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**Feruloylated Arabinoxylan and
 Oligosaccharides: Chemistry,
 Nutritional Functions, and
 Options for Enzymatic
 Modification**

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cereal bran, arabinoxylan, bioactivity, enzymatic modification,
 endo-1,4- β -xylanase

Abstract

Cereal brans and grain endosperm cell walls are key dietary sources of different types of arabinoxylan. Arabinoxylan is the main group of hemicellulosic polysaccharides that are present in the cell walls of monocot grass crops and hence in cereal grains. The arabinoxylan polysaccharides consist of a backbone of β -(1 $\rightarrow$ 4)-linked xylopyranosyl residues, which carry arabinofuranosyl moieties, hence the term arabinoxylan. Moreover, the xylopyranosyl residues can be acetylated or substituted by 4-*O*-methyl-D-glucuronic acid. The arabinofuranosyls may be esterified with a feruloyl group. Feruloylated arabinoxyloligosaccharides exert beneficial bioactivities via prebiotic, immunomodulatory, and/or antioxidant effects. New knowledge on microbial enzymes that catalyze specific structural modifications of arabinoxylans can help us understand how these complex fibers are converted in the gut and provide a foundation for the production of feruloylated arabinoxyloligosaccharides from brans or other cereal grain processing sidestreams as functional food ingredients. There is a gap between the structural knowledge, bioactivity data, and enzymology insight. Our goal with this review is to present an overview of the structures and bioactivities of feruloylated arabinoxyloligosaccharides and review the enzyme reactions that catalyze specific changes in differentially substituted arabinoxylans.

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INTRODUCTION

Arabinoxylans are the dominant hemicellulosic polysaccharides present in the plant cell walls of all main cereal food grains. Because the cell walls are thicker in the cereal bran fraction than in the endosperm, arabinoxylans notably accumulate in the cereal brans. The contents of arabinoxylans in cereal brans depend on the cereal type and range from ~5–25% (weight/weight). The arabinoxylan thus typically constitutes approximately 30% of the total dietary fiber, as there is 24% arabinoxylan in corn bran, 10–16% in rye, barley, and wheat bran, 7% in rice bran, and 5% in oat bran (Wang et al. 2020). In accordance with the current nutritional emphasis on high dietary fiber intake and whole-grain products, also manifesting via the emergence of new whole-grain versions of bread and pasta, the significance of food arabinoxylans and arabinoxylan-oligosaccharides on health remains profound.

Arabinoxylan molecules consist of a linear backbone of β -(1→4)-linked xylose residues [xylopyranosyl form (Xylp)], which can be mono- or disubstituted with arabinose moieties [arabinofuranosyl (Araf)] at the O2 and/or O3 position and/or carry acetyl groups or 4-*O*-methyl-D-glucuronic acid (MeGlcA) at these positions (Mendis et al. 2016). The structural complexity of arabinoxylans with regard to substitutions varies widely and notably depends on the source; the arabinofuranosyl:xylopyranosyl ratio in different types of cereal brans thus ranges from ~0.5–1.2, is highest in corn bran, and is lower in endosperm cell walls than in bran (Munk et al. 2020, Niño-Medina et al. 2010, Wang et al. 2020).

An important aspect of the chemical structure of arabinoxylans is that some of the Araf residues may be esterified with a hydroxycinnamate at the O5 position, usually, i.e., in more than 90% of the cases, the esterification is to a *trans*-feruloyl (ferulic acid) group, hence the term feruloylated arabinoxylans, which are the focus of this review. This possible O5 feruloyl acylation of the Araf substituents is a key, unique feature of arabinoxylans; structural details are discussed in the next section. The feruloyl esterifications on cereal arabinoxylans are mainly on the α -(1→3)-linked Araf residues. In fact, interestingly, there appear to be no reports of feruloyl substitutions on O2-linked Araf substituents in arabinoxylans. The highest feruloyl levels in brans are 2.6–3.3% in corn bran, 0.5–1.5% in wheat bran, and 0.3% in rye bran (Zhao & Moghadasian 2008). This means that, notably, corn bran, wheat bran, and whole corn and wheat products are particularly important natural sources of dietary feruloylated arabinoxylans. Although the feruloyl substitutions constitute a maximum of 10% of the arabinoxylan, they impact the physical and bioactivity properties of the cell walls and the arabinoxylans and are hypothesized to partially hinder enzymatic depolymerization of the arabinoxylans. As a particularly unique feature, a portion, approximately 5–50%, of the feruloyl groups may form different types of covalent dimers, i.e., diferuloyls, that function to cross-link, stabilize, and strengthen the arabinoxylan chains in the plant cell walls (**Figure 1**).

As already hinted, the arabinoxylans from corn bran are particularly highly substituted and structurally complex and are the most highly cross-linked. For instance, the Xylp residues in corn bran are highly disubstituted with both α -(1→2)-linked and α -(1→3)-linked Araf residues, partially substituted by α -(1→2)-linked MeGlcA, and may carry either L- or D-galactosyls and be acetylated at the O2 and/or O3 position (Agger et al. 2010, Appeldoorn et al. 2013, Wang et al. 2020). Lastly, for the sake of completion, it should be noted that some of the Araf residues in corn bran are substituted with an additional Xylp and/or a galactosyl residue (Appeldoorn et al. 2013, Saulnier et al. 1995).

A growing body of evidence suggests that feruloylated arabinoxylo-oligosaccharides (FAXOs) exert important gut functional prebiotic, antioxidant, and immuno-stimulating effects. Furthermore, new data show that FAXOs extracted from corn bran attenuate diabetes in rats—most likely by modulating the composition and metabolism of the gut microbiome (Song et al. 2020). In this review, data on nutrifunctional effects of FAXOs are systematically examined. Recent progress has

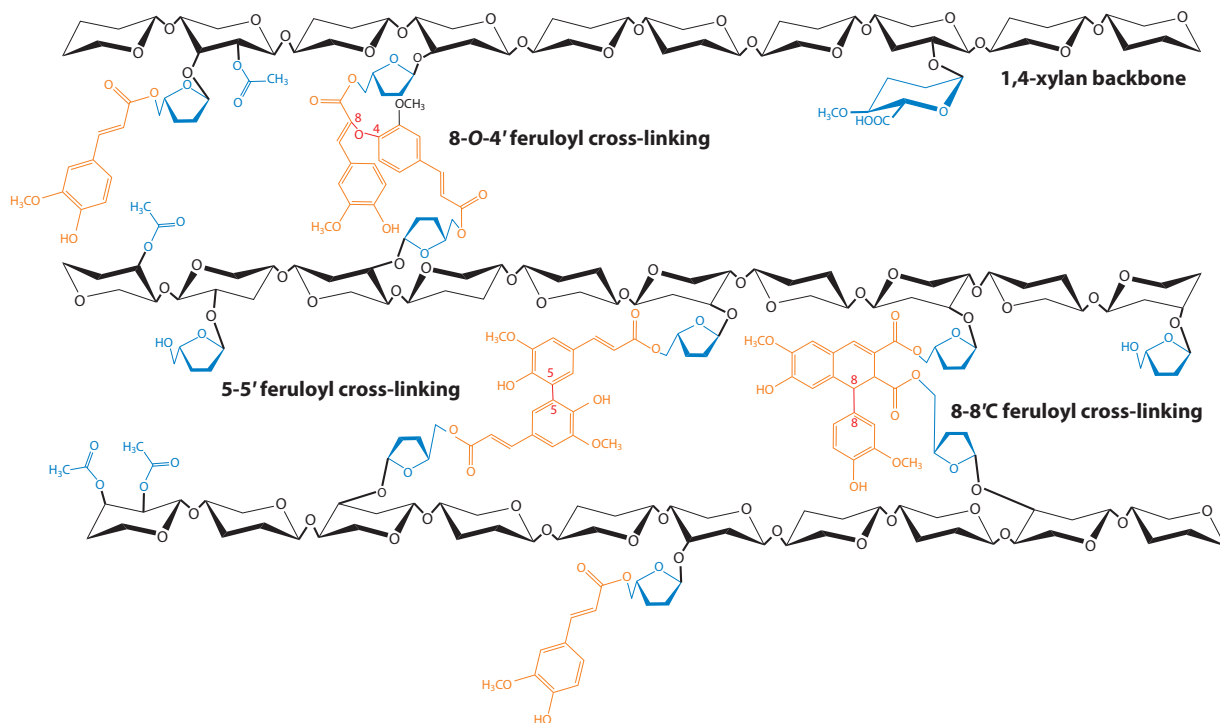


Figure 1

Stylized structural sketch of feruloyl-substituted and feruloyl dimer cross-linked arabinoxylan.

also advanced our understanding of how various microbial endo-1,4- β -xylanases [Enzyme Commission (EC) number: 3.2.1.8] can catalyze degradation of differently substituted and feruloylated arabinoxylans. We outline the enzyme reaction details focusing on the molecular substrate attack sites in arabinoxylans and the oligomer products obtained. The objective of spotlighting the enzymes and enzyme reactions is to provide an expanded foundation on which continued scientific and technological advancements can be made via selective modifications to explore the nutritional and functional significance of dietary feruloylated arabinoxylan and arabinoxylo-oligosaccharides.

STRUCTURAL DETAILS

The simplest and most common type of feruloylated arabinoxylan structure, one found in all dietary bran types, is an oligomeric moiety in which the arabinosyl-feruloyl is linked to a small fragment of 2–3 Xylp residues, i.e., Oligosaccharide 1 [5-*O*-(*trans*-feruloyl)-L-arabinofuranosyl-(1 $\rightarrow$ 3)- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-D-xylan] (Table 1). Obviously, the number of backbone Xylp residues and the molecular weight of FAXOs vary depending on the origin and the method used for extraction and analysis of the FAXOs. A structure having two neighboring feruloylated Araf's has never been reported, but in wheat and wheatgrass, the arabinosyl-feruloyl substitutions may also occur next to Xylp residues that are substituted by arabinosyls on either the O2 or O3 position of the Xylp residues, i.e., Oligosaccharides 2 and 3 (Table 1) (Schendel et al. 2015, 2016b).

