Characterization and functionalities study of hemicellulose and cellulose components isolated from sorghum bran, bagasse and biomass

Running Title: Hemicellulose and cellulose fractions isolated from sorghum sources

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Abstract

This study was undertaken to isolate and characterize three carbohydrate-rich fractions: Hemicellulose A (Hemi. A), Hemicellulose B (Hemi. B) and cellulose-rich residue (CRF) from sorghum bran (SBR), sorghum bagasse (SBA) and sorghum biomass (SBI). The monosaccharide composition of the Hemi. A and Hemi. B fractions was determined, indicating that SBR has a highly branched structure. The analysis of insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF) showed that TDF and IDF contents in original sorghum materials were in the following order: SBA>SBI>SBR. CRF from SBA was rich in IDF. Hemi. B fractions were completely soluble in water and so they were rich in SDF. CRFs from all sorghum sources show high water holding capacity (22.76 to 35.27 g water/g CRF). The emulsion stability study showed that the Hemi. B from all sorghum sources had a better emulsion stability than the well-studied corn fiber gum (CFG).

Keywords: Sorghum bran, sorghum bagasse, sorghum biomass, arabinoxylan, cellulose, emulsion stability

37 Introduction

38 Among the cultivated sorghums, *Sorghum bicolor* represents types that have been selected not
 39 only for grain production but also for sugar, fiber and forage production. Based on its
 40 conventional uses, it can be divided into grain sorghum, sweet sorghum, forage/biomass sorghum.
 41 Sorghum grain is one of the staple cereals in the world. Sorghum bran (SBR) is a by-product
 42 obtained from the commercial sorghum milling process. Sorghum bran from certain cultivars
 43 contains significant quantities of anthocyanins and phenolic acids (Carbonneau et al., 2014).
 44 Some sorghum varieties have extremely high contents of phenolic compounds that aid in the
 45 natural defense of plants against pests and diseases (Carbonneau et al., 2014). Sweet sorghum is
 46 a food and bioenergy crop that produces large amount of soluble sugar in its stems and yields
 47 significant amounts of bagasse as a lignocellulosic feedstock (Zegada-Lizarazu & Monti, 2012).
 48 Both its grain and stalk are ideal raw material for bioethanol production due to its high biomass
 49 and bio-based chemicals yields (Billa et al., 1997). The juice extracted from the sweet sorghum
 50 is composed of sucrose, glucose, and fructose and can therefore be readily fermented to alcohol.
 51 The solid fraction left behind after juice extraction, the so-called bagasse, is a lignocellulosic
 52 residue which consisted of 25% hemicelluloses (Sipos, et al., 2009). In contrast to sweet
 53 sorghum juice, which can be used in the food industry, the bagasse fraction has only non-food
 54 utilization alternatives (Sipo, et al., 2009). Earlier, it was used as animal feed or as soil fertilizer
 55 after composting with other wastes. Nowadays, bagasse is mainly used for energy production by
 56 combustion. Sorghum bagasse (SBA) is a renewable, cheap and widely available resource. Both
 57 forage sorghum and fiber sorghum [Sorghum biomass (SBI)] are non-food plants and used as
 58 silage for many years. They are predominantly composed of structural carbohydrates and
 59 harvested for fodder or paper production and producing second generation biofuels.

60 Cereal cell wall polysaccharides comprise mainly arabinoxylans, (1,3), (1,4)- β -D-glucans and
 61 cellulose. Arabinoxylans (AXs) are major dietary fiber components of many cereals such as
 62 wheat, rye, corn, barley, oat, rice, and sorghum (Fincher & Stone, 1986, Yadav et. al., 2007a).
 63 They have been isolated from various tissues of cereal grains, and are most abundant in the thick
 64 cell wall of the outer layers of kernels. Although the most commonly studied arabinoxylans are
 65 isolated from cereal endosperm and brans, they are also found in other plant sources such as rye
 66 grass, bamboo shoots, switch grass and miscanthus (Izydorczyk & Biliaderis, 1995). They consist
 67 of backbone chains of (1,4)-linked β -D-xylopyranose (Xyl) residues substituted primarily with α -
 68 L-arabinofuranose (Ara) units at O-3 or at both O-2 and O-3 of certain Xyl units and they are
 69 often named as pentosans. Moreover, they contain considerable amounts of uronic acid residues,
 70 predominantly D-glucuronic acid (GlcA) attached at O-2 of Xyl residues (Verbruggen, et al.,
 71 1998). The ferulic acid and other hydroxycinnamic acids are usually attached at O-5 of arabinose.
 72 Covalent linkages, ionic and hydrogen bonding between the cell wall components have been
 73 claimed by Verbruggen, et al (1998). The possible linkages between hemicellulose and the cell
 74 wall components are mentioned in detail by Saulnier et al. (1995b), explaining that ferulic,
 75 diferulic and p-coumaric acids play a very important role in cross linking them together (Saulnier
 76 et al., 1995b). In the cell wall, arabinoxylan and protein are also closely associated as supported
 77 by the finding of a stable linkage between hemicelluloses and protein in corn bran and rye bran
 78 (Saulnier et al., 1995a).

