Production and evaluation of enzymatically processed oat fibres as potential prebiotics

Elin Johansson

Supervisors: Siri Norlander & Carl Grey

Examiner: Patrick Adlercreutz

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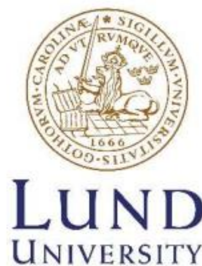

Abstract

Insoluble oat bran fibres, with substantial arabinoxylan (AX) content, are obtained as a by-product from industrial processing of oat. By further processing it is possible to solubilise and hydrolyse AX into (arabino)xylo-oligosaccharides ((A)XOS). (A)XOS derived from other crops have shown to have prebiotic potential by stimulation of probiotic bacteria (Falck et al., 2013). In this study the enzymatic production of (A)XOS from insoluble oat fibres and their prebiotic potential was examined.

Heat extraction and alkali extraction was separately investigated as two methods for solubilising AX. Both methods were shown to be equally efficient with 49 % and 44% recovery of AX, but heat extraction led to a higher purity. Hydrolysis of AX into (A)XOS was investigated by different enzymes, individually and in pairs. No positive synergy in (A)XOS production could be observed with enzymes in pair compared to their individual effect. Rather, Pentopan 500 BG was found to be the most efficient enzyme as a wide range of (A)XOS was produced. Thus Pentopan 500 BG was used for further production of (A)XOS.

Lactobacillus brevis (DSM 1269) and *Bifidobacterium adolescentis* (DSM 20083) was utilised to investigate the prebiotic potential of the produced (A)XOS. It was shown that both strains consumed (A)XOS while producing short chain fatty acids (SCFA). Specifically, *B. adolescentis* utilised both XOS and AXOS. *L. brevis* utilised XOS with DP 2-3, possibly utilisation of A^3X was seen. It was hence concluded that (A)XOS derived from insoluble oat fibres have prebiotic potential.

Populärvetenskaplig sammanfattning

Havrerester, en win-win-situation?

Tänk om man både kunde förbättra människors hälsa och samtidigt göra en insats för miljön? Genom att bearbeta havrerester som fås ur havreproduktionen kan det vara möjligt! Havreresterna har ett högt fiberinnehåll som skulle kunna gynna de goda bakterierna i våra tarmar.

De goda bakterierna i våra tarmar, också kända som probiotika, har visat sig ha en positiv effekt på vår hälsa. En hög förekomst av probiotika i tarmarna bidrar till exempel till att minska risken för bland annat hjärt- och kärlsjukdomar, en av våra vanligaste folksjukdomar. När probiotika växer produceras diverse fettsyror och det är delvis bildningen av dem som gynnar vårt välmående. Probiotika trivs inte bara i våra tarmar utan finns också naturligt i viss mat vi äter, bland annat i yoghurt och surdegsbröd. Genom att vi äter sådana livsmedel kan vi alltså öka förekomsten av probiotika i vår kropp. Ett annat sätt att gynna tillväxten av probiotika är att variera vår kost. Probiotika kan nämligen utnyttja vissa komponenter i vår kost som vi inte klarar av att bryta ner själva.

Havrerester har ett högt innehåll av fibrer som vi inte kan bryta ner. Restprodukten utgör därför en möjlig källa för att utvinna komponenter som stimulerar tillväxten av probiotika. Den främsta fibern som finns i havrerester är den olösliga polysackariden arabinoxylan. Genom bearbetning är det möjligt att bryta ner arabinoxylan till mindre, vattenlösliga kostfibrer. Det är dessa kostfibrer som skulle kunna gynna tillväxten av probiotika. I den här studien undersöktes hur effektiv olika typer av bearbetning var för att ta fram mindre och vattenlösliga kostfibrer. Sedan undersöktes det hur väl två olika probiotiska bakterier kunde växa på kostfibrerna som togs fram. Liknande forskning som gjorts på arabinoxylan från andra sädesslag har visat att det är möjligt att framställa kostfibrer som probiotika kan utnyttja. Beroende på sädesslag ser dock arabinoxylan och kostfibrerna som fås fram olika ut. Eftersom olika probiotika kan vara olika kräsna är det intressant att titta på vilka kostfibrer som kan utvinnas från just havre.

Här undersöktes två vanliga probiotiska bakterier som har hög förekomst både i livsmedel och i tarmarna. Genom att förse dem med kostfibrer som utvunnits ur havrerester kunde man se att de inte bara växte utan även bildade de eftersträvade fettsyrorna som gynnar vår hälsa. Bearbetningen som krävdes var först lösliggörning av arabinoxylan från övriga komponenter som finns i havreresterna. Både lösliggörning genom värme samt basiska förhållanden visade sig vara effektiva metoder för att frigöra arabinoxylan. De lösliga fibrerna klövs sedan upp i de mindre kostfibrerna genom enzymatisk behandling.

Det betyder alltså att havrerester kan få ett ökat värde om vi bearbetar dem och sedan äter dem. På så vis gynnas probiotikan som i förlängningen gynnar oss samtidigt som vi gynnar miljön genom att ta vara på de resurser som finns.

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List of abbreviations

AX	Arabinoxylan
(A)XOS	(Arabino)xylo-oligosaccharides
GH	Glycoside hydrolase
DP	Degree of polymerisation
SCFA	Short chain fatty acid
A/X	Arabinose to xylose ratio
MOS	Maltooligosaccharides

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1 Introduction

Industrial processing of oat results in insoluble oat bran fibres as a by-product. One type of fibre with high abundance in this fraction is arabinoxylan (AX). AX is by nature primarily insoluble but by further processing such as extraction and enzymatic treatment AX may be solubilised and hydrolysed into shorter (arabino)xylo-oligosaccharide ((A)XOS). The raw material and the specific treatment will influence the length of produced (A)XOS. Probiotic bacteria, which produces health beneficial metabolites in our gut, have been found to utilise (A)XOS derived from different raw materials (Falck et al., 2013). By examining the production of (A)XOS from insoluble oat fibres it would thus be possible to increase the value of this by-product and simultaneously promote human health.

This master thesis strives to deepen the knowledge of fibres produced from oats by examining the production of (A)XOS from oat and their potential as prebiotics. More specifically the possibility to use pre-treatment as a method to solubilise AX will be studied as well as the effect of enzyme synergy on specific (A)XOS production. Finally, the growth of probiotic bacteria on (A)XOS produced from insoluble oat bran fibres will be examined.

2 Background

2.1 Arabinoxylan

Arabinoxylan (AX) is one of the major hemicelluloses in the cell walls of cereals such as oat. AX constitutes of a xylan backbone built of xylose units linked with β-(1-4)-linkages. The backbone is substituted with α-linked arabinose units as depicted in Figure 1. A xylose unit can be either non, mono or di-substituted and the substitutions occur either at the C2 or C3 position of the xylose unit (Bastos et al., 2018). Both the substitution pattern and the occurrence of AX varies with factors such as species and tissue type. The substitution pattern can vary along the polymeric chains and AX from oat grains has previously been reported to have regions with both high and low occurrence of substitutions (Tian et al., 2015). Bran is usually the tissue with highest AX content but one cannot designate a specific structure of AX (Bastos et al., 2018). The degree of arabinose substituents can be described by the arabinose to xylose (A/X) ratio. That is a measure of the branching of AX and impacts both solubility and viscosity. Other substituents readily occurs at the backbone such as acetyl groups, uronic acids and other sugar units (Izydorczyk, 2009). In addition phenolic acids such as ferulic acid can be linked with ester linkages to the arabinose units (Bastos et al., 2018).

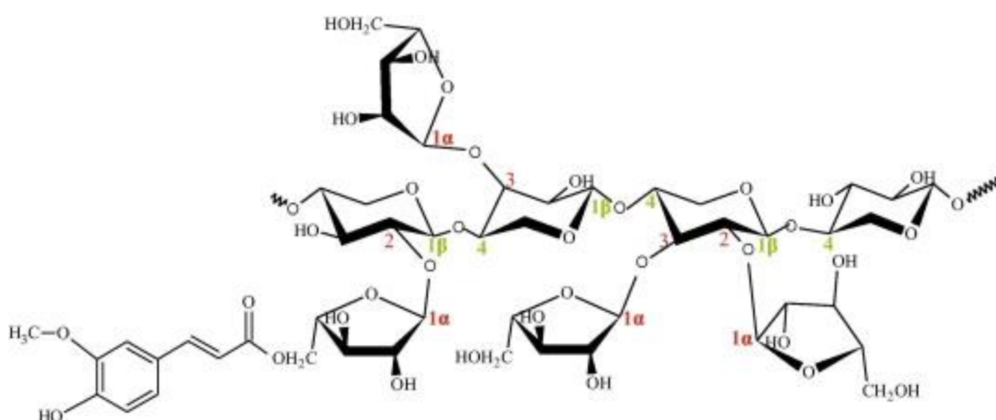

Figure 1. General structure of arabinoxylan obtained from Reis et al. (2015).

2.2 Extraction

The majority of AX is naturally insoluble due to complex crosslinking with cellulose and lignin and other constituents in the plant cell wall, as depicted in Figure 2 (Izydorczyk, 2009). The solubility is partly determined by the substitutions as highly substituted xylan reduces interactions with cellulose

(Scheller and Ulvskov, 2010). On the other hand presence of arabinose substituents and especially arabinose linked to ferulic acids may increase the covalent linkages with lignin and thereby decrease the solubility (Bastos et al., 2018). Pre-treatment can be applied in order to solubilise a larger fraction of AX by disrupting the linkages as schematically described in Figure 2.

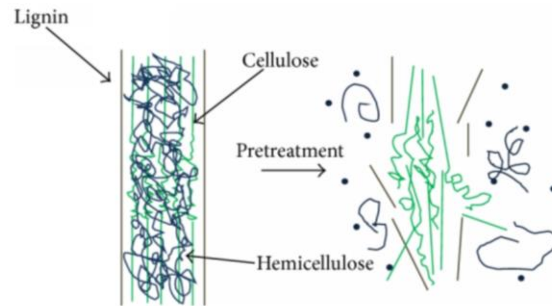

Figure 2. Schematic structure of lignocellulosic material and the effect of pre-treatment obtained from Lee et al. (2014)

Pre-treatment can be achieved in different ways; two common types are chemical and heat extraction. The method used will impact the composition and properties of extracted AX. Chemical extraction is often performed under alkaline conditions. The alkaline environment causes disruption of both covalent and hydrogen bonds. In addition, linkages between substituents such as ferulic acid and acetyl groups breaks, altering the substitution pattern of AX. Glycosidic linkages are however resistant to alkali conditions and degradation of the xylan backbone occurs from the reducing ends. Alkali extraction can be combined with ultrasound-assisted extraction to disrupt the cell walls which increase the accessibility of the hemicellulose (Bastos et al., 2018). One drawback with alkali extraction is the need of neutralisation which leads to formation of salts that decreases the purity (Falck, 2014).

During heat extraction, also called autohydrolysis, AX is exposed to high temperatures under high pressure, often either water or steam is used. Under such conditions water autoionizes and forms hydronium ions which catalyse the formation of acetic acid from acetyl groups in lignin and hemicellulose. The reaction causes an acidic environment which leads to degradation of the cell wall and depolymerisation of the hemicellulose. Under optimal conditions the hemicellulose breaks down to oligosaccharides, such as (A)XOS but if too harsh conditions are applied the oligosaccharides in turn break down to monosaccharides (Carvalheiro et al., 2016). Compared to alkali extraction, heat extraction is a milder treatment in terms of substituents reaming and a larger fraction of substituents usually remains, including both ferulic acids and acetyl groups. Independent of pre-treatment method, harsher conditions, in terms of temperature, time and pH, usually leads to higher yields but often at the expense of formation of several monosaccharides. The severity of a pre-treatment method also influences the A/X ratio, generally the harsher treatment the lower A/X ratio. However it has been shown that under alkaline conditions the arabinose substitutes are protected hence the A/X ratio is unchanged while pH increases (Roos et al., 2009).

