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# Production of feruloylated arabinoxylo-oligosaccharides from maize (*Zea mays*) bran by microwave-assisted autohydrolysis

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ABSTRACT

Maize bran was treated with microwave irradiation (160–200 °C for 2–20 min) to release feruloylated arabinoxylo-oligosaccharides (AXOS). Lower temperatures and shorter treatment times were consistent with low AXOS yields, while higher temperatures and longer reaction times also resulted in low yields, which were contaminated with increasing levels of monosaccharides, free ferulic acid, and furfural. Maximum release of AXOS, accompanied by low production of monosaccharides, free ferulic acid, and furfural, occurred with treatment at 180 °C for 10 min or 200 °C for 2 min. Under these conditions, about 50% of the initial arabinoxylan content could be released as AXOS, containing a wide variety of molecular weights. AXOS were highly feruloylated, containing 6.62 and 8.00 g of esterified ferulate/100 g of AXOS. These feruloylated AXOS may provide health benefits, including prebiotic effects and prevention of detrimental oxidation reactions. The evaluation of these benefits will be the subject of future research.

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## 1. Introduction

Whereas it was once thought that dietary fibres were only of nutritional importance insofar as they prevented constipation, it is now recognised that these polymers induce numerous beneficial effects on human health, including the prevention of heart disease, diabetes, obesity, and many gastrointestinal diseases (Anderson et al., 2009). Furthermore, cereal dietary fibres often contain high levels of phenolic antioxidants, which may provide additional health benefits (Vitaglione, Napolitano, & Fogliano, 2008).

Maize bran is a rich source of dietary fibre and phenolic antioxidants (Vitaglione et al., 2008). In fact, of the major cereal brans, maize bran is the highest in both of these constituents. The chief portion of maize bran is a hemicellulose, which is, more specifically, a complex heteroxylan consisting mainly of a xylan backbone with arabinosyl side units (Saulnier & Thibault, 1999). Esterified to some of the arabinosyl units is ferulic acid, a phenolic antioxidant, which may form cross-links via oxidative dimerisation (Saulnier & Thibault, 1999). This results in an insoluble dietary fibre that is largely inaccessible to digestion or fermentation in the digestive tract, leading to >90% recovery of ingested maize bran in the faeces (Dintzis et al., 1979). Because many functional properties and health benefits of dietary fibre rely on solubility and microbial fermentation (Anderson et al., 2009; Dikeman & Fahey, 2006; Rose, Demeo, Keshavarzian, & Hamaker, 2007), maize bran in its native state offers few applications.

A plethora of chemical and physical treatments have been suggested to break up the cross-linked polymers in maize bran and other types of agricultural biomass to partially release hemicellulosic material into solution. One processing procedure that offers substantial promise is autohydrolysis. This treatment utilises hydronium ions and *in situ* generated organic acids at high processing temperatures (160–220 °C) to partially hydrolyse hemicellulosic polymers and yield soluble hydrolysates (Moure, Gullon, Dominguez, & Parajo, 2006). The major products of autohydrolysis are referred to as oligosaccharides, even though they often represent broad distributions of molecular weights that do not necessarily fall within the traditional definition of an oligosaccharide (Moure et al., 2006).

Autohydrolysis is advantageous because it does not require the use of harsh chemicals or enzymes that can contribute substantially to cost. Furthermore, many distinguishing structural features of the native dietary fibre, such as the presence of esterified phenolic antioxidants, are retained with autohydrolysis (Kabel et al., 2002), as opposed to other treatments, which may remove these potentially valuable products (Hartley & Morrison, 1991).

The disadvantage of autohydrolytic treatment is that some objectionable compounds can be produced under the extreme temperatures, necessitating refining steps such as solvent precipitation, freeze drying and solvent extraction, and ion exchange (Vegas, Alonso, Dominguez, & Parajo, 2005). The production of undesirable compounds can be minimised by introducing a pre-treatment step, during which the biomass is heated at a lower

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temperature (120–150 °C) and then washed to remove many of the constituents that contribute to the production of offending compounds. Additionally, careful optimisation and control of processing conditions can minimise production of undesirable compounds. The purpose of this research was to use microwave irradiation to initiate autohydrolysis of maize bran and release partially depolymerised, water-soluble, feruloylated arabinoxylo-oligosaccharides (AXOS).

