10^3, 11.8×10^3, and 5.9×10^3.

Ferulic Acid Content. Ferulic acid contents of the WE-AX populations were determined according to Izydorczyk et al. (*21*). This is based on liberation of ferulic acid by treating the samples with 2.0 M NaOH (30 min, 35 °C), after which they were acidified to pH 2.0 with HCl (2.0 M). After extraction of the samples with hexane, free ferulic acids in the water phase were extracted with ethyl acetate and quantified by determining the absorbance at 320 nm. Free ferulic acid was used as the standard.

Farinograph Water Absorption. Farinograph water aborptions of mixtures of flour and different levels of LMW-WE-AX, MMW-WE-AX, HMW-WE-AX, WU-AX (0−1.4%), and guar gum (0−0.5%) were determined with a Brabender Farinograph E (Duisburg, Germany) according to AACC method 54-21 (*17*).

Wheat Flour Gluten−Starch Separation with AX or Guar Gum Addition. The laboratory scale fractionation procedure is based on Frederix et al. (*22*). Wheat flour (250 g, 14% moisture base) or a mixture of wheat flour and AX or guar gum (250 g including 0−1.4% AX or 0−0.5% guar gum, 14.0% moisture base) was mixed with water (Farinograph water absorption) in a KitchenAid mixer (K5SS/KPM5, St. Joseph, MI) equipped with a dough hook (4 min). The percentages of the added AX fractions are expressed as percentages AX, thus taking into account the purity of the fractions. Following a dough rest (8 min), additional water (250 mL) was added, the suspension was stirred (flat beater, 25 min), extra water (1000 mL) was added again, and the suspension was further stirred (35 min). It was then brought over vibrating sieves with decreasing pore sizes (400, 250, and 125 μm). The gluten on the sieves was washed with extra water (1000 mL), recovered from the sieves, lyophilized, and ground to pass a 250 μm sieve.

Analysis of Protein Contents. Protein contents were determined using an adaptation of the AOAC Official Dumas Method to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands) (*18*). The coefficient of variation of the analysis was less than 1%.

Several parameters (*22*) were defined as follows:

gluten protein recovery (GPR_{xxx}), that is, g of protein (dm) agglomerated on the sieve with a pore diameter of xxx μm per 100 g of flour protein (dm);

total gluten protein recovery (GPR_{tot}), that is, the total amount of protein (dm) agglomerated on the three consecutive sieves (400, 250, and 125 μm) per 100 g of flour protein (dm); and

gluten protein agglomeration index (GPAI), that is, the ratio (in percent) of protein agglomerated on the 400 μm sieve to the total amount of protein recovered from the three consecutive sieves.

The coefficient of variation of the GPR was less than 5%, 15%, and 10% for the 400, 250, and 125 μm sieves, respectively. The GPR and

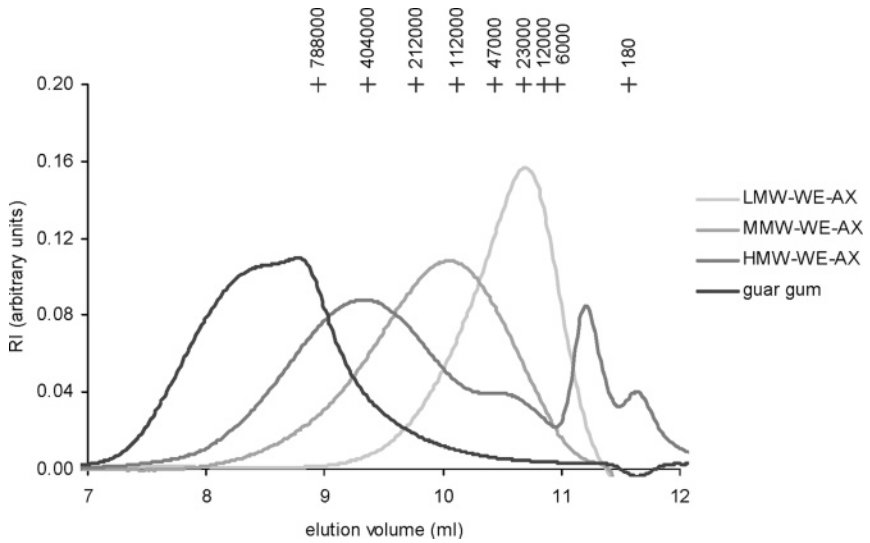

Figure 1. Molecular weight profiles (HPSEC) of the different WE-AX populations and guar gum. Pullulan MW markers are indicated with crosses.

the distribution of proteins over the different sieves are a measure of the agglomeration behavior and reflect the processing properties of the flour. The GPAI is a measure of the tendency of gluten to agglomerate and form large gluten aggregates.

