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Fractionation of carbohydrate polymers from Indonesian sorghum by-products

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ABSTRACT

Extraction of cellulose and hemicellulose from natural resources has attracted considerable interest. In comparison to other cereals, there is relatively little knowledge on the cellulose, hemicellulose and lignin content of sorghum by-products. As such, the aims of this study were to develop a multi-step process for sorghum by-products (bran, stalk and panicles) fractionation and characterise them with regards to their physicochemical properties. In terms of chemical composition, sorghum panicles were found similar to bran, featuring a significant presence of arabinoxylans as well. A sequential alkaline extraction with varying concentrations of NaOH was applied, resulting in the generation of three fractions (residue, hemicellulose and alkali soluble lignin). Fractionation of sorghum stalks with 1.0 M NaOH at 50 °C for 3 h obtained ~64 % (w/w) cellulose rich fraction and 52 % (w/w) of xylose, predominant in the hemicellulose fraction. In the bran, the fractionation with 0.75 M NaOH at 50 °C for 3 h obtained ~40 % (w/w) cellulose rich fraction. Approximately 76 % of the glucose was in hemicellulose fraction reflecting starch solubilisation in the case of bran, while nearly 2 % (w/w) alkaline-soluble lignin was extracted. Overall, this study demonstrated an effective approach for the fractionation and the recovery of key components in sorghum by-products.

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1. Introduction

The depletion of fossil fuels, global environmental issues and the increasing interest for utilising renewable resources as feedstocks for the production of a variety of chemicals are the main drivers for the valorisation of agricultural food waste and by-products. Cereal grain by-products such as straw account for approximately 3000 million tons per year worldwide (Sun and Ren, 2010), with a significant proportion being produced in Low-to-Middle-Income countries, such as Indonesia. In Indonesia, agricultural residues and by-products are estimated to be approximately 147 million tons of biomass per year, and include residues from plantations such as rice, maize and sugar cane (Tajalli, 2015). Sorghum [*Sorghum bicolour* (L.) Moench] represents the fifth most important cereal in the world after maize, rice, wheat and barley (Taylor and Duodu, 2010). As reported by the Indonesian Agency for Agricultural Research and Development (IAARD), sorghum has become an important crop in recent years and its plantation continues to increase on an annual basis (IAARD, 2013).

Among the sweet sorghum varieties, Kawali and Numbu are the most popular ones in Indonesia. According to the Indonesian Cereals Research Institute (ICERI), these sweet sorghum varieties have a short harvesting time of around 100–110 days, a very high carbohydrate content (> 80 %), and a low tannin content (0.18–0.21 %) (Subagio and Aqil, 2013).

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Moreover, their production generates approximately 4–10 million tons of biomass (including bran, stalk and panicles) per year. In Indonesia, sorghum biomass is usually used for feed and for land spreading, and to a lesser extend for electricity production, whereas in some cases it is either discarded or burnt (IAARD, 2013). However, particulate combustion from burning biomass is associated with health and environmental issues (Gadde et al., 2009). Therefore, developing novel strategies for the valorisation of sorghum by-products, such as bran, stalks and panicles is a priority in order to minimise the environmental impact of the current disposal methods, utilise nutrient-rich natural resources and generate additional economic value for the Indonesian farmers and the whole economy. Sorghum bran, stalks and panicles are lignocellulosic materials that contain high amounts of cellulose, hemicellulose, lignin and polyphenols, and as such they could have applications in various sectors including the food, agricultural and packaging sector.

Bran is the main by-product of the sorghum milling operation, accounting for 10–11.5 % (w/w) of the whole grain. It is derived from the decortication process which separates the kernel of sorghum and the aleurone layer to obtain high-quality food products including flour, kafirin or products for other purposes, such as livestock feed (Ayala-soto et al., 2015). The stalk is the above-ground part of the sorghum plant that remains after the leaves and the panicles are removed and is the part that is pressed to obtain sugar juice. Following pressing, the residual stalks are dried; this biomass represents around 30 % (w/w) of the fresh stalks (Thanapimmetha et al., 2011) and could be potentially used for bioethanol production (Yu et al., 2012).

In comparison to other cereals, there is relatively little knowledge on the cellulose, hemicellulose and lignin content of sorghum by-products. In recent years, extraction of cellulose and hemicellulose from natural resources has attracted a lot of interest and as a result a number of potential applications are being explored, including their use as dietary fibre and functional ingredients in food products (Pronyk et al., 2011). The hemicellulosic component is of particular interest as the hemicellulose structure depends significantly on the type of plant cell wall extracted from and consequently impacts its physicochemical properties (Sun and Ren, 2010). Cereal hemicellulose may include heteroxylans such as glucuronoarabinoxylans in barley husks (Krawczyk et al., 2008), mixed linked-glucans in the endosperm of barley and oat, arabinoxylans in the endosperm of wheat, barley and oat (Muralikrishna and Subba Rao, 2007), as well as in wheat bran which can also contain small amounts of arabinogalactan originating form aleurone and endosperm cells (Maes et al., 2002; Mandalari et al., 2005), xyloglucans in rice grain (Shibuya and Iwasaki, 1985), whereas glucomannans, xyloglucans, callose and uronic acids are minor constituents in barley endosperm cell walls.

