1 **Integrated processing of sugarcane bagasse:**
2 **Arabinoxylan extraction integrated with ethanol production.**
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16
17 **Abstract**
18
19 A proof-of-concept project compared extraction of arabinoxylans (AX) from sugarcane bagasse and
20 wheat bran via alkaline hydrogen peroxide followed by enzyme-assisted extraction with
21 combinations of feruloyl esterases and a xylanase. Bagasse contains comparable amounts of AX to
22 wheat bran, but with a much lower arabinoxylan substitution on the xylan backbone (A:X ratio of
23 around 0.2 compared with 0.6 for wheat bran), hence offering AX products with distinctive
24 functionality and potential end uses. In the current work, bagasse released its AX more readily than
25 wheat bran, and released a wider range of molecular weights. Use of feruloyl esterase and xylanase
26 enzymes on their own or following alkaline peroxide extraction did not enhance AX release
27 substantially; however, the xylanase appeared to be effective at reducing the size of AX molecules,
28 and there is scope to optimise the effects of enzymes to produce specific AX product fractions. As
29 bagasse frequently arises within the context of bioethanol production, integration of AX extraction
30 with ethanol production could allow economic production of a portfolio of AX products, as has been
31 demonstrated in principle for AX co-production in a wheat ethanol plant.
32
33
34 **Keywords:** sugarcane bagasse, wheat bran, arabinoxylans, bioethanol, biorefinery integration.
35
36
37 **Abbreviations**
38
39 AX   Arabinoxylan
40 UoH   University of Huddersfield
41 UoL   University of Lincoln
42 UoY   University of York
43 UoStA   University of St Andrews
44 BDC   Biorenewables Development Centre
45 LBNet   Lignocellulosic Biorefinery Network
46 P2PNet   Plants to Products Network
47 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 (*C. mixtus*, PRO-E0051, Prozomix UK)
52	FE-E0355	Feruloyl esterase (*A. cellulolyticus*, PRO-E0355, Prozomix UK)
53	FE-E0356	Feruloyl esterase (*A. cellulolyticus*, PRO-E0356, Prozomix UK)

54

55 **Declarations of interest:** None.

56

57

58 **1. 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 the
75 company AB Sugar wrote "Something valuable from sugarcane bagasse (not furfural, we already do
76 that)". Seeing these notes together prompted the idea that arabinoxylans might similarly be
77 extracted from bagasse in an integrated biorefinery producing ethanol from sugarcane, and that the
78 residual bagasse following AX extraction might reveal a suitable source of lignin for further
79 processing. A proof-of-concept project was constructed, to demonstrate the feasibility of AX
80 extraction from sugarcane bagasse, and to examine the residual lignin, following partial
81 deconstruction during the AX extraction process, for its suitability as a feedstock for conversion into
82 a phenolic monomer using an established reaction sequence [2]. A parallel activity studying wheat
83 bran was included, to extend the scope and make the findings more immediately applicable to the
84 UK context (where bioethanol production is largely from wheat). Enzyme enhancement of the AX
85 release, a scale-up component and a techno-economic analysis of AX production were also included
86 in the project, to give a consortium comprising the Universities of Huddersfield, St Andrews, York,
87 Lincoln and 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
96 the investigation of the residual bagasse following AX extraction, in terms of the nature of its lignin
97 and its suitability for further processing, including further integration opportunities with ethanol and
98 butanol.
99
100 Global   sugarcane   production   is   around   1.9   billion   tonnes   per   annum
101 (https://www.statista.com/statistics/249604/sugar-cane-production-worldwide/),   resulting   in
102 around 570 million tonnes wet bagasse or half this amount if dried. Sugarcane bagasse thus
103 represents a major waste stream arising from sugar and alcohol industries, typically containing
104 around 40-50% cellulose, 25-35% hemicelluloses (predominantly xylans) and 20-30% lignin [5,6]. A
105 focus of previous work has been to deploy feruloyl esterases and xylanases to assist the
106 saccharification of bagasse to increase recovery of fermentable sugars, by removing the
107 hemicelluloses that (to put it simply) link lignin and cellulose, thus increasing accessibility of the
108 latter to cellulases and hence the release of glucose [7]. A difference in the current work is that the
109 intention was not to hydrolyse arabinoxylan hemicelluloses to their constituent sugars, but rather to
110 release and recover them as intact large AX molecules, in which form they have potential as
111 functional food ingredients and non-food products including film forming, emulsifiers and stabilisers
112 in the food, pharmaceutical and cosmetic industries [8,9]. The context of the ethanol biorefinery
113 gives scope for AX co-production to be economic as a result of integration with ethanol production
114 (used for precipitating the AX), as has been shown previously for AX production from wheat bran in
115 a wheat ethanol biorefinery [1]. Wheat bran typically contains 20-30% AX [10,11], similar to
116 bagasse, but with a much higher ratio of arabinose to xylose units; in wheat bran the A:X ratio is
117 typically in the range 0.5-0.6 [12,13]), while in sugarcane bagasse it is much lower at around 0.2
118 typically [14]. This "cleaner" xylan backbone with fewer arabinose substitutions is likely to exhibit
119 different functional properties compared with wheat bran AX, including reduced solubility and
120 greater susceptibility to enzyme action, as well as effects on viscosity and gel formation, and
121 performance in food products or animal feed.
122
123
124 **2. Materials and Methods**
125
126 Sugarcane bagasse (25 kg) and wheat bran (50 kg) were sourced commercially by AB Sugar and
127 provided to the project. The bagasse and bran were milled at the Biorenewables Development
128 Centre (BDC) using a Retsch Cutting Mill SM 300 (Retsch GmbH, Germany) with a 2.00 mm screen,
129 and AX extraction studies undertaken at the University of Huddersfield (UoH). Proximate analysis
130 and arabinoxylan (AX) content measurements were performed at the University of York (UoY).
131 Lignin studies, to be presented in a future paper, were undertaken at the University of St Andrews
132 (UoStA).
133
134 Figure 1 describes the chemical extraction process using alkaline hydrogen peroxide applied to the
135 milled wheat bran and sugarcane bagasse, based on the work of Hollmann and Lindhauer [15] and
136 Du et al. [16]. Dilute alkaline peroxide reacts with lignin, breaking the association with
137 polysaccharides and facilitating the release of arabinoxylans [10]. The residue material was then
138 subjected to enzyme treatment to see if further release of AX resulted, using selected combinations
139 of β-Xylanase (*C. mixtus*, PRO-E0051, referred to here as XYL) and two types of Feruloyl esterase (*A.
140 cellulolyticus*, PRO-E0355 and PRO-E0356, referred to here as FE-E0355 and FE-E0356) from
141 Prozomix UK. The hypothesis was that the feruloyl esterases, by breaking links between AX and

142 lignin, might enhance AX release, while the xylanase, which breaks the AX chains themselves, might
143 similarly release AX fragments to enhance overall extraction, while also altering the size of the
144 released molecules. Enzyme-assisted extraction on its own was also investigated, along with
145 chemical extraction followed by further extraction with buffer solution, and buffer extraction on its
146 own.