2.3 Enzymatic hydrolysis of arabinoxylan

Arabinoxylan can be degraded into oligosaccharides. Depending on their substitution they are called arabinoxylan-oligosaccharides (AXOS) alternatively xylan-oligosaccharides (XOS), i.e., fractions of AX without arabinose substitutions. (A)XOS refers to the combination of both XOS and AXOS. The specific name depends on the degree of polymerisation (DP) and the substitution. The (A)XOS considered in this study is presented with their name and abbreviation in Table 1.

Degradation of AX can be achieved by enzymatic hydrolysis with xylanases. Xylanases belong to the glycoside hydrolase (GH) families, i.e., it breaks glycosidic bonds found between xylose units. Xylanases differ in their structures and their affinity for substrates and substitutions and are somewhat

grouped based on their functionalities. For (A)XOS production xylanases with endo-(1-4)-β-activity belonging to GH10, GH11 and GH5 are among the most investigated groups (Nordberg Karlsson et al., 2018). It has been shown that the same enzymatic treatment can yield different (A)XOS depending on the raw material and what pre-treatments it has been subjected to (Falck et al., 2014).

Table 1. Full names and abbreviations of investigated (A)XOS.

Abbreviation	Full name
X2, X3, X4, X5, X6	Xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose
A^2XX	2^3-α-L-Arabinofuranosyl-xylotriose
A^3X	3^2-α-L-Arabinofuranosyl-xylobiose
XA^2XX	2^3-α-L-Arabinofuranosyl-xylotetraose
XA^3XX	3^3-α-L- Arabinofuranosyl-xylotetraose
$A^{2\text{-}3}XX$	2^3,3^3-di-α-L-Arabinofuranosyl-xylotriose

Xylanases belonging to the GH10 family have been found to have strong affinity towards short substrate resulting in substantial number of oligosaccharides with DP 2-3. If the hydrolysis time is too long on the other hand it results in large formation of monosaccharides (Falck et al., 2014). GH10 are inefficient on water insoluble AX, enhancing the need of a pre-treatment step. The activity of GH10 enzymes is generally not inhibited by arabinose substituents, but two subsequent unsubstituted xylose units are required for cleavage. Enzymes belonging to family GH11 are on the contrary more inhibited by arabinose substituents but are more efficient on insoluble AX. The increased efficiency can be explained by the in general smaller size of GH11 enzymes, which facilitates the penetration through the cell wall to the substrate. For comparison three subsequent unsubstituted xylose units are needed for cleavage (Nordberg Karlsson et al., 2018). Enzymes from GH11 is best suited for longer substrates with a higher degree of polymerisation, with few arabinose substituents. (Linares-Pasten et al., 2018). Due to the difference in preference, enzymes from GH11 usually leads to formation of (A)XOS with higher DP than enzymes from GH10 (Nordberg Karlsson et al., 2018).

Among the enzymes belonging to GH5 there are subgroup which require arabinose substitutions. These enzymes are therefore useful for hydrolysing highly substituted xylan, which is poorly hydrolysed by GH10 and GH11 enzymes (Nordberg Karlsson et al., 2018). Additionally, these specific enzymes have shown to produce solely AXOS and no unsubstituted XOS (Falck et al., 2018).

Apart from enzymes from the GH family, enzymes with other functionalities could possibly aid the hydrolysis by removing substituents and increase the availability of the substrate by detaching it from other materials in the cell wall. For instance, laccases that can degrade lignin and ferulic esterase which may cleave the ester linkages between arabinose and phenolic acids (Chaturvedi and Verma, 2013, Hunt et al., 2017).

2.4 Probiotics and Prebiotics

Probiotics refers to microorganisms that improve the health and well-being of the host. Probiotic bacteria thrive in our intestines and may modify the composition of our gut microflora and thereby impact the human health (Andrew L. Wells, 2008). As a result of probiotic fermentation, short chain fatty acids (SCFA) are produced. SCFA lowers the pH which creates a hostile environment for several pathogens and thereby reduces risks of infections (Gibson, 2004). Further, absorbance of

micronutrients is enhanced (Andrew L. Wells, 2008). Other health improvements caused by probiotic bacteria are for instance improved gut barrier function and reduced risk of cardiovascular disease (O'Toole and Cooney, 2008).

For bacteria to be considered as probiotic it should, among others be able to adhere to epithelial cells, survive in the gastro intestinal tract and possess antagonistic activity against pathogens (Saarela et al., 2000) Two of the most studied probiotic genera are *Lactobacillus* and *Bifidobacterium*, which are commonly found in fermented food (O'Toole and Cooney, 2008). The gut microflora can thus be altered by consuming foods containing probiotics, either naturally or enriched. Another method is to promote the growth of probiotic bacteria in the gut by consuming prebiotics. Prebiotics are since 2017 defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). That would include dietary fibres resistant to human digestion but fermentable by probiotic bacteria, such as oligosaccharides (Andrew L. Wells, 2008).

The human genome lacks enzymes for degrading (A)XOS which makes it a putative prebiotic (Nordberg Karlsson et al., 2018). Previous studies have shown that both XOS and AXOS extracted from cereals can stimulate growth of probiotics as for instance, *Lactobacillus brevis* and *Bifidobacterium adolescentis.* It has been shown that XOS with a DP 2-3 can be consumed by both species whereas AXOS exclusively have been utilised by *B. adolescentis.* Suggesting that consumption of AXOS is more specific than consumption of XOS (Falck et al., 2013).

3 Methods

3.1 Raw materials

Insoluble fibres obtained as a by-product from processing of oat bran, from 2019, were provided from Lantmännen and stored at -20 °C until usage. The insoluble fibres were treated in several steps and an overview of the processing can be seen in Figure 3.

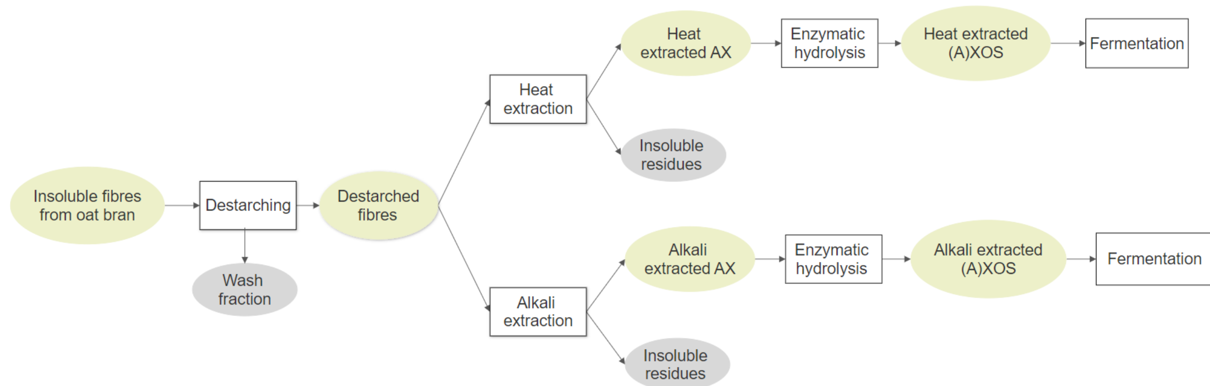

Figure 3. Schematic overview of the experiments

3.2 Pre-treatment

Starch was removed from all insoluble fibres which were then subjected to either alkali extraction or heat extraction as described below.

3.2.1 Removal of starch

The insoluble fibres were thawed and directly treated to remove starch. Tap water was added to the insoluble fibres to reach a dry content of 5 %. The suspension was heated to 70 °C for 5 min with continuous stirring after which it was cooled to 40 °C. Amyloglucosidase (36 U/mg) and α-amylase (500 U/mg) purchased from Megazyme, were both added in a volume corresponding to 2.4 mL/100 g insoluble fibres and the suspension was kept at 40 °C for 2 h with continuous stirring. Thereafter the suspension was centrifuged at 3000 rpm for 5 min. The obtained pellet was washed twice with tap

water and filtered through a cloth, whereupon the solid fraction was pre-treated further as described below and the wash fractions and supernatant were discarded.

3.2.2 Alkali extraction

Alkali extraction of destarched material was performed according to the suggestion of optimal treatment for oat hull reported by Schmitz et al. (2021) with some modifications. The destarched fibres were first subjected to ultrasonication and thereafter incubated under alkaline conditions. Modifications of the method was done in the following ways, 2.5 M of NaOH was used and the incubation temperature was 65 ℃. After recovering the soluble fibres and neutralising the suspension as described by Schmitz et al. (2021) the fibres were precipitated by addition of 4 volumes of 99.5% ethanol. Precipitation was performed overnight at 4 ℃. The precipitate was then recovered by centrifugation at 4000*x*g for 10 min and the pellet was collected. The product was freeze-dried before further usage.

3.2.3 Heat extraction

Destarched fibres were mixed with Milli-Q water to obtain a dry content of 10%. The suspension was autoclaved at 121 ℃ for 15 h. Thereafter, equal amounts of Milli-Q water were added again to aid dispersion and the suspension was centrifuged at 8000*x*g for 10 min. The soluble fibres in the supernatant were precipitated and recovered as described in section 3.2.2 and the pellet was discarded.

3.3 Enzymatic treatment

Enzymatic hydrolysis of pre-treated material was performed with the enzymes presented in Table 2. The effect of enzymes was investigated both as single enzymes and in pairs, all possible combinations were examined. Sodium phosphate buffer with a volume of 1 mL, (100 mM, pH 6.0) was used to dissolve 50 mg of pre-treated fibres. Enzymes with volumes corresponding to 100 U/g arabinoxylan was added, a control without any enzymes was included. Reactions were prepared in duplicates. Once the enzymes were added the reactions was incubated in a thermoshaker at 40 ℃ with shaking, 600 rpm for 24 h. Termination of the reactions was performed by heating them up to 110 ℃ for 5 min and thereafter letting them cool on ice. Reactions were saved in the freezer upon analysis.

Table 2. Compiled information about enzymes used for enzymatic treatment. All enzymes were used in volumes corresponding to 100 U/g arabinoxylan.

Enzyme	Type	Supplier
E-FAERU	Ferulic acid esterase	Megazyme
NS51003	Laccase	Novozymes
E-XYNBS	GH 10	Megazyme
Pentopan Mono BG	GH 11, (Sajib et al., 2018)	Novozymes
Feed xylanase	Xylanase	Novozymes
Pentopan 500 BG	Multicomponent xylanase, (Sajib et al., 2018)	Novozymes
CtXyl5A	GH5-34, (Nordberg Karlsson et al., 2018)	nzytech

3.4 Composition analysis

The composition of the fibres was analysed in its different states; the raw material, destarched fibres and pre-treated fibres. Enzymatically treated fibres i.e., (A)XOS were solely subjected to analysis of oligosaccharides. All analyses were performed in duplicates.

3.4.1 Analysis of monosaccharides

Fibres were hydrolysed completely into monosaccharides with sulphuric acid as described by Sluiter A. (2008). A downscaled version of the protocol was however applied for preparation of destarched fibres. In that case 10 mg of destarched fibres were hydrolysed with 175 µL 72 % sulphuric acid during 60 min at 30 °C. Thereafter the sulphuric acid concentration was decreased to 4 % by addition of 4.9 mL Milli-Q water and incubated at 100 °C for 3 h. $Ba(OH)_2$ was added to neutralise the sample. All samples were analysed using HPAEC-PAD (ICS-5000, Dionex, Thermo Scientific), equipped with a CarboPac PA20 column, as described by Falck et al. (2014). The following standards were used for identification and quantification; arabinose, glucose, galactose and xylose, purchased from Sigma.

3.4.2 Determination of Lignin

The lignin content was analysed according to the protocol provided by Sluiter A. (2008). In summary the samples were hydrolysed with sulphuric acid before filtering them through crucibles. The crucibles were dried at 105 °C overnight and then burned in an incinerator at 525 °C during 20 h to determine the acid insoluble lignin content. Acid soluble lignin was determined by directly measuring the absorbance at 320 nm of the filtrate in a spectrophotometer.

