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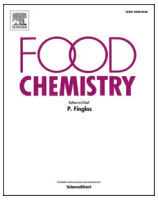
Food Chemistry 413 (2023) 135660

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Arabinoxylan supplemented bread: From extraction of fibers to effect of baking, digestion, and fermentation

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ARTICLE INFO

Keywords:
Dietary fibers
Feruloylated-arabinoxylans
Bread
Upper tract digestion
Probiotic fermentation

ABSTRACT

The intake of dietary fibers is related with important benefits for human health. We produced two different arabinoxylan fibers with (FAX) and without ferulic acid linked (AX), 12.5 and 0.1 mg g^{-1} of ferulic acid respectively, by subcritical water extraction of wheat bran. Both FAX and AX fibers were used as supplement in bread production, while non-supplemented bread was used as control. Through an enzymatic deconstruction process we investigated the effect of bread making on the fibers, the preservation of their molecular structure (A/X ratio of 0.13 and Mw of 10^5 Da) and the interaction with other macromolecules in the bread. By mimicking the upper track digestion, we could confirm the non-digestability of the fibers and we used them for the fermentation with B. ovatus and B. adolescentis. The presence of AX fibers during fermentation showed specific substrate adaptation by the probiotic bacteria in correlation with its potential prebiotic effect.

1. Introduction

Dietary fibers (DF) are known for its potential health benefits when consumed, which includes regulatory fuctions and microbial modulation of the gut as well as prevention and management of diseases (Gill, Rossi, Bajka, & Whelan, 2020; O'Grady, O'Connor, & Shanahan, 2019). By definition, DFs are components of plants, mostly carbohydrates, that are not digested or absorbed in the small intestine but can be completely or partially fermented in the large intestine (AACC, 2001). Fibers that additionally result in change on the composition and activity of the gut microbiota are considered as prebiotics (Pandey, Naik, & Vakil, 2015; Slavin, 2013). Several different types of DFs exist within families of macromolecules present in plants, and these include cellulose, hemicellulose, pectin, gums, resistant starch and lignin.

Among different natural fibers, arabinoxylan (AX) is largely present in our cereal-based diets. AX is present in wheat, rye, oat, barley, rice, corn, sorghum and millets, where it is mainly localized in the endosperm cell wall, aleurone layer and pericarp of the cereal grains (Roubroeks, Andersson, & Åman, 2000). The understanding and preservation of the fiber structure is essential to assess their potential as dietary fiber after digestion. Cereal AXs structure is composed of a (1 → 4)-linked-β-D-xylopyranosyl backbone with substitutions of α-L-arabinofuranose at the C(O)-3 and/or C(O)-2 position (M. S. Izydorczyk & Biliaderis, 2000). AXs can be further esterified to hydroxycinnamic acids, like ferulic acid and *p*-coumaric acid, at the C(O)-5 position of the arabinofuranose (Ayala-Soto, Serna-Saldívar, & Welti-Chanes, 2016). The hydroxycinnamic acid moieties provide antioxidant properties, which enhances the fiber property (Katapodis, Vardakou, Kalogeris, Kekos, Macris, & Christakopoulos, 2003). The ferulic acids, however, reduce the extractability of the AX, as they form junction points in the plant cell wall, crosslinking AX to other AXs, lignin and proteins (Carpita & McCann, 2000).

For the extraction of structuraly preserved AX fibers with ferulic acid still attached, as feruloylated arabinoxylan (FAX), subcritical water extraction (SWE) can be implemented maintaining both the ferulic acid moities and high molar mass of the polysaccharides (Ruthes, Martinez-Abad, Tan, Bulone, & Vilaplana, 2017). In SWE, water is heated and pressurized below the critical point (374 °C, 22 MPa), resulting in

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https://doi.org/10.1016/j.foodchem.2023.135660
Received 25 July 2022; Received in revised form 27 January 2023; Accepted 6 February 2023
Available online 10 February 2023

physicochemical changes, such as lowered polarity and viscosity, that allows for improved mass transfer and extractability of the targeted compounds (Mustafa & Turner, 2011). SWE is considered as a green technology, due to its sole use of water, short extraction times, and minimal downstream processing. The feasibility of the technology to be upscaled (Rudjito, Ruthes, Jiménez-Quero, & Vilaplana, 2019) also enables sufficient production of FAX for development of fiber-supplemented applications.

In baking industry, the addition of DFs in bread has become an attractive approach as more and more consumers opt for a healthier diet rich in fibers with a recommended intake of at least 25 g DFs a day by the FAO (WHO/FAO, 2003). Knowledge how bread-making and digestion can affect the integrity of DFs is important in understanding their potential health effects and how fiber-based products can be improved in the future. Normally, DFs are not digested in the upper gastrointestinal tract, but tests that confirm the structural integrity of the fibers are still important to perform. Here, an *in vitro* approach was used to test the upper gastrointestinal digestion, as it is under a controlled enviroment, of low cost and poses no ethical considerations (Chen et al., 2011).

Following digestion in the upper gastrointestinal tract, DFs can be totally or partially fermented by gut microbiota in the large intestine. During fermentation, bacterial growth increases, resulting in the colonic pH to decrease and antioxidant components to be produced, while the number of pathogenic bacteria decreases and the immune system is activated (Galanakis, 2019). In general, soluble DFs are more easily utilized by the gut microbiota than insoluble fibers. These fermentable fibers provide the main source of energy for the microbiota, while non-fermentable fibers can reduce the transit time of food and dilute the concentration of toxic and carcinogenic components in the food consumed. There are over 400 bacterial species comprising the gut microbiome (Junjie et al., 2012), and over 10 different phyla can contribute to beneficial health effects. This includes *Firmicutes* (10–50 % of bacteria in gut microbiota) and *Bacteroidetes* (up to 75 %), where both dominate the gastrointestinal tract (Walker et al., 2011). Studies have shown that *Bacteroidetes* encode enzymes that degrade complex plant polysaccharides and secretions of the gastrointestinal tract, such as the *N*-glycans (Salyers, Vercellotti, West, & Wilkins, 1977). Meanwhile, the *Bifidobacterium* from the phylum *Actinobacteria*, accounting for about 10 % in the human colon (Walker et al., 2011), utilize the energy from complex carbohydrates that are indigestible by human. During fermentation of DFs, short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate are produced as metabolites (Junjie et al., 2012), subsequently contributing to various health implications, such as homeostasis of the gut (Donohoe et al., 2011).

In this study, FAX and AX from wheat bran were used as supplements into bread, with the aim to investigate the effect of processing (bread making) and digestion (*in vitro* upper tract digestion and further probiotic fermentation) on their molecular structure (composition, molecular weight, and substitution pattern). Using enzymatic approaches, we showed the possibility to isolate 'endogenous' AX populations already present in wheat flour and compared them with the 'added' FAX and AX. The mixed AX populations (endogenous and added) were monitored throughout the baking and different digestion steps as well as during *in vitro* fermentation using selected probiotic bacteria (*Bacteroides ovatus* and *Bifidobacterium adolescentis*). We also compared FAX and AX, as preservation of ferulic acid for its antioxidant activity is generally favored in DFs. Our hypothesis was that the AXs will largely maintain their structural integrity during baking and upper tract digestion, whilst differences in AX structure will consequently affect their prebiotic effect and so the production of secondary metabolites by the selected bacteria. This study thoroughly investigated the effect of bread processing, upper tract digestion, and fermentation on AX dietary fibers, providing insight into their structural integrity and prebiotic effects.

2. Materials and methods

2.1. Materials

The flour sample was provided by Lantmännen (Stockholm, Sweden), it is commercialized as *Vetemjöl* Special (Nutrient content is provided in Supplementary Table S1). The wheat bran used to source the feruloylated arabinoxylan (FAX) and arabinoxylan (AX) was also provided by Lantmännen (Stockholm, Sweden). The bacterial strains *Bacteroides ovatus* CCUG 4943 and *Bifidobacterium adolescentis* CCUG 18363 were procured from the Culture Collection University of Gothenburg (Gothenburg, Sweden). All the chemicals were of analytical grade and obtained from Sigma-Aldrich (Stockholm, Sweden), unless otherwise stated.