147

148 In total eight extractions were performed for each feedstock:
149 1. Direct buffer extraction (control for all trials, particularly Trials 7-8);
150 2. Chemical extraction followed by buffer extraction (control for Trials 3-6);
151 3. Chemical extraction followed by FE-E0355 and XYL;
152 4. Chemical extraction followed by FE-E0356 and XYL;
153 5. Chemical extraction followed by FE-E0355;
154 6. Chemical extraction followed by FE-E0356;
155 7. Direct enzyme extraction with FE-E0355 and XYL;
156 8. Direct enzyme extraction with FE-E0356 and XYL.

157

158 The supernatant following treatment, centrifugation and filtering was separated by ultrafiltration
159 over a 10 kDa membrane (see below for details). The chemical extraction thus yielded High cut-off
160 (CE-High) and Low cut-off (CE-Low) fractions and a pellet of residual material; enzyme extractions
161 directly on the raw material or on the pellet following chemical extraction similarly yielded High cut-
162 off (EE-High) and Low cut-off (EE-Low) fractions and a pellet. A single chemical extraction was
163 performed to produce residual material for subsequent enzyme extraction in Trials 3-6; a second
164 chemical extraction was performed for subsequent buffer extraction (Trial 2).

165

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

180

181 The high molecular weight material in the supernatant was concentrated by ultrafiltration using a
182 Vivaflow™ 200 system (Sartorius Stedim Biotech GmbH, Germany) with polyethersulfone
183 membranes with a molecular weight cut-off of 10 kDa. (Ultrafiltration would be done as part of a
184 commercial process to reduce the amount of ethanol needed subsequently to precipitate the AX
185 [1,15]; in the current work the fractions were not precipitated. The ultrafiltration served to separate
186 the released AX into larger MW and smaller MW fractions.) Retentate was recycled until the volume
187 was reduced to one fifth of the original. The retentate (High cut-off) and permeate (Low cut-off)
188 fractions were freeze-dried using a Christ Freeze Dryer Alpha 1-4 LDplus, (Martin Christ

189   Gefriertrocknungsanlagen GmbH, Germany) at –47.8°C and 0.35 mbar. Samples of freeze-dried High
190   cut-off and Low cut-off material and oven-dried pellets were sent to UoY for AX analysis (see below).
191   Samples of the dried pellet material were also sent to the University of St Andrews (UoStA) for
192   assessment of its lignin, to be described in a future paper.
193
194   For enzyme-assisted extraction trials on raw wheat bran or sugarcane bagasse or on pellets after
195   chemical extraction, material (30 g for the wheat bran and 6 g for the sugarcane bagasse) was
196   weighed into a 1 L Duran bottle with a magnetic stirrer. 300 mL of a buffer (composed of 107.4 mL
197   of 0.2 M disodium phosphate, 42.6 mL of citric acid and 150 mL of water) at pH 6.5 was added, the
198   bottles placed in a 37°C water bath and the pH of the solution readjusted to 6.5 with citric acid when
199   the temperature reached 37°C. The different enzymes were added at a level of 10 µg/10 g for the
200   XYL and FE-E0355 and 5 µg/10 g for the FE-E0356, and the solution was stirred for 2 h. As above,
201   samples were centrifuged, filtered and washed, in this case using 100 mL of water for each washing,
202   and the residual solid pellet oven-dried overnight at 50°C. Again the supernatant was passed
203   through ultrafiltration over 10 kDa and the retentate (High cut-off, EE-H) and permeate (Low cut-off,
204   EE-L) freeze dried and sent to UoY for analysis along with the oven-dried pellet, with pellet samples
205   also sent to UoStA for lignin analysis.
206
207   Due to the limited scope of this small proof-of-concept project, replicate enzyme extractions were
208   not performed, the aim being to demonstrate broad effects in relation to AX yields from wheat bran
209   and bagasse and the potential effects of enzyme-assisted extraction. Five replicate chemical
210   extractions were performed, as enzyme extraction Trials 3-6 and buffer Trial 2 each required a
211   chemical extraction first, although High and Low cut-off fractions were subsequently produced for
212   only two of these (2 and 3), and only these two pellets were sent for lignin analysis. Subsequent
213   similar work with replication has confirmed the broad trends reported here and shown sufficient
214   reproducibility to have confidence in the trends, which are reported and discussed here within the
215   limits of the acknowledged lack of replication.
216
217   Proximate analysis of the wheat bran and sugar cane bagasse was undertaken as follows:
218

219   *Lignin determination: acetyl bromide method*
220   Biomass powder was weighed out (4 mg) into 2 mL tubes. The biomass was heated at 50°C for 3
221   hours after adding 250 µL of acetyl bromide solution (25% acetyl bromide and 75% glacial acetic acid
222   by volume) and vortexing every 15 minutes. After the samples were cooled to room temperature,
223   the contents were transferred into 5 mL volumetric flasks. A further 1 mL of NaOH (2 mol L^{-1}) was
224   used to rinse the tubes pouring the NaOH into the 5 mL flasks. 175 µL of hydroxylamine HCl (0.5 mol
225   L^{-1}) was added to the volumetric flasks and, after vortexing, the latter were filled up to 5 mL with
226   glacial acetic acid and mixed several times. Finally, in order to measure the 280 nm UV adsorption
227   by spectrophotometer, 100 µL of each sample was diluted in 900 µL of glacial acetic acid. The
228   amount of lignin was calculated using the following formula: [absorbance/(coefficient pathlength)] ·
229   [(total volume · 100%)/biomass weight], where coefficient = 15.69, pathlength = 1, total volume = 5,
230   biomass weight = 4.
231

232   *Non-cellulosic monosaccharide determination*
233   Following the method of Fry [17], biomass dry powder (4 mg) was partially hydrolysed by adding 0.5
234   mL of trifluoroacetic acid (TFA, 2 mol L^{-1}). Then, the vials were flushed with dry argon, mixed and
235   heated at 100°C for 4 hours, mixing periodically. The vials were then cooled to room temperature

