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Abstract: A proof-of-concept project compared extraction of arabinoxylans (AX) from sugarcane bagasse and wheat bran via alkaline hydrogen peroxide followed by enzyme-assisted extraction with combinations of feruloyl esterases and a xylanase. Bagasse contains comparable amounts of AX to wheat bran, but with a much lower arabinoxylan substitution on the xylan backbone (A:X ratio of around 0.2 compared with 0.6 for wheat bran), hence offering AX products with distinctive functionality and potential end uses. In the current work, bagasse released its AX more readily than wheat bran, and released a wider range of molecular weights. Use of feruloyl esterase and xylanase enzymes on their own or following alkaline peroxide extraction did not enhance AX release substantially; however, the xylanase appeared to be effective at reducing the size of AX molecules, and there is scope to optimise the effects of enzymes to produce specific AX product fractions. As bagasse frequently arises within the context of bioethanol production, integration of AX extraction with ethanol production could allow economic production of a portfolio of AX products, as has been demonstrated in principle for AX co-production in a wheat ethanol plant.

Dr James Winterburn
Guest Editor *Biochemical Engineering Journal*
– “Bioprocesses Development” Special Issue

17th November 2018

Dear Dr Winterburn,

We are pleased to submit to the *Biochemical Engineering Journal* a manuscript entitled:

“Integrated processing of sugarcane bagasse:
Arabinoxylan extraction integrated with ethanol production.”

for consideration for the upcoming Special Issue entitled “Bioprocess Development”. The work arose from a proof-of-concept project funded by the Lignocellulosic Biorefinery Network (LBNet). We are aware that the small scope of the project limits the findings; however, the findings are sufficiently robust to support the objectives and conclusions, while the paper contains additional value in terms of some specific technical outcomes as well the broader perspectives it illustrates that are well aligned with the Bioresources and Biorefinery Engineering theme and with the momentum of research in this area. We therefore hope that the manuscript will be considered well suited to publication in this Special Issue of the *Biochemical Engineering Journal*.

Declaration of interests: None.

Yours sincerely,



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**Integrated processing of sugarcane bagasse:
Arabinoxylan extraction integrated with ethanol production.**

Highlights

- Bagasse released arabinoxylan more readily than bran, with a wider range of sizes
- Feruloyl esterase and xylanase enzymes could help to produce specific AX fractions
- Integration of AX extraction with ethanol production may allow economic production

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**Integrated processing of sugarcane bagasse:
Arabinoxylan extraction integrated with ethanol production.**

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Abstract

A proof-of-concept project compared extraction of arabinoxylans (AX) from sugarcane bagasse and wheat bran via alkaline hydrogen peroxide followed by enzyme-assisted extraction with combinations of feruloyl esterases and a xylanase. Bagasse contains comparable amounts of AX to wheat bran, but with a much lower arabinoxylan substitution on the xylan backbone (A:X ratio of around 0.2 compared with 0.6 for wheat bran), hence offering AX products with distinctive functionality and potential end uses. In the current work, bagasse released its AX more readily than wheat bran, and released a wider range of molecular weights. Use of feruloyl esterase and xylanase enzymes on their own or following alkaline peroxide extraction did not enhance AX release substantially; however, the xylanase appeared to be effective at reducing the size of AX molecules, and there is scope to optimise the effects of enzymes to produce specific AX product fractions. As bagasse frequently arises within the context of bioethanol production, integration of AX extraction with ethanol production could allow economic production of a portfolio of AX products, as has been demonstrated in principle for AX co-production in a wheat ethanol plant.

Keywords: sugarcane bagasse, wheat bran, arabinoxylans, bioethanol, biorefinery integration.

Abbreviations

AX	Arabinoxylan
UoH	University of Huddersfield
UoL	University of Lincoln
UoY	University of York
UoStA	University of St Andrews
BDC	Biorenewables Development Centre
LBNet	Lignocellulosic Biorefinery Network
P2PNet	Plants to Products Network
CE-High	High cut-off fraction (from ultrafiltration over 10 kDa) following chemical extraction

48	CE-Low	Low cut-off fraction (<10 kDa) following chemical extraction
49	EE-High	High cut-off fraction following enzyme-assisted extraction
50	EE-Low	Low cut-off fraction following enzyme-assisted extraction
51	XYL	β -Xylanase (<i>C. mixtus</i> , PRO-E0051, Prozomix UK)
52	FE-E0355	Feruloyl esterase (<i>A. cellulolyticus</i> , PRO-E0355, Prozomix UK)
53	FE-E0356	Feruloyl esterase (<i>A. cellulolyticus</i> , PRO-E0356, Prozomix UK)

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55 **Declarations of interest:** None.

56 57 58 **Introduction**

59
60 For a reaction to take place, entities must come together and interact under the right conditions.
61 The interactions leading to the current work arose from a workshop organized in September 2014 by
62 the Lignocellulosic Biorefinery Network (LBNet, <https://lb-net.net>), a Biotechnology and Biological
63 Sciences Research Council Network in Industrial Biotechnology and Bioenergy (BBSRC NIBB). At this
64 workshop, one of those who eventually formed a consortium asked of another, “If I want to use a
65 source of lignin for a chemical conversion process I have developed, what lignin would make a good
66 source?”, to which the other advised “One that already arises naturally within existing biorefineries,
67 such that transportation costs are negated and the infrastructure and integration opportunities are
68 in place to enable the lignin processing to be undertaken economically, and in which the raw
69 material might already be partially degraded through earlier processing, to give more ready access
70 to the lignin.” At a later point in the workshop, the participants were asked to write on pieces of
71 paper what we would like to research, and place them on the floor. One of us wrote
72 “Arabinoxylans”, based on a long-standing interest in this subject as a promising co-product of
73 ethanol biorefineries, as the ethanol is used to precipitate the arabinoxylans, making the production
74 of AX potentially economically viable in that context [1]. Meanwhile a representative of AB Sugar
75 wrote “Something valuable from sugarcane bagasse (not furfural, we already do that)”. Seeing
76 these notes together prompted the idea that arabinoxylans might similarly be extracted from
77 bagasse in an integrated biorefinery producing ethanol from sugarcane, and that the residual
78 bagasse following AX extraction might reveal a suitable source of lignin for further processing. A
79 proof-of-concept project was constructed, to demonstrate the feasibility of AX extraction from
80 sugarcane bagasse, and to examine the residual lignin, following partial deconstruction during the
81 AX extraction process, for its suitability as a feedstock for conversion into a phenolic monomer using
82 an established reaction sequence [2]. A parallel activity studying wheat bran was included, to
83 extend the scope and make the findings more immediately applicable to the UK context (where
84 bioethanol production is largely from wheat). Enzyme enhancement of the AX release, a scale-up
85 component and a techno-economic analysis of AX production were also included in the project, to
86 give a consortium comprising the Universities of Huddersfield, St Andrews, York, Lincoln and
87 Nottingham along with the Biorenewables Development Centre in York
88 (<http://www.biorenewables.org/>). Later a student project supported by the Plants to Products
89 Network (another BBSRC NIBB, <http://www.nibbp2p.org>) extended the project by undertaking a
90 bioethanol pinch analysis, following the approaches of Martinez et al. [3], to minimise ethanol usage
91 while producing a range of AX products including arabinoxylan-oligosaccharides (AXOS) [4].

92
93 This paper describes the proof-of-concept work undertaken to demonstrate AX extraction from
94 sugarcane bagasse and to compare it with extraction from wheat bran in terms of yield, composition

95 and responsiveness of the extraction process to enzyme enhancement. A future paper will describe
1 96 the investigation of the residual bagasse following AX extraction, in terms of the nature of its lignin
2 97 and its suitability for further processing, including further integration opportunities with ethanol and
3 98 butanol.
4 99

5 100 Global sugarcane production is around 1.9 billion tonnes per annum
6 101 (<https://www.statista.com/statistics/249604/sugar-cane-production-worldwide/>), resulting in
7 102 around 570 million tonnes wet bagasse or half this amount if dried. Sugarcane bagasse thus
8 103 represents a major waste stream arising from sugar and alcohol industries, typically containing
9 104 around 40-50% cellulose, 25-35% hemicelluloses (predominantly xylans) and 20-30% lignin [5,6]. A
10 105 focus of previous work has been to deploy feruloyl esterases and xylanases to assist the
11 106 saccharification of bagasse to increase recovery of fermentable sugars, by removing the
12 107 hemicelluloses that (to put it simply) link lignin and cellulose, thus increasing accessibility of the
13 108 latter to cellulases and hence the release of glucose [7]. A difference in the current work is that the
14 109 intention was not to hydrolyse arabinoxylan hemicelluloses to their constituent sugars, but rather to
15 110 release and recover them as intact large AX molecules, in which form they have potential as
16 111 functional food ingredients and non-food products [8,9]. The context of the ethanol biorefinery
17 112 gives scope for AX co-production to be economic as a result of integration with ethanol production
18 113 (used for precipitating the AX), as has been shown previously for AX production from wheat bran in
19 114 a wheat ethanol biorefinery [1]. Wheat bran typically contains 20-30% AX [10,11], similar to
20 115 bagasse, but with a much higher ratio of arabinose to xylose units; in wheat bran the A:X ratio is
21 116 typically in the range 0.5-0.6 [12,13]), while in sugarcane bagasse it is much lower at around 0.2
22 117 typically [14]. This “cleaner” xylan backbone with fewer arabinose substitutions is likely to exhibit
23 118 different functional properties compared with wheat bran AX, including reduced solubility and
24 119 greater susceptibility to enzyme action, as well as effects on viscosity and gel formation, and
25 120 performance in food products or animal feed.
26 121
27 122

