Influence of Phenolic Acid Content on the Antioxidant Capacity of Hemicellulose from Sorghum Plant Fractions

Ryan J. Stoklosa,* Renee J. Latona, Michael J. Powell, and Madhav P. Yadav

Recovered hemicellulose fractions from biorefineries have the potential to improve overall process economics during the production of biofuels or other high value chemicals. A common hemicellulose found in many agricultural feedstocks is arabinoxylan (AX). This work investigated the influence of ferulic and *p*-coumaric acids on the antioxidant capability of AX hemicellulose recovered from sorghum bran, biomass, and bagasse. Sorghum bagasse and sorghum biomass AX contained the largest quantities of bound ferulic and *p*-coumaric acids at 13.1 mg/100 g and 6.3 mg/100 g, respectively. Antioxidant performance showed that sorghum bagasse AX hemicellulose produced the best reducing capability, while sorghum biomass and sorghum bran AX hemicellulose performed better as free radical scavengers. A reduction in free radical scavenging, as determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, occurred for sorghum bagasse and sorghum biomass AX hemicellulose at higher polysaccharide concentrations, which was either caused by the solution properties of the AX hemicelluloses or DPPH reaction reversibility in the presence of phenolic compounds with methoxy content. Alternatively, H_2O_2 scavenging by the AX hemicellulose revealed a dose-dependent response. Although scavenging effect was reduced at higher concentrations, sorghum bagasse AX hemicellulose functioned as having the best antioxidant capacity with respect to total reducing capability.

Keywords: Sorghum; Hemicellulose; Antioxidant; Ferulic acid; p-Coumaric acid

Contact information: Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, USDA, ARS, 600 East Mermaid Lane, Wyndmoor, PA, 19038, USA;
*† Mention of trade names or commercial products in this publication is solely for providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture; USDA is an equal opportunity provider and employer; *****Corresponding author: ryan.stoklosa@usda.gov*

INTRODUCTION

The transition to a sustainable bioeconomy requires the development of commercially viable biorefineries that convert agricultural feedstocks to biofuels or co-products. Because the profit margins for producing ethanol as a biofuel are small, a greater emphasis has been placed on simultaneously generating value added co-products to improve revenue and process economics (Lau *et al.* 2012). These co-products can range from biochemically generated building block chemicals, such as succinic acid or xylitol, (Bozell and Petersen 2010; Gao *et al*. 2016), recovered polymeric lignin for applications, such as an adhesive/coating (El Mansouri and Salvadó 2006), or recovered hemicellulose for functional food applications (Otieno and Ahring 2012). Moreover, hemicellulose can be recovered from different plant fractions as they can accumulate in lignified plant cell

walls or seeds (Saha 2003). Processing agricultural feedstocks in a biorefinery can allow for hemicellulose polysaccharides to be recovered after multiple processing steps and from different feedstock fractions.

One of the highest hemicellulose fractions that can be found in feedstocks, ranging from cereal grains to hardwoods, is arabinoxylan (AX) (Ebringerová 2005). This hemicellulose can be recovered in large quantities from corn fiber, a byproduct of the corn wet milling or dry grind process; it has been investigated as a replacement for gum arabic due to its favorable emulsification properties (Yadav *et al.* 2008). Both cereal grains and grasses contain phenolic acids, mainly ferulic and *p*-coumaric acid, that can be esterified to the 5-hydroxyl spot of arabinose in the AX hemicellulose chain (Lapierre *et al.* 2018; Peng and She 2014). Due to the presence of these bound phenolic acids, AX hemicellulose has been identified to possess antioxidant properties that are stronger than unassociated phenolic acids (Rondini *et al.* 2004). Barley and wheat AX hemicellulose with bound ferulic acid were identified to protect against free radicals and were hypothesized to be functional in the gastrointestinal tract (Malunga and Beta 2015). The ability of AX hemicellulose to provide possible defense against free radicals makes them a potential food additive that could improve nutritional content.

This research investigated AX hemicelluloses recovered from different sorghum fractions to identify antioxidant capacity through the presence of ferulic and *p*-coumaric acid. Sorghum was chosen for this work as its multiple cultivars can provide grain and lignocellulosic material for food, foraging, or biochemical conversion to biofuels. Studies that focus on the antioxidant capability from AX hemicelluloses have mostly focused on cereal grains such as millet, oats, rice, rye, or wheat (Masisi *et al.* 2016). A lack of information on the function and applications of AX hemicellulose from sorghum exists in the research literature. However, some recent studies have evaluated whole sorghum brans for antioxidant capability or AX hemicellulose recovered from sorghum for film applications (Ayala-Soto *et al.* 2015; Stoklosa *et al.* 2019). AX hemicelluloses from sorghum bran, biomass, and bagasse were isolated and recovered, and each fraction was characterized for ferulic and *p*-coumaric acids content, along with antioxidant capacity.

EXPERIMENTAL

Materials

Feedstocks and chemicals

All chemicals utilized were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of reagent grade. Three separate *Sorghum bicolor* feedstock fractions were utilized to isolate AX hemicellulose. Sorghum grains were purchased from Bob's Red Mill Natural Foods (Milwaukie, OR, USA). Because the grain sorghum was purchased from a commercial supplier, the grain sorghum cultivar utilized in this work is proprietary information. Delta BioRenewables (Memphis, TN, USA) provided sorghum bagasse. The bagasse fraction is a mixture containing sweet sorghum cultivars that are proprietary. Sorghum biomass was grown and provided by the United States Department of Agriculture (USDA)-Agricultural Research Service (ARS), Southern Plains Area (SPA), Cropping Systems Research Laboratory (Lubbock, TX, USA). The sorghum biomass fraction contained the cultivars PaceSetter BMR and Sorghum Partners 1990.

Arabinoxylan Hemicellulose Isolation

Sorghum grains were decorticated according to previous methods to produce sorghum bran (Hums *et al.* 2018). The recovered bran was dried overnight in an oven at 60 °C. The sorghum biomass and bagasse were dried overnight at 60 °C to remove additional moisture followed by grinding in a Thomas Model 4 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA, USA) to pass through a 20-mesh screen. After grinding the sorghum biomass, the bagasse was dried further at 60 °C overnight to remove excess moisture. All three fractions were de-oiled according to prior methods (Moreau *et al.* 1996). All three sorghum fractions were subjected to de-starching, and AX hemicellulose from all three sorghum fractions was isolated and recovered as described previously (Stoklosa *et al.* 2019). In brief, each sorghum fraction was subjected to NaOH extraction at 85 °C and a pH of 11.5. The NaOH concentration utilized for extraction was 0.1 M. After cooling to room temperature, the suspension was centrifuged at 14,000 *g* for 10 min. The supernatant was recovered, and the solution pH was adjusted to 4.0 to 4.5 by adding concentrated HCl. The precipitate was centrifuged at 10,000 *g* for 30 min. The acidic supernatant was recovered, while the acid-insoluble fraction was discarded. The acid-insoluble fraction was shown to be high in ash and protein content, thus reducing the overall polysaccharide mass fraction (Qiu *et al.* 2017). Next, the acidic supernatant was mixed with two volumes of ethanol to precipitate the remaining AX hemicellulose. This fraction was collected by filtration, washed, and dried in vacuum at 50 °C overnight.

