

Arabinofuranosidases: the revolution to boost the efficacy of xylanases

Arabinofuranosidases appeared to be a revolution in the efficacy of xylanases to break down hemicellulose in plants. They allow to boost the total feed digestibility from energy to protein. To improve the expression of those targeted enzymes, a new method was developed.

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Carbohydrates represent the main source of dietary energy in animal feeds. They are essentially provided by cereals or cereal by-products. However, besides starch, the main energy-rich carbohydrate, cereals contain non-starch polysaccharides (NSP), which are not readily digested within the small intestine of birds and pigs due to the absence of the required enzyme activities and thus interfere with feed digestibility and intestinal physiology.

Arabinoxylans, main NSP in cereals

NSP are $\beta(\alpha)$ polymers, present mainly in the cell walls of the endosperm but also in the bran. They include cellulose, hemicelluloses and pectin and can also be divided according to their nutritional value into water-soluble and water-insoluble fractions (Bach Knudsen, 2014). Soluble NSP are known to possess anti-nutritional properties by either encapsulating nutrients and/or depressing overall nutrient digestibility via modifications within the gastro-intestinal tract.

Among the cereals, corn and wheat grains are the mostly used. The cell walls of the outer tissues of the kernel mainly contain cellulose and complex xylans, together with significant amount of lignin. Conversely in the endosperm, cell walls contain almost exclusively arabinoxylans (AX) and β -glucans. Arabinoxylan is composed of a β -(1-4)-linked xylose backbone carrying arabinose residues at the C(O)2 and/or C(O)3 positions of the xylose residues. Arabinose residues might even be substituted by ferulic acid or even more complex side chains including (feruloylated) arabinofuranose, xylose, galactose and α -glucuronic acid. The arabinose:xylose ratio and the content of no-, mono and di-substituted xylose are variable between cereal grains and also between the different tissues

within the same grain. The heteroxylans of corn are more substituted (80%) than those of wheat (70%) and contain more glucuronic acid (8.3% vs 2.6% dry matter). The most substituted soluble AX is found in rice with an arabinose:xylose ratio from 2.8 to 5.6. This complexity will affect the susceptibility to exogenous enzymes. Even if a high degree of substitution preclude from any viscous issue, breaking down such structures will improve feed digestibility.

Xylanases and arabinofuranosidases, diversity and complementarity to be more efficient

Polysaccharides can be degraded by hydrolytic enzymes which are part of a larger group called Carbohydrate Active enZymes (CAZymes – www.cazy.org). They have been classified into 5 groups: glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and finally auxiliary activities (AA), according to their functional domains and catalytic modules. They are involved in the degradation of the different polymers present in the plant cell wall.

The GH group is further subdivided into 133 families, based on the amino-acid sequence similarity of the enzymes. Affinity to specific substrate conformations can vary from one group to another such as in xylanases from GH 10 or 11 families.

When considering endo-1,4- β -xylanase, this term covers a range of proteins with different molecular sizes, and different specificities toward xylan-like substrates. Indeed, the various endoxylanases identified in *Talaromyces versatilis* differ in their molecular size, their optimum pH and their selectivity or affinity to insoluble or soluble substrates. Among the different endoxylanases from *Talaromyces versatilis*, Xyn D belongs to the GH 10 while XynB and XynC belong to GH 11 families.




Liaison type	Chemical structure	Abf 1	Abf 2	Abf 3	Abf 4
C(O)2		+++	+++	+++	+++
C(O)3		++	++	+	-
C(O)2 & C(O)3		-	-	++	+++

Figure 1: A range of Abf is required to breakdown all types of xylose substitution (Abf 1 & 2 from family GH 62; Abf 3 & 4 from family GH 54).