2.2. Experimental processes

2.2.1. Production of FAX at pilot scale

Feruloylated arabinoxylans were obtained from wheat bran using a cascade process that included a pretreatment step (destarching), subcritical water extraction (SWE) and precipitation with ethanol. In the pretreatment step, 4 kg of wheat bran was added to pre-heated tap water (37 °C) in a 50 L reactor (Hastelloy C-22, Örnalp Unozon AB, Sweden) with a solid to liquid ratio of 1:10 (w/v). An alpha amylase was added to the mixture (120 mL of RONOZYME® HiStarch at 100 U/ml, DSM Nutritional Products, Switzerland) and the mixture was stirred at 150 rpm for 4 h. The mixture was then filtered through a filter bag (Nylon mesh filter, HRFilters, UK) with a pore size of 100 μm and pressed using a filter press (stainless basket, MacIntosh, USA). The destarched wheat bran was then washed with 40 L of cold tap water, followed by a second filtration step. The destarched wheat bran was composed of 35–40 % AXs (w/w) dry weight. The cascade process was performed in triplicate.

In the same reactor, tap water was added to the destarched wheat bran to a solid to liquid ratio of 1:10 (w/v) and preheated to 95 °C. The reactor was sealed, and the temperature was increased to 150 °C at a rate of 0.7 °C/min. Once the reactor had reached 150 °C, a cooling step followed at a rate of 1.1 °C/min. The hot mixture was then filtered in one filter bag with a pore size of 40 μm and pressed using the filter press as quickly as possible to avoid the cooling of the solvent during the dewatering step. The filtrate was cooled to 4 °C overnight in a plastic 20 L barrel.

Precipitation using ethanol was performed in a 100-L glass-lined reactor (chemReactor CR, Bûchi AG, Switzerland). The subcritical water extract was added to the reactor with 99.7 % (v/v) ethanol at a ratio of 1:4 (extract: ethanol). The slurry was stirred for 2 h, then the temperature was decreased to 4 °C and the polysaccharides were left to precipitate overnight. The precipitate was then filtered, washed 3 times with 99.7 % (v/v) at an equal volume to the extract and dried in a vacuum drying cabinet (VT 6025 Standard, Thermo; MA, USA) at 40 °C.

2.2.2. Removal of ferulic acid from FAX to produce AX

To produce arabinoxylans free of ferulic acid (AX), mild saponification was performed on the FAX extracts. FAX obtained after SWE was directly mixed with NaOH to a concentration of 0.5 M in a reactor (chemReactor CR, Bûchi AG, Switzerland) and stirred for 4 h at 20 °C. The solution was then neutralized using acetic acid until pH 7 and ethanol 99.7 % (v/v) was added at a ratio of 1:4 (NaOH solution: ethanol). The solution was then stirred for 2 h and cooled to 4 °C overnight to precipitate the ferulic acid free AX. The precipitate was filtered through a porous metal plate (pore size of 40 μm) and washed 3 times with 75 % (v/v) ethanol and dried in a vacuum drying cabinet (VT 6025 Standard, Thermo; MA, USA) at 40 °C. The removal of ferulic acid was performed in triplicate.

2.2.3. Baking of bread samples

The wheat pan breads were prepared with a farinograph via the

straight-dough procedure (AACC Method 10–10.03). The weight ratio of ingredients for the bread included: wheat flour or flour plus fiber fraction (1 0 0), oil (2.5), sugar (5), salt (1.5), yeast (4.6), microencapsulated sorbic acid (0.15), and water (variable, between 50 and 60). Part of the flour was replaced by fiber fractions to obtain an AX content of 1.0 % (w/w) of flour. Fiber fractions (FAX and AX) were dissolved in water before baking by stirring while heating to 80 °C and cooling down to room temperature (RT). Three bread samples were analyzed in this study, which included bread control (B-C), bread containing 1 % (w/w) FAX (B-FAX) and Bread containing 1 % (w/w) AX (B-AX).

2.2.4. Enzymatic deconstruction (ED)

Enzymatic deconstruction was performed using α-amylase 14 U/mg from porcine pancreas type VI-B, amyloglucosidase 3300 U/mL from *Aspergillus niger* (Megazyme, Wicklow, Ireland), lichenase 250 U/mL from *B. subtilis* (Megazyme Wicklow, Ireland) and protease K 30 U/mg from *Tritirachium album* to isolate arabinoxylans from other biomacromolecules. 100 mg samples (flour or breads) were suspended with 10 mL phosphate buffer and boiled for 15 min. After cooling down, α-amylase (11.4 mg) and lichenase (8 μL) were added and the mixture was incubated overnight at 37 ℃ with stirring. The next day, the pH value of the mixture was adjusted to 4, and amyloglucosidase (21 μL) was added and the mixture was further incubated for 3 h at 37 ℃ with stirring. The pH value of the sample solution then was re-adjusted to 7–8 before adding protease (0.33 mg) and the mixture was incubated again for 4 h at 37 ℃ with stirring. The solution was boiled for 15 min for enzyme inactivation and the digested sample was dialyzed against deionized water through a 6–8 kDa membrane (Spectrum Laboratories Inc., CA, USA) for 24 h and freeze-dried until further analysis.

2.2.5. In vitro upper tract digestion (UTD)

The mimic of the upper gastrointestinal tract digestion was divided into three steps which simulated the food digestion in the mouth, stomach, and small intestine, respectively (Tuncil, Thakkar, Arioglu-Tuncil, Hamaker, & Lindemann, 2018). 200 mg sample was suspended in 20 mL phosphate buffer (20 mM, pH 6.9). 2 mL of salivary α-amylase (57.1 U/ml in 1 mM $CaCl_2$) (Megazyme, Wicklow, Ireland) was added and incubated for 15 min at 37 °C with stirring. The pH value of the sample solution was adjusted to 2 by 6 M HCl, and 2 mL of pepsin (2.5 mg/ml in 15 mM HCl) was added and incubated for 2 h at 37℃ with stirring. The pH value was re-adjusted to 7 using 6 M NaOH, then 6 mL of pancreatic juice (12.5 g/L NaHCO3, 5 g/L bile salts, 0.9 g/L pancreatin in phosphate buffer) was mixed and incubated for 2 h at 37 °C with stirring. Inactivation of the enzymes was performed by boiling for 20 min, followed by dialysis against deionized water through a 6–8 kDa membrane (Spectrum Laboratories Inc., CA, USA) for 24 h. The digested product was freeze-dried for further analysis.

2.2.6. Fermentation by gut microorganisms

The fermentation process was modified for studying the bread samples (Zeybek, Rastall, & Buyukkileci, 2020). *Bacterial activation*. 30 μL of *Bacteroides ovatus* was inoculated into 3 mL BHI-S medium containing heat sensitive components (150 μL L-cysteine 10 g/L, 30 μL hemin, 3 μL resazurin 0.1 % w/v, 60 μL $NaHCO_3$ 10 % w/v). 30 μL of *Bifidobacterium adolescentis* was inoculated into 3 mL MRS medium with 150 μL L-cysteine at 10 g/L. The inoculated media were incubated for 48 h under anaerobic atmosphere at 37℃. *Fermentation with digested bread sample (from UTD)*. For *B. ovatus*, 3 mL fermentative medium (2 g/l peptone water, 2 g/l yeast extract, 0.1 g/l NaCl, 0.04 g/l KH_2PO_4, 0.04 g/l $MgSO_4{\cdot}7H_2O$, 0.01 g/l $CaCl_2{\cdot}6H_2O$, 0.5 g/l L-cysteine hydrochloride, 0.05 g/l hemin, 0.001 g/L resazurin, 2 g/L $NaHCO_3$ and 1 mL of digested product 5 mg/mL) were boiled and later mixed with heat sensitive components as described above, then inoculated with 40 μL of activated *B. ovatus* culture. For *B. adolescentis*, 40 μL of activated culture was inoculated into pre-boiled 3 mL fermentative medium and 1 mL of digested product with heat sensitive components. *Enumeration of bacteria*. At 8 h, 24 h and 48 h, 1 mL aliquot of the cultured mixture was taken and diluted using 0.85 % (w/v) NaCl. Then 100 μL of diluted culture was inoculated into the solid media in triplicates and incubated for 48–72 h. Two different solid media were used for the enumeration of the bacteria. Wilkins-Chalgreen agar + 2 % defrinated horse blood (ThermoFisher Scientific, Stockholm, Sweden) was used for enumerating the growth of *B. ovatus*, while Reinforced Clostridial agar (ThermoFisher Scientific, Stockholm, Sweden) was applied for *B. adolescentis*. The colonies were presented in terms of colony forming units (CFU).

2.3. Analysis methods

2.3.1. Yields determination

The yields obtained from the subcritical water extraction, enzymatic deconstruction and upper tract digestion were determined gravimetrically and conducted based on the dry weight.