However, the fractionation of lignocellulosic biomass and the recovery of hemicellulose and cellulose with high yields and purity, which would be required for a commercial process, is challenging due to the tight structure of the cell wall. The variations often observed in the chemical composition of cereal by-products reflect the differences in the source of the raw material (crop variety, primary processing) and the extraction methods used to separate the various components. It is hypothesised that a multi-step alkali fractionation would be suitable for the solubilisation and recovery of carbohydrate-rich fractions from sorghum by-products. Therefore, this study focuses on the development of an efficient multi-step alkali fractionation process for the separation of sorghum bran, stalks and panicles, into three fractions, a cellulose-rich, a hemicellulose-rich and a soluble lignin fraction. The proposed concept is supported by detailed compositional and mass balance analyses of the different fractions and could lead to the design of a novel value chain and to added-value applications for sorghum by-products.

2. Materials and methods

2.1. Materials

The sorghum varieties used in this study were *Sorghum bicolour* var. Kawali and var. Numbu. The sorghum bran, which constitutes a milling by-product, was obtained from the Indonesian Cereals Research Institute, whereas the sorghum stalks were obtained from the farm of a national Indonesian company [PTPN XII (PERSERO)]. The bran was sieved through a 60-mesh size sieve. The leaves and the panicles were removed from the stalks. The juice was then squeezed from the stalks using a roller mill; then the panicles and residual stalks were collected and dried separately under sunlight and then cut into small pieces. They were then ground and sieved through a 60-mesh size sieve and stored at – 20 °C for further use. All chemicals used were of analytical grade.

2.2. Methods

2.2.1. Proximate analysis of sorghum by-products

Protein and lipid analyses were performed using standard AOAC methods. More specifically, the protein content was measured using the Kjeldahl method and by multiplying the nitrogen (N) content by 5.7 (Mosse, 1990). The lipid content was determined gravimetrically using the Soxhlet method (Horwitz and Latimer),The ash content was measured by weighing 5 g of sample into a pre-weighted crucible and placing it in a furnace at 600 °C for 4 h. Samples were cooled down in a desiccator, and the weight of the samples was measured to calculate the ash content (Escarnot et al., 2011).

2.2.2. Carbohydrate analysis

Carbohydrate analysis of the raw materials and the collected fractions was performed according to the Laboratory Analytical Procedure (LAP) protocol on Structural Carbohydrate Determination by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2004). Approximately 300 mg of dried samples were mixed with 3 mL (72 %, v/v) of H_2SO_4 and incubated at 30 °C for 1 h. The mixture was then diluted to 4 % (v/v) H_2SO_4 with deionised water and autoclaved at 121 °C for 30 min. Samples were left to cool down and filtered. The pH of the supernatants was adjusted to pH 5.0 using anhydrous solid $CaCO_3$ (1–2 g) and the supernatants were filtered through 0.2 µm filters into 2 mL vials. Monosaccharide analysis was carried out by High Pressure Liquid Chromatography (HPLC) using an Agilent Infinity HPLC system (Agilent 1200 series), equipped with an Aminex HPX-87 H analytical column at 65 °C and a refractive index (RI) detector. The mobile phase used was 0.005 M sulphuric acid and the flow rate was set at 0.6 mL/min. For sugar quantification, known concentrations of sugars and sugar acids (glucose, xylose, arabinose, galacturonic acid, glucuronic acid) were used to construct calibration curves.

Table 1 – Compositional analysis of sorghum by-products (%, g/100 g dry weight).

Components	Bran		Stalk		Panicles	
	var. Kawali	var. Numbu	var. Kawali	var. Numbu	var. Kawali	var. Numbu
Moisture	5.2 ± 0.0^{b}	6.7 ± 0.1^{a}	4.1 ± 0.0^{a}	4.7 ± 0.1^{a}	2.4 ± 0.0^{a}	2.1 ± 0.1^{a}
Ash	3.8 ± 0.1^{b}	4.3 ± 0.0^{a}	4.4 ± 0.1^{a}	5.4 ± 0.1^{b}	3.8 ± 0.2^{a}	3.8 ± 0.1^{a}
Protein	12.1 ± 0.1^{a}	13.9 ± 0.1^{a}	1.9 ± 0.1^{a}	1.3 ± 0.1^{b}	12.9 ± 0.1^{a}	14.1 ± 0.1^{a}
Fat	10.4 ± 0.1^{a}	9.8 ± 0.1^{a}	4.8 ± 0.2^{a}	5.1 ± 0.3^{a}	9.8 ± 0.2^{a}	8.5 ± 0.2^{b}
Starch	25.1 ± 1.3^{a}	34.2 ± 0.2^{a}	3.9 ± 0.1^{a}	2.4 ± 0.4^{a}	18.4 ± 0.1^{a}	27.4 ± 1.0^{b}
Cellulose and β-glucan	14.9 ± 1.7^{b}	23.6 ± 1.7^{a}	29.4 ± 0.7^{a}	28.8 ± 0.04^{a}	12.7 ± 0.0^{a}	8.5 ± 0.7^{b}
Hemicellulose	37.5 ± 1.2	20.9 ± 1.1	23.4 ± 0.9	24.6 ± 0.3	27.2 ± 0.2	28.8 ± 0.4
Arabinose	10.4 ± 0.2^{a}	3.5 ± 1.2^{a}	2.2 ± 0.1^{a}	2.0 ± 0.0^{a}	7.3 ± 0.1^{a}	5.9 ± 0.0^{b}
Xylose	15.4 ± 0.3^{a}	4.5 ± 0.0^{b}	20.9 ± 0.5^{a}	21.5 ± 0.1^{a}	14.0 ± 0.0^{a}	11.9 ± 0.1^{b}
Glucuronic acid	8.0 ± 0.5^{a}	0.7 ± 0.3^{a}	0.1 ± 0.3^{a}	0.5 ± 0.3^{a}	5.8 ± 0.2^{a}	0.7 ± 0.1^{a}
Galacturonic acid	3.7 ± 0.3^{a}	12.2 ± 0.5^{a}	0.2 ± 0.2^{a}	0.6 ± 0.1^{a}	0.1 ± 0.1^{a}	10.3 ± 0.2^{a}
Acid Soluble Lignin	0.9 ± 0.1^{a}	1.4 ± 0.0^{a}	1.9 ± 0.1^{a}	1.7 ± 0.1^{a}	1.6 ± 0.0^{a}	1.8 ± 0.2^{a}

Mean ± standard deviation; values with the same letters in the same rows did not differ significantly, based on the Duncan's new multiple range test at a 95 % significance level (p = 0.05).