28 123 **Materials and Methods**

29 124
30 125 Sugarcane bagasse (25 kg) and wheat bran (50 kg) were sourced commercially by AB Sugar and
31 126 provided to the project. The bagasse and bran were milled at the Biorenewables Development
32 127 Centre (BDC) using a Retsch Cutting Mill SM 300 (Retsch GmbH, Germany) with a 2.00 mm screen,
33 128 and AX extraction studies undertaken at the University of Huddersfield (UoH). Proximate analysis
34 129 and arabinoxylan (AX) content measurements were performed at the University of York (UoY).
35 130 Lignin studies, to be presented in a future paper, were undertaken at the University of St Andrews
36 131 (UoStA).
37 132

38 133 Figure 1 describes the chemical extraction process using alkaline hydrogen peroxide applied to the
39 134 milled wheat bran and sugarcane bagasse, based on the work of Hollmann and Lindhauer [15] and
40 135 Du et al. [16]. The residue material was then subjected to enzyme treatment to see if further
41 136 release of AX resulted, using selected combinations of β -Xylanase (*C. mixtus*, PRO-E0051, referred to
42 137 here as XYL) and two types of Feruloyl esterase (*A. cellulolyticus*, PRO-E0355 and PRO-E0356,
43 138 referred to here as FE-E0355 and FE-E0356) from Prozomix UK. Enzyme-assisted extraction on its
44 139 own was also investigated, along with chemical extraction followed by further extraction with buffer
45 140 solution, and buffer extraction on its own.
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142 In total eight extractions were performed for each feedstock:

- 1 143 1. Direct buffer extraction (control for all trials, particularly Trials 7-8);
- 2 144 2. Chemical extraction followed by buffer extraction (control for Trials 3-6);
- 3 145 3. Chemical extraction followed by FE-E0355 and XYL;
- 4 146 4. Chemical extraction followed by FE-E0356 and XYL;
- 5 147 5. Chemical extraction followed by FE-E0355;
- 6 148 6. Chemical extraction followed by FE-E0356;
- 7 149 7. Direct enzyme extraction with FE-E0355 and XYL;
- 8 150 8. Direct enzyme extraction with FE-E0356 and XYL.

9 151
10 152 The supernatant following treatment, centrifugation and filtering was separated by ultrafiltration
11 153 over a 10 kDa membrane (see below for details). The chemical extraction thus yielded High cut-off
12 154 (CE-High) and Low cut-off (CE-Low) fractions and a pellet of residual material; enzyme extractions
13 155 directly on the raw material or on the pellet following chemical extraction similarly yielded High cut-
14 156 off (EE-High) and Low cut-off (EE-Low) fractions and a pellet. A single chemical extraction was
15 157 performed to produce residual material for subsequent enzyme extraction in Trials 3-6; a second
16 158 chemical extraction was performed for subsequent buffer extraction (Trial 2).

17 159
18 160 For the chemical extraction, 50 g bran or 11 g bagasse (because of the lower bulk density of the
19 161 latter restricting the amount that could be processed in a bottle) was weighed into a 1 L Duran
20 162 bottle. Foaming is a problem during AX extraction, so 15 drops of anti-foaming agent (Dimeticon
21 163 SILFAR® SE 4, Wacker Chemie AG, Germany) were added, then 400 mL of 2% hydrogen peroxide
22 164 (Fisher Scientific UK Limited, analytical grade) was added very slowly, with stirring with a magnetic
23 165 stirrer. 5 more drops of anti-foaming agent were added, followed by the final 100 mL of hydrogen
24 166 peroxide solution. The pH was adjusted to 11.5 with 50% NaOH. The bottle was placed in a 50°C
25 167 water bath and the solution stirred for 4 h. The pH was controlled every hour and readjusted to 11.5
26 168 if needed. After cooling to room temperature the pH of the mixture was adjusted to 7 with
27 169 concentrated sulphuric acid. The solution was centrifuged for 15 minutes at 4000 rpm using a
28 170 Beckman GS-6S centrifuge (Beckman Coulter Life Sciences, USA). The supernatant was filtered and
29 171 the remaining solids washed with 150 mL of water, then centrifuged, filtered, washed with 150 mL
30 172 water and centrifuged again. The final supernatant was filtered and pooled with the previous two.
31 173 The residual solid (the pellet) was placed in the oven to dry overnight at 50°C.

32 174
33 175 The high molecular weight material in the supernatant was concentrated by ultrafiltration using a
34 176 Vivaflow™ 200 system (Sartorius Stedim Biotech GmbH, Germany) with polyethersulfone
35 177 membranes with a molecular weight cut-off of 10 kDa. (Ultrafiltration would be done as part of a
36 178 commercial process to reduce the amount of ethanol needed subsequently to precipitate the AX
37 179 [1,15]; in the current work the fractions were not precipitated. The ultrafiltration served to separate
38 180 the released AX into larger MW and smaller MW fractions.) Retentate was recycled until the volume
39 181 was reduced to one fifth of the original. The retentate (High cut-off) and permeate (Low cut-off)
40 182 fractions were freeze-dried using a Christ Freeze Dryer Alpha 1-4 LDplus, (Martin Christ
41 183 Gefriertrocknungsanlagen GmbH, Germany) at -47.8°C and 0.35 mbar. Samples of freeze-dried High
42 184 cut-off and Low cut-off material and oven-dried pellets were sent to UoY for AX analysis (see below).
43 185 Samples of the dried pellet material were also sent to the University of St Andrews (UoStA) for
44 186 assessment of its lignin, to be described in a future paper.

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188 For enzyme-assisted extraction trials on raw wheat bran or sugarcane bagasse or on pellets after
1 189 chemical extraction, material (30 g for the wheat bran and 6 g for the sugarcane bagasse) was
2 190 weighed into a 1 L Duran bottle with a magnetic stirrer. 300 mL of a buffer (composed of 107.4 mL
3 191 of 0.2 M disodium phosphate, 42.6 mL of citric acid and 150 mL of water) at pH 6.5 was added, the
4 192 bottles placed in a 37°C water bath and the pH of the solution readjusted to 6.5 with citric acid when
5 193 the temperature reached 37°C. The different enzymes were added at a level of 10 µg/10 g for the
6 194 XYL and FE-E0355 and 5µg/10 g for the FE-E0356, and the solution was stirred for 2 h. As above,
7 195 samples were centrifuged, filtered and washed, in this case using 100 mL of water for each washing,
8 196 and the residual solid pellet oven-dried overnight at 50°C. Again the supernatant was passed
9 197 through ultrafiltration over 10 kDa and the retentate (High cut-off, EE-H) and permeate (Low cut-off,
10 198 EE-L) freeze dried and sent to UoY for analysis along with the oven-dried pellet, with pellet samples
11 199 also sent to UoStA for lignin analysis.

16 200
17 201 Due to the limited scope of this small proof-of-concept project, replicate enzyme extractions were
18 202 not performed, the aim being to demonstrate broad effects in relation to AX yields from wheat bran
19 203 and bagasse and the potential effects of enzyme-assisted extraction. Five replicate chemical
20 204 extractions were performed, as enzyme extraction Trials 3-6 and buffer Trial 2 each required a
21 205 chemical extraction first, although High and Low cut-off fractions were subsequently produced for
22 206 only two of these (2 and 3), and only these two pellets were sent for lignin analysis. Subsequent
23 207 similar work with replication has confirmed the broad trends reported here and shown sufficient
24 208 reproducibility to have confidence in the trends, which are reported and discussed here within the
25 209 limits of the acknowledged lack of replication.

26 210
27 211 Proximate analysis of the wheat bran and sugar cane bagasse was undertaken as follows:

28 212 29 213 *Lignin determination: acetyl bromide method*

30 214 Biomass powder was weighed out (4 mg) into 2 mL tubes. The biomass was heated at 50°C for 3
31 215 hours after adding 250 µL of acetyl bromide solution (25% acetyl bromide and 75% glacial acetic acid
32 216 by volume) and vortexing every 15 minutes. After the samples were cooled to room temperature,
33 217 the contents were transferred into 5 mL volumetric flasks. A further 1 mL of NaOH (2 mol L⁻¹) was
34 218 used to rinse the tubes pouring the NaOH into the 5 mL flasks. 175 µL of hydroxylamine HCl (0.5 mol
35 219 L⁻¹) was added to the volumetric flasks and, after vortexing, the latter were filled up to 5 mL with
36 220 glacial acetic acid and mixed several times. Finally, in order to measure the 280 nm UV adsorption
37 221 by spectrophotometer, 100 µL of each sample was diluted in 900 µL of glacial acetic acid. The
38 222 amount of lignin was calculated using the following formula: [absorbance/(coefficient pathlength)] ·
39 223 [(total volume · 100%)/biomass weight], where coefficient = 15.69, pathlength = 1, total volume = 5,
40 224 biomass weight = 4.