236 and dried in centrifugal evaporator with fume extraction overnight. The pellets were washed twice
237 with 500 μL of 2-propanol and vacuum dried. Finally, the samples were resuspended in 200 μL of
238 deionised water, filtered with 0.45 μm PTFE filters, and analysed by HPAEC (see below).
239
240 It became evident that these hydrolysis conditions were inadequate to release all the AX in the
241 original wheat bran (see below, where the mass balance indicates more AX in the extracted fractions
242 than appeared to be present initially in the bran). The wheat bran was therefore hydrolysed under a
243 range of conditions, to investigate the effects on AX measurement and to draw conclusions about
244 the most appropriate conditions for AX analysis in wheat bran. The bran samples were hydrolysed in
245 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
246 at 120°C.   In addition, samples were pretreated in 98% TFA for 1, 2, 4 and 6 hours at room
247 temperature, then diluted to 4M and boiled for 1 hour at 120°C.
248
249 *Crystalline cellulose*
250 Biomass dry pellets after TFA hydrolysis were washed once with 1.5 mL of water, and twice using 1.5
251 mL of acetone. The dried pellets were left to air dry overnight before complete hydrolysis by adding
252 90 μL of 72% w/w sulphuric acid, incubating at room temperature for 4 hours. 1.89 mL of water was
253 subsequently added and the sample was heated for 4 hours at 120°C. The glucose content of the
254 supernatant was assessed using the colorimetric Anthrone assay, using a glucose standard curve.
255
256 Analysis of the sugar compositions of the wheat bran and bagasse samples and of fractions and
257 residues following the various chemical and enzyme extractions was undertaken as follows:
258
259 Monosaccharide analysis was performed by high performance anion-exchange chromatography
260 (HPAEC) (Dionex IC 2500) on a Dionex Carbopac PA-10 column with integrated amperometry
261 detection [18]. The separated monosaccharides were quantified using external calibration with an
262 equimolar mixture of nine monosaccharide standards (arabinose, fucose, galactose, galacturonic
263 acid, glucose, glucuronic acid, mannose, rhamnose, and xylose), which were subjected to TFA
264 hydrolysis in parallel with the samples.
265
266
267 **3. Results and Discussion**
268
269 Table 1 shows the compositions of the wheat bran and sugarcane bagasse used in the current work.
270 The wheat bran had a significant starch content; therefore for the scale-up work undertaken at BDC,
271 the wheat bran was washed with water to remove starch prior to extraction, in line with the
272 recommendation of Du et al. [16]. The bagasse had 28% lignin and nearly 20% AX, broadly in line
273 with literature [5,6,14], and suggesting it was a promising candidate for recovery of both materials.
274 The analytical procedure used indicated 10% lignin in the wheat bran, although subsequent NMR
275 work suggested a much lower lignin content, in line with other recent reports that wheat bran
276 contains less lignin than previously thought. These results also suggest an AX content of only 8.64%
277 in the wheat bran; this figure is lower than the 20-30% generally expected for wheat bran, and later
278 proved to be incompatible with the mass balance for AX recovered in the various fractions and
279 residues, which suggested an AX content in the original material of around 24% (see below). The A:X
280 ratio for the wheat bran was 0.57, and for the bagasse 0.21, in line with typical values expected from
281 the literature, and showing the much "cleaner" xylan backbone for the bagasse AX, with fewer
282 arabinose substitutions compared with the wheat AX.

283
284   Tables 2 and 3 report the crude yields, AX concentrations and hence AX yields from 50 g wheat bran
285   and 11 g sugarcane bagasse, respectively, in the starting materials and in the fractions following the
286   various chemical, enzyme and buffer treatments. The second column in each table is for chemical
287   extraction only, showing the data from two replicates, with good agreement. Considering the wheat
288   bran results first, chemical extraction of around 50.6 g of bran (moisture content 9.5%) yielded a
289   residual dry pellet weighing 29.94 g (averaged from Trials 2 and 3), a High cut-off (CE-High) of 8.21 g,
290   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).
291   The AX concentration in the pellet was 35.81% and in the High and Low cut-off fractions was 16.32%
292   and 1.25%, respectively. This implies a total amount of AX in the pellet and two fractions of
293   29.94×35.81% + 8.21×16.32% + 4.40×1.25% = 10.72 + 1.34 + 0.06 = 12.12 g. The mass balance
294   therefore implies an AX concentration of 12.12/50.6 = 24% in the original wheat bran, higher than
295   the 8.64% reported in Table 1, and more in line with the expected AX content of wheat bran,
296   suggesting that the 8.64% figure is erroneous.
297
298   It is well known that the appropriate hydrolysis conditions for this sort of analysis depend on the
299   nature of the sample, with different samples requiring different combinations of time, temperature
300   and acidity to get an optimum balance between release and degradation of monosaccharides
301   [19,20]. The bran sample was therefore reanalysed under a range of hydrolysis conditions as
302   described above. Figure 2 shows the AX contents calculated for each of the hydrolysis regimes.
303   Under similar conditions to the original analysis (2M for 4 hours, but at a higher temperature,
304   120°C), the measurement was similar at 8.79%, while 2M for 6 hours released more AX to give
305   14.04%. 4M for 1 or 2 hours released even more (18.58-19.71%), but 4M for 4 hours appears to give
306   substantial degradation and a final measurement of only 5.50%. Pretreatment in concentrated acid
307   appeared effective at releasing more AX for effective hydrolysis, giving measurements of around
308   26.7% after 1 or 2 hours of pretreatment, decreasing to 22.8 and 20.6% after 4 and 6 hours,
309   suggesting degradation at the high acid concentration despite the low temperature. Overall, these
310   results demonstrate that an AX content of around 24%, as implied by the mass balance, is plausible,
311   but that measuring the AX content of raw wheat bran requires different hydrolysis conditions
312   compared to extracts or the residual pellet following extraction.
313
314   Thus, of a total of around 12 g AX in the original 50 sample, 1.4 g or 11.5% was released by the
315   alkaline hydrogen peroxide extraction process. This is much lower than the recoveries of 46-50%
316   reported by Hollmann and Lindhauer [15] and Du et al. [16] for alkali-extracted AX from wheat bran
317   using similar conditions. In those studies the wheat bran was boiled in 70% ethanol at 80°C for 4
318   hours prior to alkaline H_2O_2 treatment, which Hollmann and Lindhauer [15] advised was necessary to
319   achieve high yields; this step was omitted in the current work (for safety and cost reasons in relation
320   to the planned scale-up work, and because related unpublished work from our labs on extraction
321   from maize meal had found that this step was not needed, as also confirmed by work from Doner
322   and Hicks [21] on AX extraction from maize fibre). The omission of this ethanol boiling step is
323   possibly the reason for the lower yields than in this previously reported work.
324
325   Considering the sugarcane bagasse results, chemical extraction of around 11.5 g of bagasse
326   (moisture content 9.3%) yielded a residual dry pellet weighing 5.43 g (averaged from Trials 2 and 3),
327   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
328   17.34 g, c.f. about 10.5 g solids in the original material; the mass balance does not give good
329   agreement in this case. This is probably because the Low cut-off was extrapolated from the solids

330 left after freeze-drying dilute samples (and the freeze-dried samples may not have been completely
331 dry), such that the 8.70 g figure is not accurate, while overall the mass balance from just 11 g of
332 bagasse is inherently less accurate than that from 50 g wheat bran, and the contribution from salts
333 formed on neutralisation relatively greater.