Moreover, recent work by Schendel et al. (2016a) has emphasized the common occurrence in cereal arabinoxylans of structural elements in which the feruloylated Araf is attached at the C1 of

Xylp residues at the reducing end, i.e., Oligosaccharide 4 [β -D-xylapyranosyl-(1 $\rightarrow$ 2)-5-*O*-(*trans*-feruloyl)-L-arabinofuranose] (**Table 1**), and has underlined the apparent ubiquitous presence in cereal grains of a galactose-substituted version of Oligosaccharide 4, α -L-galactopyranosyl-(1 $\rightarrow$ 2)- β -D-xylapyranosyl-(1 $\rightarrow$ 2)-5-*O*-*trans*-feruloyl-L-arabinofuranose, a structure that until recently was presumed to be present only in corn bran (Allerdings et al. 2006, Saulnier et al. 1995,

Table 1 Molecular structures and major food sources of feruloylated oligosaccharides

Nomenclature	Molecular structure	Food source	References
Oligosaccharide 1 5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabinofuranosyl-(1 $\rightarrow$ 3)- β -D-xylapyranosyl-(1 $\rightarrow$ 4)-D-xylan		Wheat, rice, rye, corn, oat, barley, millet, bamboo shoot, pineapple fruit	Ishii & Hiroi 1990; Schendel et al. 2015, 2016a,b; Smith & Harris 2001
Oligosaccharide 2 5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabinofuranosyl-(1 $\rightarrow$ 3)-[α -L-arabino-furanosyl (1 $\rightarrow$ 3)]- β -D-xylapyranosyl-(1 $\rightarrow$ 4)-D-xylan		Wheatgrass	Schendel et al. 2015
Oligosaccharide 3 5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabinofuranosyl-(1 $\rightarrow$ 3)-[α -L-arabino-furanosyl (1 $\rightarrow$ 2)]- β -D-xylapyranosyl-(1 $\rightarrow$ 4)-D-xylan		Wheat	Schendel et al. 2016b
Oligosaccharide 4 β -D-xylapyranosyl-(1 $\rightarrow$ 2)-5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabino-furanose		Wheat, rice, rye, spelt, oat, corn, barley, millet	Schendel et al. 2016a

(Continued)

Table 1 (Continued)

Nomenclature	Molecular structure	Food source	References
Oligosaccharide 5 5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabinofuranosyl-(1→3)-(2- <i>O</i> -acetyl)- <i>O</i> -β-D-xylopyranosyl-(1→4)-β-D-xylan		Corn	Arai et al. 2019
Oligosaccharide 6 5- <i>O</i> -(<i>trans</i> -feruloyl)-L-arabinofuranosyl-(1→3)-[3- <i>O</i> -acetyl-xylopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→4)-β-D-xylan		Wheat	Schendel et al. 2016b
Cross-linked FAXOs			
Oligosaccharide 7 8–8C DiFA cross-link		Corn	Bunzel et al. 2008, Saulnier et al. 1999
Oligosaccharide 8 5–5 DiFA cross-link		Bamboo, corn	Allerdings et al. 2005, Ishii 1991
Oligosaccharide 9 8- <i>O</i> -4 DiFA cross-link		Corn	Allerdings et al. 2005, Bunzel et al. 2008

Abbreviation: DiFA, feruloyl dimer; FAXOs, feruloylated arabinoxylo-oligosaccharides.

Schendel et al. 2016a). There are also other variations of galactose-substituted Oligosaccharide 4 (Appeldoorn et al. 2013). Additional acetylation of the O2 or O3 position of the Xylp carrying the feruloylated Ara_f provides additional complex structural FAXO moieties, e.g., Oligosaccharides 5 and 6 (**Table 1**) (Appeldoorn et al. 2013, Arai et al. 2019, Schendel et al. 2016b).

Between 50% and 95% of the feruloyls that are ester-linked to arabinoxylan are in their monomeric form, i.e., single feruloyl moieties (Schendel et al. 2016a), but, as mentioned above, an important and unique aspect is that these feruloyls can form feruloylate dimers (and in corn bran, even trimers and tetramers). The feruloyl dimers (DiFAs) result from enzyme-assisted oxidative coupling reactions and cross-link the arabinoxylan polysaccharides in the plant cell wall (**Figure 1**). The diferulate cross-linking affects cell wall strength and microbial degradability (Mnich et al. 2020). Increased ferulate cross-linking increases viscosity and gelation and can be used to valorize bran and other cereal-processing residues, as forced enzymatic oxidative cross-linking creates strong odorless and taste-neutral hydrogels from solubilized FAXOs in vitro (Munk et al. 2020, Niño-Medina et al. 2010).

In cereals, arabinoxylans are most often cross-linked via 5–5, 8-*O*-4 or 8–8C coupled DiFAs (Oligosaccharide 7–9) (**Figure 1**) (Allerdings et al. 2005, Bunzel et al. 2008, Ishii 1991, Saulnier et al. 1999). Ferulate trimers and tetramers have been identified in corn bran and function to create stronger cross-links of polysaccharide chains than diferulates (Burr & Fry 2009a,b; Mastrangelo et al. 2009).

POTENTIAL NUTRITIONAL FUNCTIONS OF FERULOYLATED ARABINOXYLO-OLIGOSACCHARIDES

Prebiotic Effects

A prebiotic is generally defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” and includes nondigestible carbohydrates and polyols (de Paulo Farias et al. 2019). Prebiotics play a vital role in modulating the microbiota in the gut and may therefore prevent or affect various diseases such as obesity, inflammatory bowel disease, cardiovascular diseases, and metabolic syndrome associated with the gut microbiome function (Han & Xiao 2020). Most prebiotics work by selectively stimulating the growth and activity of beneficial bacteria in the gut and/or simultaneously suppressing the proliferation of harmful bacteria, and via the production of short-chain fatty acids (SCFAs). FAXOs are fermented by gut microbes (**Table 2**) and modulate the gut microbiota composition via selective microbial conversion of the arabinoxyloligosaccharides and presumably also via effects of the ferulic acid released by microbial feruloyl esterase. The prebiotic effects of FAXOs are therefore regarded as resulting from both the arabinoxylan structure (degree and types of substitutions) and the feruloyl moieties, and several recent studies affirm that FAXOs exert prebiotic and potentially gut-beneficial effects (**Table 2**). Some of the more significant results are that corn bran FAXOs supplemented to high-fat diets in rats significantly increase *Bacteroidetes* and decrease *Firmicutes*. At the same time, body weight and amount of adipose tissue are reduced compared with rats on a similar diet without FAXOs (Yang et al. 2016) (**Table 2**). Other studies have confirmed that the ratio of *Firmicutes* to *Bacteroidetes* is reduced—i.e., relative amounts of *Bacteroidetes* increase—in both healthy (Ou et al. 2016) and diabetic rats with the administration of corn bran FAXOs (Song et al. 2020) (**Table 2**).

The results of modulating the ratio of *Bacteroidetes* to *Firmicutes* are important because *Firmicutes* and *Bacteroidetes* constitute the most abundant bacterial phyla in the intestinal microbiome. The balance between them is believed to play a role in obesity; hence, lean body weight is associated with a relatively higher *Bacteroidetes* to *Firmicutes* ratio than that associated with obesity, which is accompanied by a decreased abundance of *Bacteroidetes* and a proportional elevated level

Table 2 Potential nutritional functions of FAXOs from food

Type of feruloyl-arabino-oligos	Food source	Functions	Reference
Prebiotic effects			
Mixture of feruloyl-arabinose and feruloyl-arabinose-1,3-xylose	Corn bran	In vivo: modulate gut microbiota composition in diabetic rats; balance the ratio of obesity-related bacteria; ↑growth of beneficial gut bacteria; ↑production of SCFAs	Song et al. 2020
Mixed FAXOs	Wheat bran	In vitro: ↑fermentation; ↑production of SCFAs; ↑growth of beneficial bacteria; ↓growth of harmful bacteria	Gong et al. 2019
FAXOs and cross-linked FAXOs	Corn bran	In vitro: ↑fermentation; ↑production of SCFAs; ↑growth of butyrogenic gut bacteria	Zhang et al. 2019
Mixed FAXOs	Rice bran	In vitro: ↑fermentation; ↑production of SCFAs; ↑growth of beneficial bacteria; ↓growth of harmful bacteria	Pham et al. 2017
Mixed FAXOs	Corn bran	In vivo: modulate gut microbiota composition of high-fat-diet-fed rats; balance ratio of obesity-related bacteria; ↑production of SCFAs	Yang et al. 2016
Mixture of feruloyl-arabinose and feruloyl-arabinose-1,3-xylose	Corn bran	In vivo: modulate gut microbiota composition of normal rats; ↑growth of beneficial gut bacteria; balance the ratio of obesity-related bacteria	Ou et al. 2016
Mixture of short- and long-chain FAXOs	Wheat bran, corn bran	In vitro: ↑fermentation; ↑production of SCFAs	Yang et al. 2014
Antioxidant activity			
Mixed FAXOs (of avDP 3–11)	Wheat bran	In vitro: ↑ABTS, DPPH, hydroxyl, and superoxide radical scavenging; ↑metal ion chelation	Zhao et al. 2018
Mixed FAXOs	Wheat bran	In vitro: ↑ABTS and DPPH radical scavenging	Ruthes et al. 2017
Mixed FAXOs	Wheat bran	In vivo: ↑antioxidant capacity of AAPH-induced rats	Zhang et al. 2017
FAXOs with avDP 3, A:X of 0.4	Wheat bran	In vitro: ↑antioxidant capacity of AAPH-induced HepG2 cells	Zhang et al. 2016
Mixture of FAXOs and small xylo-oligomers	Wheat bran	In vitro: ↓erythrocytic osmotic fragility and hemolysis (rat); ↓malonic dialdehyde in rat liver homogenate; ↑antioxidant capacity of serum from tumor-bearing rats	Yu et al. 2015
Mixture of small FAXOs	Wheat aleurone	In vitro: ↑DPPH and ABTS radical scavenging; ↑ORAC capacity	Malunga & Beta 2015
Mixed FAXOs	Wheat bran	In vivo: ↑antioxidant capacity in heart, liver, and kidney from normal rats	Zhang et al. 2015
Mixture of feruloyl-arabinose and feruloyl-arabinose-1,3-xylose	Corn bran	In vitro: ↑DPPH radical scavenging; ↑ORAC capacity; ↓oxidative stress in neuronal cells induced by H ₂ O ₂	Yao et al. 2014
Feruloyl-arabinose	Corn bran	In vitro: ↑DPPH and superoxide radical scavenging; ↑metal ion chelation	Q. Lin et al. 2014
Mixed FAXOs and xylo-oligomers	Wheat bran	In vivo: ↑antioxidant capacity of serum from tumor-bearing rats	Yu & Gu 2013
Mixed FAXOs	Wheat bran	In vivo: ↑antioxidant capacity of plasma from AAPH-induced rats	Wang et al. 2010

(Continued)

Table 2 (Continued)

