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Isolation and identification of feruloylated arabinoxylan mono- and oligosaccharides from undigested and digested maize and wheat

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Abstract

Feruloylated arabinoxylan mono- and oligosaccharides (F-AXOS) are a subject of interest because of their prebiotic and antioxidant properties. We aimed at isolating and identifying F-AXOS from maize, wheat, wheat bran and wheat aleurone using HPLC and LC-MS/MS. Prior to extraction of F-AXOS, samples were subjected to either simulated gastric fluid with enzymes (gastric) or without enzymes (pH) or water (aqueous) at 37 °C. F-AXOS present in all samples were identified as 5-O-feruloyl-α-L- arabinofuranose and possibly 5-O-feruloyl-α-L-arabinofuranosyl-(1 → 3)-O-β-D-xylopyranose. Their mean content, measured as esterified ferulic acid (FA), was 2.5 times higher in maize (10.33 ± 2.40 μg/g) compared to wheat. Digestion under gastric or pH conditions resulted in a two-fold increase in F-AXOS in all samples. The level of F-AXOS produced during gastric or pH condition was positively correlated to the insoluble bound FA content of the sample (R^2 = 0.98). 5-O-Feruloyl-α-L- arabinofuranose was the only identifiable F-AXOS released during gastric digestion. Our results suggest feruloyl arabinose is the most abundant form of F-AXOS in maize and wheat.

Keyword: Food science

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1. Introduction

Arabinoxylan oligosaccharides (AXOS) are a subject of interest due to their perceived health benefits (Ou and Sun, 2014). AXOS have been shown to possess prebiotic (Damen et al., 2012; Yang et al., 2014; Yuan et al., 2005a) and antioxidant properties (Katapodis et al., 2003; Lin et al., 2014; Ohta et al., 1997; Yuan et al., 2005b) both *in vivo* and *in vitro* studies. Consumption of 2.4 g AXOS per day for 3 weeks significantly increased bifidobacteria and short chain fatty acid concentrations in healthy individuals (Damen et al., 2012). Moreover, dietary supplementation with 1% (w/w) feruloylated arabinoxylan oligosaccharides (F-AXOS) increased the antioxidant potential of plasma in rats (Wang et al., 2009a). The F-AXOS antioxidant potential is dependent on ferulic acid content, degree of substitution and polymerization (Malunga and Beta, 2015a; Snelders et al., 2014). Thus, F-AXOS obtained from ragi, rice, maize, and wheat brans had significantly different antioxidant potential (Veenashri and Muralikrishna, 2011).

F-AXOS are produced through enzymatic (endoxylanase) or mild acid hydrolysis of arabinoxylan polysaccharides. In general, arabinoxylans contain a linear (1–4)-β-D-xylopyranose chain that is substituted with L-arabinofuranose at O-2 and/or O-3 position (Lequart et al., 1999). The xylan chain can also be substituted with α-(1,2)-glucuronic acid and/or α-(1,2)-4-O-methylglucuronic acid branches (Ma et al., 2012). The arabinoxylans may be feruloylated with ferulic acid at the O-5 position of the arabinose units (Bunzel et al., 2005). The length and sugar constituents of feruloylated side chain varies, however, the most common ones are mono- and disaccharides of arabinose and xylose (Ishii, 1997; Smith and Hartley, 1983; Wende and Fry, 1997).

A method to classify ferulic acid in cereal grain was recently proposed by Vaidyanathan and Bunzel (2012). Among four of those classes was ferulic acid esterified to mono-/oligosaccharides which is identical to conjugated or soluble bound ferulic acid. Soluble bound or conjugated ferulic acid has been studied for over three decades, however, the nature or type of mono-/oligosaccharide to which the ferulic acid is bound is not well documented. Feruloyl oligosaccharides reported in literature are usually a product of mild acid (Ohta et al., 1997; Wende and Fry, 1997) or enzyme treatment (Ahluwalia and Fry, 1986).

Arabinoxylans are generally considered as dietary fibers which are expected to transverse the digestive system unaltered chemically unless fermented by the resident microbes in the colon. Arabinose linkages of the arabinoxylan are susceptible to low gastric pH during digestion (Zhang et al., 2003). Thus exposure of cereal grains to low gastric pH may partially release feruloyl arabinose since ferulic acid is bound to O-5 position of the arabinose units. Feruloyl arabinose may be bound to glycoprotein (Obel et al., 2003). It is likely that low gastric pH and/or

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protein hydrolysis will increase F-AXOS content during gastric digestion. However, data on release of F-AXOS during gastric digestion is limited.

Wheat and maize are among the highly consumed cereal grains in the world. Maize arabinoxylans have higher ferulic acid, di-ferulic acid, tri-ferulic acid (Bunzel, 2010; Jilek and Bunzel, 2013) and degree of substitution (Knudsen, 1997) compared to wheat arabinoxylans. The percentage of unsubstituted, mono-substituted, and di-substituted xylose in maize arabinoxylans may be 24, 52 and 24, respectively (Rumpagaporn et al., 2015). In contrast, water extractable arabinoxylans from wheat contains about 60–65% unsubstituted xylose (Saulnier et al., 2007). Hence maize is considered to have a more complex arabinoxylan structure compared to wheat (Pedersen et al., 2015). Moreover among grain botanical fractions, arabinoxylans from wheat bran have higher degree of substitution and di-ferulic acid compared to that of aleurone (Antoine et al., 2003; Saulnier et al., 2007). The complexity of the structure affects susceptibility of arabinoxylans to microbial fermentation (Pedersen et al., 2015) or hydrolysis by endoxylanase (Biely et al., 1997).