2.2.3. *Acid soluble lignin analysis*

The acid soluble lignin content of the samples was determined according to a published protocol (Sluiter et al., 2004). Approximately 50 mL of the acid hydrolysed sample produced from the analysis of monosaccharides were vacuum filtered and the absorbance of the aliquot was measured at 320 nm using a spectrophotometer. The acid soluble lignin (ASL) was then calculated and determined as follows:

$$ASL\ (\%) = [UV_{abs} \times Volume\ filtrate \times Dilution / \varepsilon \times DW_{sample} \times Pathlength] \times 100,$$

where:

UV_{abs} = average absorbance for the sample at 320 nm.

ε = Absorptivity of biomass at specific wavelength.

DW_{sample} = Dry weight of sample in milligrams.

Pathlength = optical pathlength of sample in UV-Vis square cell (cm).

2.2.4. *FT-IR spectroscopy*

The functional groups of the different fractions after extraction were analysed by Fourier transform infrared spectroscopy (FTIR) equipped with universal-attenuated total reflectance (ATR) scanning accessory to remove the need for transmission cells and KBr pellets when performing measurements on liquid, semi-solid and solid materials. The Atmospheric Vapour Compensation (AVC) software was used to remove spectral interferences caused by water and carbon dioxide. The spectra were obtained with a FT-IR spectrophotometer (Perkin Elmer Spectrum 100) and were recorded in the range of 4000–600 cm^{-1} at 4 cm^{-1} resolution, with 16 scans per sample.

2.2.5. *X-ray diffraction*

A powdered X-ray diffractometer (Bruker D8 Advance, Germany) was used to record the residue and hemicellulosic fractions from sorghum by-products diffractogram at 25 °C. The X-ray source was Cu Kα radiation at 40 kV and 35 mA (λ = 1.54 Å). The samples were mounted on a sample holder, and the pattern was recorded in the reflection mode at an angle of 2θ over a range of 5.000–80.030° at a speed of 10°/min. The crystallinity index (Cr.I) was calculated as follows:

$$Cr{\cdot}I{\cdot}(\%) = (Sc/St) \times 100,$$

where: Sc – area of the crystalline domain, St – area of the total domain.

2.2.6. *Starch analysis*

The starch content of sorghum samples was measured enzymatically using a total starch test kit (Megazyme). The method has been validated by AOAC (Official Method 996.11 and Method 76.13.01).

2.2.7. *Fractionation of sorghum by-products*

The fractionation of dried sorghum stalks and bran was carried out under alkaline conditions according to previous protocols with some modifications (Xiang et al., 2014; Xiao et al., 2001). Briefly, 5 g of dry-milled sample were mixed with 200 mL NaOH solution (0.75, 1.0 and 1.5 M) at 50 °C, in a 1:20 solid to liquid ratio. The mixture was incubated at 50 °C and stirred at 200 rpm for 3 h, followed by centrifugation at 17,105×*g* for 20 min. The collected residue was washed with deionised water and freeze-dried (Virtis SP scientific model 2KBTES, USA) (fraction R). The alkali-soluble supernatant was adjusted to pH 5.5 using 6 M HCl and concentrated to about one-third of its original volume in a rotary evaporator. Then, three volumes of ethanol (95 %, v/v) were slowly poured into the solution under constant stirring. The precipitated solid was separated and washed with 95 % ethanol using a filter paper and was designated as alkali-soluble hemicellulose fraction (H). The ethanol solution was designated as the soluble lignin fraction (SL) and was collected as a solid after evaporating the ethanol.

2.2.8. *Statistical analysis*

A one-way analysis of variance (ANOVA) was used to compare the mean differences of the components of the sorghum by-products. Duncan's Multiple Range Test was applied whenever a difference was detected ($p < 0.05$). The SAS 9.1.3 software was used for the statistical analysis. Results are presented as mean ± standard deviation.

3. Results and discussion

3.1. *Chemical composition of sorghum by-products*

Compositional analysis was carried out to characterise the sorghum by-products (Table 1). The bran of both sorghum varieties contained considerable amounts of starch (25–34 %, w/w) and protein (12–13 %, w/w), which was comparable to previous studies reporting the presence of approximately 33 % w/w starch (Qiu et al., 2017) and 10 % w/w of protein

Table 2 – Content of monomeric sugars (% dry matter) of fractions extracted from sorghum Kawali's stalk and bran.