Type of feruloyl-arabino-oligos	Food source	Functions	Reference
Mixed FAXOs	Wheat bran	In vitro: ↓lipid peroxidation; delayed hemolysis of human erythrocytes	Wang et al. 2009a
Mixed FAXOs	Wheat bran	In vivo: ↑antioxidant capacity in alloxan-induced diabetic Sprague-Dawley rats	Ou et al. 2007
Mixed FAXOs	Corn bran	In vitro: ↑DPPH and ABTS radical scavenging; ↓rat erythrocyte hemolysis	Yuan et al. 2005
FAXOs with A:X of 1:3	Wheat flour	In vitro: ↑DPPH radical scavenging and ↑inhibition of LDL oxidation	Katapodis et al. 2003a
Immunomodulatory effects			
Mixture of feruloyl-arabinose and feruloyl-arabinose-1,3-xylose	Corn bran	In vivo: attenuated induced colitis in rats; restored immune response balance via ↓production of proinflammatory cytokines IL-23, IL-6; ↑anti-inflammatory cytokine TGF-β1	Xia et al. 2019
FAXOs and small xylo-oligomers	Wheat bran	In vitro: ↓cancer cell growth In vivo: ↓tumor growth; ↑cytokines IFN-γ and IL-3	Yu et al. 2014
Mixed FAXOs	Rice bran	In vitro: ↑proliferation of T cells; ↑Th1 response; ↑production of proinflammatory cytokines TNF-α, IL-6, IL-10, and IL-12	C. Lin et al. 2014
Mixed FAXOs	Rice bran	In vitro: ↑proinflammatory cytokines TNF-α, IL-1β, IL-6, NO, and PGE ₂ in macrophages; ↑in vitro anti-inflammation by suppressing proinflammatory cytokines and inducing anti-inflammatory cytokine IL-10 in LPS-induced macrophages	Fang et al. 2012
Other effects			
Mixture of feruloyl-arabinose and feruloyl-arabinose-1,3-xylose	Corn bran	In vivo hypoglycemic, hypolipidemic, and antioxidant effects in streptozotocin-induced type 2 diabetic rats	Huang et al. 2018
Mixed FAXOs, feruloyl arabinose	Corn bran/wheat aleurone	In vitro: inhibit activities of mammalian intestinal α-glucosidase and glucose transporters and inhibit uptake of glucose in human Caco-2 cells	Malunga et al. 2016

Abbreviations: AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); avDP, average degree of polymerization; A:X, arabinose to xylose ratio; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FAXOs, feruloylated arabinoxylo-oligosaccharides; LPS, lipopolysaccharide; ORAC, oxygen radical absorbance capacity; SCFAs, short-chain fatty acids.

of *Firmicutes* (Turnbaugh et al. 2006). Recently, three antiobesity and antidiabetic strains of *Bacteroides*, *Faecalibacterium*, and *Bifidobacterium* were shown to increase when FAXOs are administered both in vivo and in vitro (Ou et al. 2016, Pham et al. 2017, Song et al. 2020). There is also an abundance of in vitro fermentation data on different types of FAXOs that support the putative gut-beneficial effects of FAXOs (Table 2) (Pham et al. 2017, Yang et al. 2014).

FAXOs have also recently been shown to modulate the growth of other types of gut bacteria in various animal experiments. FAXOs decreased the level of *Desulfovibrio* in diabetic rats, which is considered beneficial, as *Desulfovibrio* is positively related to weight gain (Song et al. 2020). *Akkermansia*, an antiobesity and antidiabetic strain, was promoted by FAXOs in diabetic rats and high-fat-diet-fed rats (but did not increase significantly in healthy models) (Song et al. 2020, Yang et al. 2016). Growth and abundance of an (undesirable) obesity-related *Dorea* strain were significantly reduced by FAXOs in rats fed high-fat diets as well as in in vitro fermentation of fecal

samples (Gong et al. 2019, Pham et al. 2017, Yang et al. 2016). *Lactobacilli* spp. abundance, which may also help prevent obesity (Cao et al. 2019), was promoted by FAXOs in healthy rats (Ou et al. 2016). In contrast, a recent report with rats claims that elevated *Lactobacillus* levels may correlate positively with type-2 diabetes and that *Lactobacillus* levels are inhibited by FAXOs in diabetic rats (Song et al. 2020). This latter result highlights that several aspects of the bioactivity and potential beneficial effects of the prebiotic–microbial growth interactions remain to be understood.

Short-Chain Fatty Acids

The major beneficial fermentation products resulting from a healthy gut microbiome are SCFAs, including acetate, propionate, butyrate, and lactate. The SCFAs reduce the pH of the intestinal environment, improve absorption of calcium and magnesium, and inhibit the growth of some harmful bacteria (de Paulo Farias et al. 2019). Butyrate, the preferred energy source for the colon epithelial cells, exerts a range of beneficial effects, including protecting against colon cancer and colitis, improving gut barrier function by stimulation of the formation of mucin, antimicrobial peptides, and tight-junction proteins, and reducing oxidative stress and inflammation in the colon (Rivière et al. 2016). Both propionate and butyrate are involved in protection against diet-induced obesity, and propionate reduces the proliferation of cancer cells in the liver (Bindels et al. 2012, Lin et al. 2012). FAXOs have been convincingly shown to significantly increase the total content of SCFAs, including acetate, propionate, and butyrate, in both in vitro and in vivo gut microbiota fermentations (Gong et al. 2019; Pham et al. 2017; Song et al. 2020; Yang et al. 2014, 2016). Notably, FAXOs increased the total SCFAs in diabetic rats (Song et al. 2020). Increased propionate has also been observed with reductions in fasting glucose and insulin levels, the index of insulin resistance, and plasma leptin levels in fat rats fed a diet containing FAXO supplements (Yang et al. 2016).

Branched SCFAs, such as isobutyric acid and isovaleric acid, are the major products of protein fermentation in the gut and appear to contribute to obesity-associated insulin resistance. FAXOs were recently shown to significantly reduce the levels of isobutyric acid and isovaleric acid in the gut microbiota during in vitro fermentation (Gong et al. 2019).

In accord with the understanding that arabinoxylan substitutions, including feruloylation, provide resistance to enzymatic and microbial degradation, it is evident that fermentation of substituted arabinoxylans by gut microbes decreases with increasing structural complexity and size of the arabinoxylan, e.g., higher arabinose to xylose ratio (A:X) and higher average degree of polymerization (avDP). Simple arabinoxylo-oligosaccharides with low avDP (≤ 3) are known to increase the production of acetate and butyrate and boost the growth of *Bifidobacteria* during human fecal fermentation (Van Craeyveld et al. 2008). Likewise, in vitro fermentations have shown that pure, singly substituted arabinoxylo-oligosaccharides are utilized almost completely by a mixture of only a few bifidobacterial species (as well as by the fecal microbiota). However, pure arabinoxylo-oligosaccharides with a doubly substituted xylose residue, i.e., disubstituted with arabinose moieties in the O2 and O3 position, were found to be fermented only by the fecal microbiota and not by the mix of *Bifidobacteria* that easily converted the singly substituted arabinoxylo-oligosaccharides (Pastell et al. 2009).

Structure–Function Effects Regarding Prebiotic Effects

Despite the detailed structural chemical knowledge available on different types of substituted arabinoxylans, the current bioactivity studies have mainly been conducted using mixtures of FAXOs and sometimes even included nonferuloylated xylan-oligomers (Table 2). Hence, despite the likely significance of the intricate structural details on the putative prebiotic and SCFA-generating effects of FAXOs, e.g., arabinosyl substitutions, acetylation, molecular size, and degree

of feruloylation, there are not yet enough data to derive any specific structure–function effects; this may be because the FAXOs used in bioactivity studies are not particularly well defined (also evident from the fact that mainly FAXO mixtures have been used) (Table 2). However, it is known that arabinoxylo-oligosaccharides with higher avDP than 12 maintain a decent fermentation rate in mixed gut microbiota fermentation, although lower avDP substrates consistently ferment at a higher rate (Snelders et al. 2014).

The role of the feruloyl substitutions on the prebiotic effects of FAXOs appears important. Recently, both feruloylated arabinoxylans and feruloyl cross-linked arabinoxylans have been demonstrated to be fermentable by the gut microbiota and to modulate the gut microbiome composition and increase the production of SCFAs in in vitro fermentations (Yang et al. 2014, Zhang et al. 2019). A high degree of feruloylation of FAXOs reduces the rate of fermentation by the gut microbiota (Snelders et al. 2014). Recently, it has been shown that diferuloylation (feruloyl cross-linking) of FAXOs significantly decreases fermentation rates in vitro and results in less acetate, propionate, and total SCFAs (Zhang et al. 2019). Nevertheless, the proportion of butyrate was significantly higher with cross-linked FAXOs than with non-cross-linked FAXOs in fermentations in which several unassigned butyrate-producing bacteria, a *Ruminococcaceae* sp., *Blautia* sp., *Clostridium* sp., and *Faecalibacterium prausnitzii*, were promoted (Zhang et al. 2019). The significance of feruloylation may be due to the liberation of free ferulic acid. Although it was recently affirmed that free ferulic acid does not affect the ratio of *Firmicutes* to *Bacteroidetes* in diabetic rats (Song et al. 2020), free ferulic acid modulates the growth of several individual putative obesity- and diabetes-related strains in the gut; hence, growth of *Akkermansia*, *Bacteroides*, *Blautia*, and *Phascolarctobacterium* was promoted, *Desulfovibrio*, *Lactobacillus*, and *Dorea* species were diminished (Gong et al. 2019, Song et al. 2020), and total SCFAs, notably propionic acid, was significantly increased by ferulic acid in diabetic rats (Song et al. 2020). However, free ferulic acid was also reported to decrease the production of acetic acid, propionic acid, and butyric acid in fecal samples of healthy individuals (Gong et al. 2019).

On the basis of the data reported, we can infer that the A:X, avDP, and degree of feruloylation of FAXOs affect the fermentation of gut microbiota and the degradation of FAXOs. Liberation of ferulic acid depends on the microbial enzymes produced by the gut microbiota, and the content of released ferulic acid affects the growth of gut microbiota, which in turn affects the production of SCFAs and branched SCFAs such as isobutyric acid and isovaleric acid. The availability of more well-defined FAXO structures would allow a much more detailed understanding of the functionality and possible prebiotic and gut functional bioactivity of specific structural FAXO elements.

Antioxidant and Possible Immunomodulatory Activities of Feruloylated Arabinoxylo-Oligosaccharides

In vitro experiments have indicated that FAXOs exert antioxidant radical scavenging activity in DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assays as well as hydroxyl and superoxide radical scavenging capacity and metal ion-chelating capacity (Katapodis et al. 2003a, Ruthes et al. 2017, Snelders et al. 2013, Yuan et al. 2005, Zhao et al. 2018) (Table 2). Clearly, the feruloyl moieties of FAXOs are considered to play a vital role in antioxidant activities both in vitro and in vivo compared with nonferuloylated arabinoxylo-oligosaccharides. A higher content of feruloyl usually results in stronger antioxidant capacity in vitro (Q. Lin et al. 2014, Malunga & Beta 2015, Ruthes et al. 2017, Snelders et al. 2013, Yao et al. 2014), and therefore many studies have shown that FAXO antioxidant activity is dose dependent (Q. Lin et al. 2014, Ruthes et al. 2017, Yao et al. 2014, Yu et al. 2015, Zhao et al. 2018). However, the

antioxidant activity of FAXOs does not appear to take place through the liberation of ferulic acids. FAXOs have thus been reported to exert greater *in vivo* antioxidant activity than the equivalent amount of free ferulic acid (Ou et al. 2007); however, no significant difference in antioxidant capacity *in vivo* was observed between FAXOs and sodium ferulate in rat models (Zhang et al. 2017). Interestingly, in the presence of arabinoxylo-oligosaccharides, free ferulic acid shows higher *in vitro* radical scavenging and oxygen radical absorbance capacity than the same amount of individual ferulic acid. However, with the same molecular structure and ferulic acid concentration of FAXOs, a lower *in vitro* antioxidant capacity was observed in arabinoxylo-oligosaccharides bound with ferulic acid compared with arabinoxylo-oligosaccharides rich in free ferulic acid (Snelders et al. 2013). It should be noted that results differ in different types of antioxidant test systems. For instance, compared to FAXOs, free ferulic acid exerted stronger DPPH radical scavenging capacity but had weaker inhibitory effects on oxidation of low-density lipoproteins (Katapodis et al. 2003a).