Arabinoxylan Hemicellulose Solution Preparation

The AX hemicellulose solution for each fraction was prepared by dissolving 1 g AX in 200 mL deionized (DI) water to produce a 5 g/L polysaccharide solution. The solution was heated at 90 °C for 15 min while stirring. After 15 min the solution was removed from the heater and cooled to room temperature. After cooling, the solution was centrifuged in an Allegra X-14 (Beckman Coulter, Brea, CA, USA) centrifuge at 3724 *g*. The AX hemicellulose sample solutions were then prepared by serial dilutions to obtain specific concentrations for antioxidant testing (*i.e.* 2.5 g/L, 1.0 g/L, and 0.1 g/L).

Arabinoxylan Hemicellulose Analysis for Ferulic and *p*-Coumaric Acid

The AX hemicellulose fractions were analyzed for ferulic and *p*-coumaric acid components according to prior methods with slight modification (Yadav *et al.* 2007). First, 0.5 g of sample material was mixed in 10 mL of 1.5 M methanolic KOH and 500 µL of DI water. Sample tubes were capped and heated to 70 °C for 1 h with mixing performed every 5 to 10 min. Samples were cooled after extraction. Next, 6 mL of methanol and 8 mL of chloroform were mixed into the alkali extracted samples. The filtrate was collected by filtering each sample under gentle vacuum. Each sample filtrate was acidified by adding 5 mL of 3 N HCl. An additional 2.5 mL DI water and 8 mL chloroform was added and mixed following acidification. The final proportion of chloroform-to-methanol-to-water was 2:2:1 (volumetric ratio). Each sample was centrifuged at 70 *g* for 10 min to assist with phase separation. The lower phase was removed, collected, and dried under nitrogen for analysis. Samples were prepared for HPLC analysis at 5 mg/mL in ethyl acetate and syringe filtered.

A Thermo UltiMate 3000 high-performance liquid chromatography (HPLC) system from Thermo Fisher Scientific (Waltham, MA, USA) was utilized to separate and

quantify ferulic and *p*-coumaric acid. Detection was made with a Thermo UltiMate diode array detector (Waltham, MA, USA) (DAD) at 205, 254, 280, and 320 nm, and a Thermo Scientific Dionex Corona Veo charged aerosol detector (Waltham, MA, USA) (CAD). Ferulic and *p*-coumaric acid were quantified at 320 nm. A LiChrosorb 5-micron DIOL column (MilliporeSigma, Burlington, MA, USA) heated to 30 °C was utilized for separation. A gradient mobile phase was used for separation and consisted of a 0.1% (volumetric ratio) acetic acid solution in hexane (mobile phase A) and isopropyl alcohol (mobile phase B) at a flow rate of 0.5 mL/min. The operating pressure of the HPLC was 30 bar. The gradient program utilized can be found in the information under Table 1.

Table 1. Mobile Phase Gradient Program Used for Separation of Ferulic and *p*-Coumaric Acid

Time (min)	Solvent A (% Volume)	Solvent B (% Volume)
0	99	1
10	99	1
40	90	10
50	90	10
51	99	1
60	99	1

Total Phenolic Acid Content Analysis of Arabinoxylan Hemicellulose

The total phenolic content of the AX hemicellulose fractions was analyzed according to a previous method (Hodge *et al.* 2009). The Folin-Ciocalteu reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). Next, 0.2 mL of AX hemicellulose at 5 g/L was mixed with 2.6 mL of DI water and 0.2 mL of 2 N Folin-Ciocalteu reagent. Samples were incubated at room temperature for 6 min followed by the addition of 2 mL of a 7% (w/v) Na_2CO_3 solution. Samples were vortexed, followed by incubation at room temperature for 90 min. The samples were then read on a Shimadzu Scientific UV-1800 ultraviolet-visible (UV-vis) spectrophotometer (Somerset, NJ, USA) at 750 nm. A calibration curve was developed using gallic acid at a concentration range of 0.1 g/L to 1.0 g/L. A water blank was analyzed during testing. Samples were measured in triplicate.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of AX Gums

The AX hemicellulose was analyzed by FTIR on a Bruker Alpha II FTIR (Billerica, MA, USA). The AX hemicellulose fractions were made into a transparent pellet with KBr. A total of 128 scans were recorded between 4000 and 400 cm^{-1} with a spectral resolution of 4 cm^{-1}.

Ferric Ion Reducing/Antioxidant Power (FRAP) Assay

The FRAP assay to assess the antioxidant power of the AX hemicellulose was performed according to previous methods (Benzie and Strain 1996; Chattopadhyay *et al.* 2010). The following solutions were prepared for testing: 10 mM solution of 2,4,5-tri(2-pyridyl-5-triazine) (TPTZ) prepared in 40 mM HCl, 0.3 M acetate buffer, and 20 mM $FeCl_3$. The FRAP testing reagent was prepared by mixing 2.5 mL of TPTZ solution, 25 mL acetate buffer, and 2.5 mL $FeCl_3$.

For testing, the FRAP reagent was warmed to 37 °C. A reagent blank was read on a Shimadzu Scientific UV-1800 ultraviolet-visible (UV-vis) spectrophotometer (Somerset, NJ, USA) at 593 nm. A test sample volume of 30 µL was mixed with 90 µL of DI water and added to 900 µL of FRAP reagent. The absorbance was read at 0 min and 30 min. The absorbance change was determined according to Eq. 1,

$$\Delta A = A_{30\ \text{min}} - A_{0\ \text{min}} \quad (1)$$

where ΔA is the change in absorption units (AU) calculated from the difference at 30 min ($A_{30\ \text{min}}$) relative to 0 min ($A_{0\ \text{min}}$). Using Eq. 1, the ΔA was related to an Fe(II) calibration standard. Calibration curves were developed from standards utilizing $FeSO_4$. A stock solution at 2000 µM was prepared and serial dilutions performed to prepare a calibration curve ranging from 100 to 1000 µM. Analyses were performed in triplicate.

DPPH Scavenging

The antioxidant scavenging capability of the AX hemicellulose was performed according to previous methods using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Chattopadhyay *et al.* 2010; Xiong *et al.* 2013). Next, a 0.5 mM DPPH solution was prepared in a 50% (volumetric ratio) ethanol solution. To 4 mL of sample solution, 1 mL of DPPH reagent was added and mixed thoroughly. The test solutions were incubated for 30 min at room temperature in the dark. After 30 min, the samples were read on the spectrophotometer at 517 nm. A reference curve using butylated hydroxyanisole (BHA) was created to compare scavenging capability. A stock solution of BHA was prepared at 5 g/L. Serial dilutions were made to test BHA at concentrations of 2.5 g/L and 1.0 g/L. Samples were measured in triplicate. Scavenging capability was calculated according to Eq. 2,

$$DPPH\ Scavenging\ Effect\ (\%) = \left[1 - \frac{A_{\text{Sample at 517 nm}}}{A_{\text{Control at 517 nm}}}\right] \times 100 \quad (2)$$

where $A_{\text{Sample at 517 nm}}$ (AU) is the sample absorbance after 30 min reaction and $A_{\text{Control at 517 nm}}$ (AU) is the control absorbance of the reagent containing water after 30 min reaction.