2.3.2. Monosaccharide analysis by HPAEC-PAD

Two common acid hydrolysis methods were utilized in this study. *Sulfuric acid hydrolysis*. 4 mg sample was suspended with 125 μL 72 % (v/v) H_2SO_4 and incubated for 1 h at RT with stirring. The mixture was added with 1375 μL MilliQ water and hydrolysis was continued for 3 h at 100 ℃ in the thermo-block. After cooling down to RT, the mixture was diluted by 1:10 with deionized water and filtered through Chromacol 0.2 μm filters (Thermo Scientific, USA) into plastic vials for analysis (Saeman et al., 1954). *Trifluoroacetic acid (TFA) hydrolysis*. 1 mg sample was mixed with 1 mL of 2 M TFA. After 3 h of hydrolysis at 120℃ in the thermo-block, 100 μL of the hydrolyzed solution was transferred into a new glass tube and air dried overnight (Taylor, Taylor, Campanella, & Hamaker, 2016). The dried sample was redissolved in 1000 μL deionized water and filtered through Chromacol 0.2 μm filters into plastic vials. Separation was perfromed on the HPAEC-PAD (ICS 3000 system, Dionex, Sunnyvale, CA) with a PA1 column according to (McKee et al., 2016). Above measurements were performed in triplicate.

2.3.3. Total starch

The starch content was determined by the Megazyme Total Starch Assay Kit in two set of triplicates (K-TSTA-50A, Megazyme), with modifications. 1 mg of sample grinded in fine powder by ball milling was mixed with 20 μL of aqueous ethanol 80 % (v/v) to wet the sample and aid dispersion in an Eppendorf tube. Immediately 300 μL of thermostable α-amylase (300 U in 100 mM sodium acetate buffer pH 5.0) was added and the tube was incubated for 12 min at 100 ℃ in the thermo-block with stirring after 4, 8 and 12 min. Then the tube was put in the thermo-block at 50 ℃ and mixed with 10 μL of amyloglucosidase (330 U, Megazyme) and then incubated at 50 ℃ for 30 min with stirring. The volume was adjusted with MilliQ water to 1 mL and the tube was centrifuged at 3000 rpm for 10 min. 40 μL of the supernatant was then mixed with 960 μL GOPOD solution and incubated for 20 min at 50 ℃. Finally, the D-glucose was measured at 510 nm by spectrophotometer (Cary-50 Bio Varian, Agilent, USA). Above measurements were performed in triplicate.

2.3.4. Protein content

The protein content of the soluble extracts was determined by Bradford method in duplicates (Bradford, 1976).

2.3.5. Solid-State NMR

Solid-state CP/MAS 13C NMR experiments were performed with a Varian Inova-600 spectrometer (Varian Inc., CA, USA) operating at 14.7 T and equipped with a 3.2 mm solid-state MAS probe. Measurements were conducted at 298 K with a MAS spinning rate of 10 kHz. A CP/MAS 13C NMR pulse sequence with a SPINAL-64 decoupling sequence was used. Acquisition parameters included a 2.9 ms 1H pulse, a 900 ms CP-contact time, 10 ms acquisition time, 5 s recycle delay, and 12 000 scans. The chemical shifts were referenced to adamantane with the CH_2 signal

being set to 38.48 ppm.

2.3.6. Molar mass distribution

The molar mass distribution was determined by Size Exclusion Chromatography (SECurity 1260, Polymer Standard Services, Mainz, Germany) using a with a refractive index detector at 45 ℃. 1 mg of sample was solubilized with 300 μL of dimethyl sulfoxide (DMSO) with 0.5 % w/w LiBr. The solution was mixed at 50 °C overnight, then filtered by 0.2 μm TPFE filters to vials for *SEC* analysis. The separation was carried through GRAM Analytical columns of 100 and 10000 Å (Polymer Standard Services, Mainz, Germany) with 20 μL at a flow rate of 0.5 mL/min and 60 °C. Linear pullulan standards were used as reference (Ruthes, Martinez-Abad, Tan, Bulone, & Vilaplana, 2017).

2.3.7. Xylanase activity

The method was adapted from previous approaches (Cleemput, Bleukx, van Oort, Hessing, & Delcour, 1995). 0.5 g of sample was mixed with 2 mL sodium acetate buffer (1:4 w/v, 100 mM, pH 5). Then, the mixture was equilibrated at 37 ℃ with stirring for 5 min. A sample solution was added to the 10 mL falcon tube with pre-weighing 4 mg of AZCL-arabinoxylan (I-AZWAX, Megazyme, Wicklow, Ireland). The negative control only contained sodium acetate buffer and AZCL-arabinoxylan. Sample solution and negative control were incubated at 37 ℃ for 2 h with stirring. After incubation, 5 mL of 2 % (w/v) Tris-base solution (pH 10) was added to stop the reaction. The blank solution was prepared by adding the Tris-base solution to the sample buffer solution before adding AZCL-arabinoxylan. The mixture was centrifuged at 4000 rpm for 10 min, then 1 mL supernatant was transferred into 1 cm cuvettes and read at 590 nm by a spectrophotometer. As positive controls, *endo*-1,4-β-xylanase from GH10 (E-XYNACJ, Megazyme, Wicklow, Ireland) and an enzymatic cocktail rich in xylanase from GH11 (Ronozyme-WX2000, Novozymes, Bagsværd, Denmark) were used in this study. Above measurements were performed in triplicate.

2.3.8. Ferulic acid content

The content was analyzed by saponification of the phenolic compounds by HPLC analysis in duplicates (Rudjito et al., 2019). 10 mg of the sample was suspended with 500 μL of 2 M NaOH in amber 1.5 mL tubes. The mixture was flushed with N_2 and left overnight at 30 ℃ under dark stirring conditions. The mixture was acidified by 12 M HCl to pH 3, then the mixture was partitioned using 800 μL ethyl acetate (3 times). The ethyl acetate fraction was then transferred into clean glass tubes. The sample was dried with a constant stream of N_2 and covered with aluminum foil to avoid light. The dried sample was redissolved in 500 μL solution of 2 % acetic acid: methanol (1:1, v/v). Finally, the solution was filtered through 0.2 μm filters to vials for HPLC analysis. Analysis was performed using the ZORBAX StableBond C18 column (Agilent Technologies, Santa Clara, CA, USA) fitted to a Waters 2695 separation module (Waters Corporation, Milford, MA, USA) and a photodiode array detector (Waters Corporation, Milford, MA, USA) at 200–400 nm. Separation was achieved using a method previously described (Rudjito et al., 2019). The measurements were performed in triplicate.

2.3.9. Glycosidic linkage analysis

The glycosidic linkage analysis of fibers was performed in accordance with Pettolino, Walsh, Fincher, and Bacic (2012). In brief, samples were per-*O*-methylated, acid hydrolyzed using TFA, reduced and derivatized as alditol acetates. The per-*O*-methylated alditol acetates (PMAA) were then analyzed using the GC–MS with a separation method as described formerly (Rudjito, Ruthes, Jimenez-Quero, et al., 2019). Detected PMAAs were corrected with the monosaccharide content of the polysaccharides obtained after TFA acid hydrolysis.

2.3.10. Antioxidant activity by DPPH

The antioxidant activity of the dietary fibers and bread after upper tract digestion was measured on DPPH radicals with modification to a 96-well microtiter plate, as previously described (Brand-Williams, Cuvelier, & Berset, 1995).

2.3.11. Short chain fatty acid analysis

The short chain fatty acids (SCFA) released during fermentation were analyzed using the HPLC (Waters 2695, Milford, MA, USA) coupled to a photodiode array detector (Waters 2996, Milford, MA, USA) at 210 nm in duplicate. Separation of the acids was performed using the Aminex® HPX-87H ion exclusion column at 60 °C (Bio-Rad, CA, USA) with an isocratic flow of 5 mM H_2SO_4 at 0.6 mL/min. The measurements were performed in triplicate. For standards, a mixture of acetate, propionate, butyrate, pyruvate, lactate, fumarate and succinate were used.

2.3.12. Bioinformatic analysis

The information fo polysaccharide degrading enzymes were collocted from cited literatures, Carbohydrate-active enzymes database (CAZY, https://www.cazy.org/) and National Center for Biotechnology Information database (NCBI). (https://www.ncbi.nlm.nih.gov/). For *Bacteroides ovatus* α-glucosidase, three reported α-glucosidase from *Bacteroides thetaiotaomicron* (*sus_1, sus_2 and sus_3*) were used as reference sequences to find the homologues in *Bacteroides ovatus*. Three homologues, KAA3962140.1 (susb_1), WP_148364530.1 (susb_2), WP_149944418.1 (susb_3), were found with 99 %, 100 %, 99 % query cover and 94.1 %, 90.8 %, 88.9 % identity, respectively. Homologues searching was performed by BLASTP from NCBI website.