3.4.3 Determination of Ash

The ash content was determined by adding 500 mg of sample into pre-weighed crucibles and burning them in the incinerator at 525 °C during 24 h.

3.4.4 Analysis of phenolic acids

Phenolic acids were analysed as described by Sajib et al. (2018) using a Dionex HPLC system (Ultimate-3000 RSLC, Dionex). Standards used for quantification and identification were ferulic acid, caffeic acid and coumaric acid, purchased from Sigma.

3.4.5 Beta-glucan analysis

The beta-glucan content was analysed using the kit “Mixed-linkage beta-glucan, K-BGLU 01/21” purchased from Megazyme. Buffers and reagents solutions were prepared according to the appurtenant protocol. Solid samples were milled and 15 mg of fine powder was dispersed in 40 µL of 50 % ethanol and mixed with 800 µL of sodium phosphate buffer (20 mM, pH 6.5) and incubated at 95 °C for 25 min. Thereafter 40 µL of lichenase was added to each sample which were mixed thoroughly and incubated at 50 °C for 1 h. Sodium acetate buffer, (50 mM, pH 4.0) was added with a volume of 500 µL into the samples which was centrifuged at 16000*xg* for 8 min. Betaglucosidase was mixed with the supernatant, 50 µL of each. Additionally, a reaction blank was prepared by mixing 50 µL of supernatant with 50 µL of sodium acetate buffer (200 mM, pH 4.0). All tubes were incubated 10 min at 50 °C, after which 1.5 mL of GOPOD reagent was added and the tubes were incubated 20 min at 50 °C. Glucose standards in triplicate were prepared by mixing 10 µL Milli-Q water, 40 µL D-glucose standard solution and 50 µL sodium acetate (200 mM, pH 4.0). A reagent blank was prepared by mixing 50 µL Milli-Q water with 50 µL sodium acetate (200 mM, pH 4.0). Both blanks and standards were incubated 20 min at 50 µL with 1.5 mL GOPOD reagent. The absorbance was measured at 510 nm against the reagent blank. In the case that the absorbance of the sample was above the absorbance of the standards, the sample was diluted prior to the addition of betaglucosidase and then incubated anew.

3.4.6 Analysis of oligosaccharides

Oligosaccharides were analysed by HPAEC-PAD (ICS-5000, Dionex, Thermo Scientific), equipped with a CarboPac PA200 column as described by Berger et al. (2014). Standards used for quantifications were arabinose, X1-X6, A^2XX, A^3X, XA^2XX, XA^3XX, $XA^{2\text{-}3}XX$ and maltooligosaccharides, MOS, with one to six glucose units, all purchased at Megazyme.

3.5 Fermentation

The two known probiotics *L. brevis* (DSM 1269) and *B. adolescentis* (DSM 20083) were used to examine the prebiotic potential of produced (A)XOS. Both strains were purchased from DSMZ. Additionally, *Escherichia coli* (BL21 DE3) was used as a negative control strain. Each strain was cultivated on both alkali and heat extracted (A)XOS, separately. A positive control with *L. brevis* and *B.* adolescentis growing on glucose and *E. coli* on yeast extract as well as a negative control without any carbon source were included.

3.5.1 Production of substrate

Based on the analysis of oligosaccharides from the enzymatic treatment the enzymes yielding the most interesting (A)XOS profiles with respect on both pre-treated fibres was chosen to produce hydrolysates to be used as substrates in the fermentation. The enzymes were chosen based on the number of identifiable XOS and AXOS produced. The enzymatic treatment was performed as described in section 3.3 but in larger scale. After termination, the hydrolysates were freeze-dried.

Substrate stock solutions of alkali extracted, and heat extracted (A)XOS were prepared by dissolving the respective hydrolysate in Milli-Q water. The concentration of the stock solutions was 40 mg/mL, based on the total fibre content. Sterilization was achieved by filtering the solutions through 0.2 µm filters under sterile conditions. The substrate solutions that were used for cultivation of *B. adolescentis* were performed anaerobically by boiling the Milli-Q water before dissolving the fibres and flushing the vacant headspace with nitrogen gas under sterile conditions.

A glucose solution was prepared by dissolving glucose in Milli-Q water to a concentration of 400 g/L in a serum bottle. Vacant head space in the bottle was flushed with nitrogen gas before sealing and autoclaving the bottle at 121 °C for 15 min.

3.5.2 Preparation of growth media

B. adolescentis was cultivated on Bifidobacterium medium which was prepared by mixing the ingredients as described in recipe no 58 Bifidobacterium medium from DSMZ (2008). with the exceptions that glucose was not added and bacto casitone was utilized instead of bacto soytone. Deionized water was used to dissolve the ingredients and the suspension was boiled by microwaving it for 10 min at 800 W. The medium was cooled on ice simultaneously as it was bubbled with carbon dioxide during 20 min. pH was adjusted to 6.8 with addition of NaOH. The media was distributed accurately in serum bottles. Nitrogen gas was used to flush the vacant head space in the bottles for 2 min, to remove oxygen before the bottles were sealed with a rubber and metallic lid.

L. brevis was cultivated on MRS which was prepared in accordance with recipe no 11 MRS from DSMZ (2007). The media was prepared as described for the Bifidobacterium medium, with the exception that it was not cooled under carbon dioxide. The pH of MRS media was 6.8. Additionally, purchased MRS broth with a glucose of 20 g/L was prepared by dissolving the powder in distilled water and otherwise handled as described above. The pH of this broth was not adjusted, hence the pH was 6.2. This media was used as the positive control of cultivation of *L. brevis.*

LB media was prepared for cultivation of *E. coli* by mixing 4.5 g of tryptone and 4.5 g of NaCl with 450 mL deionized water. The pH was adjusted to 7 by addition of NaOH. Similarly purchased LB broth, with yeast extract, was prepared to be used as the positive control. The media was accurately distributed in E-flask with baffles.

All prepared media were autoclaved at 121 °C for 15 min.

3.5.3 Cultivation

Pre-inoculum of *B. adolescentis* was prepared by adding 100 µL of glycerol stock to 20 mL of Bifidobacterium medium with glucose, 10 g/L. The pre-inoculum was incubated in serum bottles, for 36 h statically in 37 °C. The actual cultivations were prepared by adding each carbon source into the media to a concentration of 10 g/L, based on the total fibre content, and a final volume of 55 mL. Pre-inoculum with a volume corresponding to 2 % (v/v) was added.

Pre-inoculums on respective carbon source was prepared for *L. brevis*. That is, substrate stock solution of alkali and heat extracted (A)XOS was added to a concentration of 10 g/L, based on the total fibre content, and a volume of 13 mL. A pre-inoculum on the MRS with glucose was also included. To each pre-inoculum 100 µL of glycerol stock was added whereupon they were incubated, in falcon tubes, statically at 37 °C overnight. The actual cultivations were prepared by adding 2 % (V/V) of respective inoculum into 60 mL of cultivation media. For the negative control, pre-inoculum grown on glucose was added. Alkali and heat extracted (A)XOS was added to reach a concentration of 10 g/L, into respective cultivation.

Pre-inoculums on respective carbon source was prepared for *E. coli*. That is, substrate stock solution of alkali and heat extracted (A)XOS was added to a concentration of 10 g/L, based on the total fibre content, and a volume of 5 mL. A pre-inoculum on the MRS with glucose was also included. To this *E. coli* was added with an injection loop. The pre-inoculums were incubated, in falcon tubes, overnight shaking at 37 °C. The actual cultivations were prepared as described for *L. brevis* above, expect for that *E. coli* was cultivated aerobically in cultivation flasks.

All cultures were incubated at 37 °C for 48 h with sampling at 0, 8, 24 and 48 h. *L. brevis* and *B. adolescentis* were cultivated anaerobically in a static incubator and *E. coli* aerobically in a shaking incubator. All cultivations were performed in duplicates.

3.5.4 Analysis of fermentation samples

OD at 620 nm and pH was measured immediately of all cultivation samples. As a blank respective media without any carbon source or bacteria was used. The samples were stored in -20 °C until analysis of oligosaccharides and short chain fatty acids. Prior to analysis of the samples were centrifuged 2 min in a bench centrifuge. For analysis of oligosaccharides the supernatant was diluted 10 times with Milli-Q water and analysed as described in section 3.4.6. Analysis of SCFA was performed as described below.

3.5.4.1 Analysis of SCFA

Prior to analysis of SCFA the samples were diluted to a total volume of 2 mL with 20 µL of 20% sulphuric acid and Milli-Q water. Samples from timepoint 0 were diluted 10 times and all other samples were diluted 2 times. A HPLC system equipped with the column Aminex HPX87H was used for analysis. A continuous flow of 0.5 mL/min of 5 mM of sulphuric acid was applied. Detection was made with refractive index. Standards used for identification and quantification were glucose, acetate, D-lactate, butyrate, propionate, succinate and ethanol.

4 Results

4.1 Effect of pre-treatments

In Figure 4 the compositions of the materials throughout the processing are shown. The composition is expressed in w/w % based on the dry weight. Polymeric sugars have been hydrolysed into monosaccharides through acidic hydrolysis and the reported sugar content represents the amount of polymeric sugar. Hence, all xylose and arabinose detected is thought to inherit from arabinoxylan (AX) and the total AX content expressed in percentage is reported in Table 3 along with the A/X ratio.

Measured glucose comes from cellulose, starch and beta-glucan of which the latter was measured and reported in Table 3 as well.

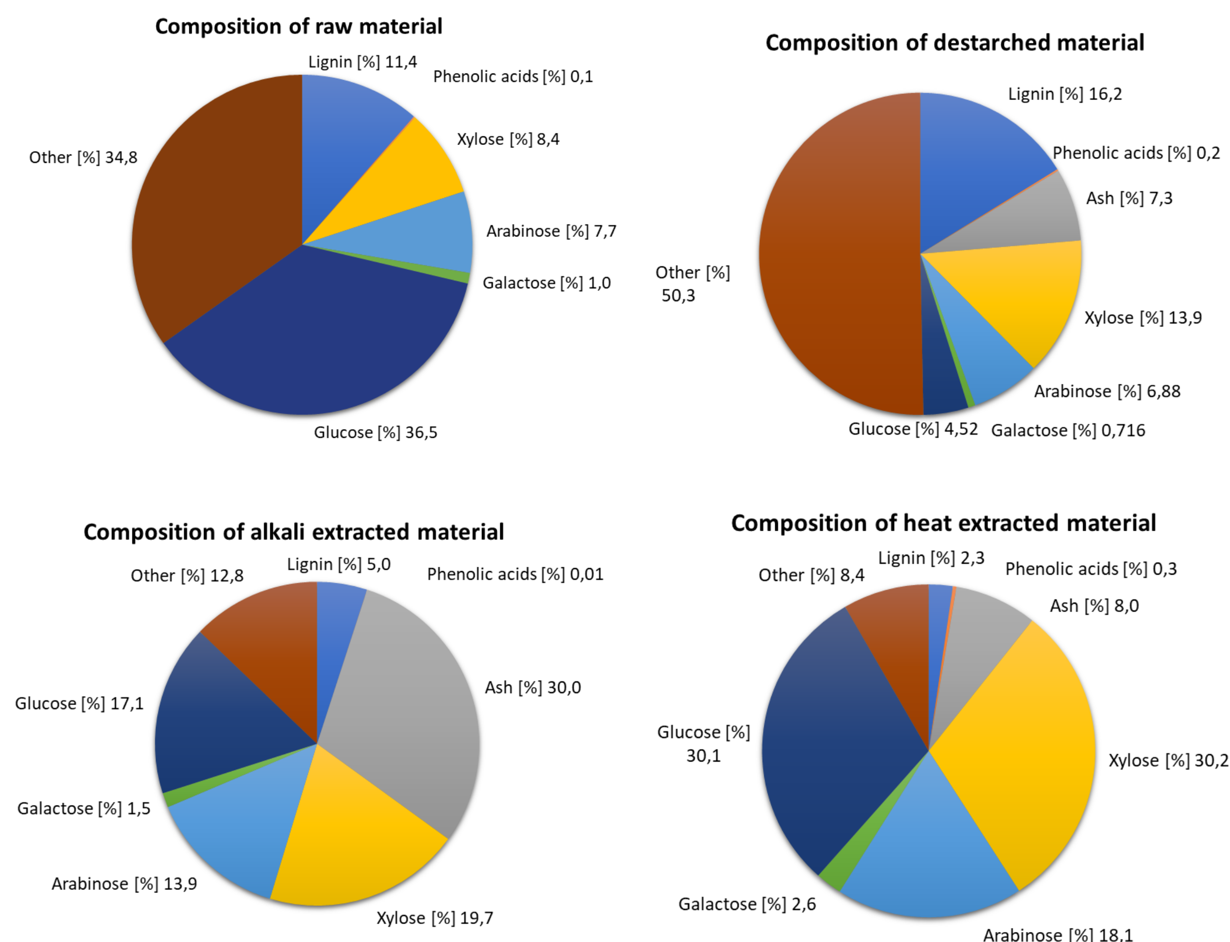

Figure 4. Composition of materials throughout the processing. Top left shows the composition of the raw material, right destarched material. Alkali and heat extracted material is depicted in the bottom. Values are reported as means of duplicates, standard deviation less than 4%. The ash content in the raw material was not analysed.

