Corresponding Author: Professor Grant Murray Campbell, PhD

Corresponding Author's Institution: University of Huddersfield

First Author: Grant Murray Campbell, PhD

Order of Authors: Grant Murray Campbell, PhD; Nikolina Cukelj; Mohammad
Alyassin; Leo Gomez; Rachael Simister; James Flint; Darren Phillips; Mark
Gronnow; Nicholas Westwood

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,

Professor Grant Campbell
Professor of Chemical Engineering

Professor Grant Campbell PhD, CEng, CSci, FIChemE, FIFST, SFHEA
Professor of Chemical Engineering, School of Applied Sciences. Tel: +44(0)1484 472 134; Email: grant.campbell@hud.ac.uk

Queensgate, Huddersfield, HD1 3DH, UK
+44 (0) 1484 422288   +44 (0) 1484 516151
Patron: HRH The Duke of York, KG
Vice-Chancellor: Professor Bob Cryan DL MBA DSc CEng FIET FHEA

INVESTORS IN PEOPLE

theguardian UNIVERSITY AWARDS Winner 2013

THE QUEEN'S AWARDS FOR ENTERPRISE

CUSTOMER SERVICE EXCELLENCE CSE

2012 THE AWARDS WINNER Entrepreneurial University of the Year

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

1   **Integrated processing of sugarcane bagasse:**

2   **Arabinoxylan extraction integrated with ethanol production.**

3

4   Campbell GM[a*], Čukelj N[a1], Alyassin, M[a], Gomez LD[b], Simister R[b], Flint J[c], Philips DJ[d], Gronnow MJ[d]

5   and Westwood NJ[e]

6

7   a. School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield

8   b. Centre for Novel Agricultural Products, Department of Biology, University of York, Heslington,

9   York, UK

10   c. College of Science, Joseph Banks Laboratories, University of Lincoln

11   d. Biorenewables Development Centre, 1 Hassacarr Close, Chessingham Park, Dunnington, York, UK

12   e. School of Chemistry and Biomedical Sciences, University of St Andrews

13   1. Currently at: Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

14   * Author for correspondence, g.campbell@hud.ac.uk

15

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

CE-Low	Low cut-off fraction (<10 kDa) following chemical extraction
EE-High	High cut-off fraction following enzyme-assisted extraction
EE-Low	Low cut-off fraction following enzyme-assisted extraction
XYL	β-Xylanase (*C. mixtus*, PRO-E0051, Prozomix UK)
FE-E0355	Feruloyl esterase (*A. cellulolyticus*, PRO-E0355, Prozomix UK)
FE-E0356	Feruloyl esterase (*A. cellulolyticus*, PRO-E0356, Prozomix UK)

Declarations of interest: None.

Introduction

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

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

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

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

Materials and Methods

Sugarcane bagasse (25 kg) and wheat bran (50 kg) were sourced commercially by AB Sugar and provided to the project. The bagasse and bran were milled at the Biorenewables Development Centre (BDC) using a Retsch Cutting Mill SM 300 (Retsch GmbH, Germany) with a 2.00 mm screen, and AX extraction studies undertaken at the University of Huddersfield (UoH). Proximate analysis and arabinoxylan (AX) content measurements were performed at the University of York (UoY). Lignin studies, to be presented in a future paper, were undertaken at the University of St Andrews (UoStA).

Figure 1 describes the chemical extraction process using alkaline hydrogen peroxide applied to the milled wheat bran and sugarcane bagasse, based on the work of Hollmann and Lindhauer [15] and Du et al. [16]. The residue material was then subjected to enzyme treatment to see if further release of AX resulted, using selected combinations of β-Xylanase (*C. mixtus*, PRO-E0051, referred to here as XYL) and two types of Feruloyl esterase (*A. cellulolyticus*, PRO-E0355 and PRO-E0356, referred to here as FE-E0355 and FE-E0356) from Prozomix UK. Enzyme-assisted extraction on its own was also investigated, along with chemical extraction followed by further extraction with buffer solution, and buffer extraction on its own.

142 In total eight extractions were performed for each feedstock:

143 1. Direct buffer extraction (control for all trials, particularly Trials 7-8);

144 2 Chemical extraction followed by buffer extraction (control for Trials 3-6);

145 3. Chemical extraction followed by FE-E0355 and XYL;

146 4. Chemical extraction followed by FE-E0356 and XYL;

147 5. Chemical extraction followed by FE-E0355;

148 6. Chemical extraction followed by FE-E0356;

149 7. Direct enzyme extraction with FE-E0355 and XYL;

150 8. Direct enzyme extraction with FE-E0356 and XYL.

