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Behavior of endo-xylanases on wheat milling products in relation with variable solid loading conditions

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ABSTRACT

To investigate the incubation conditions encountered by enzymes in cereal-based product transformation processes, this study aims to provide comprehensive information on the effect of low (18 %) to high (72 %) solid loading on the behavior of bacterial and fungal xylanases towards wheat grain fractions, i.e. white flour, ground whole grain and bran. Both enzymes are effective from 30 % water content. A water content of 50 % appears as the threshold for optimal arabinoxylan solubilisation. The specificity of enzymes was influenced by low hydration conditions, particularly in wheat bran, which contains arabinoxylan with diverse structures. Especially the bacterial xylanase became more tolerant to arabinose substitution as the water content decreased. Time Domain-NMR measurements revealed four water mobility domains in all the fractions. The water populations corresponding to 7.5 nm to 15 nm pores were found to be the most restrictive for enzyme activity. These results define the water content limits for the optimal xylanase action in cereal products.

1. Introduction

Cereals are the dietary staple of the world population and are utilized in diverse food and feed applications. Wheat holds the most prominent position among cereals, with an annual production of over 788.5 million tons (FAO, 2023), of which food usage is estimated at 69 % (Prückler et al., 2014). The nutritional and technological significance of wheat lies in its major components: starch and proteins. While cell walls constitute 12–18 % of the grain and offer dietary fiber benefits, they also present challenges for food and feed applications (Barron et al., 2020). Cereal grains are formed of distinct tissues performing different physiological functions such as protection or storage, resulting in specific cell wall compositions, structures and organizations (Saulnier, 2019). From a technological point of view, wheat bran is produced as the main co-product when wheat grain is processed to produce refined flours. The former consists of the outer tissues, i.e. the pericarp, testa, hyaline layer and most of the aleurone layer, while the later contain endosperm and part of the aleurone layer depending on the degree of refining. The outer layers are primarily composed of cellulose and complex arabinoxylans (AX) that are substituted by ferulic acid, which enables them to crosslink and form ester and ether bonds with lignin (Saulnier et al., 2012). The AX in these outer layers are highly substituted by arabinose, glucuronic acid and more complex side-chains compared to those in the aleurone layer and starchy endosperm (Saulnier et al., 2012). In the endosperm, AX account for about 60 % the cell walls, while mixed-linked glucans comprise about 5–10 % (Burton & Fincher, 2014; Saulnier et al., 2012). Endosperm AX is characterized by a high amount of single arabinose side-chains relative to AX in other tissues. While cellulose is typically reported in low level in the cell wall of the endosperm (<5 %), a recent study reported a higher proportion of cellulose, up to 20 % (Gartaula et al., 2018). About 25 % of AX in wheat grain is found in the starchy endosperm, which is by weight the main tissue of the grain. Another 25 % of AX is found in the aleurone layer. The remaining 50 % are present in the outermost protective tissues of the grain (Marion & Saulnier, 2020).

AX are therefore the major components of wheat grain cell walls, and *endo*-xylanases are widely used in cereal processing to improve the quality and help the processing of cereal products. A spectacular example of their efficiency is demonstrated in breadmaking where a limited solubilisation of AX improves dough behavior and bread quality

Abbreviations: AX, Arabinoxylan; A/X, Arabinose/Xylose ratio; GH, Glycoside Hydrolase; LMwAX, Low Molecular Weight Arabinoxylan; TD-NMR, Time Domain Nuclear Magnetic Resonance; XAa, *Aspergillus aculeatus* xylanase; XBs, *Bacillus subtilis* xylanase.

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(Courtin & Delcour, 2002). Endo-xylanases are also commonly included in the enzyme preparations used to enhance the growth performance of animals fed with cereals, with particular effectiveness in poultry production (Bedford & Cowieson, 2012). In this case, an extensive degradation of AX lowers the bolus viscosity and produces oligosaccharides having prebiotic effects. Endo-xylanases hydrolyze the beta 1,4 glycosidic linkage between xylose residues and belong to different glycosyl hydrolases families, mostly the GH10 and GH11. Numerous xylanases have been very well characterized (Paës et al., 2012). Depending on the sources they have different specificities regarding pH and optimal temperature, sensitivity towards xylanase inhibitors, sensitivity towards the presence of substitution on the xylan backbone, efficiency towards soluble or insoluble substrate.

Despite extensive research on *endo*-xylanases, the vast majority of work has been carried out under diluted conditions, which may not accurately reflect real-world processing conditions. How endo-xylanases operate in high-solids loading scenarios (≥15 % dry matter *w*/w, Sant'Ana da Silva et al., 2020), commonly encountered in feed and food processing (about 70 % for animal feeding, 40–45 % for breadmaking), remains insufficiently explored.

Water is essential in enzymatic processes as it provides the solvent medium for the reaction, controls the physicochemical conditions of the reaction (such as pH, ionic strength…), ensures the mass transfer of insoluble and soluble products, and is a reagent of the hydrolysis reaction. Most of the studies reported in the literature on the effect of high solid loading on enzymatic action focus on cellulases acting on lignocellulosic biomasses. A compilation of previous investigations revealed that decreasing conversion at increasing solids content is a widespread phenomenon (Kristensen et al., 2009). This phenomenon has been attributed, in part, to a limited adsorption of the enzymes on its substrate and non-productive complexes (Kristensen et al., 2009), the initial viscosity of the starting slurry (Sant'Ana da Silva et al., 2016), and the potential inhibition of the enzyme by its own hydrolysis products (Chen & Liu, 2017). In this respect, controversial results were obtained depending on the enzymes used (Kristensen et al., 2009; Sant'Ana da Silva et al., 2016). Moreover, the concentration in soluble compounds seemed to shift the distribution of water away from the surface of the insoluble matrix (Selig et al., 2012).

In addition, the enzymes may be confronted to a high solid content in two manners: the solid phase can be constituted predominantly by their substrate, or the substrate can be diluted in a solid matrix of other polymers on which enzymes are either inactive or prevented from accessing the substrate. These two situations are especially encountered when xylanases are applied to wheat fractions. In white flour, AX is embedded within a matrix primarily composed of starch and proteins. Conversely, AX is the main component in bran. High solid loading conditions offer advantages like energy and water conservation, but pose challenges due to limited water availability. Insufficient water affects reagent diffusion (Hodge et al., 2008), mixing (Du et al., 2017), and hydrolysis, potentially altering enzyme specificity and mode of action.

In view of the previous findings, we hypothesize that the behavior of xylanases is influenced by water mobility in high-solids loading conditions. Therefore, our study aims at determining the extent to which low water content limits the enzyme action. Three wheat fractions, i.e. white flour, and ground whole grain and bran, were chosen. They allowed investigating the impact of variability in AX structures. Moreover, they offered different situations of high-loading conditions regarding the AX exposure of enzymes to AX. Two xylanases with different selectivities were used at varying hydration levels (30–80 % *w*/w). The liberation of AX and their subsequent structural modifications were monitored, in connection with water mobility distribution, characterized by NMR relaxometry.

This study seeks to enhance our understanding of enzymatic hydrolysis under realistic conditions and specifically to determine the extent to which low moisture content limits the enzyme action. By elucidating the interplay between enzyme action, substrate composition, solid loading, and water mobility, our research aims to provide valuable insights applicable to the optimization of enzymatic transformation steps in food and feed processing.

2. Materials and methods

2.1. Plant material, polysaccharides and enzymes

White flour, ground whole grain and bran, subsequently referred to as flour, grain and bran, respectively, were produced from wheat (*Triticum aestivum*) grains on an industrial mill using a unique grain lot and were gifted by Petits Moulins de France (Minoterie Giraudineau, 44310 Saint-Colomban, France). Bran and whole grain were ground in small scale laboratory mill (Retsch GmbH, Haan, Germany) to pass through 0.5 mm screen. The particle size distribution was measured in the 3 samples using an automated dynamic morphological analyzer QICPIC (SympaTec GmbH, Clausthal, Germany, see Supplementary information).

