The role of arabinoxylan in determining the non-linear and linear rheology of bread doughs made from blends of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) flour

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Abstract

Part of the role which arabinoxylan (AX) plays in bread making relates to its impact on the bulk rheology of dough. However, the actual mechanisms by which it influences dough rheology are still not well understood. An evident research approach is to use endo-β-1,4-xylanases (XYLs) to hydrolyze AX and hence increase their solubility. Here, the AX population in (i) wheat dough and (ii) mixed cereal doughs made from blends of wheat flour (WF) and rye flour (RF) was subjected to hydrolysis by XYL from *Aspergillus aculeatus* (XAA) or *Bacillus subtilis* (XBS). Non-linear and linear dough rheology was studied by uniaxial extensional and small-amplitude oscillatory shear measurements, respectively. Low dosages of XBS or XAA in wheat or mixed cereal doughs changed neither their non-linear nor their linear extensional rheological responses, presumably because limited AX hydrolysis did not result in high losses of water binding and/or because dough constituents bound the liberated water. A high dosage of XBS or XAA led to extensive hydrolysis of AX and considerably decreased the linear extensional viscosity. This decrease resulted from an altered dough water distribution.

Keywords: Dough rheology; Wheat flour-rye flour dough; Non-linear extensional rheology; Linear shear rheology; Arabinoxylan; Xylanase

1. Introduction

Wheat (*Triticum aestivum* L.) gluten proteins play an essential role in bread making. Upon flour hydration and kneading, they develop into a continuous network which provides the resultant dough with the ability to strain harden under extension (van Vliet, 2008). This implies that the stress required to stretch dough increases super-linearly with increasing deformation. A result of gluten protein strain hardening is that dough films between adjacent gas cells incorporated during mixing thin where they have the greatest thickness (Dobraszczyk & Roberts, 1994; van Vliet, Janssen, Bloksma, & Walstra, 1992). This supposedly promotes uniform expansion of gas cells during fermentation and baking. As such, strain hardening plays a crucial role in determining wheat bread loaf volume and crumb structure (Dobraszczyk & Roberts, 1994; Dobraszczyk & Salmanowicz, 2008; Dobraszczyk, Smewing, Albertini, Maesmans, & Schofield, 2003; van Vliet, Janssen, Bloksma, & Walstra, 1992).

Some health benefits have been ascribed to mixed cereal bread products (Bushuk, 2001; Dewettinck et al., 2008). An example is that partially replacing wheat flour (WF) by rye (*Secale cereale* L.) flour (RF) increases bread dietary fiber (Andersson, Fransson, Tietjen, & Aman, 2009; Angioloni & Collar, 2011) and lysine (Delcour & Hoseney, 2010; Dewettinck et al., 2008) levels. However, RF proteins lack the ability to form a viscoelastic network upon kneading which then results in RF dough being poorly extensible (Beck, Jekle, Selmair, Koehler, & Becker, 2011; Gellrich, Schieberle, & Wieser, 2004; Koehler & Wieser, 2000; Verwimp, Courtin, & Delcour, 2006). RF doughs do not display strain hardening (Meeus, Janssen, Wouters, Delcour, & Moldenaers, 2020). The protein-starch matrix in such doughs can therefore be expected to generate gas cells with less structural support during bread making than in WF dough. Indeed, rye bread loaves generally have lower volume and/or crumb with larger and more

heterogeneously distributed gas cells than wheat bread loaves (Angioloni & Collar, 2011; Buksa, Ziobro, Nowotna, & Gambuś, 2013; Janssen, Wouters, Pauly, & Delcour, 2018).

Blending WF and RF provides opportunities to produce breads of high nutritional quality (Angioloni & Collar, 2011). In earlier work (Meeus et al., 2020), the rheology of mixed WF-RF bread doughs was assessed via uniaxial extensional measurements. An increased portion of RF in the blend considerably increased the linear extensional viscosity, as assessed by extrapolating the region of linear extensional viscosity to maximum strain [$\eta_{e0}^{+}(\varepsilon_{max})$], whilst the transient extensional viscosity at maximum strain [$\eta_{e}^{+}(\varepsilon_{max})$] was not affected.

The higher concentration of arabinoxylan (AX) in RF [typically 3.6 (Buksa et al., 2013) to 5.0 % (Vinkx & Delcour, 1996) w/w of flour dry matter (dm)] than in WF [typically 1.4 to 2.8 % w/w of flour dm (Gebruers et al., 2008)] may in part be at the basis of these observations. AX has a backbone of β-1,4-D-xylopyranosyl units and α-L-arabinofuranosyl units which are attached to some of the C(O)-2 and/or C(O)-3 position(s) (Perlin, 1951b, 1951a). A distinction is generally made between water-extractable AX (WE-AX) and water-unextractable AX (WU-AX). While WE-AX can account for up to 40% w/w of the total AX (TOT-AX) content in RF (Vinkx & Delcour, 1996), its relative amount in WF is typically lower (15 - 31% w/w of TOT-AX)(Gebruers et al., 2008). WU-AX molecules can (partially) be solubilized by alkaline treatment (AS-AX) or by enzymatic hydrolysis (Courtin & Delcour, 2002; Delcour, Vanhamel, & De Geest, 1989; Gruppen, Hamer, & Voragen, 1991; Nilsson, Saulnier, Andersson, & Åman, 1996; Ordaz-Ortiz & Saulnier, 2005)

Both WE-AX and WU-AX populations are structurally heterogeneous and vary considerably in molecular weight (MW), degree of substitution [typically assessed by the arabinose-to-xylose (A/X) ratio] and substitution pattern (Courtin & Delcour, 2002; Vinkx & Delcour, 1996). Readers interested in a detailed description of AX structural heterogeneity are referred to earlier studies on wheat (Barron, Surget, &

Rouau, 2007; Cleemput, Booij, Hessing, Gruppen, & Delcour, 1997; Dervilly-Pinel, Rimsten, Saulnier, Andersson, & Åman, 2001; Gruppen, Kormelink, & Voragen, 1993; Izydorczyk, Biliaderis, & Bushuk, 1991) and rye (Buksa, Nowotna, Ziobro, & Praznik, 2014; Cyran & Dynkowska, 2014; Izydorczyk & Biliaderis, 1995; Vinkx, Stevens, Gruppen, Grobet, & Delcour, 1995) AX.

Endo-β-1,4-xylanases (EC 3.2.1.8) (further referred to as XYLs) catalyze the hydrolysis of β-1,4 glycosidic bonds in the xylopyranosyl backbone of AX, thereby decreasing their average molecular weight (Dekker & Richards, 1976). Over the past decades, XYLs have been extensively used to study the WF AX population (Courtin, Gelders, & Delcour, 2001; Courtin, Roelants, & Delcour, 1999; Gruppen et al., 1992; Hoffmann, Geijtenbeek, Kamerling, & Vliegenthart, 1992; Hoffmann, Leeflang, de Barse, Kamerling, & Vliegenthart, 1991; Leys, De Bondt, Bosmans, & Courtin, 2020). They are also often used in commercial bread making and may also be endogenously present in WF (Cleemput, Bleukx, van Oort, Hessing, & Delcour, 1995) and RF (Rasmussen, Boskov Hansen, Hansen, & Melchior Larsen, 2001). Different XYLs exist, each with different substrate specificities. XYL from *Bacillus subtilis* (XBS) and *Aspergillus aculeatus* (XAA) preferentially hydrolyze WF WU-AX and WE-AX, respectively (Courtin et al., 2001).

Literature on the role of AX in determining the rheology of WF or RF doughs is scarce. Döring et al. (2015) investigated the effect of a commercial preparation of rye AX (90% pure) in gluten-starch doughs (0.0%, 2.5%, 5.0%, 7.5% or 10.0% w/w of a gluten-starch blend was replaced by AX) using oscillatory shear and creep-recovery tests. They noted a gradual decrease in dough elasticity with increasing AX levels up to 7.5% w/w, after which dough elasticity remained constant. Grossmann & Koehler (2016) used a fractionation-reconstitution approach to investigate the role of RF WE-AX and WU-AX in RF model doughs. With similar tests they noted that WE-AX and WU-AX lead to a decreased and increased dough elasticity, respectively. Döring et al. (2017) showed that AX influences rye dough viscoelasticity. While these studies have merits, some remarks should be made. First, the oscillatory shear

measurements have only been performed at a frequency of 1.0 Hz, which does not cover the whole range of relaxation mechanisms and associated time-scales in dough. Frequency sweeps would provide a more accurate picture of the overall linear rheological response of dough. Second, the WE-AX and WU-AX contents of the AX preparation or the AX fraction in RF, used by Döring et al. (2015) and Döring et al. (2017), respectively, were not mentioned. In both studies the water content of the doughs was adjusted to keep their firmness constant. Finally, bread dough during fermentation and baking undergoes extensional rather than shear deformations. These factors may well be relevant when investigating the role of AX in bread making.

Against this background, it is investigated in the present study how enzymatic hydrolysis of WE-AX and/or WU-AX influences the linear and non-linear extensional rheology of (i) WF dough and (ii) mixed doughs prepared from various blends of WF and RF. Doughs were treated either with single (XBS_1 or XAA_1) or tenfold higher (XBS_{10} or XAA_{10}) dosages of XBS or XAA. The extent of AX hydrolysis was evaluated with gas chromatography (GC). The non-linear uniaxial extensional rheology of the doughs was studied with an extensional viscosity fixture (EVF), while their linear rheological response was probed with small-amplitude oscillatory shear (SAOS) measurements. The findings of this paper will contribute to grasping the mechanisms by which AX influences dough rheology and therefore to optimizing the production processes of mixed cereal breads.