151

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

159

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

174

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

187

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

200

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

210

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

212

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

225

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

233

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

242
243   *Crystalline cellulose*
244   Biomass dry pellets after TFA hydrolysis were washed once with 1.5 mL of water, and twice using 1.5
245   mL of acetone. The dried pellets were left to air dry overnight before complete hydrolysis by adding
246   90 μL of 72% w/w sulphuric acid, incubating at room temperature for 4 hours. 1.89 mL of water was
247   subsequently added and the sample was heated for 4 hours at 120°C. The glucose content of the
248   supernatant was assessed using the colorimetric Anthrone assay, using a glucose standard curve.

249
250   Analysis of the sugar compositions of the wheat bran and bagasse samples and of fractions and
251   residues following the various chemical and enzyme extractions was undertaken as follows:

252
253   Monosaccharide analysis was performed by high performance anion-exchange chromatography
254   (HPAEC) (Dionex IC 2500) on a Dionex Carbopac PA-10 column with integrated amperometry
255   detection [18]. The separated monosaccharides were quantified using external calibration with an
256   equimolar mixture of nine monosaccharide standards (arabinose, fucose, galactose, galacturonic
257   acid, glucose, glucuronic acid, mannose, rhamnose, and xylose), which were subjected to TFA
258   hydrolysis in parallel with the samples.

259
260
261   **Results and Discussion**

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

276
277   Tables 2 and 3 report the crude yields, AX concentrations and hence AX yields from 50 g wheat bran
278   and 11 g sugarcane bagasse, respectively, in the starting materials and in the fractions following the
279   various chemical, enzyme and buffer treatments. The second column in each table is for chemical
280   extraction only, showing the data from two replicates, with good agreement. Considering the wheat

281 bran results first, chemical extraction of around 50.6 g of bran (moisture content 9.5%) yielded a
282 residual dry pellet weighing 29.94 g (averaged from Trials 2 and 3), a High cut-off (CE-High) of 8.21 g,
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).
284 The AX concentration in the pellet was 35.81% and in the High and Low cut-off fractions was 16.32%
285 and 1.25%, respectively. This implies a total amount of AX in the pellet and two fractions of
286 29.94×35.81% + 8.21×16.32% + 4.40×1.25% = 10.72 + 1.34 + 0.06 = 12.12 g. The mass balance
287 therefore implies an AX concentration of 12.12/50.6 = 24% in the original wheat bran, higher than
288 the 8.64% reported in Table 1, and more in line with the expected AX content of wheat bran,
289 suggesting that the 8.64% figure is erroneous.

290

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

306

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
308 alkaline hydrogen peroxide extraction process. This is much lower than the recoveries of 46-50%
309 reported by Hollmann and Lindhauer [15] and Du et al. [16] for alkali-extracted AX from wheat bran
310 using similar conditions. In those studies the wheat bran was boiled in 70% ethanol at 80°C for 4
311 hours prior to alkaline H_2O_2 treatment, which Hollmann and Lindhauer [15] advised was necessary to
312 achieve high yields; this step was omitted in the current work (for safety and cost reasons in relation
313 to the planned scale-up work, and because related unpublished work from our labs on extraction
314 from maize meal had found that this step was not needed, as also confirmed by work from Doner
315 and Hicks [21] on AX extraction from maize fibre). The omission of this ethanol boiling step is
316 possibly the reason for the lower yields than in this previously reported work.

317

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

327

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

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

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

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

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

Figure 6 shows the A:X ratios in the various wheat bran and bagasse extracts. In line with the starting material and as expected from literature reports, the wheat bran extracts have much higher A:X ratios than those from bagasse. Given the limitations of the work, not much more can be read into the fine detail of Figure 6, beyond noting that for the bagasse extracts, the smaller molecular weight material (Low cut-off fractions) consistently had lower A:X ratios than the corresponding larger MW fractions, whereas the wheat-derived AX presents a more mixed picture. It is well established that in general different parts of the biomass structure contain AX with different molecular weights and A:X ratios, reflective of different botanical functions of AX in different parts of the plant [22]. For the bagasse extracts, it appears to be consistently the case that material initially released by whichever means (chemical extraction, enzymes or buffer) has higher A:X ratios than material released subsequently via further extraction with buffer or enzymes, again reflecting differences in the nature of AX material given up easily compared with that released on further processing. The picture is less consistent for the wheat bran extracts, reflecting that the initial release was less extensive from the wheat bran than from the bagasse, such that comparisons are less dominated by that initial release; for the bagasse, so much was released initially that the remaining AX material is understandably quite different, whereas for the wheat bran, so little was released at all that what was released at any point was similar in structure. The A:X ratios greater than 1 for some of the Low cut-off wheat bran fractions (Trials 1 and 2) are probably erroneous, arising from errors in measuring very low concentrations of A and X in these samples (see Figure 2(b)), although some components of wheat outer layers (cross-cells and pericarp) can have A:X ratios great than 1 [22].