41 225 42 226 *Non-cellulosic monosaccharide determination*

43 227 Following the method of Fry [17], biomass dry powder (4 mg) was partially hydrolyzed by adding 0.5
44 228 mL of trifluoroacetic acid (TFA, 2 mol L⁻¹). Then, the vials were flushed with dry argon, mixed and
45 229 heated at 100°C for 4 hours, mixing periodically. The vials were then cooled to room temperature
46 230 and dried in centrifugal evaporator with fume extraction overnight. The pellets were washed twice
47 231 with 500 µL of 2-propanol and vacuum dried. Finally, the samples were resuspended in 200 µL of
48 232 deionised water, filtered with 0.45 µm PTFE filters, and analyzed by HPAEC (see below).

49 233

234 It became evident that these hydrolysis conditions were inadequate to release all the AX in the
1 235 original wheat bran (see below, where the mass balance indicates more AX in the extracted fractions
2 236 than appeared to be present initially in the bran). The wheat bran was therefore hydrolysed under a
3 237 range of conditions, to investigate the effects on AX measurement and to draw conclusions about
4 238 the most appropriate conditions for AX analysis in wheat bran. The bran samples were hydrolysed in
5 239 4M TFA (2 mL per 4 mg bran) for 1, 2 and 4 hours, and in 2M (2 mL per 4 mg bran) for 4 and 6 hours
6 240 at 120°C. In addition, samples were pretreated in 98% TFA for 1, 2, 4 and 6 hours at room
7 241 temperature, then diluted to 4M and boiled for 1 hour at 120°C.
8 242

12 243 *Crystalline cellulose*

13 244 Biomass dry pellets after TFA hydrolysis were washed once with 1.5 mL of water, and twice using 1.5
14 245 mL of acetone. The dried pellets were left to air dry overnight before complete hydrolysis by adding
15 246 90 µL of 72% w/w sulphuric acid, incubating at room temperature for 4 hours. 1.89 mL of water was
16 247 subsequently added and the sample was heated for 4 hours at 120°C. The glucose content of the
17 248 supernatant was assessed using the colorimetric Anthrone assay, using a glucose standard curve.
18 249

19 250 Analysis of the sugar compositions of the wheat bran and bagasse samples and of fractions and
20 251 residues following the various chemical and enzyme extractions was undertaken as follows:
21 252

22 253 Monosaccharide analysis was performed by high performance anion-exchange chromatography
23 254 (HPAEC) (Dionex IC 2500) on a Dionex CarboPac PA-10 column with integrated amperometry
24 255 detection [18]. The separated monosaccharides were quantified using external calibration with an
25 256 equimolar mixture of nine monosaccharide standards (arabinose, fucose, galactose, galacturonic
26 257 acid, glucose, glucuronic acid, mannose, rhamnose, and xylose), which were subjected to TFA
27 258 hydrolysis in parallel with the samples.
28 259

36 260 **Results and Discussion**

37 261
38 262
39 263 Table 1 shows the compositions of the wheat bran and sugarcane bagasse used in the current work.
40 264 The wheat bran had a significant starch content; therefore for the scale-up work undertaken at BDC,
41 265 the wheat bran was washed with water to remove starch prior to extraction, in line with the
42 266 recommendation of Du et al. [16]. The bagasse had 28% lignin and nearly 20% AX, suggesting it was
43 267 a promising candidate for recovery of both materials. The analytical procedure used indicated 10%
44 268 lignin in the wheat bran, although subsequent NMR work suggested a much lower lignin content, in
45 269 line with other recent reports that wheat bran contains less lignin than previously thought. These
46 270 results also suggest an AX content of only 8.64% in the wheat bran; this figure is lower than the 20-
47 271 30% generally expected for wheat bran, and later proved to be incompatible with the mass balance
48 272 for AX recovered in the various fractions and residues, which suggested an AX content in the original
49 273 material of around 24% (see below). The A:X ratio for the wheat bran was 0.57, and for the bagasse
50 274 0.21, in line with typical values expected from the literature, and showing the much “cleaner” xylan
51 275 backbone for the bagasse AX, with fewer arabinose substitutions compared with the wheat AX.
52 276

53 277 Tables 2 and 3 report the crude yields, AX concentrations and hence AX yields from 50 g wheat bran
54 278 and 11 g sugarcane bagasse, respectively, in the starting materials and in the fractions following the
55 279 various chemical, enzyme and buffer treatments. The second column in each table is for chemical
56 280 extraction only, showing the data from two replicates, with good agreement. Considering the wheat
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281 bran results first, chemical extraction of around 50.6 g of bran (moisture content 9.5%) yielded a
1 282 residual dry pellet weighing 29.94 g (averaged from Trials 2 and 3), a High cut-off (CE-High) of 8.21 g,
2 283 and a Low cut-off (CE-Low) of 4.40 g (total 42.55 g, c.f. about 45 g solids in the original material).
3 284 The AX concentration in the pellet was 35.81% and in the High and Low cut-off fractions was 16.32%
4 285 and 1.25%, respectively. This implies a total amount of AX in the pellet and two fractions of
5 286 $29.94 \times 35.81\% + 8.21 \times 16.32\% + 4.40 \times 1.25\% = 10.72 + 1.34 + 0.06 = 12.12$ g. The mass balance
6 287 therefore implies an AX concentration of $12.12/50.6 = 24\%$ in the original wheat bran, higher than
7 288 the 8.64% reported in Table 1, and more in line with the expected AX content of wheat bran,
8 289 suggesting that the 8.64% figure is erroneous.

290
13 291 It is well known that the appropriate hydrolysis conditions for this sort of analysis depend on the
14 292 nature of the sample, with different samples requiring different combinations of time, temperature
15 293 and acidity to get an optimum balance between release and degradation of monosaccharides
16 294 [19,20]. The bran sample was therefore reanalysed under a range of hydrolysis conditions as
17 295 described above. Figure 2 shows the AX contents calculated for each of the hydrolysis regimes.
18 296 Under similar conditions to the original analysis (2M for 4 hours, but at a higher temperature,
19 297 120°C), the measurement was similar at 8.79%, while 2M for 6 hours released more AX to give
20 298 14.04%. 4M for 1 or 2 hours released even more (18.58-19.71%), but 4M for 4 hours appears to give
21 299 substantial degradation and a final measurement of only 5.50%. Pretreatment in concentrated acid
22 300 appeared effective at releasing more AX for effective hydrolysis, giving measurements of around
23 301 26.7% after 1 or 2 hours of pretreatment, decreasing to 22.8 and 20.6% after 4 and 6 hours,
24 302 suggesting degradation at the high acid concentration despite the low temperature. Overall, these
25 303 results demonstrate that an AX content of around 24%, as implied by the mass balance, is plausible,
26 304 but that measuring the AX content of raw wheat bran requires different hydrolysis conditions
27 305 compared to extracts or the residual pellet following extraction.

28 306
29 307 Thus, of a total of around 12 g AX in the original 50 sample, 1.4 g or 11.5% was released by the
30 308 alkaline hydrogen peroxide extraction process. This is much lower than the recoveries of 46-50%
31 309 reported by Hollmann and Lindhauer [15] and Du et al. [16] for alkali-extracted AX from wheat bran
32 310 using similar conditions. In those studies the wheat bran was boiled in 70% ethanol at 80°C for 4
33 311 hours prior to alkaline H₂O₂ treatment, which Hollmann and Lindhauer [15] advised was necessary to
34 312 achieve high yields; this step was omitted in the current work (for safety and cost reasons in relation
35 313 to the planned scale-up work, and because related unpublished work from our labs on extraction
36 314 from maize meal had found that this step was not needed, as also confirmed by work from Doner
37 315 and Hicks [21] on AX extraction from maize fibre). The omission of this ethanol boiling step is
38 316 possibly the reason for the lower yields than in this previously reported work.

39 317
40 318 Considering the sugarcane bagasse results, chemical extraction of around 11.5 g of bagasse
41 319 (moisture content 9.3%) yielded a residual dry pellet weighing 5.43 g (averaged from Trials 2 and 3),
42 320 a High cut-off (CE-High) of 3.11 g, and a Low cut-off (CE-Low) of 8.70 g. The total appears to be
43 321 17.34 g, c.f. about 10.5 g solids in the original material; the mass balance does not give good
44 322 agreement in this case. This is probably because the Low cut-off was extrapolated from the solids
45 323 left after freeze-drying dilute samples (and the freeze-dried samples may not have been completely
46 324 dry), such that the 8.70 g figure is not accurate, while overall the mass balance from just 11 g of
47 325 bagasse is inherently less accurate than that from 50 g wheat bran, and the contribution from salts
48 326 formed on neutralisation relatively greater.