2. Materials and methods

2.1. Materials

Bilux WF was from Dossche Mills (Deinze, Belgium) and is produced from different cultivars. Rye kernels (cultivar Dukato) were from AVEVE (Merksem, Belgium), conditioned to 16.0 % moisture and subsequently milled in-house using a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill (Delcour et al., 1989) to obtain RF. Blends (all expressed on dry matter basis, see section 2.2) were obtained by mixing WF and RF overnight by means of an overhead shaker. Sugar and salt were from a local supermarket. L-arabinose, β-D-Allose, D-mannose, D-xylose, D-glucose, D- galactose, ethyl acetate, benzoic acid, 2-octanol, and sodium borohydride were from Sigma-Aldrich (Bornem, Belgium). Trifluoroacetic acid, absolute ethanol, 1-methylimidazole, and bromophenol blue were from Thermo Fisher Scientific (Aalst, Belgium). Acetic acid, ammonia, acetic anhydride, potassium hydroxide, and anhydride sodium sulfate were from VWR International (Oud-Heverlee, Belgium). All reagents, solvents, and chemicals were at least of analytical grade.

WF and RF moisture levels and protein contents (N x 5.7) were determined as in Meeus et al. (2020) using AACCI Method 44-19.01 and AOAC Method 990.03 (AOAC International, 1995), respectively.

A. aculeatus (Shearzyme 500L) was obtained from Novozymes (Bagsvaerd, Denmark) as a liquid xylanase preparation and is throughout this manuscript referred to as XAA. A solid xylanase preparation from *B. subtilis* (XBS) was kindly donated by AB Mauri (Merelbeke, Belgium). Before XBS use, an aqueous XBS solution (containing 20 mg XBS preparation/mL deionized water) was prepared by mechanical shaking (150 rpm, 4 °C, 30 min) and subsequent centrifugation (4,000 *g*, 21 °C, 5 min). This allowed removing insoluble carrier material and accurately dosing low XBS amounts in dough making. The aqueous extract

was filtered over paper and stored at -20 °C. XBS (0.5 ± 0.1 U/ml) and XAA (608.9 ± 4.9 U/ml) activity levels were determined with the Megazyme (Bray, Ireland) XylX6 method. In general, a substrate (XylX6) is converted into 4-nitrophenol and D-xylose by subsequent action of XAA or XBS and β-xylosidase. Next, alkalization converts the released 4-nitrophenol into 4-nitrophenolate which is quantified colorimetrically at 400 nm. One unit of activity is the amount of enzyme required to release one µmol of 4-nitrophenol from the XylX6 substrate per min under the defined assay conditions.

2.2. Dough preparation

Doughs were made by mixing flour [8.6 g dry matter (dm)], deionized water (**Table 1**), sugar (6.0% w/w on a flour weight basis), and salt (1.5% w/w on a flour weight basis) in a pin mixer (National Manufacturing, Lincoln, NE, USA). Doughs were prepared from the following: pure WF (WF100), 80% WF and 20% RF (WF80-RF20), 60% WF and 40% RF (WF60-RF40), 40% WF and 60% RF (WF40-RF60), 20% WF and 80% RF (WF20-RF80), and pure RF (RF100). Optimal dough water absorptions were determined using a Farinograph (Brabender, Duisburg, Germany) according to AACC method 54-21.02 (AACC International, 2000) (**Table 1**). Optimal dough mixing times were determined using a Mixograph according to AACC method 54-40.02 (AACC International, 2000) (**Table 1**). To investigate the role of AX in altering the water binding dynamics in the different doughs, doughs with modified water content, indicated as '*+ 0.5 water*' or '*- 0.5 ml* water', were prepared by respectively adding or subtracting 0.5 ml of water from the optimal water absorptions (**Table 1**).

Table 1 Optimal water absorptions (% w/w on flour basis) and mixing times (s) of doughs prepared with systematically varying compositions: 100% wheat flour (WF) (WF100), 80% WF and 20% rye flour (RF) (WF80-RF20), 60% WF and 40% RF (WF60-RF40), 40% WF and 60% RF (WF40-RF60), 20% WF and 80% RF (WF20-RF80) and 100% RF (RF100). Reprinted with permission from Meeus et al. (2020).

Sample	*Water absorption [% w/w on flour basis]*	*Mixing time [s]*
WF100	59.5	180
WF80 – RF20	57.5	165
WF60 – RF40	57.0	165
WF40 – RF60	56.6	90
WF20 – RF80	56.0	90
RF100	55.0	90

XBS or XAA were added to doughs as aqueous solutions, which were prepared by diluting aqueous extracts of XBS or a liquid preparation of XAA (see section 2.1) in deionized water. XBS was added at dosages corresponding to 2 ppm or 20 ppm of XBS preparation on flour basis, whilst XAA was added at dosages corresponding to 10 ppm or 100 ppm of its preparation on flour basis. These dosages are further referred to as XBS_1 and XBS_{10}, and XAA_1 and XAA_{10}, respectively. The XBS and XAA dosages were chosen based on preliminary experiments. While XBS_1 was the lowest dosage which caused substantial solubilization of the WU-AX in WF100 and RF100 doughs, XAA_1 was the lowest dosage which produced a substantial hydrolysis of the WE-AX population in WF100 and RF100 doughs. For both XBS and XAA, the high dosage was ten times the low dosage. When XAA or XBS was used, the water dosage of each respective control dough was corrected for the liquid added upon dosage of the enzyme to keep the total water content constant.

2.3. Characterization of arabinoxylan in dough

The total AX (TOT-AX) levels of WF100 and RF100 control doughs were determined by the GC procedure described by Loosveld et al. (1997). All results are means of single measurements on each of two separate dough batches which were flash frozen with nitrogen, freeze-dried and gently ground to powders which were subjected to acid hydrolysis by adding 5.0 ml 2.0 M trifluoroacetic acid to 10 mg of

the ground powder. The released monosaccharides were reduced to alditols with sodium borohydride and subsequently converted to alditol peracetates (Englyst & Cummings, 1984). The latter were analyzed with an Agilent (Wilmington, DE, USA) gas chromatograph (6890 Series) equipped with a flame ionization detector and an auto sampler. A polar column (Supelco SP 2380, 0.32 mm inner diameter, 30.0 m length, 0.2 µm film thickness, Bellefonte, PA, USA) was used for separation using helium as carrier gas. Separation was at 225°C, while injection port and detector temperatures were 270°C. TOT-AX levels were calculated as 0.88 [a correction factor to account for the uptake of water by D-xylose/D-arabinose during hydrolysis, i.e. the ratio of the molecular weight of anhydro-D-xylose/anhydro-D-arabinose (132.11 g/mol) to that of D-xylose/D-arabinose (150.13 g/mol)] times the sum of xylose and arabinose levels (Gebruers, Courtin, & Delcour, 2009). Arabinose levels were corrected for arabinose in the water extracts originating from arabinogalactan peptide (arabinose to galactose ratio 0.7) in wheat (Loosveld et al., 1997) and rye (Van den Bulck et al., 2005) flours. TOT-AX levels of WF80 – RF20, WF60 – RF40 , WF40 – RF60 and WF20 – RF80 control doughs were estimated from the values obtained for WF100 and RF100 control doughs. TOT-AX levels of enzyme-treated doughs were evidently the same as in the corresponding control doughs.

WE-AX was extracted from control WF100 and control RF100 doughs, as well as from enzyme-treated WF100, WF80-RF20, WF60-RF40, WF40-RF60, WF20-RF80 and RF100 doughs. To this end, freeze-dried and ground dough powders (1.0 g) were suspended in 10 ml 80 % (v/v) ethanol. The solvent was evaporated at 95 °C, which ensured inactivation of the XYLs. Subsequently, the residues were suspended in 20.0 ml water, mechanically shaken (30 min, 4 °C), centrifuged (1,000 g, 10 min, 7 °C) and the resultant supernatants were filtered. WE-AX levels in the aqueous extracts were determined with a procedure similar to that used for the TOT-AX content, the only difference being that the acid hydrolysis step was performed by adding 2.5 ml 4.0 M trifluoroacetic acid to a 2.5 ml aliquot of the aqueous

extract. WE-AX levels of WF80 – RF20, WF60 – RF40 , WF40 – RF60 and WF20 – RF80 control doughs were estimated from the values obtained for WF100 and RF100 control doughs.

The A/X ratios of TOT-AX and WE-AX in WF100 and RF100 control dough were used to estimate those of WF80 – RF20, WF60 – RF40, WF40 – RF60 and WF20 – RF80 control doughs. WE-AX A/X ratios of enzyme-treated WF100, WF80-RF20, WF60-RF40, WF40-RF60, WF20-RF80 and RF100 doughs in this case were calculated as the sum of arabinose originally present in WE-AX and in the enzymatically solubilized WE-AX divided by the sum of xylose originally present in WE-AX and in the enzymatically solubilized WE-AX. WU-AX levels were calculated by subtracting WE-AX from TOT-AX levels. The number average degrees of polymerization ($avDP_n$s) of WE-AX in WF100 and RF100 control doughs and in enzyme-treated WF100, WF80-RF20, WF60-RF40, WF40-RF60, WF20-RF80 and RF100 doughs were calculated by expressing the levels of WE-AX relative to the levels of reducing end xylose residues in the aqueous extracts. WE-AX reducing end xylose levels were determined as in Courtin et al. (2000) after reduction to alditols, acid hydrolysis using trifluoroacetic acid and conversion to alditol peracetates. Doing so allows distinguishing alditol peracetates originating from reducing end monosaccharides from those derived from non-reducing monosaccharide residues. WE-AX $avDP_n$s of WF80 – RF20, WF60 – RF40, WF40 – RF60 and WF20 – RF80 control doughs were estimated from the values obtained for WF100 and RF100 control doughs.

2.4. Rheological measurements

Rheological measurements were performed on control and enzyme-treated WF100, WF80 – RF20, WF60 – RF40, WF40 – RF60 doughs (extensional and shear measurements), and on control and enzyme-treated WF20 – RF80 and RF100 doughs (only shear measurements) as in Meeus et al. (2020). Doughs were prepared as described in section 2.2. By pressing the mixed doughs between two plates for 1 min a uniform sample thickness of 4 mm was obtained. Prior to measurements, pressed dough samples were

placed in a closed container to rest at 21 °C for half an hour to ensure relaxation of stresses caused by mixing and pressing.