335 The AX concentration in the pellet was 14.02% (averaged from Trials 2 and 3, although higher in
336 Trials 4-6) and in the High and Low cut-off fractions was 23.64% and 7.23%, respectively. This
337 implies a total amount of AX in the pellet and two fractions of 5.53×14.02% + 3.11×23.64% +
338 8.70×7.23% = 0.775 + 0.735 + 0.629 = 2.139 g, compared with 11.54×19.58% = 2.260 g of AX in the
339 original sample. This mass balance appears reasonable, although the uncertainty over the Low cut-
340 off contribution is acknowledged. In this case it appears that the AX left in the pellet (assuming this
341 to be a more accurate figure) is about one-third of the AX in the original material (0.775/2.25 = 34%),
342 implying nearly two-thirds has been released, compared with only 11.5% for the wheat bran. This
343 suggests sugarcane bagasse may be very amenable to AX extraction, as it appears to yield its AX
344 more readily than wheat bran (although the higher water:solids ratio used for the bagasse extraction
345 is noted). The readiness of the bagasse to release its AX compared with wheat bran suggests the
346 expensive and hazardous ethanol boiling step advised by Hollmann and Lindhauer [15] for wheat
347 bran may not be needed in a bagasse-based AX extraction process. As noted above, Doner and Hicks
348 [21] advised that dewaxing with toluene-ethanol was unnecessary for AX extraction from maize
349 fibre.

351 Figure 3 shows the crude yields of High and Low cut-off material following the various wheat bran
352 extractions, the AX concentrations in the fractions and hence the absolute yields of AX in each
353 fraction. Trial 1 shows the recovery of High and Low fractions from just extraction with Buffer.
354 Clearly, while quite a lot of small molecular weight material (<10 kDa) was recovered in the Low cut-
355 off fraction, it contained very little AX, while some high MW AX was extracted just with the use of
356 Buffer. Chemical extraction is shown in Trials 2 and 3, with good agreement, showing roughly twice
357 as much High cut-off material as Low was recovered, but that the latter contained very little AX, such
358 that the majority of the recovered AX was in the High cut-off fraction, giving a yield of around 2.6%
359 compared with only 0.62% with Buffer. Thus chemical extraction using alkaline hydrogen peroxide
360 was somewhat successful at releasing high MW AX from wheat bran. Further extraction with Buffer
361 (Trial 2) recovered a little more high MW AX.

363 Turning to Trials 7 and 8, use of FEA enzymes in combination with XYL appeared to enhance release
364 of AX a little compared with just Buffer, but not substantially. Trials 3-7 taken together indicate that
365 enzyme treatment following chemical extraction was able to release a little more AX, with the
366 balance changing towards low MW material when the xylanase was included. This makes sense,
367 although the absence of any detectable AX in the fractions from Trial 6 is unexpected. However, in
368 general the enzymes did not dramatically enhance the further extraction of AX, and it is not possible
369 to infer any meaningful differences in the performance of the different enzyme combinations.

371 Figure 4 shows the equivalent results for sugarcane bagasse. Clearly the patterns are overall quite
372 contrasting to those for the wheat bran. Most obviously, from Figure 4(c), the recovery of AX by
373 chemical extraction was much greater than for wheat bran for both High and Low cut-off material,
374 with yields of around 6-7% for high MW AX and 5.5% for low MW AX, compared with 2.6% and 0.1%,
375 respectively, for wheat bran. Thus the bagasse released its AX much more readily following chemical
376 extraction, and released a more balanced profile of AX between large and small MW molecules; this

377 is also apparent in Figure 4(a) which shows much greater crude yields of Low cut-off material than
378 High, in contrast to the yields from wheat bran.

379

380 A consequence of the ready release of AX under chemical extraction is that there is therefore less
381 material for the enzymes subsequently to work on, hence the subsequent enzyme treatments yield
382 very little extra AX, although again there is evidence that the presence of the xylanase shifts the
383 balance towards smaller molecules, as expected; this is clearer in Figure 5 which presents an
384 expanded view of the Absolute yields, to allow the effects of the enzyme treatments, such as they
385 are, to be seen more clearly. Even on their own, however, without prior chemical extraction, the
386 enzymes release little more than Buffer alone (Trials 7-8 *c.f.* Trial 1), with the extra being entirely
387 small MW material. It is recognised, however, that this small study did not explore a wider range of
388 enzyme dosages and incubation conditions; it is likely that the effects of the enzymes could be
389 enhanced under optimised conditions.

390

391 Figure 6 shows the A:X ratios in the various wheat bran and bagasse extracts. In line with the
392 starting material and as expected from literature reports, the wheat bran extracts have much higher
393 A:X ratios than those from bagasse. Given the limitations of the work, not much more can be read
394 into the fine detail of Figure 6, beyond noting that for the bagasse extracts, the smaller molecular
395 weight material (Low cut-off fractions) consistently had lower A:X ratios than the corresponding
396 larger MW fractions, whereas the wheat-derived AX presents a more mixed picture. It is well
397 established that in general different parts of the biomass structure contain AX with different
398 molecular weights and A:X ratios, reflective of different botanical functions of AX in different parts of
399 the plant [22]. For the bagasse extracts, it appears to be consistently the case that material initially
400 released by whichever means (chemical extraction, enzymes or buffer) has higher A:X ratios than
401 material released subsequently via further extraction with buffer or enzymes, again reflecting
402 differences in the nature of AX material given up easily compared with that released on further
403 processing.

404

405 The picture is less consistent for the wheat bran extracts, reflecting that the initial release was less
406 extensive from the wheat bran than from the bagasse, such that comparisons are less dominated by
407 that initial release; for the bagasse, so much was released initially that the remaining AX material is
408 understandably quite different, whereas for the wheat bran, so little was released at all that what
409 was released at any point was similar in structure. The A:X ratios greater than 1 for some of the Low
410 cut-off wheat bran fractions (Trials 1 and 2) are probably erroneous, arising from errors in measuring
411 very low concentrations of A and X in these samples (see Figure 2(b)), although some components of
412 wheat outer layers (cross-cells and pericarp) can have A:X ratios great than 1 [22].

413

414 As noted already, the cleaner xylan chains of bagasse AX would offer somewhat different properties
415 compared to those of wheat AX. This is an important consideration in developing commercial
416 products; the challenge is to understand the functional performance and potential uses of AX
417 fractions as affected by molecular weight and A:X ratio, and hence to understand which feedstocks
418 and extraction processes are suitable for producing specific fractions. Even then, the likely scenario
419 is not that specific fractions would be targeted for exclusive production, but rather that processing
420 would co-produce a range of AX fractions, each suitable for different end-use applications, including
421 small AXOS fractions with prebiotic functionality in food and in animal feed, alongside mid-range and
422 large molecular weight fractions offering gradations of product functionality in relation to viscosity,
423 gel formation and interaction with other food components [4]. In this respect, commercialisation of

AX-based products is likely to follow the fractionation paradigm of crude oil cracking, to produce a range of products and to find markets for each. The use of enzymes would form part of the approach for creating specific fractions with targeted end-uses.