Feruloyl cross-linked arabinoxylo-oligosaccharides have decreased *in vitro* antioxidant capacity (Snelders et al. 2013), hinting that the antioxidant activity via abstraction of electrons from the phenoxyl-group (–OH) in the ferulic acid is affected by the DiFA cross-linking or that the antioxidant activity may rely on not only classic abstraction of phenoxyl electrons followed by resonance stabilization but also steric availability, solubility, and other factors in the microenvironment surrounding the arabinoxylo-oligosaccharides. Interestingly, the antioxidant effect appears to depend on the type of diferulate; hence, 8–5C DiFA exerts weaker *in vitro* antioxidant activity than monomeric ferulic acid, but feruloyl cross-linked 8-*O*-4 DiFA, 5–5 DiFA, and 8–8C DiFA show better antioxidant capacity than monomeric ferulic acid (Jia et al. 2018).

FAXOs may directly protect against oxidative damage *in vivo*. Hence, in normal or oxidatively induced rat models, and even in diabetic and tumor-bearing rats, intake of FAXOs has been shown to significantly increase the total antioxidant capacity of the serum (Ou et al. 2007, Wang et al. 2010, Zhang et al. 2017) and in general to suppress the levels of oxidation, e.g., via reducing levels of the oxidative marker malonic dialdehyde (Yu et al. 2015) (**Table 2**). In relation to the protective, potentially disease-preventing nutritional effects of FAXOs, it is particularly interesting to note that FAXOs extracted from wheat bran have been found to increase the antioxidant capacity of samples from the heart, liver, and kidney of normal rats (Zhang et al. 2015) (**Table 2**).

In an *in vitro* cellular experiment using human HepG2 cells (a human liver cancer cell line), the addition of mixed low-molecular-weight FAXOs from wheat bran not only increased the overall antioxidant capacity of the cells (Zhang et al. 2016) (**Table 2**) but also promoted expression of several antioxidant-related enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and decreased formation of the oxidative marker malonic dialdehyde (Zhang et al. 2016). Mixed wheat-bran-derived FAXOs were also reported to suppress lipid peroxidation and delay hemolysis of human erythrocytes induced by addition of the free-radical azo-compound AAPH [2,2'-azobis(2-amidinopropane) dihydrochloride] (Wang et al. 2009a) (**Table 2**). Other studies have substantiated these results by showing that mixtures of FAXOs (mix of FAXOs and low-molecular-weight xylo-oligomers) from wheat bran inhibit hemolysis and osmotic fragility of oxidatively stressed erythrocytes (Yu et al. 2015) (**Table 2**).

Although attempts to explain the possible cellular signaling pathways and cascade events behind the FAXO effects via antioxidant activity *in vivo* are available in the literature (Zhang et al. 2015, 2016, 2017), the mechanistic details and the possible structure–function relations of the FAXOs in this regard are still unclear. Part of the problem is that mainly mixtures of various types of FAXO structures and even blends including low-molecular-weight xylo-oligomers have been used in the functional bioactivity studies (see **Table 2**).

Immunomodulatory and Other Nutritional Functions

FAXOs also appear to exert a range of immunomodulatory and other beneficial effects (**Table 2**). The effects are complex, have mainly been demonstrated in various in vitro systems, and include both enhanced production of proinflammatory cytokines in macrophages and induction of anti-inflammatory cytokine IL-10 in specific lipopolysaccharide-induced macrophages (Fang et al. 2012). Also, activation of dendritic cells, proliferation of T cells (C. Lin et al. 2014) (by rice bran FAXOs), and growth reduction of human gastric cancer cells and human lung adenocarcinoma cells and tumor growth in vitro have been investigated (Yu et al. 2014) (**Table 2**). In vivo, growth of tumors in tumor-bearing rats has been shown to be inhibited by treatment with wheat bran–derived FAXOs and xylo-oligomers, and the effect may be associated with elevated production of antitumor-related cytokines INF- γ and IL-3 (Yu et al. 2014). The relationship between immunomodulatory effects and FAXO structure is not clear, but elevated antitumor activity and immunostimulatory effects were achieved with higher avDP of FAXOs (Yu et al. 2014). A higher content of ferulic acid may also promote stronger activity of natural killer cells and release more INF- γ (Ma et al. 2016). Antidiabetic effects of FAXOs have also been demonstrated; notably, FAXOs from corn bran were found to exert hypoglycemic, hypolipidemic, antioxidant, and antiglycation effects in vivo on type-2 diabetic rats (Huang et al. 2018) (**Table 2**). In addition, feruloyl arabinose and FAXOs from corn bran and wheat inhibited glucose transporters and reduced glucose uptake in cells in vitro (Malunga et al. 2016). FAXOs may also protect against hepatocyte and myocardial injuries induced by diabetes by lowering the activity of myocardial enzymes. The formation of advanced glycation end-products (one of the biomarkers of diabetes) and oxidative stress induced by hyperglycemia was inhibited in the liver, kidney, and heart of diabetic rats following intake of FAXOs (Huang et al. 2018, Ou et al. 2007). Recently, it was suggested that the antidiabetic effects of FAXOs might be related to their regulation of gut microbes (Song et al. 2020).

ENZYMATIC RELEASE AND MODIFICATION OF FERULOYLATED ARABINOXYLO-OLIGOSACCHARIDES

Releasing Feruloylated Arabinoxyloligosaccharides by Endo-1,4- β -Xylanases

Endo-1,4- β -xylanases (EC 3.2.1.8) (endoxylanases) catalyze xylan degradation. This enzyme type catalyzes cleavage of the β -(1 $\rightarrow$ 4)-glycosidic linkages between Xylp residues in the xylan backbone of arabinoxylans, resulting in the production of different substituted and unsubstituted xylo-oligosaccharides. The specificity of different endoxylanases toward substituted substrates results in different products. According to the gene sequences of their catalytic domain, endoxylanases are classified into glycoside hydrolase (GH) families 5, 8, 10, 11, 30, 98, and 141 in the carbohydrate-active enzymes database (<http://www.cazy.org>). It is mainly microbial—both bacterial and fungal—endoxylanases from families GH10, GH11, and, recently, GH30 that have been used to enzymatically release (solubilize) FAXOs from various biomass substrates (**Table 3**).

GH10 and GH11 Endo-1,4- β -Xylanases

GH10 and GH11 endoxylanases are the most widely used endoxylanases in industry. Each one attacks both substituted and unsubstituted xylan regions and has been used in hydrolysis of xylan chains and to generate FAXOs (**Table 3**). New discoveries and application options of GH10 and GH11 endoxylanases constantly emerge. Recently, for instance, two new fungal GH10 and GH11 endoxylanases (*EPXyn1* and *EPXyn3*, derived from the *Penicillium*-like fungus *Eupenicillium parvum* and recombinantly expressed in *Pichia pastoris*) (**Table 3**) were found to release

Table 3 Endo- β -1,4-xylanases used for liberating FAXOs from various brans and other fibrous substrates^a

Enzyme	Organism of origin	MW (kDa)	pH _{opt}	T _{opt} (°C)	Activity (U/mg)	Assay substrate	Biomass reaction and notes	References
<i>CmXyn10</i> ^b	<i>Cellvibrio mixtus</i>	41.7	6.5	50	32	WAX (assay at 40°C, pH 6.5)	Used with <i>TXyn11</i> and novel ferulic acid esterases on insoluble WAX	Wong et al. 2019
<i>SoXyn10A</i>	<i>Streptomyces olivaceoviridis</i> E-86	45.0	5.5–6.0	56	ND	<i>p</i> -Nitrophenyl- β -D-xylobioside	CC-xylan; reaction at 37°C, pH 5.5	Arai et al. 2019
<i>EpXyn1</i>	<i>Eupenicillium parvum</i>	40.8	5.5	75	384	BEX/(BIX/OSX)	WB	Long et al. 2018
<i>TmXyl10</i> ^b	<i>Thermotoga maritima</i>	41.7	5.0	80	96	WAX	WB	Schendel et al. 2016b
<i>XylIn-SH1</i>	Not known (bacteria from a Holstein cattle rumen metagenomics library)	41.5	6.5	40	13,000	BIX	WS	Cheng et al. 2012
<i>XylIn-SH1</i>	Not known (bacteria from a Holstein cattle rumen metagenomics library)	41.5	6.5	40	896	OSX	WS	Cheng et al. 2012
<i>GcXT6</i>	<i>Geobacillus stearothermophilus</i> T6	46.8	ND	77–87	ND (k _{cat} = 1,356 s ⁻¹)	BIX (assay at 50°C, pH 7.6)	CS A thermostable, recombinantly produced mutant origin enzyme was used FAXOs release only tested together with a feruloyl esterase	Zhang et al. 2013
<i>TaXyn10A</i>	<i>Thermascus aurantiacus</i>	35.7	4.0–4.5	70–75	358	OSX (assay at 50°C, pH 5.0)	CC Reaction at 50°C, pH 5.0 A purified enzyme from the origin organism was used	Katapodis & Chris-takopoulos 2008
<i>TXyn11</i> ^b	<i>Trichoderma longibrachiatum</i>	20.0	6.0	50	135	WAX	Used with <i>CmXylB</i> and novel ferulic acid esterases on insoluble WAX	Wong et al. 2019
<i>SoXyn11A</i> (GXYN)	<i>S. olivaceoviridis</i> E-86	23.0	6.0	60	2716	BIX (assay at pH 5.7, 56°C)	CC-xylan Reaction at 37°C, pH 5.5 A purified enzyme from the origin organism was used	Arai et al. 2019

(Continued)

Table 3 (Continued)

Enzyme	Organism of origin	MW (kDa)	pH _{opt}	T _{opt} (°C)	Activity (U/mg)	Assay substrate	Biomass reaction and notes	References
BsXyn (maybe more than one xylanase enzyme)	<i>Bacillus subtilis</i>	ND	5.0–7.0	50	ND	BIX	WB Optimum reaction conditions not documented Crude enzyme preparation used at 50°C, pH 5.0	Yuan et al. 2006, Zhang et al. 2017
EpXyn3	<i>E. parvum</i>	21.6	5.0	55	214	BEX/(BIX/OSX)	WB	Long et al. 2018
AnXyn11A	<i>Aspergillus niger</i> BE-2	25.0	5.0	50–60	1,600	BEX (assay at 50°C, pH 5.0)	WB	Wu et al. 2017
TcXyn2A ^b	<i>Trichoderma viride</i>	20.5	ND	30–50	100–300	Uncertain	WA/WB	Malunga & Beta 2015
NpXyn11A ^b	<i>Neocallimastix patriciarum</i>	25.8	6.0	40	600	WAX	WB/WA/CB	Malunga et al. 2016, Schendel et al. 2016b
AoXyn11A	<i>Aspergillus oryzae</i>	24.6	5.5	50	2,415	BIX	WB	Zeng et al. 2014
AuXyn11A	<i>Aspergillus usamii</i> E001	24.1	ND	ND	ND	ND	WB	Gong et al. 2013
TxXyl11	<i>Thermobacillus xylanilyticus</i> D3	20.7	6.0	75	2,000	BIX	WB/WS	Rakotoarivonina et al. 2011
StXylA	<i>Sporotrichum thermophile</i>	25.0	5.0	70	875	BIX	WB	Vardakou et al. 2004
DcXyn30A	<i>Dickeya chrysanthemi</i>	45.2	5.5	45	ND	ND	CB	Munk et al. 2020

^a An activity unit is generally measured as the amount of enzyme required to release one μ mole of reducing sugar (xylose) equivalents per minute at the assay conditions. Unless specified otherwise, recombinantly expressed enzymes have been used.

