Characterisation of Fractions Extracted from Sorghum Byproducts

Prima Luna, Afroditi Chatzifragkou, Dimitris Charalampopoulos

Abstract—Sorghum byproducts, namely bran, stalk, and panicle are examples of lignocellulosic biomass. These raw materials contain large amounts of polysaccharides, in particular hemicelluloses, celluloses, and lignins, which if efficiently extracted, can be utilised for the development of a range of added value products with potential applications in agriculture and food packaging sectors. The aim of this study was to characterise fractions extracted from sorghum bran and stalk with regards to their physicochemical properties that could determine their applicability as food-packaging materials. A sequential alkaline extraction was applied for the isolation of cellulosic, hemicellulosic and lignin fractions from sorghum stalk and bran. Lignin content, phenolic content and antioxidant capacity were also investigated in the case of the lignin fraction. Thermal analysis using differential scanning calorimetry (DSC) and X-Ray Diffraction (XRD) revealed that the glass transition temperature (T_g) of cellulose fraction of the stalk was ~78.33 °C at amorphous state (~65%) and water content of ~5%. In terms of hemicellulose, the T_g value of stalk was slightly lower compared to bran at amorphous state (~54%) and had less water content (~2%). It is evident that hemicelluloses generally showed a lower thermal stability compared to cellulose, probably due to their lack of crystallinity. Additionally, bran had higher arabinose-to-xylose ratio (0.82) than the stalk, a fact that indicated its low crystallinity. Furthermore, lignin fraction had T_g value of ~93 °C at amorphous state (~11%). Stalk-derived lignin fraction contained more phenolic compounds (mainly consisting of *p*-coumaric and ferulic acid) and had higher lignin content and antioxidant capacity compared to bran-derived lignin fraction.

Keywords—Alkaline extraction, bran, cellulose, hemicellulose, lignin, sorghum, stalk.

I. INTRODUCTION

SORGHUM byproducts, namely the bran, the stalk, and the panicles constitute types of lignocellulosic biomass. Lignocellulosic residues comprise heterogeneous materials that attract much research interest as renewable resources. After cellulose, hemicellulose is the second most abundant polysaccharide family deriving from plant materials, forest and agricultural byproducts. The properties of fractions from sorghum by products are very important in order to identify and develop suitable approaches for the exploitation of these abundant materials and convert them to added-value products. These differences in composition are possibly related to the function of these macromolecules in the different parts of the sorghum plant [1]. Compared to other cereal byproducts,

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sorghum bran contains less lignin, less cellulose and hemicellulose, and more starch compared to maize bran. This is probably a result of the differences in processing technologies used for bran extraction and due to the intrinsic differences in grain composition between maize and sorghum. The starch content in sorghum bran is relatively high (~30%), most likely because of the considerable portion of the endosperm extracted during the decortication process.

Lignin is a non-polysaccharide cell wall component that is often bound to cellulose fibre. The bran contains very low amounts of lignin whereas the stalk contains 7-20%. There are three monomers of lignin, methoxylated to various degrees: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [2]. These monolignols biosynthesis generates lignin through shikimate pathway which started after enzymatic deamination of phenylalanine. This product of synthesis is integrated into the polymer, which consists of guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S). A lot of *Poaceae* families have mostly G, while some plants have mainly S [3]. Lignin can be used as an intrinsic resin used for the production of binder-less boards. Lignin is commonly extracted from hardwood and softwood, as well as cereal straw and stalk.

Xylan is the main hemicellulose in cereal plants, consisting of a linear backbone of (1,4)-linked β-D-xylopyranose backbone with varying degree and type of substitution. Xylans form hydrogen bonds with cellulose, covalent bonds (mainly a-benzyl ether linkages) with lignin and ester linkages with acetyl units and hydroxyl cinnamic acids. The main chain can be substituted in positions 2 or 3 or both with R-L-arabinofuranosyl, D-glupyranosyluronic acid, or D-xylopyranosyl residue; the xylopyranosyl units can be mono- or di-substituted and the arabinofuranosyl units can be further substituted with other sugars. Some naturally occurring xylans, particularly in wheat, also carry O-acetyl and feruloyl substituents. The degree of substitution varies significantly between xylans from different species, but also between different parts of the plant and between plant materials grown in different seasons. For example, xylans in sorghum, finger millet, rice and maize have more complex side chains than in wheat, oat and barley, which include xylopyranose, galactopyranose, and α-D-glucuronic acid or 4-O-methyl-α-D-glucuronic residues [4]. The degree and type of substitution have a great impact on the physicochemical properties of the xylan polymer (e.g. solubility, viscosity, water binding capacity) and consequently on its applications. Currently, arabinosylated xylans (AXs), primarily from wheat and maize, are used as thickening agents and as improvers of dough properties in bread processing. AXs are added since they have