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

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. 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.

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.

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

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

468　**Acknowledgements**

478

479

480　**References**

481

482　[1]　N. Misailidis, G.M. Campbell, C. Du, J. Sadhukhan, M. Mustafa, F. Mateos-Salvador, R.M.

483　　　　Weightman, Evaluating the feasibility of commercial arabinoxylan production in the context

484　　　　of a wheat biorefinery principally producing ethanol. Part 2. Process simulation and economic

485　　　　analysis, Chem. Eng. Res. Des. 87 (2009). doi:10.1016/j.cherd.2008.12.028.

486　[2]　C.S. Lancefield, O.S. Ojo, F. Tran, N.J. Westwood, Isolation of functionalized phenolic

487　　　　monomers through selective oxidation and CO bond cleavage of the β-O-4 linkages in Lignin,

488　　　　Angew. Chemie - Int. Ed. (2015). doi:10.1002/anie.201409408.

489　[3]　E. Martinez-Hernandez, J. Sadhukhan, G.M. Campbell, Integration of bioethanol as an in-

490　　　　process material in biorefineries using mass pinch analysis, Appl. Energy. 104 (2013).

491　　　　doi:10.1016/j.apenergy.2012.11.054.

492　[4]　E. Martinez-Hernandez, A. Tibessart, G.M. Campbell, Conceptual design of integrated

493　　　　production of arabinoxylan products using bioethanol pinch analysis, Food Bioprod. Process.

494　　　　(2018). doi:10.1016/j.fbp.2018.08.005.

495　[5]　Z.C. Geng, J.X. Sun, S.F. Liang, F.Y. Zhang, Y.Y. Zhang, F. Xu, R.C. Sun, Characterization of

496　　　　water- and alkali-soluble hemicellulosic polymers from sugarcane bagasse, Int. J. Polym. Anal.

497　　　　Charact. (2006). doi:10.1080/10236660600649095.

498　[6]　P. Feng, R. Jun-Li, X. Feng, B. Jing, P. Pai, S. Run-Cang, Comparative study of hemicelluloses

499　　　　obtained by graded ethanol precipitation from sugarcane bagasse, J. Agric. Food Chem. 57

500　　　　6305-6317. (2009). doi:10.1021/jf900986b.

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,

502　　　　Addition of feruloyl esterase and xylanase produced on-site improves sugarcane bagasse

503　　　　hydrolysis, Bioresour. Technol. (2014). doi:10.1016/j.biortech.2014.07.115.

504　[8]　M. Aguedo, H.A. Ruiz, A. Richel, Non-alkaline solubilization of arabinoxylans from destarched

505　　　　wheat bran using hydrothermal microwave processing and comparison with the hydrolysis by

506　　　　an endoxylanase, Chem. Eng. Process. Process Intensif. (2015).

507　　　　doi:10.1016/j.cep.2015.07.020.

508　[9]　L. Jacquemin, A. Mogni, R. Zeitoun, C. Guinot, C. Sablayrolles, L. Saulnier, P.Y. Pontalier,

509　　　　Performance evaluation of a semi-industrial production process of arabinoxylans from wheat

510　　　　bran, Process Biochem. (2015). doi:10.1016/j.procbio.2015.01.015.

511　[10]　C. Maes, J.A. Delcour, Alkaline hydrogen peroxide extraction of wheat bran non-starch

512　　　　polysaccharides, J. Cereal Sci. (2001). doi:10.1006/jcrs.2001.0377.

513　[11]　C. Maes, J.A. Delcour, Structural characterisation of water-extractable and water-

514　　　　unextractable arabinoxylans in wheat bran, J. Cereal Sci. 35 (2002) 315–326.

515　　　　doi:10.1006/jcrs.2001.0439.

516　[12]　C.M. Courtin, J.A. Delcour, Arabinoxylans and endoxylanases in wheat flour bread-making, J.

517　　　　Cereal Sci. (2002). doi:10.1006/jcrs.2001.0433.

518　[13]　J.J. Ordaz-Ortiz, L. Saulnier, Structural variability of arabinoxylans from wheat flour.

519　　　　Comparison of water-extractable and xylanase-extractable arabinoxylans, J. Cereal Sci. 42

520 (2005) 119–125. doi:10.1016/j.jcs.2005.02.004.

521 [14] A.P. de Souza, D.C.C. Leite, S. Pattathil, M.G. Hahn, M.S. Buckeridge, Composition and
522 Structure of Sugarcane Cell Wall Polysaccharides: Implications for Second-Generation
523 Bioethanol Production, Bioenergy Res. 6 (2013) 564–579. doi:10.1007/s12155-012-9268-1.