Extensional measurements

Uniaxial extensional measurements were conducted at 21 °C using an ARES-G2 rheometer (TA Instruments, New Castle, DE, USA) equipped with an EVF. The EVF consisted of (i) a stationary drum and (ii) a drum revolving around both the stationary drum and its own axis. Dough strands were prepared using a hollow stamp to obtain fixed dimensions (length L_0 = 18 mm, height H_0 = 4 mm, thickness B_0 = 4 mm) and subsequently gently attached to the drums using a set of clamps. Extension took place at an effective rate of 0.100 s^{-1}. Device limitations limit the EVF setup to a maximum attainable strain of 2.89. The ratio of the transient extensional viscosity at maximum strain $\eta_e^+(\varepsilon_{max})$ to the linear extensional viscosity extrapolated to this maximum strain $\eta_{e0}^+(\varepsilon_{max})$ was used to calculate the strain hardening index (SHI), which is a measure for the degree of strain hardening in doughs. A more detailed explanation of the extensional measurements and the concept of strain hardening can be found elsewhere (Meerts, Cardinaels, Oosterlinck, Courtin, & Moldenaers, 2017b; Meeus et al., 2020).

Extensional data were obtained by calculating the mean of the measurement on seven different dough strands of a single batch and the procedure was executed for three different dough batches. Variability within the measurements was estimated with a pooled standard deviation (s_p) (McNaught & Wilkinson, 1997):

$$s_p = \sqrt{\frac{(n_1 - 1) \times s_1^2 + (n_2 - 1) \times s_2^2 + (n_3 - 1) \times s_3^2}{n_1 + n_2 + n_3 - N}} \quad (1)$$

with s_1, s_2, and s_3 the standard deviations obtained from the seven measurements on strands of dough batches 1, 2, and 3, respectively. n_1, n_2, and n_3 represent the amount of measurements (i.e., seven) per

dough batch. *N* is the total number of dough batches (i.e., three). The relative standard deviation was approximately 10 % and agreed well with previous reports (Meerts et al., 2017b; Meeus et al., 2020).

Shear measurements

SAOS measurements were performed at 25 °C on a stress-controlled MCR501 rheometer (Anton Paar, Graz, Austria) using a 40-mm parallel plate. A solvent trap combined with wet cotton wool prevented dehydration of the dough samples. Slip was prevented by applying sandpaper to the top and bottom plates.

After loading a time sweep with an angular frequency of 1 rad.s^{-1} and a strain of 0.04 % (well within the linear region as verified by strain sweeps) was carried out for 15 min to allow samples to reach a steady-state. Following the time sweep a frequency sweep was performed (0.1 – 100 rad.s^{-1}, strain of 0.04 %).

The storage *G'(ω)* [Pa] and loss *G''(ω)* [Pa] moduli were determined, with *G'* representing the elastic behavior and *G''* the viscous behavior. The tangent of the phase angle *tan* (*δ*) is defined as the ratio of *G''* to *G'*:

$$\tan(\delta) = {}^{G''}/_{G'} \qquad (2)$$

The SAOS data in this work are means of single measurements on each of three different doughs. Relative standard deviations did not exceed 5 %.

Statistical analysis

Data were analyzed with the Statistics Toolbox of MATLAB (Release 2020b, The MathWorks, Natick, MA, USA). Significant differences ($P < 0.05$) were found using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference test. Normality of the data was verified using Shapiro-Wilk tests. Homogeneity of variance of the data was assessed using Bartlett tests.

3. Results and discussion

3.1 Characterization of arabinoxylan in dough

The XBS and XAA dosages were chosen based on preliminary experiments. While an XBS_1 level corresponding to 2 ppm of XBS preparation on flour basis was the lowest dosage at which the WU-AX content in WF100 and RF100 doughs significantly ($P < 0.05$) decreased, an XAA_1 level corresponding to 10 ppm of XAA preparation on flour basis was the lowest dosage at which a significant ($P < 0.05$) reduction of the $avDP_n$ of the WE-AX population in WF100 and RF100 doughs was observed. For both XBS and XAA, the high dosage was ten times the low dosage (i.e. 20 ppm of XBS preparation on flour basis and 100 ppm of XAA preparation on flour basis).

Table 2 Total arabinoxylan (TOT-AX) levels and arabinose-to-xylose (A/X) ratios, water-extractable AX (WE-AX) levels and A/X ratios and number-average degree of polymerization ($avDP_n$), water-unextractable AX (WU-AX) contents and A/X ratios for doughs prepared with 100% wheat flour (WF) (WF100), 80% WF and 20% rye flour (RF) (WF80 - RF20), 60% WF and 40% RF (WF60 - RF40), 40% WF and 60% RF (WF40 - RF60), 20% WF and 80% RF (WF20 - RF80) or 100% RF (RF100), treated with or without xylanase (XYL) from *Bacillus subtilis (*XBS_1 and XBS_{10}, respectively) or from *Aspergillus aculeatus* (XAA_1 and XAA_{10}, respectively). Doughs prepared without enzyme additions served as control. TOT-AX levels of enzyme-treated doughs were evidently those measured for the corresponding control doughs. The numbers between brackets represent the average relative decrease in WE-AX $avDP_n$ or WU-AX levels from those of the respective control doughs. Error values represent the deviation from the mean for single measurements of each of two separate doughs. Where no standard deviation is provided, the number represents the weighted average of the values obtained for WF100 and RF100 doughs.

	TOT-AX		WE-AX				WU-AX	
Sample	**Level [%]**	**A/X-ratio [-]**	**Level [%]**	**$avDP_n$ [-]**		**A/X-ratio [-]**	**Level [%]**	**A/X-ratio [-]**
WF100								
Control	2.40 ± 0.22	0.65 ± 0.03	0.56 ± 0.03	161 ± 10		0.49 ± 0.01	1.84	0.71
XBS_1			0.70 ± 0.02	11 ± 1	(-93 %)	0.51 ± 0.01	1.70 (-7%)	
XBS_{10}			0.95 ± 0.01	10 ± 1	(-94 %)	0.52 ± 0.01	1.45 (-22%)	
XAA_1			0.60 ± 0.04	4 ± 1	(-97 %)	0.51 ± 0.01	1.80 (-2%)	
XAA_{10}			0.96 ± 0.02	7 ± 1	(-96 %)	0.50 ± 0.01	1.44 (-21%)	
WF80 – RF20								
Control	2.52	0.66	0.64	187		0.51	1.88	0.71
XBS_1			0.85 ± 0.01	9 ± 1	(-95 %)	0.50 ± 0.01	1.67 (-11 %)	
XBS_{10}			1.12 ± 0.02	10 ± 1	(-95 %)	0.50 ± 0.01	1.40 (-26 %)	
XAA_1			0.71 ± 0.02	13 ± 1	(-93 %)	0.52 ± 0.01	1.81 (-4 %)	
XAA_{10}			1.12 ± 0.02	10 ± 1	(-95 %)	0.51 ± 0.01	1.40 (-25 %)	
WF60 – RF40								
Control	2.64	0.66	0.72	213		0.53	1.92	0.72

XBS_1			0.92 ± 0.02	11 ± 1	(-95 %)	0.53 ± 0.01	1.72	(-10 %)	
XBS_{10}			1.27 ± 0.06	12 ± 1	(-94 %)	0.54 ± 0.01	1.37	(-29 %)	
XAA_1			0.84 ± 0.02	12 ± 1	(-94 %)	0.52 ± 0.02	1.80	(-6 %)	
XAA_{10}			1.29 ± 0.06	12 ± 1	(-94 %)	0.54 ± 0.02	1.35	(-30 %)	
WF40 – RF60									
Control	2.76	0.67	0.81	238		0.55	1.95		0.72
XBS_1			1.05 ± 0.02	13 ± 1	(-95 %)	0.57 ± 0.01	1.71	(-12 %)	
XBS_{10}			1.37 ± 0.03	15 ± 1	(-94 %)	0.55 ± 0.01	1.39	(-29 %)	
XAA_1			0.98 ± 0.08	13 ± 1	(-95 %)	0.54 ± 0.02	1.78	(-9 %)	
XAA_{10}			1.34 ± 0.02	11 ± 1	(-95 %)	0.55 ± 0.01	1.42	(-27 %)	
WF20 – RF80									
Control	2.88	0.68	0.90	264		0.57	1.98		0.73
XBS_1			1.19 ± 0.04	15 ± 1	(-94 %)	0.55 ± 0.01	1.68	(-15 %)	
XBS_{10}			1.47 ± 0.04	20 ± 2	(-92 %)	0.54 ± 0.02	1.41	(-29 %)	
XAA_1			1.02 ± 0.02	12 ± 1	(-95 %)	0.56 ± 0.01	1.86	(-6 %)	
XAA_{10}			1.34 ± 0.08	11 ± 1	(-96 %)	0.55 ± 0.01	1.54	(-22 %)	
RF100									
Control	3.00 ± 0.18	0.68 ± 0.01	0.98 ± 0.08	290 ± 25		0.59 ± 0.01	2.02		0.73
XBS_1			1.30 ± 0.08	17 ± 1	(-94 %)	0.55 ± 0.01	1.70	(-15 %)	
XBS_{10}			1.62 ± 0.02	17 ± 1	(-94 %)	0.57 ± 0.01	1.38	(-32 %)	
XAA_1			1.17 ± 0.06	15 ± 1	(-95 %)	0.57 ± 0.01	1.83	(-9 %)	
XAA_{10}			1.63 ± 0.01	17 ± 1	(-94 %)	0.57 ± 0.01	1.37	(-32 %)	

A first notable observation is that the $avDP_n$ of the WE-AX population in control RF100 dough (290) was considerably higher than that of the control WF100 dough WE-AX population (161) (**Table 2**). Still, these values were much lower than those reported earlier for WF (Cleemput, Roels, van Oort, Grobet, & Delcour, 1993; Dervilly, Saulnier, Roger, & Thibault, 2000; Morales-Ortega et al., 2013) and RF (Girhammar & Nair, 1992; Rakha, Åman, & Andersson, 2010) WE-AX. Cleemput et al. (1997) also noted a higher WE-AX apparent molecular weight in WF than in doughs prepared thereof. It was speculated that during dough making WE-AX molecules are partially hydrolyzed by flour endogenous XYL, although other phenomena could not be excluded (Cleemput et al., 1997). That Cyran & Dynkowska (2014) observed a higher apparent molecular weight for RF WE-AX than for WE-AX in the resulting breads seems to support this reasoning.