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water binding properties [5]. Moreover, AXs have also been shown to exert potential biological activities, in particular being effective hypocholesterolemic agents, and effective agents to suppress blood pressure, bind hydrophobic mutagens in the diet, and inhibit the growth rate of tumors [1].

The study will focus on properties of fractions extracted from sorghum bran and stalk. Physicochemical properties were studied to determine their applicability as food-packaging materials.

II. MATERIALS AND METHODS

A. Xylan Extraction

Extraction of hemicelluloses from dried sorghum stalk was performed under alkaline conditions [6], [7]. Briefly, 5 g of dry-milled stalk 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 maintained at 50 °C and stirred at 200 rpm for 3 h, followed by centrifugation at 10000 rpm for 20 min. The collected alkaline insoluble residue was washed with deionised water and freeze-dried prior to further characterization. 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. The ethanol solution was designated as the alkali-lignin fraction and was collected as a solid after evaporating the ethanol.

B. Lignin Analysis

The acid soluble lignin content of the samples was determined according to a published protocol [8]. Approximately 50 mL of the acid hydrolysed solution produced from the analysis of monosaccharides were vacuum filtered and the absorbance of the aliquot was measured at 320 nm in a spectrophotometer.

C. X-Ray Diffraction

A single-crystal Oxford diffraction Gemini Ultra diffractometer was used to record the film diffractogram at 25 °C. The X-ray source was Ni-filtered Cu Kα radiation (40 kV and 35 mA). The composite films were mounted on a sample holder, and the pattern was recorded in the reflection mode at an angle 2θ over a range of 5.000° to 80.030° at a speed of 10°/min.

D. Thermal Analysis

Structural and thermal characterization of cellulose and xylan were carried out through DSC. DSC scans were performed at a heating rate of 5 °C/min from -50 °C to 250 °C under nitrogen environment.

E. Antioxidant Capacity

Scavenging activity of phenolic compounds against DPPH radicals was assessed according to the method of [9], [10] with some modifications. Briefly, 0.2 mL of various concentrations

of lignin was mixed with 2 mL of 2 mM DPPH-methanol solution. These samples were stored in the darkness for 30 minutes at 25 °C and the absorbance was measured at 517 nm. 0.2 mL water mixed with 2 mL of 2 mM DPPH-methanol solution was used as control while blank samples contained distilled water. Antioxidant activity is expressed by % inhibition and calculated using:

Percent (%) inhibition = $(A_0-A_1)/A_0$ x 100

where Ao = optical density of the blank and A_1 = optical density of the sample.

III. RESULTS AND DISCUSSION

Alkaline extraction disrupts the hydrogen and covalent bonds and loosens up the cell wall matrix, consequently releasing various polysaccharides. Hydroxyl ions disrupt the hydrogen bonds between cellulose and hemicellulose, and also break ester linkages, and hence solubilise part of the hemicellulose material [11]. An increase in the strength of alkali results in greater hemicellulose release. The yield of alkali-extracted xylan by cereal byproducts is varied around 16-31% (w/w) [4].

Alkali treatment of lignocellulosic materials, such as cereal straw, occurs by hydrolysing uronic and acetic esters, and by cellulose swelling. This decreases the crystallinity of cellulose. In addition, the treatment also cleaves the α-ether linkages between lignin and hemicelluloses and the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as p-coumaric and ferulic acids [7]. The results obtained by DSC are shown in Table I.

As can be observed, DSC thermograph revealed that the glass transition temperature (T_g) of the stalk (76.06 °C) was slightly lower than the cellulose fraction from the stalk (78.33 °C). This is probably related to an amorphous structure which is caused by alkaline extraction. Hydroxyl groups also can develop amorphous structure [12]. The glass transition value reflects a state transition between the rubbery state and the glassy state (or inversely) of a material as it is affected by temperature or moisture change, which is characterized by the glass transition temperature, also known as T_g [13]–[17].