79 As the main component of the cell wall, it is difficult to extract all AX from cereal bran easily. A
 80 large portion of AX can't be dissolved in water. The extent of physical entanglement, covalent

ester bonding between carboxyl groups of uronic acids and the hydroxyl groups of AX, as well as the formation of diferulic acid bridges between adjacent AX chains, might be related to its solubility. Use of alkaline solutions is efficient for extracting AX from cell wall materials. The solutions of NaOH, KOH, Ba(OH)₂ and Ca(OH)₂ are frequently used for AX isolation (Benamrouche, et al., 2002) and sometimes H₂O₂ is added (Maes & Delcour, 2001) for its bleaching. In the alkaline extraction, AXs are the major polysaccharides other than some starch and β -glucans.

AXs from cereal grains, such as wheat endosperm, corn bran, rye bran and sorghum bran have been widely studied, particularly in terms of their isolation, characterization and molecular properties. Wheat AXs have been proven to have significant influences on the water balance and rheological properties of dough (Izydorczyk & Biliaderis, 1995). However, the functionalities and industrial applications of hemicelluloses are still not well studied and have long been neglected, perhaps due to its property of making a low viscous solution, and a property not seen as desirable by many food industries. Recently, it has been suggested that corn AX could be produced commercially, and it could be used as an adhesive, thickener and stabilizer and as a film former and emulsifier (Yadav, et al. 2007a,b; Kale, et al., 2015). In our previous study, AX isolated from corn fiber by an alkaline hydrogen peroxide technology is called corn fiber gum, CFG (Yadav, et al. 2007a,b). CFGs isolated from coarse and fine corn fiber contain functional protein, lipid and nutraceutical phenolic compounds, and they exhibited different functionalities (Yadav, et al., 2007c). CFG, with its low solution viscosity, has been proposed as an ideal stabilizer for oil-in-water emulsions. Moreover, AX from cereals have generated research interest (thanks to their nutraceutical properties) for the control of diabetes mellitus, cardiovascular disorders, the improvement of calcium and magnesium absorption and prevention of colon cancer (Saeed et al., 2011).

Sorghum arabinoxylan is an alkaline extract of sorghum bran and bagasse, which are the main low value by-products of the sorghum grain and sweet sorghum processing respectively. Sorghum arabinoxylan can also be extracted from sorghum biomass, which is usually grown for biofuel production. Sorghum arabinoxylan, also called sorghum hemicellulose B (Hemi. B), has a unique high solubility property making a low viscous solution at high concentration. To the best of our knowledge, the functionalities of hemicellulose B and cellulose rich fraction of sorghum bran, bagasse and biomass have not been reported. In this study, three fractions (Hemi. A, Hemi. B and cellulose rich component) from sorghum bran, sorghum bagasse and sorghum biomass have been isolated and characterized. The functional properties of Hemi. B and cellulose rich fraction (CRF) are studied.

2 Materials and methods

2.1 Materials

Sorghum grains, bagasse and biomass were obtained from USDA, ARS, NPA Manhattan, KS; Delta BioRenewables, Memphis, TN and USDA, ARS, PA, Cropping Systems Research Lab., Lubbock TX respectively. Sorghum grains were decorticated for 15 seconds using an electric laboratory seed scarifier (Dayton Electric Mfg. Co., IL, USA) with a 40-grit abrasive surface and the SBR was obtained from the resulting material using a 1.4mm sieve.

SBA and SBI samples were ground to a 20-mesh particle size using a Wiley mill. All three grounded SBR, SBA and SBI were extracted with hexane to remove oil (Moreau, et al. 1996). Starch was removed from all three de-oiled SBR, SBA and SBI by treating them with heat stable

Termamyl α -amylase (Novozymes, Inc., Davis, CA).

2.2 Standard proximate analyses

Moisture, protein and ash contents of all samples were determined using “AACC Approved Methods” 44-19, 46-30 and 08-01 respectively (AACC International, 1995). For determining protein ($N \times 6.25$) by AACC Approved Method 46-30.01, Flash 2000 protein Analyzer (CE Elantech, Inc., NJ) was used. In brief, the sample was weighed in a tin capsule and introduced into the combustion reactor via autosampler together with a proper amount of pure oxygen. After combustion, the produced gases were carried by a constant helium flow to a second reactor filled with copper reducer, then swept through CO_2 and H_2O traps, a GC column and finally detected by a thermal conductivity detector. A complete N/Protein report was automatically generated by the Eager Xperience dedicated software. The analysis conditions were as follows: Combustion temperature 900 °C, reduction temperature 680 °C, oven temperature 50 °C, Helium flow rate 140 ml/min, Oxygen flow rate 100 ml/min and total run time about 5 minutes.