The most abundant monosaccharide in the raw material is glucose, which decreases after removal of starch whilst the arabinoxylan content increases. Both alkali extraction and heat extraction lead to higher proportions of arabinoxylan, of which heat extraction caused the largest increase. The lignin content decreased after both the pre-treatments.

The fraction Other, in Figure 4 refers to the fraction of the material which was not identified and accounts for uncertainties in the performed analyses, and other compounds such as protein and lipids. Ferulic acid was the dominating phenolic acid of the three quantified, however the amount was very low (µg/g) and here the total sum of quantified phenolic acid is reported.

According to the A/X ratio presented in Table 3 more arabinose than xylose is lost during the different treatments as the A/X ratio decreases throughout. The beta-glucan content increases, both when comparing the actual dry weight and the proportion of glucose that constitutes of beta-glucan, which after both pre-treatments is 0.62, compared to 0.22 in the starting material.

Table 3. Total AX content, A/X ratio and beta glucan content based on dry weight, in each process step, n.a. for not available. Values of AX and beta glucan are reported as means of duplicates, standard deviation below 4%

	Raw material	Destarched fibres	Alkali extracted fibres	Heat extracted fibres
AX [%]	16.1	20.8	33.7	48.3
A/X	0.91	0.68	0.70	0.59
Beta-glucan [%]	7.9	n.a.	10.9	18.7

The yield of alkali extracted fibres with respect of insoluble fibres was 26.9 % in terms of recovered mass and 43.5 % in terms of recovered AX. Similarly, the yields of heat extracted fibres were 21.0 % on mass basis and 48.7 % of AX.

4.2 Enzymatic treatment

The effect of different enzyme combinations was evaluated qualitatively by comparing the different chromatograms after analysis of (A)XOS. By considering the size and number of both known and unknown peaks no positive synergy could be observed with the examined enzyme combinations for either of the pre-treated material, i.e., neither the amount nor size of the peaks were increased when enzymes were combined compared to the effect of the enzymes alone. A selection of chromatograms showing the effects of Pentopan 500 BG, Feed xylanase, E-XYNBS and Ferulic acid esterase on heat extracted fibres is shown in Figure 5 - Figure 7 to exemplify this. Both the individual effect of mentioned enzymes and the combined effect is shown.

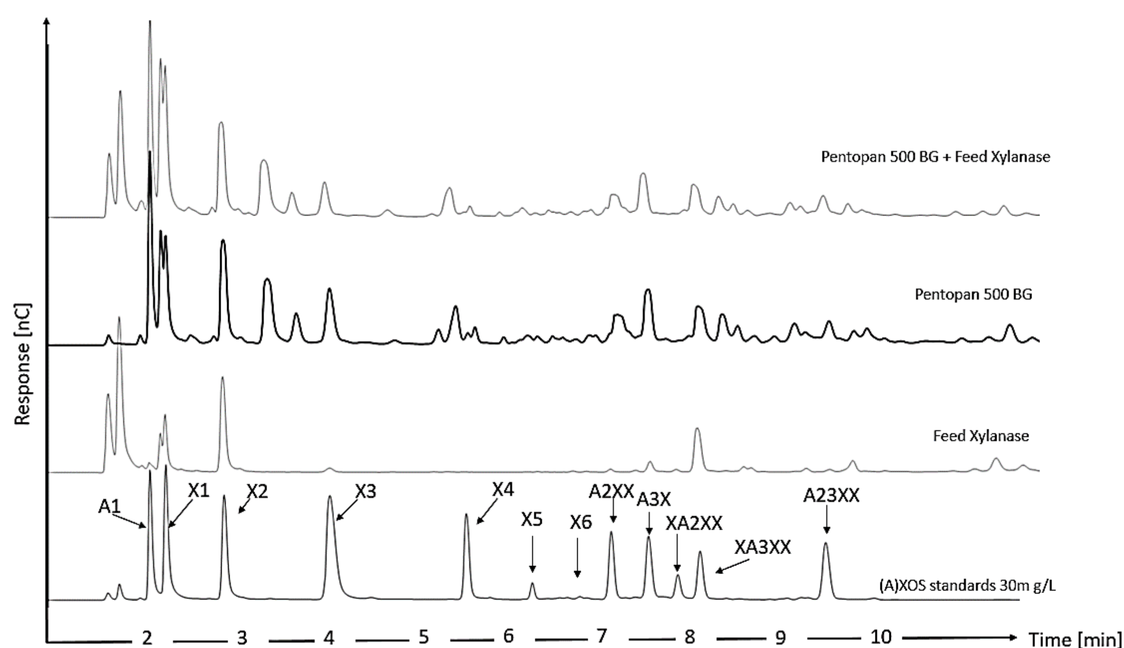

Figure 5. (A)XOS profiles, from bottom and up: (A)XOS standards, Feed xylanase, Pentopan 500 BG and Feed xylanase and Pentopan 500 BG in combination acting on heat extracted fibres. The identity of the peaks is shown in the bottom chromatogram.

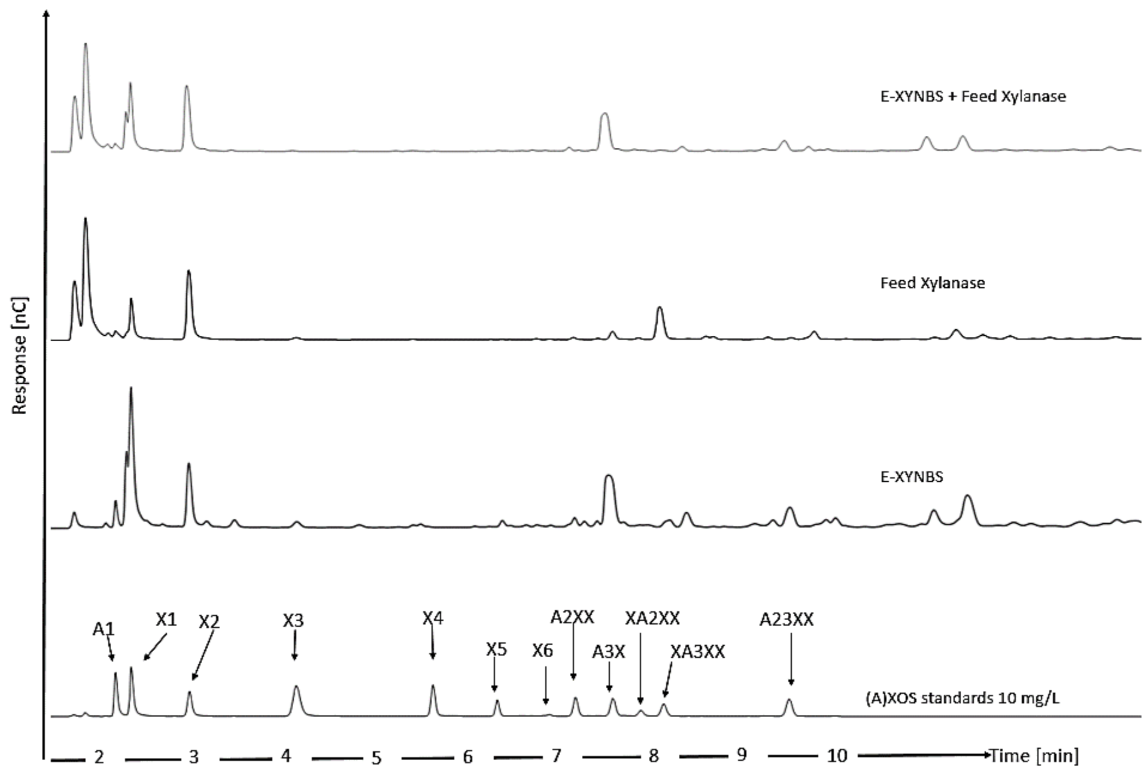

Figure 6. (A)XOS profiles, from bottom and up: (A)XOS standards, E-XYNBS, Feed Xylanase and E-XYNBS and Feed Xylanase in combination acting on heat extracted fibres. The identity of the peaks is shown in the bottom chromatogram.

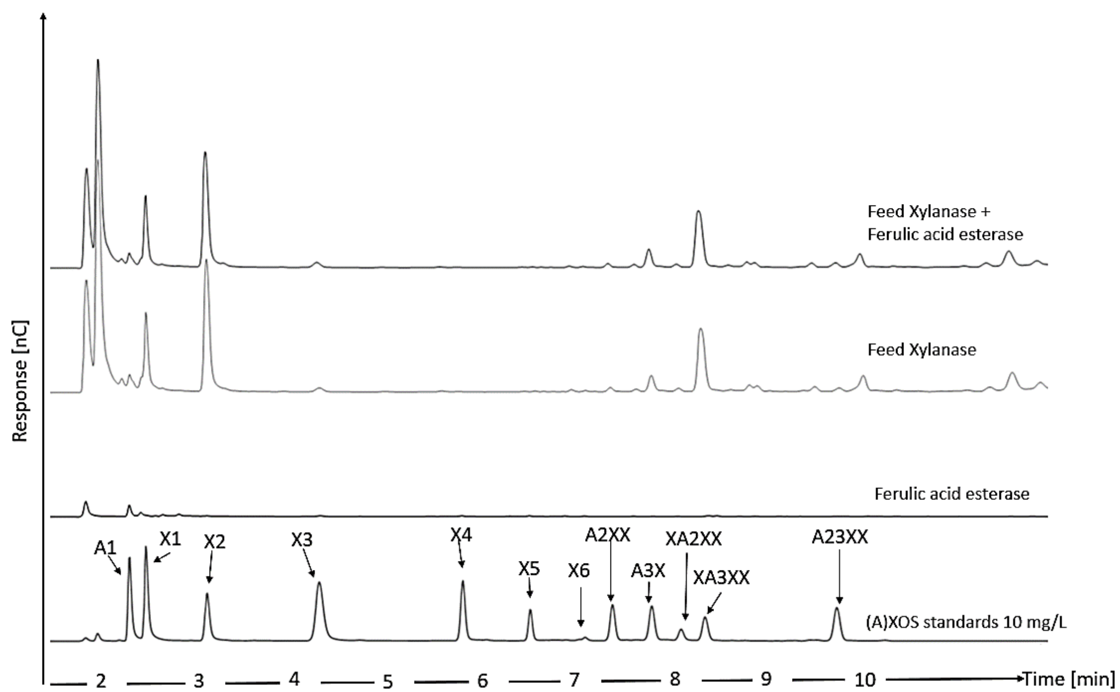

Figure 7. (A)XOS profiles, from bottom and up: (A)XOS standards, Ferulic acid esterase, Feed xylanase and of Ferulic acid esterase and Feed xylanase in combination acting on heat extracted fibres. The identity of the peaks is shown in the bottom chromatogram.