In the hemicellulose fraction extracted from the stalk, the glass transition temperature was 40.79 °C, which is much lower than cellulose fraction. This could be linked to the structure of xylan since it has less hydrogen bonds than cellulose. The xylan fractions extracted from sorghum bran and stalk had almost similar T_g to arabinoxylan and commercial xylan samples, was and specifically in the range of 31.72-44.19 °C.

In term of the bran, the moisture content in the cellulose bran may have caused to the lower glass transition temperature (T_g), compared to the initial bran. Tg value will decrease in correlation to the increment of water activity [15], [16]. In a temperature of storage above Tg, water content and temperature highly affect the rate of crystallisation of amorphous sugar [2]. Concerning water content, the molecular mobility rises as a result of the increment of it and

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consequently, give effect on Tg temperature. Thus, by declining Tg and water plasticisation promotes crystallisation in amorphous sugar [2], [3]. Branching in xylan or the ratio arabinose and xylose may also lead to lower Tg. The ratio of arabinose and xylose in the bran was 0.82, which indicated its low crystallinity. Amorphous substances will absorb water which bound in the material system. Due to this fact, water constitutes a good plasticiser. Therefore, water content and

glass transition temperature are essentially considered in this matter. These phenomena have also been reported by Heikkenen et al. [18]. A drop of glass transition temperature occurs when the dry material of amorphous and partially solid matters absorb the water, and afterward, the higher water content may lead to a gradual decline of Tg temperature [19].
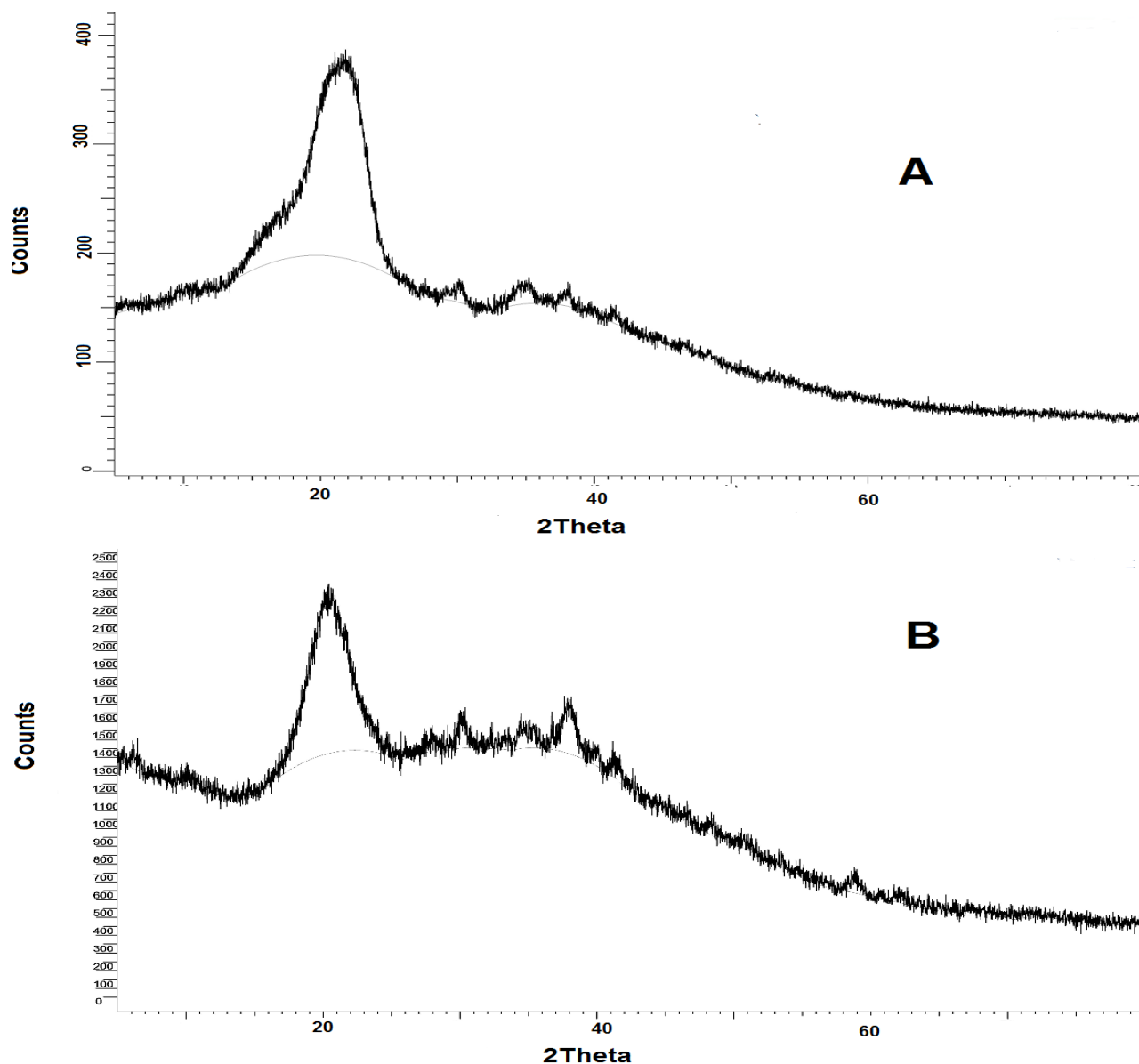