## 2. Materials and methods

### 2.1. Production and composition of maize bran insoluble material

Maize bran insoluble material was produced according to Yuan, Wang, and Yao (2006) with modifications. One hundred grams of finely milled maize bran (Bunge Milling, Danville, IL, USA) were suspended in 1 l of water and autoclaved at 121 °C for 45 min. The pH was adjusted to 7.0 with NaOH, 4 ml of thermostable α-amylase (A-3403, Sigma–Aldrich, St. Louis, MO, USA) were added, and the slurry was boiled under constant stirring for 30 min. After cooling to 50 °C, the pH was adjusted to 7.5 with NaOH, 3 ml of protease (P-4806, Sigma–Aldrich) were added, and protein was hydrolysed for 30 min at 50 °C. The pH was then adjusted to 4.5 with HCl, 3 ml of amyloglucosidase (A-7095, Sigma–Aldrich) were added, and digestion continued for 30 min at 50 °C. Following digestion, the maize bran was filtered (Whatman No. 1) and suspended in 500 ml of water. After mixing, the slurry was filtered again, and the washing and filtration steps were repeated with 95% (v/v) ethanol and acetone, followed by air drying at room temperature, and fine grinding in a coffee mill to obtain maize bran insoluble material.

Total starch, protein, and moisture were determined on maize bran and maize bran insoluble material. Total starch was quantified using an assay kit [total starch (AA/AMG) method, Megazyme, Wicklow, Ireland]. Protein was determined by the combustion method with a protein correction factor of $\%N \times 6.25$ (approved method 46-30, AACC International, 2000), and moisture was determined as loss in weight upon drying (approved method 44-15A, AACC International, 2000).

Maize bran insoluble material was also assayed for neutral sugar and total ferulic acid contents. Neutral monosaccharide composition was determined according to Theander, Aman, Westerlund, Andersson, and Petersson (1995). Total ferulic acid in maize bran insoluble material was determined according to Shin, McClendon, Le, Taylor, and Chen (2006) with some modifications. A 20 mg sample was shaken (150 rpm) with 1 ml of 2 M sodium hydroxide in the dark for 2 h at 45 °C. The pH was then brought to about 2 with 0.6 ml of 3.5 M phosphoric acid, and the tubes were extracted five times with 1.6 ml of ethyl acetate each. Centrifugation at 1000*g* for 5 min was necessary to separate the aqueous and organic layers between extractions. The combined ethyl acetate layers were evaporated to dryness under partial vacuum at 45 °C, and the extracts were re-dissolved in 2 ml methanol. Fifteen microlitres were then injected onto a Shimadzu 10A HPLC system (Columbia, MD, USA) equipped with an Inertsil ODS-3 reverse-phase C-18 column (5 μM, 250 × 4.6 mm, with a Metaguard guard column, Varian, Palo Alto, CA, USA). The initial conditions were 20% methanol and 80% 0.01 M phosphoric acid (v/v), at a flow rate of 1 ml/min for 2 min, followed by development to 100% methanol in a linear gradient over 53 min. Peaks were monitored using a Hewlet–Packard 1040A photodiode array detector running under the HP Chemstation software version A.02.05. Quantification of ferulic acid was accomplished by comparison of retention time and peak area with ferulic acid standards of known concentration (F-3500, Sigma–Aldrich).

### 2.2. Microwave treatment

Three grams of maize bran insoluble material were weighed into a 100 ml perfluoroalkoxy, Teflon reactor vessel and 27 g of water were added. Vessels were then sealed with special lids that were equipped with a temperature sensor and placed in an Advanced Microwave System (Ethos 1600, Milestone Inc., Monroe, CT), which was controlled and monitored by EasyWave software (version 3.5.4.1, Milestone Inc., Monroe, CT). During treatment, the vessels were stirred magnetically at 320 rpm, and microwave power was continually varied to maintain set temperature parameters. Set temperatures were 160, 180, and 200 °C, and holding times were 2, 5, 10, and 20 min. Come-up times for 160, 180, and 200 °C were 8, 9, and 10 min, respectively.