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328 The AX concentration in the pellet was 14.02% (averaged from Trials 2 and 3, although higher in
1 329 Trials 4-6) and in the High and Low cut-off fractions was 23.64% and 7.23%, respectively. This
2 330 implies a total amount of AX in the pellet and two fractions of $5.53 \times 14.02\% + 3.11 \times 23.64\% +$
3 331 $8.70 \times 7.23\% = 0.775 + 0.735 + 0.629 = 2.139$ g, compared with $11.54 \times 19.58\% = 2.260$ g of AX in the
4 332 original sample. This mass balance appears reasonable, although the uncertainty over the Low cut-
5 333 off contribution is acknowledged. In this case it appears that the AX left in the pellet (assuming this
6 334 to be a more accurate figure) is about one-third of the AX in the original material ($0.775/2.25 = 34\%$),
7 335 implying nearly two-thirds has been released, compared with only 11.5% for the wheat bran. This
8 336 suggests sugarcane bagasse may be very amenable to AX extraction, as it appears to yield its AX
9 337 more readily than wheat bran. The readiness of the bagasse to release its AX compared with wheat
10 338 bran suggests the expensive and hazardous ethanol boiling step advised by Hollmann and Lindhauer
11 339 [15] for wheat bran may not be needed in a bagasse-based AX extraction process. As noted above,
12 340 Doner and Hicks [21] advised that dewaxing with toluene-ethanol was unnecessary for AX extraction
13 341 from maize fibre.
14 342

15 343 Figure 3 shows the crude yields of High and Low cut-off material following the various wheat bran
16 344 extractions, the AX concentrations in the fractions and hence the absolute yields of AX in each
17 345 fraction. Trial 1 shows the recovery of High and Low fractions from just extraction with Buffer.
18 346 Clearly, while quite a lot of small molecular weight material (<10 kDa) was recovered in the Low cut-
19 347 off fraction, it contained very little AX, while some high MW AX was extracted just with the use of
20 348 Buffer. Chemical extraction is shown in Trials 2 and 3, with good agreement, showing roughly twice
21 349 as much High cut-off material as Low was recovered, but that the latter contained very little AX, such
22 350 that the majority of the recovered AX was in the High cut-off fraction, giving a yield of around 2.6%
23 351 compared with only 0.62% with Buffer. Thus chemical extraction using alkaline hydrogen peroxide
24 352 was somewhat successful at releasing high MW AX from wheat bran. Further extraction with Buffer
25 353 (Trial 2) recovered a little more high MW AX.
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27 355 Turning to Trials 7 and 8, use of FEA enzymes in combination with XYL appeared to enhance release
28 356 of AX a little compared with just Buffer, but not substantially. Trials 3-7 taken together indicate that
29 357 enzyme treatment following chemical extraction was able to release a little more AX, with the
30 358 balance changing towards low MW material when the xylanase was included. This makes sense,
31 359 although the absence of any detectable AX in the fractions from Trial 6 is unexpected. However, in
32 360 general the enzymes did not dramatically enhance the further extraction of AX, and it is not possible
33 361 to infer any meaningful differences in the performance of the different enzyme combinations.
34 362

35 363 Figure 4 shows the equivalent results for sugarcane bagasse. Clearly the patterns are overall quite
36 364 contrasting to those for the wheat bran. Most obviously, from Figure 4(c), the recovery of AX by
37 365 chemical extraction was much greater than for wheat bran for both High and Low cut-off material,
38 366 with yields of around 6-7% for high MW AX and 5.5% for low MW AX, compared with 2.6% and 0.1%,
39 367 respectively, for wheat bran. Thus the bagasse released its AX much more readily following chemical
40 368 extraction, and released a more balanced profile of AX between large and small MW molecules; this
41 369 is also apparent in Figure 4(a) which shows much greater crude yields of Low cut-off material than
42 370 High, in contrast to the yields from wheat bran. A consequence of the ready release of AX under
43 371 chemical extraction is that there is therefore less material for the enzymes subsequently to work on,
44 372 hence the subsequent enzyme treatments yield very little extra AX, although again there is evidence
45 373 that the presence of the xylanase shifts the balance towards smaller molecules, as expected; this is
46 374 clearer in Figure 5 which presents an expanded view of the Absolute yields, to allow the effects of
47 375

375 the enzyme treatments, such as they are, to be seen more clearly. Even on their own, however,
1 376 without prior chemical extraction, the enzymes release little more than Buffer alone (Trials 7-8 *c.f.*
2 377 Trial 1), with the extra being entirely small MW material. It is recognised, however, that this small
3 378 study did not explore a wider range of enzyme dosages and incubation conditions; it is likely that the
4 379 effects of the enzymes could be enhanced under optimised conditions.
5 380

8 381 Figure 6 shows the A:X ratios in the various wheat bran and bagasse extracts. In line with the
9 382 starting material and as expected from literature reports, the wheat bran extracts have much higher
10 383 A:X ratios than those from bagasse. Given the limitations of the work, not much more can be read
11 384 into the fine detail of Figure 6, beyond noting that for the bagasse extracts, the smaller molecular
12 385 weight material (Low cut-off fractions) consistently had lower A:X ratios than the corresponding
13 386 larger MW fractions, whereas the wheat-derived AX presents a more mixed picture. It is well
14 387 established that in general different parts of the biomass structure contain AX with different
15 388 molecular weights and A:X ratios, reflective of different botanical functions of AX in different parts of
16 389 the plant [22]. For the bagasse extracts, it appears to be consistently the case that material initially
17 390 released by whichever means (chemical extraction, enzymes or buffer) has higher A:X ratios than
18 391 material released subsequently via further extraction with buffer or enzymes, again reflecting
19 392 differences in the nature of AX material given up easily compared with that released on further
20 393 processing. The picture is less consistent for the wheat bran extracts, reflecting that the initial
21 394 release was less extensive from the wheat bran than from the bagasse, such that comparisons are
22 395 less dominated by that initial release; for the bagasse, so much was released initially that the
23 396 remaining AX material is understandably quite different, whereas for the wheat bran, so little was
24 397 released at all that what was released at any point was similar in structure. The A:X ratios greater
25 398 than 1 for some of the Low cut-off wheat bran fractions (Trials 1 and 2) are probably erroneous,
26 399 arising from errors in measuring very low concentrations of A and X in these samples (see Figure
27 400 2(b)), although some components of wheat outer layers (cross-cells and pericarp) can have A:X
28 401 ratios great than 1 [22].
29 402

38 403 As noted already, the cleaner xylan chains of bagasse AX would offer somewhat different properties
39 404 compared to those of wheat AX. This is an important consideration in developing commercial
40 405 products; the challenge is to understand the functional performance and potential uses of AX
41 406 fractions as affected by molecular weight and A:X ratio, and hence to understand which feedstocks
42 407 and extraction processes are suitable for producing specific fractions. Even then, the likely scenario
43 408 is not that specific fractions would be targeted for exclusive production, but rather that processing
44 409 would co-produce a range of AX fractions, each suitable for different end-use applications, including
45 410 small AXOS fractions with prebiotic functionality in food and in animal feed, alongside mid-range and
46 411 large molecular weight fractions offering gradations of product functionality in relation to viscosity,
47 412 gel formation and interaction with other food components [4]. In this respect, commercialisation of
48 413 AX-based products is likely to follow the fractionation paradigm of crude oil cracking, to produce a
49 414 range of products and to find markets for each. The use of enzymes would form part of the
50 415 approach for creating specific fractions with targeted end-uses.
51 416

57 417 The above observations and comments regarding the results from the current work are made in full
58 418 recognition of the limits of replication and accuracy of the study; nevertheless, the overall patterns
59 419 are clear, relative to the objectives of the work and the wider commercial context, and lead
60 420 confidently to the following conclusions:
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- 422 1. Arabinoxylans can be extracted from sugarcane bagasse via similar protocols previously used for
423 wheat bran, yielding AX with a lower A:X ratio than for wheat bran.
- 424 2. Bagasse released its AX more readily than wheat bran, and released a wider range of AX
425 molecules, with a greater proportion of small MW (<10 kDa) molecules. In the current work,
426 around two-thirds of the AX in the bagasse was released by chemical extraction, more or less
427 equally divided between High and Low cut-off material, compared with just 11% of mostly large
428 MW AX initially in the wheat bran.
- 429 3. Within the conditions used, feruloyl esterase and xylanase enzymes had small effects on
430 releasing AX from raw wheat bran or sugarcane bagasse or from residues following chemical
431 extraction, with the xylanase tending to shift the balance from large to small MW molecules.