In all doughs, the addition of XBS_1 solubilized at least 7 % WU-AX. At the same time, a strong decrease in the WE-AX $avDP_n$ was observed (**Table 2**). Courtin & Delcour (2001) found that in the presence of both WE-AX and WU-AX XBS affects both populations (Courtin & Delcour, 2001). Thus, that XBS_1 here led to a strong decrease of the WE-AX $avDP_n$ (**Table 2**) indicates that not only some WU-AX was solubilized, but also that the WE-AX population and the enzymatically solubilized AX was hydrolyzed.

In line with previous findings (Courtin & Delcour, 2001), the higher dosage of XBS (XBS_{10}) resulted in more extensive WU-AX solubilization (22 – 32%) than was the case for the lower dosage (XBS_1) (7 – 15%) (**Table 2**). Similar to what was noted for XBS_1, at XBS_{10} a pronounced decrease of the WE-AX $avDP_n$ was observed (**Table 2**), indicating that the WE-AX population was extensively hydrolyzed. For WF100, WF80 – RF20 and WF60 – RF40 doughs containing XBS_{10} the estimated decrease in both absolute and relative WU-AX amounts was more pronounced when the portion of RF in the blend increased (**Table 2**). A similar trend was observed for the estimated decrease in absolute amount of WU-AX in WF40 – RF60,

WF20 – RF80 and RF100 doughs containing XBS_{10}. These observations suggest that WF WU-AX is more resistant to enzymatic hydrolysis by XBS than RF WU-AX.

Under the present experimental conditions less WU-AX was solubilized when doughs contained XAA_1 than when they contained XBS_1 (**Table 2**). This is in line with the study of Courtin & Delcour (2001), who for mixtures of a WE-AX isolate and a squeegee starch fraction (as a source of WU-AX) incubated with XAA or XBS observed that XBS solubilized a greater portion of WU-AX than XAA. In addition, the use of XAA_1 led to a considerable decrease in the $avDP_n$ of the WE-AX population in all doughs (**Table 2**). A more important observation was that the extents of WU-AX solubilization and the degrees to which the WE-AX $avDP_n$ were decreased by the highest dosages of both enzymes (XAA_{10} and XBS_{10}) were similar (**Table 2**). This agrees well with the observation that a high XAA dosage solubilizes significant amounts of WU-AX in WF doughs (Courtin et al., 2001).

Interestingly, neither addition of XBS nor that of XAA resulted in changes in the A/X ratios of the sum of the WE-AX and enzymatically solubilized population of the different doughs as compared to that of the control doughs (**Table 2**). That this was the case even when part of the WU-AX [which in all cases had considerably higher A/X ratios than the respective WE-AX population (**Table 2**)] was solubilized by the enzyme action illustrates how the enzymes preferentially released rather unsubstituted WU-AX regions (Pollet, Delcour, & Courtin, 2010).

In conclusion, at sufficiently high concentrations XBS and XAA solubilize a substantial portion of the WU-AX present and likely hydrolyze both the originally present and enzymatically released AX molecules in each of the studied doughs. To what extent these changes in AX structure and extractability affect the linear and non-linear extensional rheology of dough is discussed in the next section.

3.2 Non-linear uniaxial extensional rheology of doughs with standard water content

The transient extensional viscosity as a function of strain of control and enzyme-treated WF100, WF80 – RF20, WF60 – RF40 and WF40 – RF60 dough strands (see section 2.4) is shown in **Figures 1** and **2,** when using XBS and XAA respectively. **Tables 3** and **4** list the three main parameters [$\eta_{e0}^{+}(\varepsilon_{max})$, $\eta_{e}^{+}(\varepsilon_{max})$ and the SHI] obtained from these measurements for doughs made with WF100 or WF40 – RF60, treated with XBS_{10} or XAA_{10}. Extensional rheology data of doughs prepared with WF80 – RF20 and WF60 – RF40 treated with XBS_{10} or XAA_{10}, and of all doughs treated with XBS_{1} or XAA_{1}, are included as **supplementary information**.

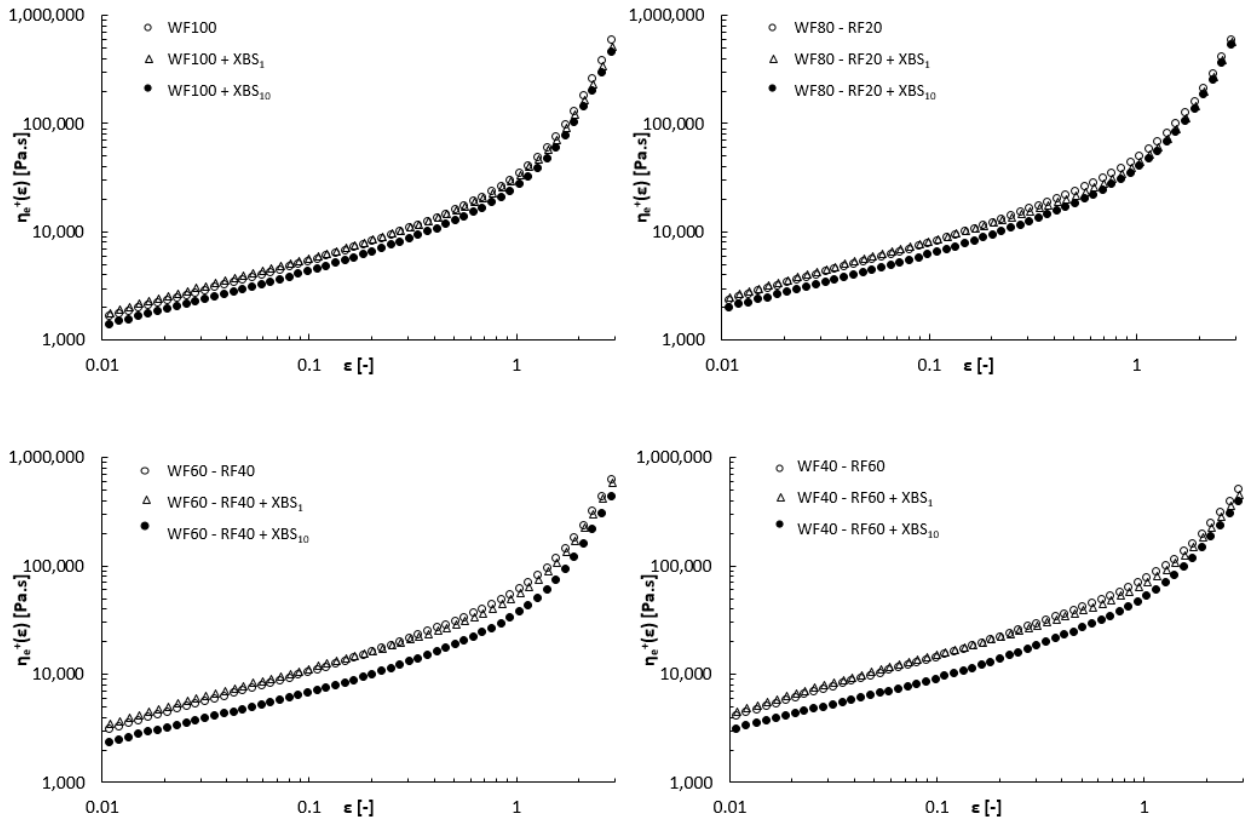


Figure 1 Transient extensional viscosity η_{e}^{+} as a function of strain of control and enzyme-treated dough strands prepared with 100% wheat flour (WF) (WF100), 80% WF and 20% rye flour (RF) (WF80 - RF20), 60% WF and 40% RF (WF60 - RF40), and 40% WF and 60% RF (WF40 - RF60). *Bacillus subtilis* xylanase

(XYL) dosages (XBS_1 and XBS_{10}) are explained in the text. The data represent means of seven measurements of each of three separate dough batches.