Sugars in Fractions of Stalk (%)	$R_{0.75}$[a]	R_1	$R_{1.5}$	$H_{0.75}$[b]	H_1	$H_{1.5}$
Xylose	12.9 ± 0.3	10.6 ± 10.8	7.1 ± 1.1	49.3 ± 0.8	52.1 ± 0.5	32.0 ± 1.6
Arabinose	1.4 ± 0.3	1.2 ± 0.2	0.9 ± 0.1	8.0 ± 0.0	7.6 ± 0.3	7.1 ± 0.5
Glucose	55.4 ± 0.1	63.8 ± 2.3	62.5 ± 6.9	11.4 ± 1.3	11.6 ± 1.2	4.9 ± 0.0
Glucuronic acid	nd[d]	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.3 ± 0.3	0.8 ± 0.1
Galacturonic acid	0.2 ± 0.0	nd	nd	1.0 ± 0.2	0.8 ± 0.9	0.2 ± 0.1
Total sugars	69.9	75.7	70.6	69.7	73.4	44.9
Sugars in Fractions of Bran (%)	**$R_{0.75}$**	**R_1**	**$R_{1.5}$**	**$H_{0.75}$**	**H_1**	**$H_{1.5}$**
Xylose	8.7 ± 0.6	5.6 ± 0.8	5.1 ± 0.6	5.9 ± 0.5	6.0 ± 1.0	3.9 ± 0.3
Arabinose	6.9 ± 0.6	4.2 ± 0.6	4.1 ± 0.4	5.5 ± 0.6	6.0 ± 0.1	3.5 ± 0.2
Glucose	39.9 ± 0.6	35.4 ± 5.6	37.3 ± 2.0	75.9 ± 0.9	59.1 ± 0.7	40.8 ± 3.1
Glucuronic acid	0.5 ± 0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.2 ± 0.3	0.4 ± 0.1
Galacturonic acid	nd	0.6 ± 0.1	nd	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.0
Total sugars	56.0	46.1	46.9	88.0	71.5	48.9

Mean ± standard deviation; [a] $R_{0,75}$, R_1, $R_{1.5}$ represent residue fractions generated by alkaline extraction with 0.75, 1, and 1.5 M NaOH; [b]$H_{0.75}$, H_1, $H_{1.5}$ represent hemicellulose-rich fractions generated by alkaline extraction with 0.75, 1, and 1.5 M NaOH, respectively; [d]nd, not detected.

(Ayala-soto et al., 2015). The amount of starch and protein in the sorghum grain may vary depending on the species, or the genetic and environmental variations within the same species (Serna-Saldivar, 2016), whereas for the bran it is primarily liked to the debranning process used. The total carbohydrate content of the two sorghum bran varieties was 61–68 %. The Kawali bran had a higher xylose and arabinose content (15.4 % and 10.4 % respectively) compared to the Numbu bran (4.5 % and 3.5 % respectively), indicating a higher amount of arabinoxylans in the former (calculated as the sum of arabinose and xylose content). On the other hand, the Kawali bran had a lower glucose content (14.9 %) compared to the Numbu bran (23.6 %) indicating a lower amount of cellulose and potentially mixed-linkage glucans (calculated by the total glucose content). The soluble lignin content of both varieties (< 1.5 % w/w) was lower compared to a previous report of 4 % (Ayala-soto et al., 2015). The lipid content of bran was approximately 10 % for both varieties; this was higher than that reported (Qiu et al., 2017) which was 5 % w/w. The ash content was approximately 4 % (w/w) for both bran varieties; ash consists mainly of vitamin B and minerals such as iron, and zinc, magnesium, manganese and copper (Serna-Saldivar, 2016).

The stalks of both sorghum varieties contained considerably smaller amounts of starch (2.4–3.9 %) and protein (~ 1.5 % w/w) compared to bran. The glucose content was ~29 %, indicating the presence of cellulose and mixed linkage-glucans, and the xylose and arabinose were ~23 % in total, indicating the presence of hemicellulose polymers such as arabinoxylan (Table 1). The cellulose and hemicellulose contents of the stalks were comparable to the data reported by Matsakas et al. (2014), i.e. 20 % of cellulose, and Sun et al. (2013), i.e. 21.8 % of hemicellulose (w/w). However, She et al. (2010). reported a higher hemicellulose content of 31.4 % in sorghum stalks; this was most likely due to the de-waxing and de-lignification treatment prior to the hemicellulose extraction process, which removed lipid soluble compounds present in the cell wall (e.g. waxes, pigments, and chlorophyll), and thus concentrated the sample.

The panicles of the two varieties consisted of starch (18–27 %), hemicellulose (16–21 %), cellulose and mixed linkage β-glucans (8.5–12 %), protein (12–14 %), and acid soluble lignin (1.58–1.77 %) (Table 1). Overall, the composition of the panicles was similar to that of bran and indicates the significant presence of arabinoxylans as well. To our knowledge, this is the first time that the chemical composition of sorghum panicles is reported. A possible reason for this is that higher value applications for biomass including panicles have not been considered thus far; and the main disposal route of this by-product is incineration in the field (Zhong et al., 2020). Worth also mentioning is the fact that significant amounts of galacturonic acid were detected in the bran and panicles of the Nimbu variety, whereas glucuronic acid was mainly detected in Kawali. Galacturonic acid is the main constituent of pectins, which are found in minute concentrations in cereal grains. Glucuronic and galacturonic acid have been reported to be linked to the xylan-backbone in sorghum bran (Qiu et al., 2017). The compositional analysis demonstrated that there is significant potential for the valorisation of panicles targeting the production of added value products. However, the rest of this study is focused on the sorghum bran and stalks, as currently there is significant activity and interest in Indonesia in these particular by-products. Based on the results from the two varieties, the Kawali variety was selected for further research in this study as its bran contains higher amounts of arabinoxylan and is also a more popular cultivar in Indonesia than the Numbu variety.