Hydrogen Peroxide Scavenging

Hydrogen peroxide scavenging by the AX hemicellulose fractions was performed according to previous methods (Ruch *et al.* 1989; Xiong *et al.* 2013). A 40 mM H_2O_2 solution in a 0.2 mM sodium phosphate buffer solution (pH = 7.40) was prepared. Different concentrations of dissolved AX hemicellulose were tested (0.1 to 1.0 g/L) by mixing with 0.6 mL of 40 mM H_2O_2 solution. The absorbance for each sample was determined at 230 nm on a spectrophotometer after 10 min measured against a blank solution (phosphate buffer without hydrogen peroxide). The percent scavenging effect was calculated according to Eq. 3,

$$H_2O_2\ Scavenging\ Effect\ (\%) = \left[1 - \frac{A_{\text{Sample at 230 nm}}}{A_{\text{Control at 230 nm}}}\right] \times 100 \quad (3)$$

where $A_{\text{Sample at 230 nm}}$ is the absorbance (AU) of the sample at 230 nm and $A_{\text{Control at 230 nm}}$ is the absorbance (AU) of the phosphate buffer with hydrogen peroxide. A solution of ascorbic acid was analyzed to compare hydrogen peroxide scavenging performance.

RESULTS AND DISCUSSION

Phenolic Acid Content of Arabinoxylan Hemicellulose

The AX hemicelluloses isolated from sorghum bran, biomass, and bagasse plant fractions were analyzed for phenolic acid content to determine how these components could influence antioxidant capacity. The total overall yield for each hemicellulose fraction on a dry basis was found to be 5.57%, 4.13%, and 3.14% for sorghum bran, sorghum biomass, and sorghum bagasse, respectively, as determined by a previous study (Qiu *et al*. 2017). The monomeric sugar composition of each sorghum hemicellulose fraction was quantified in a previous study (Stoklosa *et al.* 2019). The xylose and arabinose mass fractions (relative mass %) reported for sorghum bran AX were 41.31 and 48.66%, sorghum biomass AX was 71.53% and 16.86%, and sorghum bagasse was 54.8% and 21.75%, respectively. The remaining sugar fractions of glucose and galactose were present at around 10% of the total mass fraction, except for sorghum bagasse AX hemicellulose, which had a larger fraction of glucose approaching 16% relative mass. The high fraction of glucose that was found in these sorghum AX hemicellulose fractions is not an uncommon characteristic (Verbruggen *et al.* 1995; Nandini and Salimath 2001). Other studies have confirmed the co-extraction of glucose with AX hemicellulose from sorghum kernels primarily as *β*-D-glucan and from sweet sorghum stem using alkali (Verbruggen *et al.* 1995; Sun *et al*. 2013). The high arabinose-to-xylose ratio in sorghum bran AX was previously shown to provide a high degree of branching based on rheological behavior of AX solutions (Qiu *et al.* 2020). The branching structure of sorghum bran AX was confirmed when preparing flexible hemicellulose films, as opposed to more brittle films prepared from sorghum biomass and bagasse AX that exhibit less branching due to a lower arabinose-to-xylose ratio (Stoklosa *et al.* 2019).

Phenolic acids linked to arabinose moieties can influence the antioxidant potential of AX hemicellulose from fractionated cereal grains or lignocellulosic feedstocks (Bijalwan *et al.* 2016). Total phenolic content, ferulic, and *p*-coumaric acid for sorghum AX hemicelluloses are quantified and displayed in Fig. 1 for each AX hemicellulose fraction. In Fig. 1A, the total phenolic content is displayed for sorghum bran, biomass, and bagasse AX hemicellulose. The total phenolic content is presented based on mg of gallic acid equivalents per 100 g AX hemicellulose, as measured by the Folin-Ciocalteu assay. Sorghum bran and biomass AX hemicelluloses have almost equal amounts of total phenolic content at around 220 mg/100 g AX, while sorghum bagasse AX hemicellulose contains over 300 mg/100 g AX. Although measuring phenolic content by this method was robust, the reaction conditions utilized can oxidize any reducing substance that is present (Singleton *et al.* 1999). Proteins that can be present are easily oxidized under the reaction conditions for this assay. Previous research conducted on AX hemicellulose fractions recovered from sorghum found that protein was approximately 3% by mass, which would indicate negligible interference (Qiu *et al.* 2017).

As displayed in Fig. 1B, sorghum bagasse AX hemicellulose was determined to have the highest ferulic and *p*-coumaric acid content at 13.1 mg/100 g AX. Both the ferulic and *p*-coumaric acid contents of sorghum bagasse in AX hemicellulose were found to be almost equal in composition. The content of ferulic and *p*-coumaric acids content in sorghum biomass AX hemicellulose was lower by about half at 6.3 mg/100 g AX in comparison to sorghum bagasse AX. The sorghum biomass AX hemicellulose was more

enriched in *p*-coumaric acid than ferulic acid. Opposite the results for sorghum bagasse and sorghum biomass AX was the low content for sorghum bran AX hemicellulose that only contained 0.53 mg/100 g AX. The sorghum bran AX hemicellulose contained a much larger fraction of ferulic acid at 0.48 mg/100 g AX compared to *p*-coumaric acid at 0.05 mg/100 g AX.