Soluble and insoluble AX from wheat were used to quantify the xylanase activity. Soluble AX was purchased from Megazyme (LMwAX, Bray, Ireland). It contained 862 mg/g AX with A/X:0.58. The insoluble AX was extracted as the endosperm cell wall material from Soissons flour as previously described (Bonnin et al., 2006). The AX content was 530 mg/g with A/X: 0.56, and it also contained glucose (220 mg/g), mannose (34 mg/g), galactose (13 mg/g) and proteins (67 mg/g).

The GH10 *endo*-xylanase from *Aspergillus aculeatus* (XAa) was graciously provided as a liquid preparation by Novozyme A/S (Shearzyme 500 L, Bagsværd, Denmark), and the GH11 endo-xylanase from *Bacillus subtilis* (XBs, Sibbesen & Sorensen, 2001) was gifted as a powder preparation by DuPont Nutrition Biosciences (Brabrand, Denmark). The XBs powder was dissolved in MilliQ water at 10 mg/mL and mixed on a wheel at 4 °C for 15 min. The mixture was centrifuged and enzyme-containing supernatant was collected.

All the reagents and standards were of highest purity and obtained from reputed suppliers.

2.2. Enzyme characterisation

The activity of the two xylanases was measured thanks to the appearance of reducing ends (Nelson, 1944 adapted to microplate). Soluble AX (LMwAX, Megazyme, Bray, Ireland) and insoluble AX (from laboratory collection) from wheat were used as the substrates to measure the enzyme activity. Precisely weighed 5.4 mg wheat AX was suspended in 540 µL of MilliQ water to which 60 µL of enzyme was added. The reaction mixture was incubated at 40 °C for 10 min. Each assay was performed in triplicate and the activity was expressed in nkat/mL. One nkat corresponds to the amount of enzyme necessary to release 1 nmol of reducing ends per second. The protein content of the enzymes was measured using Bradford Assay (Bradford, 1976). The specific activity was calculated by dividing the enzymatic activity by the protein content in the enzyme solution.

2.3. Endogenous enzyme and inhibitor activities

The activity of endogenous enzymes and xylanase inhibitors present in wheat samples were measured through Nelson method (Nelson, 1944) adapted to microplate. This measurement was carried out to account for any interference from the endogenous enzymes or xylanase inhibitors in wheat during the enzyme treatment of fractions. For this purpose, a water extract was prepared from ground whole grain (1 g suspended in 4 mL water, Bonnin et al., 2005) and 50 µL ground whole grain extract was added to the enzyme-LMwAX mixture followed by incubation at 40 °C for 20 min. The blanks comprising boiled xylanases or boiled whole grain extract were run to account for endogenous xylanase activity and reducing power of the extract, respectively.

2.4. Enzymatic treatment of wheat fractions

The wheat fractions and enzymes were thoroughly mixed with variable hydration conditions in a 2 g-Mixograph (National Manufacturing, Lincoln, NE, USA). Purposely, precisely weighed 2 g sample was added in the Mixograph bowl and the enzyme-water premix was added (corresponding to specified enzyme activity and targeted hydration level, i.e. from 0.5 to 8.0 mL corresponding to ≈ 28–82 % hydration taking into account the water content on the starting materials). In the first set of experiments, fixed amount of 40 nkat/g xylose was applied to each sample. After mixing 10 min at 70 rpm at 40 °C, the content was quickly collected in a plastic tube with cap (preheated to 40 °C) and incubated in water bath at 40 °C for 4 h. At water content ≥70 %, the enzyme and fraction were directly mixed in a plastic tube on vortex mixer and incubated for 4 h.

For kinetic study, 25 g sample was mixed with the enzymes in a Farinograph bowl thermostated at 40 °C (Brabender GmbH, Duisburg, Germany) for 5 min. The solution of enzyme in water was added drop wise in <1 min with the help of a burette while mixing the sample to ensure uniform distribution of the enzyme solution. After mixing, the sample was placed in a plastic jar preheated to 40 °C and incubated in water bath at 40 °C. An aliquote of the sample was taken immediately after mixing (referred as 0 h incubation), and after 0.5, 1, 2, 4, 8 and 24 h of incubation. The enzymatic reaction was stopped by placing the tubes at −18 °C overnight, immediately after incubations. The samples were freeze-dried, followed by grinding using FastPrep-24 instrument (MP Biochemicals, Illkirch-Graffenstaden, France) at a frequency of 6.5 m/s for two cycles of 20 s. The milled samples were put in the vacuum oven at 40 °C under P_2O_5 for 2 h. The solubilized/degraded AX was extracted with water in 5 mL Eppendorf tube for 30 min at 4 °C agitated on a rotating wheel. The amount and volume used were set as follows: 2.5 mL for 500 mg for flour, 3 mL for 500 mg for grain, 3 mL for 350 mg for bran. The suspension was centrifuged at 4 °C (10 min at 5000 g) and the supernatant obtained was then boiled for 10 min to inactivate the enzymes. The extract was frozen at −18 °C for further analysis. All the enzymatic treatments of the fractions and the controls without enzyme were run in duplicate.

2.5. Starch analysis

Starch was analyzed according to the enzymatic AOAC method 996.11 (McCleary et al., 1997) where starch is solubilized by a thermostable α-amylase into soluble branched and unbranched maltodextrins, which are further quantitatively hydrolyzed in glucose by an amyloglucosidase. Both enzymes were purchased to Megazyme (Bray, Ireland). The released glucose was quantified by HPAEC using a CarboPack PA-1 column as previously described (Guillon et al., 2011). The analysis was performed in duplicate.

2.6. Monosaccharide composition

The wheat fractions and extracts were analyzed for monosaccharides composition through gas chromatography (Englyst & Cummings, 1988). The cryo-milled (SPEX® SamplePrep, Metuchen, NJ-US) wheat fractions were treated with 12 mol.L^{-1} H_2SO_4 for 30 min followed by hydrolysis with 1 mol.L^{-1} H_2SO_4 at 100 °C for 2 h along with centrifuged extracts. The alditol acetates of the hydrolysate and sugars standard were injected on a BP-225 fused-silica capillary column (Optima225, Macherey-Nagel GmbH, Düren, Germany; 25 m × 0.32 mm) mounted on a Perkin Elmer Gas Chromatography system (Clarus® 580, Perkin Elmer France, Villebon/Yvette, France). The analysis was performed in triplicate. The xylose content of the enzyme-treated samples was expressed as a weight percentage of the initial xylose content in the fraction.

2.7. Water mobility through time domain-NMR (TD-NMR)

The white flour, ground grain and bran were subjected to NMR analysis to characterize the mobility of water at variable solid loadings. Purposely, 20–25 g sample (20 g for bran, 25 g for flour) was mixed at 40 °C with requisite amount of water (0.5, 1.0, 1.5, 2.0 mL/2 g flour corresponding to ≈ 28–82 % hydration). After dry mixing for 15 s, the water was added dropwise, and sample was mixed for a total time of 5 min using farinograph bowl. The sample was then transferred in a plastic container, which was tightly closed and incubated at 40 °C for requisite time. Thereafter, the sample was transferred in the NMR tubes (10 mm diameter) at room temperature to a maximum height of 1 cm. The sample was weighed, the NMR tube was clogged with a cylinder and capped with para-film to avoid moisture evaporation. Three replicates of the sample were weighed at the same time and run sequentially on NMR. The samples were put on NMR tube heaters to equilibrate temperature of 40 °C before analysis. TD-NMR analyses were carried out using a Minispec mq20 spectrometer (Bruker Biospin, Wissembourg, France) operating at 0.47 T (20 MHz proton resonance frequency) and equipped with a thermostated (± 0.1 °C) ^{1}H probe. The transverse T_2 relaxation curves were acquired using a Carr–Purcell–Meiboom–Gill (CPMG) sequence. The 180° pulse separation was 0.2 ms, 700 even echoes were collected, and 512 scans were acquired with a recycle delay of 1 s, resulting in a total acquisition time of about 10 min. An inverse Laplace transformation (ILT) was applied to convert the relaxation signal into a continuous distribution of T_2 relaxation components (Berman et al., 2013). To extract quantitative information from the ILT continuous analysis, T_2 distribution envelopes were modelled using a sum of peaks with a normal asymmetrical shape using an in-house routine developed in Matlab® software. Relative peak area or population (P_{2i}), and transverse relaxation time (T_{2i}) mean values of each peak "i" were determined in this way.