432
433 Following these results, larger scale extractions of AX from bran and bagasse were performed at the
434 Biorenewables Development Centre (BDC) using alkaline hydrogen peroxide extraction. Bran was
435 washed with water prior to extraction to remove the starch. 25 kg of bran and bagasse, in 5 kg
436 batches, were subject to chemical extraction, centrifugation, ultrafiltration, ethanol precipitation,
437 recovery and drying. A total of 1250 g of wheat bran extract (5% yield) of 54% purity and 848 g of
438 bagasse extract (17% yield) at 52% purity were produced, with much of the rest being analysed as
439 glucose, either from residual starch or from cellulose.

440 441 442 **Conclusions**

443
444 The hypotheses that arabinoxylans could be extracted from sugarcane bagasse using similar
445 protocols used for wheat bran, and that enzyme treatment might enhance the extraction, were
446 investigated in a small proof-of-concept project. Bagasse was shown to be a promising source of AX
447 in terms of its content (around 20%) and structure (with a low A:X ratio) and the readiness with
448 which it yielded its AX to give a balanced release of both large (>10 kDa) and small (<10 kDa)
449 molecules. Use of feruloyl esterase and xylanase enzymes on their own or following alkaline
450 peroxide extraction was not particularly effective at enhancing AX release; however, there was
451 evidence that the xylanase was effective at reducing the size of AX molecules, and there is scope to
452 optimise the action of the enzymes through a more comprehensive study of dosage and incubation
453 effects.

454
455 Thus, the metaphorical reactions that arose from the LBNet workshop were successful in
456 demonstrating the proof of concept, but the literal reactions in relation to optimising AX extraction
457 from wheat bran and sugarcane bagasse retain some scope for further enhancement.

458
459 As bagasse frequently arises within the context of bioethanol production, integration of AX
460 extraction with ethanol production could allow economic production of AX products, as has been
461 demonstrated in principle for AX co-production in a wheat ethanol plant [1]. Further processing of
462 the now lignin-rich residue could give even further opportunities within the biorefinery, both for co-
463 production of additional products and for further integration (particularly if the lignin processing
464 also involves ethanol). The nature and additional processing of the lignin in the residual fractions
465 from the current work were therefore studied further, and will be the topic of a future paper.

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15 47916 480 **References**

- 17 481
18
19 482 [1] N. Misailidis, G.M. Campbell, C. Du, J. Sadhukhan, M. Mustafa, F. Mateos-Salvador, R.M.
20 483 Weightman, Evaluating the feasibility of commercial arabinoxylan production in the context
21 484 of a wheat biorefinery principally producing ethanol. Part 2. Process simulation and economic
22 485 analysis, *Chem. Eng. Res. Des.* 87 (2009). doi:10.1016/j.cherd.2008.12.028.
- 23
24 486 [2] C.S. Lancefield, O.S. Ojo, F. Tran, N.J. Westwood, Isolation of functionalized phenolic
25 487 monomers through selective oxidation and CO bond cleavage of the β -O-4 linkages in Lignin,
26 488 *Angew. Chemie - Int. Ed.* (2015). doi:10.1002/anie.201409408.
- 27 489 [3] E. Martinez-Hernandez, J. Sadhukhan, G.M. Campbell, Integration of bioethanol as an in-
28 490 process material in biorefineries using mass pinch analysis, *Appl. Energy*. 104 (2013).
29 491 doi:10.1016/j.apenergy.2012.11.054.
- 30
31 492 [4] E. Martinez-Hernandez, A. Tibessart, G.M. Campbell, Conceptual design of integrated
32 493 production of arabinoxylan products using bioethanol pinch analysis, *Food Bioprod. Process.*
33 494 (2018). doi:10.1016/j.fbp.2018.08.005.
- 34 495 [5] Z.C. Geng, J.X. Sun, S.F. Liang, F.Y. Zhang, Y.Y. Zhang, F. Xu, R.C. Sun, Characterization of
35 496 water- and alkali-soluble hemicellulosic polymers from sugarcane bagasse, *Int. J. Polym. Anal.*
36 497 *Charact.* (2006). doi:10.1080/10236660600649095.
- 37
38 498 [6] P. Feng, R. Jun-Li, X. Feng, B. Jing, P. Pai, S. Run-Cang, Comparative study of hemicelluloses
39 499 obtained by graded ethanol precipitation from sugarcane bagasse, *J. Agric. Food Chem.* 57
40 500 6305-6317. (2009). doi:10.1021/jf900986b.
- 41 501 [7] C.M.P. Braga, P. da S. Delabona, D.J. da S. Lima, D.A.A. Paixão, J.G. da C. Pradella, C.S. Farinas,
42 502 Addition of feruloyl esterase and xylanase produced on-site improves sugarcane bagasse
43 503 hydrolysis, *Bioresour. Technol.* (2014). doi:10.1016/j.biortech.2014.07.115.
- 44 504 [8] M. Aguedo, H.A. Ruiz, A. Richel, Non-alkaline solubilization of arabinoxylans from destarched
45 505 wheat bran using hydrothermal microwave processing and comparison with the hydrolysis by
46 506 an endoxylanase, *Chem. Eng. Process. Process Intensif.* (2015).
47 507 doi:10.1016/j.cep.2015.07.020.
- 48
49 508 [9] L. Jacquemin, A. Mogni, R. Zeitoun, C. Guinot, C. Sablayrolles, L. Saulnier, P.Y. Pontalier,
50 509 Performance evaluation of a semi-industrial production process of arabinoxylans from wheat
51 510 bran, *Process Biochem.* (2015). doi:10.1016/j.procbio.2015.01.015.
- 52
53 511 [10] C. Maes, J.A. Delcour, Alkaline hydrogen peroxide extraction of wheat bran non-starch
54 512 polysaccharides, *J. Cereal Sci.* (2001). doi:10.1006/jcrs.2001.0377.
- 55 513 [11] C. Maes, J.A. Delcour, Structural characterisation of water-extractable and water-
56 514 unextractable arabinoxylans in wheat bran, *J. Cereal Sci.* 35 (2002) 315–326.
57 515 doi:10.1006/jcrs.2001.0439.
- 58
59 516 [12] C.M. Courtin, J.A. Delcour, Arabinoxylans and endoxylanases in wheat flour bread-making, *J.*
60 517 *Cereal Sci.* (2002). doi:10.1006/jcrs.2001.0433.
- 61 518 [13] J.J. Ordaz-Ortiz, L. Saulnier, Structural variability of arabinoxylans from wheat flour.
62 519 Comparison of water-extractable and xylanase-extractable arabinoxylans, *J. Cereal Sci.* 42
- 63
64
65

- 520 (2005) 119–125. doi:10.1016/j.jcs.2005.02.004.
- 1 521 [14] A.P. de Souza, D.C.C. Leite, S. Pattathil, M.G. Hahn, M.S. Buckeridge, Composition and
2 522 Structure of Sugarcane Cell Wall Polysaccharides: Implications for Second-Generation
3 523 Bioethanol Production, *Bioenergy Res.* 6 (2013) 564–579. doi:10.1007/s12155-012-9268-1.
- 4 524 [15] J. Hollmann, M.G. Lindhauer, Pilot-scale isolation of glucuronoarabinoxylans from wheat
5 525 bran, *Carbohydr. Polym.* (2005). doi:10.1016/j.carbpol.2004.09.015.
- 6 526 [16] C. Du, G.M. Campbell, N. Misailidis, F. Mateos-Salvador, J. Sadhukhan, M. Mustafa, R.M.
7 527 Weightman, Evaluating the feasibility of commercial arabinoxylan production in the context
8 528 of a wheat biorefinery principally producing ethanol. Part 1. Experimental studies of
9 529 arabinoxylan extraction from wheat bran, *Chem. Eng. Res. Des.* 87 (2009) 1232–1238.
10 530 doi:10.1016/j.cherd.2008.12.027.
- 11 531 [17] S.C. Fry, *The growing plant cell wall: chemical and metabolic analysis*, The Blackburn Press,
12 532 New Jersey, USA, 1988. doi:10.1111/j.1469-8137.2003.00980.x.
- 13 533 [18] L. Jones, J.L. Milne, D. Ashford, S.J. McQueen-Mason, Cell wall arabinan is essential for guard
14 534 cell function, *Proc. Natl. Acad. Sci.* (2003). doi:10.1073/pnas.1832434100.
- 15 535 [19] S. Ranganathan, D.G. Macdonald, N.N. Bakhshi, Kinetic studies of wheat straw hydrolysis
16 536 using sulphuric acid, *Can. J. Chem. Eng.* 63 (1985) 840–844. doi:10.1002/cjce.5450630521.
- 17 537 [20] S. Willför, A. Pranovich, T. Tamminen, J. Puls, C. Laine, A. Suurnäkki, B. Saake, K. Uotila, H.
18 538 Simolin, J. Hemming, B. Holmbom, Carbohydrate analysis of plant materials with uronic acid-
19 539 containing polysaccharides-A comparison between different hydrolysis and subsequent
20 540 chromatographic analytical techniques, *Ind. Crops Prod.* 29 (2009) 571–580.
21 541 doi:10.1016/j.indcrop.2008.11.003.
- 22 542 [21] L.W. Doner, K.B. Hicks, Isolation of hemicellulose from corn fiber by alkaline hydrogen
23 543 peroxide extraction, *Cereal Chem.* (1997). doi:10.1094/CCHEM.1997.74.2.176.
- 24 544 [22] L. Saulnier, P.E. Sado, G. Branlard, G. Charmet, F. Guillon, Wheat arabinoxylans: Exploiting
25 545 variation in amount and composition to develop enhanced varieties, *J. Cereal Sci.* 46 (2007)
26 546 261–281. doi:10.1016/j.jcs.2007.06.014.
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Table 1. Compositions of wheat bran and sugarcane bagasse (%w/w dry basis).