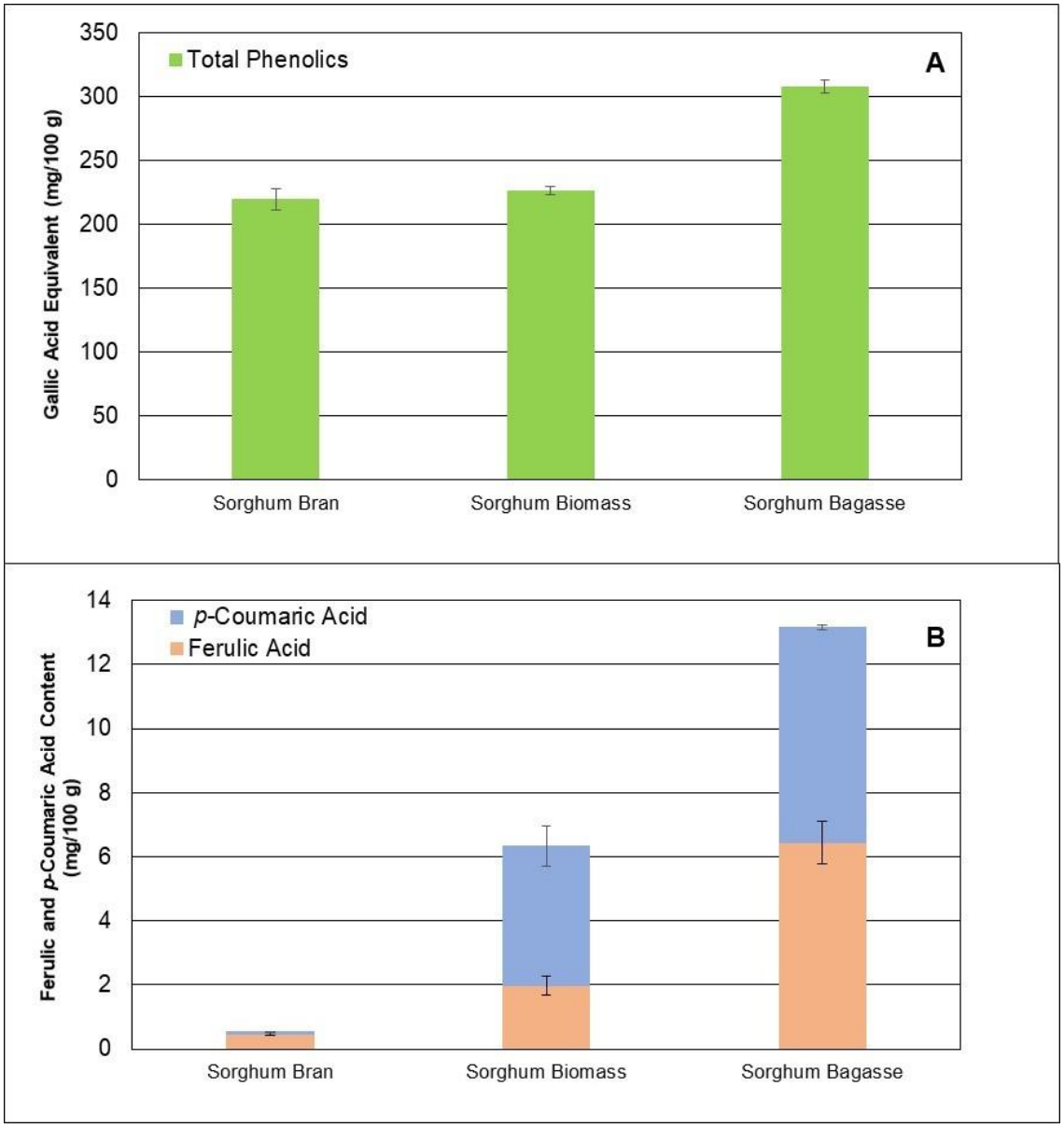

Fig. 1. (A) Total phenolic content; (B) Ferulic and *p*-coumaric acid composition of AX hemicelluloses from sorghum bran, bagasse, and biomass

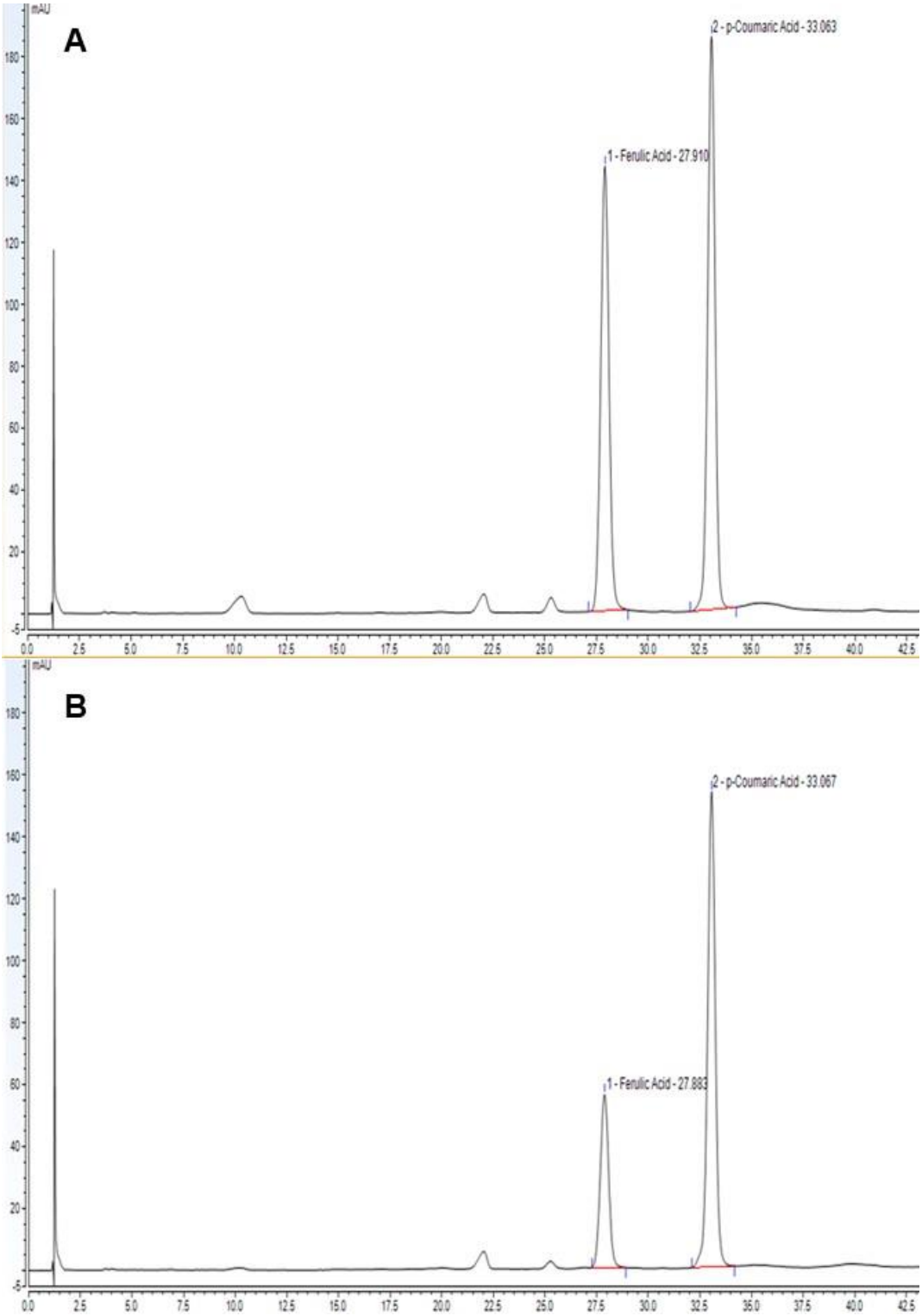

Fig. 2. HPLC Chromatogram Displaying Ferulic and *p*-Coumaric Acids Separation for (A) Sorghum Bagasse AX Hemicellulose and (B) Sorghum Biomass AX Hemicellulose