The above observations and comments regarding the results from the current work are made in full recognition of the limits of replication and accuracy of the study; nevertheless, the overall patterns are clear, relative to the objectives of the work and the wider commercial context, and lead confidently to the following conclusions:

1. Arabinoxylans can be extracted from sugarcane bagasse via similar protocols previously used for wheat bran, yielding AX with a lower A:X ratio than for wheat bran, hence offering different functional properties for end-use applications.
2. Bagasse released its AX more readily than wheat bran, and released a wider range of AX molecules, with a greater proportion of small MW (<10 kDa) molecules. In the current work, around two-thirds of the AX in the bagasse was released by chemical extraction, more or less equally divided between High and Low cut-off material, compared with just 11% of mostly large MW AX initially in the wheat bran.
3. Boiling wheat bran in ethanol prior to extraction appears to be necessary to obtain good yields of AX.
4. Within the conditions used, feruloyl esterase and xylanase enzymes had small effects on releasing AX from raw wheat bran or sugarcane bagasse or from residues following chemical extraction, with the xylanase tending to shift the balance from large to small MW molecules.

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

4. Conclusions

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

469 Thus, the metaphorical reactions that arose from the LBNet workshop were successful in
470 demonstrating the proof of concept, but the literal reactions in relation to optimising AX extraction
471 from wheat bran and sugarcane bagasse retain some scope for further enhancement.
472
473 As bagasse frequently arises within the context of bioethanol production, integration of AX
474 extraction with ethanol production could allow economic production of AX products, as has been
475 demonstrated in principle for AX co-production in a wheat ethanol plant [1]. Further processing of
476 the now lignin-rich residue could give even further opportunities within the biorefinery, both for co-
477 production of additional products and for further integration (particularly if the lignin processing
478 also involves ethanol). The nature and additional processing of the lignin in the residual fractions
479 from the current work were therefore studied further, and will be the topic of a future paper.
480
481
482 **Acknowledgements**
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494 **References**
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563 Table 1. Compositions of wheat bran and sugarcane bagasse (%w/w dry basis).
564

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
AX (=0.88×(A+X))	8.64	19.58
A:X ratio	0.57	0.21

565
566

567
568 Table 2. Crude yields, AX concentrations and AX yields from wheat bran and its fractions following extraction
569 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

570 a. The data reported for AX concentration and amount in the bran appear to be erroneous, as they indicate less AX in the
571 raw material than in the residual pellet; a starting concentration of 24% is more in line with the mass balance and with the
572 expected AX content of wheat bran.
573 b. The results for the pellet and high- and low-cutoff fractions following just buffer extraction are reported in these EE
574 rows, but these are not to be understood as having undergone enzyme treatment.
575 c. Initial weights averaged from Trials 2 and 3.
576

577
578 Table 3. Crude yields, AX concentrations and AX yields from sugarcane bagasse and its fractions following extraction under different treatments.
579

|	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

580   a. The results for the pellet and high- and low-cutoff fractions following just buffer extraction are reported in these EE

581   rows, but these are not to be understood as having undergone enzyme treatment.

582   b. Initial weights averaged from Trials 2 and 3.

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586   Figure 1. Procedure for chemical extraction of AX from wheat bran or sugarcane bagasse, yielding High
587   cut-off (CE-High) and Low cut-off (CE-Low) fractions and a residual pellet; and procedure for further
588   enzyme treatment of the pellet to yield further High (EE-High) and Low (EE-Low) cut-off fractions.
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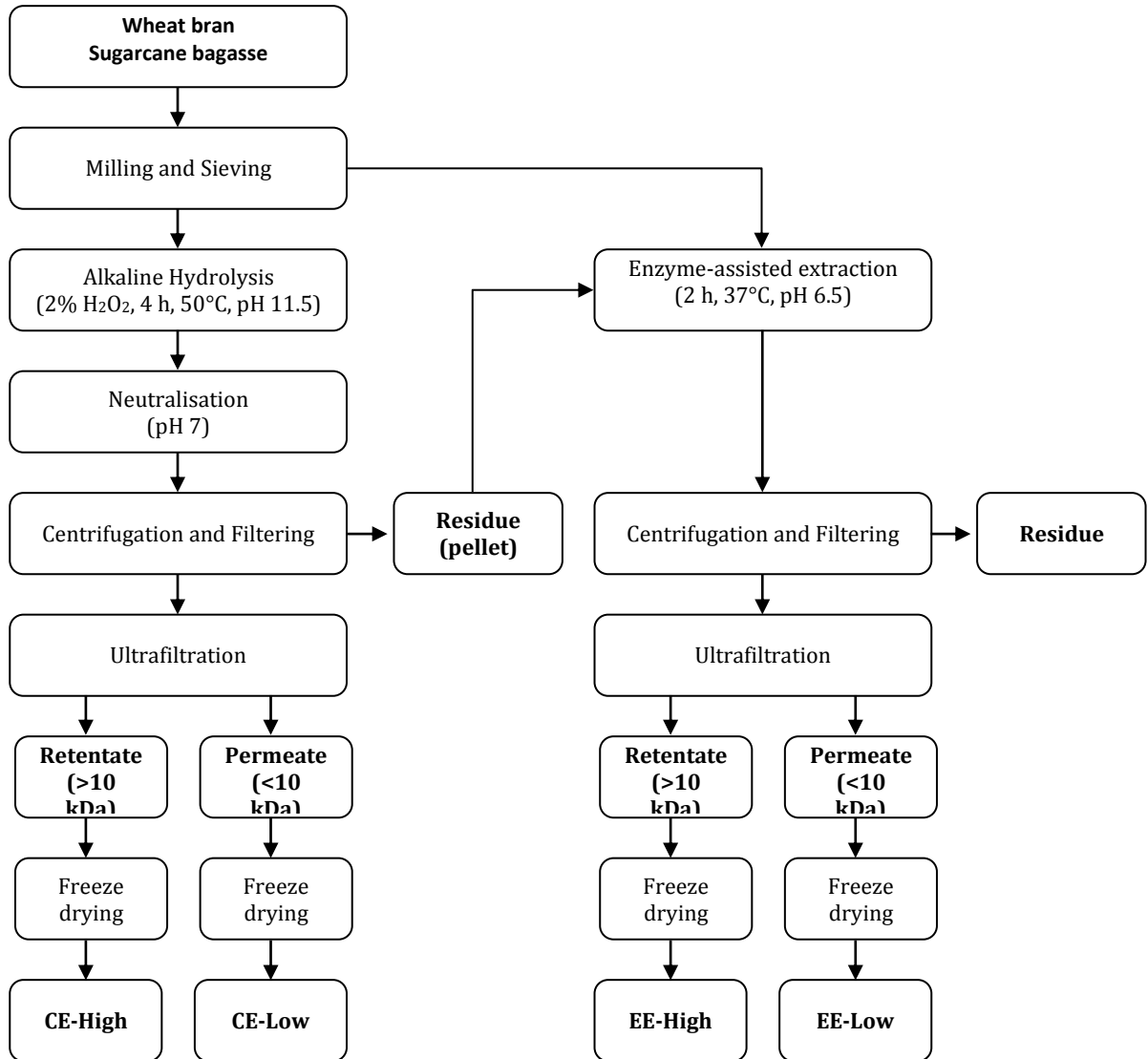
594   Figure 2. Measurement of arabinoxylan content in wheat bran following different hydrolysis conditions.