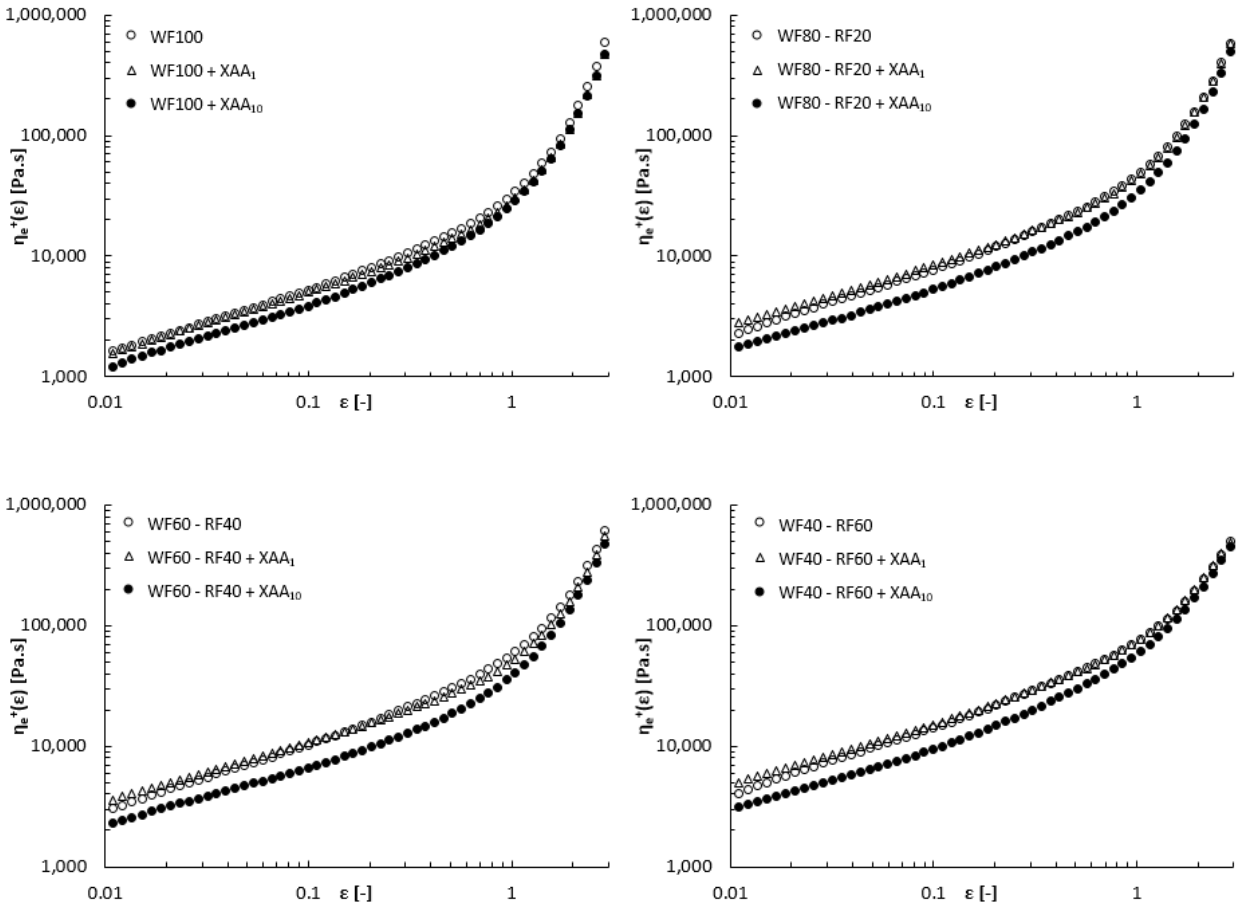


Figure 2 Transient extensional viscosity η_e^+ as a function of strain of control and enzyme-treated dough strands prepared from 100% wheat flour (WF) (WF100), 80% WF and 20% rye flour (RF) (WF80 - RF20), 60% WF and 40% RF (WF60 - RF40), and 40% WF and 60% RF (WF40 - RF60). *Aspergillus aculeatus* xylanase (XYL) dosages (XAA_1 and XAA_{10}) are explained in the text. The data represent means of seven measurements of each of three separate dough batches.

Table 3 Overview of the linear transient extensional viscosity η_{e0}^+ extrapolated to the maximum strain of 2.89, transient extensional viscosity η_e^+ at the maximum strain of 2.89, and strain hardening indices (SHIs) of doughs prepared with 100% wheat flour (WF100). The first set of doughs was prepared without

xylanase (XYL) and served as control. The second and third sets of doughs were prepared with the higher dosages of *Bacillus subtilis* (XBS_{10}) or *Aspergillus aculeatus* (XAA_{10}) (see text). In each of these three sets, doughs were also made with more (indicated as '*+ 0.5 ml water*') or less (indicated as '*- 0.5 ml* water') water. Error values represent the deviation from the mean for seven measurements of each of three separate dough batches. Column values with the same lowercase letter are not significantly different ($P > 0.05$).

WF100	$\eta_{e0}^{+}(\varepsilon_{max})$ **[10^4 Pa.s]**	$\eta_{e}^{+}(\varepsilon_{max})$ **[10^5 Pa.s]**	**SHI [-]**
Control	3.4 ± 0.3^{a}	5.9 ± 0.7^{ab}	17.2 ± 1.7^{ab}
+ 0.5 ml water	2.0 ± 0.3^{bc}	4.0 ± 0.5^{c}	19.7 ± 2.3^{bc}
– 0.5 ml water	6.3 ± 0.7^{d}	7.4 ± 1.1^{b}	11.8 ± 1.1^{d}
XBS_{10}	2.6 ± 0.2^{b}	4.5 ± 0.6^{ac}	17.2 ± 1.4^{ab}
+ 0.5 ml water	1.6 ± 0.2^{c}	3.5 ± 0.4^{c}	22.3 ± 1.7^{ce}
- 0.5 ml water	4.5 ± 0.5^{e}	6.4 ± 0.7^{b}	14.2 ± 0.8^{ad}
XAA_{10}	2.5 ± 0.3^{b}	4.7 ± 0.5^{ac}	19.2 ± 1.6^{bc}
+ 0.5 ml water	1.6 ± 0.2^{c}	3.9 ± 0.4^{c}	24.8 ± 2.1^{e}
- 0.5 ml water	4.1 ± 0.4^{ae}	6.4 ± 0.9^{b}	15.6 ± 1.7^{a}

Table 4 Overview of the linear transient extensional viscosity η_{e0}^{+} extrapolated to the maximum strain of 2.89, transient extensional viscosity η_{e}^{+} at the maximum strain of 2.89, and strain hardening indices (SHI) of doughs prepared with 40% wheat flour – 60% rye flour (WF60 - RF40). The first set of doughs was prepared without xylanase (XYL) and served as control. The second and third set of doughs were prepared with the high dosages of *Bacillus subtilis* XYL (XBS_{10}) or *Aspergillus aculeatus* XYL (XAA_{10}) (see text). In each of these three sets, doughs were also made with more (indicated as '*+ 0.5 ml water*') or less (indicated as '*- 0.5 ml water*') water. Error values represent the deviation from the mean for seven

measurements of each of three separate dough batches. Column values with the same lowercase letter are not significantly different (P > 0.05).

WF40 - RF60	**$\eta_{e0}^{+}(\varepsilon_{max})$ [10^4 Pa.s]**	**$\eta_{e}^{+}(\varepsilon_{max})$ [10^5 Pa.s]**	**SHI [-]**
Control	9.6 ± 0.7^{a}	5.0 ± 0.5^{ab}	5.2 ± 0.4^{ab}
+ 0.5 ml water	6.4 ± 0.6^{b}	3.5 ± 0.6^{d}	5.5 ± 0.8^{ab}
– 0.5 ml water	16.5 ± 1.6^{c}	7.6 ± 0.9^{e}	4.7 ± 0.4^{a}
XBS_{10}	5.0 ± 0.4^{b}	3.9 ± 0.4^{cd}	7.7 ± 0.5^{c}
+ 0.5 ml water	3.1 ± 0.3^{d}	2.5 ± 0.3^{f}	7.9 ± 0.8^{c}
- 0.5 ml water	10.0 ± 1.1^{a}	6.0 ± 0.7^{g}	5.9 ± 1.1^{b}
XAA_{10}	6.1 ± 1.0^{b}	4.5 ± 0.5^{ac}	7.5 ± 0.8^{cd}
+ 0.5 water	2.8 ± 0.5^{d}	2.1 ± 0.4^{f}	7.6 ± 1.4^{c}
- 0.5 ml water	8.7 ± 1.0^{a}	5.4 ± 0.8^{bg}	6.4 ± 1.0^{bd}

Treatment with XBS_1 did not significantly (P > 0.05) impact $\eta_{e0}^{+}(\varepsilon_{max})$, $\eta_{e}^{+}(\varepsilon_{max})$ or the SHI values of any of the doughs (**Figure 1**). In contrast, the doughs containing XBS_{10} had a significantly (P < 0.05) lower $\eta_{e0}^{+}(\varepsilon_{max})$, while $\eta_{e}^{+}(\varepsilon_{max})$ decreased only in the case of WF60 – RF40 and WF40 – RF60 doughs (**Figure 1**). That the SHIs of WF60 - RF40 and WF40 - RF60 doughs containing the higher XBS dosage were significantly (P < 0.05) higher than those of their control counterparts illustrates that the decrease of $\eta_{e0}^{+}(\varepsilon_{max})$ was more pronounced than the decrease of $\eta_{e}^{+}(\varepsilon_{max})$.

Much as what was observed when using the low XBS dosage (XBS_1), the low XAA dosage (XAA_1) resulted neither in a significantly (P > 0.05) affected $\eta_{e0}^{+}(\varepsilon_{max})$, $\eta_{e}^{+}(\varepsilon_{max})$ nor SHI of any of the doughs (**Figure 2**). In contrast, including the high XAA dosage (XAA_{10}) in the different doughs resulted in a significant (P < 0.05) decrease of $\eta_{e0}^{+}(\varepsilon_{max})$. As a result, the SHIs of WF80 – RF20, WF60 – RF40 and WF40 – RF60 doughs

treated with XAA_{10} were significantly ($P < 0.05$) higher than those of their control counterparts. Again this is similar to what was observed for high doses of XBS.

In summary, the non-linear extensional responses of XBS_{10} and XAA_{10} containing doughs were rather similar for all doughs analyzed (**Figures 1** and **2**). This similarity in rheological responses is in line with the above observation that addition of XBS_{10} and XAA_{10} to doughs resulted in similar extents of WU-AX solubilization and reduction of the $avDP_n$ of the WE-AX population.

As discussed in section 3.1, in all doughs the higher dosage of both enzymes (XBS_{10} and XAA_{10}) resulted in considerably more WU-AX solubilization than the lower one (XBS_1 and XAA_1) (**Table 2**). Despite this difference, the $avDP_n$ of the WE-AX population was pronouncedly reduced in all cases.