Therefore, this work aimed at isolating and identifying feruloylated arabinoxylan mono-/oligosaccharide (F-AXOS) from maize, wheat and wheat aleurone and bran. The effect of gastric digestion on F-AXOS content was also investigated.

2. Materials and methods

2.1. Materials

Pepsin from porcine gastric mucosa (P7000-25G), α-amylase from porcine pancreas (A3176-1MU), phenolic acid standards and XAD2 resin were purchased from Sigma-Aldrich (Milwaukee, Wisconsin, USA). Sodium hydroxide, sodium chloride, sodium sulphate, all acids and organic solvents were obtained from Fischer Scientific (Whitby, Ontario, Canada). All chemicals and solvents used were of analytical or HPLC grade.

2.2. Samples

Four whole grain wheat, five whole grain maize, one wheat aleurone and one wheat bran samples were used. One yellow Argentinian flint maize (DASCA) was grown in USA copper belt while orange (MW5021) and white maize samples were grown in a mountainous region (Dedza District) in Malawi. Another sample set of orange (MW5021) and white maize samples were also grown in a plain region (Ekwendeni, Mzimba District) in Malawi. The Malawi maize samples were all flint type. The four wheat samples (purple wheat, Caledonia, Ambassador, and MSUD8006) grown in Michigan, U.S.A. were all soft wheat varieties. A commercial wheat aleurone (Grainwise^{TM} wheat aleurone) was a gift from Cargill

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Limited from Horizon Milling (Wichita, Kansas, USA). Red winter wheat bran was purchased locally from Bulk Barn (Winnipeg, Manitoba, Canada). Samples were ground using a laboratory coffee grinder to pass through sieve number 35 (0.5 mm). Samples (Table 1) were analyzed for their protein, ash, and moisture content using AOAC methods. Samples were kept in a desiccator at −20 °C for isolation of feruloylated arabinoxylan mono-/oligosaccharides. Five subsamples from each grain or bran or aleurone were collected and used during the outlined experiments.

2.3. Isolation of feruloylated arabinoxylan mono-/oligosaccharides from untreated whole grains (maize and wheat), wheat aleurone and wheat bran

The method of Vaidyanathan and Bunzel (2012) was used with modification. Flour samples (20 g) were mixed with 20:80 water: ethanol (250 mL) and boiled under reflux for 30 minutes to deactivate endogenous enzymes. The cooled samples were centrifuged (10,000 g, 4 °C and 20 mins) and supernatant collected. The residues were washed twice with 200 mL 80% ethanol and centrifuged to collect the supernatants. Ethanol (80%) is essential to avoid solubilisation of arabinoxylan polysaccharide. The residues were air dried for further experiments and labelled as deactivated ground samples. The pooled supernatants were evaporated to dryness under vacuum using a rotary evaporator (Buchi rotavapor R − 205 (Laboratoriums Technik AG, Flawil, Switzerland)) at 40 °C. The dried supernatants were

Table 1. Proximate composition (g/100 g) of maize, wheat, wheat aleurone and wheat bran samples prior to aqueous, pH and gastric treatment for extraction of feruloylated oligosaccharides.

	Moisture	Ash	Protein	Lipid
Wheat (Caledonia)	12.1 ± 0.3	1.6 ± 0.0	6.0 ± 0.7	2.0 ± 0.1
Wheat (MSDU)	12.3 ± 0.2	1.5 ± 0.0	7.5 ± 0.4	2.3 ± 0.1
Wheat (Ambassador)	12.1 ± 0.1	1.6 ± 0.0	6.9 ± 0.1	1.9 ± 0.2
Wheat (purple)	12.9 ± 0.5	1.7 ± 0.0	6.3 ± 0.1	2.1 ± 0.2
Aleurone (wheat)	8.6 ± 0.2	7.6 ± 0.0	12.4 ± 0.1	*
Red bran (wheat)	5.8 ± 0.0	5.3 ± 0.0	11.1 ± 0.2	*
Maize − white (Ekwendeni)	13.7 ± 0.8	1.4 ± 0.1	8.8 ± 0.4	5.8 ± 0.5
Maize − orange (Ekwendeni)	14.0 ± 1.4	1.3 ± 0.0	9.0 ± 0.4	5.3 ± 0.1
Maize − white (Dedza)	13.5 ± 0.8	1.4 ± 0.1	9.7 ± 0.7	5.6 ± 0.4
Maize − orange (Dedza)	13.3 ± 0.9	1.4 ± 0.2	8.9 ± 0.6	5.4 ± 0.7
Maize (DASCA)	11.5 ± 0.3	1.2 ± 0.0	6.8 ± 1.1	6.1 ± 0.2

Values presented as mean ± standard deviation (n = 5). * means that it was not determined.