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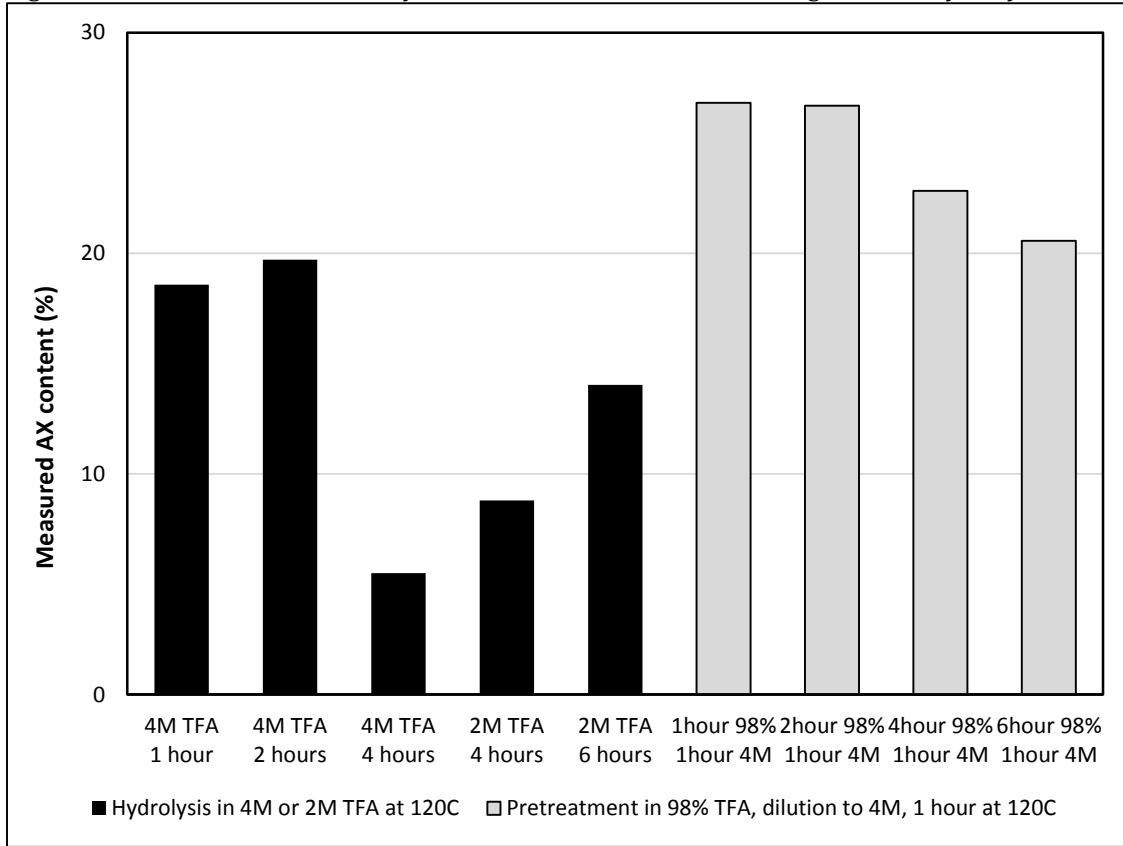
597 Figure 3. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction
598 from wheat bran.
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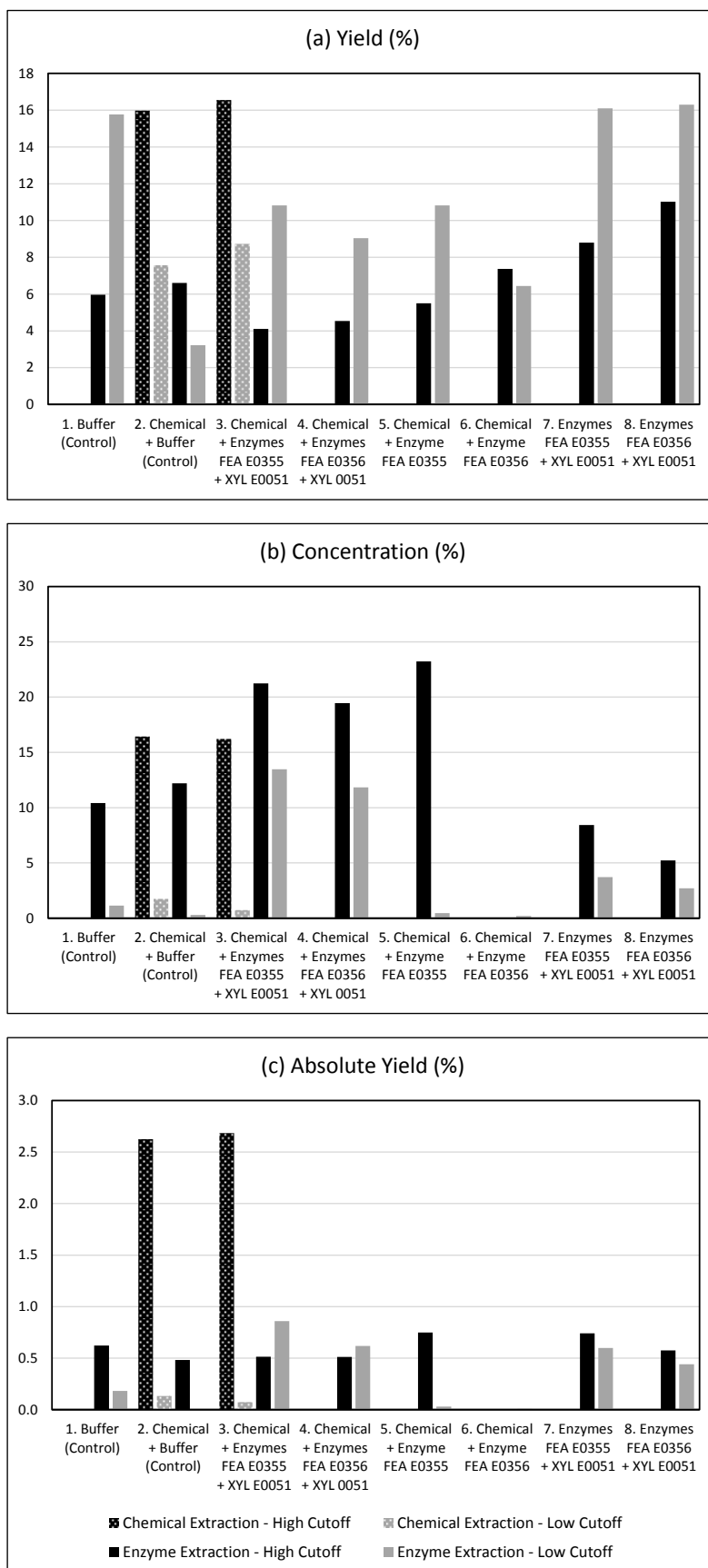
601　Figure 4. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction
602　from sugarcane bagasse.
603