Thus, that the η_e^+ vs. strain plots of doughs treated with XBS_1 or XAA_1 were quasi similar to those of their respective controls implies that solubilization of low WU-AX and strong reduction of the $avDP_n$ of the WE-AX and enzymatically solubilized population as a whole did not pronouncedly alter the extensional rheology of the wheat or mixed cereal doughs. In contrast, more pronounced WU-AX hydrolysis (at higher enzyme dosages) and simultaneous reduction of the $avDP_n$ of the WE-AX and enzymatically solubilized population in all doughs led to significantly different rheological properties. It can be argued that the WU-AX population mainly determines the extensional rheology of WF-RF doughs. However, several phenomena may mask each other. For instance, at low enzyme dosage the limited enzymatic hydrolysis of WU-AX may have generated sufficient enzyme-solubilized AX to increase the dough transient extensional viscosity and compensate for a potential decrease in dough extensional viscosity caused by the hydrolysis of WE-AX and the solubilized AX fraction.

It has recently been shown (Meerts, Cardinaels, Oosterlinck, Courtin, & Moldenaers, 2017a; Meeus et al., 2020) that the magnitude of $\eta_{e0}^+(\varepsilon_{max})$ is sensitive to (i) the dough water content and (ii) the strength of the short-range interactions between constituents. Given the effect of the XBS_{10} or XAA_{10} dosages on

$\eta_{e0}^{+}(\varepsilon_{max})$ and the associated partial solubilization of WU-AX, it seems reasonable that XYL action changed the water distribution in dough. To test this hypothesis, the extensional rheology of control and enzyme-treated doughs prepared with water contents higher and lower than those applied in control dough making was investigated. To better understand the role of AX in determining the short-range interactions in dough, the linear rheology of dough was studied in-depth by SAOS measurements.

3.3 Non-linear uniaxial extensional rheology of doughs with modified water content

To evaluate whether the changes in dough rheology as a result of XYL use can be attributed to changes in the water binding dynamics of the system, extensional measurements were executed with control and XBS or XAA containing doughs, each prepared with 0.5 ml more (indicated by $^{+}$, which resulted in more sticky and less viscous doughs) or less (indicated by $^{-}$, which resulted in stiffer doughs) water than their respective optimal dough water absorption (**Table 1**). Including the lower dosages (XBS_1 or XAA_1) in doughs prepared with modified water content did not significantly ($P > 0.05$) influence their linear and non-linear rheological response under uniaxial extension. For this reason the extensional rheology results of XBS_1 and XAA_1 containing doughs prepared with modified water content are included as **supplementary information**. As previously described, the WE-AX $avDP_n$ and WE-AX/WU-AX ratio of the AX population in XBS_{10} and XAA_{10} containing doughs were strikingly similar. Accordingly, EVF data of doughs prepared with modified water content and containing either XBS_{10} or XAA_{10} were comparable for a given dough type and water level. Here, the results for doughs treated with XBS_{10} are shown in **Figure 3**, and results for doughs treated with XAA_{10} are included as **supplementary information**. **Tables 3** and **4** list the $\eta_{e0}^{+}(\varepsilon_{max})$, $\eta_{e}^{+}(\varepsilon_{max})$ and SHIs obtained from extensional measurements performed with WF100 or WF40 – RF60 doughs, treated with XBS_{10} or XAA_{10}. The results obtained for the other dough blends are included as **supplementary information**.

In agreement with what was reported by Meerts et al. (2017a), an increased water content of control WF100 dough led to a significant ($P < 0.05$) decrease of $\eta_{e0}^{+}(\varepsilon_{max})$ (**Table 3**). This implies that once dough constituents are fully hydrated, excess water causes $\eta_{e0}^{+}(\varepsilon_{max})$ to decrease. At the same time, a decreased WF100 dough water content resulted in an increased $\eta_{e0}^{+}(\varepsilon_{max})$. A lower dough water absorption leads to a more viscous and stiffer dough due to the combined effect of a reduced amount of free water and a sub-optimal hydration of dough constituents. Similar trends were observed for all other dough blends (**Table 4** and **supplementary information**). Whilst $WF100^{+}$ and $WF40 – RF60^{+}$ doughs had significantly ($P < 0.05$) lower $\eta_{e}^{+}(\varepsilon_{max})$ values than WF100 and WF40 – RF60 doughs, respectively (**Tables 3** and **4**), such decrease was not observed for $WF80 – RF20^{+}$ and $WF60 – RF40^{+}$ doughs when compared with their respective control doughs (see **Figure 3** and **supplementary information**). Finally, $\eta_{e}^{+}(\varepsilon_{max})$ values of $WF80 – RF20^{-}$, $WF60 – RF40^{-}$ and $WF40 – RF60^{-}$ doughs were significantly ($P < 0.05$) higher than those of their control counterparts (see **Figure 3** and **supplementary information**). Although Meerts et al. (2017a) observed no significant difference in $\eta_{e}^{+}(\varepsilon_{max})$ for WF dough recipes with different water contents, in this work, the magnitude of $\eta_{e}^{+}(\varepsilon_{max})$ of mixed wheat-rye doughs increased with decreasing dough water content. This discrepancy most likely relates to the broader range of dough water contents used in the present study (an increase/decrease in dough water content of 9% on Farinograph dough water absorption basis) than in the study by Meerts et al. (2017a) (an increase/decrease in dough water content of 6% on Farinograph dough water absorption basis).

While including XBS_{10} or XAA_{10} dosages in $WF100^{+}$ doughs had no significant ($P > 0.05$) effect on their $\eta_{e0}^{+}(\varepsilon_{max})$ or $\eta_{e}^{+}(\varepsilon_{max})$, the SHIs of XAA_{10} containing $WF100^{+}$ doughs were significantly ($P < 0.05$) higher than those of $WF100^{+}$ doughs made without XAA_{10} (**Table 3**). In addition, a significant ($P < 0.05$) decrease in $\eta_{e0}^{+}(\varepsilon_{max})$ was observed when $WF80 - RF20^{+}$, $WF60 - RF40^{+}$ or $WF40 – RF60^{+}$ doughs contained XBS_{10} or XAA_{10}, the only exception being $WF60 – RF40^{+}$ doughs treated with XBS_{10} (see **Table 4** and **supplementary information**). Nevertheless, the SHIs of control $WF80-RF20^{+}$, $WF60 - RF40^{+}$ and $WF40 –$

RF60^{+} doughs were significantly ($P < 0.05$) lower than those of their XBS_{10} or XAA_{10} containing counterparts (**Table 4** and **supplementary information**).

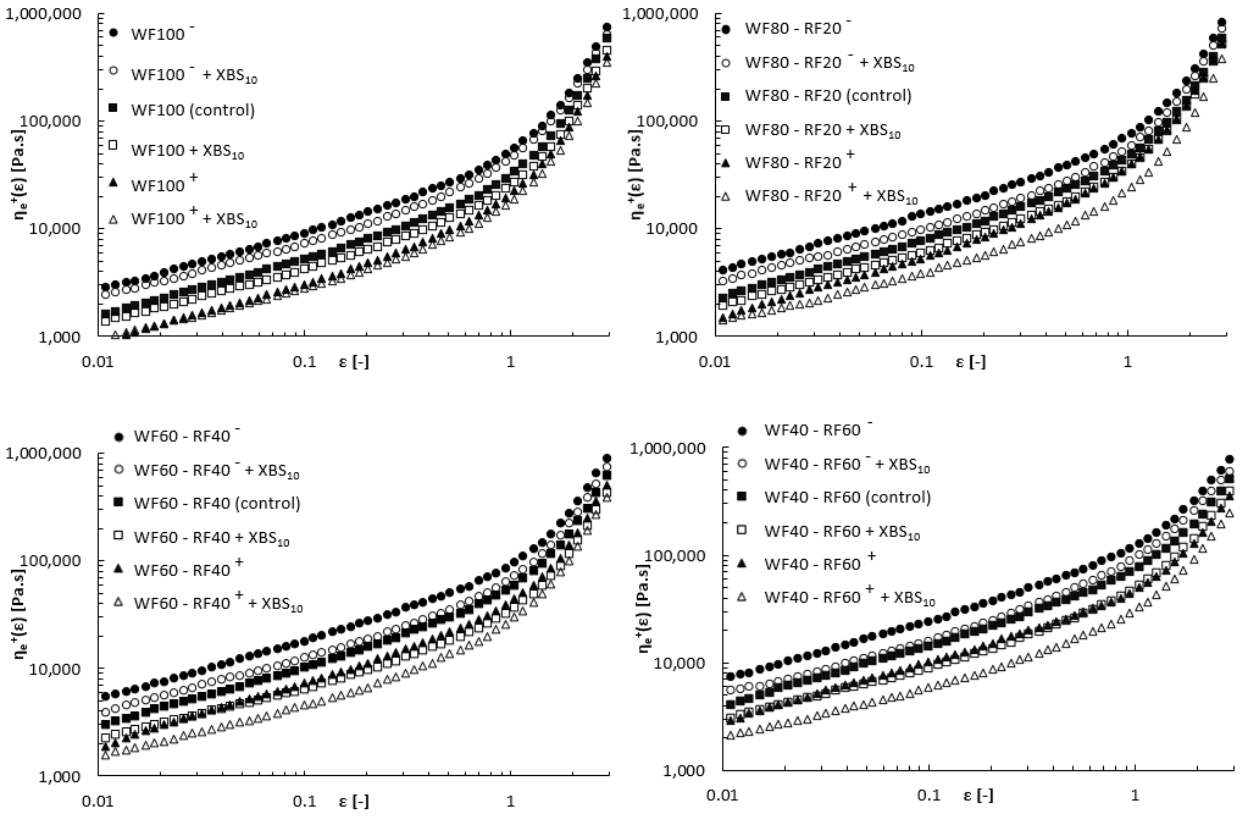


Figure 3 Transient extensional viscosity η_e^+ as a function of strain of dough strands prepared with 100% wheat flour (WF) (WF100), 80% WF and 20% rye flour (RF) (WF80 - RF20), 60% WF and 40% RF (WF60 - RF40), and 40% WF and 60% RF (WF40 - RF60). The first set of doughs was prepared without adding xylanase (XYL) and served as control. The second set of doughs was prepared with the high *Bacillus subtilis* XYL dosage (XBS_{10}) (see text). In each of these two sets, doughs were also made with more (indicated by $^{+}$) or less (indicated by $^{-}$) water. The data represent means of seven measurements of each of three separate dough batches.