2.3 Isolation of Hemi. A, Hemi. B and cellulose rich fraction

Arabinoxylans from SBR, SBA and SBI were isolated by an alkaline solution following our previously published method with some modification (Yadav, et al., 2007a-d). Each de-oiled and de-starched SBR or SBA or SBI (300 g) was mechanically stirred into water and heated to 85 °C for 1 hr. During the reaction, the suspension was kept at pH 11.5 by adding 50% (w/v) NaOH as needed. After cooling the hot reaction mixture, it was centrifuged at $6000 \times g$ for 20 min and the supernatant was separated from the residue by decantation. The obtained residue was extracted with alkali two more times by repeating above steps for complete isolation of AX. The pH of the alkaline extract (supernatant from all three centrifugation) was adjusted to 4.0-4.5 by adding concentrated HCl. The hemicellulose A (acid-insoluble arabinoxylan, “Hemi. A”) was collected by centrifugation at $10,000 \times g$ for 30 min. Two volumes of ethanol were gradually added to the supernatant with stirring to precipitate the major arabinoxylan fraction, also called hemicellulose B (Hemi. B) or sorghum arabinoxylan (SAX). The SAX was allowed to settle out as a white flocculent precipitate at the bottom of the beaker for overnight. The clear alcohol/water mixture above the precipitate was removed by decantation. The white flocculent precipitate was transferred into another beaker, stirred in 100% ethanol and filtered under vacuum. The SAX fraction obtained on the Buchner funnel was washed with 100% ethanol and dried in a vacuum oven at 50 °C for overnight. The insoluble residue left after alkaline extraction was suspended into water, pH adjusted to 5.5–6.0 and stirred for about 15 min. The undissolved residue was collected by vacuum filtration using Whatman filter paper number 1, rinsed with 100% ethanol and dried in vacuum oven. This final water insoluble residue is called cellulose rich fraction (CRF). The one half of collected CRF was drum dried and the second half spray dried separately for their water holding capacity determination.

2.4 Determination of sugar composition

The carbohydrate composition was analyzed by a HPAEC-PAD using methanolysis (Yadav, et al., 2007d) combined with trifluoroacetic acid (TFA) hydrolysis (Yadav, et al., 2007d) with some modification. The gum samples to be analyzed were dissolved in deionized water (1 mg/mL). An aliquot of 100 μ L of this solution along with 100 nmol of myo-inositol (internal standard) were dried in a Teflon-lined screw cap glass vial by blowing with filtered nitrogen followed by drying in a vacuum oven at 50 °C overnight. These samples were methanolized with 1.5 M methanolic HCl in the presence of 20% (v/v) methyl acetate for 16 h, cooled to room temperature, and dried

by blowing with filtered N₂ after adding five drops of t-butanol. The methanolized samples were hydrolyzed with 0.5 mL of 2 M TFA at 121 °C for 1 h, and evaporated by blowing with filtered N₂ at 50 °C, and the residue was washed by sequential addition and evaporation of three aliquots (0.5 mL) of methanol. In three separate glass vials were placed 100, 300, and 500 nmol of a mixture of standard sugars containing fucose, arabinose, rhamnose, galactose, glucose, xylose, glucuronic acid, and galacuronic acid. Then, 100 nmol of myo-inositol (internal standard) was added to each vial, evaporated, and dried as above. These standard samples were also methanolized and hydrolyzed as described above and used for quantification.

Hydrolyzates were analyzed for neutral and acidic sugars by HPAECPAD using a Dionex DX-500 system that included a CarboPac PA 20 column and guard column, a GP 50 gradient pump, an ED40 electrochemical detector utilizing the quadruple potential waveform (gold working electrode and pH reference electrode), an AS3550 autosampler with a thermal compartment (30 °C column-heater), and a PC10 pneumatic controller postcolumn addition system. The mobile phase consisted of isocratic 12 mM NaOH eluant for 10 min followed by 100 mM KOH and 6 mM CH₃COONa for 17 min at a flow rate of 0.5 mL/min at ambient temperature. A 0.1-min column wash with 12 mM NaOH followed by 10-min equilibration with 12 mM NaOH at a flow rate of 0.5 mL/min at ambient temperature was required to yield highly reproducible retention times for the monosaccharides. The total run time was ca. 70 min. In order to minimize baseline distortion due to change in the pH of the eluant during monosaccharide detection by PAD, 730 mM NaOH was added to the post-column effluent via a mixing tee.

2.5 Dietary fiber

Insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF) of the original SBR, SBA and SBI and their constituents were determined following the standard A2000 automated fiber analyzer (ANKOM Technology, Macedon, NY), (ANKOM Technology, 2011).

2.6 Water holding capacity (WHC)

The water holding capacity of CRF from all three sources was determined following the published procedure (Elhardallou & Walker, 1993) with some modification. CRF (0.5 g) was weighed and added into 24.5 mL water. The suspension was sheared using a Polytron bench top homogenizer (Brinkmann, Switzerland, PT 10/35) at 10,000 rpm for 2 min and at 15,000 rpm for additional 1 min. The tubes were placed on a shaker at 37 °C with moderate shaking for 24 h. The suspensions were centrifuged at 250 × g for 15 min. at room temperature. The supernatant was removed and the weight of the pellet was record. The WHC (g/g) of the sample was calculated using the following equation:

$$WHC = g_{water} / g_{sample}$$

Where g_{water} (g) and g_{sample} (g) are the weight of water held by the sample and the original weight of dry sample respectively. The WHC measurement for each sample was done in triplicate.

2.6 SAX stock solution for emulsification

The sample for emulsification (2.5 g) was prepared with 1:4 ratio of emulsifier to MCT (octanoic/decanoic acids triglycerides) containing 0.1% sodium benzoate and 0.3% citric acid. A stock solution of SAX emulsifier (27.78 mg/g solution) containing 0.1% (w/w) sodium benzoate (a preservative) and 0.3% (w/w) citric acid was prepared for emulsification study by slowly adding a calculated amount of SAX, a little at a time with vigorous stirring to a solution of

sodium benzoate and citric acid in water at room temperature and then gently stirring overnight to produce a hydrated, well dissolved and homogeneous solution. (Yadav, et al., 2007a). A control solution without SAX addition was also prepared.