(a) Yield (%)

(b) Concentration (%)
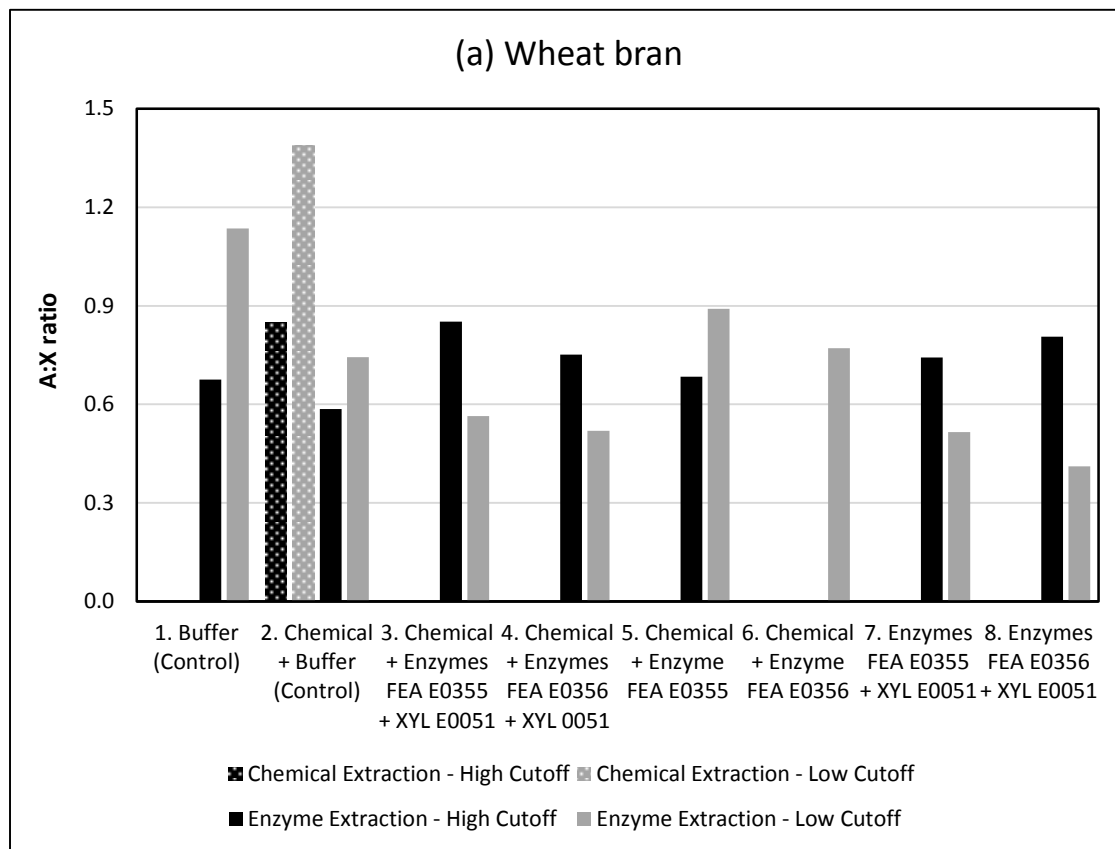
(c) Absolute Yield (%)