^b Commercial enzyme preparation (for biochemistry research).

Abbreviations: BEX, beechwood xylan; BIX, birchwood xylan; CB, corn bran; CC, corn cob; CS, corn stalk; FAXOs, feruloylated arabinoxylo-oligosaccharides; ND, not determined; OSX, oat spelled xylan; WA, wheat aleurone; WAX, wheat arabinoxylan; WB, wheat bran; WS, wheat straw.

~11–14 $\mu\text{mol/g}$ FAXOs from destarched wheat bran (Long et al. 2018). Another fungal GH10 endoxylanase, *TaXyn10A* (derived from *Talaromyces aurantiacus*), was shown to catalyze the release of FAXOs from corn cob at a yield of 12 $\mu\text{mol/g}$ (Katapodis & Christakopoulos 2008). Higher FAXO yields were obtained from wheat bran by optimized enzymatic hydrolysis using a GH11 endoxylanase from *Bacillus subtilis* (Yuan et al. 2006). The GH11 endoxylanase from *Bacillus subtilis* has indeed been used to produce FAXOs in many studies (Aguedo et al. 2014; Wang et al. 2009b, 2010; Yuan et al. 2006; Zhang et al. 2015, 2017). It is important to mention that the wild-type *B. subtilis* GH11 XynA endoxylanase is sensitive to inhibition by a prominent inhibitor, TAXI, present in cereals, notably wheat (*Triticum aestivum*); hence, the name TAXI is abbreviated from *T. aestivum* xylanase inhibitor (Debyser et al. 1997). Because of the increased use of microbial endoxylanases in baking and other applications (to, e.g., decrease viscosity), a variant of the *B. subtilis* GH11 XynA has been developed that is less prone to inhibition by TAXI. Although the overall activity of the enzyme variant is slightly decreased compared to the wild-type enzyme on various arabinoxylan substrates and wheat bran (Rasmussen et al. 2010), this enzyme variant is sold commercially and used industrially for baking. GH10 and GH11 endoxylanases derived from other bacteria, e.g., *Cellvibrio mixtus* and *Thermotoga maritima*, or from fungi, e.g., *Trichoderma viride*, *Trichoderma longibrachiatum*, and *Neocallimastix patriciarum*, can also attack complex arabinoxylan biomass materials (**Table 3**) and produce mixtures of FAXOs and xylo-oligosaccharides from a range of substrates (Dilokpimol et al. 2017, Malunga & Beta 2015, Schendel et al. 2016b, Wong et al. 2019). The general DP of FAXOs released after exhaustive enzymatic treatment ranges from 3 to 5. Most studies have focused more on the enzymatic specificity description and less on the detailed determination of product structure, but it is possible to derive some overall rules by considering the products obtained in relation to the substrate attack preferences of the enzymes and notably the substitutions on the backbone that can be accommodated by the enzymes.

The major type of FAXO released by GH10 and GH11 endoxylanases from feruloylated arabinoxylan biomass substrates such as pretreated bran, corn cob, or various types of wood xylans is Oligosaccharide 1 (**Table 1**). However, the substitution sites and oligomer backbone length on the produced oligomers differ because the enzymes in different GH families exhibit subtle differences in enzyme-attack preferences. GH10 endoxylanases, for example, accept *Araf* substituents at the -3 , -2 , $+1$, and $+2$ subsites, whereas GH11 endoxylanases are more restricted, only accepting *Araf* substituents at the -3 , -2 , and $+2$ subsites (**Table 4**). Neither GH10 nor GH11 endoxylanases accept substituents at the -1 subsite, which is why enzymatically produced FAXOs usually have unsubstituted xylose units at their reducing end (**Figure 2**).

Hence, for GH10 endoxylanases, the products are dominated by Oligosaccharide 1 as a “xylobiose” with a feruloylated *Araf* substituent at the nonreducing end (Schendel et al. 2016b, Vardakou et al. 2003) (**Figure 2**), whereas GH11 endoxylanases liberate Oligosaccharide 1 as a “xylotetraose” with a feruloylated *Araf* substituent on the second xylose from the nonreducing end (Lequart et al. 1999, Vardakou et al. 2003, Wang et al. 2009b) (**Figure 2**). This difference is a result of the fact that in the active site of GH10 endoxylanases, feruloylated *Araf* substituents can be accommodated at the -2 subsite (as shown directly for *TaXyn10A*) (**Table 4**), whereas GH11 endoxylanases (e.g., as verified for *NpXyn11A*) principally fit feruloylated-arabinosyl substitutions at the $+2$ subsite (Vardakou et al. 2005, 2008) (**Table 4**). For this reason, both GH10 and GH11 endoxylanases can release a “xylotriose” with a feruloylated *Araf* substituent on the central xylose (**Figure 2**). However, recent reports suggest that there may be exceptions to these rules; for example, xylo-oligosaccharides with feruloylated *Araf* at the nonreducing end released by a GH10 endoxylanase might suggest acceptance of feruloylated *Araf* at the $+1$ subsite (Arai et al. 2019, Schendel et al. 2016b) (**Table 4**). Accordingly, FAXOs released from wheat bran by a combination of GH10 and GH11 endoxylanases were identified as Oligosaccharide 1, 2, and 6 (**Table 1**) (Schendel et al.

Table 4 Substituents that can be accommodated or which are required in subsites of endoxylanases from different GH families^a

Endoxylanase	−3	−2	−1	+1	+2	+3
GH5_2	Araf			Araf		
GH5_34		Araf	Araf ^c	Araf	Araf	
GH10	Araf, MeGlcA	FA-Araf, Araf		FA-Araf, Araf, MeGlcA	Araf	
GH11	Araf, MeGlcA	Araf (FA-Araf) ^b			FA-Araf, Araf, MeGlcA	
GH30_7		MeGlcA, ^c Araf		MeGlcA, Araf		
GH30_8	Araf	MeGlcA ^c		Araf		

^aFA-Araf, Araf, MeGlcA indicate substitutions on the xylan backbone, and entries indicate that the enzyme can accept either one of these substitutions in a subsite in the active site (subsite numbers indicated as −3, −2, −1 and +1, +2, +3).

^bEntry in parenthesis indicates that the evidence is only indicative (i.e., not validated via structural evidence of the enzyme-substrate binding).

^cRequired for the enzymatic cleavage.

Abbreviations: Araf, O3 arabinofuranosyl; FA, feruloyl; MeGlcA, 4-O-methyl-D-glucuronic acid.

Data summarized from Arai et al. (2019), Biely et al. (2014), Correia et al. (2011), Fujimoto et al. (2004), Machara et al. (2017), Munk et al. (2020), Nakamichi et al. (2019b), Pauchet et al. (2020), Pell et al. (2004), Sakka et al. (2012), Schendel et al. (2016b), and Vardakou et al. (2005, 2008).

2016b). In another recent study, the addition of GH10 and GH11 endoxylanases from *Streptomyces olivaceoviridis* E-86 to pretreated corn cobs generated Oligosaccharide 5-type FAXOs containing both feruloylated Araf and acetylation on the same xylose (Arai et al. 2019). These data thus suggest that GH11 endoxylanase from *S. olivaceoviridis* may be able to accommodate feruloylated Araf substituents at subsite −2, although this suggestion has not been verified by a structural elucidation of the enzyme-substrate binding.

Insoluble arabinoxylans are more efficiently hydrolyzed by GH11 endoxylanase because of their relatively smaller molecular size, whereas GH10 endoxylanase preferably cleaves the shorter and soluble substrates. GH10 endoxylanases generally have higher activity than GH11 against highly Araf-substituted substrates (Pollet et al. 2010a). For example, Xyln-SH1 (GH10 xylanases) derived from a Holstein cattle rumen metagenomic library appear to have the potential to release FAXOs from lignified biomass, such as wheat straw (Cheng et al. 2012). Finally, some GH10 and GH11 endoxylanases may be able to accommodate 1,2-linked MeGlcA in distant subsites (Katapodis et al. 2003b, Vardakou et al. 2008), indicating that it is possible to use these enzymes to liberate glucuronate-substituted FAXOs from certain substrates.

GH5 Endo-1,4-β-Xylanases

Xylanases of family GH5 have not been reported to accept feruloylated Araf substituents in the active site, but GH5 of subfamily 2 and 34 are known to specifically attack the Araf-substituted regions of xylan chains and liberate highly substituted arabinoxylo-oligosaccharides (DP from 2 to 8) from arabinoxylans. Hence, a subfamily 2 (GH5_2) xylanase from the (gut of the) beetle *Apriona japonica* (A7AGH5_2–1) was recently shown to have remarkably high activity toward substituted arabinoxylans, and the enzyme accepts O2- or O3-substituted Araf at the −3 and/or at the +1 subsite (Pauchet et al. 2020). The enzyme can therefore catalyze liberation of short xylo-oligomers (xylotriose and xylotetraose) with Araf at the nonreducing end. The same is the case for GH5 subfamily 34 (GH5_34) xylanases, also named arabinoxylanase (EC 3.2.1.-) (Falck et al. 2018) (Table 4). Unfortunately, GH5_34 arabinoxylanases show no activity toward glucuronoxylan, beechwood, birchwood, or oat spelt xylan, which each lacks Araf substituents (Correia et al.