Since no combinations of enzymes displayed a positive synergy the individual effect of the enzymes was investigated. This showed that each enzyme yielded rather similar profiles on both alkali and heat extracted fibres. Both laccase and ferulic esterase possessed a low activity on the substrates and did not result in any (A)XOS but solely a minor part of monosaccharides. Likewise did the G5_34 enzyme CtXyl5A show minor activity on the substrate, with some formation of (A)XOS but in low amounts. E-XYNBS from GH10 and Pentopan Mono BG and Feed xylanase from GH11 showed similar activity on the substrates yielding high concentrations of XOS with low DP and a minor part of AXOS. Pentopan 500 BG, however, produced a substantial amount of identifiable XOS and AXOS on both substrates. Hence, this enzyme was chosen for further treatment of the fibres. In Figure 8 the (A)XOS profiles of Pentopan 500 BG acting on both alkali and heat extracted fibres can be seen. Apart from the identified (A)XOS, several peaks were detected as can be seen in the figure. Six of them could be identified as maltooligosaccharides (MOS) of different DP whereas the majority remains unidentified. The identified (A)XOS produced by this treatment were quantified and the percentages of each based on the total AX content before enzymatic treatment is presented in Table 4.

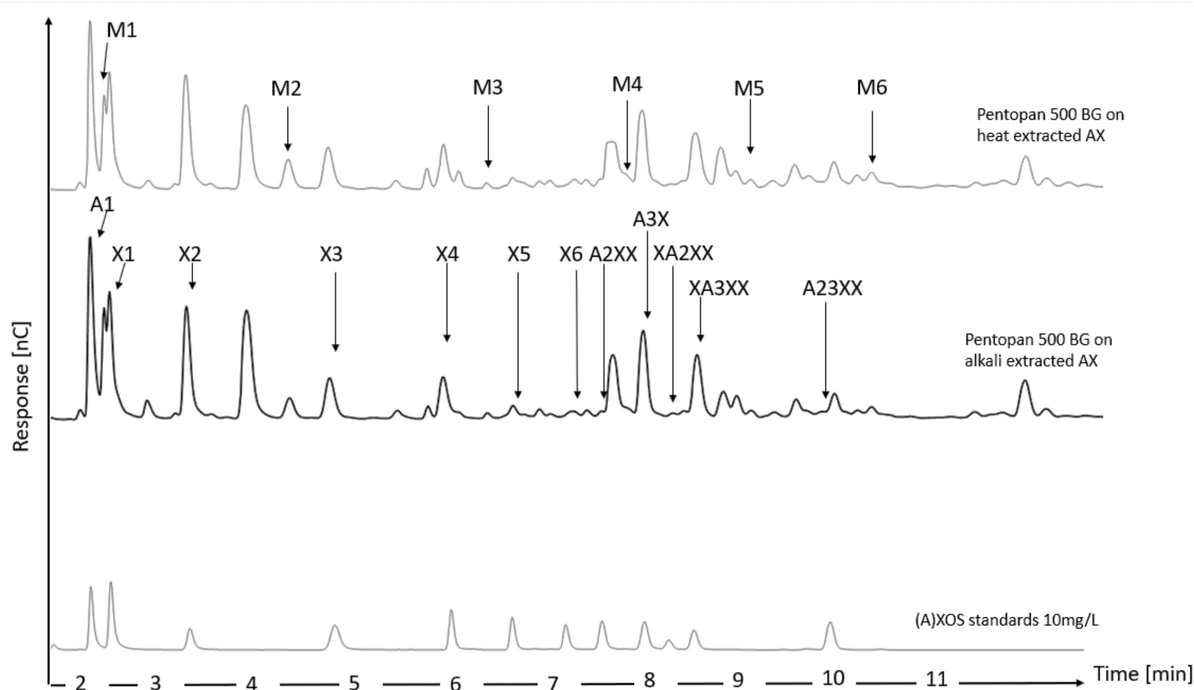

Figure 8. (A)XOS profiles of Pentopan 500 BG acting on heat and alkali extracted fibres. A chromatogram of the (A)XOS standards is included in the bottom for reference. M1-M6 indicates the different maltooligosaccharides identified, from glucose to maltohexaose.

In Table 4 it can be seen that xylobiose, X2, was the major XOS product formed and A^3X and XA^3XX the major AXOS. The conversion of AX in alkali extracted fibres into identifiable (A)XOS was 82%, the corresponding fraction of heat extracted fibres was 53 %. Taking the pre-treatment into account 36 % of AX was recovered after alkali extraction and enzymatic treatment, the recovery of heat extracted and enzymatically treated AX was 26 %. It can be seen that less AXOS has the arabinose substitutions at C2 of xylose, the majority of the quantified AXOS is substituted at the third carbon.

Table 4. Quantity of different (A)XOS obtained after pre-treatment and enzymatic treatment with Pentopan 500 BG.

Compound	Alkali extracted fibres treated with Pentopan 500 BG		Heat extracted fibres treated with Pentopan 500 BG	
	Percentage recovered from AX in pre-treated fibres	Concentration [g/L]	Percentage recovered from AX in pre-treated fibres	Concentration [g/L]
A1	13	2.2	7.6	1.8
X1	8.9	1.5	5.3	1.3
X2	18	3.0	12.6	3.0
X3	5.2	0.86	3.4	0.81
X4	0.81	0.14	1.2	0.30
X5	2.9	0.49	1.4	0.34
X6	3.3	0.55	1.7	0.41
A^2XX	0.9	0.15	0.59	0.14
A^3X	12	2.1	8.4	2.0
XA^2XX	2.7	0.45	1.2	0.3
XA^3XX	13	2.2	9.3	2.2
$A^{2-3}XX$	0.93	0.16	0.25	0.06
Sum	82		53	

4.3 Fermentation

4.3.1 Cultivation of *L. brevis*

In Figure 9 the OD overtime for cultivation of *L. brevis* on different substrates is shown. It can be seen that the growth on alkali and heat extracted (A)XOS ceases after 8h whereas growth on glucose continues for at least 24 h.

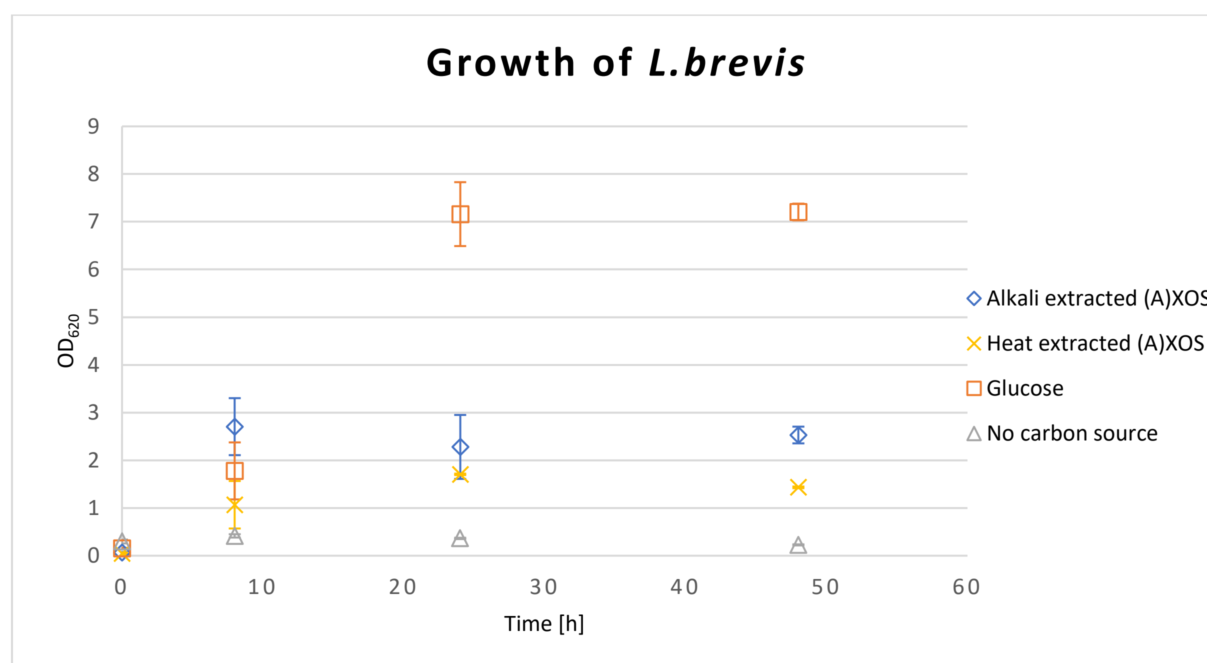

Figure 9. Growth of L. brevis on different substrates

In Table 5 the final pH, change in OD and SCFA concentration between the start point and end point of fermentation of each cultivation of *L. brevis* is presented. Cultivation on both types of (A)XOS resulted in the same final pH, the final pH in cultivation on glucose was lower, although the initial pH

of that broth was lower as well. A larger increase in ΔOD was obtained when the substrate was alkali extracted (A)XOS compared to heat extracted (A)XOS. No growth was observed in the negative control.

As for the OD the major changes in SCFA concentration occurred during the first 8h. Neither butyric acid nor ethanol could be quantified due to co-elution of the standards. Formation of a compound that was assumed to be either butyric acid or ethanol was however seen to a large extent in the positive control and slightly in cultivations on (A)XOS. Similar concentrations of succinic acid were obtained in all four cultivations. The major SCFA produced is lactic acid. Cultivation on alkali extracted (A)XOS yielded more SCFA than cultivation on heat extracted (A)XOS. In Figure 10 a chromatogram showing the end point amounts of SCFA after cultivation of *L. brevis* on alkali extracted (A)XOS is presented.

Table 5. Final pH change in OD, and SCFA concentrations, between t=0 and t=48 h, during the fermentation of L. brevis on different substrates.

Substrate	ΔOD	Final pH	Change in Succinic acid [g/L]	Change in Acetic acid [g/L]	Change in Lactic acid [g/L]	Change in Propionic acid [g/L]
Alkali extracted (A)XOS	2.5	5.6	0.12 ± 0.002	1.5 ± 0.04	2.5 ± 0.02	0.19 ± 0.01
Heat extracted (A)XOS	1.4	5.6	0.11 ± 0.004	0.60 ± 0.1	1.2 ± 0.01	0.33 ± 0.2
Glucose	7.1	3.8*	0.19 ± 0.003	0.34 ± 0.3	6.4 ± 0.01	0.26 ± 0.07
No Carbon source	0.04	6.8	0.14 ± 0.001	0.01 ± 0.2	0.3 ± 0.04	0.01 ± 0.2

** Initial pH of fermentation broth used for cultivation on glucose was 6.2, compared to 6.8 in the other cases.*

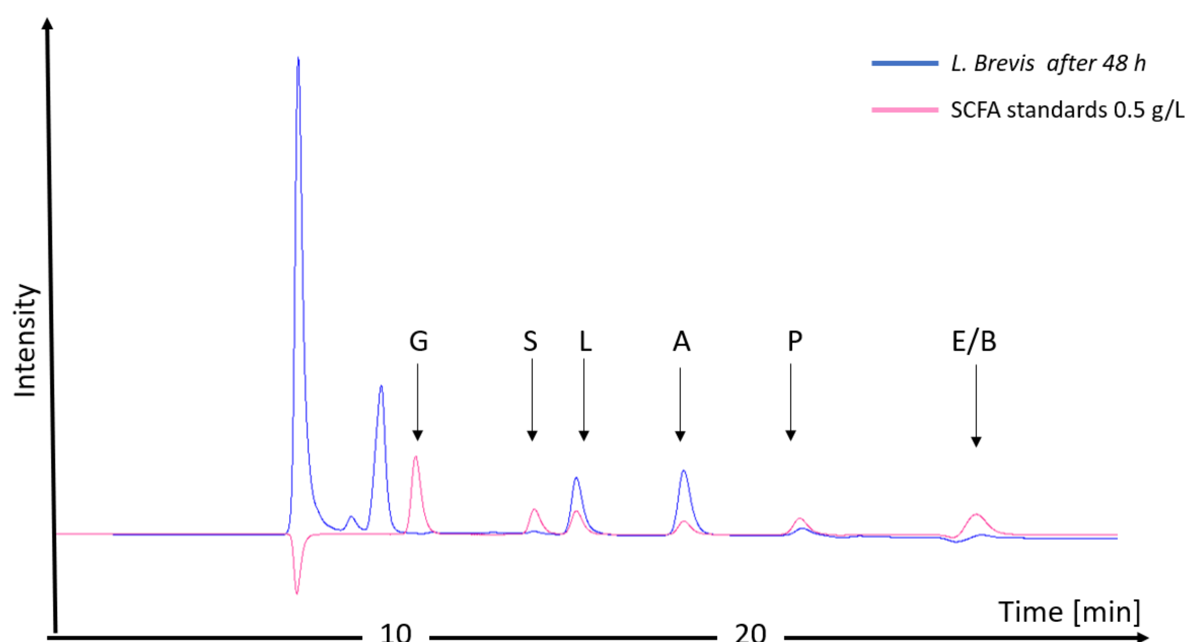

Figure 10. Chromatogram from SCFA analysis of L. brevis growing on alkali extracted (A)XOS after 48 h. G – glucose, S – succinic acid, L – lactic acid, A – acetic acid, P – propionic acid, E/B – ethanol and butyric acid.