3. Results and discussion

3.1. Pilot-scale production of feruloylated and non-feruloylated arabinoxylans

In the first part of this study, we extracted and fractionated feruloylated arabinoxylan (FAX) and non-feruloylated arabinoxylan (AX) from wheat bran using a multi-step approach. As the aim was to obtain large amounts of dietary fibers (DFs) to be supplemented into bread, a pilot scale process was performed, involving enzymatic pretreatment to remove starch and β-glucans, subcritical water extraction (SWE) and ethanol precipitation. Here, SWE was opted as the method of choice, as it could result in high molar-mass arabinoxylans with ferulic acid still attached to the AX core i.e, FAX (Rudjito et al., 2019; Ruthes et al., 2017). To obtain AX from FAX, mild saponification was additionally performed on the FAX extracts. A summary of the yields and composition of FAX and AX produced in shown in Table 1.

The process resulted in arabinoxylans yields of 12 % DW for both FAX and AX after ethanol precipitation. Due to the extensive removal of starch and β-glucans in the pretreatment step, relatively pure arabinoxylan fractions were obtained ranging at 729.9 and 789.3 mg/g, for FAX and AX, respectively (Fig. 1A). Both fibers exhibited an arabinose to xylose (A/X) ratio of 0.13, which was found somewhat low for wheat arabinoxylans (M. Izydorczyk & Biliaderis, 2007; Rudjito et al., 2019). The low substitution could be caused by prolonged exposure to heat and temperature during pilot-scale SWE, resulting in acidification of the solvent and cleavage of labile arabinoses in furanose form (Grohmann & Bothast, 1997). Nonetheless, the SWE preserved the ferulic acid on the FAX at 12.5 mg/g and both fibers exhibited a high molar mass of 10^5 Da (Fig. 1B). The presence of ferulic acid can further contribute to antioxidant properties and modulation of the gut microflora (Castelluccio, Bolwell, Gerrish, & Rice-Evans, 1996; Snelders et al., 2014), while in terms of bread making, ferulic acid can affect the extensibility of the dough (Pietiäinen, Moldin, Ström, Malmberg, & Langton, 2022). Therefore, to study the effect of ferulic acid in bread making and digestion, AX was produced from FAX, exhibiting a significantly lower ferulic acid content of 0.1 mg/g.

Table 1
Yields and composition of FAX and AX obtained from pilot scale extraction.

	Feruloylated arabinoxylan (FAX)	Non-feruloylated arabinoxylans (AX)
Yields (% dry weight) [a]		
Pretreatment of wheat bran	61.6 (6.5)	57.7 (3.1)
Pilot scale SWE	20.1 (0.8)	21.3 (2.8)
Ethanol precipitation	12.4 (0.9)	12.3 (1.3)
Composition of ethanol precipitated fraction (mg/g)		
Carbohydrate [b]	833.5 (225.6)	932.0 (55.5)
Arabinoxylan [b]	729.9 (197.1)	789.3 (46.8)
A/X [c]	0.13 (<0.01)	0.13 (<0.01)
Starch [d]	82.6 (17.6)	74.2 (15.9)
β-glucans [e]	10.5 (7.9)	62.3 (7.7)
Other sugars [b]	19.7 (5.7)	19.8 (1.7)
Proteins [f]	28.1 (1.8)	21.6 (3.9)
Ferulic acid [g]	12.5 (0.5)	0.1 (0.06)

Number in brackets show standard deviation of triplicates.
[a] Yields were determined on gravimetrical basis.
[b] Monosaccharide content was determined using TFA hydrolysis followed by HPAEC-PAD analysis.
[c] A/X ratio was determined by comparing the content Ara by Xyl.
[d] Starch content was determined enzymatically using the Total Starch Kit (Megazyme, Ireland).
[e] β-glucan content was determined as the difference between the total glucan and starch content.
[f] Protein content was determined using the Bradford method.
[g] Ferulic acid content was determined by saponification followed by HPLC analysis.

3.2. Characterization of bread samples revealed two distinct AX populations

To understand how the DFs affected the composition of the bread samples, compositional analysis was performed on the flour and bread samples (Fig. 1A). As expected, the flour contained mostly glucans at 784.4 mg/g, whereby 730.0 mg/g was composed of starch. In terms of molar mass, the presence of starch was clearly marked by the presence of high molar mass populations with distributions of 10^6-10^8 Da (Fig. 1B). The flour also contained a small amount of endogenous AX, at 30.0 mg/g, which was significantly more substituted than the extracted AX, exhibiting an A/X ratio of 0.45. This value corresponded well with AX originating from the cell walls of wheat endosperm that ranges between 0.44 and 0.46 (Gartaula, Dhital, Pleming, & Gidley, 2017).

Moreover, the carbohydrate composition of the bread samples revealed that the addition of fibers not only increased the AX content, but also decreased the A/X ratio from 0.39 in the bread control (B-C), to 0.24 and 0.27 in bread FAX (B-FAX) and bread AX (B-AX), respectively. The decrease in A/X ratio was likely caused by the mixing of endogenous and added AXs (Fig. 1C). The bread samples also showed lower glucan contents as compared to the flour. Here, interactions formed during bread making, i.e. formation of starch-gluten complexes as confirmed from the reduced soluble protein content in the breads (Fig. 1D), likely made the breads less soluble and more recalcitrant to hydrolysis with trifluoroacetic acid (TFA). It was, thus, likely that not all the glucan content was accounted for in the bread samples. In an effort to more accurately quantify the glucans, a harsher hydrolysis method by employing sulfuric acid was used on the bread samples. The harsher acid resulted in more comparable glucan contents to that of the flour, however, at the cost of the xylose residues being severely degraded and understimated (Supplementary Figure S1). As the structure of the fibers were the primary focus of this study, we opted to continue with TFA on all the following hydrolysis.

Also, the bread samples showed slightly lower starch content after the addition of fibers due to the replacement in total mass (Fig. 1A) but also by the formation of complex networks between the starch and lipids (amylose–lipid complex) and starch and fibers, which prevented the starch from being fully measured. AX can interact with starch and proteins on the starch surface, preventing swelling and dissolution of the starch (Pietiäinen, Moldin, Ström, Malmberg, & Langton, 2022). In addition, AXs also result in increased competition for water, among fibers, starch and protein during dough making, consequently influencing the formation of the gluten-starch matrix (Roozendaal, Abu-hardan, & Frazier, 2012) and induction of gluten aggregation (Nawrocka, Miś, & Niewiadomski, 2017). Such changes could have resulted in a tighter starch structure in B-FAX and B-AX, and thus stronger resistance towards hydrolysis during analysis.

During bread making, the increase in water content and temperature could result in the activation of endogenous enzymes present in flour, which include peptidases, amylases and xylanases (Arendt & Zannini, 2013). To test whether active endogenous xylanases were indeed present, which could consequently alter the polymeric structure of both the endogenous and added AXs during bread making, we analyzed the xylanase activity of the flour (Fig. 1E) (Cleemput et al., 1995). Results on the chromogenic AZCL-arabinoxylan substrate revealed that indeed xylanase activity was present in the flour, that is at a considerably lower