3. Results and discussion

3.1. Characterisation of the wheat fractions

The carbohydrate compositions of white flour, grain and bran were characterized (Table 1). Flour contained 84.3 % starch, grain, 70.3 % and bran contained only 9.6 %. The bran had the highest xylose content (14.8 %) while the flour had the lowest (1 %). Considering that arabinose and xylose come mainly from AX, an AX content was estimated using the sum arabinose+xylose. Bran had the highest AX content (23.5 %), followed by grain (5.2 %) and flour (1.7 %).

The molar ratio arabinose/xylose (A/X), a key indicator of AX structural complexity, was the highest in the white flour (0.72) and the lowest in bran (0.59), indicating the presence of linear xylan in specific cell layers of this fraction (hyaline layer). As expected, the ground whole grain, encompassing diverse tissues, exhibited an intermediate A/X value of 0.63. These overall results demonstrate that at high solids content in flour, AX are dispersed within a starch-protein matrix present in the flour. In contrast, in bran, AX constitute the predominant component of the matrix. Additionally, it is worth noting that AX in white flour possess a simpler structure compared to bran AX, which

Table 1
Water content and carbohydrate composition of the wheat fractions, expressed as a percentage of fresh weight. Results are the average of duplicates and the standard deviation is given in parenthesis.

Fraction	Water	Arabinose	Xylose	AX	A/X	Starch
Flour	11.4 (0.1)	0.72 (0.01)	1.00 (0.01)	1.72 (0.02)	0.72 (0.02)	84 (5)
Grain	12.3 (0.1)	2.00 (0.05)	3.20 (0.02)	5.20 (0.07)	0.63 (0.03)	70 (4)
Bran	10.1 (0.2)	8.8 (0.1)	14.8 (0.2)	23.6 (0.3)	0.59 (0.01)	9.6 (0.1)

originate from distinct tissues.

3.2. Enzyme characteristics

The activity towards soluble and insoluble wheat AX exhibiting similar A/X ratio (0.56) was determined in water for XBs and XAa preparations, and the specific activities were calculated (Table 2). Both enzymes were active on the soluble and on the insoluble AX. The selectivity, calculated as the ratio of activity on soluble AX/activity on insoluble AX, indicated that XBs was proportionally more active on insoluble AX than XAa. This implies that XBs acts primarily as a solubilizing *endo*-xylanase, while XAa functions as a degrading counterpart (Hardt et al., 2014).

Subsequently, the activities were assessed on insoluble AX in the presence of a water extract derived from ground whole grain (Table 3). This extract contained a comprehensive blend of both endogenous xylanases (Bonnin et al., 1998) and xylanase inhibitors (Bonnin et al., 2005) inherent to wheat grain. Indeed, it was crucial to check whether the enzymes under investigation were sensitive or not to endogenous inhibitors, given the experimental conditions featuring high loading of wheat fractions.

Since the activity in the presence of the whole grain extract was higher than in its absence, the results suggested that neither enzyme was susceptible to the potential inhibitory influence of the extract. Furthermore, the activity shown in the presence of grain extract was greater than the sum of the 'enzyme + grain extract' activities, suggesting a synergistic effect between the two. This cooperative effect could be attributed to the presence of arabinofuranosidases within the grain extract (Bonnin et al., 1998), which likely contributed to the enhanced hydrolysis process.

3.3. Xylose solubilisation at variable hydration

All three wheat fractions underwent enzymatic treatment using the two enzymes under varying hydration levels (30–80 %). The enzymatic activity was standardized based on the xylose content determined in the different starting wheat fractions (Table 1) thereby ensuring a constant enzyme/substrate ratio (40 nkat/g xylose). The xylanases solubilized AX and the content of xylose present in the soluble fraction (further expressed as solubilized xylose) was considered a relevant marker of AX solubilisation and was quantified.

In white flour, the non-enzymatic solubilisation of xylose observed in the control samples reached about 38 % to 50 % (Fig. 1a). When treated with XBs, 86 % of the xylose was solubilized at the lowest hydration level (28 %). It went up to 96 % when water content reached 50 % and did not significantly change above. Likewise, the xylose solubilisation by XAa started at 68 % in the lowest water content (28 %) and reached a maximum of 91 % at 50 % hydration. Both enzymes appeared to be equally efficient to solubilize AX from white flour. Below 50 % hydration, the sample mixing was not uniform and the formation of lumps was visible. However, almost complete AX solubilisation was attained signifying that, despite AX dispersion within the starch-protein matrix, water availability did not limit enzyme action, including diffusion and catalysis.

In grain, the xylose solubilisation in control samples without enzyme showed a slight increase from 13 % of the initial xylose content at 28 % hydration to 17–19 % from 50 % hydration. This trend is consistent with the recognized behavior of wheat AX that are partially water-extractable polymers exhibiting viscosity-inducing properties (Saulnier, 2019). The increase in dry matter content in the incubation medium appeared to negatively influence the solubilisation, likely due to the physical limitation of mass transfer. In the presence of XBs, xylose solubilisation from grain increased from 29 % at lowest level of hydration (28 %) to 51 % at 50 % hydration, and then plateaued to 56 % at highest level of hydration (82 %) (Fig. 1a). With XAa, the xylose solubilisation peaked at 50 % hydration (33 %) and then remained constant. For both enzymes, the

Table 2
Characterisation of the enzyme solutions: protein contents and activities towards soluble and insoluble wheat AX in XBs and XAa. Results are the average of triplicate and the standard deviation is given in parenthesis.

	XBs	XAa
Protein (mg/mL)	0.095 (0.003)	10.2 (0.2)
SA/LMwAX (nkat/mg)	343 (21)	535 (21)
SA/Insoluble AX (nkat/mg)	84 (8)	92 (4)
Activity/LMwAX (nkat/mL)	33 (1)	5433 (106)
Activity/Insoluble AX (nkat/mL)	8 (1)	936 (26)
Selectivity	4.1 (0.6)	5.8 (0.5)

SA: Specific activity; LMwAX: Low Molecular weight arabinoxylan. XBs was a powder prepared at 10 mg/mL, while XAa was a liquid preparation.

Table 3
Activity of XBs and XAa in the absence or presence of grain extract (nkat/mL). The activity in the grain extract alone was 1.4 nkat/mL.

	Absence of grain extract	Presence of grain extract
XBs	6.1	10.7
XAa	1463	2022

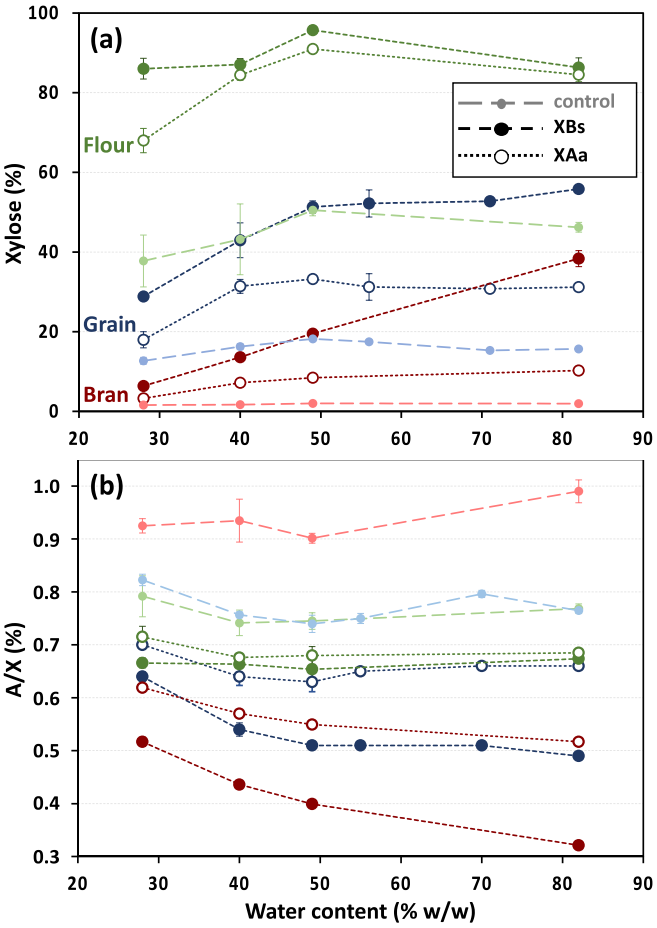

Fig. 1. Release of AX from the different wheat samples in the presence of xylanases and in increasing water content conditions. (a) Xylose solubilisation (expressed as a percentage of the initial xylose content, see Table 1) and (b) Arabinose/Xylose ratio (A/X) of the polysaccharides solubilized from white flour (Flour, green), ground grain flour (Grain, blue) and Bran (red) treated with XBs (filled circles and dotted lines) or XAa (open circles and full lines) at various water contents from 28 % to 82 %. The markers and dashed lines of lighter colours are for controls without enzyme. The results are the average of duplicates.