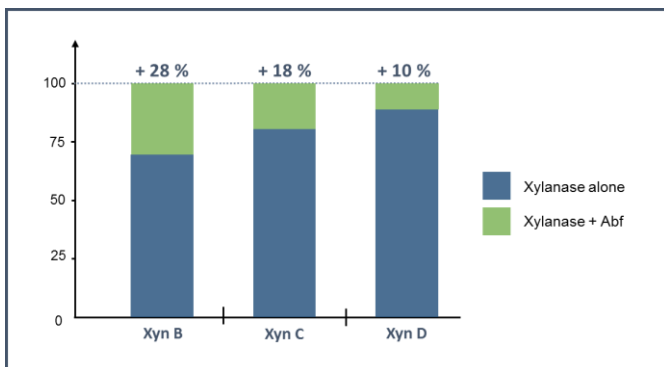


Figure 2: Complementary effect of arabino-furanosidase (Abf from family GH 51) and endo-xylanases (Xyn B, C and D from *Talaromyces versatilis*) as measured by viscometric method.

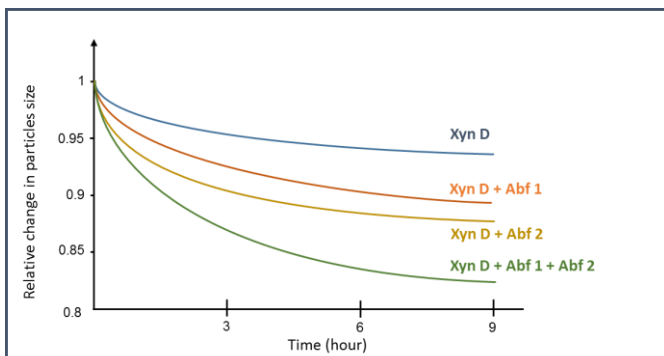


Figure 3: Effect of xylanase alone or combined with different Abf to breakdown arabinoxylan as measured by the reduction of particle size (Abf 1 from family GH54; Abf 2 from family GH62).

Due to their narrow specificity towards one linkage, different enzymes are required for the degradation of the arabinoxylans. While endoxylanases hydrolyze the xylose backbone, their activity is frequently hampered by the substitution with arabinose residues. Indeed to be efficient, most of the endo-1,4-b-xylanases need a long enough stretch of unsubstituted xyloses on the xylan backbone (Lafond et al., 2014). α -L arabinofuranosidases (ABF) are Glycosyl Hydrolases able to cleave arabinose from the backbone. Therefore, they are an important actor of the hydrolytic system to degrade hemicelluloses

such as arabinoxylans, arabinanans and arabinogalactans. Belonging to different GH families GH43, 51, 54, 62,... are able to release arabinose from mono or di-substituted xyloses in oligo or polysaccharides (Figure 1, De La Mare et al., 2015). Moreover, the main characteristic of ABF is to have a lower optimal pH than endo-xylanases. The direct consequence in the digestive tract is that the debranching action occurs in the upper part (stomach) while the endo-xylanases will act further down along the digestive tract.

Arabifuranosidases: the revolution in the efficacy of xylanases

To improve the efficacy of *Talaromyces versatilis* enzyme-based product, an original molecular biotechnology approach has recently been developed (Guais et al., 2015). The basis of the method was not to express one particular arabinoxylan degrading enzyme but rather to over-express a transcription factor (XlnR) allowing stimulating a serie of GH genes resulting in an improved expression of various new xylanases as well as new arabinofuranosidases.

Figure 2 shows the boosting effect of removing the substitution with the ABF to degrade arabinoxylans as measured by the viscometric method. Indeed, whatever the single xylanase obtained from *Talaromyces versatilis* (Xyn B, C or D), the complementation with even only one ABF (Abf 51a) improves the xylanase activity. Adding multiple arabinofuranosidases to one xylanase enhances insoluble arabinoxylan particle degradation as shown in Figure 3 thanks to a toric reactor and a CCD camera. The best results are obtained with a combination of two arabinofuranosidases to the xylanase D (Figure 3).

Conclusion

The complexity of NSP and their great variety in common feedstuffs used in poultry and swine nutrition demonstrates the need for a large range of enzyme activities in order to alleviate most of their anti-nutritional effects and to get the full availability of their nutrient contents.

The higher substitution of corn arabinoxylans further requires those combinations to get full access to the xylose backbone. To have an efficient action *in vivo*, a large spectrum of different xylanases is required to function all along the digestive tract as well a range of arabinofuranosidases to be able to efficiently break down the most common feedstuff: corn.

References are available on request.