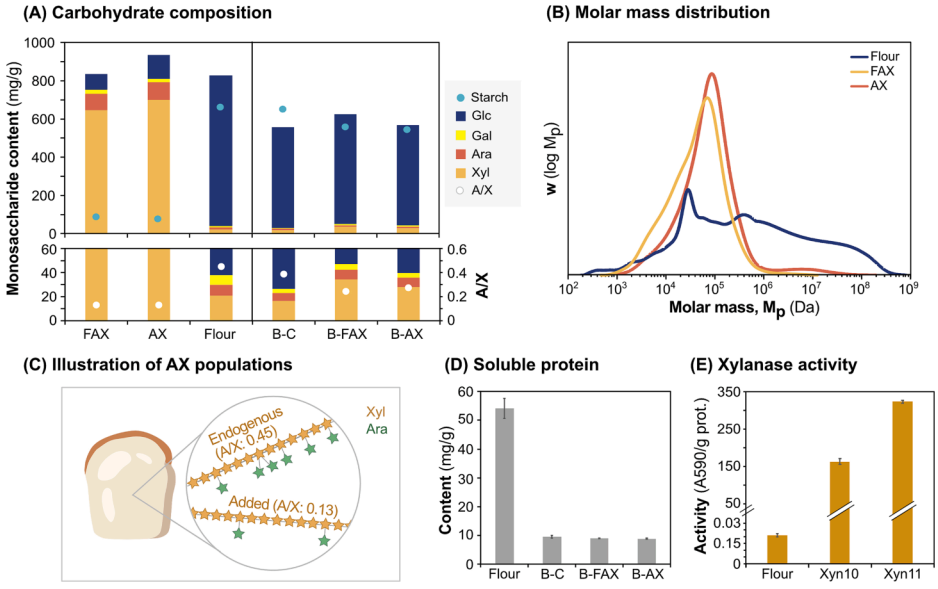


Fig. 1. Characterization of bread samples and its main ingredients. (A) Carbohydrate composition represented as monosaccharides after TFA acid hydrolysis and HPAEC-PAD analysis. Starch content was determined enzymatically. Different scales used to zoom in the fiber fraction. (B) Molar mass distribution of AXs and flour determined using *SEC*. (C) Illustration highlighting the presence of two arabinoxylan (AX) populations: 'endogenous' AX present in the flour and 'added' AX into the supplemented breads. Both AXs coexist in the supplemented breads. (D) Soluble protein content determined using the Bradford method. (E) Xylanase activity of flour in comparison to commercial enzymes determined using the AZCL-arabinoxylan assay. Note: AX – arabinoxylan, FAX – feruloylated arabinoxylan, BC – Bread control, B-FAX – Bread containing 1 % FAX and B-AX – Bread containing 1 % AX.

degree than pure xylanases. Such results implied that there is the possibility of the AXs to undergo enzymatic degradation during the bread making, although likely minimal.

3.3. Enzymatic deconstruction: A tool to isolate arabinoxylans of interest

To understand the molecular structure of the inherent AX in the flour as well as the mixture of the inherent and added AXs in the bread samples in more detail, we performed enzymatic deconstruction (ED) to remove the majority of interfering biomacromolecules (starch, mixed-linked β-glucans and proteins) present in the samples (Fig. 2A). A combination of α-amylase and amyloglucosidase was used to degrade the starch into dextrins and glucose. Meanwhile lichenase and protease were added to degrade mixed-linked β-glucans and proteins, respectively. The degraded components were dialyzed out, leaving the polymeric AX-rich fraction to remain after ED. The ED approach worked well on the flour, as we could enrich the AX content from 30 mg/g in flour to 566.1 mg/g (67.9 % of the total carbohydrate content) in flour ED (Fig. 2B). Interestingly, the AX in flour ED exhibited a higher A/X ratio (0.64) than that of the initial flour (0.45). This suggests small linear xylooligosaccharides were likely present in the flour and subsequently removed during the dialysis step in ED. Meanwhile, the other remaining components were glucose (176.1 mg/g) and galactose (93.1 mg/g), which likely originated from resistant starch, β-glucans and/or arabinogalactan. In terms of the molar mass distribution (Fig. 2D), the AX-rich flour ED displayed a dominating peak at around 10^4-10^5 Da, which likely corresponded to endogenous AX, while the shoulder peak at 10^6 could represent partly digested starch. Native starch normally exhibits two peaks, one corresponding to amylopectin and another to amylose (H. Li, Gilbert, & Gidley, 2021). Subsequently, the enzymatic digestion might affect both populations differently, and there might be some residual amylose after enzymatic digestion that would elute close to the AX peak, probably around 10^6 Da.

In the bread samples, the ED approach did not work as well as with the flour. Here, the AX content was enriched by one order of magnitude, from 22.8 to 42.5 mg/g in the initial bread samples to 364.7–392.3 mg/g in the bread ED samples. However, most of the content was still glucans that could not be effectively removed (Supplementary table S3). As mentioned previously, the formation of a strong starch-gluten matrix during bread making likely impeded the enzymes from being able to effectively cleave the starch. Hindrance could be caused by the decrease in solubility and/or physical obstruction of the starch-gluten complex. This complex seems to be present in the samples as the non-measurable fraction of the composition, which was most evident for the B-AX ED at almost 20 % (Fig. 2B).

Nevertheless, additional information could still be obtained regarding the inherent and added AXs. For instance, the A/X ratios were once again higher in the bread ED samples (0.31–0.46) compared to the

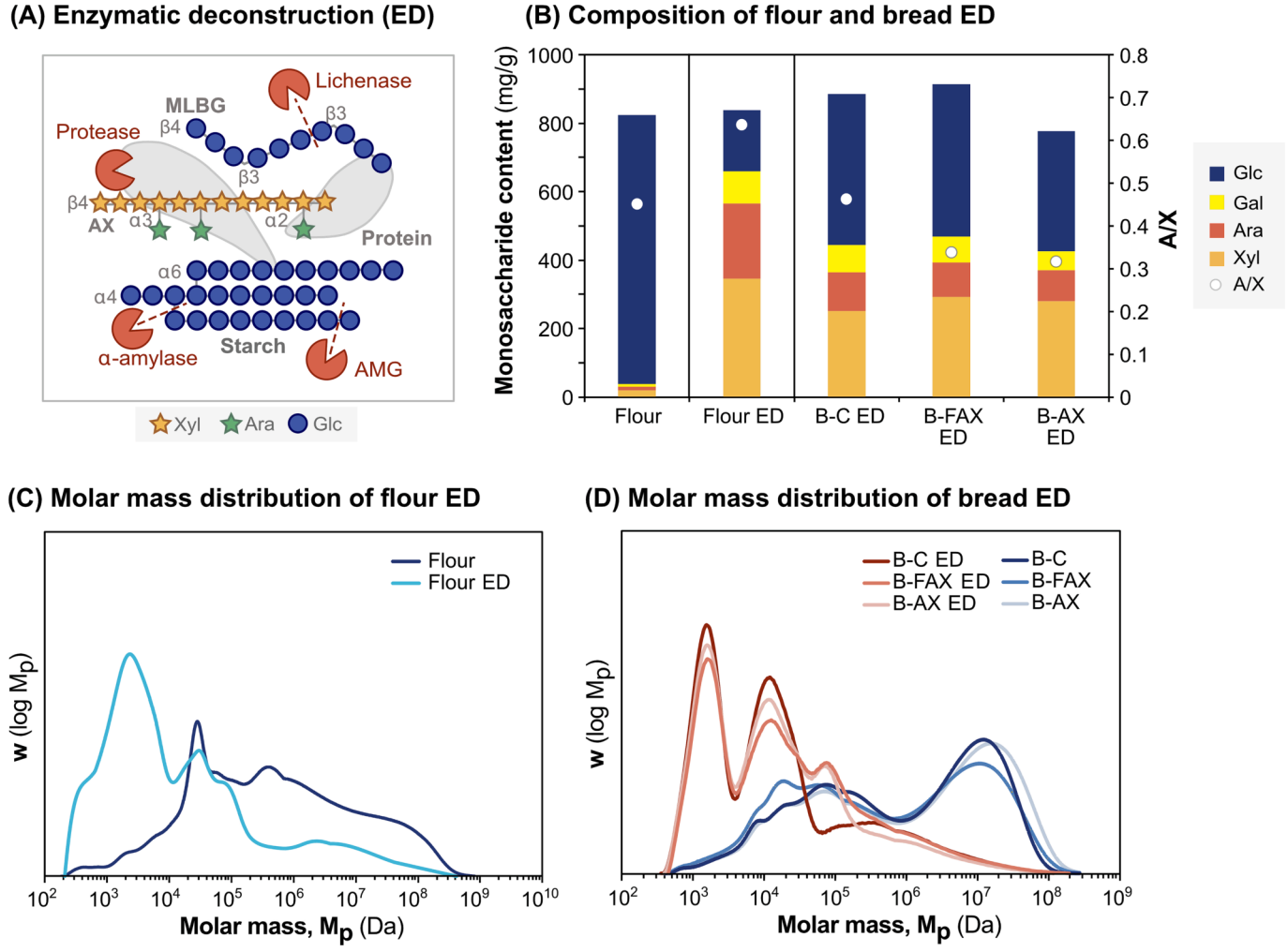


Fig. 2. Enzymatic deconstruction: isolating DF of interest. (A) Illustration of the enzymatic deconstruction (ED) process involving α-amylase, amyloglucosidase (AMG), lichenase and protease to hydrolyze starch, mixed-linked β-glucans (MLBG) and proteins, respectfully. (B) Monosaccharide composition of flour and bread after enzymatic digestion (ED) in comparison to the initial flour. White point represents the ratio between arabinose and xylose in the samples. Molar mass distribution as equivalent of pullulan of (C) flour before and after ED and (D) bread samples before and after ED.

initial breads (0.24–0.39). This supports the fact that the flour contains small linear xylooligosaccharides, which were incorporated in the bread, but were then removed during dialysis in the ED process. Regarding the molar mass distribution, ED could remove the large populations at 10^6-10^7 Da in the bread samples, corresponding to starch complexes. After ED, the molar mass distributions were enriched with smaller distinct populations at 10^3, 10^4 and 10^5 Da respectively (Fig. 2D). The peak at 10^5 Da clearly represented the added FAX and AX, as it was present in B-FAX and B-AX but not B-C, while the 10^4 Da peak represented the inherent AX, β-glucans and/or proteins. The 10^3 Da peak could correspond to partly digested glucan fibers that could not be removed during dialysis.