Fig. 1 X-Ray diffraction pattern of (A) cellulose stalk and (B) cellulose bran

Structural analysis of cellulose and hemicellulose (xylan) fractions extracted from sorghum byproducts was performed by XRD using Cu Kα radiation (40 kV and 35 mA). 2θ was measured from 5 ℃ to 70 ℃. The XRD patterns of these fractions are depicted in Figs. 1 and 2. Commercial arabinoxylan and xylan samples were also analysed as a comparison. Cellulose fractions from sorghum bran and stalk

were more crystalline than xylan fractions. The percentage of crystallinity for cellulose stalk and cellulose bran was 46.5% and 41.8%, respectively. In addition, the major peaks of the XRD patterns for both cellulose stalk and bran showed diffraction at the same point temperature for (2θ=~20 ℃). Therefore, it was confirmed that cellulose fractions in both materials are the same, and mainly glucose.

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TABLE I
THERMAL ANALYSIS PROPERTIES AND CRYSTALLINITY

Samples	Moisture Content (%)	Glass transition temperature (°C)	Crystallinity (%)
Stalk	4.13±0.02	76.06	53.4
Cellulose stalk	5.81±0.78	78.33	34.6
Hemicellulose stalk	2.88±0.14	40.79	46.5
Bran	5.18±0.04	31.72	46.5
Cellulose Bran	22.72±0.57	29.54	28.1
Hemicellulose bran	4.47±0.14	41.92	41.8
Commercial xylan	1.11±0.31	44.19	43.6
Commercial arabinoxylan	7.62±0.49	31.72	35
Lignin	1.03±0.18	93.05	89.3

In terms of the stalk, the percentage of crystallinity of the initial stalk was higher compared to its extracted cellulose fraction (53.4% and 34.6%, respectively) (Table I). Therefore, in agreement with DSC results, the T_g of cellulose stalk was slightly higher compared to the initial stalk as a result of a more amorphous structure of the extracted cellulose. In the cellulose fraction, water absorption depends largely on the availability of the free hydroxyl groups. It has been generally considered that water absorption occurs almost in all the amorphous regions of cellulose without considering the free hydroxyl groups that may be available on the surfaces of crystallites [20].