Component	Wheat bran	Sugarcane bagasse
Starch	29.8	ND
Hemicellulose	16.6	22.1
Cellulose	21.8	28.3
Lignin	10.4	22.0
Other	21.2	27.6.8
AX (=0.88×(A+X))	8.64	19.58
A:X ratio	0.57	0.21

Table 2. Crude yields, AX concentrations and AX yields from wheat bran and its fractions following extraction under different treatments.

	1. Buffer (Control)	2. Chemical + Buffer (Control)	3. Chemical + Enzymes FEA E0355 + XYL E0051	4. Chemical + Enzymes FEA E0356 + XYL 0051	5. Chemical + Enzyme FEA E0355	6. Chemical + Enzyme FEA E0356	7. Enzymes FEA E0355 + XYL E0051	8. Enzymes FEA E0356 + XYL E0051
Initial bran (g)	30.34	50.73	50.20	50.47 ^c	50.47 ^c	50.47 ^c	30.61	30.47
AX concentration (%) ^a	8.64	8.64	8.64	8.64	8.64	8.64	8.64	8.64
AX amount (g) ^a	2.62	4.38	4.34	4.36	4.36	4.36	2.64	2.63
CE Pellet (g)		30.29	29.58	29.21	29.54	30.27		
AX concentration (%)		34.05	38.50	33.67	36.98	35.87		
AX amount in CE pellet (g)		10.31	11.39	9.84	10.92	10.86		
CE High (g)		8.11	8.31					
AX concentration (%)		16.43	16.21					
AX amount in CE High (g)		1.33	1.35					
Absolute yield (%)		2.63	2.68					
CE Low (g)		3.84	4.97					
AX concentration (%)		1.75	0.74					
AX amount in CE Low (g)		0.067	0.037					
Absolute yield (%)		0.13	0.07					
EE Pellet (g)	14.63 ^b	16.02	15.28	15.04	14.10	15.06	15.88	15.71
AX concentration (%)	31.00	38.19	35.82	31.21	35.74	22.61	36.07	31.85
AX amount in EE pellet (g)	4.54	6.12	5.47	4.69	5.04	3.41	5.73	5.00
Absolute yield (%)	14.95	12.06	10.90	9.30	9.99	6.75	18.71	16.42
EE High (g)	1.81	2.00	1.21	1.32	1.62	2.23	2.69	3.36
AX concentration (%)	10.42	12.2	21.25	19.46	23.23	0.00	8.43	5.22
AX amount in EE High (g)	0.19	0.24	0.26	0.26	0.38	0.00	0.23	0.18
Absolute yield (%)	0.62	0.48	0.51	0.51	0.75	0.00	0.74	0.58
EE Low (g)	4.78	0.97	3.20	2.64	3.20	1.95	4.93	4.97
AX concentration (%)	1.15	0.29	13.47	11.83	0.47	0.21	3.72	2.70
AX amount in EE Low (g)	0.05	0.00	0.43	0.31	0.02	0.00	0.18	0.13
Absolute yield (%)	0.18	0.01	0.86	0.62	0.03	0.01	0.60	0.44

a. The data reported for AX concentration and amount in the bran appear to be erroneous, as they indicate less AX in the raw material than in the residual pellet; a starting concentration of 24% is more in line with the mass balance and with the expected AX content of wheat bran.

b. The results for the pellet and high- and low-cutoff fractions following just buffer extraction are reported in these EE rows, but these are not to be understood as having undergone enzyme treatment.

c. Initial weights averaged from Trials 2 and 3.

Table 3. Crude yields, AX concentrations and AX yields from sugarcane bagasse and its fractions following extraction under different treatments.

	1. Buffer (Control)	2. Chemical + Buffer (Control)	3. Chemical + Enzymes FEA E0355 + XYL E0051	4. Chemical + Enzymes FEA E0356 + XYL 0051	5. Chemical + Enzyme FEA E0355	6. Chemical + Enzyme FEA E0356	7. Enzymes FEA E0355 + XYL E0051	8. Enzymes FEA E0356 + XYL E0051
Initial bran (g)	6.34	11.31	11.76	11.54 ^b	11.54 ^b	11.54 ^b	6.29	6.15
AX concentration (%)	19.54	19.54	19.54	19.54	19.54	19.54	19.54	19.54
AX amount (g)	1.24	2.21	2.30	2.25	2.25	2.25	1.23	1.20
CE Pellet (g)		5.37	5.48	5.68	5.36	5.39		
AX concentration (%)		14.56	13.47	16.06	20.52	17.75		
AX amount in CE pellet (g)		0.78	0.74	0.91	1.10	0.96		
CE High (g)		2.91	3.30					
AX concentration (%)		23.23	24.05					
AX amount in CE High (g)		0.68	0.79					
Absolute yield (%)		5.98	6.75					
CE Low (g)		7.97	9.43					
AX concentration (%)		7.49	6.97					
AX amount in CE Low (g)		0.60	0.66					
Absolute yield (%)		5.28	5.59					
EE Pellet (g)	3.36 ^a	4.12	3.94	4.25	4.23	4.18	3.54	3.93
AX concentration (%)	29.81	17.37	18.42	15.95	17.07	21.25	23.84	25.95
AX amount in EE pellet (g)	1.00	0.72	0.73	0.68	0.72	0.89	0.84	1.02
Absolute yield (%)	15.80	6.33	6.17	5.88	6.26	7.70	13.42	16.58
EE High (g)	1.00	1.17	0.96	1.00	1.12	1.02	0.92	0.97
AX concentration (%)	3.86	5.05	4.56	2.82	3.88	5.26	4.17	3.72
AX amount in EE High (g)	0.039	0.06	0.04	0.03	0.04	0.05	0.04	0.04
Absolute yield (%)	0.61	0.52	0.37	0.24	0.38	0.46	0.61	0.59
EE Low (g)	3.01	3.04	3.13	3.06	2.97	3.36	2.78	2.94
AX concentration (%)	1.44	0.44	0.85	1.10	0.46	0.66	2.25	1.86
AX amount in EE Low (g)	0.043	0.01	0.03	0.03	0.01	0.02	0.06	0.05
Absolute yield (%)	0.68	0.12	0.23	0.29	0.12	0.19	1.00	0.89

a. The results for the pellet and high- and low-cutoff fractions following just buffer extraction are reported in these EE rows, but these are not to be understood as having undergone enzyme treatment.

b. Initial weights averaged from Trials 2 and 3.

Figure 1. Procedure for chemical extraction of AX from wheat bran or sugarcane bagasse, yielding High cut-off (CE-High) and Low cut-off (CE-Low) fractions and a residual pellet; and procedure for further enzyme treatment of the pellet to yield further High (EE-High) and Low (EE-Low) cut-off fractions.