Solid-state NMR spectroscopy was used to confirm the gluten protein presence and content with respect to carbohydrates present after digestion. Fig. 3 displays a magnified part of the CP/MAS 13C NMR spectra of wheat flour (blue) and B-AX (pink), respectively, with full spectra shown as inset. Carbohydrate moieties (from glucans, xylans, etc) are strictly found in the 50–110 ppm region whereas the gluten protein signals arise around 173, 130, and 30 ppm, respectively, but also to some extent in the overlapped carbohydrate region (Li et al., 1996). In the latter spectral region, within 50–110 ppm, the gluten NMR signals correspond to 30–40 % of the total gluten NMR integral intensity as calculated from previously reported spectra (S. Li, Dickinson, & Chinachoti, 1996; Uriyo, 1998). By calculating the integral intensities of the four marked spectral regions in Fig. 3, and including the influence of the overlapped 50–110 ppm gluten signals, the quantity relationship between gluten protein and carbohydrate could be estimated. It was found that the protein-to-carbohydrate ratio is approximately 4 times higher in B-AX compared to wheat flour. After baking this macromolecular interaction could be created, contributing to a less digestible glucans as shown in Fig. 2B.

3.4. Both FAX and AX remained structurally intact during upper tract digestion (UTD)

In vitro digestion is a widely accepted approach for assessing food digestion rate and its physiological effects, as the methods are efficient, low cost and cause non-ethical problems (Chen et al. 2011). In our study, we constructed an *in vitro* workflow consisting of four steps, simulating the human oral, gastric and small intestinal conditions, with aims to monitor how these conditions would affect the integrity of the bread samples (Fig. 4A). The salivary α-amylase was used to simulate starch digestion into maltose and glucose in the human oral cavity. Then, the pH was acidified to 2 and pepsin was added to simulate the acidic

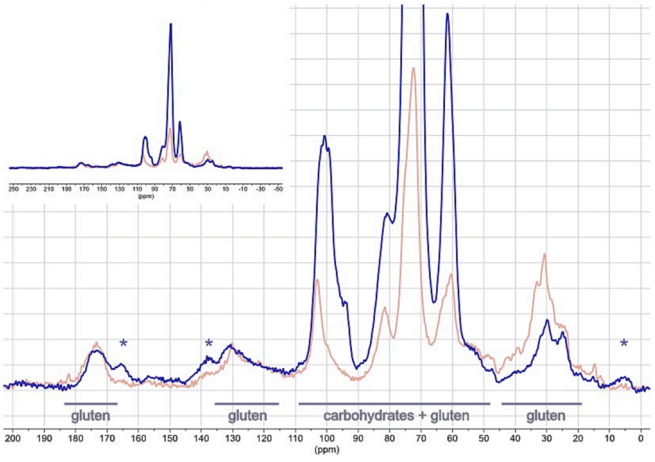


Fig. 3. Solid-state NMR of flour (blue) and B-AX ED (pink) samples. Chemical shift signal of the gluten proteins and region of gluten + carbohydrates. Spinning sidebands are denoted by *.

environment and gastric fluids in the human stomach. In the human small intestinal, both digestion and absorption occurs simultaneously. A neutral environment (pH 6–7) is needed for the activity of pancreatic enzymes, while bile salts secreted from the gallbladder can emulsify fats into tiny droplets, which improves pancreatic enzyme activity. Thus, pH was adjusted to 7, and bile salts and pancreatin were used to mimic the real digestion situation in step 3 of Fig. 4A. After food digestion in the small intestine, carbohydrates are degraded into monosaccharides, proteins into peptides and amino acids, while dietary fat into fatty acids and 2-monoglycerides (Campbell, Berry, and Liang 2019). In the last step of the workflow, a 6–8 kDa membrane was utilized for mimicking the food absorption in the small intestine.

In terms of the yields after UTD (Fig. 4B), B-C UTD was higher than both B-FAX UTD and B-AX UTD, the addition of the fibers in B-FAX and B-AX containing 1 % (w/w) FAX and 1 % (w/w) AX has decreased the efficiency of the digestion because of the formation of complexes as discussed previously. Meanwhile, in terms of the total monosaccharide content, the AX in all samples UTD increased almost 3-fold in comparison to their initial contents (Fig. 4C). The main reason was that starch was digested and removed during UTD, whereby starch contents dropped by over 50 % after UTD (Supplementary Table S4). Non-starch glucans were inversely enriched with the degradation of starch. The arabinose-to-xylose ratio in bread samples after UTD were increased compared to initial bread samples (supplementary Table S4). These results showed that monomers or oligosaccharides of xylan generated after baking could be removed during the UTD, letting only poligomeric fibers of arabinoxylans and non-starch glucans in the samples.

The protein contents in bread after UTD were measured at approximately 35 mg/g (Fig. 4D). Compared to the initial bread samples (Fig. 1D), the protein concentrations of bread after UTD increased. This increase was likely caused by the degradation of proteins, which made them more soluble and hence more measurable by the Bradford method. In the upper digestive tract, proteins are generally digested by pepsin and pancreatin into a dimer or trimer of peptides and amino acids (Campbell et al., 2019).

As the fibers were enriched after UTD, the measured content of ferulic acid after UTD remained close to their initial concentration. This discrepancy was likely attributed to an underestimation of the intact initial bread samples (Fig. 4E). Ferulic acid plays a valuable role in exhibiting antioxidant activity of fibers and in bread, as shown in Fig. 4F. The initial FAX exhibited a low EC_{50} value of 1.6 mg extract /g DPPH, which was attributed to high ferulic acid content of 12.5 mg/g. The EC_{50} value of FAX was only slightly higher compared to pure ferulic acid and ascorbic acid, exhibiting EC_{50} values of 0.4 and 0.1 mg/g, respectively. After the upper tract digestion, B-FAX UTD still maintained its antioxidant activity, exhibiting an EC_{50} value of 7.1 mg/g. This highlighted that the ferulic acid remained attached to FAX and was still able to exert antioxidant activity as part of the digested bread. The B-C and B-AX were also tested but did not result in any measured antioxidant activity, likely due to the low or negligible amounts of ferulic acid (Supplementary Table S4).

The results obtained elucidated that FAX and AX were not digested after UTD. To examine if the structure of fibers were still integrated after UTD, we determined their molar mass distribution before and after UTD (Fig. 4G). It showed that large polymers (between 10^5 – 10^8 Da), corresponding to starch and gluten complexes in B-C UTD, B-C FAX UTD, B-C AX UTD, were degraded after UTD. The peak between 10^4-10^5 Da likely represented the inherent and added AX, while the peak at 10^3 Da that emerged after digestion could represent the degraded starch or digested proteins that were not removed by dialysis. The difference among the molar mass distributions of B-C UTD, B-C FAX UTD, B-C AX UTD were not significant, highlighting that the added fibers did not influence the interactions of starch-gluten complexes.