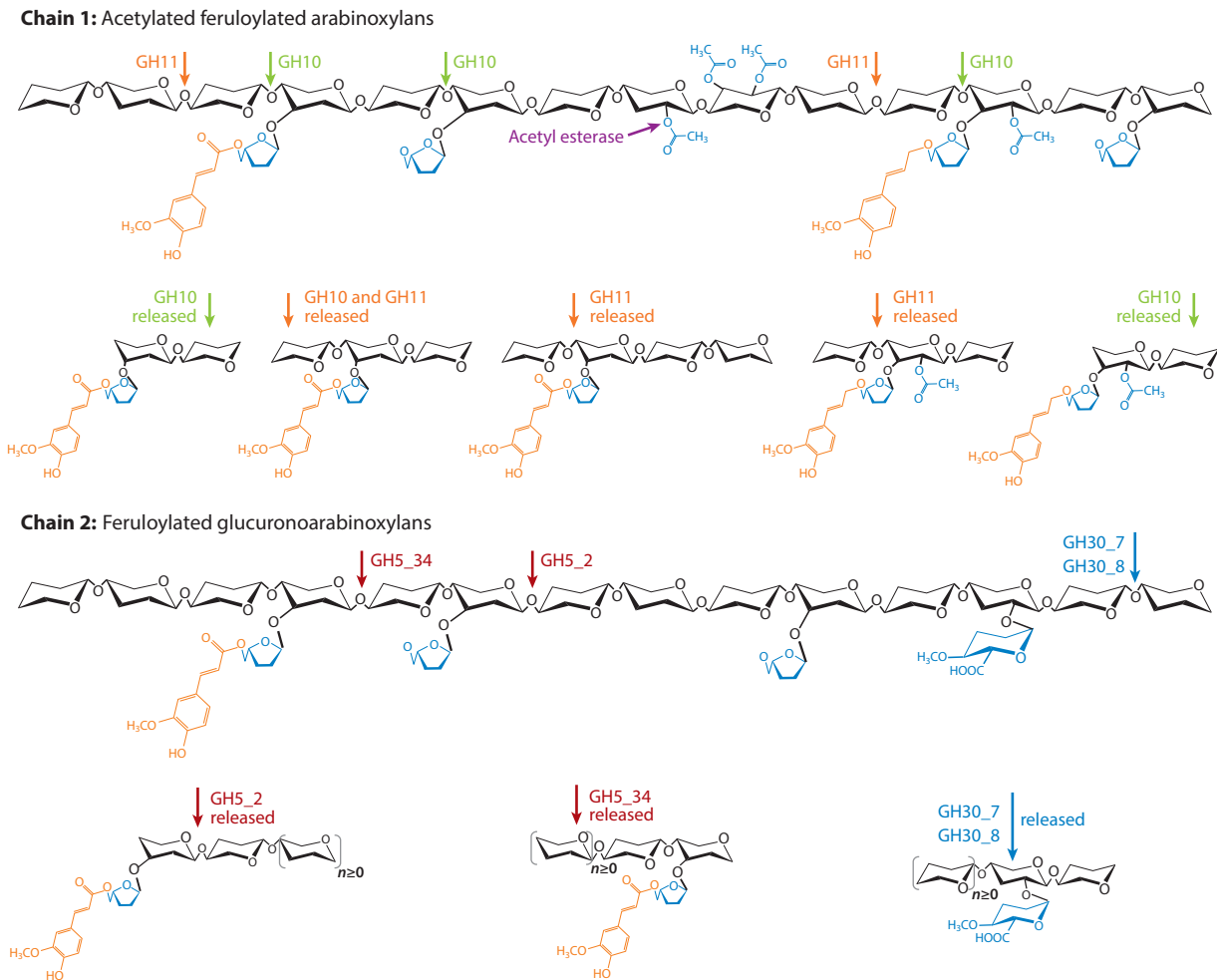


Figure 2

Sites of enzymatic attack near feruloyl substitutions to enzymatically release different feruloylated xylo-oligosaccharides (FAXOs) from complex arabinoxylan structures (e.g., from wheat or corn bran). The attack points are shown for acetyl xylan esterase and endoxylanases from various glycoside hydrolase (GH) families. Chain 1 is a stylized illustration of an acetylated feruloylated arabinoxylan chain, and chain 2 is a feruloylated glucuronoarabino-substituted xylan.

2011, Hagiwara et al. 2020). Still, because of the ability of GH5 endoxylanases to accommodate arabinosyls close to the active site, these enzymes may be interesting candidates for production of FAXOs.

GH30 Endo-1,4- β -Xylanases and Acetyl Xylan Esterase

Among the GH30 endoxylanases, several enzymes, notably some of those of subfamily 7 and 8, i.e., GH30_7 and GH30_8, are specific toward MeGlcA-substituted xylan regions in arabinoxylans and may even require the presence of an MeGlcA substitution at the -2 subsite for action (Table 4). This requirement therefore renders products with a MeGlcA substituent on the second xylosyl unit upstream from the reducing end (Biely et al. 2014). However, with

extended enzymatic treatment, the enzymes may also attack other parts of the xylan backbone to release various xylo-oligomers (**Figure 2**) and catalyze degradation of the liberated MeGlcA-substituted xylo-oligomers (Nakamichi et al. 2019b). This particular specificity has led to the classification of (some of) these endoxylanases as glucuronoxylanases (EC 3.2.1.136). A few GH30_7 and GH30_8 xylanases also appear to be able to accommodate Araf-substituted xylan moieties near the active site (**Table 4**), providing options for liberating highly arabinofuranosyl-substituted oligomers, perhaps even FAXOs, from complex arabinoxylan substrates. Because of the considerable research interest in enzyme-assisted upgrading of both plant biomass and fiber-rich sidestreams, there is a constant flow of reports on the discovery of unique enzymes that catalyze specific structural changes in highly complex, substituted xylans. For example, two novel fungal GH30_7 bifunctional glucuronoxylanase/exo-xylobiohydrolases *TcXyn30B* (from *Talaromyces cellulolyticus*) and *TtXyn30A* (from *Thermothelomyces thermophila*), which are specifically active on glucurono-substituted xylans, were recently reported (Katsimpouras et al. 2019, Nakamichi et al. 2019a); moreover, a recent report even describes the discovery of an additional GH30_7 glucuronoxylanase from *T. cellulolyticus*, coined *TcXyn30C*. This enzyme appears to prefer glucurono-substituted arabinoxylan (accommodating the MeGlcA in position -2) but can also catalyze degradation of Araf-substituted and less substituted regions in xylans (Nakamichi et al. 2019b). For the sake of completion, we note that a bacterial GH30_8 from *Clostridium acetobutylicum* has been shown to exert high activity on wheat arabinoxylan and beechwood glucurono-arabinoxylan (St. John et al. 2018). Considering that feruloylated arabinoxylans from both wheat and corn bran are also substituted with MeGlcA and in fact are feruloylated glucuronoarabinoxylans, we posit that GH30 xylanases can be used to catalyze the liberation of bioactive FAXOs from this type of complex substrate. Such enzymatic production of FAXOs may at the same time potentially help provide new processes for upcycling of agroindustrial and food-processing sidestreams. An example of this includes the use of a bacterial GH30 enzyme, *DcXyn30*, derived from the maize pathogen *Dickeya chrysanthemi* for extraction of feruloylated glucurono-arabinoxylo-oligosaccharides from corn bran (Munk et al. 2020). The enzyme belongs to subfamily 8 and is hence a GH30_8 xylanase. The *DcXyn30* catalyzed controlled release from corn bran of both single- and double-stranded highly arabinose-substituted and glucuronated FAXOs that could form strong hydrogels upon forced oxidative diferulate cross-linking (Munk et al. 2020). There are currently no data on any possible nutrifunctional effects of diferulate cross-linked FAXOs.

In addition to substitutions with Araf, feruloyl, and MeGlcA, the acetyl substitutions are also significant aspects of arabinoxylans. The acetylation impedes enzymatic xylanase hydrolysis (Agger et al. 2010, Appeldoorn et al. 2013). For this reason, the addition of acetyl xylan esterase (EC 3.1.1.72), which catalyzes deacetylation of xylan and xylo-oligomers, may improve catalytic efficiency of various endoxylanases. Although it has recently been shown that novel xylanases from *S. olivaceoviridis* E-86 can catalyze liberation of Oligosaccharide 5 FAXOs from pretreated corn bran (Arai et al. 2019) (**Figure 2**), addition of acetyl xylan esterase can be envisaged to help prepare unsubstituted and less substituted xylan regions. Although certain GH10 endoxylanases have been reported to be able to accommodate various substitutions at the +1 subsite (**Table 4**), the classical comprehension is that, especially with regard to acetylation, a minimum of two consecutive unsubstituted xylose units are necessary for most GH10 and GH11 endoxylanase to attack arabinoxylan (Nordberg Karlsson et al. 2018, Pollet et al. 2010a). Hence, enzymatic deacetylation can improve the action of most GH10 and GH11 enzymes to release shorter highly substituted xylo-oligosaccharides, including FAXOs from substituted arabinoxylans. Such a combination of enzymes creates a more diversified population mix of solubilized FAXOs and short xylo-oligosaccharides but increases yields of FAXOs from substituted arabinoxylan

substrates without hydrothermal pretreatment, which is otherwise used to unlock and deacetylate biomass substrates and fibrous sidestream substrate residues.

CONCLUSIONS AND PERSPECTIVES

Brans and whole-grain food products constitute an important dietary source of complex substituted insoluble arabinoxylans. These sources provide important dietary fiber, but recent data have shown that solubilized FAXOs derived from complex arabinoxylans provide an array of protective, health-promoting effects via the gut (**Table 2**). These effects include critically important immunomodulatory, prebiotic, antioxidant, and even possible anticancerogenic effects. In addition to whole-grain food products, potentially beneficial FAXOs can be produced from bran and other cereal-processing sidestreams. Currently, based on corn production data in the United States, the annual coprocessing production of corn bran alone is estimated to be approximately 55 million tons (USDA 2020). Upgrading this bran could create additional value and help secure the competitiveness and flexibility of corn-processing plants, e.g., corn starch plants. The progress of the structural knowledge of cereal arabinoxylans and the recent knowledge of enzymes that can selectively degrade substituted arabinoxylans have provided a favorable basis for producing well-defined FAXOs from different arabinoxylan substrates via targeted enzymatic treatment. These technological advances hint at a major avenue of new possibilities for using enzymes to produce more defined and uniform FAXO structures. The availability of defined FAXOs can help improve our understanding of specific nutrifunctional mechanisms of FAXOs.