The utilisation of specific (A)XOS was investigated by analysing the samples in HPAEC-PAD. The results can be seen in Figure 11 and Figure 12 for growth on alkali and heat extracted (A)XOS

respectively. During the first 8h arabinose, xylose, X2 and X3 were consumed as well as the unidentified compounds with similar elution times. XOS with higher DP does not seem to be consumed neither AXOS. However, after 48 h of fermentation on alkali extracted (A)XOS a reduction of A^3X and possibly XA^3XX is seen.

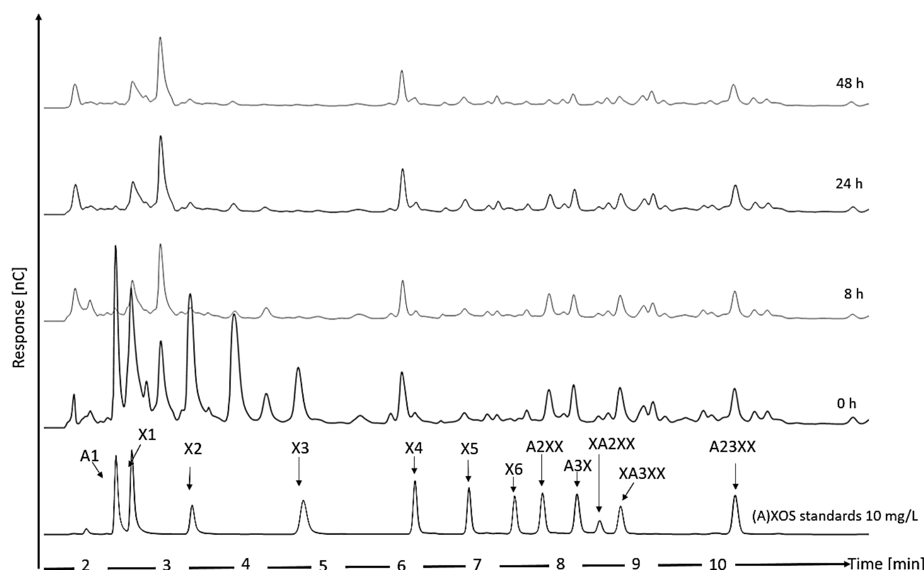

Figure 11. Alkali extracted (A)XOS utilisation pattern of L. brevis. From bottom and up: (A)XOS standards, initial fermentation broth, broth after 8, 24 and 48h, respectively.

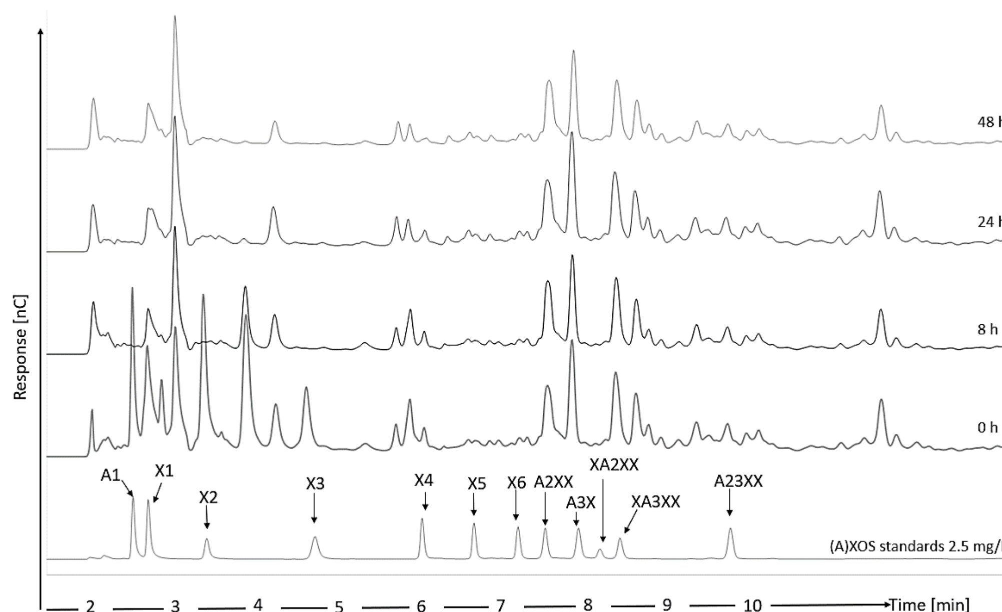

Figure 12. Heat extracted (A)XOS utilisation pattern of L. brevis. From bottom and up: (A)XOS standards, initial fermentation broth, broth after 8, 24 and 48h, respectively.

4.3.2 Cultivation of *B. adolescentis*

The growth over time of *B. adolescentis* on different substrates is displayed in Figure 13. As for *L. brevis* the growth on the different (A)XOS ceased after 8h even though a minor increase in OD can be seen later for growth on heat extracted (A)XOS. Nevertheless, cultivation on glucose resulted in the largest change in OD as can be seen in Table 6 along with the final pH and SCFA concentrations. In

contrast to cultivation of *L. brevis* the highest ΔOD was obtained during cultivation on heat extracted (A)XOS rather than on alkali extracted (A)XOS.

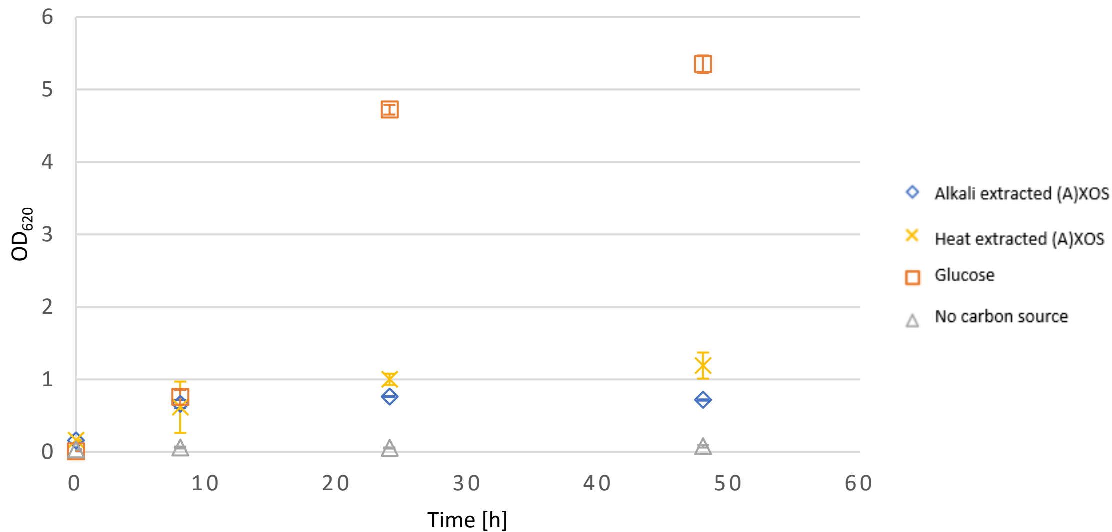

Figure 13. Growth of B. adolescentis on different substrates

It was possible to see a continuous increase in SCFA concentration during the cultivations, the results are although presented as the difference between the endpoint and the starting time. Succinic acid, acetic acid and lactic acid were the only SCFA that could be detected and quantified. As for *L. brevis* succinic acid was formed in equal amounts irrespective of carbon source. Acetic acid was the major SCFA produced. Cultivation on heat extracted (A)XOS lead to higher concentrations of SCFA than on alkali extracted (A)XOS. Regarding the pH, the final values were different between different substrates during these cultivations.

Table 6. Final pH, change in OD, and SCFA concentrations, between t=0 and t=48 h, during the fermentation of B. adolescentis on different substrates.

Substrate	ΔOD	Final pH	Change in Succinic acid [g/L]	Change in Acetic acid [g/L]	Change in Lactic acid [g/L]
Alkali extracted (A)XOS	0.56	6.1	0.18 ± 0.01	0.93 ± 0.05	0.59 ± 0.04
Heat extracted (A)XOS	1.0	5.5	0.19 ± 0.004	1.5 ± 0.2	0.84 ± 0.02
Glucose	5.3	3.8	0.13 ± 0.1	3.7 ± 0.1	2.7 ± 0.06
No Carbon source	0.046	6.8	0.24 ± 0.01	0.28 ± 0.01	0.12 ± 0.003

Specific (A)XOS utilisation was again examined, and the results are presented in Figure 14 and Figure 15. It can be seen that *B. adolescentis* not consume arabinose or xylose but it utilises both XOS and AXOS with higher DP. More specifically X2 up to X4. Additionally, it seems as if A^2XX and A^3X is consumed in both cultivations. A minor reduction of AX^3XX can also be seen. The major utilisation occurs during the first 8h.

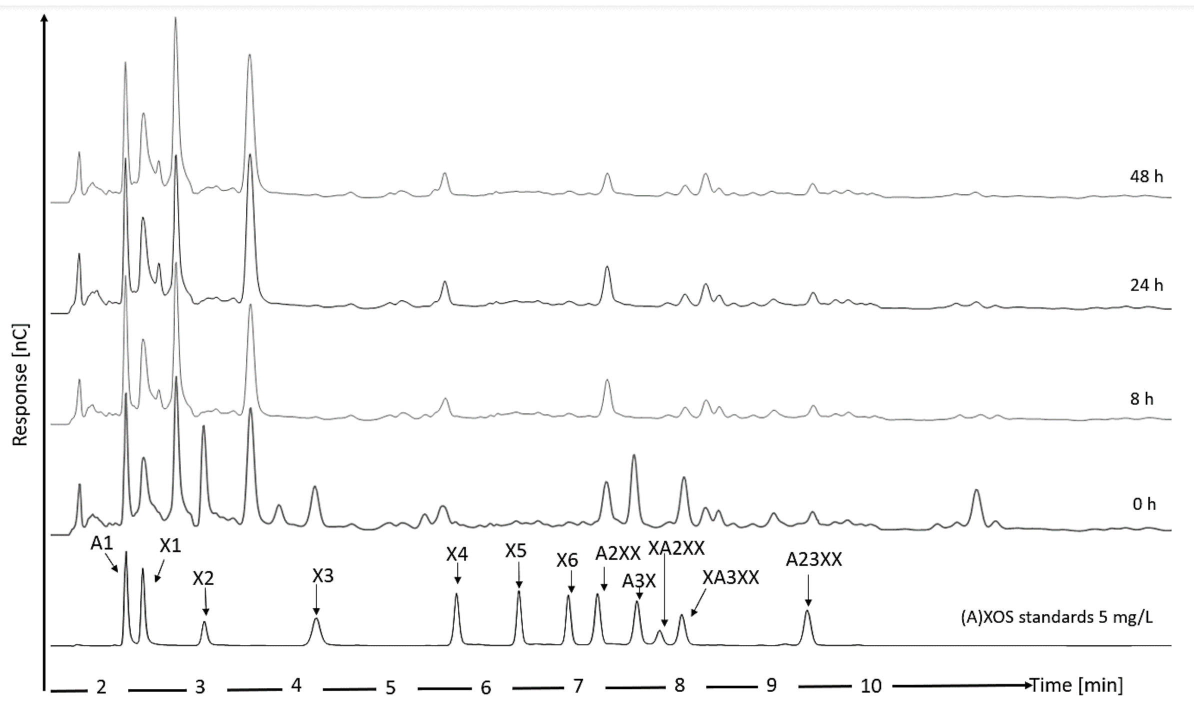

Figure 14. Alkali extracted (A)XOS utilisation pattern of B. adolescentis. From bottom and up: (A)XOS standards, initial fermentation broth, broth after 8, 24 and 48h, respectively.