The results in Fig. 1B showed that the phenolic acids content of these sorghum AX hemicellulose fractions were lower by two orders of magnitude when compared to previous

results for whole sorghum bran (Ayala-Soto *et al.* 2015), wheat bran AX (Aguedo *et al.* 2014), and whole corn kernels (Butts-Wilmsmeyer *et al.* 2018). The lower phenolic acid content in the AX fractions evaluated reflect typical variation between species and cultivar types for agricultural feedstocks. Additionally, the sorghum AX hemicellulose fractions might have lower phenolic acids due to losses during isolation and recovery. During the isolation process, de-starched material from sorghum bran, biomass, and bagasse was subjected to a boiling alkaline extraction kept at a constant pH of 11.5 *via* the addition of a 50% (w/v) NaOH solution. It is well known that grass hemicelluloses possess alkali-labile linkages with phenolic acid moieties and some degradation might be expected under the isolation conditions (Banerjee *et al.* 2012). Conversely, the phenolic acids present in sorghum bran AX hemicellulose may be easier to cleave in alkali compared to sorghum biomass and sorghum bagasse AX hemicellulose, which are obtained from lignified plant cell walls. Although ferulic and *p*-coumaric acid were the predominant phenolic acids detected for each sorghum AX hemicellulose fraction, other phenolic acids may still be present. Within grass and bran hemicellulose, phenolic acids in the form of caffeic and sinapinic acid could be present, while the presence of lignin with alkali resistant bonds could be associated with the recovered sorghum AX hemicelluloses (Stoklosa *et al.* 2019; Ruthes *et al.* 2020). Figure 2 displays the HPLC chromatogram for sorghum bagasse and sorghum biomass AX hemicelluloses showing the separation of ferulic and *p*-coumaric acids. Although these phenolic acids are both the predominant peaks in Fig. 2, there are other smaller peaks detected at much lower quantities.

AX Hemicellulose FTIR Analysis

The FTIR analysis was performed on sorghum bran, sorghum biomass, and sorghum bagasse AX hemicelluloses to further ascertain the presence of phenolic acids. The collected spectra for each sorghum AX fraction are shown in Fig. 3. Table 2 accompanies Fig. 3 and lists spectra assignments for the presence of different bonds associated with the polysaccharide or other phenolic components. The spectra in Fig. 3A and 3B for sorghum biomass and sorghum bagasse AX hemicellulose, respectively, produced similar profiles indicating both fractions contain similar structures. In Fig. 3C, the spectra for sorghum bran AX hemicellulose contained a slightly different profile after 1500 cm^{-1} when compared to sorghum biomass and bagasse AX hemicellulose. Table 2 lists spectral assignments for structures that were found to be associated with the AX hemicelluloses. Starting at 1600 cm^{-1}, a strong absorption can be identified in Fig. 3A and 3B for sorghum biomass and bagasse AX. The common signatures shared between all three AX fractions appear around 1465 cm^{-1} and are typical of recovered AX hemicellulose. The FTIR results confirmed that sorghum biomass and bagasse AX have the greatest prevalence of bound ferulic and *p*-coumaric acid content.

The most intense signals starting at 1600 and going to approximately 1020 cm^{-1} can be attributed to vibrations or deformations associated with bonds on the ferulic or *p*-coumaric acid aromatic ring. Some of these signatures appear in Fig. 3C for the sorghum bran AX hemicellulose, but they are not as intense as they are for the sorghum biomass and bagasse AX hemicellulose, which have higher phenolic acid content. This region of the FTIR spectra that indicates the presence of phenolic acids could also show bound lignin to each AX hemicellulose fraction.

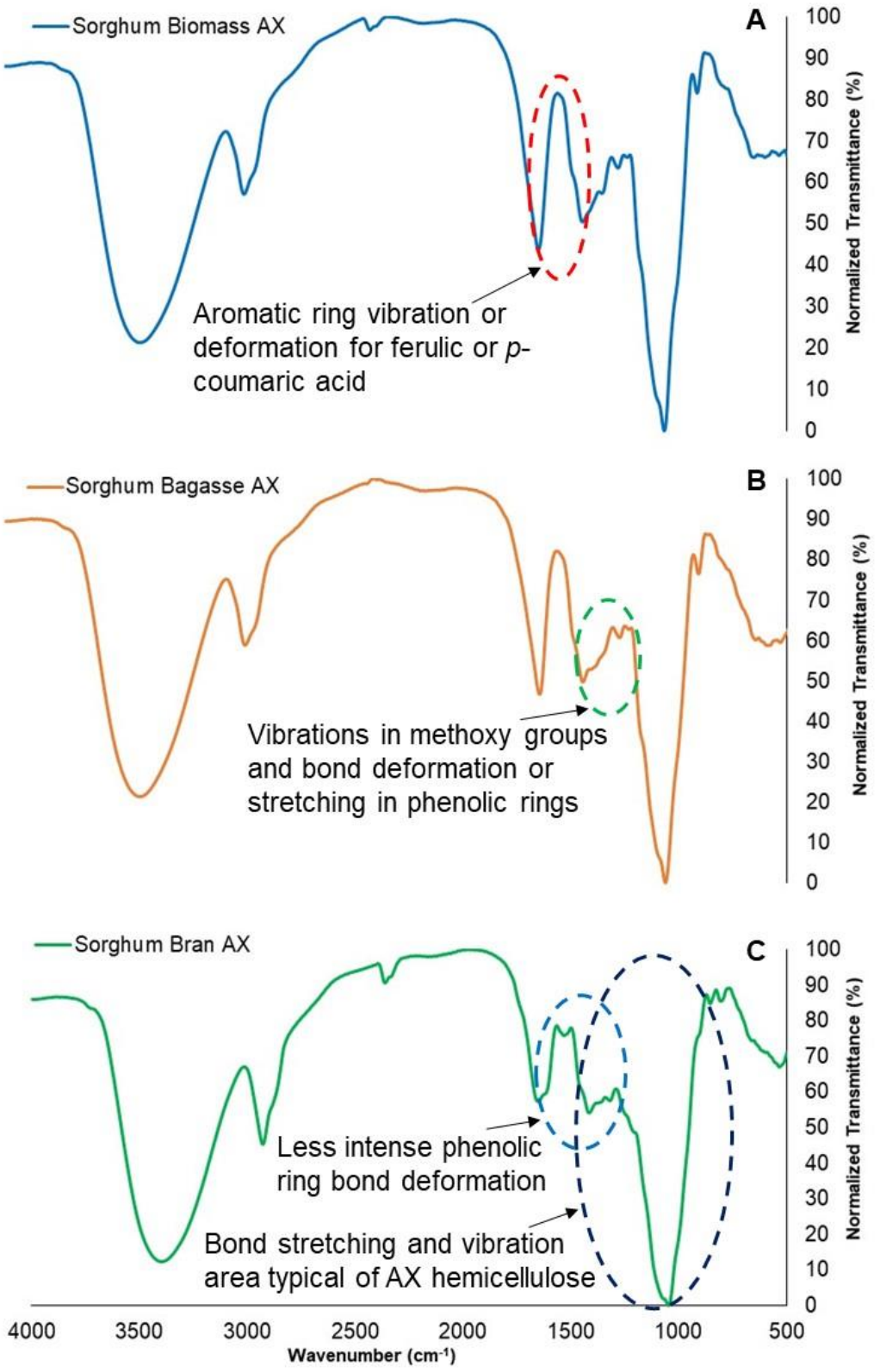

Fig. 3. FTIR spectra for (A) sorghum biomass AX, (B) sorghum bagasse AX, and (C) sorghum bran AX