2.7 Emulsion preparation

The samples for oil-in-water emulsions were prepared in triplicate for each sample by taking 2.25 g of above emulsifier stock solution and 0.25 g of Medium chain triglycerides (MCT, octanoic/decanoic acids triglycerides) oil in a glass vial. Medium chain triglycerides (density, 0.95 g/ml at 20 °C) was used as the dispersed phase since it is stable to oxidation with no bad smell or aroma and dissolves many lipophilic substances. No weighting agent was used. The solution was vortexed and then a coarse emulsion was prepared by pre-homogenization using a Polytron bench top homogenizer equipped with a 12 mm diameter head (Brinkmann, Switzerland, PT 10/35) at 20,000 rpm for 3 min. A fine emulsion was prepared by passing the above coarse emulsion through the EmulsiFlex-B3 high-pressure homogenizer (Avestin Inc., Canada) at 20,000 psi homogenization pressure 3 times. The prepared emulsion was stored at 60 °C for 0, 3 and 7 days for droplet size distribution measurement (Yadav et al., 2016).

2.8 Particle Size Determination

The droplet size distribution of the emulsions was measured by a Malvern Mastersizer 2000 particle size analyzer (Malvern Ltd., UK) using values 1.45 and 0.001 for MCT refractive index and absorption index respectively. The volume mean diameter was used for describing particle size of emulsions. The emulsification effectiveness was evaluated on the initial particle size of the emulsion at 0 time. The emulsion stability was determined after storing emulsions at 60 °C (acceleration test), (Cirre et al., 2014) for 3 and 7 days.

2.9 Data analysis

Each test was performed three times, and the reported results were obtained using the SAS system (Version 9.1 for Windows, SAS Institute Inc., Cary, NC, USA). To determine the differences among samples at different measurement points, a t-test for independent samples at a probability level of 0.05 was used ($p < 0.05$).

3 Results and discussion

3.1 Extraction of Hemi. A, Hemi. B and CRF

Hemi. A, Hemi. B and CRF of sorghum bran (SBR), sorghum bagasse (SBA) and sorghum biomass (SBI) were extracted according to the scheme shown in Fig. 1. The purpose of isolating pure SAXs (Hemi. B) from all three sources was to characterize them separately and study their structure/function relationship. The extraction procedure followed is a slight modification of the published isolation method for Corn Fiber Gum (CFG) (Yadav, et al., 2007a-d). The oil present in the three original sorghum materials was removed by hexane extraction (Moreau et al., 1996) to avoid contamination of its oxidation products in SAXs. The SBR, SBA and SBI contained 5.34, 0.43 and 1.10% (w/w) hexane extractable materials, respectively (Table 1a). The total oil content in sorghum caryopsis ranged from 0.5 to 5.2 % (w/w), with the mean value about 3.4 % (w/w) (Waniska and Rooney, 2000). Hexane extractable lipid and oil of SBR fell in the range reported by other investigators (Verbruggen, et al., 1993; 1995). The starch associated with SBR, SBA and SBI was removed by its digestion into maltodextrins with thermostable α -amylase at 90-95 °C, which was essential to avoid alkali-soluble starch or dextran contamination in SAXs. The starch content of de-oiled SBR and SBA were 32.87 and 2.71%, respectively. The presence

of starch in sorghum biomass was not expected and so it was not determined. The starch content in SBR was high like some other cereal bran (Nandini & Salimath, 2001a). During abrasive decortication, the sorghum outer layers detach from the kernel including germ tissue and endosperm, which were rich in starch, lipids and proteins (Nandini & Salimath, 2001a). That could be the reason of the higher content of lipids and starch in SBR than SBA and SBI.

Unlike our previous study (Yadav et al., 2007a), the addition of H_2O_2 was omitted for making food grade products. In this preparation, sorghum Hemi. B was extracted with only NaOH rather than a mixture of NaOH and $Ca(OH)_2$ to increase its yield (Yadav, et al., 2007c). AX was suggested to be strongly associated with cellulose (Nandini & Salimath, 2001b). The alkaline treatment released most of the cereal hemicelluloses, which were linked through phenolic or non-phenolic ester linkages or cross-linked by diferulic ester-bridge (Chanliaud, et al., 1994; Saulnier et al. 1995b). Some polyphenolics (lignin) form alkali-resistant ether linkages with hemicellulose. By repeating the isolation procedure two times as shown in scheme (Fig. 1), most of AXs which are strongly bound to the cell wall matrix were released. The yields of Hemi. A from SBI, SBA and SBR were 16.52, 13.43 and 5.34% respectively. The yields of Hemi. B from the SBR, SBA and SBI were 5.57, 3.14 and 4.13%, respectively (Table 1a), which are much lower than the yield of Hemi. B (CFG) from corn bran or corn fiber (Yadav et al., 2007b). Corredor et al. (2007) also reported that sorghum bran contained lower hemicellulose compared to corn fiber (CF), and these authors attributed differences due to grain composition and milling techniques. SBR samples were obtained from a mechanical decortication process, whereas the CF was a commercial by-product of corn wet-milling industry. The total hemicelluloses content (Hemi. A and Hemi. B) of SBR, SBA and SBI was slightly lower than the total hemicelluloses content of forage sorghum (233-277 g/Kg, Miron et al., 2007). The alkali and water insoluble material obtained after hemicelluloses extraction is called cellulose rich fraction (CRF). The yields of CRF from SBR, SBA and SBI were 11.68, 46.73 and 35.35%, respectively (Table 1a). The SBA and SBI have comparatively higher percent of CRF than SBR. These results are in good agreement with the findings of Dolciotti, et al. (1998), who reported 25.41% and 41.85 % cellulose in sweet sorghum and fiber sorghum respectively.