3.2. *Fractionation of sorghum stalks and bran by alkaline extraction*

A multi-step process based on an initial alkaline treatment was used to fractionate sorghum stalks and bran. Different NaOH concentrations (0.75, 1.0 and 1.5 M) were tested and following extraction and secondary processing, three fractions were generated, namely the residue (R), hemicellulose-rich (H), and soluble lignin (L). The results of the compositional analysis of these fractions are presented in Table 2. The rationale for using alkaline conditions for fractionation was to saponify the acetyl groups that are attached to the xylan backbone; this prevents the formation of hydrogen bonds, resulting in the solubilisation of a significant proportion of hemicelluloses. The alkali treatment also results in breaking the alkali sensitive ester linkages of ferulic acid to glucoroarabinoxylans and glucoronorabinoxylans-lignin,

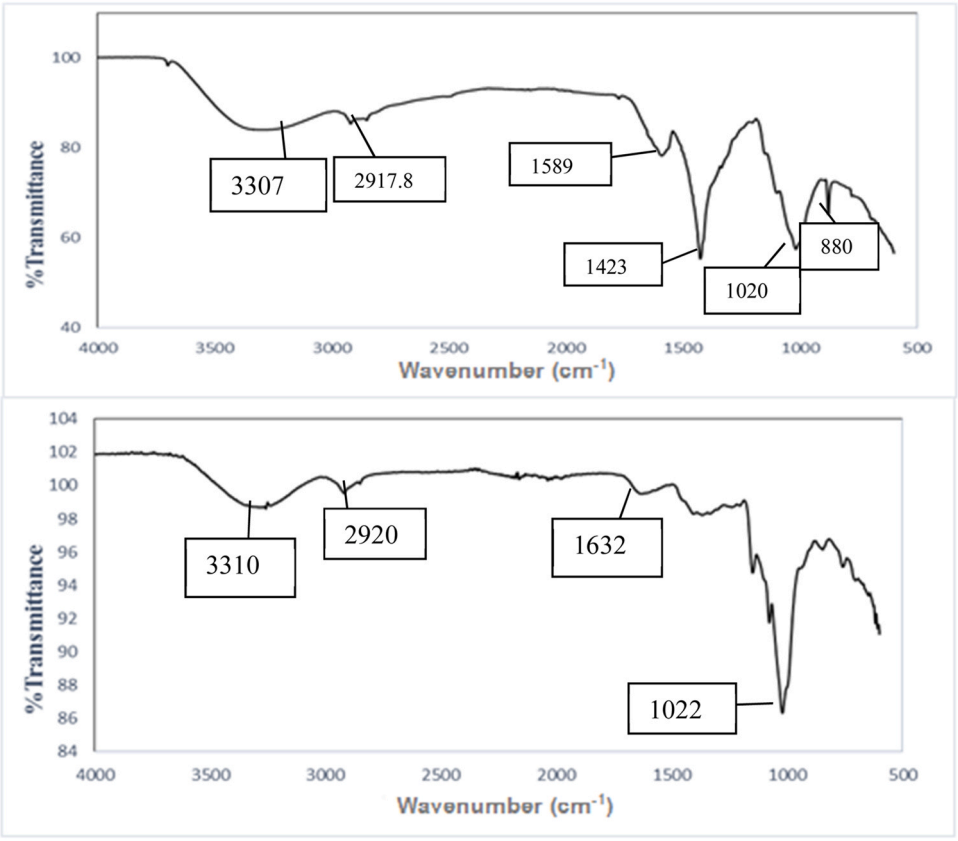

Fig. 1 – FT-IR spectra of hemicellulose fractions (H) obtained from sorghum stalk extraction with 1 M NaOH at 50 °C for 3 h (A), and sorghum bran extraction with 0.75 M NaOH at 50 °C for 3 h (B).

leaving behind a solid residue containing water-unextractable and alkali-unextractable components (Cyran et al., 2003).

The R fraction of sorghum stalk consisted primarily of glucose (55.4–63.8 %) and to a lesser extent of xylose (7.1–12.8 %), indicating the most likely presence of cellulose and a proportion of the hemicellulose, which was not alkaline soluble. The glucose content increased while the xylose and arabinose content decreased as the NaOH concentration increased from 0.75 M to 1.5 M. This indicated that the alkali treatment extracted a substantial amount of the hemicellulose present in the stalks, with the residue containing primarily cellulose. This is in line with a previous study which demonstrated that the residue fraction obtained after alkali extraction of sweet sorghum with 0.5 % and 1 % NaOH at 30 °C for 3 h consisted of 66.9 % and 57.8 % cellulose (Li et al., 2015). On the other hand, the glucose content of the R fraction of the bran was much lower than of the stalk and ranged from 35 % to 40 %; interestingly, it, was not affected by the NaOH concentration. However, the H fraction of the bran contained high amounts of glucose, due to the solubilisation of starch, which is indicated in the compositional analysis results (Table 1). The extraction yields obtained in this study are higher than those obtained in a previous study in which the cellulose-rich fractions of sorghum bran and bagasse (dried stalk) yielded 11.7 % and 46.7 %, respectively by alkaline extraction (50 % w/v NaOH) (Qiu et al., 2017). With regards to similar raw materials, sweet sorghum bagasse has been used as a starting material for extraction of crude xylan, consisting of 79 % xylose, 5 % arabinose and 2 % of glucose, using 2 M NaOH at 86 °C (Wei et al., 2018). In another study, Geng et al. (2019) used a range of hardwood and non-woody biomass (including sugarcane bagasse, wheat straw and switchgrass) to investigate hemicellulose extraction under alkaline conditions. It was noted that for non-wood materials, hemicellulose solubilisation is further influenced by lignin syringyl-to-guaicayl ratio, in that in biomass with higher syringyl-to-guaicayl ratio, xylan dissolution is greater.