Of particular interest is the similar effect on dough extensional rheology of including more water in the dough recipe or adding the higher XYL dosages (XBS_{10} or XAA_{10}). This suggests that with both enzymes an

184 excess of water was generated. Indeed, no significant ($P > 0.05$) differences in $\eta_{e0}^{+}(\varepsilon_{max})$ and $\eta_{e}^{+}(\varepsilon_{max})$
185 readings were noted when adding either more water or the higher XYL dosages (XBS_{10} or XAA_{10}) to the
186 doughs. The only exceptions to this observation were $\eta_{e0}^{+}(\varepsilon_{max})$ for WF60 – RF40 and $\eta_{e}^{+}(\varepsilon_{max})$ for WF40 –
187 RF60 when the samples contained the higher XAA dosage.

188 Including the high XYL dosages (XBS_{10} or XAA_{10}) in $WF100^{-}$, WF80 – $RF20^{-}$, WF60 – $RF40^{-}$ or WF40 – $RF60^{-}$
189 doughs resulted in significantly ($P < 0.05$) lower $\eta_{e0}^{+}(\varepsilon_{max})$ values and significantly ($P < 0.05$) higher SHIs
190 than in non-enzymatically treated $WF100^{-}$, WF80 – $RF20^{-}$, WF60 – $RF40^{-}$ and WF40 – $RF60^{-}$ doughs,
191 respectively (**Tables 3** and **4** and **supplementary information**).

192 The high XYL dosages (XBS_{10} or XAA_{10}) in $WF100^{-}$ and WF-RF^{-} dough recipes in almost all cases
193 pronouncedly decreased their $\eta_{e0}^{+}(\varepsilon_{max})$ to values similar to those obtained for their respective controls
194 (i.e. WF100 and WF-RF control doughs prepared without XYL). This suggests that enzymatic hydrolysis of
195 both WE-AX and WU-AX caused water previously bound by AX to be set free. The released water then
196 took over the role of the water present in a control dough recipe without reduced water content, which
197 resulted in similar $\eta_{e0}^{+}(\varepsilon_{max})$ values for a control dough and a XYL treated dough with lower water
198 content.

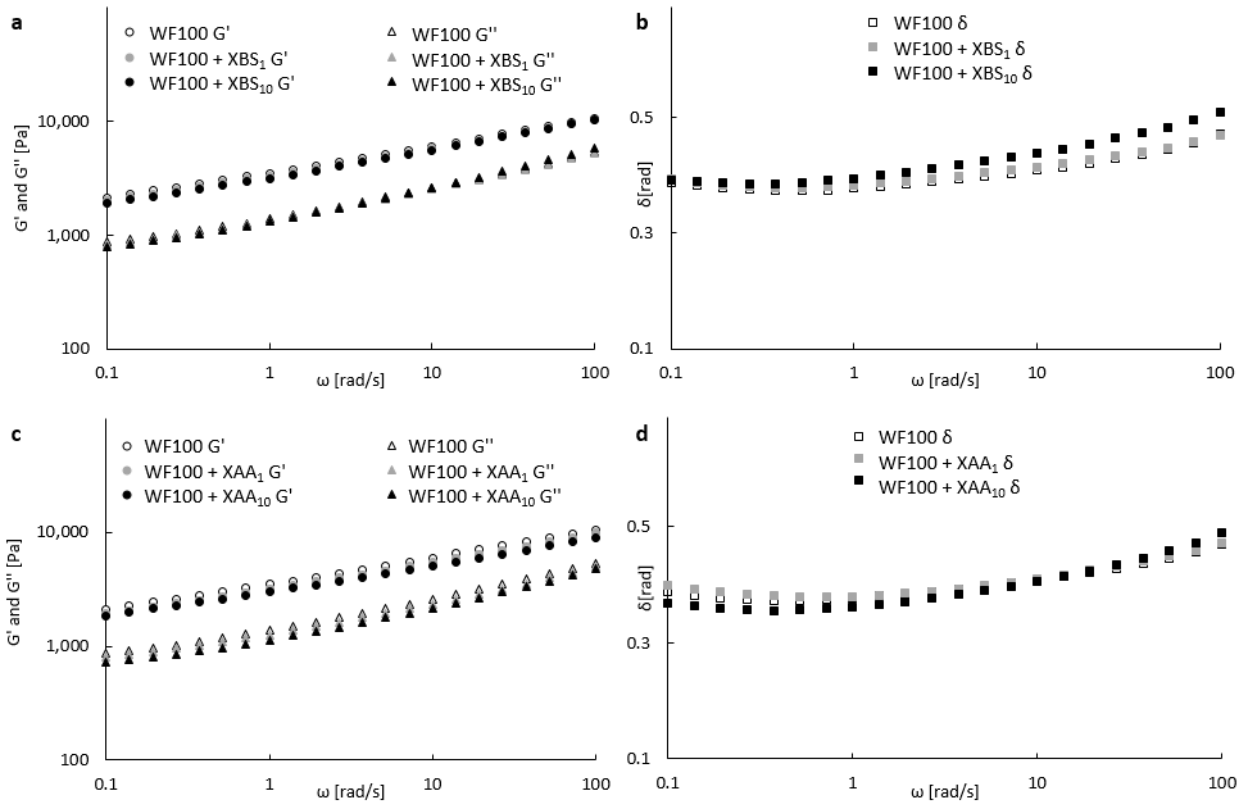
199 It was observed in several studies that enzymatic hydrolysis of WU-AX in WF doughs results in an
200 increased bulk viscosity of the dough aqueous phase (Courtin & Delcour, 2001; Rouau, El-Hayek, &
201 Moreau, 1994). Courtin et al. (1999) argued that (partial) enzymatic hydrolysis of WU-AX may result in
202 redistribution of water from WU-AX to other dough constituents. Considering that a sufficiently high XBS
203 dosage in WF dough reduces the bulk viscosity of aqueous extracts thereof (Courtin et al., 2001) and
204 leads to a decreased $avDP_n$ of WE-AX (**Table 2**), it seems logical that water bound to WU-AX redistributes
205 to other dough components. Recently, Leys et al. (2020) used ^{1}H NMR to assess the water distribution in
206 a starch-gluten-WU-AX-water model system and a WF-water model system. It was inferred that WU-AX

competes with starch and to a lesser extent with gluten for water. In addition, inclusion of XBS in such model dough system led to redistribution of water from WU-AX to other dough components. This again supports the earlier statement that the pronounced decrease of $\eta_{e0}^{+}(\varepsilon_{max})$ caused by enzymatic hydrolysis of both WE-AX and WU-AX to a large extent relates to the release of water previously bound by AX. Since only $\eta_{e0}^{+}(\varepsilon_{max})$ was significantly affected by AX hydrolysis, and given the importance of the gluten network for $\eta_{e}^{+}(\varepsilon_{max})$ (Meerts et al., 2017b; Meeus et al., 2020), the results of this study suggest that hydrolysis of AX does not significantly alter the gluten network in a way that would affect the extensional properties of WF and WF-RF dough at large strains.

In what follows, the potential impact of XBS and XAA addition during WF and WF-RF dough making on the interactions occurring between AX and other dough constituents is discussed.

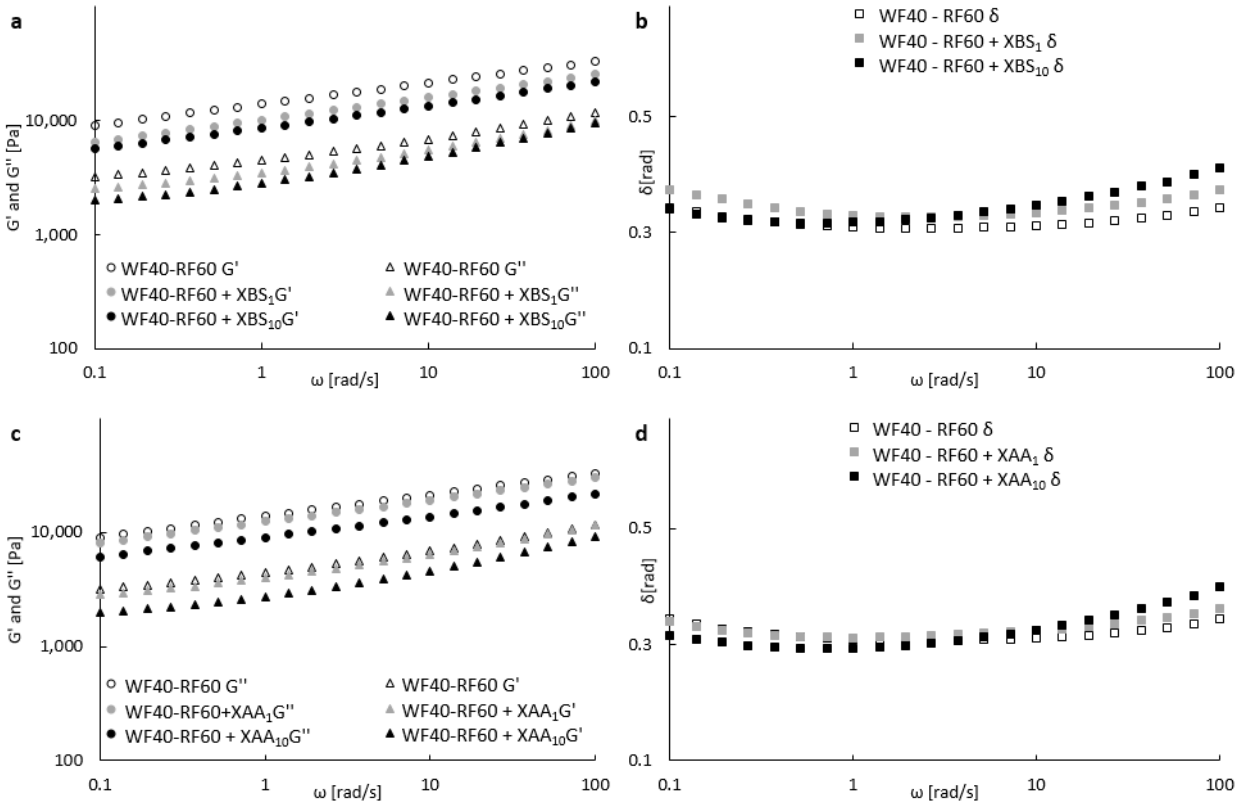
3.4 Linear rheology

SAOS measurements were performed on control and XBS_1, XBS_{10}, XAA_1 or XAA_{10} containing doughs. The evolutions of G', G'' and δ obtained from frequency sweeps carried out on WF100 and WF40-RF60 doughs are shown in **Figures 4** and **5**, respectively. The SAOS data for doughs prepared with WF80 – RF20, WF60 – RF40, WF20 – RF80 and RF100 are included as **supplementary information**.