524 [15] J. Hollmann, M.G. Lindhauer, Pilot-scale isolation of glucuronoarabinoxylans from wheat
525 bran, Carbohydr. Polym. (2005). doi:10.1016/j.carbpol.2004.09.015.

526 [16] C. Du, G.M. Campbell, N. Misailidis, F. Mateos-Salvador, J. Sadhukhan, M. Mustafa, R.M.
527 Weightman, Evaluating the feasibility of commercial arabinoxylan production in the context
528 of a wheat biorefinery principally producing ethanol. Part 1. Experimental studies of
529 arabinoxylan extraction from wheat bran, Chem. Eng. Res. Des. 87 (2009) 1232–1238.
530 doi:10.1016/j.cherd.2008.12.027.

531 [17] S.C. Fry, The growing plant cell wall: chemical and metabolic analysis, The Blackburn Press,
532 New Jersey, USA, 1988. doi:10.1111/j.1469-8137.2003.00980.x.

533 [18] L. Jones, J.L. Milne, D. Ashford, S.J. McQueen-Mason, Cell wall arabinan is essential for guard
534 cell function, Proc. Natl. Acad. Sci. (2003). doi:10.1073/pnas.1832434100.

535 [19] S. Ranganathan, D.G. Macdonald, N.N. Bakhshi, Kinetic studies of wheat straw hydrolysis
536 using sulphuric acid, Can. J. Chem. Eng. 63 (1985) 840–844. doi:10.1002/cjce.5450630521.

537 [20] S. Willför, A. Pranovich, T. Tamminen, J. Puls, C. Laine, A. Suurnäkki, B. Saake, K. Uotila, H.
538 Simolin, J. Hemming, B. Holmbom, Carbohydrate analysis of plant materials with uronic acid-
539 containing polysaccharides-A comparison between different hydrolysis and subsequent
540 chromatographic analytical techniques, Ind. Crops Prod. 29 (2009) 571–580.
541 doi:10.1016/j.indcrop.2008.11.003.

542 [21] L.W. Doner, K.B. Hicks, Isolation of hemicellulose from corn fiber by alkaline hydrogen
543 peroxide extraction, Cereal Chem. (1997). doi:10.1094/CCHEM.1997.74.2.176.

544 [22] L. Saulnier, P.E. Sado, G. Branlard, G. Charmet, F. Guillon, Wheat arabinoxylans: Exploiting
545 variation in amount and composition to develop enhanced varieties, J. Cereal Sci. 46 (2007)
546 261–281. doi:10.1016/j.jcs.2007.06.014.

547

Table

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[a]	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.

```
                    ┌─────────────────────┐
                    │   Wheat bran        │
                    │ Sugarcane bagasse   │
                    └─────────┬───────────┘
                              │
                    ┌─────────▼───────────┐───────────────────┐
                    │  Milling and Sieving│                   │
                    └─────────┬───────────┘                   │
                              │                               │
        ┌─────────────────────▼──┐          ┌─────────────────▼──────────┐
        │  Alkaline Hydrolysis   │          │  Enzyme-assisted extraction│
        │ (2% H₂O₂, 4 h, 50°C,   │◄──┐      │  (2 h, 37°C, pH 6.5)       │
        │      pH 11.5)          │   │      └─────────────┬──────────────┘
        └─────────┬──────────────┘   │                    │
                  │                   │                    │
        ┌─────────▼──────────┐       │                    │
        │  Neutralisation    │       │                    │
        │     (pH 7)         │       │                    │
        └─────────┬──────────┘       │                    │
                  │                   │                    │
  ┌───────────────▼────────┐  ┌──────┴─────┐  ┌────────────▼──────────────┐  ┌─────────┐
  │ Centrifugation and     │─►│  Residue   │  │ Centrifugation and        │─►│ Residue │
  │ Filtering              │  │  (pellet)  │  │ Filtering                 │  └─────────┘
  └───────────┬────────────┘  └────────────┘  └────────────┬──────────────┘
              │                                             │
  ┌───────────▼────────┐                       ┌────────────▼──────────┐
  │  Ultrafiltration   │                       │   Ultrafiltration     │
  └───┬───────────┬────┘                       └───┬───────────────┬───┘
      │           │                                │               │
 ┌────▼────┐ ┌────▼────┐                      ┌─────▼───┐     ┌─────▼───┐
 │Retentate│ │ Permeate│                      │Retentate│     │ Permeate│
 │(>10 kDa)│ │(<10 kDa)│                      │(>10 kDa)│     │(<10 kDa)│
 └────┬────┘ └────┬────┘                      └────┬────┘     └────┬────┘
      │           │                                │               │
 ┌────▼───┐  ┌────▼───┐                       ┌────▼───┐      ┌────▼───┐
 │ Freeze │  │ Freeze │                       │ Freeze │      │ Freeze │
 │ drying │  │ drying │                       │ drying │      │ drying │
 └────┬───┘  └────┬───┘                       └────┬───┘      └────┬───┘
      │           │                                │               │
 ┌────▼───┐  ┌────▼───┐                       ┌────▼───┐      ┌────▼───┐
 │CE-High │  │ CE-Low │                       │EE-High │      │ EE-Low │
 └────────┘  └────────┘                       └────────┘      └────────┘
```