Analysis of Carbohydrate Composition. The carbohydrate compositions of the flour, the different AX populations, guar gum, and the gluten fractions were determined by gas liquid chromatography of alditol acetates obtained after acid hydrolysis (2 M trifluoroacetic acid), reduction (sodium borohydride), and acetylation (acetic anhydride) of the samples (*23*). Derivative separation was on a Supelco SP-2380 polar column (Supelco, Bellefonte, PA) in an Agilent chromatograph (Agilent 6890 series, Wilmington, DE) equipped with a flame ionization detector. The inlet and detector temperatures were 270 °C, while separation took place at 225 °C. The carrier gas was He. The coefficient of variation of the analysis was less than 5%.

Measurement of Viscosity. A sample of the batter withdrawn immediately after sieving was centrifuged (5000*g*, 15 min, 4 °C). The supernatant was frozen with liquid nitrogen and lyophilized. Boiling water (150 mL) was then added (100 °C, 30 min) to the lyophilizate to denature protein material, and, after renewed lyophilization, the material was dispersed in water (50 mL) and centrifuged (10000*g*, 15 min, 4 °C). The viscosity of the supernatant was determined in triplicate with an Ostwald viscometer (30 °C) according to Vinkx et al. (*24*). The viscosity of the batter extracts was expressed relative to that of water under the same conditions. The coefficient of variation of the measurement was less than 2%.

Table 1. Farinograph Water Absorptions (%) of the Flour–AX Mixtures (0–1.4% AX) and Flour–Guar Gum Mixtures (0–0.5% Guar Gum) and Relative Viscosities (30 °C) of Batter Extracts Resulting from Gluten–Starch Separations of These Mixtures

		Farinograph water absorption	relative viscosity
LMW-WE-AX	0.0%	58.5	1.23
	0.4%	58.6	1.24
	0.8%	58.7	1.32
	1.4%	58.8	1.30
MMW-WE-AX	0.0%	58.5	1.23
	0.5%	61.0	1.35
	1.0%	62.3	1.49
	1.4%	63.2	1.69
HMW-WE-AX	0.0%	58.5	1.23
	0.6%	62.8	1.54
	0.9%	63.7	1.66
	1.4%	66.2	2.07
WU-AX	0.0%	58.5	1.23
	0.4%	60.8	1.26
	0.7%	62.3	1.26
	1.4%	65.7	1.32
guar gum	0.0%	58.5	1.23
	0.25%	61.0	1.44
	0.5%	62.6	1.72

RESULTS

Characterization of AX Samples and Guar Gum. The substitution degrees or arabinose-to-xylose ratios of LMW-WE-AX, MMW-WE-AX, HMW-WE-AX, and WU-AX were, respectively, 0.65, 0.45, 0.49, and 0.48. The WE-AX fractions all contained ferulic acid esterified to the arabinose residues. For LMW-WE-AX, MMW-WE-AX, and HMW-WE-AX, the ferulic acid contents were, respectively, 67, 100, and 114 mg/100 g AX.

Guar gum contained 57.4% galactose and 32.7% mannose and only minor levels of arabinose and xylose, which indicates that this fraction consists mainly of galactomannans.

The molecular weight profiles of the WE-AX fractions and guar gum (**Figure 1**) showed apparent peak MW for HMW-WE-AX, MMW-WE-AX, and LMW-WE-AX of ca. 404k, 112k, and 23k, respectively. Guar gum contained very high MW polysaccharides with an apparent peak MW exceeding 800k.