DISCLOSURE STATEMENT

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LITERATURE CITED

- Agger J, Viksø-Nielsen A, Meyer AS. 2010. Enzymatic xylose release from pretreated corn bran arabinoxylan: differential effects of deacetylation and deferuloylation on insoluble and soluble substrate fractions. *J. Agric. Food Chem.* 58(10):6141–48
- Aguedo M, Fougnyes C, Dermience M, Richel A. 2014. Extraction by three processes of arabinoxylans from wheat bran and characterization of the fractions obtained. *Carbohydr. Polym.* 105(1):317–24
- Allerdings E, Ralph J, Schatz PF, Gniechwitz D, Steinhart H, Bunzel M. 2005. Isolation and structural identification of diarabinosyl 8-*O*-4-dehydrodiferulate from maize bran insoluble fibre. *Phytochemistry* 66(1):113–24
- Allerdings E, Ralph J, Steinhart H, Bunzel M. 2006. Isolation and structural identification of complex feruloylated heteroxylan side-chains from maize bran. *Phytochemistry* 67(12):1276–86
- Appeldoorn MM, de Waard P, Kabel MA, Gruppen H, Schols HA. 2013. Enzyme resistant feruloylated xylooligomer analogues from thermochemically treated corn fiber contain large side chains, ethyl glycosides and novel sites of acetylation. *Carbohydr. Res.* 381:33–42
- Arai T, Biely P, Uhliariková I, Sato N, Makishima S, et al. 2019. Structural characterization of hemicellulose released from corn cob in continuous flow type hydrothermal reactor. *J. Biosci. Bioeng.* 127(2):222–30
- Biely P, Puchart V, Stringer MA, Krogh KBRM. 2014. *Trichoderma reesei* XYN VI: a novel appendage-dependent eukaryotic glucuronoxylan hydrolase. *FEBS J.* 281(17):3894–903

- Bindels LB, Porporato P, Dewulf EM, Verrax J, Neyrinck AM, et al. 2012. Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. *Br. J. Cancer*. 107(8):1337–44
- Bunzel M, Allerdings E, Ralph J, Steinhart H. 2008. Cross-linking of arabinoxylans via 8–8-coupled diferulates as demonstrated by isolation and identification of diarabinosyl 8–8(cyclic)-dehydrodiferulate from maize bran. *J. Cereal Sci.* 47(1):29–40
- Burr SJ, Fry SC. 2009a. Extracellular cross-linking of maize arabinoxylans by oxidation of feruloyl esters to form oligoferuloyl esters and ether-like bonds. *Plant J.* 58(4):554–67
- Burr SJ, Fry SC. 2009b. Feruloylated arabinoxylans are oxidatively cross-linked by extracellular maize peroxidase but not by horseradish peroxidase. *Mol. Plant* 2(5):883–92
- Cao S-Y, Zhao C-N, Xu X-Y, Tang G-Y, Corke H, et al. 2019. Dietary plants, gut microbiota, and obesity: effects and mechanisms. *Trends Food Sci. Technol.* 92:194–204
- Cheng F, Sheng J, Dong R, Men Y, Gan L, Shen L. 2012. Novel xylanase from a Holstein cattle rumen metagenomic library and its application in xylooligosaccharide and ferulic acid production from wheat straw. *J. Agric. Food Chem.* 60(51):12516–24
- Correia MAS, Mazumder K, Brás JLA, Firbank SJ, Zhu Y, et al. 2011. Structure and function of an arabinoxylan-specific xylanase. *J. Biol. Chem.* 286(25):22510–20
- de Paulo Farias D, de Araújo FF, Neri-Numa IA, Pastore GM. 2019. Prebiotics: trends in food, health and technological applications. *Trends Food Sci. Technol.* 93:23–35
- Debyser W, Derdelinckx G, Delcour JA. 1997. Arabinoxylan solubilization and inhibition of the barley malt xylanolytic system by wheat during mashing with wheat wholemeal adjunct: evidence for a new class of enzyme inhibitors in wheat. *J. Am. Soc. Brew. Chem.* 55:153–56
- Dilokpimol A, Mäkelä MR, Mansouri S, Belova O, Waterstraat M, et al. 2017. Expanding the feruloyl esterase gene family of *Aspergillus niger* by characterization of a feruloyl esterase, *FaeC*. *New Biotechnol.* 37:200–9
- Falck P, Linares-Pastén JA, Karlsson EN, Adlercreutz P. 2018. Arabinoxylanase from glycoside hydrolase family 5 is a selective enzyme for production of specific arabinoxylooligosaccharides. *Food Chem.* 242:579–84
- Fang H-Y, Chen Y-K, Chen H-H, Lin S-Y, Fang Y-T. 2012. Immunomodulatory effects of feruloylated oligosaccharides from rice bran. *Food Chem.* 134(2):836–40
- Fujimoto Z, Kaneko S, Kuno A, Kobayashi H, Kusakabe I, Mizuno H. 2004. Crystal structures of decorated xylooligosaccharides bound to a family 10 xylanase from *Streptomyces olivaceoviridis* E-86. *J. Biol. Chem.* 279(10):9606–14
- Gong L, Wang H, Wang T, Liu Y, Wang J, Sun B. 2019. Feruloylated oligosaccharides modulate the gut microbiota in vitro via the combined actions of oligosaccharides and ferulic acid. *J. Funct. Foods*. 60:103453
- Gong Y-Y, Yin X, Zhang H-M, Wu M-C, Tang C-D, et al. 2013. Cloning, expression of a feruloyl esterase from *Aspergillus usamii* E001 and its applicability in generating ferulic acid from wheat bran. *J. Ind. Microbiol. Biotechnol.* 40(12):1433–41
- Hagiwara Y, Mihara Y, Sakagami K, Sagara R, Bat-Erdene U, et al. 2020. Isolation of four xylanases capable of hydrolyzing corn fiber xylan from *Paenibacillus* sp. H2C. *Biosci. Biotechnol. Biochem.* 84(3):640–50
- Han Y, Xiao H. 2020. Whole food-based approaches to modulating gut microbiota and associated diseases. *Annu. Rev. Food Sci. Technol.* 11:119–43
- Huang J, Wang X, Tao G, Song Y, Ho C, et al. 2018. Feruloylated oligosaccharides from maize bran alleviate the symptoms of diabetes in streptozotocin-induced type 2 diabetic rats. *Food Funct.* 9(3):1779–89
- Ishii T. 1991. Isolation and characterization of a diferuloyl arabinoxylan hexasaccharide from bamboo shoot cell-walls. *Carbohydr. Res.* 219:15–22
- Ishii T, Hiroi T. 1990. Isolation and characterization of feruloylated arabinoxylan oligosaccharides from bamboo shoot cell-walls. *Carbohydr. Res.* 196:175–83
- Jia Y, He Y, Lu F. 2018. The structure-antioxidant activity relationship of dehydrodiferulates. *Food Chem.* 269:480–85
- Katapodis P, Christakopoulos P. 2008. Enzymic production of feruloyl xylo-oligosaccharides from corn cobs by a family 10 xylanase from *Thermoascus aurantiacus*. *LWT Food Sci. Technol.* 41(7):1239–43
- Katapodis P, Vardakou M, Kalogeris E, Kekos D, Macris BJ, Christakopoulos P. 2003a. Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan. *Eur. J. Nutr.* 42(1):55–60

- Katapodis P, Vršanská M, Kekos D, Nerinckx W, Biely P, et al. 2003b. Biochemical and catalytic properties of an endoxylanase purified from the culture filtrate of *Sporotrichum thermophile*. *Carbohydr. Res.* 338(18):1881–90
- Katsimpouras C, Dedes G, Thomaidis NS, Topakas E. 2019. A novel fungal GH30 xylanase with xylobiohydrolase auxiliary activity. *Biotechnol. Biofuels.* 12(1):120
- Lequart C, Nuzillard J-M, Kurek B, Debeire P. 1999. Hydrolysis of wheat bran and straw by an endoxylanase: production and structural characterization of cinnamoyl-oligosaccharides. *Carbohydr. Res.* 319(1–4):102–11
- Lin C, Chen H, Chen Y, Chang H, Lin P, et al. 2014. Rice bran feruloylated oligosaccharides activate dendritic cells via Toll-like receptor 2 and 4 signaling. *Molecules* 19(4):5325–47
- Lin HV, Frassetto A, Kowalik EJ Jr., Nawrocki AR, Lu MM, et al. 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLOS ONE* 7(4):e35240
- Lin Q, Ou S, Wen Q. 2014. In vitro antioxidant activity of feruloyl arabinose isolated from maize bran by acid hydrolysis. *J. Food Sci. Technol.* 51(7):1356–62
- Long L, Xu M, Shi Y, Lin Q, Wang J, Ding S. 2018. Characterization of two new endo- β -1,4-xylanases from *Eupenicillium parvum* 4–14 and their applications for production of feruloylated oligosaccharides. *Appl. Biochem. Biotechnol.* 186(4):816–33
- Ma X, Wang L, Wei H, Huo X, Wang C, et al. 2016. Adjuvant properties of water extractable arabinoxylans with different structural features from wheat flour against model antigen ovalbumin. *Food Funct.* 7(3):1537–43
- Maehara T, Yagi H, Sato T, Ohnishi-Kameyama M, Fujimoto Z, et al. 2017. GH30 glucuronoxylan-specific xylanase from *Streptomyces turgidiscabies* C56. *Appl. Environ. Microbiol.* 84(4):e01850–17
- Malunga LN, Beta T. 2015. Antioxidant capacity of arabinoxylan oligosaccharide fractions prepared from wheat aleurone using *Trichoderma viride* or *Neocallimastix patriciarum* xylanase. *Food Chem.* 167:311–19
- Malunga LN, Eck P, Beta T. 2016. Inhibition of intestinal α -glucosidase and glucose absorption by feruloylated arabinoxylan mono- and oligosaccharides from corn bran and wheat aleurone. *J. Nutr. Metab.* 2016:1932532
- Mastrangelo LI, Lenucci MS, Piro G, Dalessandro G. 2009. Evidence for intra- and extra-protoplasmic feruloylation and cross-linking in wheat seedling roots. *Planta* 229(2):343–55
- Mendis M, Leclerc E, Simsek S. 2016. Arabinoxylans, gut microbiota and immunity. *Carbohydr. Polym.* 139:159–66
- Mnich E, Bjarnholt N, Eudes A, Harholt J, Holland C, et al. 2020. Phenolic cross-links: building and deconstructing the plant cell wall. *Nat. Prod. Rep.* 37:919–61
- Munk L, Muschiel J, Li K, Liu M, Perzon A, et al. 2020. Selective enzymatic release and gel formation by cross-linking of feruloylated glucurono-arabinoxylan from corn bran. *ACS Sustain. Chem. Eng.* 8(22):8164–74
- Nakamichi Y, Fouquet T, Ito S, Watanabe M, Matsushika A, Inoue H. 2019a. Structural and functional characterization of a bifunctional GH30-7 xylanase B from the filamentous fungus *Talaromyces cellulolyticus*. *J. Biol. Chem.* 294(11):4065–78
- Nakamichi Y, Fujii T, Fouquet T, Matsushika A, Inoue H. 2019b. GH30-7 endoxylanase C from the filamentous fungus *Talaromyces cellulolyticus*. *Appl. Environ. Microbiol.* 85(22):e01442–19
- Niño-Medina G, Carvajal-Millán E, Rascon-Chu A, Marquez-Escalante JA, Guerrero V, Salas-Muñoz E. 2010. Feruloylated arabinoxylans and arabinoxylan gels: structure, sources and applications. *Phytochem. Rev.* 9:111–20
- Nordberg Karlsson E, Schmitz E, Linares-Pastén JA, Adlercreutz P. 2018. Endo-xylanases as tools for production of substituted xylooligosaccharides with prebiotic properties. *Appl. Microbiol. Biotechnol.* 102(21):9081–88
- Ou J, Huang J, Song Y, Yao S, Peng X, et al. 2016. Feruloylated oligosaccharides from maize bran modulated the gut microbiota in rats. *Plant Foods Hum. Nutr.* 71(2):123–28
- Ou S-Y, Jackson GM, Jiao X, Chen J, Wu J-Z, Huang X-S. 2007. Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. *J. Agric. Food Chem.* 55(8):3191–95
- Pastell H, Westermann P, Meyer AS, Tuomainen P, Tenkanen M. 2009. In vitro fermentation of arabinoxylan-derived carbohydrates by *Bifidobacteria* and mixed fecal microbiota. *J. Agric. Food Chem.* 57:8598–606