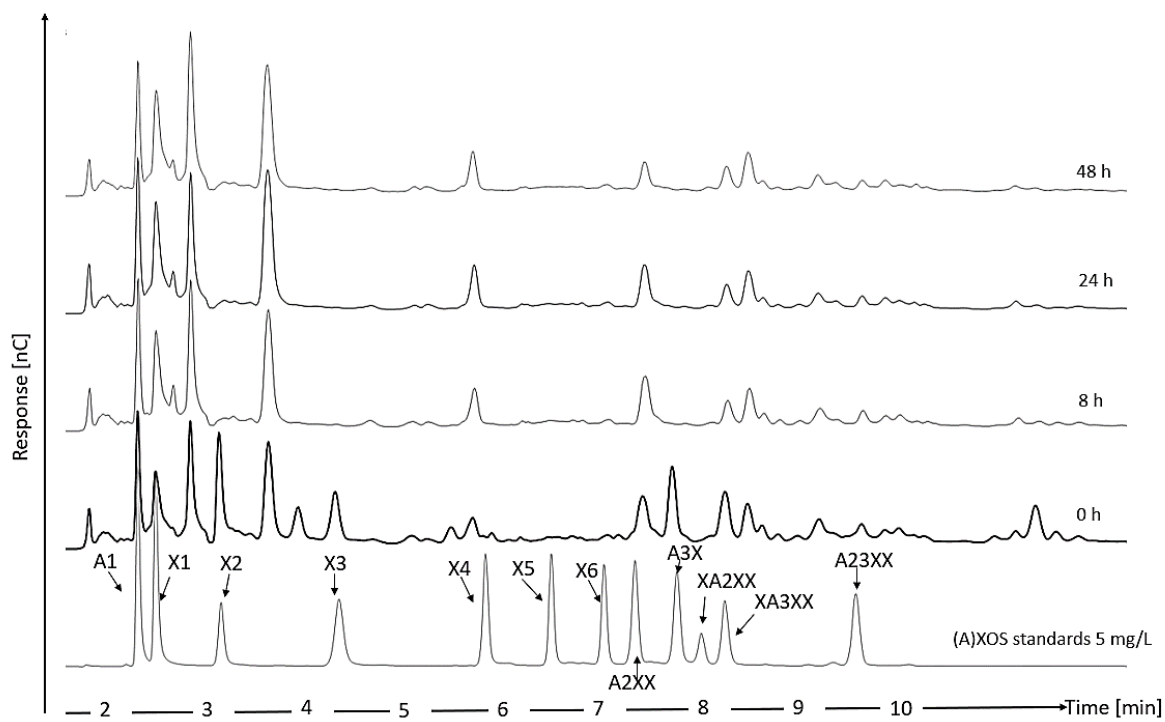

Figure 15. Heat extracted (A)XOS utilisation pattern of B. adolescentis. From bottom and up: (A)XOS standards, initial fermentation broth, broth after 8, 24 and 48h, respectively.

4.3.3 Cultivation of *E. coli*

During cultivation of *E. coli,* the pH did not change hence it is not included in Table 7 in which the ΔOD can be found for *E. coli.* Formation of SCFA was not analysed in this case. Similar results in ΔOD were obtained during cultivation on yeast extract (positive control) and alkali extracted (A)XOS. In contrast to fermentation of the other strains it can be seen in Figure 16 that the OD increases over the entire fermentation irrespectively of the substrate.

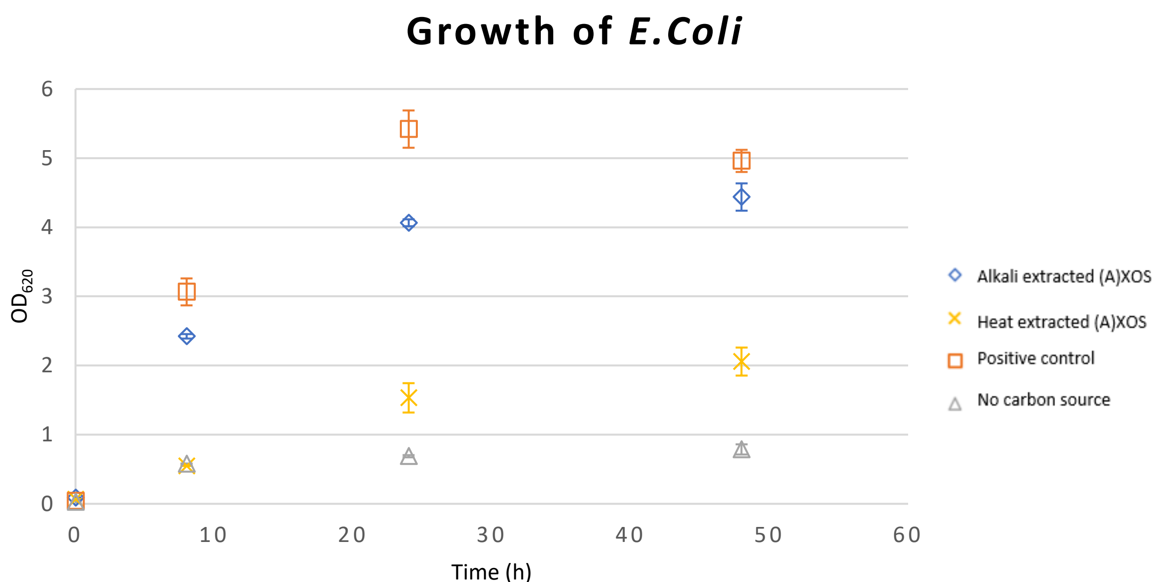

Figure 16. Growth of E. coli on different substrates

Table 7. Change in OD, between t=0 and t=48 h, during the fermentation of E. coli on different substrates.

Substrate	ΔOD
Alkali extracted (A)XOS	4.3
Heat extracted (A)XOS	2.0
Yeast extract	4.9
No Carbon source	0.76

When analysing the utilisation of (A)XOS it could be seen that neither XOS nor AXOS was consumed by *E. coli.* The growth was solely due to utilisation of monosaccharides, glucose, arabinose and xylose could all be utilised as can be seen in Figure 17 and Figure 18.

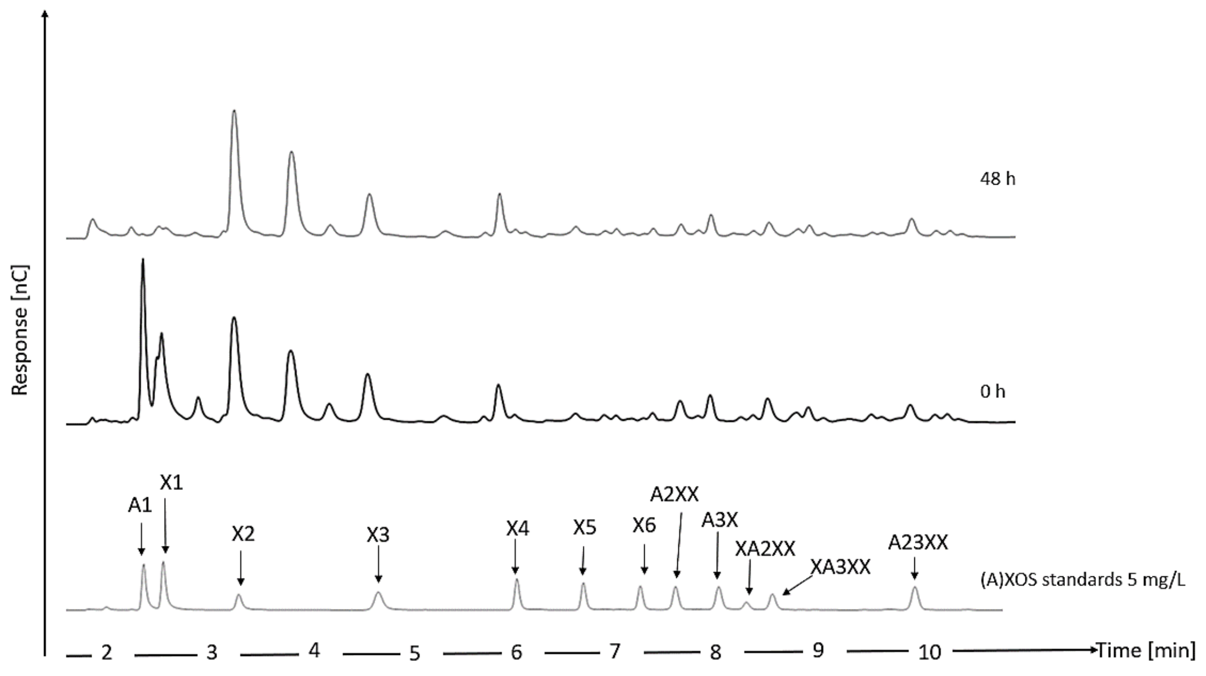

Figure 17. Alkali extracted (A)XOS utilisation pattern of E. coli. From bottom and up: (A)XOS standards, fermentation broth at 0 h and 48 h.

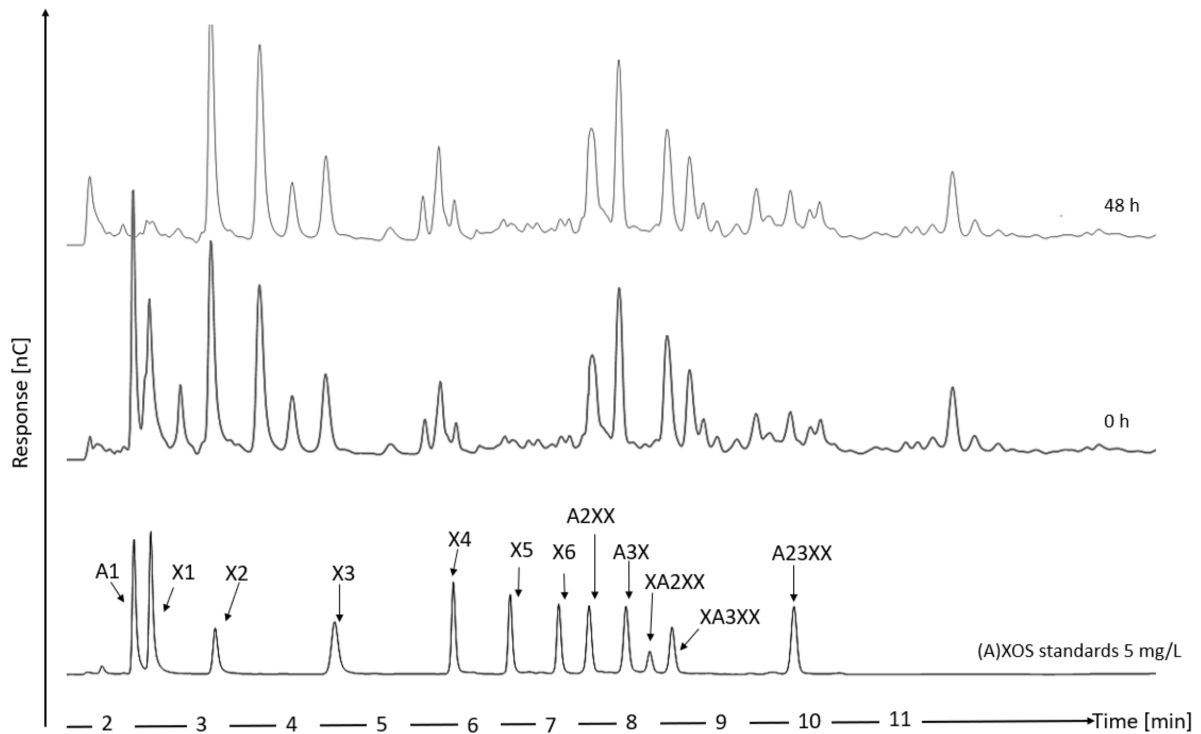

Figure 18. Heat extracted (A)XOS utilisation pattern of E. coli. From bottom and up: (A)XOS standards, fermentation broth at 0 h and 48 h.