Milling and Sieving → Alkaline Hydrolysis (2% H_2O_2, 4 h, 50°C, pH 11.5); Enzyme-assisted extraction (2 h, 37°C, pH 6.5)
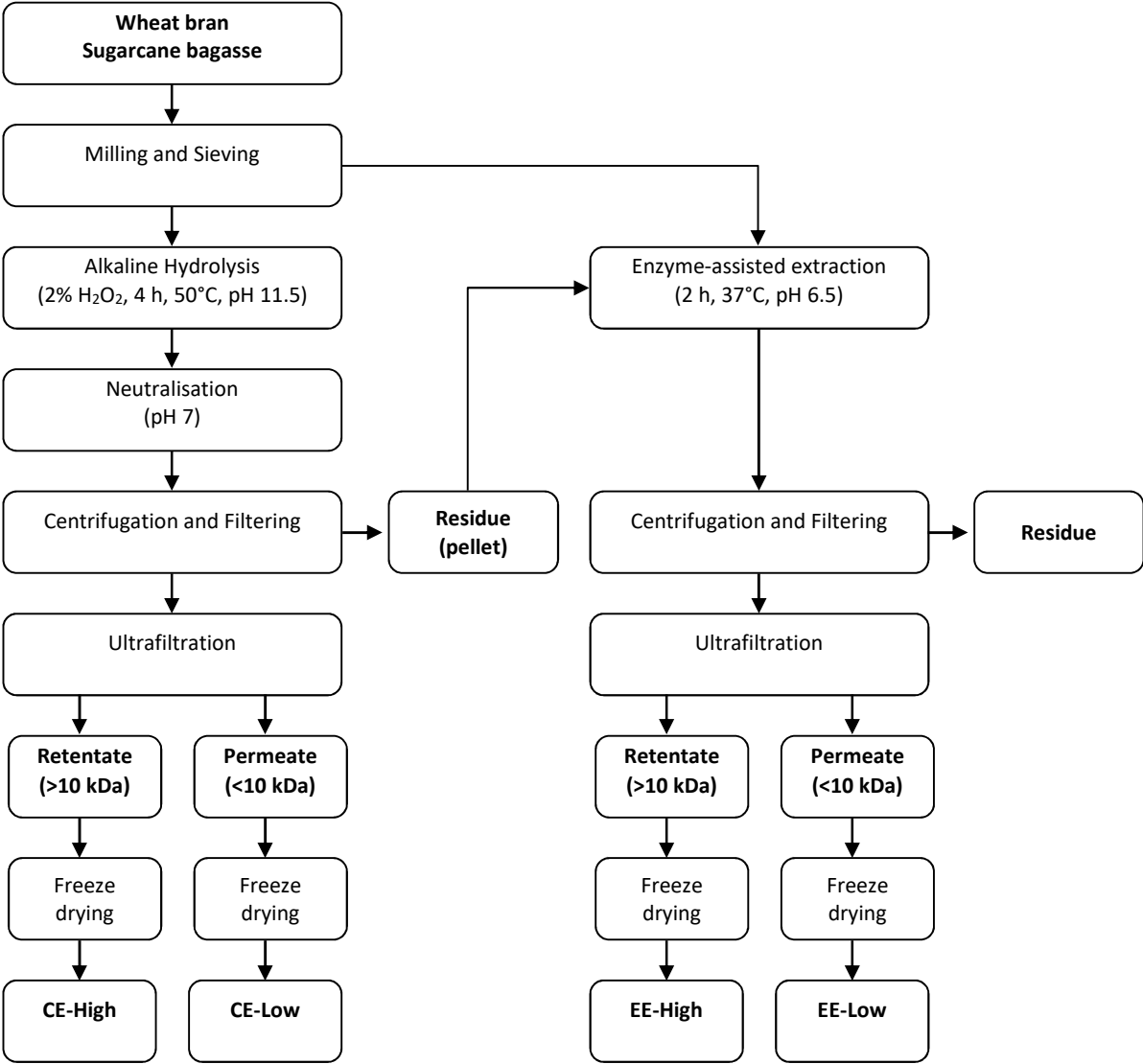
Figure 2. Measurement of arabinoxylan content in wheat bran following different hydrolysis conditions.