3.2 Proximate composition

The proximate analysis data of SBR, SBA and SBI and all the components isolated from them were given in Table 1b. Moisture content in the fractions isolated from all three sorghum sources varied from about 4.00% to 9.97% (w/w). The original sorghum materials contained 7.21-10.34% moisture, in which the SBI had the highest moisture content among all the samples. The original sorghum biomass and its Hemi. A, and Hemi. B fractions had high ash content, ranging from 11.13–17.03% (w/w). It has also been reported that the leaves, stems and reproductive organs of fiber sorghum have high ash content (Zegada-Lizarazu & Monti, 2012). Hemi. A from all three sources contain very high percent of ash (12.85-17.03%), which is due to doing alkaline hydrolysis of the original sorghum materials and adding acid to reduce pH to 4 for precipitating it. The protein content of the original sorghum materials are in the following order: SBI>SBA>SBR. A very high percentage of starch (Table 1a) might be the reason of a comparatively lower amount of protein detected in SBR. Proteins linked to hemicelluloses by covalent bonds are usually stable to alkaline and thermal treatment. It is also likely that proteins are linked to each other by covalent bonds are co-precipitated with arabinoxylans. The protein in Hemi. A (23.7-36.1%) was much higher than that in other fractions. Usually the isoelectric precipitation of protein at pH 4.5 is applied to remove it from its mixture with AX (Zhou, et al., 2010). Thus the protein present in Hemi. A isolated from all three sorghum sources might be

precipitated at pH 4.0-4.5 due to its isoelectric point falling in that range. The protein content in Hemi. A isolated from SBR is higher than Hemi. A isolated from SBA or SBI. The protein contents of Hemi. B isolated from SBR, SBA and SBI are close to each other (2.68 to 3.34 %). It is well documented that protein plays an important role for the emulsifying activities of naturally occurring emulsifiers, gum arabic (Dickinson, 1991) and CFG (Yadav et al., 2007a-d). Proteins impart an emulsifying activity to a polysaccharide, to which it is covalently attached with a glycosidic linkage (Dickinson, 1991). The hydrophobic protein associated with carbohydrate polymer makes a hydrophobic head and position to adsorb on the oil/water interface of emulsions, and the hydrophilic carbohydrate molecules extends into the aqueous phase to stabilize the oil dispersion. Thus the extraction condition of sorghum Hemi. B, which preserves its protein content, is important for making a functional AX with good emulsion stabilizing capacity. As expected, no protein was detected in CRF from all three sorghum sources.

3.3 Sugar composition

The sugar composition (relative mole %) of Hemi. A and Hemi. B isolated from SBR, SBA and SBI are given in Table 2. In Hemi. A and Hemi. B fractions from all three sources, the predominant monomeric sugar units are xylose and arabinose, followed by galactose, uronic acid, glucose and a trace amount of rhamnose. The high Ara/Xyl ratio (1.01 and 0.84) in Hemi-A and Hemi-B from SBR was reported by previous workers (Verbruggen, et al., 1993, Rumpagaporn, et al., 2015). The Ara/Xyl ratio of alkali extracted hemicellulose fractions of sorghum endosperm cell wall was found to be 0.77 to 1.64 (Nandini & Salimath, 2001a; Verbruggen, et al., 1993). Saulnier et al. (1995a,b) reported that the Ara/Xyl ratio was positively correlated to the proportion of di-substituted xylosyl residues (dXyl) and negatively correlated to the proportion of unsubstituted xylosyl residues (uXyl), whereas the proportion of mono-substituted xylosyl residues (mXyl) is independent of the A/X ratio. For this reason, the higher Ara/Xyl values observed in the SBR Hemi. A and Hemi-. B indicated a higher proportion of dXyl and a more branched structures in SBR than other two sources. The Hemi. A and Hemi. B fractions from SBA and SBI with Ara and xylose content from 14.19 to 18.02 (relative mole %) and 57.59 to 64.84 (relative mole %) respectively have a less branched structure.