After microwave treatment, the pH of the slurry was recorded, and then transferred into a tared centrifuge tube followed by centrifugation at 1500*g* for 10 min. The supernatant was retained, and the residue was washed twice with water, once with ethanol, and once with acetone, followed by air drying for 24 h and vacuum (50 °C, 100 kPa) drying for 16 h before recording the final weight. The water washings were combined with the original supernatant, and the total volume was adjusted to 100 ml to make the final autohydrolysate liquor. Total solids in the autohydrolysate liquor were determined by freeze drying (−50 °C, 20 kPa) a 5 ml aliquot, and determining the change in weight.

### 2.3. Arabinoxylo-oligosaccharide and monosaccharide quantification

Released oligosaccharides were quantified by an indirect method. The autohydrolysate from above was reduced, acetylated, and analysed according to Theander et al. (1995), without chemical hydrolysis, to determine the concentrations of free monosaccharides. To determine total neutral sugar content, a second aliquot was subjected to hydrolysis in 0.4 M (final concentration) sulphuric acid at 125 °C for 1 h before reduction, acetylation, and analysis (Theander et al., 1995). The increase in the concentration of each sugar upon hydrolysis compared to without hydrolysis and was used to determine the oligosaccharide concentration. Using this approach, saccharides of degree of polymerisation (DP) two or higher are considered oligosaccharides. This is different than the normal definition of oligosaccharides, which usually refers to a DP of 3–10 (Tungland & Meyer, 2002), but is nevertheless a common practice when referring to autohydrolysate liquors (Caparros, Garrote, Ariza, Diaz, & Lopez, 2007; Carvalheiro, Esteves, Parajo, Pereira, & Girio, 2004).

### 2.4. Molecular weight distribution

Molecular weight distribution was determined by diluting the autohydrolysate liquor to 2.5 mg of carbohydrate/ml (as determined by the sum of anhydrous neutral sugars, Theander et al., 1995), and then injecting 100 μl onto a gel permeation chromatograph (150-CV plus, Waters, Milford, MA, USA) equipped with a Shodex SB-803 HQ column preceded by a Shodex SB-G column (Showa Denko K.K., Tokyo, Japan). The column temperature was maintained at 50 °C, and the mobile phase was 0.02% sodium azide at 1 ml/min. Detection was carried out using a refractive index detector, and data were collected using Millenium$^{32}$ software (Waters, Milford, MA). Molecular weights were estimated using pullulan and glucooligosaccharide standards.

### 2.5. Free and esterified ferulic acid

Free ferulic acid and esterified ferulate in autohydrolyate liquors were measured according to Yuan et al. (2006) with some modifications. One millilitre of liquid fraction, in tandem, was

pipetted into amber vials, and 1 ml of water or 0.4 M sodium hydroxide was added. Vials were shaken for 2 h at room temperature, and then 1.5 ml of 0.4 M phosphoric acid was added followed by 1 ml of either water or sodium hydroxide, whichever had not been previously added. Fifteen microlitres of sample were then injected onto a Shimadzu 10A HPLC system according to the conditions above. The concentration of free ferulic acid was determined by the ferulic acid content of the unsaponified sample, and esterified ferulic acid was determined by the difference between the ferulic acid content of the saponified and unsaponified samples.

### 2.6. Furfural

Furfural was measured by injecting 10 µl of properly diluted autohydrolysate liquor onto a HP Series 1100 HPLC (Agilent, Santa Clara, CA, USA) equipped with a reverse-phase column (Econosil C8, 260 mm × 4.6 mm × 0.5 µm, Alltech, Nicholsville, KY, USA). HPLC conditions were according to Gomis, Alvarez, Naredo, and Alonso (1991) except the mobile phase was water:acetonitrile (96:4, v/v). Furfural was detected at 280 nm and quantified by comparison of retention time and peak area with that of known furfural standard solutions (185914, Sigma–Aldrich).

### 2.7. Data analysis

Data were analysed using SAS software (version 8, SAS Institute, Cary, NC, USA) with a mixed model analysis of variance. *p*-Values for determining significant differences between treatment combinations were determined using Tukey's multiple comparison adjustment.

## 3. Results

### 3.1. Composition of maize bran insoluble material

The maize bran starting material contained 10.91 ± 0.06% starch and 4.86 ± 0.17% protein. The starch was completely removed, and the protein was partially removed by treatment with amylolytic and proteolytic enzymes, autoclaving, and extensive washing to yield maize bran insoluble material (Table 1). Maize bran insoluble material was mainly arabinoxylan, as these two sugars constituted half of the dry weight of the material. The portion of maize bran insoluble material not accounted for in Table 1 is likely composed of uronic, acetic, and other organic acids, lignin, lipid, and ash.