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reconstituted with 20 ml ultrapure water and filtered through Whatman filter paper No. 41 to remove the gummy residues. The filtrates were kept at −20 °C for further purification and analysis of F-AXOS.

2.4. Extraction of feruloylated arabinoxylan mono-/oligosaccharides following gastric digestion and pH treatment of deactivated ground whole grains, wheat aleurone and wheat bran

Simulated gastric digestion was done according to Chandrasekara and Shahidi (2012) with modifications. Briefly, deactivated ground samples (5 g) were mixed with 45 mL ultrapure water and 30 mL 0.15 M sodium chloride solution. The mixture was incubated at 37 °C for 10 minutes under continuous shaking in a water bath. Three milliliters of porcine amylase in 5 mg/ mL sodium phosphate buffer (20 mM, pH 6.9, 1 mM calcium chloride) was then added. After 5 minutes incubation, 9.5 mL hydrochloric acid (0.15 M) was added and pH adjusted to 2. Porcine pepsin (3 mL, 40 mg/ mL 20 mM HCl) was also added prior to incubation at 37 °C for 1 hour. The samples were then boiled at 95 °C for 5 minutes to deactivate the enzymes. Incubation of samples at 95 °C for 5 minutes was verified in our laboratory not to result in release of F-AXOS. The samples were freeze dried and labeled simulated gastric digesta (SG). Control samples (aqueous treated, AG) were treated similarly to SG except that only ultrapure water was being added at every step. Another group of samples (pH treated, pH) were treated likewise except that only buffers were being added without enzymes.

Feruloylated arabinoxylan mono-/oligosaccharides (F-AXOS) were extracted thrice from each sample using 80% ethanol. Briefly, ~2.0 g freeze dried samples (AG, SG and pH treated) were mixed with 25 ml 80% ethanol and shaken continuously on a wrist shaker (250 rpm) for an hour at room temperature. The suspension was centrifuged at 10,000 g for 10 minutes at room temperature. The supernatants were pooled together and passed through Whatman filter paper (No. 41) to a round bottom evaporating flask. A Buchi rotavapor R − 205 (Laboratoriums Technik AG, Flawil, Switzerland) was used to remove ethanol at 40 °C and 200 rpm. The samples were reconstituted in 20 ml ultrapure water and kept at −20 °C for further purification and analysis of F-AXOS.

2.5. Determination of free, mono/oligosaccharide (soluble) or insoluble bound ferulic acid content

A modification of the method reported by Vaidyanathan and Bunzel (2012) was used to estimate esterified ferulic acid content in F-AXOS extracts. Five milliliter of the reconstituted samples was transferred to two 50 ml conical flasks for extraction of free ferulic acid and bound ferulic acid. The reconstituted samples in

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the first flask were acidified with 6 M hydrochloric acid to pH 1.5 and free phenolic acids were extracted thrice using 25 mL ethyl acetate. To the second flask, 4 M sodium hydroxide (5 mL) was added and incubated under nitrogen in the dark for 2 hours. Phenolic acids were extracted thrice using a total of 75 mL ethyl acetate after adjusting pH to 1.5. Ethyl acetate was evaporated under vacuum and samples reconstituted with 2 ml water: methanol (50:50). Phenolic acids were quantified using HPLC method as described by Malunga and Beta, 2015b. The difference in ferulic acid concentration between free phenolic acid (flask 1) and total phenolic acid (flask 2) was considered the amount of ferulic acid esterified to mono-/oligosaccharides (esterified FA or soluble bound FA). Insoluble bound ferulic acid was determined by first saponifying 200 mg of deactivated ground samples with 5 mL 2 M sodium hydroxide under nitrogen at 4 °C for overnight. The mixture was acidified by 6 M hydrochloric acid to pH 1.5 and centrifuged at 10,000 g for 10 minutes at 4 °C. The residue was washed twice with 5 mL ultrapure water and the supernatants pulled together. Ferulic acid was extracted 3 times using 50 mL ethyl acetate. Ethyl acetate was evaporated under vacuum and samples reconstituted in 2 ml water: methanol (50:50) for analysis of ferulic acid. The insoluble bound ferulic acid include both ferulic acid esterified to water soluble and insoluble arabinoxylan.

2.6. Identification of feruloylated arabinoxylan oligosaccharides (F-AXOS) by LC-MS

The reconstituted sample from Sections 2.3 or 2.4 (10 mL) was further purified on 80 mL XAD2 resin column as described earlier (Saulnier et al., 1995). XAD2 column was preconditioned with 1 column volume ethanol (80 mL) followed by 25 mL ultrapure water. After sample application, it was eluted with 1 column volume water to remove sugars, 1 column volume methanol: water (50:50) and 1 column volume methanol. Both free and bound ferulic acids are adsorbed to XAD2 resin (Saulnier et al., 1995). Elution with 50% methanol liberates more F-AXOS than free ferulic acid. Further purification with LH20 or preparative reverse phase HPLC is usually deployed to obtain pure F-AXOS but this was not necessary as identification was carried out on LC-MS. The 50% methanol eluent was collected and evaporated to dryness in a rotary evaporator (40 °C). F-AXOS extracts were reconstituted with 3 mL methanol: water (50:50) and kept at −20 °C. F-AXOS extracts were separated with reverse phase column (5.0-μm Phenomenex C18 column (150 × 4.6 mm)) using high performance liquid chromatograph (Waters Alliance 2695 instrument (Waters, Milford, MA)) system as described by Malunga and Beta (2015b). The F-AXOS were identified by introducing HPLC eluent into the mass spectrometer (Q-TOF MS) (Micromass, Waters Corp., Milford, MA) using electrospray ionization (ESI) in negative mode. The operating conditions were set as follows: desolvation temperature 300 °C; source temperature, 120 °C