Table 2. FTIR Spectra Assignments for Sorghum AX Fractions

Wavenumber (cm^{-1})	Common Pattern	Spectra Assignment	Reference
1600	Sorghum biomass and bagasse AX	C=C vibration associated with ferulic acid	Ram *et al.* 2003
1511	Sorghum biomass and bagasse AX	Aromatic vibrations in ferulic or *p*-coumaric acid	Li *et al.* 2015
1470 to 1435	Sorghum biomass and bagasse AX	Methoxy group asymmetric and symmetric -C-H deformation vibration	Abbas *et al.* 2017
1310 to 1210 1120 to 1020	Sorghum biomass and bagasse AX	C-O vibrations of alkyl-aryl ethers	Pei *et al.* 2008
1247 to 1242	Sorghum biomass and bagasse AX	C-O-H deformation and C-O stretching of phenolics	Corredor *et al.* 2009
1200 to 1185	Sorghum biomass and bagasse AX	Methoxy group asymmetric and symmetric -C-H rocking vibration	Abbas *et al.* 2017
1465, 1168, 1045	All three fractions	Bands are typical of arabinoxylans: C-O, C-C stretching, or C-OH bending	Li *et al.* 2015
903	All three fractions	C-1 vibration of β-glycosidic links between xylose	Li *et al.* 2015
856	All three fractions	Arabinosyl furanoid ring	Kačuráková *et al.* 1999

Because the AX hemicellulose was extracted using NaOH from the lignocellulosic portion of sorghum biomass and bagasse, it is likely that a small fraction of lignin is present in this material, given that ferulic acid bridges are known to connect hemicellulose and lignin (Sun *et al*. 2002; Torre *et al*. 2008). Alternatively, sorghum bran AX hemicellulose should contain much lower quantities of lignin, as whole sorghum bran typically contains less than 1% lignin on a dry mass basis (Corredor *et al*. 2007). The rheological properties of each sorghum AX hemicellulose fraction have been investigated to suggest that they are heterogeneous and contain prevalent feruloyl groups that crosslink to lignin, but the overall behavior of each sorghum AX fraction in solution is typical of AX polysaccharides (Qiu *et al*. 2020). While associated lignin in the sorghum AX hemicelluloses cannot be ruled out in the FTIR spectra, the bound ferulic and *p*-coumaric acids to the AX hemicellulose side chain should still provide most of the antioxidant capacity for each AX fraction.

AX Hemicellulose Reducing Capability

The FRAP assay was utilized to determine the reducing capability for each AX hemicellulose fraction. In this assay, a ferric salt is reduced in the presence of an oxidant by electron-transfer (Benzie and Strain 1996; Huang *et al*. 2005; Chattopadhyay *et al*. 2010). Table 3 presents the AX hemicellulose fractions total reducing capability measured as an increase in the reducing reaction from 0 to 30 min. Each concentration tested reported the amount of ferric salt reduced, expressed as µmol Fe(II) per mg AX hemicellulose.

Other studies have noted that short reaction times (*e.g.*, around 4 min) are not long enough to accurately determine reducing capability because the reaction can progress for certain phenolic compounds including ferulic acid (Huang *et al.* 2005). Increasing the AX hemicellulose concentration did not substantially increase reducing capability. The sorghum bran AX hemicellulose reducing capability slightly decreased at AX

concentrations higher than 1.0 g/L. Only a minor increase in reducing capability for sorghum bran AX was determined between 0.1 and 1.0 g/L. Alternatively, sorghum biomass AX hemicellulose reducing capability slightly increased beyond 1.0 g/L. A negligible decrease in reducing capability for sorghum biomass AX exists between 0.1 and 1.0 g/L as the reducing capability falls within the standard deviation. The sorghum bagasse AX hemicellulose reducing capability increased from 0.1 g/L but remained relatively unchanged between 1.0 and 5.0 g/L.

Table 3. Total Reducing Capability of AX Fractions as Measured by the FRAP Assay

AX Fraction	AX Concentration (g/L)	Total Reducing Capability (µmol Fe(II)/mg AX)
Sorghum bran	0.1	2.12 ± 0.42
	1	2.82 ± 0.13
	2.5	1.25 ± 0.19
	5	1.25 ± 0.03
Sorghum biomass	0.1	2.27 ± 0.49
	1	1.59 ± 0.18
	2.5	1.93 ± 0.05
	5	1.99 ± 0.07
Sorghum bagasse	0.1	3.20 ± 0.21
	1	5.61 ± 0.56
	2.5	5.42 ± 0.65
	5	5.13 ± 0.27

The highest total reducing capability was obtained in sorghum bagasse AX hemicellulose followed by sorghum bran AX hemicellulose at 1 g/L concentration, yet the sorghum bagasse AX hemicellulose reducing capability was doubled compared to the sorghum bran AX hemicellulose. The reducing capacity results in Table 3 are two orders of magnitude higher when compared to similar AX hemicellulose recovered from wheat bran (Hromádková *et al.* 2013). A more recent study on different cereal brans revealed that native sorghum bran produced a reducing capacity around 700 µM $FeSO_4$/g of material (Ahmad *et al.* 2019). However, the results for sorghum bran AX hemicellulose in Table 3 at the completion of 30 min reaction time are approximately two times higher than the result reported for native sorghum bran, if compared on the same per mg material basis. A higher reducing capability for sorghum bran AX hemicellulose *versus* native sorghum bran might be attributed to more accessible phenolic acid linkages present in AX hemicellulose as compared to native sorghum bran that contains a heterogenous matrix of starch, protein, hemicellulose, and other components.

AX Hemicellulose Scavenging Effect

Additional insight into the antioxidant properties of sorghum AX hemicellulose was performed by determining antioxidant scavenging capability. One of the main mechanisms for antioxidation is through proton radical scavenging (Chattopadhyay *et al.* 2010). The DPPH possesses stable free radicals, and when a radical scavenger is encountered a color change is detected that can be quantified *via* spectrophotometry (Yamaguchi *et al.* 1998). The ability of antioxidant materials to scavenge is based on their

ability to donate a proton (Xiong *et al.* 2013). Although the DPPH assay can be classified as having an electron transfer reaction mechanism, proton transfer from aromatic hydroxyl groups can occur if given long enough reaction times (Huang *et al.* 2005). Table 4 presents the DPPH scavenging effect for all sorghum AX hemicellulose fractions. Butylated hydroxyanisole (BHA) was utilized as a control.