### 3.2. Repeatability of microwave treatment

Traditional microwave heating is notoriously uneven and unpredictable; however, the temperature in the Ethos 1600 Advanced Microwave System was constantly monitored and microwave power was varied to maintain parameters set by the user. Upon investigation of heating curves between replicate runs, the heating cycle was found to be consistent (data not shown). Treatment temperatures of 160, 180, and 200 °C took 8, 9, and 10 min, respectively, to reach set temperatures. During treatment, temperature remained fairly constant, fluctuating ±2.5 °C from the set temperature (data not shown).

**Table 1**
Composition of maize bran insoluble material (g/100 g, dry weight); monosaccharides expressed as anhydrous sugars; nd = none detected; mean ± SD; $n = 2$.

| Constituent | Amount |
|---|---|
| Starch | nd |
| Protein | 2.99 ± 0.01 |
| Arabinose | 15.9 ± 0.9 |
| Xylose | 34.0 ± 2.2 |
| Mannose | 1.48 ± 0.08 |
| Galactose | 7.15 ± 0.73 |
| Glucose | 21.8 ± 2.0 |
| Ferulic acid | 2.95 ± 0.03 |
| Other (by difference) | 13.7 |

### 3.3. Change in pH and release of maize bran insoluble material

The final pH of the autohydrolysate liquors ranged from 3.05 to 4.82 (Table 2). As the temperature increased or time lengthened, the pH decreased gradually.

Solids released into solution upon autohydrolytic treatment of maize bran insoluble material ranged from 5.05 to 58.3 g/100 g maize bran insoluble material (Table 2). At 160 °C, increasing the treatment time resulted in a steady increase in the amount of material solubilised. At 180 °C, treatment times of 2–10 min resulted in increases in the release of maize bran insoluble material to a maximum of about 57 g/100 g. Further treatment for 20 min did not result in greater release of insoluble material. The residue remaining after treatment of maize bran insoluble material at 200 °C was not significantly affected by treatment times from 2 to 10 min, but decreased when treatment time increased to 20 min.

### 3.4. Arabinoxylo-oligosaccharide yield and molecular weight distribution

At 160 °C, a nearly linear increase in AXOS release was evident, ranging from 1.45 to 12.2 g/100 g of maize bran insoluble material (Table 3). A treatment temperature of 180 °C was much more effective at releasing AXOS, reaching a maximum of 25.8 g/100 g of maize bran insoluble material at a treatment time of 10 min. Further treatment at this temperature for a total of 20 min resulted in no significant change in AXOS release. A dramatic decrease in AXOS release from 23.1 to 0.846 g/100 g of maize bran insoluble material was apparent at 200 °C with treatment times of 2–20 min. Optimum treatment combinations for AXOS release were 180 °C for 10 min or 200 °C for 2 min, which resulted in statistically similar levels of released AXOS. These treatment combinations released 25.8 and 23.1 g AXOS/100 g of maize bran insoluble material, or 51.6% and 46.3% of the initial arabinoxylan content of maize bran insoluble material, respectively.

The molecular weight profiles of autohydrolysate liquors from the treatment combinations that resulted in the highest release of AXOS, 180 °C for 10 min and 200 °C for 2 min, are shown in

**Table 2**
Final pH of autohydrolysate liquors and solids released from maize bran insoluble material (g/100 g dry weight); mean ± SD; within column, like lower-case superscripts indicate no significant difference ($p > 0.05$); $n = 2$.