Impact of AX and Guar Gum on Farinograph Water Absorption. Addition of WE-AX as well as WU-AX to wheat flour increased the Farinograph water absorption of the flour−AX mixtures (**Table 1**). The MW of WE-AX preparations impacted their effect on the Farinograph absorptions. Addition of LMW-WE-AX hardly affected the water absorption, while that of 1.4% MMW-WE-AX and 1.4% HMW-WE-AX increased the Farinograph water absorption from 58.5% (control) to 63.2% and 66.2%, respectively. The water absorption of a mixture of flour and 1.4% WU-AX was 65.7%. Addition of guar gum to wheat flour increased Farinograph water absorption from 58.5% (control) to 62.6% (0.5% guar gum). These water absorptions were used during the dough-making step of the gluten−starch separation procedure. This way, a correction was made for the amount of water absorbed by the added AX, and a more optimal dough development was assured.

Impact of AX and Guar Gum on Gluten Agglomeration Behavior. *WE-AX of Different MW.* **Figure 2** shows the distribution of gluten proteins over the different sieves upon addition of different levels of LMW-WE-AX, MMW-WE-AX, and HMW-WE-AX. The figures above the bars in **Figure 2**

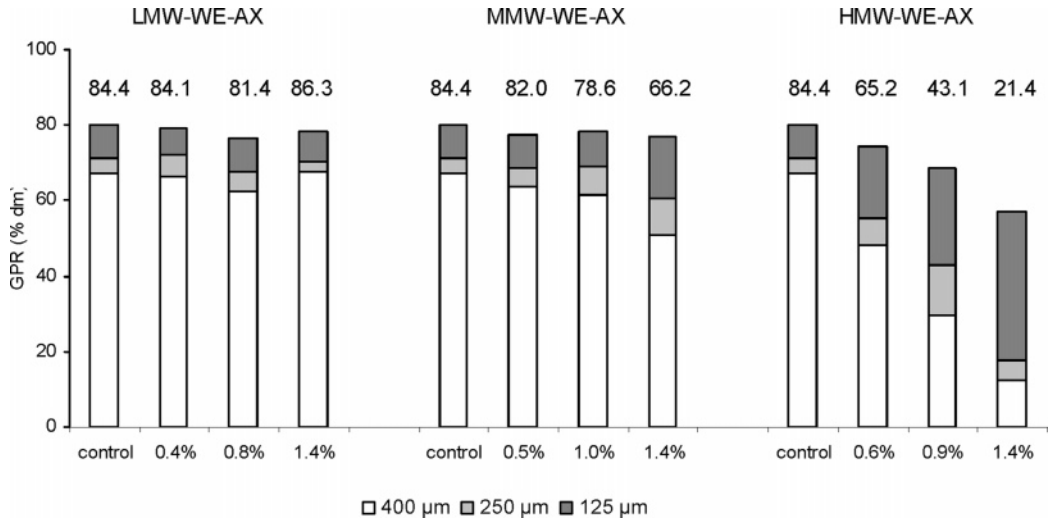

Figure 2. GPR (%) on 400, 250, and 125 μm sieves during gluten–starch separation of wheat flour to which different concentrations of LMW-WE-AX, MMW-WE-AX, and HMW-WE-AX were added. The figures above the bars are the corresponding GPAI (%).

are the corresponding GPAI and reflect the tendency to form large gluten aggregates. LMW-WE-AX hardly affected the distribution of the gluten proteins over the sieves. With addition of different concentrations of LMW-WE-AX, the GPAI remained virtually constant. Added MMW-WE-AX had a negative effect on gluten agglomeration. The proportion of large gluten agglomerates decreased, and more proteins were retained on the 250 and 125 μm sieves. The GPAI decreased from 84% to 66% with addition of 1.4% MMW-WE-AX. Added HMW-WE-AX had a more detrimental effect on gluten agglomeration. Addition of 1.4% HMW-WE-AX decreased the level of proteins on the 400 μm sieve and the GPAI drastically from 67% to 12% and from 84% to 21%, respectively. The total yield of protein on the three sieves decreased from 80% to 57%. The above indicate the importance of the MW of the added WE-AX. Higher MW AX had more negative effects on gluten agglomeration. Wang et al. (*14*), who used a modified Glutomatic system to fractionate flour, found that medium viscosity WE-AX (Megazyme) had a negative effect on gluten yield. Xylanase pretreatment of such WE-AX could not overcome the negative effect. This is not consistent with the present findings that added LMW-WE-AX had almost no effect on gluten agglomeration. Other researchers (*10*, *11*, *25*) also found that a decrease of AX MW by xylanase action improves gluten agglomeration.