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spray voltage; capillary voltage, 1.2 kV; sample cone voltage, 45 V; extraction cone voltage, 4 V; cone gas flow, 50 L/hr; and desolvation gas flow 900 L/hr. The collision energy for MS/MS was 20 V.

2.7. Statistical analysis

All analyses were performed in quintuplicate and all statistics were calculated using one way analysis of variance (ANOVA) on a JMP 10 statistical software (SAS Institute Inc., Cary, NC). Sample means were compared using Tukey HSD method and significant differences determined at $p \leq 0.05$.

3. Results and discussion

3.1. Estimation of free ferulic acid, soluble or insoluble bound ferulic acid content

Ferulic acid is the most abundant in cereal grains and found as free or soluble or insoluble bound. We analyzed free, soluble or insoluble bound ferulic acid content in maize and wheat grain and the results are presented in Table 2. Our results

Table 2. Concentration of free ferulic acid, ferulic acid bound to mono-/oligosaccharide (soluble) and insoluble bound ferulic acid (μg) per g flour of maize, wheat, wheat aleurone and wheat bran samples.

	Free	Mono-/Oligosaccharide	Insoluble bound
White maize (Ekwendeni)	2.93 ± 0.67^{cd}	9.44 ± 0.59^{b}	1273.45 ± 157.22^{c}
White maize (Dedza)	1.65 ± 0.35^{d}	13.54 ± 1.91^{a}	1582.51 ± 275.32^{c}
Orange maize (Ekwendeni)	3.47 ± 0.50^{c}	10.89 ± 1.31^{ab}	1622.36 ± 272.16^{c}
Orange maize (Dedza)	1.61 ± 0.23^{d}	10.82 ± 1.90^{ab}	1390.45 ± 212.24^{c}
Yellow maize (DASCA)	2.15 ± 0.41^{d}	6.95 ± 0.50^{bc}	1759.42 ± 300.15^{c}
Mean (maize)	2.36 ± 0.81	10.33 ± 2.40	1525.64 ± 193.08
Ambassador wheat	3.06 ± 0.23^{c}	3.91 ± 0.18^{d}	780.09 ± 80.35^{d}
Caledonia wheat	3.13 ± 0.18^{c}	3.38 ± 0.29^{d}	818.18 ± 150.76^{d}
MSDU wheat	3.92 ± 0.27^{c}	3.45 ± 0.21^{d}	773.77 ± 101.52^{d}
Purple wheat	3.81 ± 0.27^{c}	3.05 ± 0.23^{d}	584.82 ± 90.34^{d}
Mean (wheat)	3.48 ± 0.39	3.37 ± 0.35	739.21 ± 104.79
Aleurone (wheat)	6.42 ± 1.23^{ab}	13.86 ± 1.23^{a}	4064.97 ± 123.35^{a}
Red bran (wheat)	9.19 ± 0.93^{a}	15.87 ± 1.79^{a}	2377.03 ± 233.67^{b}

Values presented as mean ± standard deviation (n = 5). Data in the same column with the same superscript are not significantly different at $p \leq 0.05$.

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suggest that the content of free, soluble or insoluble bound ferulic acid did not vary significantly within flint maize nor within soft wheat samples. The mean total ferulic acid content in maize was twice that found in wheat samples. The majority of the ferulic acid (99%) was insoluble bound in both maize and wheat. Ferulic acid is mostly bound to arabinoxylans of the cell walls. This was reflected in the high content of total ferulic acid in the wheat aleurone (~4000 μg/g) and wheat bran (~2400 μg/g) samples. Our results are within the reported ranges of ferulic acid content in wheat (Li et al., 2008) and maize (Lopez-Martinez et al., 2009).

Wheat had higher free ferulic acid (1.5 times) but less esterified or soluble-bound FA (3 times) compared to maize (Table 2). Also the proportion of free ferulic acid to soluble-bound FA in wheat (1:1) is different from that of maize (1:4). Free ferulic acid has low solubility (0.6 mg/mL) under physiological conditions (Mota et al., 2008). However, the solubility of ferulic acid can increase to 10 mg/mL when it is esterified to arabinose (Fang et al., 2013). Thus having higher concentration of feruloyl mono-/oligosaccharide may be advantageous on bio-accessibility of ferulic acid. Dedza grown white maize (~13 μg/g) had highest concentration of esterified FA and yellow maize (DASCA) (~7 μg/ g) had the lowest among the maize cultivars. The mean concentration of esterified FA was not significantly different for maize (white or orange). Among wheats, the difference in mean concentration of esterified FA in wheat cultivars was not significant ($p < 0.05$). The outer layers (wheat bran or wheat aleurone) had higher content of esterified FA compared to both whole grain maize and wheat. It is well documented that wheat genotypes have wide range of ferulic acid concentration (Li et al., 2008).