most significant effect of the water content was observed up to 50 % hydration. Beyond this point, the increase in water content no longer had an impact on the solubilisation. The disparity in AX solubilisation between flour and grain can primarily be attributed to the structural complexity of AX in the outer layers present in grain. Apart from the starchy endosperm, the grain contains the aleurone layer (6–7 % of the grain) and the outer layers (6–8 % of the grain). The outer layers contain nearly 50 % of the grain's AX (Marion & Saulnier, 2020; Saulnier et al., 2012). Complexity of AX in outer layers is related to a high degree of xylan backbone substitution, reflected in a notably elevated A/X ratio compared to flour AX (Fig. S1). Moreover, covalent interactions, through ferulic acid bridges, exist between AX chains and lignin in this tissue, further hindering solubilisation when compared to flour. This complexity induces the recalcitrance of outer layers AX to xylanase degradation (Ordaz-Ortiz et al., 2005).

Very low amount of xylose was solubilized from control samples of bran whatever the water content, in the range of 1.6–1.9 % between 28 % and 82 % hydration. Upon exposure to XAa, the xylose solubilisation reached 8.5 % at 50 % hydration and remained rather stable when the water content increased (10.3 % solubilisation at 82 % hydration). Under XBs treatment, xylose solubilisation increased linearly with water content from 6 to 38 % without reaching a plateau. As bran is predominantly composed of outer layers and aleurone, AX solubilisation was restricted by their structural complexity. Compared to grain, lower AX solubilisation was observed with bran at similar water content, suggesting that additional mechanisms limit enzyme action.

Overall, this first set of results shows that the increase in solid loading of wheat fractions reduced the xylanase-catalyzed hydrolysis when the water content is below 50 %. However, distinct trends emerged in terms of AX solubilisation, with substantial variations observed in the maximum level of AX solubilisation among the three fractions, despite the consistent enzyme/substrate ratio applied across all fractions and with both enzymes. XBs outperformed XAa on both ground whole grain and bran and the difference increased with the water content. This indicates that the two enzymes behaved differently with the variation in hydration, a crucial aspect to consider when developing food or feed processes involving xylanases.

To further investigate the enzyme mechanism on the wheat fractions, the arabinose to xylose ratio (A/X) of the solubilized AX populations was measured for each enzyme and each water content. The starting values were quite close in the bran and grain (0.59 and 0.63, respectively), and somewhat higher in the white flour (0.72) (Table 1). The Fig. 1b depicts the A/X ratio of the AX populations released by both enzymes in the different water conditions. The two enzymes did not modify the A/X ratio of the AX released from white flour, where they kept it around the initial value. Indeed, the endosperm displays homogeneous AX structure, likely accounting for the similarity in populations extracted by each enzyme and the overall AX population found in white flour. In contrast, when acting on grain and bran, both enzymes released AX with A/X ratio lower than the initial ratio, indicating structural changes towards a less branched structure. The A/X ratio was significantly reduced as the water content increased and this effect was more pronounced with XBs than with XAa. Again, 50 % hydration appeared as a pivotal threshold for AX extraction from grain. Thus, maintaining the water content below 50 % not only constrained extraction but also influenced the structural characteristics of the extracted AX. Above this value, the water content had no impact on the structure of the extracted AX. As no arabinofuranosidase activity was detected in the two enzymatic preparations (data not shown), the differences in A/X ratios observed in the extracted populations reflect differences in the cleavage sites between the two enzymes and the influence of water content on the mode of action of xylanases, particularly XBs, when acting on wheat bran. However, arabinofuranosidase activity has previously been shown in wheat bran (Bonnin et al., 1998) and could help linearize extracted polysaccharides. However, it was detected on a model substrate and in highly diluted medium. Therefore, it cannot be ruled out that it may make a minor contribution under the conditions applied here. Finally, at reduced water contents xylanases might display an elevated specificity for the highly substituted regions. Consequently, limiting the water content of the reaction slurry may have repercussions on both the reaction rate and AX accessibility. This highlights the pivotal role of water content in shaping the enzymatic interactions. Altogether, these results indicated a different extracting power of the two enzymes in relation with water content and in agreement with their selectivity previously measured in the presence of an excess of water (Table 2).

The effect of water content on the action of a *Bacillus subtilis* xylanase was previously studied on wheat bran (Santala et al., 2011). The highest solubilisation of AX was observed at 40 and 90 % water content, while it was surprisingly lower for intermediate values between 50 % and 80 % water. Despite using a xylanase from the same bacteria, this effect was not observed in the present work, neither on bran nor on flour or grain. These differences may be due to the different operating conditions but also to different specificities of the enzymes, as suggested by the higher A/X ratio of the extracted AX observed by Santala et al. (2013). The *Bacillus subtilis* species contains many different subspecies and strains, each with several GH10 or GH11 xylanases (cazy.org/genomes/bacteria).

3.4. Kinetic study of enzymatic degradation of the wheat fractions

To explore the potential impact of limited water availability on the reaction rate, kinetic studies were performed using grain and bran as substrates. The flour was excluded from the study since almost complete solubilisation of xylose was observed at all tested water contents. Preliminary trials were carried out to adjust the amount of enzyme. Based on these trials, the enzyme dosage for the hydrolysis of grain was kept similar to the previous experiments (40 nkat/g xylose), while it was increased up to 825 nkat/g xylose for bran to reach a plateau. The intermediate hydration levels around 40 % and 50 % were chosen based on the marked changes previously observed in the AX solubilisation (Fig. 1a and b). For each time course, the first sample was withdrawn after 5 min of mixing in the bowl. The hydrolysis time courses were fitted as a pseudo-first order equation with an exponential law in order to detect changes over time (Table 4).

In grain treated with XBs, about 30 % and 34 % xylose were solubilized after 5 min of incubation for 40 % and 50 % hydration, respectively (Fig. 2a). Considering that non-enzymatic solubilisation reached 16 % and 18 % of initial xylose after 4 h at these two hydration levels (Fig. 1a), it can be deduced that XBs xylanase was highly efficient and quick in its action. At 50 % hydration, maximum solubilisation took place after 4 h incubation and remained rather constant afterwards. Thanks to the mathematical modelling, a solubilisation extent of 60 % was calculated at t_∞ (Table 4), confirming that the plateau had been effectively reached after 4 h incubation, where the solubilisation was

Table 4
Kinetic parameters of xylose solubilisation (XS) for whole grain (Grain) and bran treated with XBs and XAa at the two water contents 40 % and 50 %. Parameters were extracted from the curve fitting with the function: $XS(t) = (XS(t_\infty) - XS(t_5))[1 - exp(-t \times V_{XS})] + XS(t_5)$, where $XS(t_\infty)$ is the asymptotic value of XS, $XS(t_5)$ is the value of XS at 5 min, and V_{XS} is the rate of xylose solubilisation (see Fig. 2a).