The HMW-WE-AX levels (endogenous + added AX) in the wheat flour-HMW-WE-AX mixtures were negatively correlated with GPR_{tot} ($R^2 = 0.95$), indicating that the level of HMW-WE-AX determines the total gluten yield. Roels et al. (*26*) fractionated wheat flour from six different wheat varieties according to a similar dough batter procedure and obtained varying gluten agglomeration behaviors. The WE-AX contents of the flours (adjusted to constant protein level) and GPR_{tot} obtained by Roels et al. (*26*, *27*) were also negatively correlated ($R^2 = 0.70$), pointing to an important role of HMW-WE-AX in gluten agglomeration.

Taken together, these results point to a deleterious role of HMW-WE-AX present in the flour. When solely considering this fraction, the positive impact of a xylanase (*10*−*12*) with a selectivity for hydrolysis of such AX in the process is due to a reduction in MW and not to the formation of low MW AX fragments advantageous for the process.

Guar Gum. Guar gum also affected the gluten agglomeration behavior (**Figure 3**). Upon addition of 0.5%, the GPR_{400} and

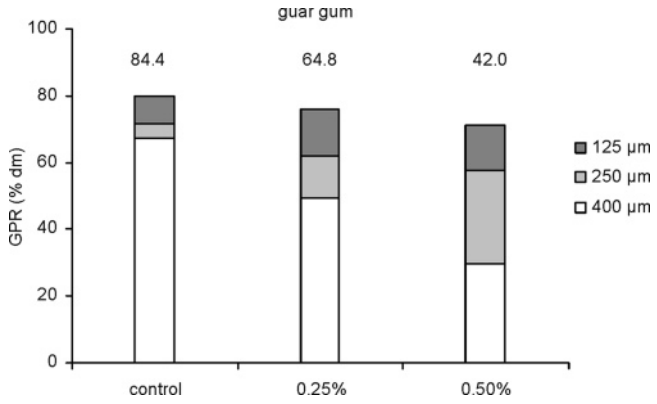

Figure 3. GPR (%) on 400, 250, and 125 μm sieves during gluten–starch separation of wheat flour to which different concentrations of guar gum were added. The figures above the bars are the corresponding GPAI (%).

GPR_{tot} decreased from 67% to 30% and from 80% to 71%, respectively. This decreased the GPAI from 84% to 42%, indicating that the amount of large gluten aggregates drastically decreased upon addition of these viscosity-increasing polysaccharides.

WU-AX. WU-AX had a negative effect on gluten agglomeration (**Figure 4**). The level of large gluten aggregates decreased, and more proteins were retained on the 250 and 125 μm sieves. This decreased GPAI from 84% to 50% with addition of 1.4% WU-AX. Wang et al. (*15*, *16*) found a detrimental impact of water-unextractable solids on gluten yield during wheat flour fractionation.

Impact of AX and Guar Gum on Viscosity of Batter Extracts. Table 1 also documents that addition of WE-AX of different MW to flour has different impacts on the relative viscosities of the batter extracts at the end of the gluten−starch separation process. Addition of LMW-WE-AX only slightly increased the viscosity of the batter extracts. Increasing concentrations of MMW-WE-AX increased viscosities of the batter extracts from 1.23 (control) to 1.69 (1.4% MMW-WE-AX), while HMW-WE-AX even more markedly affected the viscosity. Here, an increase from 1.23 to 2.07 was noted with addition of 1.4% HMW-WE-AX. The above shows the importance of the MW of the AX for the resulting viscosity. The relative viscosity of the batter extract was strongly correlated with the AX concentration. Guar gum also affected the batter extract

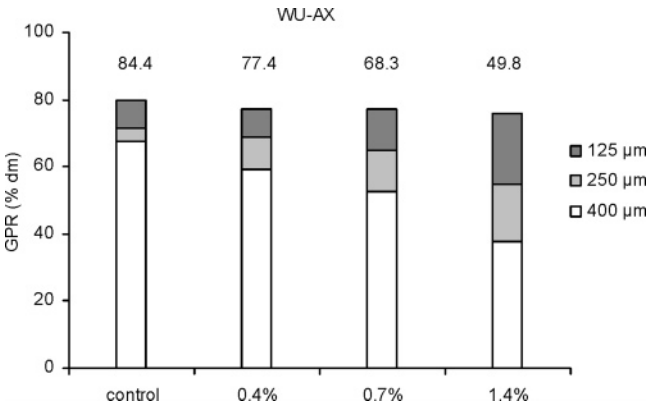

Figure 4. GPR (%) on 400, 250, and 125 μm sieves during gluten–starch separation of wheat flour to which different concentrations of WU-AX were added. The figures above the bars are the corresponding GPAI (%).

viscosity. Addition of 0.5% guar gum increased the viscosity from 1.23 to 1.72.