To further confirm that the UTD did not significantly affect the structure of the AXs, we performed glycosidic linkage analysis on the fibers before and after UTD. Here, the fibers were analyzed

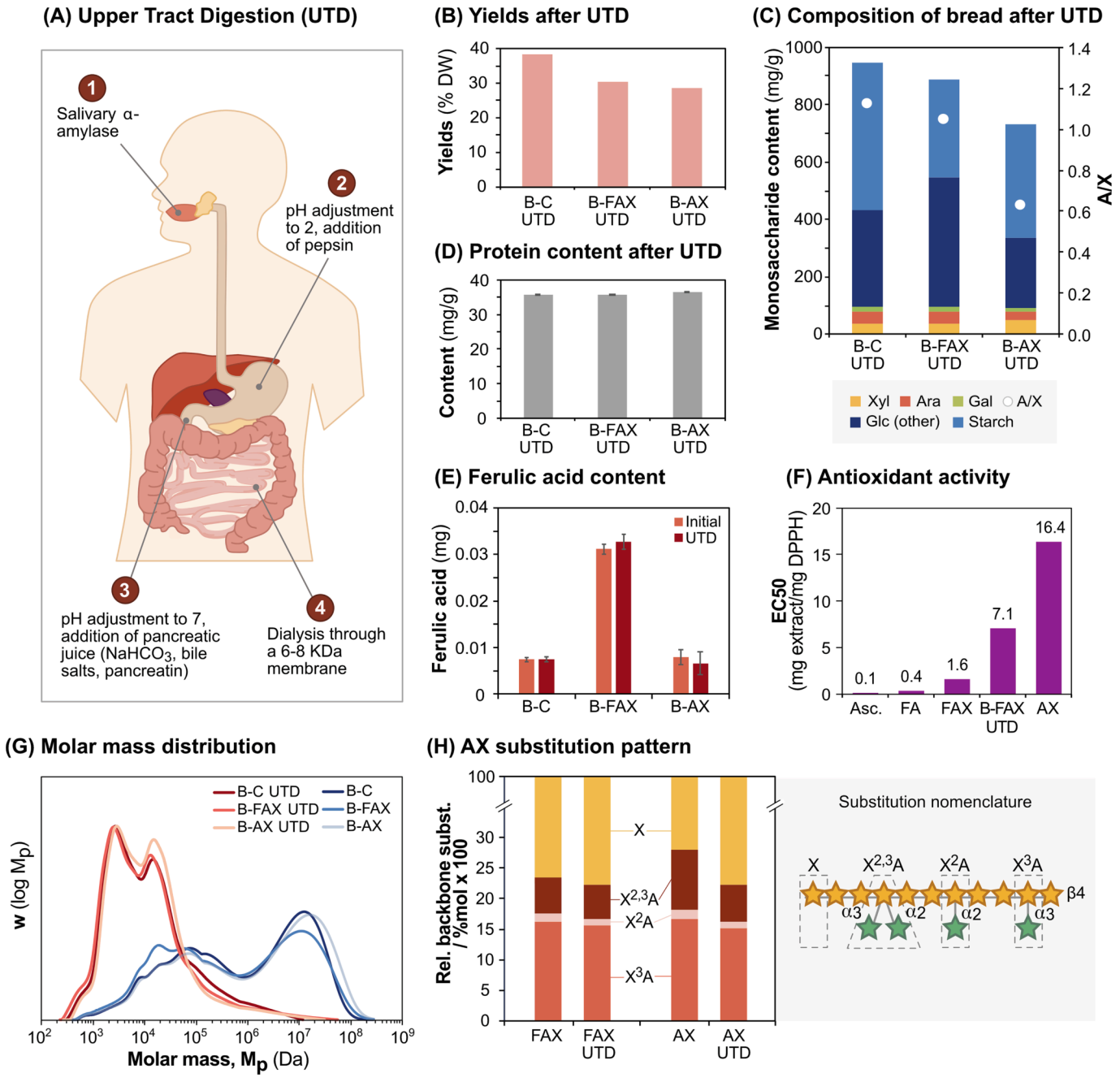
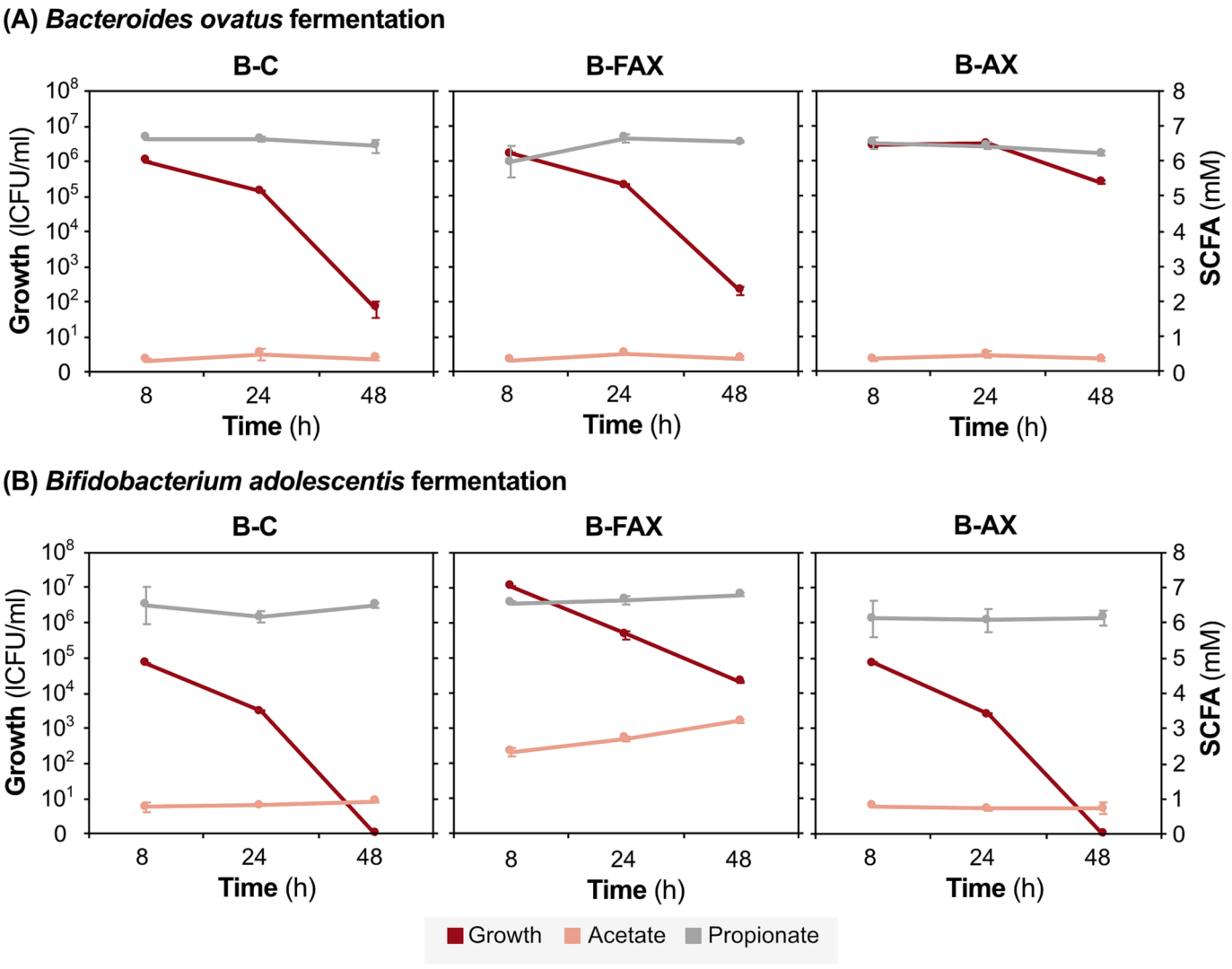


Fig. 4. Effect of upper tract digestion (UTD) on bread and AX. (A) Scheme of the upper tract digestion (UTD) mimic used in this study. (B) Yields of bread samples after the mimic of UTD and (C) their monosaccharide composition. (D) Measured ferulic acid content of bread samples before and after UTD. (F) Antioxidant activity of fibers and bread UTD against DPPH radical. (G) Molar mass distribution of bread samples before and after UTD. (H) Substitution pattern of AX fibers before and after UTD.

independently and not part of the breads (Fig. 4H), as we wanted to minimize interference from other biomacromolecules. The results showed that the arabinose substitution pattern on both FAX and AX were not considerably affected by UTD. This marked an important note that to qualify as a DF, the structural integrity of the AXs should remain during UTD (AACC, 2001). The glycosidic linkage analysis showed that almost no difference was observed on FAX, while for AX, UTD resulted in a slightly lower $X^{2,3}A$ motif which can indicate the prevention of arabinose loss when FA is further linked.