Enzyme	Fraction	Water content (%)	$XS(t_5)$ (%)	$XS(t_\infty)$ (%)	V_{XS} (min^{-1})
XBs	Grain	40	30	47	28
		50	34	60	35
	Bran	40	15	33	53
		50	18	42	63
XAa	Grain	40	23	28	19
		50	25	43	6
	Bran	40	6.8	20	27
		50	8.9	36	12

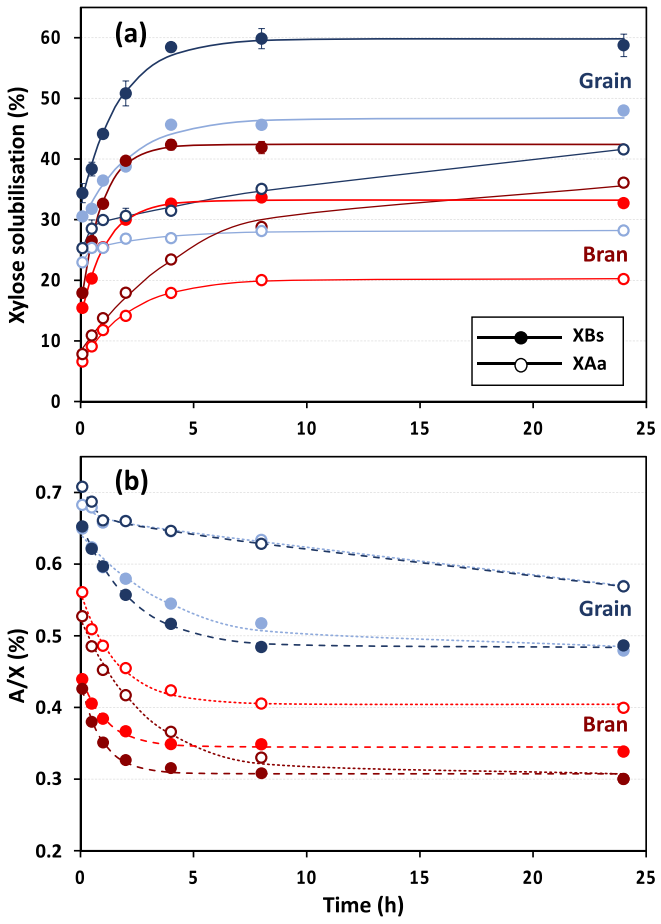

Fig. 2. Time course of AX release from grain and bran in the presence of xylanases and in 2 hydration levels. (a) Time course of xylose solubilisation (XS) and (b) evolution of Arabinose/Xylose ratio (A/X) from ground grain (Grain, blue) and Bran (red) treated with XBs (filled circles) or XAa (open circles) at two water contents, 40 % (lighter markers) and 50 % (darker markers). The results are the average of duplicates. Solid lines in (a) result from the adjustment of experimental points with a pseudo first order exponential law (see Table 4).

already of 58.5 %. With XAa, the solubilisation after 5 min incubation was lower than with XBs but higher than the non-enzymatic solubilisation. As for XBs, higher extent of solubilisation was achieved at 50 % than at 40 % hydration.

At 40 % hydration, solubilisation extent changed little over time, starting at 23 % and increasing slightly to 28 % for t_∞ (Table 4). At 50 % hydration, the final plateau was not reached at 24 h incubation and the calculation at t_∞ showed a maximum of 43 % solubilisation. In contrast with XBs, the apparent solubilisation rate of xylose (V_{XS}, Table 4) was lower at 50 % than at 40 %.

The xylose solubilisation from wheat bran treated with XBs exhibited similar pattern at the two levels of hydration tested (Fig. 2a), with relatively lower kinetic parameters (XS and V_{XS}, Table 4) at 40 % hydration. After 5 min incubation, a xylose solubilisation of 15 % and 18 % was observed at 40 % and 50 % hydration, respectively. Compared to the non-enzymatic solubilisation where <2 % xylose were solubilized (Fig. 1a), this was again an indicator of the rapid action of XBs. At t_∞, a maximum solubilisation of 33 % and 42 % was calculated at 40 % and 50 % hydration, respectively. XAa-mediated xylose solubilisation was significantly lower. At 40 % hydration, it increased from 6.8 % to 20 % between 5 min and t_∞. At 50 % hydration, the plateau took longer to reach, as it was observed with the same enzyme in the same water condition on grain. The xylose solubilisation ended at 36 % at 24 h incubation and corresponded to the final solubilisation (Table 4). As observed on grain with the same enzyme, the apparent solubilisation rate of xylose (V_{XS}, Table 4) was lower at 50 % than at 40 %.

Despite the application of a higher enzyme dosage, the final solubilisation of wheat bran AX was much lower than in grain. As the degradation by XAa increased between 8 h and 24 h, the enzyme was still active. Therefore, this observation was likely attributable to the more complex structure of AX and cell walls in the outer tissues, as well as interlinkages of AX with other cell wall components (Saulnier, 2019), inducing a restricted enzyme accessibility.

The A/X ratio of the AX extracted from grain and bran was calculated along the time course of incubation. The A/X ratio of AX extracted from grain treated with XBs and XAa decreased continuously as the incubation time increased (Fig. 2b). The reduction was notably more pronounced with XBs (at 24 h, 0.49 and 0.57 with XBs and XAa, respectively). For both enzymes, the A/X ratio all along the time course was not affected by the levels of hydration between 40 % and 50 %. However, it is interesting to note that with XAa at 50 % hydration, the value at 5 min incubation (0.71) was higher than the starting value in the native fraction (0.63, Table 1). This suggests that XAa was preferentially active on the branched AX at the very beginning of the incubation. As degradation progressed, XAa extracts maintained a significantly higher A/X ratio than XBs extracts. As no arabinofuranosidase activity was detected in the enzymes, this suggests that enzyme action was affected by the degree of branching of AX xylose backbone. Additionally, XBs may have a higher affinity for linear xylan present in the hyaline layer.

Conversely to ground grain, the A/X ratio in the AX populations extracted from bran remained significantly lower throughout the incubation period when the hydration level was higher (Fig. 2b). With XBs, the values of the control samples (0.44 at 40 % hydration and 0.43 at 50 % hydration) were already much lower than the starting value in the native bran (0.59, Table 1) showing the very rapid action of XBs on the less branched AX.

To summarize, the comparison of the two enzymes shows that XBs was the most efficient in all cases. This bacterial enzyme belongs to the GH11 hydrolase family and thus is smaller than the GH10 XAa (Paës et al., 2012). As a consequence, it may penetrate more easily the plant matrix.

3.5. Water mobility distribution

Water plays three essential roles in enzymatic catalysis of a solid matrix by hydrolases, i) as a swelling agent, ii) as a diffusion medium for the enzyme and the products, and iii) as a reagent for the catalysis. NMR relaxometry allows to distinguish different water environments in a sample by determining T_2 relaxation time distribution. Therefore, TD-NMR relaxometry was used to investigate changes in the different wheat fractions according to hydration levels and enzyme action.

As illustrated in Fig. 3, the analysis of T_2 relaxation curves led to T_2 distributions with multiple distinct peaks indicating that no simple direct relationship exists between T_2 components and specific morphological compartments or constituents in bio-based samples. Each T_2 peak is preferentially assigned to a pool of water at a given range of mobility, corresponding to specific molecular environments and/or interactions. Regardless of hydration status, four different water peaks were observed in flour, grain and bran, as previously observed by other authors on similar wheat flour/water systems (Dufour et al., 2023; Leys et al., 2020; Parenti et al., 2021). They were referenced from "*a*" for the less mobile component (shortest relaxation time ~ 1.5 ms, Table 5), to "*d*" for the most mobile component (longest relaxation time: 40–100 ms).

Fig. 4 clearly shows that T_2 distribution shifted towards higher values of T_{2i} when water content increased from 28 % to 55 %, especially for intermediate components "*b*" and "*c*".