The crystallinity of xylans is depicted in Fig. 2; it can be seen that the crystallinityies of commercial xylan, commercial arabinoxylan, xylan bran, and xylan stalk were 43.6%; 35%; 41.8%, and 46.5%, respectively. Additionally, with regards to xylan samples, the amorphous peaks of xylan-sorghum bran, xylan-sorghum stalk, commercial xylan and arabinoxylan were defined at approximately at 20 °C, which denotes mainly an amorphous state of the material but contains small crystalline peaks in the region.
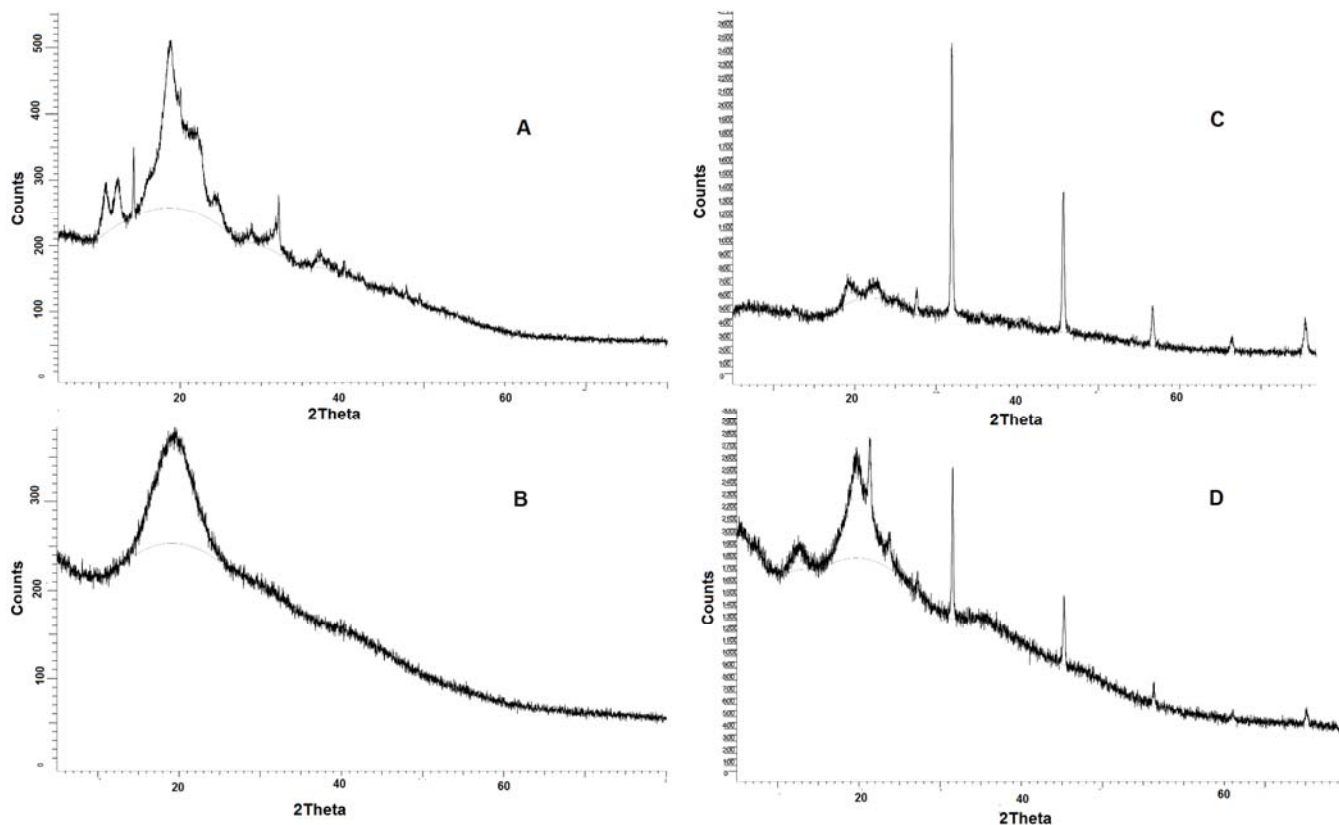

Fig. 2 X-Ray diffraction pattern of (A) commercial xylan, (B) commercial arabinoxylan, (C) xylan extracted from sorghum bran and (D) xylan extracted from sorghum stalk

In terms of xylans' crystallinity, the ratio of arabinose/ xylose affects their amorphous or crystalline structure as reported by Hoije et al. [21]; for instance, when AX was debranched with α-l-arabinofuranosidase, its crystallinity increased. Furthermore, this change in the structure was correlated to mechanical performance and oxygen permeability, when applied in films [18], [22]. The presence of larger unsubstituted regions in the xylan chains can give rise to strong hydrogen bonds, causing interchain aggregation, and can also render the isolated material partly crystalline [23]. The use of different solvents and processing conditions may alter the crystal habit of raw material, leading to variations in its physicochemical properties such as melting point, solubility, true density, dissolution profile, flowability, compressibility, structure, and thermal stability [24]. Xylan stalk had the lowest intensity among tested samples. The diffractogram showed that a semicrystalline xylan peak was present, an observation in agreement with Hoije et al. [21].