Unlike the presence of a low level of glucose in CFG (1.14-3.66 relative mole %) due to residual alkali-soluble starch or xyloglucan polymer (Yadav, et al, 2007b), the Hemi. A and Hemi. B fractions from all three sorghum sources contain high percent of glucose (6.22 to 21.20, relative mole %), which is in agreement with the previous reports (Nandini and Salimath, 2001b; Verbruggen et al. 1995). The linkage of glucose to AX was determined by neutral sugar and methylation analyses (Verbruggen et al., 1995). The co-extracted glucose was also reported in the alkali extract of sorghum flour, mainly in the form of (1,3), (1,4)- β -D-glucans (Gruppen et al., 1992; Verbruggen et al., 1995). The β -D-glucans were also reported to be present in cell walls of sweet sorghum stem. Comparatively small amount of galactose (2.47-6.12%) was detected in all Hemi. A and Hemi. B fractions. The galactose might be due to presence of galactoarabinoxylans and arabinogalactans in these fractions (Nandini & Salimath, 2001a). Glucuronic acid (GlcA) and galacturonic acid (GalA) were reported to linked to the xylan-backbone (Verbruggen et al., 1995). The proportion of GlcA and GalA in the Hemi. A and Hemi. B fractions of SBR (1.23:7.87 and 0.30:11.81 respectively) agreed well with the extraction results of sorghum endosperm cell wall material, which reported that GlcA accounted for 90% (on molar basis) of the uronic acids, whereas GalA accounted for only 5% (Verbruggen, et al., 1995). Total uronic acid levels of SAXs were reported to be the highest when compared to wheat bran, corn bran and

rice bran (Rumpagaporn, et al., 2015).

3.4 Dietary Fiber

Dietary fibers (DFs) are known to be beneficial against a variety of diseases, including colon cancer and diabetes (Goni & Martin, 1998). Insoluble dietary fibers (IDFs) provide the mass needed for peristaltic action of the intestine, reducing the gastrointestinal transit time, while the soluble dietary fibers (SDFs) have a high water-holding capacity, forming a gel that reduces the absorption of intestinal lipids and glucose, becoming a substrate for the formation of rich bacterial flora. The DF method is used to determine the contents of cellulose and hemicelluloses. The main DFs of sorghum SBR, SBA and SBI are generally cellulose (linear structural polysaccharide homogeneously composed of D-glucose units) and hemicelluloses. The monosaccharidic components (D-xylose, L-arabinose, D-glucose, D-mannose, and D-galactose) of hemicelluloses are heterogeneously expressed as their polymeric forms (xylan, arabinan, hemicellulosic glucan, mannan, and galactan, respectively). Analysis of DFs from different fractions of different sorghum sources is reported in Table 3. The TDF and IDF contents in original sorghum sources were in the following order: SBA>SBI>SBR. The IDF, SDF and TDF in all three sorghum sources and their isolated constituents were much higher than reported in the whole sorghum grain (6.5-11.0%, 0.7-1.2% and 7.5-12.3%, respectively) (Knudsen & Munck, 1985). All three CRF isolated from SBR, SBA and SBI sources were very rich in IDF containing 89.12, 97.01 and 87.89 % (w/w) respectively. The CRF was insoluble and also it was resistant to alkaline treatment during extraction. Thus, most of the IDF retained in the final cellulose rich fraction (CRF). The acid-insoluble Hemi. A fraction from all three sorghum sources contained only about 0.41-4.6% (w/w) IDF. The Hemi. B from SBA, SBI and SBR had only 0.0 to 0.9% IDF, as it was highly soluble in water. On the contrary, the SDF was very high in the Hemi. B fraction isolated from SBR, SBA and SBI (83.92%, 77.70% and 63.60% respectively). Among the three Hemi. A isolated from three difference sources, Hemi. A with the highest Ara/Xyl ratio (highly branched structure) from SBR had the highest level of SDF (39.84%) in comparison to SDF from other two Hemi. A sources. Only a trace amount of SDF (0.49-3.46%) was found in CRF from all three sources. Comparatively a low percent of TDF (18.20% to 40.32%) was detected in in Hemi. A from all three sources. But Hemi. B and CRF from all these sources contained very high percent (63.60-97.01%) of TDF. As expected, the samples with higher ash and protein content (Hemi. A in Table 1b) had lower percent of TDF in comparison to the samples with low ash and protein.

3.5 Water holding capacity

A base of sufficient strength is capable of disrupting hydrogen bonding in cellulose microfibrils in aqueous solutions, leading to water uptake and swelling of cellulose (Immergut, 1963). The WHC results of drum dry and spray CRFs from sorghum bran, bagasse and biomass are depicted in Fig. 2. CRF samples from three sorghum sources could hold 22.76 to 35.27 times water to their dry weight at room temperature. According to Mcburney et al. (1985) and Jacobs, et al. (2015), the WHC of sorghum CRFs was much higher than common dietary fiber including wheat bran, lucerne, cabbage, pectin and wood cellulose (0.67 to 15.81, g/g). Drum dried CRF holds slightly more water than spray dried CRF from three sorghum sources. The WHC of both drum and spray dried sorghum CRFs was in the order SBA > SBR > SBI. In fact, the high WHC of cellulose was due to its very porous nature and branched structure. The variation in the WHC of these CRF samples could be attributed to their respective fibril arrangement, porosity and surface areas (Gelin et al., 2007). The water resided inside the pores and was bound to the cellulose

fibrils through hydrogen bonding (Gelin et al., 2007). The higher WHC of SBA-CRF than SBR-CRF and SBI-CRF might be due to its loose fibril arrangement, more branched structure, the larger pore size, the higher surface area per unit mass and the higher hydrophilic nature than the CRF from other two sources (Dahman, 2009).