In the current study, the R fraction of sorghum stalk was rich in cellulose and has therefore the potential to be used as a functional natural fibre for food and non-food applications, e.g. in textiles, paints and composite materials. A study investigating the functional properties of a drum dried and spray dried cellulose-rich fraction (CRF) from sorghum bran showed that it has a high water holding capacity (22–35-fold its dry weight) at room temperature due to its very porous nature (Qiu et al., 2017). Moreover, cellulose is amenable to chemical modification yielding a variety of derivatives (e.g. ethers, esters) that can be used as food casings, membranes, sponges, and natural organic sorbents (Sun and Ren, 2010), further expanding the range of potential applications.

3.3. *FTIR analysis of hemicellulosic fractions extracted from sorghum stalk and bran*

The FTIR spectra of the hemicellulosic fractions (H) of both sorghum stalk and bran are depicted in Fig. 1. It can be observed that the spectral profiles and intensities were different. The absorption bands at 3307 cm^{-1} in the H fraction of sorghum stalk and at 3310 cm^{-1} in the H fraction of sorghum bran can be assigned to the stretching vibration of -OH groups. The absorption band at 2917 cm^{-1} in the H fraction of sorghum stalk was assigned as C-H stretching of aliphatic groups. The absorption at 1589 cm^{-1} in the H fraction of

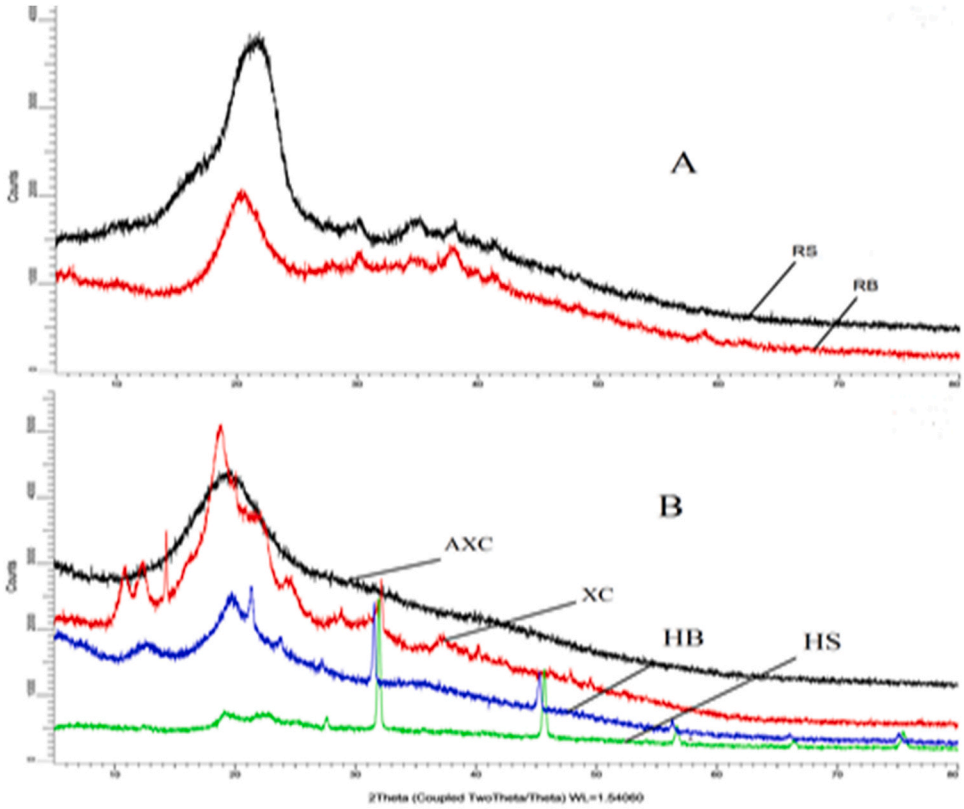

Fig. 2 – X-ray diffraction pattern of cellulose R fraction (A) and Hemicellulose H fraction (B) extracted from sorghum stalk and bran. Extraction of stalk was conducted with 1 M NaOH at 50 °C for 3 h whereas extraction of bran with 0.75 M NaOH at 50 °C for 3 h. Residue stalk (RS); Residue bran (RB); Commercial xylan (XC); Commercial arabinoxylan (AXC); hemicellulosic fraction of bran (HB); hemicellulosic fraction of the stalk (HS).

sorghum stalk is associated with absorbed water, since hemicellulose are potentially easy to be hydrated (Li et al., 2015); however, this peak was not seen in the H fraction of sorghum bran. The peak at 1632 cm^{-1} in the H fraction of sorghum bran is assigned to the bending vibrations of the –OH groups.

Additionally, a sharp peak at 1423 cm^{-1} was only available in the H fraction of the sorghum stalk and could be assigned as O-C-H-plane bending vibration of xylan. The prominent peak at 1020 cm^{-1} of the H fraction of the stalk can be assigned to glycosidic C-O, C-C stretching or C-OH bending, and could indicate the presence of xylan (Xiao et al., 2001). In the H fraction of the sorghum bran, this peak type appeared at 1022, 1077 cm^{-1}. The sharp band at 880 cm^{-1} in the H fraction of the stalk indicates that most linkages between the sugar units were β-1,4 glycosidic bonds, while in the H fraction of the sorghum bran this peak came out at 847 cm^{-1} with a weak intensity.