Interestingly, the relaxation time T_{2d} decreased from 80 to 100 ms for bran to about 40 ms for flour, and reached values associated with pore

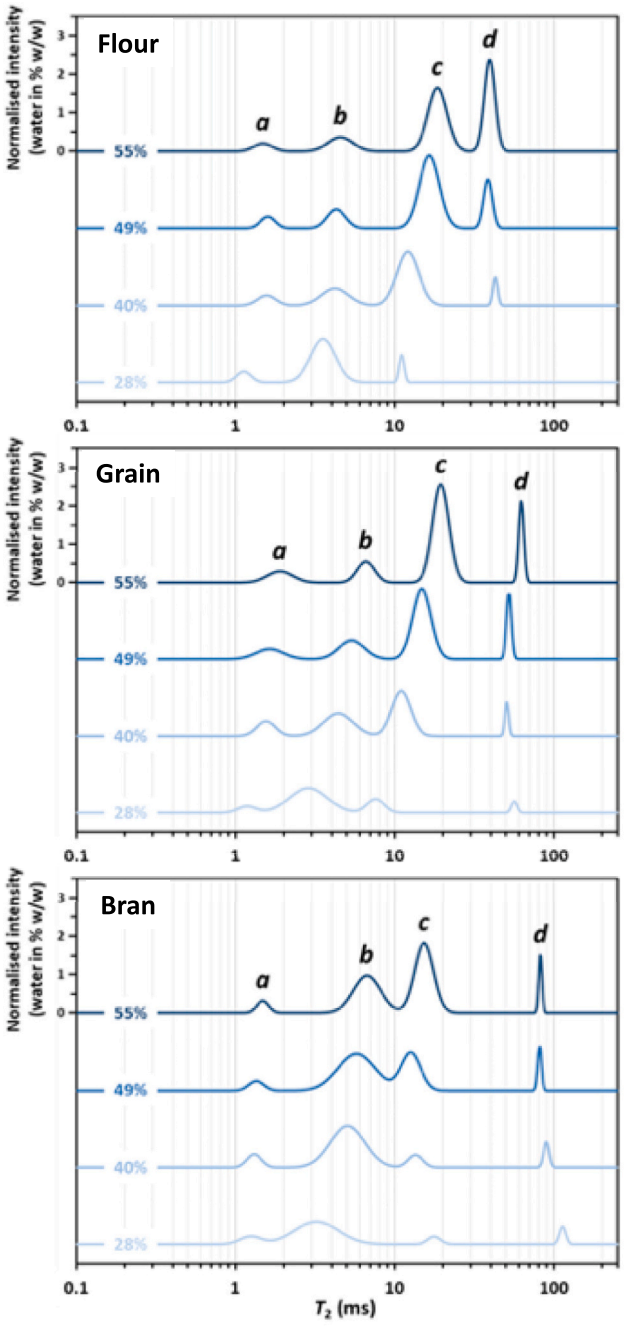

Fig. 3. T_2 water distributions characterized by time-domain relaxometry of flour, ground grain flour and bran at various water contents from 28 % (lightest blue line) to 55 % (darkest blue line). The letters *a*, *b*, *c*, *d* define the different water peaks having different T_{2i} relaxation times from the shortest ($i = a$, less mobile water) to the longest ($i = d$, more mobile water). Distributions are representative of triplicate samples.

size around 15–20 nm (Barron et al., 2021) whatever the water content. Different behaviours of T_{2i} and P_{2i} were observed for flour, grain and bran from 28 % to 55 % hydration. At 28 % hydration, except the lack of the "*d*" component for flour and the longest T_{2d} value for bran (≈ 110 ms, Table 5), relaxation distribution was very similar for the three fractions and dominated by the "*b*" peak ($T_{2b} \approx 3.2$ ms, $P_{2b} \approx 21$ %). Above 28 % hydration, the shortest relaxation component "*a*" exhibited nearly the same mobility for all samples ($T_{2a} \approx 1.5$ ms), indicating that the physical–chemical environment associated with the highest constrained water molecules was relatively unaffected in the range of 40–55 % moisture contents. However, grain differed by its proportion P_{2a} almost twice as high (≈ 5.7 %) as for flour and bran (≈ 3.2 %). For water contents >28 %, peak "*c*" became the main water component in grain and flour but not in bran still dominated by the less mobile component "*b*" until 50 %. T_{2b} values were fairly stable for flour on the range of hydration level, while they clearly increased for grain and bran. Finally, the most mobile component "*d*", whose population P_{2d} clearly increased with hydration level (except in bran), exhibited quite different values and pattern of evolution in flour and grain. Peak "*d*" was absent in flour at the lower water content (28 %) and shifted towards higher or lower water mobility modes, which may reflect a swelling variability of the particles in bran, flour and grain. However, considering the relaxation time of pure water at the working temperature ($T_{2w} \approx 4$ s at 40 °C), the T_{2d} values observed, ranging from 40 to 100 ms, indicated a restricted mobility, especially in flour.

Flour and grain mainly contain starch and a substantial amount of storage proteins (gluten) while bran mainly consists of cell wall components, particularly AX, with minimal amount of starch. Despite the huge difference in composition and in particle size between flour and grain on the one hand, and bran on the other hand, all samples exhibited four water peaks of similar domains of mobility were observed for all samples. As for any porous particle systems, components "*a*" of the T_2 distribution is expected to be dominated by water from the hydration shell of macromolecules and by water whose mobility is strongly limited in the small pores, typically 4–5.0 nm (Barron et al., 2021), therefore poorly accessible to enzymes. The "*b*" component is most likely associated with water in strong interaction with the starch and proteins in flour and grain, but also with other flour components such as cell wall AX especially in grain (Dufour et al., 2023). Conversely, "*a*" and "*b*" components in bran are most likely tightly interacting with the cell wall. Interestingly the "*b*" component in bran remained the major one up to 50 % hydration and was still high at 55 % water content, compared to flour and grain. In other words, in bran most of the water was associated to environment poorly accessible to enzymes. This has to be related to the micro- and nano-porosity present in bran, the former resulting from the hollow cells of the pericarp and the spacing between cell layers, and the latter being due to the thick walls of bran cells (Jacobs et al., 2015). In these tissues, water binding would take place at the nano- or even molecular scale. As a result, enzyme diffusion and consequently AX degradation in bran would be limited, distinctly from the relatively more favorable conditions in grain. Conversely, the main water populations "*c*" in flour and grain, relaxing within the 10–20 ms T_2 relaxation time domain, belonged to water that interacted with matrix components in larger pores, which are likely more accessible to enzymes. "*c* like" component reported in previous studies (Bosmans et al., 2012; Parenti et al., 2021) has been attributed to the water associated with the gluten network. However, in the present study the conditions of mixing and hydration may have limited the formation of a fully developed gluten network. As reported in previous papers on highly hydrated dough system (50–60 %) using ground grain or white flour incorporating milling fractions (Parenti et al., 2021) the relaxation times of the water population in grain and flour were not significantly different, except for "*d*" component, which was less mobile in flour than in grain.

The effect of the enzymes on water distribution in flour, grain and bran was studied at the two extremes water content of 28 % and 55 %. The evolution of relaxation parameters T_{2i} and $P_{2i}$are displayed on Fig. 5 and their values are gathered in Table 6.

At the lowest water content 28 %, the two enzymes did not affect the same water populations than at 55 %. In flour, the addition of enzymes resulted in an increase in the less mobile "*a*" component, and this trend was even more pronounced in bran. In grain the two enzymes had opposite effects: while XAa had similar impact as in flour and bran, leading to an increase of the less mobile "a" component, XBs shifted the major water population towards more mobile water (component "*c*"). A water content of 28 % was clearly limiting for the complete hydration of the macromolecular components of grain, flour and bran. Nevertheless,

Table 5
T_{2i} relaxation times and associated populations P_{2i} for white flour (Flour), whole grain (Grain) and bran at various water contents from 28 % to 55 %. The letters *a*, *b*, *c* and *d* denote the "*i*" water component having T_{2i} relaxation time value, from the shortest (*a*) to the longest (*d*). Values in parentheses are standard errors; n = 3.