3.2. Identification of the feruloylated mono- and oligosaccharides

This work aimed at isolating and identifying F-AXOS from maize, wheat and wheat aleurone and bran. XAD2 resins was used to isolate F-AXOS. The LC-MS was used to identify F-AXOS present in our extract. The presence of ferulic acid esterified to sugars enables detection at 325 nm using photodiode array detector. Peaks having a maximum absorbance of 320–325 were considered as belonging to F-AXOS. Two peaks (Fig. 1a) with retention times of 21.05 and 28.52 minutes met this criterion and were labeled F1 and F2, respectively. However, we observed that peak F1 was not present in UV spectra for F-AXOS extracts from digestion or pH treated samples (Fig. 1b). F2 was the most prevailing compound in our extract judging by its highest peak area (Fig. 1a).

The negative ESI mass spectra for F1 and F2 showed a compound with m/z 457 (Fig. 2a) and m/z 325 with some traces of m/z 457 (Fig. 2b), respectively. Thus compound F1 had a molecular weight of 458 and F2 of 326 Da. The generally

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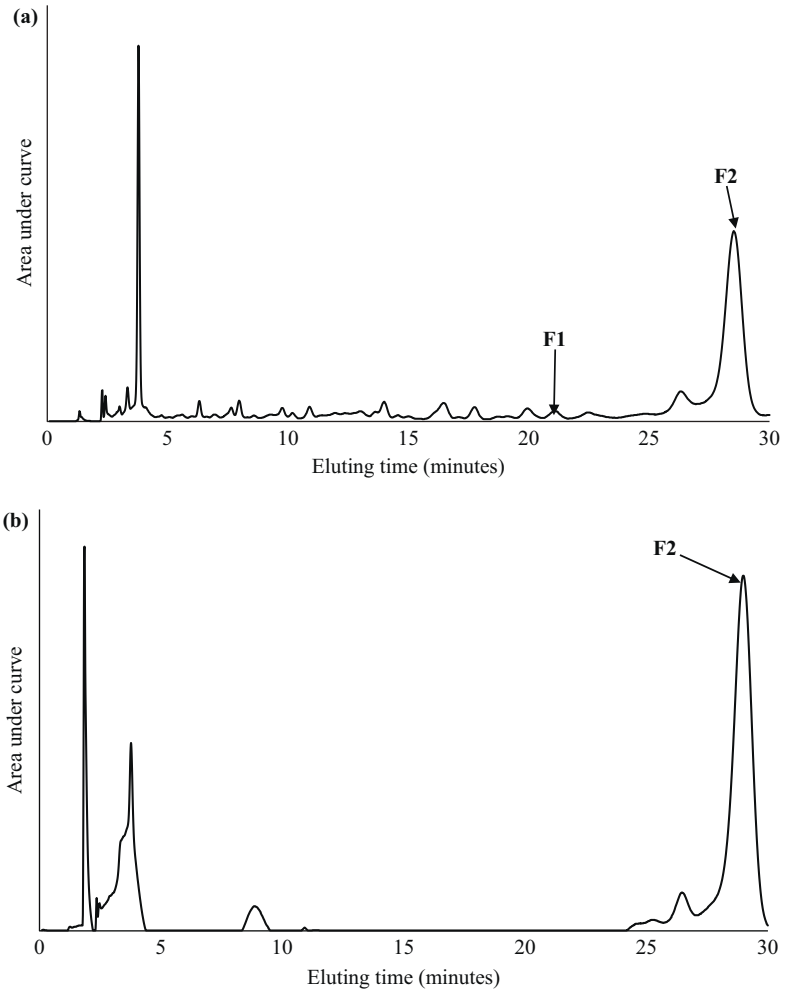

Fig. 1. Typical HPLC chromatograph of feruloyl arabinoxylan mono- and oligosaccharide extracts from wheat and maize samples. (a) Untreated sample; and (b) gastric digested sample. Peaks (F1 and F2) with maximum UV absorption spectra of 320–325 were considered feruloylated arabinoxylan mono-/oligosaccharide.

accepted structure of arabinoxylan is that ferulic acid is always attached to the O-5 position of the arabinose units (Izydorczyk and Biliaderis, 1995). Our results suggest that the predominant compound in our extract was a feruloyl arabinose (F2). Furthermore, the mass spectra for F1 suggest that our extract contained a disaccharide (xylose or arabinose) esterified to ferulic acid. Feruloyl arabinose (5-O-feruloyl-L-Ara*f*) and feruloyl arabinofuranosyl xylose have been isolated from maize or wheat bran through acid or enzymatic hydrolysis (Lin et al., 2014; Saulnier et al., 1995; Smith and Hartley, 1983).