As shown in **Table 1**, added WU-AX had little if any impact on the relative viscosity of the batter extracts. The very slight increase observed for addition of 1.4% WU-AX probably resulted from mixing-induced mechanical solubilization of WU-AX.

Impact of AX and Guar Gum on Gluten Composition. Apart from protein, gluten fractions retained on the different sieves also contained saccharide material (**Table 2**), lipids, and ash. Glucose, mainly present as starch, was quantitatively the most important carbohydrate constituent present. Besides glucose, arabinose, xylose, galactose, and small levels of mannose were present. Arabinose is a building block of both AX and AGP, while galactose is mainly part of AGP and galactolipids. The exact calculation of the AX content of the gluten samples was not possible because it is unclear how much arabinose in gluten originates from AX and how much originates from AGP. The xylose level is used as a measure for the AX content of the gluten fractions. In all cases and in line with earlier results (*22, 28*), the xylose content of the gluten fractions increased with decreasing pore size.

Addition of AX resulted in an increase of the xylose content of the 400 and 250 μm gluten fractions. This can indicate that part of the extra added AX is retained in the gluten network.

The galactose level of the gluten fractions did not depend on AX-addition and fluctuated between 0.8% and 1.1%.

The glucose content of the 400 μm gluten fractions varied between 16% and 26%. The 250 μm gluten fractions contained a lower glucose content ranging from 15% to 23%. The highest glucose levels were present in the 125 μm sieve gluten fractions (between 22% and 37%). For addition of MMW-WE-AX, HMW-WE-AX, and guar gum, the glucose content of the 400 μm gluten fractions decreased as the level of addition increased. The worsened agglomeration behavior upon AX or guar gum addition is probably responsible for a decreased level of starch entrapment in the gluten matrix.

DISCUSSION

Addition of WE-AX with different MW during gluten−starch separation showed that the MW of these polysaccharides determines their effect on gluten agglomeration. LMW-WE-AX hardly affected the distribution of gluten proteins over the different sieves, whereas MMW-WE-AX and HMW-WE-AX negatively influenced the formation of large gluten aggregates. HMW-WE-AX had the most detrimental impact on the agglomeration behavior. The MW of these AX populations is very predictive for their effect on the batter extract viscosity. While addition of LMW-WE-AX only caused an insignificant rise in batter extract viscosity, HMW-WE-AX drastically increased it.

This brings us to the possible relation between viscosity and gluten agglomeration. **Figure 5** shows a plot of the GPR_{tot} and the GPAI as a function of the relative viscosity of the batter extracts for addition of the different WE-AX populations. An increased batter extract viscosity coincided with a decreased GPR_{tot} and GPAI. This indicates the predictive value of viscosity for the gluten interactions during gluten−starch separation and is in line with earlier results (*10, 11, 25*). Frederix et al. (*11*) changed the native AX population in wheat flour during dough and batter gluten−starch separation by addition of xylanases with different substrate selectivity and also obtained a correlation between batter extract viscosity and GPAI. Decreasing viscosity of the batter extract by xylanase action improved the gluten yield. Solubilization of WU-AX, on the contrary, increased the batter extract viscosity and had a negative effect on gluten agglomeration. However, when these S-AX were degraded to low MW fragments at elevated enzyme concentration, an improvement of the gluten agglomeration was observed again.

Added HMW-WE-AX may exert a role during dough making and/or during the batter phase of the process. Because xylanase can be added in the first steps of the batter process and hence does not need to be present at the dough stage (*11*), it is reasonable to assume that the negative impact of AX in the process is mainly exerted in the early phases of the batter process and, hence, when much higher water to dry mass ratios are present. During the batter phase, starch is washed out of the gluten matrix and gluten agglomerate. This process is negatively influenced by high batter viscosities, such as those resulting from addition of AX. The increased viscosity probably decreases the mobility of the components in the batter. The fact that viscosity is an important factor for the effect of AX on gluten agglomeration is also indicated by addition of other viscosity-increasing polysaccharides and their effect on gluten interactions. Addition of guar gum increased the batter extract viscosity and clearly had a negative effect on the gluten agglomeration during the gluten−starch separation as indicated by the decreased GPR_{tot} and GPAI. These values fit within the correlation between viscosity and gluten agglomeration for addition of WE-AX as shown in **Figure 5**. The correlation coefficient indicated in **Figure 5** was hardly affected when the points corresponding to the guar gum additions were incorporated in the calculation.