		T_{2i} (ms)				P_{2i} (% w/w)			
Sample	Water content	T_{2a}	T_{2b}	T_{2c}	T_{2d}	P_{2a}	P_{2b}	P_{2c}	P_{2d}
Flour	28%	1.2 (0.1)	3.5 (0.1)	11.8 (2.2)	-	3.5 (0.4)	22.0 (0.7)	2.6 (0.9)	-
	40%	1.5 (0.1)	4.0 (0.2)	11.9 (0.1)	42.6 (0.5)	3.3 (0.6)	9.5 (0.2)	24.5 (0.6)	2.6 (0.1)
	49%	1.5 (0.1)	4.2 (0.1)	16.3 (0.2)	38.1 (0.4)	3.3 (0.1)	7.4 (0.3)	29.7 (0.4)	8.2 (0.6)
	55%	1.4 (0.2)	4.3 (0.4)	18.7 (2.4)	38.9 (2.6)	2.7 (0.5)	6.7 (1.2)	24.3 (1.3)	21.3 (1.1)
Grain	28%	1.1 (0.1)	2.9 (0.1)	8.1 (0.6)	58.1 (4.4)	2.3 (0.2)	21.3 (1.8)	3.1 (1.6)	1.3 (0.1)
	40%	1.5 (0.1)	4.4 (0.1)	10.9 (0.2)	50.0 (0.2)	5.8 (0.3)	13.8 (0.1)	17.6 (0.4)	2.8 (0.1)
	49%	1.7 (0.1)	5.4 (0.3)	14.9 (0.2)	52.4 (0.5)	5.6 (0.5)	9.6 (0.1)	26.5 (0.2)	6.9 (0.2)
	55%	1.8 (0.1)	6.6 (0.2)	19.7 (0.4)	63.0 (3.0)	5.6 (0.2)	7.8 (0.4)	33.0 (0.2)	8.5 (0.3)
Bran	28%	1,2 (0.2)	3.1 (0.2)	17.9 (0.8)	113.4 (3.1)	3.4 (1.2)	20.3 (1.2)	2.0 (0.1)	2.3 (0.1)
	40%	1,2 (0.1)	4.6 (0.5)	13.4 (2.4)	89.6 (5.4)	3.5 (0.4)	29.7 (1.0)	3.8 (1.0)	3.0 (0.2)
	49%	1,3 (0.0)	5.6 (0.2)	12.2 (0.4)	80.2 (1.1)	3.1 (0.2)	25.5 (2.0)	16.4 (1.9)	3.6 (0.1)
	55%	1,5 (0.1)	6.6 (0.1)	15.3 (0.2)	82.2 (0.6)	3.0 (0.2)	22.0 (1.1)	25.8 (1.4)	4.2 (0.2)

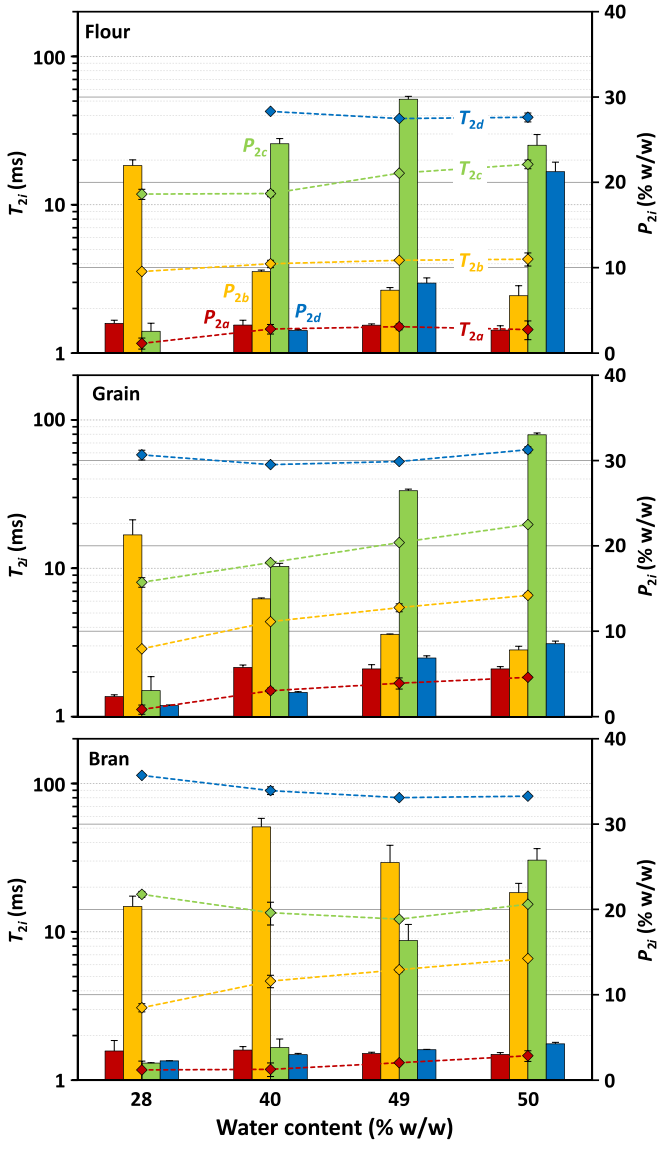

Fig. 4. T_2 time domain-NMR results of the different wheat fractions at 4 hydration levels. T_{2i} relaxation times (left logarithmic y-axis, diamonds) and associated populations P_{2i} (right y-axis, bin categories) of water for white flour (Flour); ground grain flour (Grain) and Bran at various water contents from 28 % to 55 %. The letters *a*, *b*, *c* and *d* define the different water components having different T_{2i} values, from the shortest (*a*, red) to the longest (*d*, blue). Profiles are representative of triplicate samples.

xylanases were able to work and allowed a release of the water bound to AX to the other grain components e.g. starch and proteins for flour and grain and cellulose for bran. Combined with the lower enzyme efficacy for AX extraction observed at a water content of 28 % for the two enzymes, it is likely that the water constraints observed limited enzyme action, especially in bran. At the higher water content (55 %) in flour both enzymes induced a redistribution of the water population "*d*" towards less mobile "c" population in a similar way. Again, the two enzymes had quite limited and fairly similar effects on the water distribution and constraints in grain. The most obvious change was observed for the most mobile component "*d*", whose proportion was reduced by XAa, while its mobility was increased. Conversely, no significant change was observed for the other T_2 components. Finally, the action of both enzymes in bran resulted in a limited redistribution of water. At a water content of 55 %, water is clearly not a limiting factor for enzyme action, and the enzymatic action on cell wall AX in flour, grain and bran only affected the proportion of the most mobile water populations available for enzyme diffusion and reactivity.

The action of *Bacillus subtilis* xylanase (Grindamyl) and *Aspergillus aculeatus* xylanase (Shearzyme) has been previously investigated for water contents between 34 % and 45 % in a wheat-based dough system (Hardt et al., 2014). The action of the enzymes mainly resulted in the release of water that was found to be quite similar for the two enzymes despite their different selectivity. In the present work, XBs and XAa have different effects on water redistribution at low water content, especially in grain. This difference is possibly related to distinct impacts of the physicochemical features of the matrix (flour vs dough) and AX, due to the formation of the visco-elastic gluten network in the dough system.

The relationship between reduced cellulose hydrolysis and biomass-water interaction was previously demonstrated on pretreated wheat straw (Weiss et al., 2019). Measurements of biomass-water interactions were even used for predicting wheat straw recalcitrance after different pre-treatments (Thomsen et al., 2020). In this later study, NMR relaxometry suggested that the most constrained pool of water may interact with hemicelluloses. This is in agreements with our results on wheat bran, that has a high AX content and where, up to 50 % water content, 60–80 % of the water was highly bound to the matrix (water components "a" and "b"). Conversely, the sum of populations associated to components "c" and "d" was the highest in flour (Table 5), where the enzymatic degradation was the most efficient (Fig. 1a). Moreover, their relaxation time was in a mobility domain compatible with pore size between 7.5 and 15 nm, that can favor the enzyme mobility (Barron et al., 2021). Thus, the more mobile water fractions "*c*" and "*d*" could be considered as critical for enzyme diffusion in the cell wall network.

