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Isolation and characterization of lignin from okra (*Abelmoschus esculentus*) fibre and stick

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Abstract

The okra (*Abelmoschus esculentus*) plant consists of bast fiber and core (stick). The bast fibre of okra plant was characterized with high α -cellulose (56.7%) and low lignin (12.6%) and stick with low α -cellulose (34.3%) and high lignin (25.2%) content. Lignin was isolated from the fiber and stick by acidolytic dioxane method and characterized for elemental analysis, methoxyl analysis and FTIR and ¹H-NMR spectroscopy. The C₉ formulas for okra stick and fibre lignin were C₉H_{9.61}O_{4.63}(OCH₃)_{1.24} and C₉H_{8.61}O_{4.66}(OCH₃)_{1.49}, respectively. Both of the lignins were of the guaiacyl-syringyl type. The bands of FT-IR spectrum at 1327 cm⁻¹, 1122 cm⁻¹ and 837 cm⁻¹ associated with syringyl unit was higher in okra fibre lignin than in the okra stick lignin. The structural analysis revealed that the average numbers of proton of β -O-4 (Ha & H β) per C₉ unit in okra stick and fibre lignin were 1.53 and 1.20, respectively. The β -O-4 units in these lignins had predominately erythro stereochemistry type.

Keywords: Okra plant; Fibre and stick; Dioxane lignin; Syringyl unit; Guaiacyl unit; Erythro type

Introduction

Over the last few years, forest preservation and rational use of forest and agricultural residues has received attraction. The world consumption of paper, especially fine paper is expected to increase, which indicates increased demand of fibre. Traditionally, hardwood meets the demand for short fibre in fine paper production. The wood is being gradually replaced by nonwood; although about 10 % of the world's overall pulp is obtained from nonwood. In many countries, like Bangladesh, the wood supplies availability will not continue to meet the rising demand for fibre. Research shows that agricultural wastes could be a potential source of fibre for papermaking. Okra plant (*Abelmoschus esculentus*) is one of the major vegetable wastes in Bangladesh which is an annual or perennial plant of about 2 m tall. *Abelmoschus esculentus* is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits consumed as vegetables. Okra plant consists of short fiber stick and long bast fibre like jute plant (Jahan *et al.*, 2008). Chemical characteristics of this plant have been presented in another article (Jahan *et al.*, 2012). The holocellulose and α -cellulose content are higher and lignin content is lower in the bast fibre than that of stick. The α -cellulose content in bast fibre is 56.7% while stick contains as little as 34.4%. The lignin

content in the bast fibre is similar to jute fibre (Jahan *et al.*, 2007), while lignin present in the okra stick is similar to hardwoods. In order to use the agricultural waste as pulping feedstock, it is needed to characterize the raw material, and also to gain knowledge of delignification and characteristics of its lignin. Isolation and characterization of lignin from lignocelluloses is a fundamental step for investigations of suitable pulping process and conditions. Lignin offers significant opportunity and presents many challenges for enhancing the operation of a lignocellulosic biorefinery. Lignin is a promising feedstock for generating renewable substitutes for fossil fuel.

Lignin is a heterogeneous and highly cross-linked macromolecule that represents the second most abundant natural polymeric material on earth (Fengel and Wegener, 1989). It is known that the most of the lignin in lignocelluloses consists of nonphenolic aryl-glycerol-b-O-aryl ether units. Other units, such as phenylcoumaran (b-5), resinol (b-b), and dibenzodioxocins (5-5/b-O-4, α -O-4) are also present within the lignin macromolecule (Fengel and Wegener 1989; Ralph *et al.*, 2000). Furthermore, lignin is covalently linked to carbohydrates forming a lignin-carbohydrate network made

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up of benzyl-ether (Eriksson *et al.*, 1980; Lowoko *et al.*, 2003; Yaku *et al.*, 1981), benzyl-ester (Eriksson *et al.*, 1980), and phenyl-glycoside (Yaku *et al.*, 1976) bonds. The characteristics of lignin obtained from biomass depend on both the kind of species, location and on the types. Still there is no perfect technique that would provide extraction of high lignin yield without chemical modification, and recovery of pure product (Tamminen and Hortling, 1999). A great variation is observed in various species in regards to ultimate analysis, methoxyl and hydroxyl content as well as syringyl-guaiacyl ratio. The major problem of elucidating lignin structure is total isolation of lignin in unaltered form. Björkman developed a method known as “milled wood lignin (MWL)” for the isolation of lignin by neutral solvent at room temperature, which is considered as unaltered lignin. But the yield is less, certainly does not represent whole lignin (Björkman, 1957). Acidic dioxane methods give much better yield than MWL (Tamminen and Hortling, 1999), although this method may alter lignin structure due to acidic condition. Therefore, the main objectives of this study were to isolate lignin by dioxane/ HCl mixture from the okra stick and bast fibre, and the isolated lignin characterized by elemental, methoxyl, FTIR and ¹H-NMR spectroscopy.