In analogy with the relation between relative viscosity of the batter extracts and the gluten agglomeration behavior, a similar correlation exists between the Farinograph absorptions of the flours and the gluten agglomeration behavior (results not shown). This relation is not necessarily causal, but it does indicate that the Farinograph absorption of the flour also has a predictive value for the resulting gluten agglomeration during the dough-batter process.

An alternative explanation is that HMW-WE-AX disturb the agglomeration of gluten proteins through protein−AX interactions (*25*) and that steric hindrance of these molecules is responsible for the negative effect on the interactions between gluten proteins (*29*). In the latter interpretation, the viscosity is not the factor responsible for impaired gluten agglomeration but only a quantitative indicator of the presence of HMW-WE-AX.

Whatever be the case, the data in the present work cannot rule out that steric hindrance of WE-AX or guar gum can also

Table 2. Carbohydrate Compositions (Arabinose, Xylose, Galactose, and Glucose, % dm) of Gluten Fractions Recovered on the Different Sieves during Gluten–Starch Separation of Wheat Flour to Which Different Concentrations of LMW-WE-AX, MMW-WE-AX, HMW-WE-AX, WU-AX, and Guar Gum Were Added[a]

	400 μm				250 μm				125 μm			
	ara	xyl	gal	glc	ara	xyl	gal	glc	ara	xyl	gal	glc
control	0.41	0.39	0.93	23.05	0.78	0.88	0.99	18.43	4.22	6.67	0.88	29.28
0.4% LMW-WE-AX	0.44	0.41	0.99	25.90	0.62	0.65	1.03	16.89	4.23	6.69	0.81	36.89
0.8% LMW-WE-AX	0.45	0.47	0.96	23.27	0.92	1.25	0.97	20.29	3.70	6.28	0.96	24.01
1.4% LMW-WE-AX	0.58	0.61	1.02	26.00	0.76	0.81	1.06	16.83	4.28	6.75	0.95	28.22
0.5% MMW-WE-AX	0.39	0.35	1.00	22.12	0.96	1.17	0.99	21.58	3.70	5.95	0.81	36.24
1.0% MMW-WE-AX	0.45	0.43	1.02	20.15	1.09	1.41	0.97	19.60	4.22	6.81	0.81	32.74
1.4% MMW-WE-AX	0.46	0.45	1.03	19.93	1.20	1.58	1.08	18.98	3.16	5.08	0.91	33.59
0.6% HMW-WE-AX	0.42	0.41	0.90	19.90	0.77	0.90	0.91	17.38	2.38	3.66	0.78	30.85
0.9% HMW-WE-AX	0.42	0.42	0.91	18.72	1.00	1.25	0.93	16.70	2.43	3.88	0.86	24.66
1.4% HMW-WE-AX	0.49	0.55	0.93	16.15	1.10	1.40	0.94	15.06	2.02	3.15	0.89	22.27
0.4% WU-AX	0.42	0.41	0.96	22.22	0.88	1.06	0.98	21.06	3.61	5.75	0.87	33.84
0.7% WU-AX	0.50	0.60	0.93	23.86	0.85	1.17	0.93	21.58	3.71	6.63	0.80	34.01
1.4% WU-AX	0.59	0.70	0.96	23.87	0.96	1.24	0.97	22.61	2.61	4.09	0.97	24.87
0.25% guar gum	0.40	0.42	0.89	22.96	0.67	0.81	0.96	19.45	2.77	4.59	0.90	30.79
0.5% guar gum	0.44	0.43	0.98	16.91	1.25	1.68	1.11	21.36	3.84	6.31	1.09	24.14

[a] ara, arabinose; xyl, xylose; gal, galactose; glc, glucose.