4. Conclusion

In this work, a GH10 and a GH11 *endo*-xylanases of fungal and

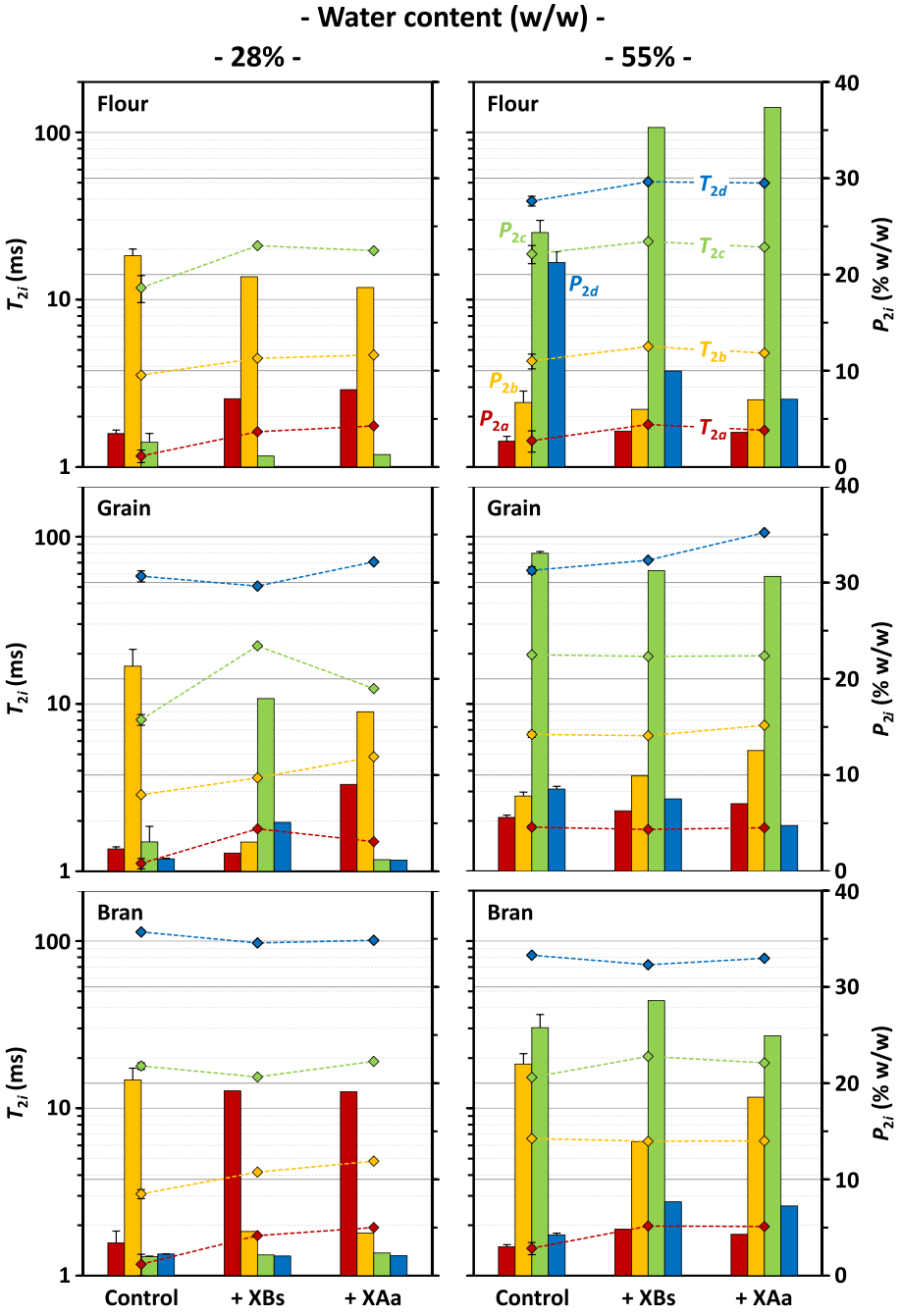

Fig. 5. T_2 time domain-NMR results of the different wheat samples at 2 hydration levels and in the presence or not of the two xylanases. T_{2i} relaxation times (left logarithmic y-axis, diamonds) and associated populations P_{2i} (right y-axis, bin categories) of water for flour, ground grain flour and bran at two water contents, 28 % and 55 %, in the absence of enzyme (control) and in the presence of enzymes XBs or XAa. The letters *a*, *b*, *c* and *d* define the different water components having different T_{2i} values, from the shortest (*a*, red) to the longest (*d*, blue). Profiles are representative of triplicate samples.

bacterial origins, respectively, were compared in their ability to degrade AX in wheat grain flour and bran at high solid concentrations up to 72 %.

Water distribution in wheat fractions has major effects on xylanase action. In this respect bran, ground grain and flour exhibit markedly different water distributions at similar water content. Interestingly, there is no major changes in water distribution above a water content of 50 %. The efficacy of both enzymes was negatively affected when the water content decreased below 50 %, which also corresponded to a decrease in the proportion of the more mobile water pools across all fractions, especially in bran. Moreover, the enzymes still displayed activity at water content as low as 30 % in these matrices, whatever their GH families. In all cases, the bacterial GH11 xylanase was more efficient than the fungal GH10. Its smaller size probably contributes to this greater efficiency.

In validation of the initial hypothesis, it becomes evident that water mobility is a pivotal factor governing enzyme action, particularly underscored in bran where it is related to particle porosity. Furthermore, AX in the most outer layers of the grain are resistant to xylanase degradation due to their structural complexity, which contributes to the low solubilisation rate of AX observed for bran when compared to grain or flour.

The potential for xylose solubilisation by xylanases at low hydration offers promising prospects of its applications in low hydration processing systems. Our results pave the way for optimizing the enzymatic

Table 6
T_{2i} relaxation times and associated populations P_{2i} for white flour (Flour), whole grain flour (Grain) and wheat bran (Bran) at two water contents of 28% and 55%. The letters *a*, *b*, *c* and *d* denote the "*i*" water component having T_{2i} relaxation time value, from the shortest (*a*) to the longest (*d*).

			T_{2i} (ms)				P_{2i} (% w/w)			
Enzyme	Sample	Water content (%)	T_{2a}	T_{2b}	T_{2c}	T_{2d}	P_{2a}	P_{2b}	P_{2c}	P_{2d}
Control	Flour	28	1.2	3.5	12	–	3.5	22.0	2.6	–
		55	1.4	4.3	19	39	2.7	6.7	24.3	21.3
	Grain	28	1.1	2.9	8	58	2.3	21.3	3.1	1.3
		55	1.8	6.6	20	63	5.6	7.8	33.0	8.5
	Bran	28	1,2	3.1	18	113	3.4	20.3	2.0	2.3
		55	1,5	6.6	15	82	3.0	22.0	25.8	4.2
XBs	Flour	28	1.6	4.5	21	–	7.1	19.8	1.1	–
		55	1.8	5.3	22	51	3.7	6.0	35.3	10.0
	Grain	28	1.8	3.6	22	51	1.9	3.1	18.0	5.1
		55	1.8	6.5	19	72	6.3	9.9	31.0	7.5
	Bran	28	1.7	4.2	15	97	19.2	4.6	2.2	2.1
		55	2.0	6.4	20	72	4.8	13.9	28.6	7.7
XTv	Flour	28	1.8	4.7	20	–	8.0	18.7	1.3	–
		55	1.7	4.8	21	50	3.6	7.0	37.4	7.0
	Grain	28	1.5	4.8	12	71	9.0	16.6	1.2	1.2
		55	1.8	7.5	19	106	7.0	12.5	30.6	4.8
	Bran	28	1.9	4.8	19	101	19.1	4.4	2.4	2.1
		55	2.0	6.4	19	79	4.3	18.5	24.9	7.3

degradation steps in such processes. Such utilization not only underscores energy efficiency but also aligns with environmentally conscious practices by reducing water consumption. Further research is needed to investigate how water- and polymer-mobility affect the enzyme action in matrices with variable rheological properties and diverse structural features. The role of polymers structure and their interaction in governing the water and polymer mobility need to be part of research explorations.

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CRediT authorship contribution statement

Allah Rakha: Writing – original draft, Investigation, Funding acquisition. **Loïc Foucat:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis. **Luc Saulnier:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Estelle Bonnin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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