| Time (min) | Final pH | Solids released |
|---|---|---|
| *160 °C* | | |
| 2 | $4.82 \pm 0.01^{a}$ | $5.05 \pm 0.10^{f}$ |
| 5 | $4.53 \pm 0.01^{b}$ | $7.72 \pm 0.32^{ef}$ |
| 10 | $4.13 \pm 0.08^{c}$ | $14.9 \pm 0.13^{de}$ |
| 20 | $3.85 \pm 0.03^{de}$ | $29.5 \pm 1.91^{c}$ |
| *180 °C* | | |
| 2 | $3.98 \pm 0.03^{cd}$ | $21.4 \pm 1.19^{d}$ |
| 5 | $3.71 \pm 0.02^{ef}$ | $40.0 \pm 1.41^{b}$ |
| 10 | $3.52 \pm 0.06^{fg}$ | $57.6 \pm 1.08^{a}$ |
| 20 | $3.36 \pm 0.12^{ghi}$ | $57.3 \pm 5.87^{a}$ |
| *200 °C* | | |
| 2 | $3.44 \pm 0.04^{gh}$ | $56.6 \pm 1.02^{a}$ |
| 5 | $3.29 \pm 0.00^{hi}$ | $58.3 \pm 0.66^{a}$ |
| 10 | $3.18 \pm 0.02^{ij}$ | $50.8 \pm 0.13^{a}$ |
| 20 | $3.05 \pm 0.01^{j}$ | $36.3 \pm 2.14^{bc}$ |

**Table 3**
Arabinoxylo-oligosaccharides (AXOS) and esterified ferulate released from maize bran insoluble material (g/100 g dry weight); mean ± SD; within column, like lower-case superscripts indicate no significant difference ($p > 0.05$); $n = 2$.

| Time (min) | AXOS | Esterified ferulate |
|---|---|---|
| *160 °C* | | |
| 2 | 1.45 ± 0.19$^{f}$ | 0.140 ± 0.010$^{h}$ |
| 5 | 2.04 ± 0.31$^{ef}$ | 0.254 ± 0.025$^{gh}$ |
| 10 | 4.88 ± 0.35$^{de}$ | 0.451 ± 0.001$^{fg}$ |
| 20 | 12.2 ± 0.5$^{c}$ | 0.721 ± 0.060$^{e}$ |
| *180 °C* | | |
| 2 | 7.48 ± 0.53$^{d}$ | 0.555 ± 0.003$^{ef}$ |
| 5 | 16.8 ± 0.9$^{b}$ | 1.44 ± 0.05$^{d}$ |
| 10 | 25.8 ± 0.5$^{a}$ | 1.71 ± 0.00$^{c}$ |
| 20 | 23.0 ± 0.8$^{a}$ | 0.633 ± 0.047$^{ef}$ |
| *200 °C* | | |
| 2 | 23.1 ± 1.5$^{a}$ | 1.85 ± 0.04$^{bc}$ |
| 5 | 17.9 ± 1.2$^{b}$ | 2.13 ± 0.05$^{a}$ |
| 10 | 10.8 ± 0.9$^{c}$ | 1.94 ± 0.14$^{ab}$ |
| 20 | 0.846 ± 0.715$^{f}$ | 0.716 ± 0.054$^{e}$ |

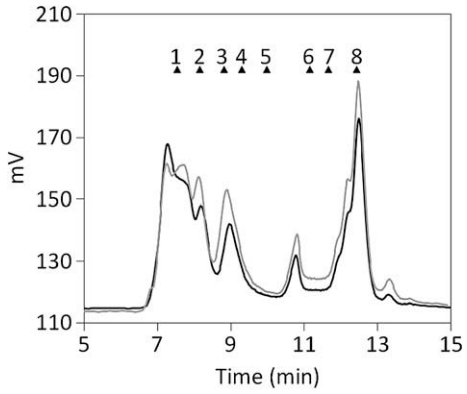


**Fig. 1.** Size-exclusion chromatography elution profiles of autohydrolysate liquors; black line = treatment combination of 180 °C for 10 min; grey line = treatment combination of 200 °C for 2 min; peak retention times of glucan standards of molecular weight $1.00 \times 10^5$, $4.80 \times 10^4$, $2.37 \times 10^4$, $1.12 \times 10^4$, $5.80 \times 10^3$, $1.15 \times 10^3$, $5.04 \times 10^2$ and $1.80 \times 10^2$ are marked 1–8, respectively, with triangles along the top of the figure.

Fig. 1. These treatments resulted in a broad range of molecular weights, but were remarkably similar between the two treatments. The most substantial differences were between the peaks at 7.3 min (peak molecular weight >100,000) and 9.1 min (peak molecular weight 14,660), where 180 °C for 10 min resulted in normalised peak areas of 21.1 ± 1.7% and 14.5 ± 0.7%, respectively, while 200 °C for 2 min resulted in normalised peak areas of 14.0 ± 0.6% and 18.2 ± 0.6%, respectively.