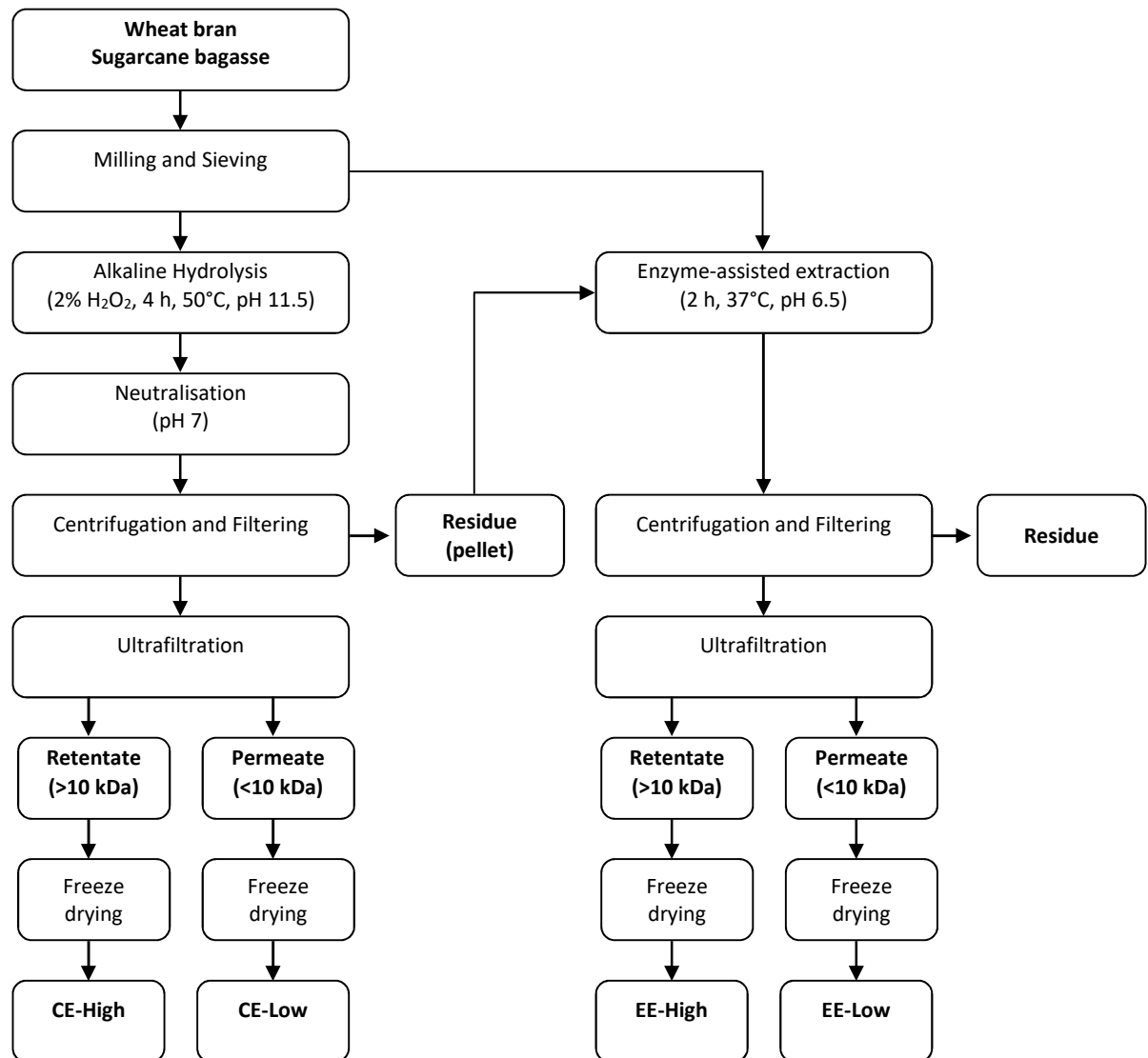


Figure 2. Measurement of arabinoxylan content in wheat bran following different hydrolysis conditions.

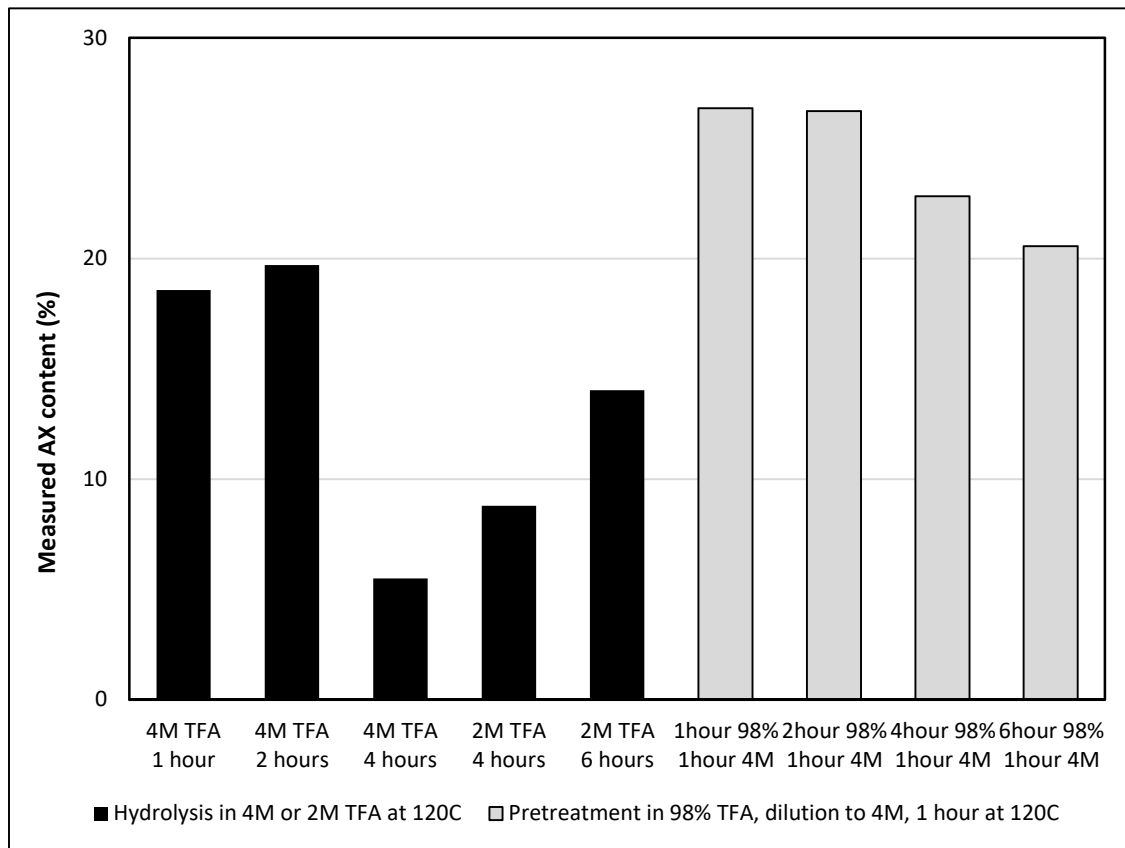


Figure 3. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction from wheat bran.

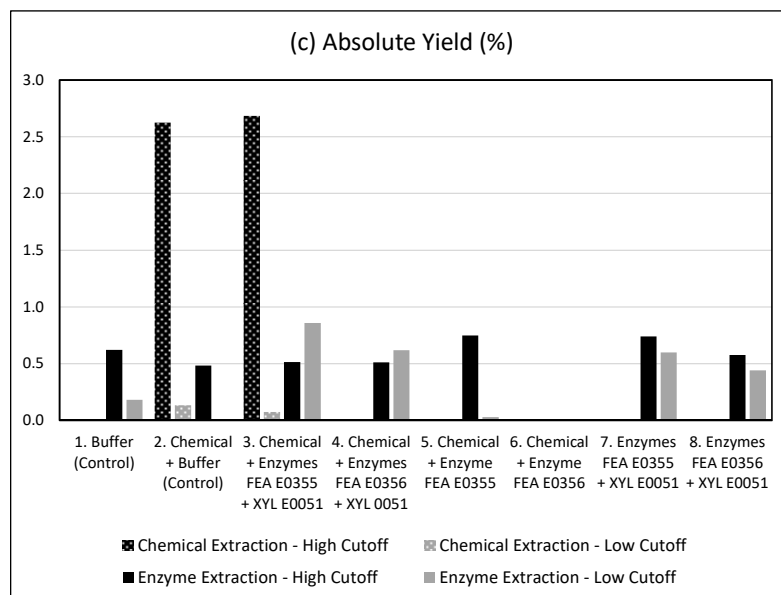
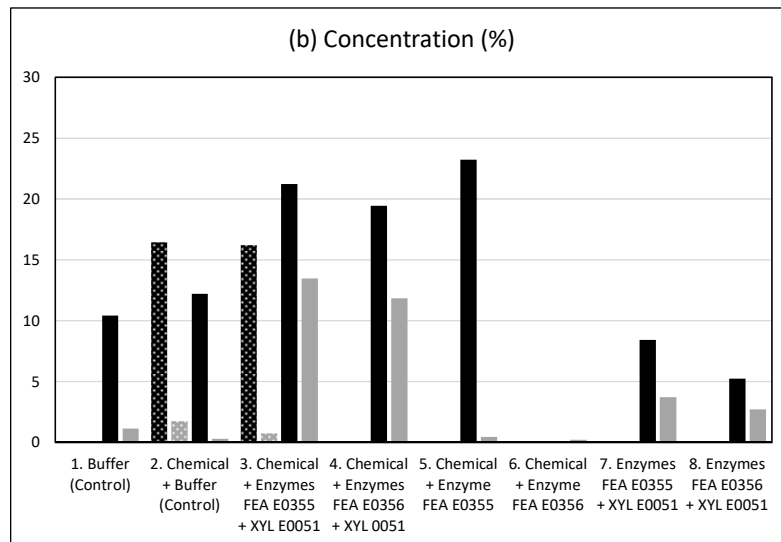
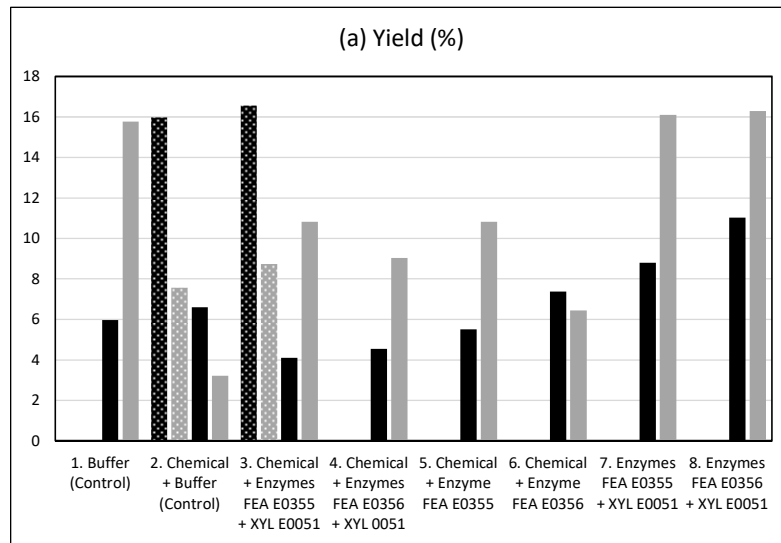


Figure 4. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction from sugarcane bagasse.