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This indicates that some of the xylan is present in its crystalline form. It is probable that unsubstituted regions crystallize and that substituted regions are amorphous.

Lignin is the most abundant renewable source, after cellulose, and is composed of aromatic units. According to previous studies, the chemical structure of lignin renders it a sustainable candidate feedstock for aromatic chemicals [25]. In this study, lignin fraction was also characterised in terms of phenolic content and its antioxidant capacity. It can be observed that lignin stalk had higher phenolic content (9.35 mg/g) compared to bran (Table II). In lignin stalk, ρ-coumaric acid was found in greater amounts than ferulic acid (Fig. 3), contrary to that of lignin bran. This finding is in agreement with the fact that coumaryl alcohol is the most abundant aromatic compound in plants belonging to *Poaceae* family.

Due to the amount of monolignol compounds in lignin fraction which break after alkaline extraction, the antioxidant capacity of the lignin fractions was determined. Free radical scavenging activity was evaluated *in vitro* using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The antioxidant capacity of lignin stalk, expressed as an inhibition percentage, was much higher than lignin bran. This can be explained by the fact that lignin stalk had also a higher concentration of phenolics compared to the bran, mainly in the form of ρ-coumaric and ferulic acid (Fig. 3). Lignin is contained in considerable amounts in stalk since lignin plays a key role in the rigidity and strength of the plant structure [25]. Thus, lignin could be a source of aromatic chemicals for the chemical industry.

TABLE II
PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY IN LIGNIN FRACTION

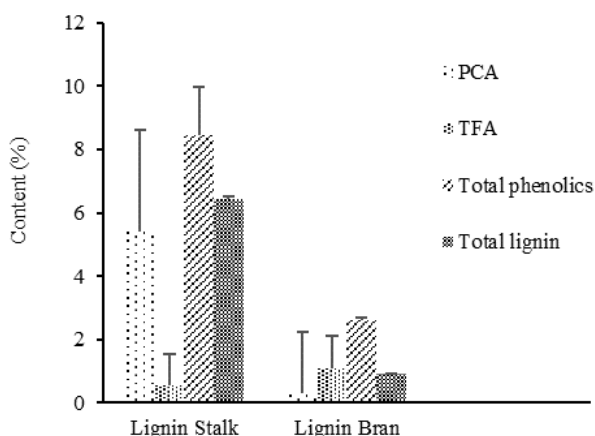| Samples	Phenolic content (mg/g)	Antioxidant capacity (%)
Lignin Bran	1.57±1.45	23.67±0.56
Lignin Stalk	9.35±1.02	54.57±0.48

Fig. 3 Phenolic and lignin content in lignin fractions

The antioxidant capacity of the lignin fractions could be attributed to the presence of ferulic acid, ferulates (which form linkages between hemicellulose and lignin), coniferaldehyde, sinapaldehyde, 5-hydroxyconiferyl alcohol, and acylated monolignols containing acetate, p-hydroxybenzoate, or p-coumarate moieties. These compounds are part of the lignin structure. Alkali treatment in lignocellulosic materials, such as cereal straw, results in the hydrolysis of uronic and acetic esters. In addition, the treatment also cleaves the α-ether linkages between lignin and hemicelluloses and the ester bonds between lignin and/or hemicelluloses, which in turn lead to the release of hydroxycinnamic acids, such as *p*-coumaric and ferulic acid [7].

IV. CONCLUSION

In this paper, the characterisation of fractions extracted from sorghum byproducts, namely bran and the stalk was performed to determine their applicability as food-packaging materials. Cellulose, hemicellulose, and lignin fractions of sorghum bran and stalk had different glass transition temperature, which is related to their crystallinity properties. Water content and crystal structure of polymers are important factors to be considered when investigating potential applications. Lignin fractions contained phenolic compounds that have potential to be used as antioxidants, antimicrobial and preservative agents, in packaging industry or other non-food industries.

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