### 3.5. Esterified ferulate yield

The highest levels of esterified ferulate were discovered in the liquors corresponding to treatment times of 5 and 10 min at 200 °C (Table 3). At the optimal treatments for AXOS release, 180 °C for 10 min and 200 °C for 2 min, slightly lower levels of esterified ferulate were evident, corresponding to 58.0% and 62.8% of the initial ferulic acid content, respectively. At treatment times of 20 min, all temperatures resulted in low levels of esterified ferulic acid, which were not significantly different.

### 3.6. Monosaccharide, free ferulic acid, and furfural production

The autohydrolysate liquors contained free monosaccharides (Table 4). The majority of these sugars were arabinose and xylose, but small quantities of galactose and glucose were also detected. At each treatment temperature, monosaccharide contamination increased as a function of treatment time, except when comparing the longest treatment times at 200 °C. In general, 160 °C resulted in the lowest levels of monosaccharides, followed by 180 °C, and then 200 °C. Autohydrolysate liquors corresponding to the optimum treatment combinations for AXOS release, 180 °C for 10 min or 200 °C for 2 min, were contaminated with 8.83 ± 0.24 and 8.85 ± 0.53 g total neutral monosaccharides/100 g maize bran insoluble material. These levels were intermediate among all treatment combinations (range: 0.97–17.01).

In comparison with esterified ferulic acid, free ferulic acid content of autohydrolysate liquors was much lower, with the exception of the treatment combination of 180 °C for 20 min, which resulted in 1.41 g/100 g maize bran insoluble material released as free ferulic acid and only 0.633 g/100 g maize bran insoluble material released as esterified ferulate (Tables 4 and 3, respectively). Treatment times of 2 and 5 min at 160 °C showed no detectable ferulic acid, while 10 and 20 min showed steady increases (Table 4). At 180 °C, a dramatic increase in ferulic acid was evident between 10 and 20 min of treatment. The ferulic acid content of autohydrolysate liquors treated at 200 °C was not substantially affected by treatment time.

All treatment temperatures showed nearly linear increases in furfural production with increasing treatment time (Table 4). The slope of the increase was a function of treatment temperature, with the steepest slope occurring at the highest temperature (200 °C). At 180 °C for 10 min and 200 °C for 2 min, 0.469 and

**Table 4**
Neutral monosaccharides (as free sugars), free ferulic acid, and furfural released from maize bran insoluble material (g/100 g dry weight); nd = none detected; mean ± SD; within column, like lower-case superscripts indicate no significant difference ($p > 0.05$); $n = 2$.

| Time (min) | Arabinose | Xylose | Galactose | Glucose | Ferulic acid | Furfural |
|---|---|---|---|---|---|---|
| *160 °C* | | | | | | |
| 2 | 0.967 ± 0.108$^{g}$ | nd | nd | nd | nd | nd |
| 5 | 1.66 ± 0.02$^{f}$ | 0.519 ± 0.153$^{f}$ | nd | nd | nd | 0.008 ± 0.003$^{d}$ |
| 10 | 2.62 ± 0.02$^{e}$ | 1.01 ± 0.01$^{ef}$ | nd | nd | 0.030 ± 0.002$^{e}$ | 0.039 ± 0.001$^{d}$ |
| 20 | 3.85 ± 0.10$^{d}$ | 1.58 ± 0.01$^{de}$ | 0.600 ± 0.005$^{d}$ | nd | 0.412 ± 0.009$^{b}$ | 0.182 ± 0.049$^{d}$ |
| *180 °C* | | | | | | |
| 2 | 2.65 ± 0.13$^{e}$ | 1.07 ± 0.05$^{ef}$ | 0.788 ± 0.064$^{cd}$ | 0.909 ± 0.198$^{a}$ | 0.107 ± 0.007$^{d}$ | 0.064 ± 0.001$^{d}$ |
| 5 | 3.80 ± 0.06$^{d}$ | 1.72 ± 0.10$^{d}$ | 0.923 ± 0.168$^{cd}$ | 0.732 ± 0.152$^{a}$ | 0.155 ± 0.017$^{d}$ | 0.208 ± 0.030$^{d}$ |
| 10 | 5.09 ± 0.20$^{b}$ | 2.80 ± 0.03$^{c}$ | 0.938 ± 0.011$^{cd}$ | nd | 0.311 ± 0.007$^{c}$ | 0.469 ± 0.040$^{d}$ |
| 20 | 5.86 ± 0.00$^{a}$ | 4.79 ± 0.24$^{b}$ | 1.82 ± 0.04$^{b}$ | nd | 1.41 ± 0.05$^{a}$ | 1.21 ± 0.05$^{c}$ |
| *200 °C* | | | | | | |
| 2 | 4.65 ± 0.19$^{c}$ | 3.12 ± 0.22$^{c}$ | 1.08 ± 0.12$^{c}$ | nd | 0.125 ± 0.003$^{d}$ | 0.492 ± 0.144$^{d}$ |
| 5 | 5.18 ± 0.11$^{b}$ | 5.31 ± 0.02$^{b}$ | 1.94 ± 0.14$^{b}$ | nd | 0.123 ± 0.008$^{d}$ | 1.29 ± 0.15$^{c}$ |
| 10 | 5.07 ± 0.13$^{bc}$ | 8.87 ± 0.00$^{a}$ | 2.79 ± 0.12$^{a}$ | 0.272 ± 0.385$^{a}$ | 0.281 ± 0.010$^{c}$ | 2.38 ± 0.32$^{b}$ |
| 20 | 3.52 ± 0.02$^{d}$ | 8.98 ± 0.28$^{a}$ | 2.49 ± 0.05$^{a}$ | 0.844 ± 0.089$^{a}$ | 0.172 ± 0.017$^{d}$ | 3.99 ± 0.31$^{a}$ |