Table 4. DPPH Scavenging Effect of AX Fractions

Component	Concentration (g/L)	Scavenging Effect (%)
Sorghum bran	0.1	71.7 ± 0.43
	1	76.9 ± 0.22
	2.5	75.5 ± 0.51
	5	73.6 ± 0.16
Sorghum biomass	0.1	73.5 ± 0.28
	1	76.7 ± 0.30
	2.5	71.7 ± 0.08
	5	60.3 ± 0.08
Sorghum bagasse	0.1	58.8 ± 0.33
	1	60.1 ± 0.38
	2.5	48.4 ± 0.87
	5	17.3 ± 0.25
BHA	0.1	39.6 ± 0.36
	1	45.8 ± 4.90
	2.5	45.5 ± 6.50
	5	86.6 ± 0.14

As shown in Table 4, the AX hemicellulose fractions outperformed BHA at 0.1, 1, and 2.5 g/L concentrations in terms of free radical scavenging. Sorghum bran and sorghum biomass AX hemicellulose performed the best scavenging at 0.1, 1, and 2.5 g/L concentrations by producing a scavenging effect over 70%, while the scavenging effect of BHA was approximately 45% at the same concentration. Opposite this result was the performance by sorghum bagasse AX hemicellulose that showed the lowest scavenging effect of any of the sorghum AX hemicellulose samples. Sorghum bagasse AX hemicellulose exhibited reduced scavenging efficiencies at higher polysaccharide concentrations.

The scavenging effect for sorghum bran AX and sorghum biomass AX hemicellulose, at their respective concentrations, generally outperformed polysaccharides isolated from *Turbinaria conoides* (a brown seaweed) and xanthan oligosaccharides up to 5 g/L (Chattopadhyay *et al.* 2010; Xiong *et al.* 2013). In the case of xanthan oligosaccharides, two different types of xanthan oligosaccharides were prepared and produced drastically different results: one fraction could produce an 80% scavenging effect at 5 g/L, surpassing both sorghum bran AX and sorghum biomass AX hemicellulose, but the other fraction at 5 g/L was below a 20% effect, falling more in line with sorghum bagasse AX hemicellulose (Xiong *et al.* 2013). As mentioned above, the antioxidant's ability to donate a proton is an important parameter for scavenging capability. Apart from overall composition of phenolic acids, a structural component aspect related to the AX fractions might determine overall scavenging capability more heavily. The AX hemicellulose is known to be more susceptible to aggregation based on lower arabinose-

to-xylose (A/X) ratios because the unsubstituted polysaccharides are more likely to have interactions with one another (Shrestha *et al.* 2019).

In Table 4 the scavenging effect for each AX hemicellulose fraction started to decrease at a concentration greater than 1 g/L. The lowest scavenging effect was observed for sorghum bagasse AX hemicellulose at 5 g/L, followed by sorghum biomass AX hemicellulose and sorghum bran AX hemicellulose. This order tracked exactly with the A/X ratio determined previously for each sorghum fraction; *i.e.*, sorghum bagasse and biomass AX hemicellulose have the lowest A/X ratio, while sorghum bran AX hemicellulose has the highest A/X ratio (Stoklosa *et al.* 2019). It is probable that an aggregation effect between the AX polysaccharides took place as they were solubilized. This type of aggregation has been shown to form due to ferulic acid moieties on the polysaccharide chain producing di- and tri-ferulic acid crosslinks that improve overall gel and rheological properties (Marquez-Escalante *et al.* 2018). This mechanism could reduce the effectiveness of the radical scavenging capacity of the AX hemicellulose fractions.

Additionally, the presence of other phenolic compounds in the AX hemicellulose fractions may produce low DPPH scavenging output due to reaction reversibility. Eugenol, a phenolic compound that can be derived from lignin, has been shown to cause a reversible reaction in the presence of DPPH (Bondet *et al*. 1997). Similar *o*-methoxyphenol structures could also influence low readings in the DPPH assay due to reaction reversibility (Huang *et al*. 2005). As sorghum bagasse AX hemicellulose contains the highest content of ferulic acid, a compound containing a methoxy group on the phenolic ring, a scavenging output reduction at higher concentration levels is probable. A contrasting result can be seen with the scavenging effect by sorghum biomass AX hemicellulose. This AX hemicellulose fraction contains lower quantities of ferulic acid but is more enriched in *p*-coumaric acid, which lacks methoxy content on the phenolic ring. By having more *p*-coumaric acid content the decrease in scavenging effect is not as substantial in sorghum biomass AX hemicellulose.

The sorghum AX hemicellulose scavenging capability was also determined by scavenging H_2O_2. Figure 4A presents the H_2O_2 scavenging effect for each sorghum AX hemicellulose fraction. Figure 4B displays the scavenging effect by ascorbic acid as a control. The ability to scavenge H_2O_2 is assumed to occur by antioxidant inhibiting oxidation through direct reaction with H_2O_2, in part due to phenolic acid side chains interacting with the H_2O_2 molecule (Huang *et al.* 2005; Shahidi and Chandrasekara 2010). Unlike DPPH scavenging, the H_2O_2 scavenging by sorghum AX hemicellulose showed a dose-dependent response that increased with AX hemicellulose concentration.

Sorghum bran AX hemicellulose slightly underperformed H_2O_2 scavenging when compared to sorghum biomass and sorghum bagasse AX hemicellulose, but the lower scavenging effect was minimal. At 1 g/L AX hemicellulose concentrations all three fractions could produce greater than a 90% scavenging effect. Higher concentrations of AX hemicellulose would saturate the spectrophotometer absorbance signal. Figure 4B displays H_2O_2 scavenging by ascorbic acid. The ascorbic acid showed a dose-dependent response for scavenging effect that increased with higher concentrations, however, the concentration range for ascorbic acid were an order of magnitude lower when compared to sorghum AX hemicellulose. Although lower concentrations of ascorbic acid were more efficient H_2O_2 scavengers, all three sorghum AX hemicellulose fractions showed better H_2O_2 scavenging at their respective concentrations than xanthan oligosaccharides (Xiong

et al. 2013). Each AX hemicellulose fraction at 1 g/L performed better at scavenging H_2O_2 when compared to previous results for wheat bran and oat bran (Martínez-Tomé *et al.* 2004).