3.5. Composition of supplemented bread samples after digestion affected probiotic fermentation.

Bacteroidetes and *Bifidobacterium* are common phyla involved in the fermentation of DFs (Walker et al. 2011). For the fermentation of DFs, *Bacteroides ovatus* had been used for detecting the utilization of plant polysaccharide due to its efficient xylan degrading system (Martens et al., 2011). While most microorganisms belonging to *Bifidobacterium* genus are saccharolytic, utilizing the energy from complex carbohydrates that are indigestible by humans. It was found that *B. ovatus* could promote the growth of *Bifidobacterium adolescentis* strains by its polysaccharide breakdown products (Rogowski et al., 2015). In Fig. 5c some of the most important enzyme genes that could be involved in the bread



(C) Polysaccharide degrading enzymes present in *B. ovatus* and *B. adolescentis*

	Arabinoxylan							MLBG		Starch		
	Xyn		β-Xyl		α-Abf		FAE	β-Gln	β-Glc	α-Amy	AMG	
	GH8	GH10	GH43	GH120	GH43	GH51	CE1/6	GH16	GH3	GH13	GH13	GH97
B. ovatus												
B. adolescentis												

Number of polysaccharide degrading enzymes
0 1 2 3 4 5

Fig. 5. Fermentation of digested bread by selected gut bacteria. (A) Fermentation of *B. ovatus* and (B) *B. adolescentis* of digested bread samples after UTD and the production of short chain fatty acids (SCFA) during fermentation. (C) Overview of polysaccharide degrading enzymes present in both *B. ovatus* and *B. adolescentis*. The information of enzymes is listed in Supplementary Table S6–7. Note MLBG: mixed-linked β-glucan, Xyn: *endo*-1,4-β-xylanase, β-Xyl: *exo*-1,4-β-xylosidase, α-Abf: α-L-arabinofuranosidase, FAE: acetyl/feruloyl esterase, β-Gln: 1,3:1,4- β-glucanase, β-Glc: 1,4-β-glucosidase, α-Amy: α-amylase and AMG: amyloglucosidase.

digestion are presented for both species with dependence on the number of different enzymes types. To evaluate how DFs regulated the growth of gut microbiota, we cultured selected two probiotic bacteria, i.e., *B. ovatus* and *B. adolescentis*, on the respective bread samples for up to 48 h under anaerobic conditions. The growth of bacteria and the produced SCFAs (acetate and propionate) were determined after 8 h, 24 h and 48 h fermentation of the digested bread samples.

In terms of the fermentation by *B. ovatus* with the bread samples after UTD (Fig. 5A), the bacteria adaptation to B-AX was best among the other bread samples. After 48 h, the colonies with B-AX samples were over 10^5

CFU/ml, while the colonies with B-C and B-FAX dropped to 10^2 CFU/ml. According to the composition of bread after UTD (Fig. 4C), the content of xylose in B-AX UTD was higher than others, which could have promoted a better adaptation of *B. ovatus* to B-AX UTD as this specie has different enzymes that degrade xylans like GH10 and GH43 (Fig. 5C). In terms of SCFA release, previous studies have reported the release of acetate and propionate by *B. ovatus* at 1.3 and 0.7 mM, respectively, after 16 h culture in ZMB1 fully defined media (Horvath et al., 2022). Compared with our result, the yields of acetate with bread samples were lower, at<1 mM after 48 h but in a minimal media only supplemented by UTD bread as carbon source. However, the saccharides in bread samples UTD, such as arabinose, xylose, glucose, galactose, showed an improvement of the propionate yield by fermentation with *B. ovatus*. The produced propionate reached to 6 mM after 8 h. Especially with the addition of FAX, the yield of propionate with B-FAX UTD was increased to 7 mM after 48 h fermentation.

The growth of *B. adolescentis* was different with that of *B. ovatus* (Fig. 5B). B-FAX after UTD favored the survival of *B. adolescentis* compared to other bread samples. With B-FAX, the colonies of *B. adolescentis* were still over 10^4 CFU/ml after 48 h, whereas no colonies were counted with B-C and B-AX after 48 h. *B. adolescentis* generally prefers glucose as carbon source (Palframan, Gibson, & Rastall, 2003), and high amount of glucose was present in B-FAX UTD (Fig. 4C), mostly β-glucans, that could lead to better bacterial adaptation. Indeed, this specie presents genes encoding for different glucanase enzymes as shown in Fig. 5C. Additionally, the acetic acid yields of *B. adolescentis* using β-glucans from barley as single nutrient was up to 96 mM/mL after 72 h fermentation, whereas the yield using xylose, glucose, xylooligosaccharides as single carbon source were 1 mM/ml after 72 h fermentation (Palframan et al., 2003; Zhao & Cheung, 2011). In our results, the acetate yields with B-C and B-AX stayed at the low level of 1 mM after 48 h, which was consistent with previous reported results. Noticeably, the acetate concentration with B-FAX was higher compared to other bread samples, which reflected the better growth of *B. adolescentis*. The produced propionate by *B. adolescentis* with the breads UTD stayed between 6 and 7 mM, which was similar with that by *B. ovatus*, highlighting that the better adaptation of *B. adolescentis* to B-FAX UTD as carbon source did not improve the yield of propionate.

Both *B. ovatus* and *B. adolescentis* could used the bread samples UTD as carbon source. The higher amount of xylose in B-AX UTD increased the growth of *B. ovatus*, while the higher β-glucan content in B-FAX UTD supported the growth of beneficial *B. adolescentis*. The substrate adaptation of these probiotic bacteria is directly related with the presence of specific enzymes that can degrade fibers and use then as carbon source and further metabolites production. After fermentation with bread samples containing AX, it benefited *B. adolescentis* producing acetate that has shown a beneficial heath effect in human by keeping the gut microbiota stable and serve to nourish other bacteria species in the colon. Moreover, some studies proved the effect of higher acetate concentration in colon with a better body weight control and a preventing effect of hypertension (Anderson et al., 2009). The variety of polymers, AXs and glucans in bread after UTD, increased the yields of propionate from both *B. ovatus* and *B. adolescentis*, which showed a great potential for the good balance on SCFA production of gut microbiota.

4. Conclusions

We presented in this work an integral process for the production and addition of dietary fibers into bread and a comprehensive study of the effect of such supplementation on the digestion and fermentation by probiotic bacteria. Pilot extraction of feruloylated-arabinoxylans (FAX) from wheat bran was performed followed by removal of ferulic acid by mild acid treatement to obtain arabinoxylans (AX). The process yielded FAX and AX with similar A/X ratio of 0.13, an average molecular weight of 10^5 DA and high purity of 75 % AXs. Both types of fibers were supplemented as much as 1 % in bread and the baking process was found not to significantly affect the structure of the fibres. The use of an enzymatic approach for selective degradation the bread components led to a deeper understanding of the effect of baking on the fibers. Enzymatic deconstruction revealed (ED) that the baking process only led to the generation of oligomeric xylan fractions that consequently led to a minor decrease of the A/X from the polymeric FAX and AXs. Furthermore, the stability of their molecular structure after upper tract digestion (UTD) makes them potential dietary fibers for human consumption. The molecular linkage of FA to the fibers also showed the preventing effect of arabinose side residue loss and could maintain after baking and digestion the potential antioxidant property of the FAX fibers, 7.1 mg of B-FAX UTD to scavenge 1 mg of DPPH.

The UTD fibers with the presence of resistant glucans and partial starch-gluten complexes served as carbon source for the anaerobic fermentation of two probiotic bacteria, *B. ovatus* and *B. adolescentis*. While the bread control did not display any difference in the growth or SCFA production by the bacteria, the addition of the AX and FAX samples tuned the SCFA produced by the species. This change is related to the differential enzyme cluster present in each bacteria. As hypothesized previously the specific structure of the AXs is preserved during food processing and digestion and it is directly connected to how the fibers can be fermented and the secondary products generated by probiotic bacteria. This differential production of such health beneficial metabolites showed the potential of these dietary fibers as prebiotics in baking products, together with the antioxidant capacity in the case of FAX supplementation. With this study we could follow the whole process of dietary fibers production, addition in bread and digestion, to finish concluding the beneficial influence in gut microbiota. The approach can be inferred to different fibers or conditions to understand the potential of natural fibers as food additives and prebiotics for human.

CRediT authorship contribution statement

Dongming Zhang: Investigation, Validation, Formal analysis, Writing – review & editing. **Reskandi C. Rudjito:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing, Visualization, Supervision. **Solja Pietiäinen:** Formal analysis, Resources. **Shu-Chieh Chang:** Formal analysis, Validation, Writing – review & editing. **Alexander Idström:** Formal analysis, Validation. **Lars Evenäs:** Validation. **Francisco Vilaplana:** Resources, Funding acquisition. **Amparo Jiménez-Quero:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing, Supervision, Project administration.

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.

Acknowledgements

This work was funded by the Lantmännen Research Foundation (Project 2016F008 and Project 2018H021) and the Swedish farmers' foundation for agricultural research (Stiftelsen Lantbruksforskning; Project O-17-20-962). The NMR measurements were carried out at the Swedish NMR Center, Gothenburg, Sweden.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.135660.

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