3.6 Emulsion Stability

The emulsion stability (ES) of Hemi. B isolated from all three sorghum resources was determined in oil-in-water emulsion system and compared with well-studied emulsifier, CFG. The ES can be defined as the maintenance of a homogeneous flavor dispersion in the system. Fig. 3 shows the visual observation of emulsion stabilized by these samples studied after storage at 60 °C for 0, 3 and 7 days. Each sample was prepared in triplicate. The sample without any emulsifier was labeled as control. The mean particle size of these samples has been investigated through the formulation of 10% MCT in water emulsions at pH 3.2 with 4:1 MCT to SAX ratio by taking an aliquot from the middle of the emulsion after 0, 3 and 7 days.

As shown in Fig. 3D, the average oil-droplet size in the freshly-made emulsions of control was larger than all other samples containing sorghum Hemi. B or CFG. The emulsions stabilizing ability of all three Hemi. B samples are similar. The emulsions stabilized by Hemi. B from SBI showed slightly smaller mean particle size (1.19 μm) than the emulsions stabilized by Hemi. Bs from other two sorghum sources. However, the emulsions without emulsifier broke down and the oil droplets started floating at the top of the solution in a few days (Fig. 3B). Oil droplets of the control merged together to form a larger droplet. It led to decrease the number of oil droplets and eventually caused the breakdown of the emulsion. Larger droplet size would form during longer term storage (Fig. 3D). It has been well studied and reported that both gum Arabic (arabinogalactan) and CFG contain a small amount of hydrophobic polypeptide chains which can adsorb on the surface of oil droplets and act as anchors in their oil-in-water emulsion system. Their hydrophilic polysaccharide chains extend into the aqueous phase and form a stable barrier by causing steric and electrostatic repulsion among the oil droplets (Yadav, et al., 2007c; Dickinson, 1991). High pressure homogenization leads to the unfolding of the aggregates to fully utilize the hydrophobic components, which adsorb on to the oil droplets (Cirre et al., 2014). Thus, gum arabic and CFG can prevent aggregation and coalescence of the droplets, and so prevents breakdown of the emulsions. After storage for 3 or 7 days at 60 °C, sorghum Hemi. B from SBR, SBA and SBI showed a better ES than CFG and control. The mean particle size of the emulsions prepared from the Hemi. B from SBR, SBA and SBI changed from 1.97, 1.45 and 1.19 μm to 2.06, 2.39 and 1.83 μm respectively after storage for 7 days at 60 °C. But, in the case of CFG stabilized emulsions, it increased from 1.45 to 9.27 μm after 7 days storage at 60 °C. It has been reported that the functional corn bran arabinoxylan fractions, with higher protein and lipid contents, have a higher emulsifying ability than the fractions with less protein and lipid in O/W emulsion system (Yadav et al., 2007b, c). The lower protein content (2.68 %) of Hemi. B of SBA- agrees very well with its lower emulsion stabilizing capacity in comparison to the other two Hemi. B samples. In addition to protein content, the microstructure characteristics like higher average molecular weight, more compact structure and lower intrinsic viscosity of corn bran arabinoxylan made it a better emulsifier as well (Yadav et al., 2009). Also, it has been demonstrated for gum arabic that an improvement of emulsifying capacity can be achieved by controlling its processing time, which leads to an increase in its molecular weight and radius of gyration values (R_g) (Dickinson, et al., 1991). Thus, it is also likely that the lower degree of branching of the AX from SBA- might have resulted in a larger mean particle size of its emulsion after 3 and 7 days storage. It is well known that the absorption of high molecular

weight polysaccharide on the surface of oil droplets forms a thick and highly effective long-term steric stability on emulsions. For control, the mean particle size increased from 5.16 to 108.4 μm during that period. It also looks very clear from the Fig. 3D that Hemi. B from all three sorghum sources showed a better ES than the gold standard emulsifier, CFG during 7 days storage at 60 °C temperature (Yadav, et al. 2007a-d).

Conclusions

The **Hemi. A, Hemi. B and CRF from SBR, SBA and SBA** have been isolated and characterized. The present study has shown that the Hemi. A and Hemi. B fractions extracted from SBR, SBA and SBA differed slightly in their sugar composition. The sugar composition analysis suggested that Hemi. A and Hemi. B from SBR were more branched than Hemi. A and Hemi. B from other two sources. The dietary fiber analysis of three fractions indicated that isolated fractions are rich in total dietary fiber (from 18.2% to 97.0 %), as they are mainly composed of cellulose and hemicellulose. Acid-insoluble fraction (Hemi. A) from all three sources contained only 0.41-4.6% IDF. As Hemi-B was highly soluble in water, only 0 to 0.9 % of IDF was detected in them. The high level ash content in original SBI and its Hemi. A and Hemi. B resulted to less amount of TDF. CRF samples from three sorghum sources holds 22.76 to 35.27 times water to their dry weight at room temperature. The O/W Emulsions prepared with Hemi. B from all three sources (SBR, SBA and SBI) had comparatively higher stability than the corresponding standard emulsifier CFG during 7-day emulsification study. Hemi. B from all three sorghum sources produced very stable emulsions with an average particle size of less than 2.4 μm during 7-day emulsification study at 60 °C. Thus, it has been concluded that the Hemi. B and CRF fractions obtained from these three sorghum sources are useful as oil-in-water emulsifier and water holding agent respectively. The production of Hemi. B and CRF is considered economical as they are isolated from the by-products of sorghum grains milling and sweet sorghum processing for making biofuel and also directly from the sorghum biomass. The further research for studying the molecular characteristics and rheological properties of Hemi. B from all these sources is in progress.