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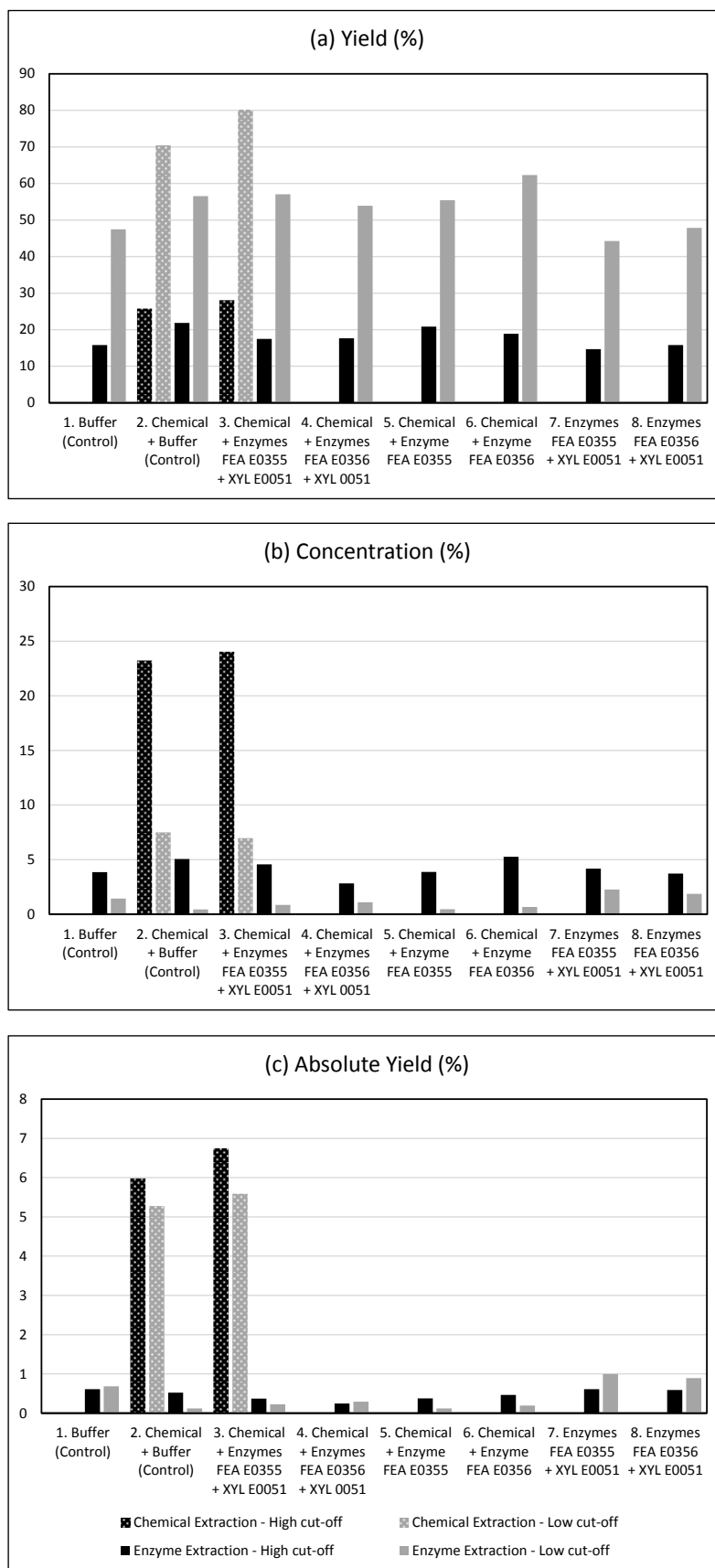
606   Figure 5. Expanded view of Absolute Yields in fractions following arabinoxylan extraction from sugarcane
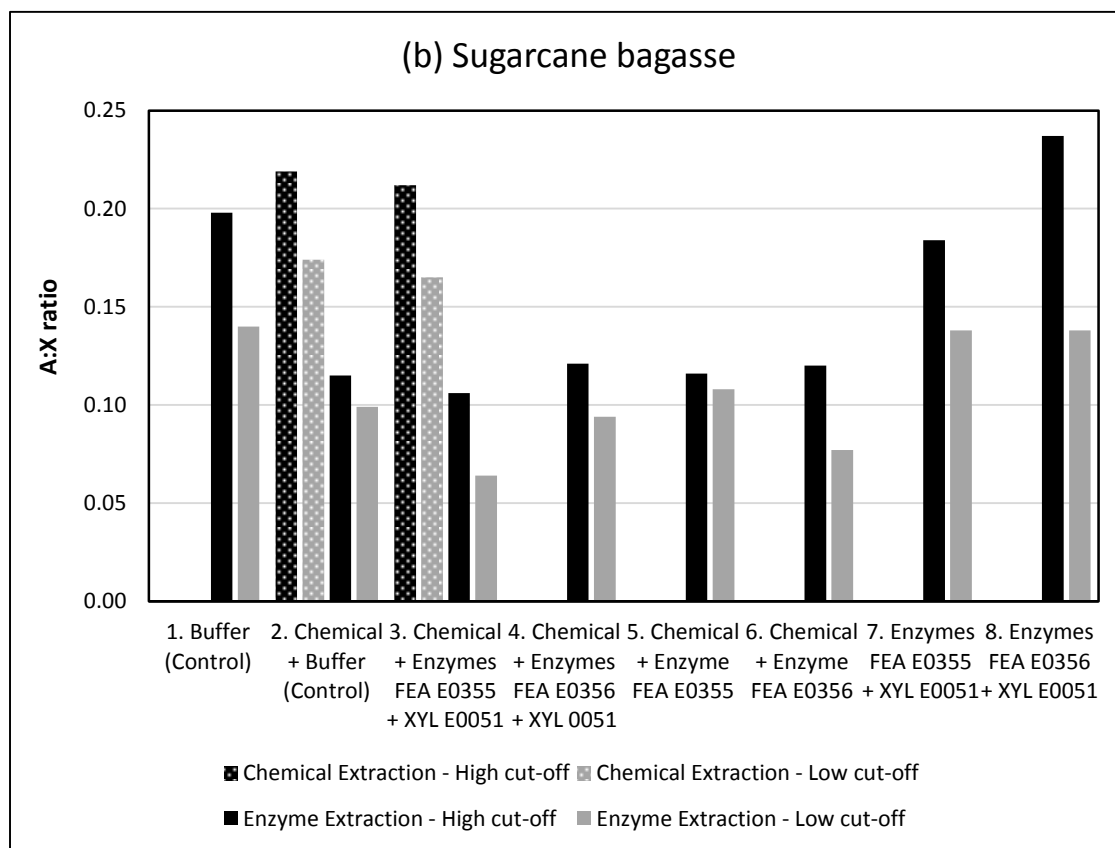607   bagasse under various chemical and enzyme treatments.
608
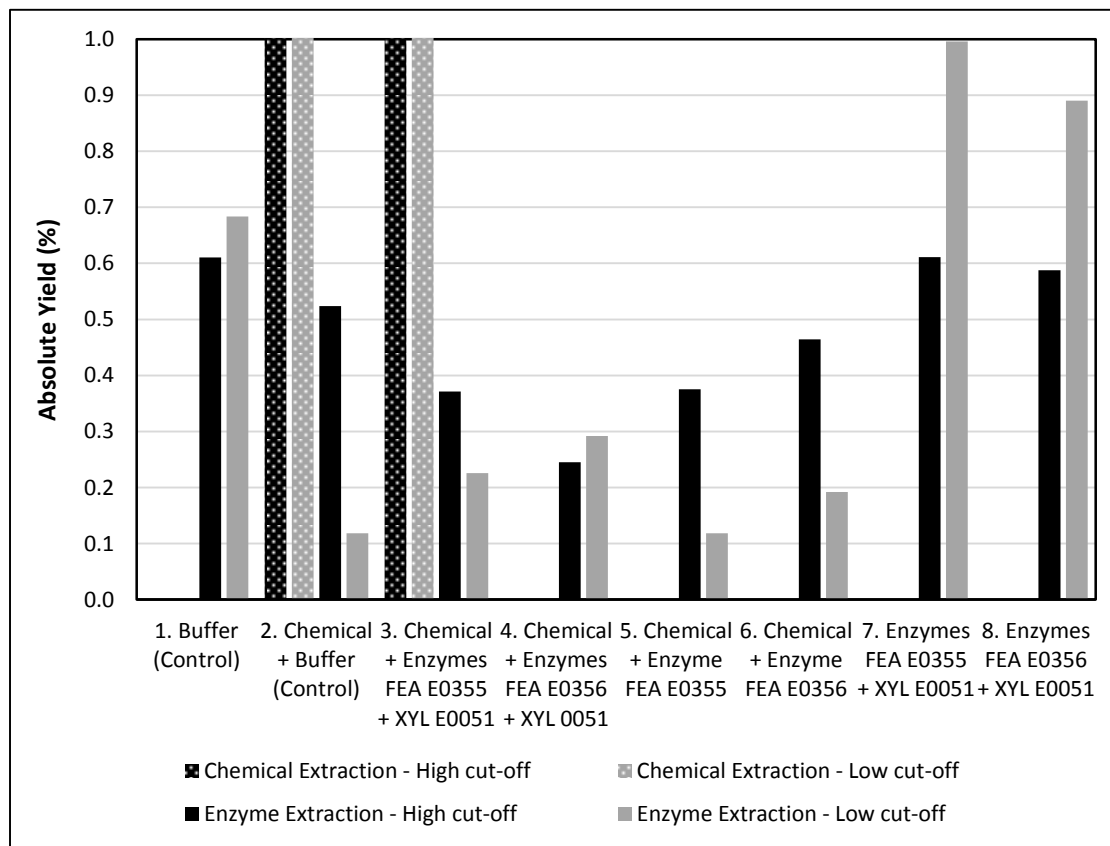
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611 Figure 6. Arabinose:Xylose ratios in fractions following arabinoxylan extraction from (a) wheat bran and
612 (b) sugarcane bagasse.
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