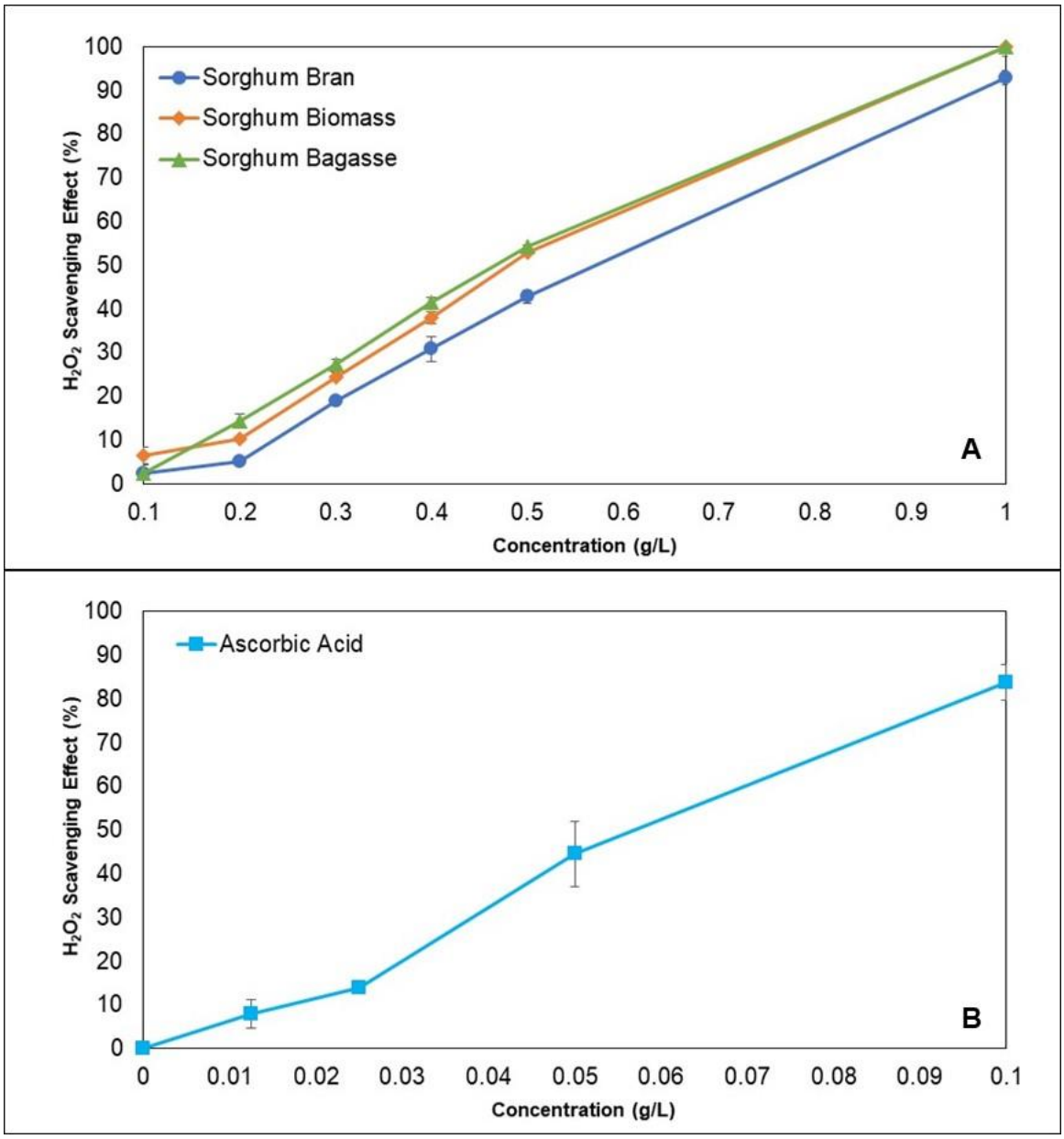

Fig. 4. (A) H_2O_2 scavenging effect for sorghum AX hemicellulose fractions; (B) H_2O_2 scavenging effect for ascorbic acid as a control

Prior research considered that bound phenolic acids to hemicellulose can provide more potent antioxidant protection *in vivo*, as they can be released under low pH conditions that are typical in gastrointestinal tracts (Van Hung 2016). For DPPH scavenging, it should be expected that at higher AX hemicellulose concentrations in the solution, a greater antioxidant scavenging capacity should be the result. This expected rationale has been

demonstrated by free ferulic acid's scavenging capability of DPPH by hydrogen donation (Ruthes *et al.* 2017). For these AX hemicellulose fractions, with the exception for sorghum bran AX, that did not occur. A reduction in DPPH scavenging could be caused by the polysaccharides ability to form gels and increase viscosity at higher AX concentrations. Mechanistically this can occur in the presence of free radical agents promoting AX hemicellulose formation into a three-dimensional network through the cross-linking of two adjacent ferulic acid residues (Izydorczyk and Biliaderis 1995). If fewer ferulic acid residues are available for scavenging this would reduce the overall ability of the AX hemicellulose to donate protons for the scavenging of DPPH. Conversely, a drop off in the H_2O_2 scavenging effect was not found at higher AX hemicellulose concentrations. At concentrations greater than 1 g/L, the scavenging effect for sorghum AX hemicellulose saturated the spectrophotometer absorbance signal. Moreover, the mechanism for H_2O_2 scavenging differs from DPPH scavenging through non-radical reactions (Shahidi and Chandrasekara 2010). These various aspects of AX hemicellulose phenolic acids composition along with the polysaccharides properties in solution appear to have an interconnected effect on the overall antioxidant performance for sorghum AX hemicelluloses.

CONCLUSIONS

1. The arabinoxylan (AX) hemicelluloses isolated from sorghum bran, sorghum biomass, and sorghum bagasse exhibited different quantities of bound ferulic and *p*-coumaric acid. Sorghum bagasse AX hemicellulose contained the largest total quantity of ferulic and *p*-coumaric acid at 13.1 mg/100 g AX, while sorghum biomass AX hemicellulose contained 6.3 mg/100 g AX. Sorghum bran AX hemicellulose exhibited the lowest quantity at 0.53 mg/100 g AX. The FTIR analysis of the AX fractions showed similar results.
2. The reducing capability, as measured by the ferric ion reducing/antioxidant power (FRAP) assay, indicated that sorghum bagasse AX hemicellulose provided the best performance. Sorghum biomass AX and sorghum bran AX hemicellulose produced similar results, but the reducing capability for both were less than the sorghum bagasse AX hemicellulose.
3. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effect by sorghum bran AX and sorghum biomass AX hemicellulose at concentrations of 1 and 2.5 g/L produced over a 70% scavenging capacity. A noticeable reduction in scavenging effect was determined at higher AX solution concentrations, primarily for sorghum bagasse AX hemicellulose. It is likely that this reduction in scavenging effect at higher concentrations of sorghum bagasse AX hemicellulose might originate from DPPH reaction reversibility in the presence of phenolic compounds containing a methoxy group on the aromatic ring.
4. The H_2O_2 scavenging effect by the sorghum AX hemicellulose fractions showed a dose-dependent response. Sorghum biomass and sorghum bagasse AX hemicellulose showed higher H_2O_2 scavenging when compared to sorghum bran AX hemicellulose, but the difference was only minimal. The difference in AX hemicellulose scavenging

performance was hypothesized to be influenced by differing reaction mechanisms for scavenging and the solution properties of AX hemicellulose.

5. Although sorghum bagasse AX hemicellulose had a reduced DPPH scavenging effect at higher concentrations, this fraction can be considered as having the best potential antioxidant applications due to higher phenolic acid content and producing higher total reducing capacity.

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