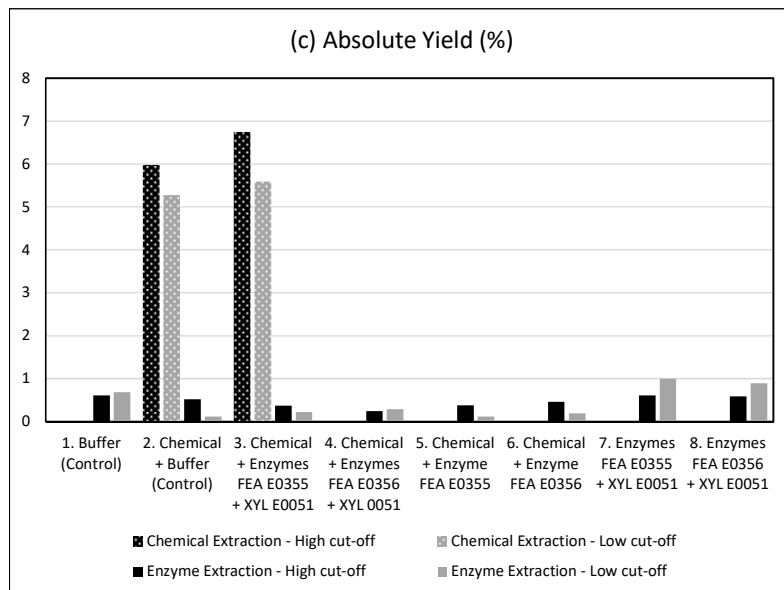
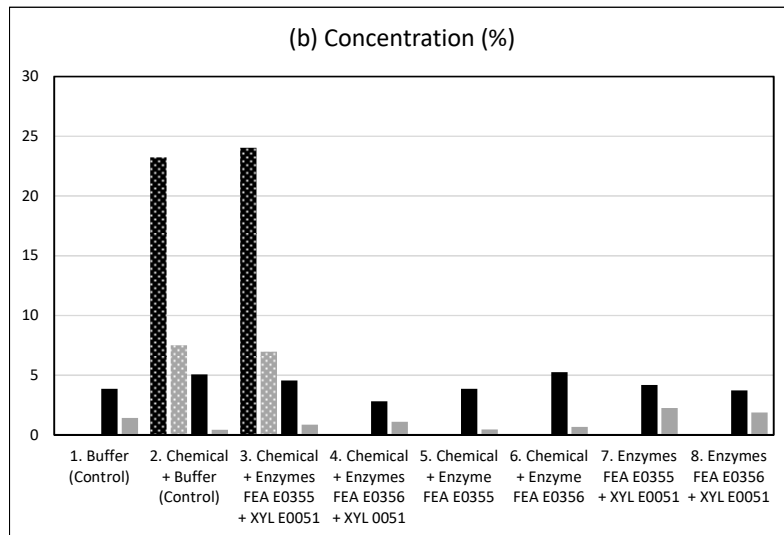
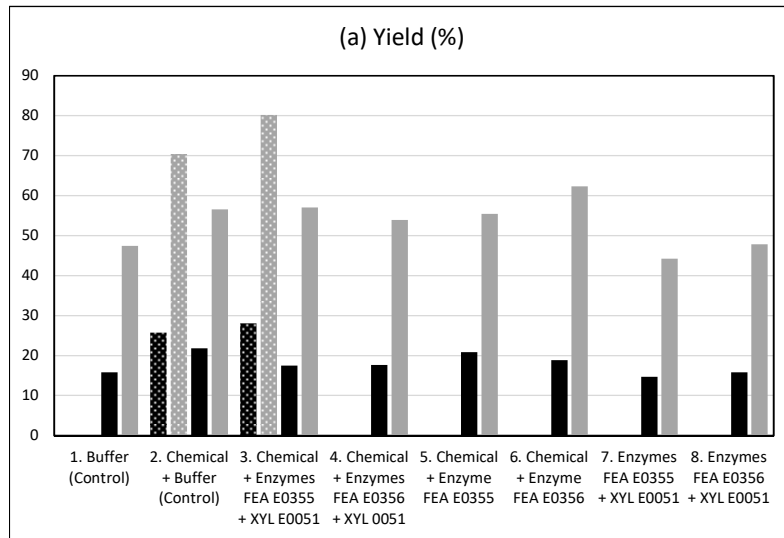


Figure 5. Expanded view of Absolute Yields in fractions following arabinoxylan extraction from sugarcane bagasse under various chemical and enzyme treatments.

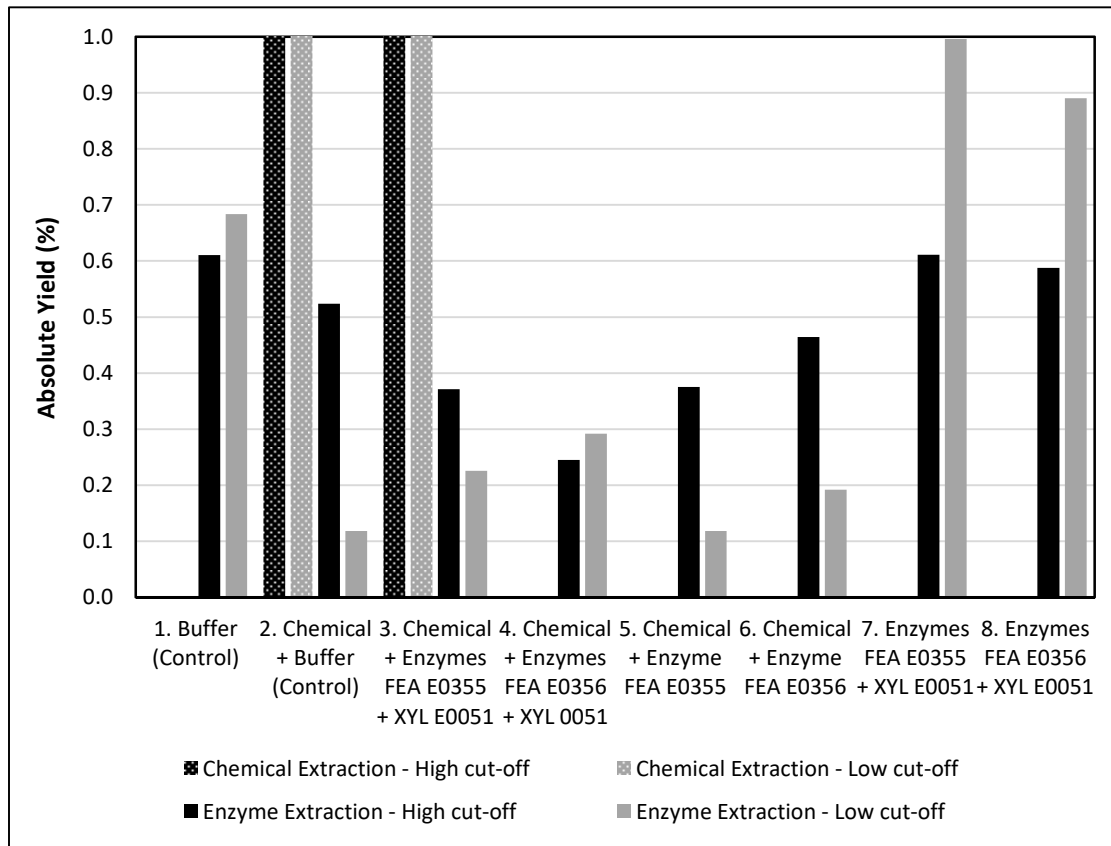


Figure 6. Arabinose:Xylose ratios in fractions following arabinoxyylan extraction from (a) wheat bran and (b) sugarcane bagasse.