Compound F1 was fragmented under negative ion mode to confirm the structural identity and the resulting ms/ms spectrum is shown in Fig. 3a. Fragment ions m/z 397 and 367 indicated cross link cleavage at $^{0,2}A_2$ and $^{2,4}A_2$ of the xylopyranose

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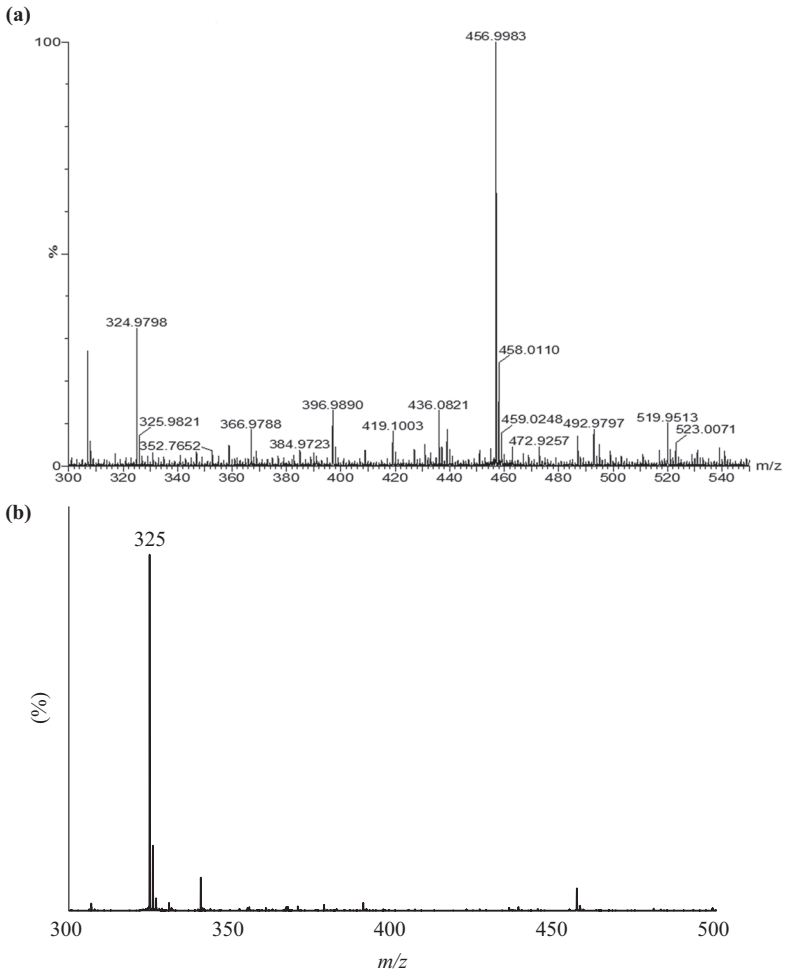

Fig. 2. Negative ion mass spectra of compound eluting at 21.05 minutes labeled F1, (a); and compound eluting at 28.52, F2, (b).

resulting in loss of $C_2H_4O_2$ and $C_3H_6O_3$, respectively as illustrated in Fig. 3b. Secondly, fragment ion m/z 325 suggests glycosidic cleavage occurred at $C_{1\alpha}$ and consequently losing a dehydrated xylopyranose ion (m/z 132). Cross ring and glycosidic cleavage is a common phenomenon during negative ion fragmentation of sugars (Wang et al., 2009b). Occurrence of cross link cleavage resulting in loss of both $C_2H_4O_2$ and $C_3H_6O_3$ may suggest (1 → 3) arabinose − xylose linkage. Finally, ferulic acid fragment ion (m/z 193) was observed and typified by its daughter ions m/z 175, 159 and 134 (Sun et al., 2007). Thus we concluded that compound F1 was a feruloyl arabinofuranosyl xylose. The fragmentation pattern and literature, F1 is likely to be a 5-O-feruloyl-α-L-arabinofuranosyl-(1 → 3)-O-β-D-xylopyranose.

We also fragmented the parent ion for compound F2 (m/z 325) in order to confirm our proposed identity (5-O-feruloyl-L-Ara*f*). Fig. 4 shows the MS/MS spectra of