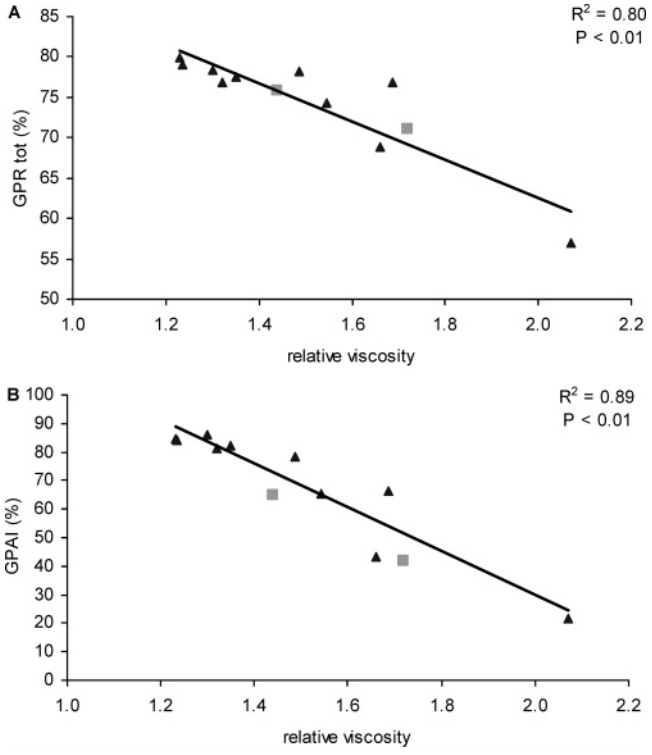

Figure 5. Correlation between GPR_{tot} (%) and relative viscosity of the batter extracts (**A**) and between GPAI (%) and relative viscosity of the batter extracts (**B**) for addition of the different WE-AX populations (points indicated by ▲). The points indicated by the gray ■ correspond to the guar gum additions and are not included in the calculation of the correlation coefficient.

be responsible for the effect on gluten interactions, but they do suggest that viscosity is a good indicator for the gluten agglomeration behavior.

Wang et al. (*14*) ascribed the negative impact of WE-AX on gluten interactions in part to a ferulic acid-mediated effect. They postulated that direct linking of WE-AX to gluten proteins through the esterified ferulic acid is responsible for the effect of WE-AX on gluten agglomeration. However, contradictory results have been mentioned about the exact role of ferulic acid in a possible cross-linking between AX and proteins, and the findings are often speculative (*7*, *30*−*34*). Because other viscosity-increasing polysaccharides such as galactomannans also negatively influence gluten agglomeration during the dough and batter gluten−starch separation, the negative effect of AX on gluten interactions is probably not an AX specific effect and viscosity probably is a much more important factor than a ferulic acid-mediated effect.

WU-AX had a negative impact on gluten agglomeration, as indicated by a decreased amount of large gluten aggregates. These WU-AX, present as discrete cell wall fragments, probably interfere with gluten agglomeration through steric hindrance and form a physical barrier for the interaction between gluten particles. In analogy with the effect of WE-AX, Wang et al. (*15*, *16*) attributed part of the effect of water-unextractable solids on gluten agglomeration to a ferulic acid-mediated interaction. Xylanase addition during gluten−starch separation also revealed the negative impact of WU-AX on gluten interactions (*11*, *12*). At very high enzyme concentrations, when extensive degradation of the total AX population to low MW was observed, the gluten agglomeration was more successful than the control and even more successful than with an enzyme treatment primarily resulting in WE-AX degradation.

ABBREVIATIONS USED

AGP, arabinogalactan-peptides; AX, arabinoxylan(s); GPAI, gluten protein agglomeration index; GPR, gluten protein recovery; GPR_{tot}, total gluten protein recovery; GPR_{xxx}, gluten protein recovery on the sieve with a pore diameter of xxx μm; HMW-WE-AX, high molecular weight water-extractable arabinoxylan(s); HPSEC, high-performance size exclusion chromatography; LMW-WE-AX, low molecular weight water-extractable arabinoxylan(s); MMW-WE-AX, medium molecular weight water-extractable arabinoxylan(s); MW, molecular weight; S-AX, solubilized arabinoxylan(s); SQF, squeegee fraction; WE-AX, water-extractable arabinoxylan(s); WPC, wheat pentosan concentrate; WU-AX, water-unextractable arabinoxylan(s).

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