0.492 g of furfural/100 g maize bran insoluble material were produced, respectively. These levels were among the lowest of all treatment combinations.

## 4. Discussion

Maize bran is a byproduct of the maize dry-milling industry. The main component of maize bran is a highly substituted heteroxylan with a molecular weight of 270,000–370,000 (Chanliaud, Roger, Saulnier, & Thibault, 1996). This heteroxylan consists of a highly substituted β(1 → 4)-linked D-xylopyranosyl backbone, with single arabinosyl units as the main side groups, and glucuronic acid or oligomeric side chains of arabinose, xylose, and galactose also occurring to a lesser degree (Allerdings, Ralph, Steinhart, & Bunzel, 2006; Kennedy, Methacanon, & Lloyd, 1999; Saulnier & Thibault, 1999). Maize bran also contains a relatively high amount of ferulic acid, which is attached to the heteroxylan via an ester linkage to arabinosyl units (Allerdings et al., 2006; Kennedy et al., 1999). Esterified ferulic acid forms oxidative cross-linkages, which, along with other intramolecular interactions, yields an insoluble polymer (Saulnier & Thibault, 1999).

The decrease in pH and release of insoluble material into solution (Table 2) support the occurrence of autohydrolysis of maize bran insoluble material upon microwave treatment (Benko et al., 2007). The major products discovered in maize bran autohydrolysate liquors were arabinose and xylose, which occurred as oligomers, along with esterified ferulic acid. Because esterified ferulate in maize bran is attached to arabinosyl units (Allerdings et al., 2006; Kennedy et al., 1999), the major products obtained in this study were referred to as feruloylated AXOS. The aim of this study was to determine conditions which yielded the highest levels of soluble, feruloylated AXOS with minimal release of undesirable products such as monosaccharides, free ferulic acid, and furfural.

The Advanced Microwave System employed herein was found to produce reliable, reproducible reaction conditions. Previous research in our lab comparing extraction of phenolic compounds by heating with microwave irradiation have shown little differences when compared to the same conditions using a traditional water bath as the heat source (Inglett, Rose, Stevenson, Chen, & Biswas, in press). Therefore, it is unlikely that the results obtained in this experiment are unique to microwave heating *per se*; they might also be produced by heating using traditional means, provided the treatment conditions, including the come-up times, are identical.