222

223 **Figure 4** Storage modulus (*G'*), loss modulus (*G''*) and phase angle (*δ*) as a function of angular frequency
224 (*ω*) of doughs prepared from 100% wheat flour (WF) (WF100). The first set of doughs was prepared
225 without xylanase (XYL) and served as control (**a**, **b**, **c** and **d**). The second set of doughs was prepared with
226 the low dosages of *Bacillus subtilis* (XBS_1) (**a** and **b**) or *Aspergillus aculeatus* (XAA_1) XYL (**c** and **d**). The
227 third set of doughs were prepared with the high dosages of these enzymes [(XBS_{10}) (**a** and **b**) or XAA
228 (XAA_{10}) (**c** and **d**)] respectively (see text). The data represent averages of single measurements of each of
229 three separate dough preparations.



230

231 **Figure 5** Storage modulus (*G'*), loss modulus (*G''*) and phase angle (*δ*) as a function of angular frequency
232 (*ω*) of doughs prepared from 40% wheat flour (WF) and 60% rye flour (RF) (WF40 – RF60). The first set of
233 doughs was prepared without e (XYL) and served as control (**a**, **b**, **c** and **d**). The second set of doughs was
234 prepared with the low dosages of *Bacillus subtilis* (XBS_1) (**a** and **b**) or *Aspergillus aculeatus* (XAA_1) XYL (**c**
235 and **d**). The third set of doughs were prepared with the high dosages of these enzymes [(XBS_{10}) (**a** and **b**)
236 or XAA (XAA_{10}) (**c** and **d**)], respectively (see text). The data represent averages of single measurements of
237 each of three separate dough preparations.

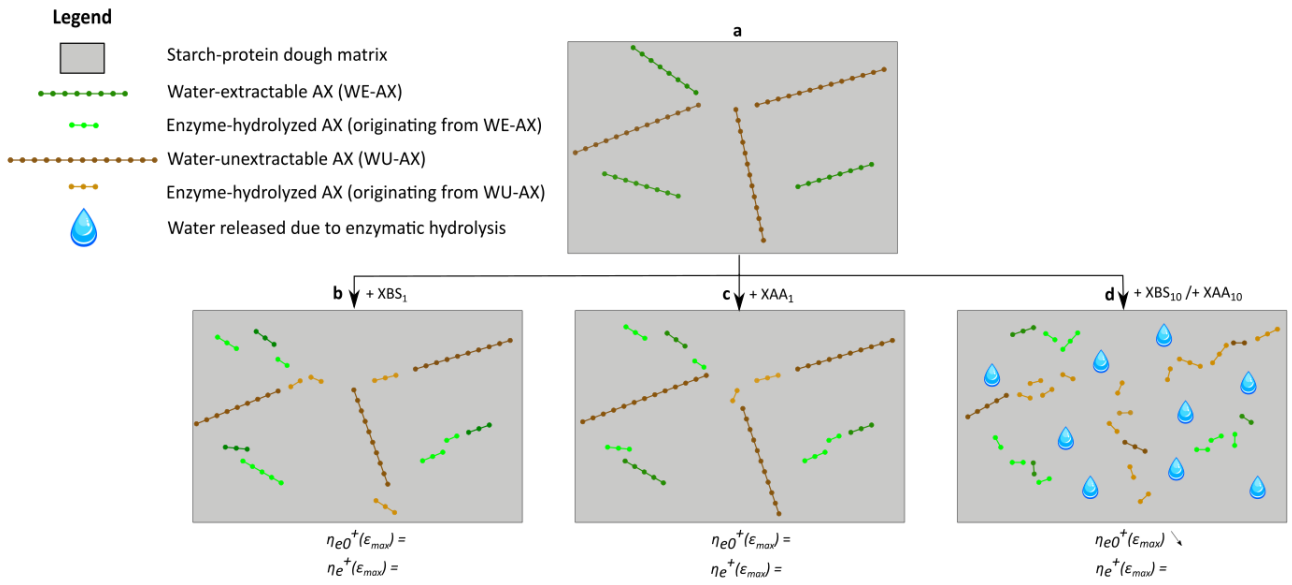
238 The *G'* vs. *ω* and *G"* vs. *ω* plots of XBS_1, XBS_{10}, XAA_1 or XAA_{10} containing WF100 and WF80 – RF20 doughs
239 were similar to those of their control counterparts. While the low dosage of XAA (XAA_1) in WF60 - RF40,
240 WF40 – RF60, WF20 – RF80 and RF100 doughs had no effect on *G'* or *G''*, the low dosage of XBS (XBS_1) in
241 said doughs resulted in a decrease of both moduli. Including the higher dosages of the XYLs (XBS_{10} or
242 XAA_{10}) in WF60 - RF40, WF40 – RF60, WF20 – RF80 and RF100 dough recipes resulted in substantial

decreases of both *G'* and *G''*. Irrespective of the dough type, including the high XYL dosages (XBS_{10} or XAA_{10}) in the recipe led to an increase in δ in the ω range of 10 – 100 rad.s^{-1} , (**Figures 4** and **5 b** and **d**). The lower dosages (XBS_1 and XAA_1) resulted in a less pronounced increase of δ for all doughs except for those prepared with WF100. Thus, at both dosages the decrease of G' was more pronounced than that of G" in the 10 – 100 rad.s^{-1} ω range. For WF dough, Meerts et al. (2017a) demonstrated that the magnitude of δ does not depend on the dough water content. The δ is indicative for the molecular interactions in a material (Létang, Piau, & Verdier, 1999). Thus, the increase of δ at higher ω implies that partial enzymatic hydrolysis of WU-AX and WE-AX (**Table 2**) weakens the short-range interactions between gluten, starch and AX in dough, leading to a more liquid-like behavior. Such short-range interactions have earlier been noted for rye model breads and have been identified as mainly mediated via ferulic acid cross-links (Buksa, 2016; Buksa & Krystyjan, 2019) and hydrogen bonds (Buksa, 2016).

To conclude, the SAOS results strongly suggest that AX interact with other AX, starch and/or gluten which then affect the linear shear rheology of dough. Apparently, it seems that these interactions do not exert a notable effect on the non-linear extensional rheology of dough. This illustrates that in the presence of a sufficiently high XYL dosage possible effects caused by changes in interactions between AX and other dough constituents is masked by the altered dough water distribution.

4. Conclusions

A schematic representation of our findings is shown in **Figure 6**. Treating doughs with a high XBS or XAA dosage caused a significant portion of WU-AX to become water extractable, as evidenced by a decrease and increase of the WU-AX and WE-AX populations, respectively, while simultaneously leading to a pronounced reduction of the $avDP_n$ of the WE-AX population as a whole (including hydrolyzed WU-AX fragments). These structural changes were accompanied by considerably lower dough $\eta_{e0}^{+}(\varepsilon_{max})$ values in extensional measurements. SAOS measurements showed that including a high dosage of XBS or XAA in WF and mixed WF-RF dough recipes increased δ, indicating that AX affects the linear shear rheology of dough through intermolecular interactions with other dough constituents. Even more important was that extensional measurements on doughs prepared with a modified water content supported the hypothesis that the observed decrease in dough $\eta_{e0}^{+}(\varepsilon_{max})$ values resulted from release of water previously bound by AX. In conclusion, this study provides a better understanding of the impact of including XBS or XAA in WF or mixed WF-RF doughs on their rheology under linear and subsequent non-linear extension and can serve as basis for further optimizing mixed cereal bread recipes.



274

275 **Figure 6** Schematic representation of the effect of including a high or low dosage *Bacillus subtilis* (XBS)
276 or *Aspergillus aculeatus* (XAA) in wheat flour and mixed wheat flour – rye flour bread doughs. (**a**)
277 Structures of water-extractable arabinoxylan (AX) (WE-AX) and water-unextractable AX (WU-AX) in
278 control dough (i.e. dough prepared with an optimal water absorption). (**b**) A low XBS dosage (XBS_1) in
279 dough results in partial enzymatic hydrolysis of both WE-AX and WU-AX but has no impact on the
280 doughs' rheological behavior under linear and non-linear extension. (**c**) As in **b**, treating doughs with the
281 low XAA dosage (XAA_1) does not change its resistance to extension despite the excessive hydrolysis of
282 WE-AX. (**d**) The high dosage of either XBS or XAA (XBS_{10} or XAA_{10}, respectively) leads to strong hydrolysis
283 of both WE-AX and WU-AX. Such hydrolysis provokes the release of water previously bound by AX which
284 decreases the resistance of dough to linear extensional deformations.

Appendix A. Supplementary data

CRediT authorship contribution statement

Yannick Meeus: Conceptualization, Methodology, Investigation, Writing - original draft

Frederik Janssen: Conceptualization, Methodology, Writing - review & editing

Arno G.B. Wouters: Conceptualization, Writing - review & editing

Jan A. Delcour: Writing - review & editing

Paula Moldenaers: Writing - review & editing

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