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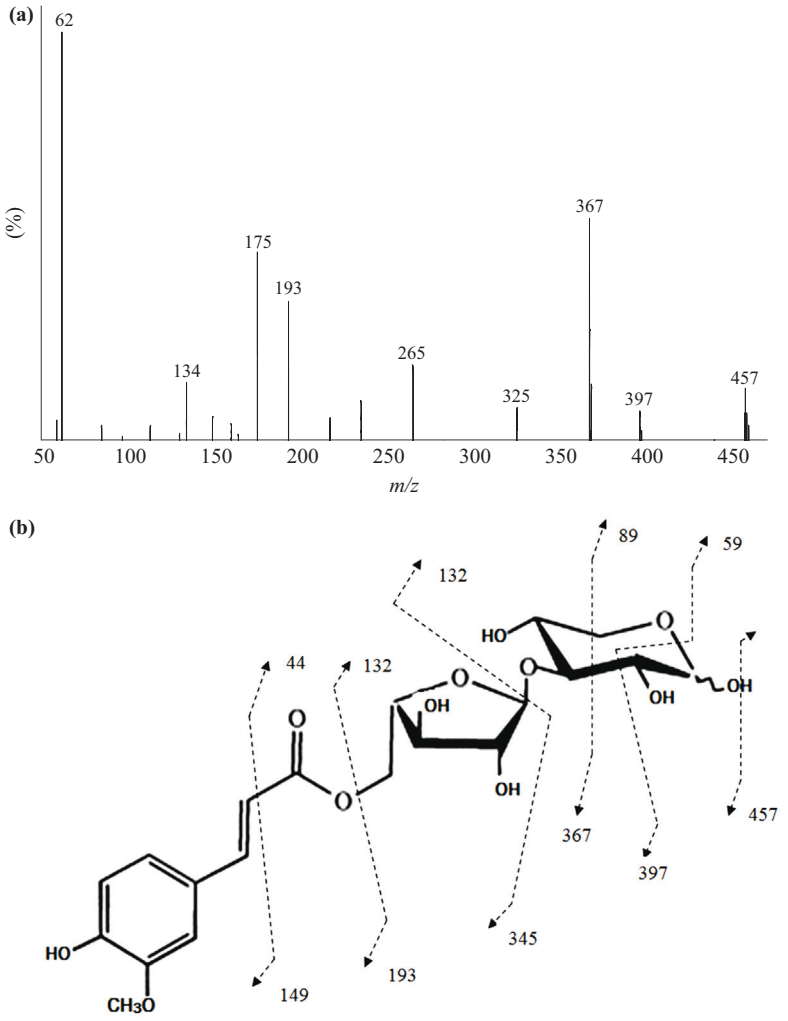

Fig. 3. (a) MS/MS spectrum of compound eluting at 21.05 minutes labeled F1 (m/z 457); (b) an illustration of fragmentation pattern of compound F1.

the daughter ions for compound F2. Cross ring cleavage of the sugar moiety was observed giving fragment ions of m/z 265 and m/z 59 (Fig. 4b). Our data also suggested further dissociation of daughter ion (m/z 265) at sugar-ferulic acid ester linkage resulting in ferulic acid (m/z 193) and a remaining cross ring fragment of arabinose (m/z 71). It appears that ferulic acid was further fragmented as shown by the presence of ions with m/z 175, 149 and 134. Ferulic acid may lose CO_2 from carboxylic acid and/or CH_3 during fragmentation (Sun et al., 2007). Thus we concluded that the compound F4 is in fact 5-O-feruloyl-α-L- arabinofuranose.

Our results suggest that the 5-O-feruloyl-α-L-arabinofuranosyl-(1 → 3)-O-β-D-xylopyranose as the feruloyl oligosaccharide present in maize or wheat grains but at low concentrations. Although the concentration of 5-O-feruloyl-α-L-arabinofuranose is relatively high, it does not meet the criterion for oligosaccharides as it only

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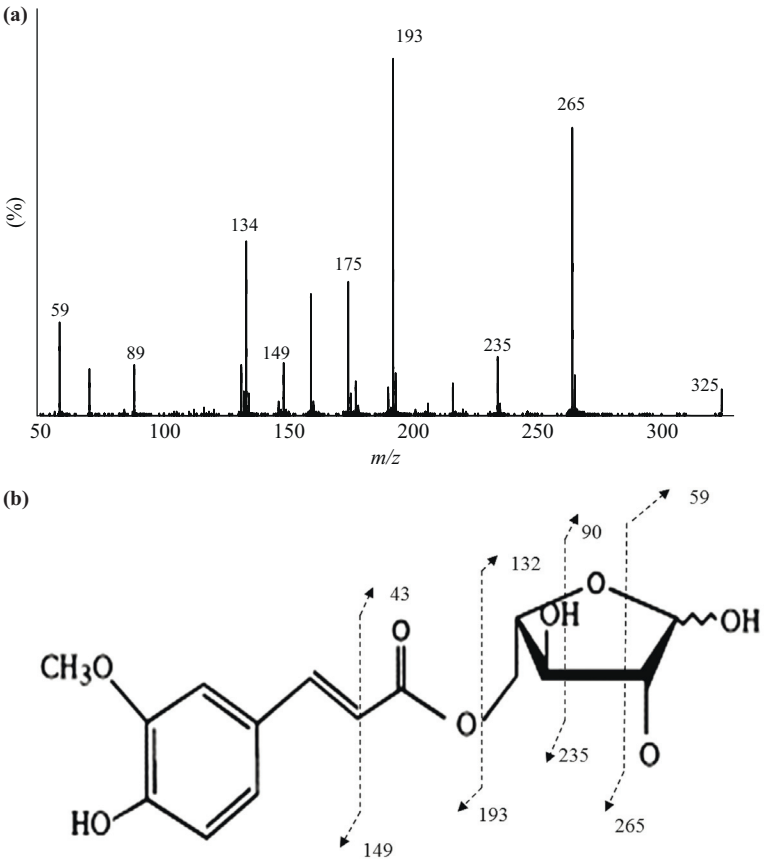

Fig. 4. (a) MS/MS spectrum of compound eluting at 28.52 minutes, labeled F2 (m/z 325); (b) an illustration of fragmentation pattern of compound F2.

has one sugar moiety. Feruloyl arabinoxylan oligosaccharides are feruloylated sugars with degree of polymerization of 2–10. Cereal grains contain endogenous xylanase enzymes that would act on arabinoxylan polysaccharides to produce arabinoxylan mono-/oligosaccharide (Cleemput et al., 1995). However, in this experiment, the samples were heat inactivated with ethanol to ensure that the observed F-AXOS were present naturally in cereal grain.