5 Discussion

5.1 Pre-treatment

Both alkali and heat treatment increased the content of AX in the materials, 33 and 48% respectively compared to 16% in the raw material.

A remarkable amount of ash was detected in the alkali treated material, this is thought to be due to salts formed during the neutralisation step. This enhances the need of either optimising the ethanol precipitation or including a desalting step if the method is to be used for feed purposes. Desalting was not performed in this study, but perhaps should have been considered to avoid possible inhibition effects. Neglecting the amount of ash, the compositions was rather similar. Including a desalting step after the alkali extraction would increase the purity of the fibres and the percentage of AX%. Given that no losses occur during such a step the AX% of alkali extracted fibres would presumably be more like that of the heat extracted fibres. Alkali treated material contains less phenolic acids which goes in line with theory as this method is known to be harsher for the substituents and especially phenolic acids (Bastos et al., 2018). The difference in phenolic acids could possibly lead to different behaviour of the materials in later processing as the presence of phenolic acid may influence both the accessibility of enzymes and the fermentability. Comparing the monosaccharides, heat extracted fibres did result in a higher AX %. Additionally, the glucose content increased to almost equal that of xylose. More than 50% of this glucose was found to come from beta-glucan, which was also increased after the pre-treatments, due to the extensive removal of starch.

More lignin was removed after heat extraction than alkali extraction in addition to the higher AX content in heat treated fibres suggesting that heat extraction leads to a more desirable and specific removal of unwanted compounds whereas alkali extraction degrades AX.

The yield on mass basis was rather low which is reasonable as the purpose was to remove all unwanted compounds which constitutes the majority in the raw material. After heat extraction 49 % of the available AX was recovered, similar results were reported after heat extraction of rye bran by Falck et al. (2014). The recovery of AX after alkali extraction was somewhat lower, 44 %, which in combination with higher purity indicates that heat extraction may be a better pre-treatment, but the differences are too small to say anything for certain. A considerable amount of AX was lost during the treatment illustrating the need for further purification of the residual or optimisation of the extraction methods.

According to the A/X ratio arabinose was lost already after the destarching step, and later after heat extraction more arabinose was removed. The decrement of arabinose after destarching is somewhat surprising as that step is thought to remove glucose. A possible explanation could be that the arabinose that was removed was present as monosaccharides in the insoluble fibres and thereby was washed away in the washing procedure. Another likely explanation is the uncertainties in the measurement. Previously it has been indicated that ethanol precipitation favours precipitation of xylan with less substituents resulting in a lower A/X ratio (Falck et al., 2014). However, the destarched fibres was analysed before ethanol precipitation was applied, but it is possible that it affects the A/X ratio of the pre-treated fibres. A lower A/X ratio was found in the heat extracted fibres than in the alkali extracted which is supported by previous studies in which the A/X ratio has been less affected under alkaline conditions (Roos et al., 2009). The behaviour can be explained by the fact that hydrolysis of the polymeric backbone occurs during heat extraction (Carvalheiro et al., 2016).

5.2 Enzymatic treatment

The diverse behaviour of GH enzymes from and within different groups suggest that combination of different enzymes could lead to an enhanced hydrolysis and formation of specific products. This was however not seen among the enzymes investigated in this study. There are several possible reasons for that. Firstly, most of the GH enzymes studied belongs to GH11, with only one enzyme belonging in

GH10 and one in GH5-34. That is, several of the enzymes are thought to have similar activity and affinity towards the substrate. With other words synergy should not be expected between the combination of two enzymes belonging in the same group. However, no positive synergy was seen in combinations with enzymes belonging to GH10 and GH5-34 either. A synergistic effect was not expected with CtXyl5A (GH5-34) though as it did display very little activity individually which potentially is due to the rather low degree of arabinose substitution, which will not increase in combination with other enzymes. The low activities obtained with GH enzymes in combination of laccase and ferulic acid esterase indicates that the lignin and ferulic acid present is not a hindrance for the GH enzymes.

Pentopan 500 BG that yielded the most products has previously been described as a multicomponent xylanase with a mixture of different GH functionalities (Ingrid Paula, 2010, Sajib et al., 2018). One could argue that the superior product profile was obtained due to this multifunctionality. Supporting that enzymes from different glycosidic hydrolyses may have a synergistic effect on the hydrolysis even though that was not seen with any of the investigated enzymes in this study. Rather it seems like the mixed functionality of Pentopan 500 BG already is optimised towards this substrate, compared to the other enzymes and combinations examined.

Less AX was converted to identifiable (A)XOS of heat extracted fibres. A possible explanation could be that the polymeric chain was more protected due to remaining substituents such as acetyl groups an ferulic acids which lowered the activity somewhat. Although, the lower A/X ratio in heat extracted fibres suggest that the substrate is more accessible for the enzyme with more unsubstituted xylose units, why opposite results would have been expected. Regardless of the reason, the low conversion of heat extracted AX indicates that a majority of the AX remains unhydrolyzed. That is, if the purpose is to promote growth of the probiotic strains examined in this study a lot of the AX will be wasted as it will be too long to be utilised. Additionally, both treatments resulted in a high fraction of monosaccharides which do not stimulates growth selectively of probiotics. Highlighting the need of tailoring the combination of pre-treatment and enzymatic hydrolysis to optimise the production of (A)XOS without too large losses in terms of monosaccharides or remaining AX polymers. That is further enhanced by the low total conversion of AX if both pre-treatment and enzymatic hydrolysis is taken in account which was 36% and 26% respectively. Since those numbers are based on the identified (A)XOS the actual conversion of AX probably was higher.

Interestingly less AXOS with substitutions at the C2 position in xylose was produced. That could possibly indicate that insoluble fibres from oat bran have little or no arabinose substitution at this position. Dominating substitution at C3 in oat grains has previously been reported by Tian et al. (2015) supporting these findings. However, similar results have also been reported by Mathew et al. (2017) which was explained by the enzymes disability to cleave close to this substitution, which would be a possible explanation in this case as well.

5.3 Fermentation

Both *L. brevis* and *B. adolescentis* could utilise (A)XOS whereas no consumption of the fibres was observed by *E. coli,* reinforcing the prebiotic potential of (A)XOS. The growth of *E. coli* can be explained by the utilisation of monosaccharides including both arabinose and xylose. It was clear that xylobiose, X2, and xylotriose, X3, was consumed by both probiotic species. The broader consumption by *B. adolescentis* which utilised both longer XOS and AXOS goes in line with previous research (Falck et al., 2013).

The degradation of A^3X after 48h of cultivation of *L. brevis* on alkali extracted (A)XOS is surprising as utilisation of AXOS previously has not been shown. It has been shown that *L. brevis* (DSM 1269) has genes coding for an arabinofuranosidase but no activity towards AXOS has previously been detected (Linares-Pastén et al., 2017). Although, a different strain of *L. brevis,* DSM 200054 has been reported to code for an arabinofuranosidase which acts on α-1-3 arabinose linkages, which opens up

the possibility that *L. brevis* degraded and consumed A^3X (Michlmayr et al., 2013). Although, as no other indications of growth was observed when degradation of A^3X was observed it is not very likely. A possible explanation would be that A^3X has been degraded, but that was not seen in the other cultivations. Furthermore, if the compound were degraded one would have expected to see an increase of other compounds for instance arabinose and xylose, unless those are utilised by the bacteria.

Since the growth conditions are rather different it is not possible to strictly compare the growth between different species or substrates. However, one can see that the growth on alkali extracted (A)XOS was larger than growth on heat extracted (A)XOS for both *E. coli* and *L. brevis,* compared to *B. adolescentis* where the opposite trend was observed. This may be due to that the concentration of monosaccharides was fairly higher in alkali extracted (A)XOS which promotes the growth of both *E. coli* and *L. brevis,* but not of *B. adolescentis.* Possibly *B. adolescentis* was inhibited by the high salt concentration in alkali extracted fibres.

Apart from utilisation of identified (A)XOS the unidentified compounds to some extents are consumed as well. Thus, to investigate the growth on specific (A)XOS it could have been wise to produce a hydrolysate with less unidentified compounds. However, consumption of these unidentified compound was not seen by *E. coli,* suggesting that those compounds may have prebiotic potential as well.

The large production of lactic acid is expected of *L. brevis* as it is one of the foremost characteristics of a lactic acid bacteria. *L. brevis* is a heterofermentative bacteria which means that it degrades carbon sources to almost equal amounts of lactic acid and ethanol or acetate depending on the presence of oxygen (Adams et al., 2016). The larger production of acetic acid seen in in the culture with alkali extracted (A)XOS can hence be explained by oxygen in the cultivation, whereas more anaerobic conditions seem to be maintained in the other cultures.

Overall, both species produces lactic acid and SCFA which amplifies the prebiotic potential of (A)XOS produced from insoluble oat bran fibres. Similar differences in production of SCFA and growth between different substrates can be observed and is probably mainly due to differences in concentration of each specific (A)XOS in each cultivation.

6 Conclusions

In this study the possibility to solubilise AX from insoluble oat bran fibres by pre-treatment was investigated. Enzymatic hydrolysis with enzymes in pairs were studied to investigate whether a synergistic effect on production of (A)XOS can be achieved. The prebiotic potential of produced (A)XOS was investigated by cultivation two probiotic strains, *L. brevis* and *B. adolescentis.*

Both alkali extraction with 2.5 M NaOH during 8 h at 65 ℃ and heat treatment for 15 h at 121 ℃ were applied as pre-treatment. It was shown that both pre-treatments indeed solubilised AX with rather similar recovery. The purity was however higher after heat extraction than alkali extraction, mainly due to the extensive amounts of salts that was added during neutralisation. More than 50 % of the AX was lost in this step why it might be interesting to optimise the treatments to reduce the losses.

No positive synergy in (A)XOS production was found with the studied enzyme combinations. Rather Pentopan 500 BG was found to be the most efficient enzyme probably due to its multicomponent functionality. One cannot exclude that enhanced hydrolysis can be achieved by combinations of enzymes, but it is a matter of finding the optimal reaction conditions and enzymes on the specific substrate.

B. adolescentis consumed XOS with a DP of 2-4, A^2XX, A^3X and to some extent AX^3XX. The consumption was similar on both heat and alkali extracted (A)XOS. *L. brevis* utilised XOS with DP 2-3 from both substrates. Additionally, degradation of alkali extracted A^3X was seen. To assure if A^3X was consumed by *L. brevis* additional cultivations could be done to exclude the possibility that

degradation occurred due to other reasons. In addition, sequencing of the specific strain can be done to understand its possible ways of degrading A^3X.

To conclude by processing of oat bran can indeed lead to fibres that selectively stimulates growth of probiotics. Further optimisation and processing are however needed to assure that the AX is used as efficiently as possible.

7 References

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