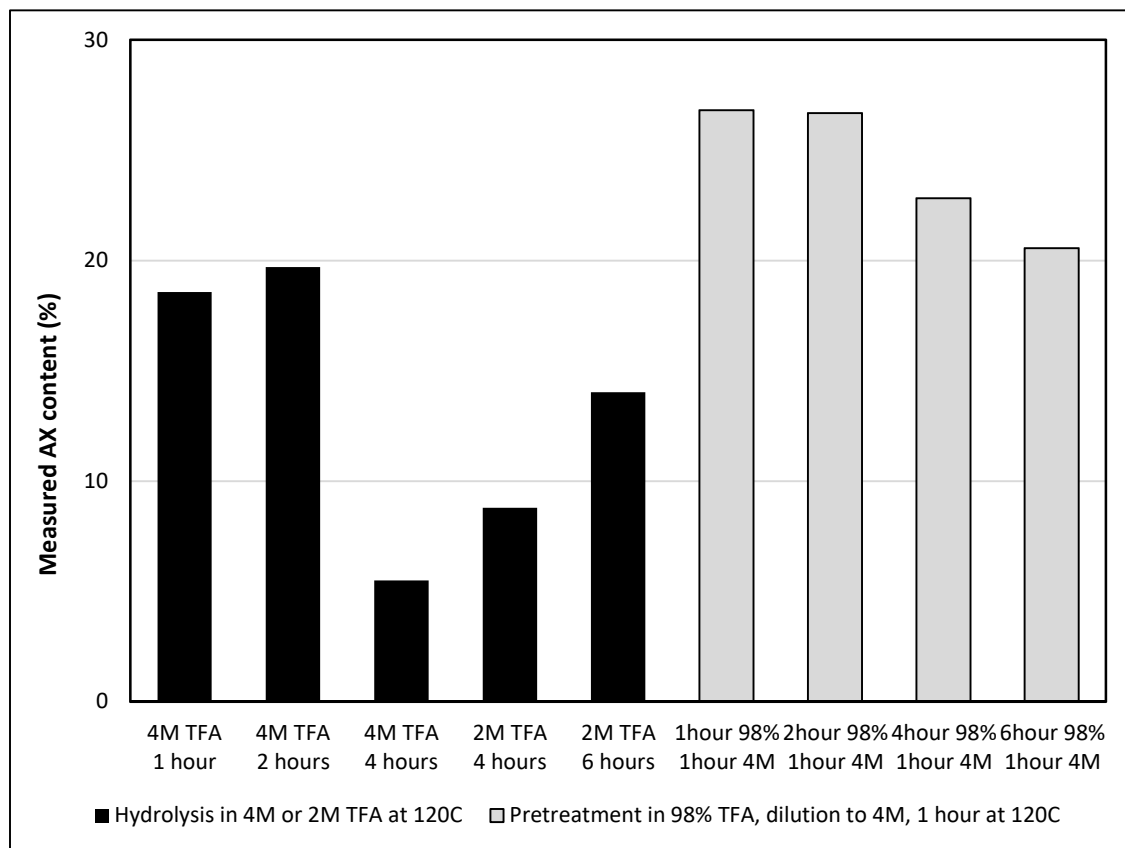
Figure 3. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction from wheat bran.
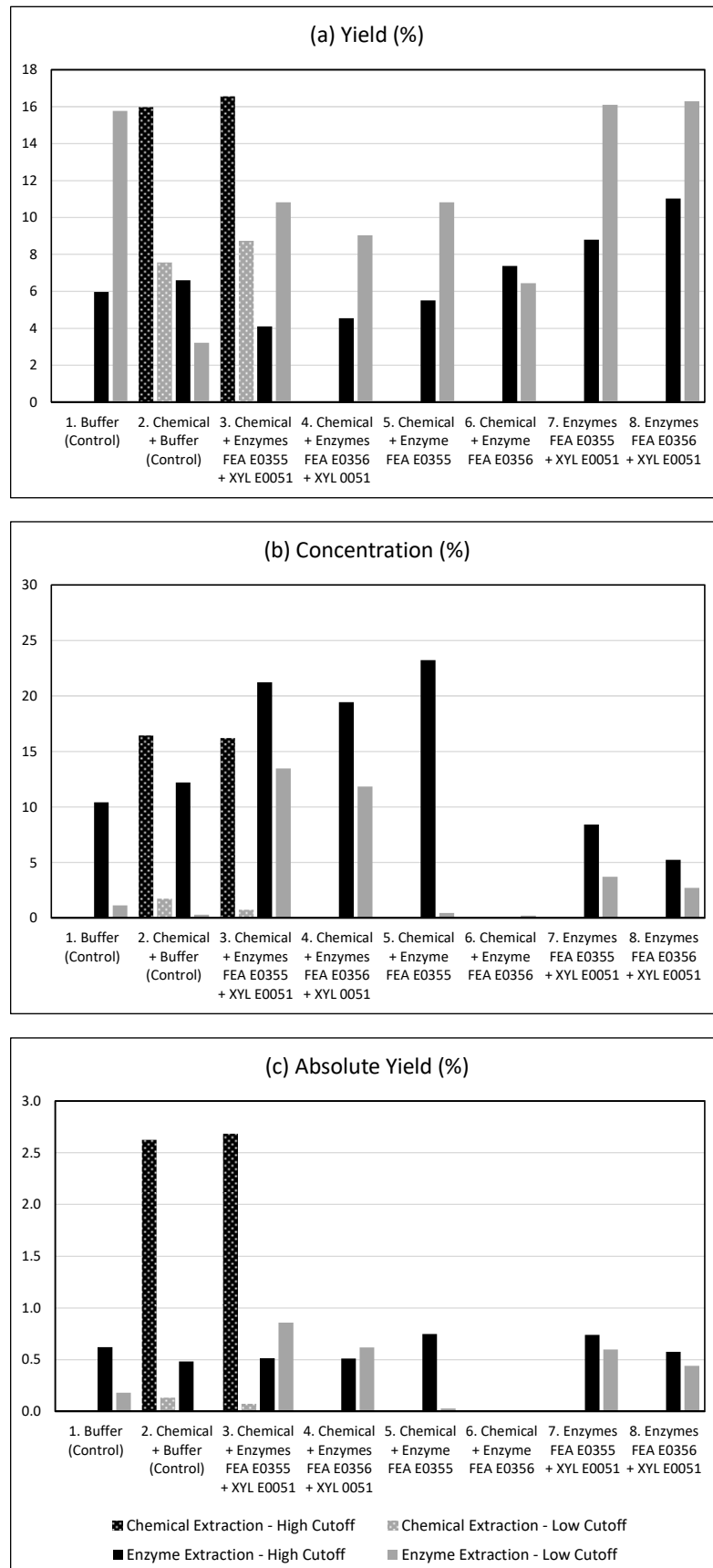
Figure 4. (a) Yield, (b) Concentration and (c) Absolute Yield in fractions following arabinoxylan extraction from sugarcane bagasse.