3.4. *X-ray diffraction of R and H fractions from sorghum stalk and bran*

The X-ray diffraction (XRD) profiles of the fractions extracted from sorghum by-products are depicted in Fig. 2. The crystallinity values for the cellulose fraction of the stalk and the bran were 34.6 % and 28.1 %, respectively. In addition, the major peaks of the XRD patterns for both cellulose fraction of the stalk and the bran showed a broad diffraction peak at the same 2θ= ~20 °C, which confirmed that the cellulose fractions in both materials were very similar. The amorphous regions (broad peaks) of the cellulose fraction of the stalk and the bran after extraction with NaOH increased significantly compared to the initial stalk and bran, by approximately 35 % and 39 %, respectively. This can be explained by the fact that alkaline extraction cleaved the hydrogen bonds between the intra- and inter-molecular bonds in cellulose and increased the porosity and surface area. A previous study reported that the Crystallinity Index (CI) of cellulose, analysed by four different techniques including XRD, varied from 39 % to 67 % (Thygesen et al., 2005). This is in line with the results from this study. Cellulose crystallinity is an important property in enzymatic hydrolysis. An amorphous region is easier for enzymes to digest compared to a crystalline region. This information is also important for identifying potential applications for the extracted cellulose. For example, amorphous cellulose can be used as an absorbent, whereas crystalline cellulose can be used as a reinforcing filler to increase the mechanical properties of products.

The crystallinity of the hemicellulosic fractions is depicted in Fig. 2B; it can be seen that the crystallinity of commercial xylan (XC), commercial arabinoxylan (AXC), the hemicellulosic fraction of bran (XB), and the hemicellulosic fraction of the stalk (XS) were 43.6 %, 35.0 %, 41.8 %, and 46.5 %, respectively. Additionally, with regards to the hemicellulosic samples, the 2θ position of XB, XS, XC and AXC was similar at 20 °C, which mainly denotes an amorphous state of the material that contains small crystalline peaks in this particular region. The substitution pattern of arabinoxylans, which is reflected by the ratio of arabinose/xylose, affects the amorphous or crystalline structure of arabinoxylans; for instance, when arabinoxylan is de-branched with α-L-arabinofuranosidase, its crystallinity increased (Heikkinen et al.,

Table 3 – Recovery of neutral sugars compared to raw material - % dry weight in the residue (R), hemicellulose (H), and soluble lignin (SL) fractions.

NaOH	Sugar	Recovery in Stalk (%)			Total recovery (%)	Recovery in Bran (%)			Total recovery (%)
		R[a]	H[b]	SL[c]		R[a]	H[b]	SL[c]	
0.75 M	Xylose	29.5	31.0	nd	60.5	19.2	43.7	0.3	63.2
	Arabinose	31.1	57.2	nd	88.3	15.9	32.0	nd	47.9
	Glucose	79.7	6.4	4.6	90.1	14.5	92.3	nd	106.8
1 M	Xylose	26.1	76.8	nd	102.9	8.2	53.0	nd	61.2
	Arabinose	31.5	49.6	nd	81.1	10.6	60.8	nd	71.4
	Glucose	87.8	5.7	1.9	95.4	11.2	57.8	1.5	69.0
1.5 M	Xylose	15.2	57.0	nd	72.2	8.1	27.5	0.9	36.5
	Arabinose	18.1	77.1	nd	95.2	6.8	25.7	nd	32.5
	Glucose	82.1	8.0	1.9	92.0	9.9	47.6	nd	57.5

*Sequential alkali treatment reaction was conducted at 50 °C for 3 h.
[a] R: Residue fraction.
[b] H: hemicellulose fraction.
[c] SL= soluble lignin fraction; nd = not detected.

2013). Moreover, the use of chemical reagents, such as NaOH, and processing conditions (e.g. temperature, time) might affect the crystallinity of the material (Haque et al., 2012) and thus influence its physicochemical properties. The hemicellulosic fraction of the bran had the lowest intensity among the tested samples. The diffractogram showed that a semi-crystalline hemicellulose peak was present. This indicates that some of the xylan is present in its crystalline form (narrow and sharp peaks). It is likely that unsubstituted regions crystallise and that substituted regions are amorphous, as depicted in Fig. 2.

3.5. *Sugar recovery and mass balance*

Table 3 lists the recovery of sugars from the sorghum stalks and bran in the extracted fractions. Overall, the recovery for arabinose ranged from 33 % to 49 % in bran and 95–100 % in stalk, while the recovery for xylose ranged from 37 % to 63 % in bran and 64–99 % in stalk. In addition, the recovery for glucose ranged from 57 % to 100 % in bran and 95–100 % in stalk.

Overall, the extraction process reflected the extractability of sorghum carbohydrate polymers. The stalk showed a higher sugar recovery due to the simpler structure of arabinoxylans compared to that of the bran. It can be inferred from the ratio A/X that the structure of hemicellulose was low branching which allowed NaOH to work more effectively to dissolve hemicellulose and break the bonds between polysaccharides in the cell wall matrix. The bran structure was more complex compared to stalk, most likely due to interaction between macromolecules including hemicellulose/cellulose, proteins and lipids (the latter two were in much higher percentage in bran). The hemicellulose content of bran was different to stalk, which resulted in different