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Conflict of interest statement

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, in whole or in part. This manuscript is not under consideration for publication in any other journal.

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Figure Caption:

Fig. 1. Basic scheme for isolating constituents of sorghum bran, bagasse and biomass

Fig. 2. The water holding capacity (in g water/g dry matter) of CRFs drum dried and spray dried from sorghum bran, bagasse and biomass. Each point was plotted in an average of three trials $\pm$ SD.

Fig. 3. Visual observation of emulsions stabilized by Hemi. B from SBR, SBA, and SBI at 60 °C for 0 day (A), 3 days (B), and 7 days (C).

Fig. 3D. Mean particle size of emulsions stabilized by Hemi. B from SBR, SBA, and SBI at 60 °C for 0, 3 and 7 days (CFG was used as reference). Lower mean particle size is an indication of greater emulsion stability. Each plotted point in an average of three trials $\pm$ SD.

Table 2. Carbohydrate composition (Relative Mole %) of constituents isolated from sorghum bran, bagasse and biomass

Sample Name	Rha	Ara	Gal	Glc	Xyl	GalA	GlcA	Total	Ara/Xyl Ratio	Ara/Gal Ratio
SBR-Hemi. A	0.14±0.04 ^a	35.74±0.73 ^a	2.47±0.08 ^a	17.07±1.21 ^a	35.49±4.57 ^a	1.23±1.04 ^a	7.87±3.50 ^a	100	1.01±0.17 ^a	14.47±0.42 ^a
SBR-Hemi. B	0.63±0.05 ^b	35.08±2.48 ^a	3.60±0.07 ^{ab}	7.07±0.29 ^b	41.52±4.49 ^b	0.30±0.42 ^b	11.81±2.71 ^b	100	0.84±0.22 ^b	9.74±1.71 ^b
SBA-Hemi. A	0.70±0.40 ^b	14.19±1.02 ^b	5.10±1.04 ^b	13.42±1.04 ^c	57.59±4.34 ^c	5.92±1.80 ^c	3.09±2.15 ^c	100	0.25±0.03 ^c	2.78±0.23 ^c
SBA-Hemi. B	1.41±0.11 ^c	18.02±1.16 ^b	6.12±0.25 ^b	9.63±0.25 ^{bc}	58.47±2.00 ^c	5.15±2.57 ^c	1.21±1.71 ^d	100	0.31±1.16 ^c	2.95±1.10 ^c
SBI-Hemi. A	0 ^a	9.33±0.36 ^c	2.86±0.16 ^a	21.20±0.16 ^d	64.84±0.66 ^d	0 ^d	1.78±0.35 ^d	100	0.14±0.21 ^d	3.26±0.03 ^c
SBI-Hemi. B	0.87±0.05 ^d	17.33±0.45 ^b	4.26±1.64 ^{ab}	6.22±0.26 ^b	64.24±1.64 ^d	3.51±0.11 ^{ac}	3.59±1.56 ^c	100	0.27±0.17 ^c	4.07±2.11 ^d

Note: Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; GalA, galactose acid; GlcA, glucuronic acid.

Data are means ± SD of triplicate; values in the same column with different superscripts are significantly different (*p* < 0.05)

Table 3. Dietary fiber of sorghum bran, bagasse, biomass and their constituents (wt. %, dry weight basis)

Sample Name	% IDF	% SDF	% TDF
Original SBR	31.40±1.27 ^a	3.70 ± 2.01 ^a	35.10±2.12 ^a
SBR Hemi. A	0.48±0.31 ^b	39.84± 1.11 ^b	40.32±4.05 ^b
SBR Hemi. B	0.90±0.52 ^b	77.70±2.13 ^c	78.60±1.23 ^c
SBR CRF	85.67±2.10 ^c	3.46±0.08 ^{ad}	89.12±4.88 ^d
Original SBA	71.40±1.26 ^d	2.50±0.44 ^d	73.90±0.34 ^c
SBA Hemi. A	4.60±1.07 ^e	13.60±1.03 ^e	18.20±1.18 ^e
SBA Hemi. B	0.00 ^b	83.92±2.11 ^f	83.92±1.42 ^{cd}
SBA CRF	94.91±5.01 ^f	2.10±0.29 ^d	97.01±0.49 ^f
Original SBI	54.00±2.22 ^g	1.90±0.06 ^{dh}	55.90±2.42 ^g
SBI Hemi. A	0.41±0.03 ^b	17.80±0.34 ^e	18.21±1.66 ^e
SBI Hemi. B	0.00 ^b	63.60±3.10 ^g	63.60±3.17 ^{cg}
SBI CRF	87.40±0.91 ^c	0.49±0.17 ^h	87.89±2.97 ^d

Data are means ± SD of triplicate; values in the same column with different superscripts are significantly different ($p < 0.05$)

Figure(s)

Fig. 1.

Fig. 2

Fig. 3A

Fig. 3B.

Fig. 3C

Fig. 3D.

Fig. 3.