3.3. Effect of gastric digestion on feruloyl mono- and oligosaccharides content

Bio-accessibility of phenolic compounds increases as the food passes through gastro-intestinal tract following hydrolysis of the macronutrients (Chandrasekara and Shahidi, 2012; Gawlik-Dziki et al., 2009). Table 3 shows the content of F-AXOS (measured as esterified FA) following gastric digestion of wheat and maize sample. Esterified FA were present in all samples following gastric digestion but not in aqueous treated samples. The observed F-AXOS could be due to release of those bound to glycoproteins (Obel et al., 2003) and/or partial acid hydrolysis

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Table 3. Concentration of mono-/oligosaccharide esterified ferulic acid (μg) per g flour of maize, wheat, wheat aleurone and wheat bran samples following aqueous, pH and gastric treatment.

		Esterified ferulic acid (μg/g)*	
Treatment type	Aqueous	pH	Gastric digestion
White maize (Ekwendeni)	nd	8.37 ± 1.89	6.94 ± 1.33
White maize (Dedza)	nd	10.24 ± 1.83	11.34 ± 1.64
Orange maize (Ekwendeni)	nd	10.38 ± 1.77	11.57 ± 2.08
Orange maize (Dedza)	nd	8.49 ± 0.62	11.62 ± 1.89
Yellow maize (DASCA)	nd	10.29 ± 0.90	8.27 ± 1.20
Mean (maize)		9.55 ± 1.02	9.95 ± 2.19
Ambassador wheat	nd	3.01 ± 0.25	3.58 ± 0.17
Caledonia wheat	nd	4.23 ± 0.15	4.61 ± 0.30
MSDU wheat	nd	3.52 ± 0.18	6.33 ± 0.24
Purple wheat	nd	3.59 ± 0.42	4.65 ± 0.37
Mean (wheat)		3.59 ± 0.43	4.69 ± 1.01
Aleurone (wheat)	nd	23.51 ± 2.32	17.83 ± 1.25
Red bran (wheat)	nd	13.28 ± 1.37	13.48 ± 1.93

Values presented as mean ± standard deviation (n = 5); nd − not detected.

(Zhang et al., 2003) of feruloylated arabinoxylan. Thus another set of sample were treated with gastric pH conditions at 37 °C. Our results show no significant difference ($p \leq 0.05$) between the content of esterified FA of pH treated and gastric digested samples (Table 3). This does suggests that protein hydrolysis did not affect the esterified FA content but rather the low gastric pH. Arabinose linkages of arabinoxylans are susceptible to gastric pH (Zhang et al., 2003). We used 3 types of flint maize (white, orange and yellow) and 4 soft wheats (ambassador, Caledonia, MSDU and purple) in our study. The structure of arabinoxylans in maize and wheat has been reported to vary greatly in degree of substitution (Knudsen, 1997) and FA cross linkages (Bunzel, 2010). The mean degree of substitution of whole grain maize arabinoxylan is 0.7 (Huisman et al., 2000; Knudsen, 1997) and that for wheat is 0.6 (Gebruers et al., 2008; Knudsen, 1997). We observed a ~2 fold increase in esterified FA in all samples regardless of grain type.

The mean concentration of esterified FA produced during simulated gastric digestion of maize (9.5 μg/g) was about 2.5 times that of wheat. The difference in esterified FA concentration was not significant among the maize or wheat samples (Table 3). The order of esterified FA content produced was aleurone > bran >

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maize > wheat suggesting a possible association with insoluble bound ferulic acid in samples. Thus we performed a Pearson correlation analysis to establish the association between the insoluble bound ferulic acid and esterified FA produced during simulated gastric digestion. A strong positive correlation ($R^2 = 0.98$) was observed between the content of insoluble bound ferulic acid and esterified FA. Cereal grains or their fractions with highest insoluble bound ferulic acid are likely to result in high concentration of esterified FA when exposed to gastric conditions.

Also considering that peak F1 was not present in pH or gastric treated samples, our result may suggest that feruloyl arabinose is the F-AXOS released during gastric digestion. Hence we concluded that gastric pH released the feruloyl arabinose from the feruloylated arabinoxylans present in the cereal grains. Also assuming that all the esterified FA originated from F-AXOS, we concluded that F-AXOS may be doubled during gastric digestion.

4. Conclusion

In this work, feruloylated arabinoxylan mono- and oligosaccharides were isolated from maize and wheat. These were identified to be 5-O-feruloyl-α-L-arabinofuranosyl-(1 → 3)-O-β-D-xylopyranose and 5-O-feruloyl-α-L- arabinofuranose using HPLC-MS/MS. F-AXOS content (measured as esterified FA) was significantly higher in maize compared to that of wheat. Gastric pH conditions resulted in over two-fold increase in F-AXOS content. 5-O-feruloyl-α-L- arabinofuranose was the only identifiable F-AXOS released during gastric digestion.

Declarations

Author contribution statement

Lovemore N. Malunga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Trust Beta: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

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Additional information

No additional information is available for this paper.

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