PROTEOMICS OF MULTIGRAIN™

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Proteomics is defined as a broad-based analysis of proteins within a proteome in a biological system. A proteome is a set of proteins produced by an organism, cell or tissues at a certain time – or, a proteome is those proteins common within a defined system. And since enzymes are proteins, proteomics can be used to identify enzymes produced by individual bacteria or fungi.

This assay accomplishes two goals. First, we can identify the proteins present, and second, we can quantitate their abundance. Liquid chromatography mass spectrometry-based proteomics has proteome analysis for MultiGrain, an enzyme composite produced by *T. reesei*. This fungal source of MultiGrain was previously recognized as an exceptional producer of carbohydrases that synergistically degrade fiber (Herpoel-Gimbert et al., 2008). A total of 22 different protein species or enzymes was revealed with two-dimensional electrophoresis that included cellulases, xylanases and become a routine method for this purpose (Karpievitch et al., 2010).

Using this technology, Novozymes recently completed the debranching enzymes (Herpoel-Gimbert et al., 2008).

This process allows us to predict which non-starch polysaccharides (NSPs) are most likely to be degraded, or the ingredient for which this group of enzymes could most effectively improve. In a nutshell, proteomics gives a comprehensive insight into the enzyme degradation potential of MultiGrain and guides us toward ingredients this group of enzymes best serves.

Key Enzymes Found in MultiGrain

This proteomics analysis revealed the existence of at least 26 different carbohydrases present in MultiGrain (Table 1), some of which are highlighted below.

- ***β-1,4-endoxylanase*** – Often referred to as xylanase, this enzyme attacks the most abundant NSP in corn – arabinoxylan. There are six different xylanases identified in MultiGrain. Although proteomics does not characterize the pH optima, activity, etc., of the different xylanases, it is likely they differ in these characteristics. Consequently, several different xylanases improve the opportunity for substrate degradation as the feed travels down the intestinal tract facing different pH and conditions in each section.

- ***α-1,4-galactosidase, acetyl xylan esterase, and α-arabinofuranosidase.*** These debranching enzymes are essential to cleave structures found attached primarily to arabinoxylan. Their presence can expedite the degradation of arabinoxylan. Each of the three is responsible to remove specific structures from NSPs.

- ***β-1,4-endoglucanase (cellulase), cellulohydrolase and β-1,4-glucosidase*** – All three enzymes work synergistically to dismantle cellulose (Watanabe and Tokuda, 2001), the 2nd most abundant NSP in corn. Each acts upon certain bonds to ultimately dismantle cellulose into individual glucose units.

- ***β-1,3(4)-endoglucanase*** – Usually referred to as β-glucanase. β-Glucans comprise a group of β-glucose polysaccharides present in the cell walls of cereals and is one of the more predominant NSPs in corn. β-glucans contain both β-1,3 and β-1,4 backbone bonds.

- ***β-1,4-endomannanase*** – Galactomannan, the substrate for this enzyme, is an endosperm polysaccharide found in most leguminous seeds.

- ***Glutamic proteases*** – This protease falls within a distinct group of peptidases thought to be found only in fungi. Named because of the presence of glutamic acid in the active site, they function best at an acidic pH.

Table 1. Proteomic Summary of MultiGrain of Key Enzyme Composition[a]

Enzyme	Substrate
β-1,4-endoxylanase[b]	Arabinoxylan
Debranching enzymes • α-1,4-galactosidase • acetyl xylan esterase • α-arabinofuranosidase	• Removes galactose on polysaccharides • Removes acetyl groups • Removes arabinose groups
β-1,4-endoglucanase (cellulase) **Cellulohydrolase** **β-1,4-glucosidase**	Synergistic degradation of cellulose
β-1,3(4)-endoglucanase	β-glucans
β-1,4-endomannanase	Galactomannan
Glutamic proteases	Proteins

[a]In several cases, multiple species of the same enzyme were found providing 26 different enzymes
[b]Six unique β-1,4-endoxylanases identified

What This Means

Celluloses, hemicelluloses and pectins are the three main categories of plant NSPs that comprise 88% to 90% of cell walls (Caprita et al., 2010). These NSPs are tightly interwoven with various sugar residues, crosslinks, and branches that encumber the main structure – especially for the hemicelluloses and pectins (Brink and Vries, 2011). Their presence consequently generates a configuration highly resilient to enzyme attack. Thus, several different carbohydrases are necessary to take part in this deconstruction process of NSPs in feed ingredients.

Consider corn, which is recognized as the most complicated of cereal grains for this reason (Knudsen, 1997; Pedersen et al., 2015). The primary NSP in corn is arabinoxylan, whose main structure is comprised of individual xyloses linked linearly to one another. By itself, this xylan chain is a simple structure and easily serves as the substrate for xylanase.

But in corn, the arabinoxylan is far more complicated. Numerous attachments are present that include galactose, xylose, arabinose, and glucuronic acid and others. These attachments and crosslinks confer significant resistance to pure xylanase, providing very few regions on the arabinoxylan where several contiguous xyloses are unsubstituted and accessible to attack (Biely et al., 2016; Agger and Meyer, 2011; Knudsen, 2019). Current feed grade xylanases are comprised of the GH 11 family and require at least four adjacent xyloses to be free of any attachments before breaking this xylan chain (Goesaert et al., 2011).

Conclusion

Proteomic analysis of MultiGrain reveals a wide range of carbohydrases essential to dismantle key NSPs in corn and other cereals, including the important debranching enzymes. MultiGrain offers opportunities to improve the nutritional value of corn.

References available upon request.

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