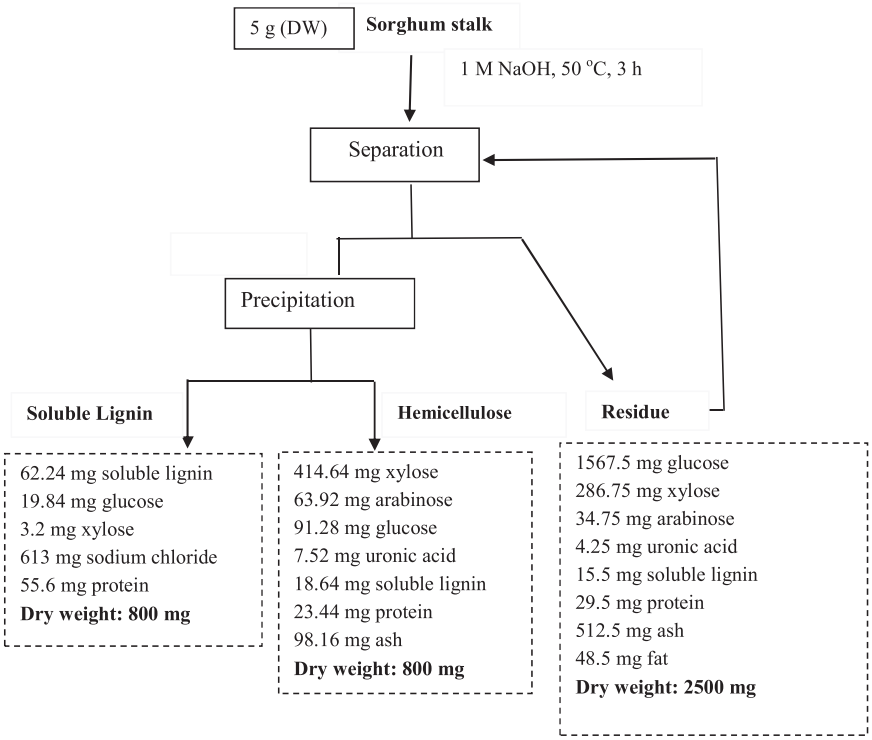

Fig. 3 – Process scheme and mass balances for the valorisation of sorghum stalk.

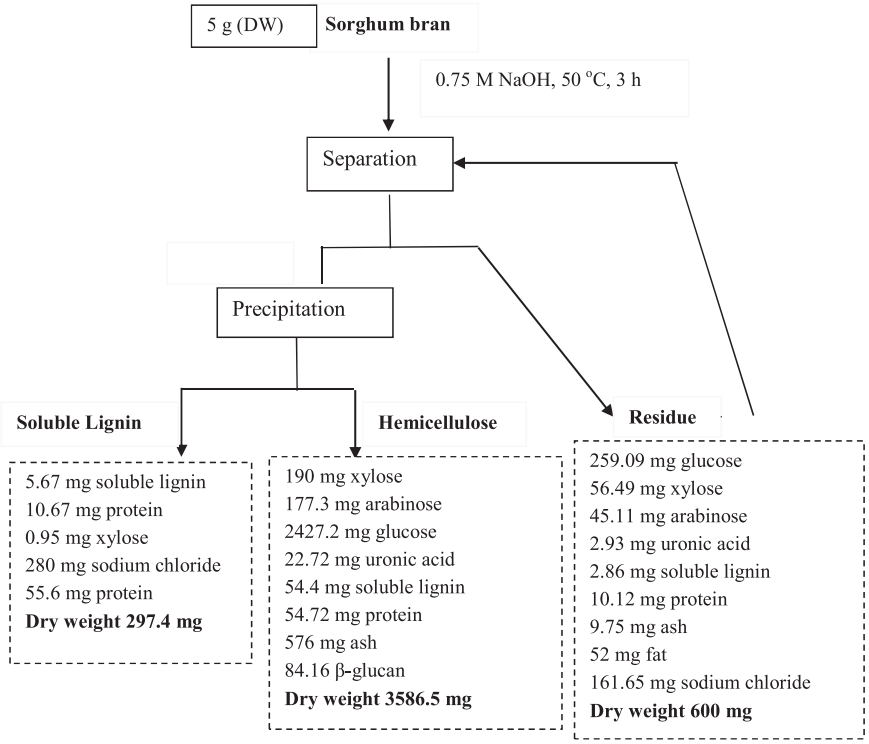

Fig. 4 – Process scheme and mass balances for the valorisation of sorghum bran.

types of interactions. A notable point is that the hemicellulosic fraction at 0.75 and 1.0 M of NaOH had overall good recovery, whereas at 1.5 M it did not.

The recovery yields indicated that conceptually using sorghum bran as the starting raw material, the main end product is a hemicellulose-rich fraction (Fig. 3); on the other hand, using stalk, the main product is a cellulose-rich fraction (Fig. 4). Both fractions are relatively pure (> 70 %) which is an advantage for further applications such as for biodegradable plastics and thickening agents. In both schemes, the overall process included a two-stage (sequential) alkaline extraction, where the residue after the first extraction was re-extracted to recover all the hemicellulosic fraction. This improved the fractionation efficiency and increased the recovery yield compared to a single alkaline extraction. The overall process, which includes the use of relatively low temperatures and concentrations of NaOH, requires relatively low amounts energy and does not utilise harsh chemicals, thus reducing its potential environmental impact. Enzymatic strategies may constitute viable alternatives to such chemical methods and are more acceptable from an environmental point of view; however, they are less effective in the deconstruction of complex biomass and are generally more expensive. Considering the proposed process, membrane filtration could be used to obtain the key products, i.e. arabinoxylans and cellulose, with a greater degree of purity, targeting higher value applications.

4. Conclusions

Stalk, bran, and panicles are underutilised lignocellulosic by-products of the sorghum plant. This study demonstrated an effective approach utilising sequential extractions with NaOH for the fractionation of sorghum stalk and bran and the recovery of key macromolecules. Overall, 79.4 % of the initial mass of the stalk and 89.6 % of the initial mass of the bran were recovered and distributed among the obtained three fractions. The residue fractions contained primarily cellulose rich insoluble fraction, hemicellulose rich fraction containing most arabinoxylan in sorghum stalk. Based on the detailed characterisation of the obtained fractions, a range of potential applications could be explored within the food and agricultural sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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