(a) Yield (%)

(b) Concentration (%)

(c) Absolute Yield (%)

■ Chemical Extraction - High cut-off   ▨ Chemical Extraction - Low cut-off
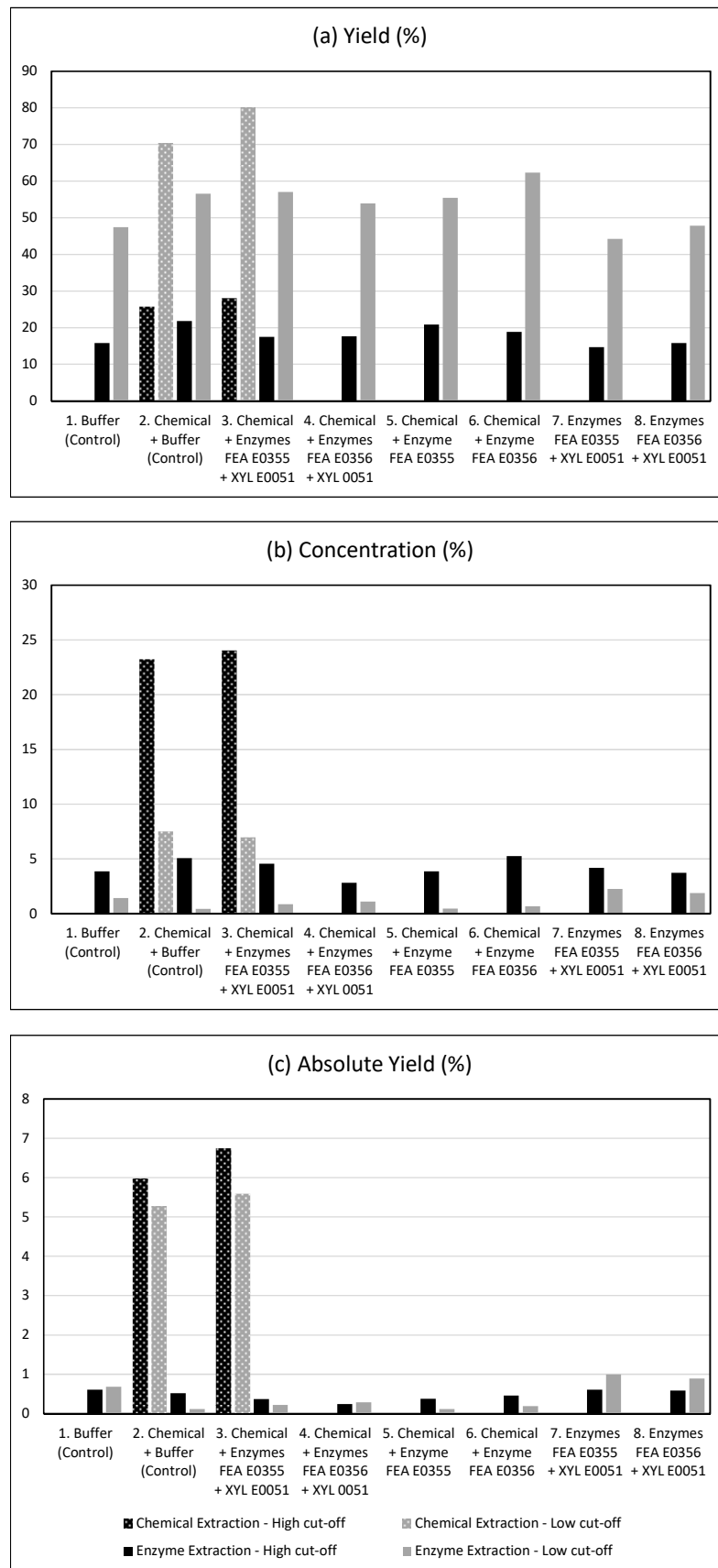■ Enzyme Extraction - High cut-off   ■ Enzyme Extraction - Low cut-off