The optimum treatment times for AXOS release were 180 °C for 10 min or 200 °C for 2 min. Under these conditions, 51.6% and 46.3% of the initial arabinoxylan content in maize bran insoluble material was released into solution. This is slightly lower, but comparable with previous reports using other agricultural biomass, which have yielded releases of up to 77% of the initial xylan content (Carvalheiro et al., 2004; Garrote, Dominguez, & Parajo, 2004). Further treatment at 180 °C did not yield an increase in AXOS recovery, and at 200 °C, further treatment resulted in significant decreases in AXOS yield. The decrease in AXOS yield at 200 °C was most likely due to hydrolysis of the oligosaccharides and decomposition of the sugar moieties. Indeed, there was a marked increase in monosaccharides from 2 to 10 min of treatment time and furfural (from monosaccharide degradation) increased dramatically among all treatment times at this temperature (Table 4). Interestingly, Benko et al. (2007) used microwave-assisted autohydrolysis to release the arabinoxylan fraction from corn fibre. Corn fibre is the term used for the bran fraction obtained from corn wet-milling, as opposed to the maize bran obtained from dry-milling used in this study. Treatment temperatures in the study by Benko et al. (2007) ranged from 160 to 210 °C, with a maximum yield of 30% at 210 °C for 5 min. Our results suggest that the maize bran from dry-milling may be a better candidate for AXOS release than corn fibre, due to the higher yields.

Autohydrolysate liquors also contained high levels of esterified ferulic acid (Table 3). Under the optimal conditions for AXOS release from maize bran 58.0–62.8% of the initial ferulic acid content occurred as esterified ferulate. This corresponds to 6.62 and 8.00 g of esterified ferulate/100 g of AXOS, although some of the ferulate may be esterified to monomeric arabinose. Higher levels of esterified ferulate were obtained by longer treatment times at 200 °C, but since AXOS yield decreased and monosaccharide yield was significantly higher, it was assumed that a large percentage of the ferulate in these samples was esterified to monomeric arabinose, and thus of limited interest. Furthermore, these treatments also produced high amounts of undesirable furfural (Table 4).

The soluble arabinoxylan fragments obtained in this study potentially possess a number of health benefits. Gel permeation chromatographic profiles of released arabinoxylans showed a broad range of molecular weights (Fig. 1). Short chain AXOS, are classified as potential prebiotics pending further research (Rastall & Maitin, 2002). Prebiotics include indigestible oligosaccharides that selectively stimulate the growth of beneficial bacteria (e.g., bifidobacteria) in the colon, thus improving colonic health. Longer chain AXOS fractions are likely to ferment more slowly for distal colonic fermentation (Moura et al., 2008); thus supplying carbohydrate to a region of the bowel that is particularly prone to disease and chronically starved for fermentative substrate (Rose et al., 2007). Additionally, feruloylated AXOS obtained by autohydrolysis of maize bran, are likely to exhibit high antioxidant activity, as has been demonstrated previously with autohydrolytic products from other agricultural biomass (Moure, Dominguez, & Parajo, 2005). Notably, of the common grains, maize bran contains at least 10 times more ferulic acid (Vitaglione et al., 2008). Thus, with maize bran, the potential exists for harvesting feruloylated AXOS with much higher antioxidant activity than those obtained when other cereal brans are used as starting materials.

The realisation of these health benefits depends upon purification of autohydrolysate liquors such that they may be consumed as food ingredients. Undesirable contaminants include monosaccharides, free ferulic acid, furfural, and other components. The most effective means of removing free ferulic acid and furfural and other coloured compounds from autohydrolysate liquors has been ethyl acetate extraction followed by ionic exchange, which may result in a product containing 70–90% carbohydrate (mono- and oligosaccharides; Vegas, Alonso, Dominguez, & Parajo, 2004; Vegas et al., 2005). The monosaccharides may be removed by chromatography (Jacobs, Palm, Zacchi, & Dahlman, 2003), ultrafiltration (Akpinar, Ak, Kavas, Bakir, & Yilmaz, 2007), or solvent precipitation (Vegas et al., 2004, 2005), although some loss of the shortest oligomers would be expected.

In conclusion, microwave irradiation was an effective and reliable means of producing soluble feruloylated AXOS from maize bran. Maximum yields of about 50% of the original arabinoxylan content were obtained with processing conditions of 180 °C for 10 min or 200 °C for 2 min. These feruloylated AXOS contained about 60% of the initial ferulic acid content as esterified ferulate. Upon purification, feruloylated AXOS may possess a number of health benefits, including prebiotic and other fermentative benefits, and antioxidant activity. These will be the subjects of future studies.

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