- Pauchet Y, Ruprecht C, Pfrengle F. 2020. Analyzing the substrate specificity of a class of long-horned-beetle-derived xylanases by using synthetic arabinoxylan oligo- and polysaccharides. *ChemBioChem* 21(10):1517–25
- Pell G, Taylor EJ, Gloster TM, Turkenburg JP, Fontes CMGA, et al. 2004. The mechanisms by which family 10 glycoside hydrolases bind decorated substrates. *J. Biol. Chem.* 279(10):9597–605
- Pham T, Teoh K, Savary B, Chen M-H, McClung A, Lee S-O. 2017. In vitro fermentation patterns of rice bran components by human gut microbiota. *Nutrients* 9(11):1237
- Pollet A, Delcour JA, Courtin CM. 2010a. Structural determinants of the substrate specificities of xylanases from different glycoside hydrolase families. *Crit. Rev. Biotechnol.* 30(3):176–91
- Rakotoarivonina H, Hermant B, Chabbert B, Touzel J-P, Remond C. 2011. A thermostable feruloyl-esterase from the hemicellulolytic bacterium *Thermobacillus xylanilyticus* releases phenolic acids from non-pretreated plant cell walls. *Appl. Microbiol. Biotechnol.* 90(2):541–52
- Rasmussen LE, Sørensen JF, Meyer AS. 2010. Kinetics and substrate selectivity of a *Triticum aestivum* xylanase inhibitor (TAXI) resistant D11F/R122D variant of *Bacillus subtilis* XynA xylanase. *J. Biotechnol.* 146:207–14
- Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. 2016. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front. Microbiol.* 7:979
- Ruthes AC, Martínez-Abad A, Tân H-T, Bulone V, Vilaplana F. 2017. Sequential fractionation of feruloylated hemicelluloses and oligosaccharides from wheat bran using subcritical water and xylanolytic enzymes. *Green Chem.* 19(8):1919–31
- Sakka M, Tachino S, Katsuzaki H, van Dyk JS, Pletschke BI, et al. 2012. Characterization of Xyn30A and Axb43A of *Bacillus licheniformis* SVD1 identified by its genomic analysis. *Enzyme Microb. Technol.* 51(4):193–99
- Saulnier L, Crépeau M-J, Lahaye M, Thibault J-F, Garcia-Conesa MT, et al. 1999. Isolation and structural determination of two 5,5'-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydr. Res.* 320(1–2):82–92
- Saulnier L, Vigouroux J, Thibault J-FF. 1995. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res.* 272(2):241–53
- Schendel RR, Becker A, Tyl CE, Bunzel M. 2015. Isolation and characterization of feruloylated arabinoxylan oligosaccharides from the perennial cereal grain intermediate wheat grass (*Thinopyrum intermedium*). *Carbohydr. Res.* 407:16–25
- Schendel RR, Meyer MR, Bunzel M. 2016a. Quantitative profiling of feruloylated arabinoxylan side-chains from graminaceous cell walls. *Front. Plant Sci.* 6:1249
- Schendel RR, Puchbauer A-K, Britscho N, Bunzel M. 2016b. Feruloylated wheat bran arabinoxylans: isolation and characterization of acetylated and O-2-monosubstituted structures. *Cereal Chem.* 93(5):493–501
- Smith BG, Harris PJ. 2001. Ferulic acid is esterified to glucuronoarabinoxylans in pineapple cell walls. *Phytochemistry* 56(5):513–19
- Snelders J, Dornez E, Delcour JA, Courtin CM. 2013. Ferulic acid content and appearance determine the antioxidant capacity of arabinoxylan oligosaccharides. *J. Agric. Food Chem.* 61(42):10173–82
- Snelders J, Olaerts H, Dornez E, Van de Wiele T, Aura A-M, et al. 2014. Structural features and feruloylation modulate the fermentability and evolution of antioxidant properties of arabinoxylan oligosaccharides during in vitro fermentation by human gut derived microbiota. *J. Funct. Foods.* 10:1–12
- Song Y, Wu M, Tao G, Lu M, Lin J, Huang J. 2020. Feruloylated oligosaccharides and ferulic acid alter gut microbiome to alleviate diabetic syndrome. *Food Res. Int.* 137:109410
- St. John FJ, Dietrich D, Crooks C, Balogun P, de Serrano V, et al. 2018. A plasmid borne, functionally novel glycoside hydrolase family 30 subfamily 8 endoxylanase from solventogenic *Clostridium*. *Biochem. J.* 475(9):1533–51
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444(7122):1027–31
- USDA (U.S. Dep. Agric.). 2020. *Grain: world markets and trade*. World Prod. Mark. Trade Rep., Foreign Agric. Serv., Washington, DC. <https://www.fas.usda.gov/data/grain-world-markets-and-trade>

- Van Craeyveld V, Swennen K, Dornez E, Van de Wiele T, Marzorati M, et al. 2008. Structurally different wheat-derived arabinoxylooligosaccharides have different prebiotic and fermentation properties in rats. *J. Nutr.* 138(12):2348–55
- Vardakou M, Dumon C, Murray JW, Christakopoulos P, Weiner DP, et al. 2008. Understanding the structural basis for substrate and inhibitor recognition in eukaryotic GH11 xylanases. *J. Mol. Biol.* 375(5):1293–305
- Vardakou M, Flint J, Christakopoulos P, Lewis RJ, Gilbert HJ, Murray JW. 2005. A family 10 *Thermoascus aurantiacus* xylanase utilizes arabinose decorations of xylan as significant substrate specificity determinants. *J. Mol. Biol.* 352(5):1060–67
- Vardakou M, Katapodis P, Samiotaki M, Kekos D, Panayotou G, Christakopoulos P. 2003. Mode of action of family 10 and 11 endoxylanases on water-unextractable arabinoxylan. *Int. J. Biol. Macromol.* 33(1–3):129–34
- Vardakou M, Katapodis P, Topakas E, Kekos D, Macris BJ, Christakopoulos P. 2004. Synergy between enzymes involved in the degradation of insoluble wheat flour arabinoxylan. *Innov. Food Sci. Emerg. Technol.* 5(1):107–12
- Wang J, Bai J, Fan M, Li T, Li Y, et al. 2020. Cereal-derived arabinoxylans: structural features and structure-activity correlations. *Trends Food Sci. Technol.* 96:157–65
- Wang J, Sun B, Cao Y, Tian Y. 2009a. Protection of wheat bran feruloyl oligosaccharides against free radical-induced oxidative damage in normal human erythrocytes. *Food Chem. Toxicol.* 47(7):1591–99
- Wang J, Sun B, Cao Y, Wang C. 2010. Wheat bran feruloyl oligosaccharides enhance the antioxidant activity of rat plasma. *Food Chem.* 123(2):472–76
- Wang J, Yuan X, Sun B, Cao Y, Tian Y, Wang C. 2009b. On-line separation and structural characterisation of feruloylated oligosaccharides from wheat bran using HPLC-ESI-MSn. *Food Chem.* 115(4):1529–41
- Wong DWS, Chan VJ, Liao H. 2019. Metagenomic discovery of feruloyl esterases from rumen microflora. *Appl. Microbiol. Biotechnol.* 103(20):8449–57
- Wu H, Li H, Xue Y, Luo G, Gan L, et al. 2017. High efficiency co-production of ferulic acid and xylooligosaccharides from wheat bran by recombinant xylanase and feruloyl esterase. *Biochem. Eng. J.* 120:41–48
- Xia X, Zhu L, Lei Z, Song Y, Tang F, et al. 2019. Feruloylated oligosaccharides alleviate dextran sulfate sodium-induced colitis in vivo. *J. Agric. Food Chem.* 67(34):9522–31
- Yang J, Bindels LB, Segura Munoz RR, Martínez I, Walter J, et al. 2016. Disparate metabolic responses in mice fed a high-fat diet supplemented with maize-derived non-digestible feruloylated oligo- and polysaccharides are linked to changes in the gut microbiota. *PLOS ONE* 11(1):e0146144
- Yang J, Maldonado-Gómez MX, Hutkins RW, Rose DJ. 2014. Production and in vitro fermentation of soluble, non-digestible, feruloylated oligo- and polysaccharides from maize and wheat brans. *J. Agric. Food Chem.* 62(1):159–66
- Yao S, Wen X, Huang R, He R, Ou S, et al. 2014. Protection of feruloylated oligosaccharides from corn bran against oxidative stress in PC 12 cells. *J. Agric. Food Chem.* 62:668–74
- Yu X, Gu Z. 2013. *Aureobasidium pullulans* fermented feruloyl oligosaccharide: optimization of production, preliminary characterization, and antioxidant activity. *BioResources.* 9(1):241–55
- Yu X, Yang R, Gu Z, Lai S, Yang H. 2014. Anti-tumor and immunostimulatory functions of two feruloyl oligosaccharides produced from wheat bran and fermented by *Aureobasidium pullulans*. *BioResources.* 9(4):6778–90
- Yu X, Zhu X, Gu Z, Lai S. 2015. Antioxidant activity in vivo and in vitro of two feruloyl oligosaccharides preparations produced from wheat bran and fermented by *Aureobasidium pullulans*. *BioResources.* 10(2):2167–76
- Yuan X, Wang J, Yao H. 2006. Production of feruloyl oligosaccharides from wheat bran insoluble dietary fibre by xylanases from *Bacillus subtilis*. *Food Chem.* 95(3):484–92
- Yuan X, Wang J, Yao H, Chen F. 2005. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT Food Sci. Technol.* 38(8):877–83
- Zeng Y, Yin X, Wu M-C, Yu T, Feng F, et al. 2014. Expression of a novel feruloyl esterase from *Aspergillus oryzae* in *Pichia pastoris* with esterification activity. *J. Mol. Catal. B* 110:140–46
- Zhang H, Wang J, Liu Y, Gong L, Sun B. 2016. Wheat bran feruloyl oligosaccharides ameliorate AAPH-induced oxidative stress in HepG2 cells via Nrf2 signalling. *J. Funct. Foods.* 25:333–40

- Zhang H, Wang J, Liu Y, Sun B. 2015. Wheat bran feruloyl oligosaccharides modulate the phase II detoxifying/ antioxidant enzymes via Nrf2 signaling. *Int. J. Biol. Macromol.* 74:150–54
- Zhang H, Zhang S, Wang J, Sun B. 2017. Wheat bran feruloyl oligosaccharides protect against AAPH-induced oxidative injury via p38MAPK/PI3K-Nrf2/Keap1-MafK pathway. *J. Funct. Foods.* 29:53–59
- Zhang S-B, Zhai H-C, Wang L, Yu G-H. 2013. Expression, purification and characterization of a feruloyl esterase A from *Aspergillus flavus*. *Protein Expr. Purif.* 92(1):36–40
- Zhang X, Chen T, Lim J, Xie J, Zhang B, et al. 2019. Fabrication of a soluble crosslinked corn bran arabinoxylan matrix supports a shift to butyrogenic gut bacteria. *Food Funct.* 10(8):4497–504
- Zhao W, Chen H, Wu L, Ma W, Xie Y. 2018. Antioxidant properties of feruloylated oligosaccharides of different degrees of polymerization from wheat bran. *Glycoconj. J.* 35(6):547–59
- Zhao Z, Moghadasian MH. 2008. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: a review. *Food Chem.* 109:691–702