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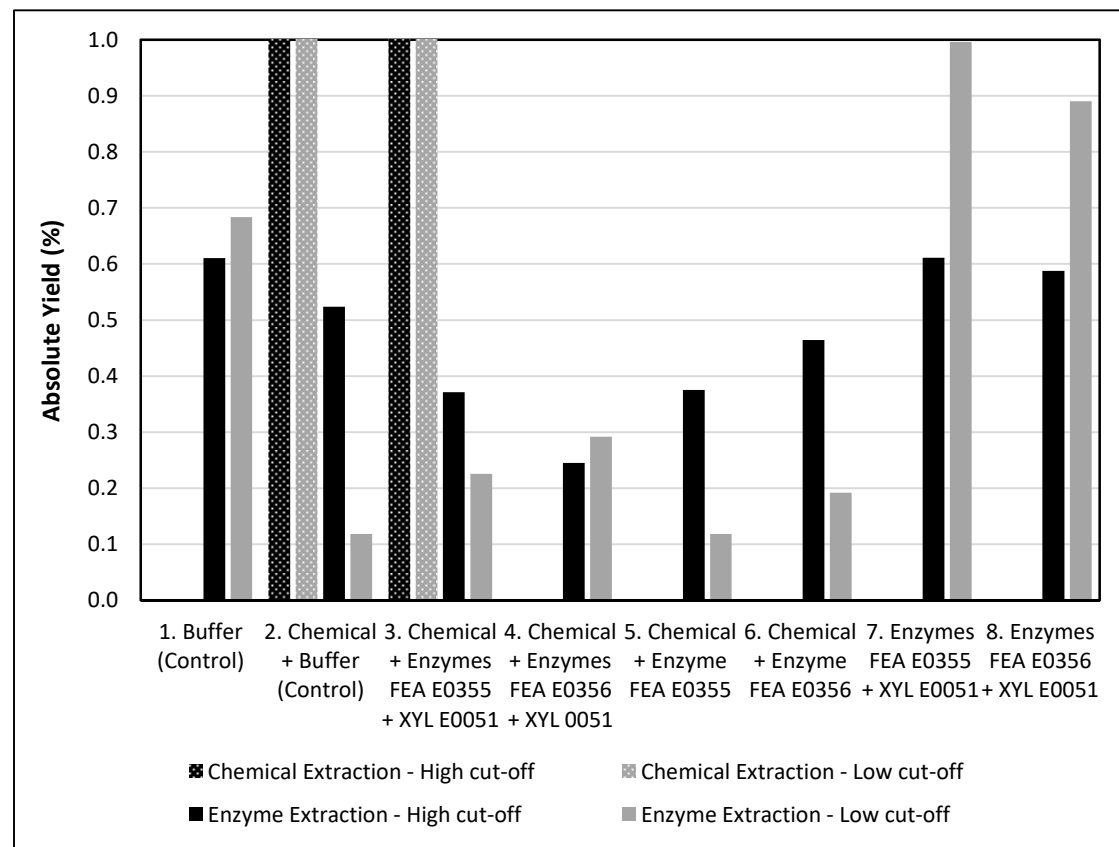
Figure 6. Arabinose:Xylose ratios in fractions following arabinoxylan extraction from (a) wheat bran and (b) sugarcane bagasse.

(a) Wheat bran

(b) Sugarcane bagasse