Materials and methods

Materials

A farmer supplied okra plants after harvesting the crop in the region of Savar, Dhaka. Subsequently, okra plant was retted for a month and the fibre and stick separated. Afterwards, these were properly sun dried. The stick and fibre were separately ground in a Willey Mill and screened to 40/60 mesh for lignin isolation.

Isolation of lignin

Okra stick and fibre meals (40-60 mesh) were refluxed with acetone solvent for 12 h. The acetone extractive free meals were refluxed with acidic dioxane (9:1) solution. The concentration of HCl in dioxane solution was 0.2 N. The dioxane to meal ratio was 8:1. The meal was refluxed with dioxane solution for 1 h at N₂ atm. The N₂ flow was maintained at 50 ml/min. After completing reflux time, wood meal dioxane mixture was filtered in a Buckner funnel using Whatmann filter paper no. 2. The residue was washed with dioxane solution. The filtrate was concentrated in a vacuum evaporator at 40°C. Then conc. dioxane soln. was added drop wise to deionized water to precipitate lignin. Precipitated lignin was centrifuged and washed frequently until reaching pH around 7 and dried. Dried crude lignin was dissolved in dioxane (9:1), and again precipitated in ether with constant stirring by magnetic bar. The precipitated lignin was dried under vacuum over P₂O₅ and weighed.

Acetylation of lignin

A mixture of 100 mg purified lignin and 1.5 ml pyridine-acetic anhydride (1:1) was kept for 72 h with frequent stirring. The solution was added to a 10-fold volume of ice-cold water whereupon the acetylated sample was recovered as a precipitate, which was purified by successive washing with water and dried in vacuum desiccators over P₂O₅.

Elemental analysis

Analysis of C and H in okra stick and fibre lignin was carried out by Analytical Research Division of BCSIR Laboratories, Dhaka, and the oxygen was determined by difference. The methoxyl content in lignin was determined in accordance to Japan International Standard Methods (JIS P8013 1972).

Spectroscopy

FTIR: Infrared spectra were recorded by using a Shimadzu FTIR spectrometer model 8201PC. The dried samples were embedded in KBr pellets in the concentration of about 1 mg/ 100 mg KBr. The spectra were recorded in the absorption band mode in the range 4000-400 cm⁻¹. ¹H NMR: Spectra of lignin samples (100 mg of acetylated lignin contained in 0.5 ml CDCl₃) were recorded in a Bruker 400 spectrometer. CDCl₃ solvent was used as internal standard (ppm 7.25). For quantification of protons, the signal in specified region of the spectrum was integrated with respect to a spectrum-wide baseline drawn at the level of the background noise, and the results were referred to the signal for methoxyl protons. The average number per C₉ unit was established as described above.

Results and discussion

The yield of dioxane lignin from okra fibre was 70.5% and stick 68.7%, (Table I). The acidic isolation conditions employed for the lignin extraction process may be the result of the hydrolysis of lignin-carbohydrate complex (LCC) linkages allowing the release of lignin fragments into the aqueous dioxane solution from the okra fibre and stick (Gellerstedt *et al.*, 1994).

Table I. Yields of lignin

Sample	Lignin yield, % (based on Klason lignin)
Okra stick	68.7
Bast fibre	70.5

Elemental analysis and C₉ formula

Table II. shows the elemental analysis, methoxyl content, and their average C₉ units as calculated from these analytical data for the lignin. Since lignin always contains associated

carbohydrates and impurities, the average C₉ formula was calculated on the assumption of pure lignin. The number of methoxyl group per C₉ unit in okra fibre and okra stick's lignin was 1.49 and 1.24, respectively. The lower methoxyl group content of okra stick lignin indicates the lower content

Table II. Elemental analyses, methoxyl contents and per-C₉-unit formula of okra fibre and stick lignin

Lignin sample	Elemental analysis, %				C ₉ formula
	C	H	O	OCH ₃	
Okra stick	53.50	5.75	40.75	16.61	C ₉ H _{9.61} O _{4.63} (OCH ₃) _{1.24}
Okra fibre	53.02	5.51	41.47	19.48	C ₉ H _{8.61} O _{4.66} (OCH ₃) _{1.49}

Table III. Assignment of FTIR spectra of lignin from okra fibre and stick.

Peak location range cm ⁻¹	Assignment	Okra fibre	Okra stick
3412 -3460	O-H stretching	3421.7	3425.3
3000 -2842	C-H stretch in methyl and methylene group	2936.7	2939.2
1738 -1709	C=O stretch in unconjugated ketone, carbonyl and ester groups	1720.5	1712.5
1675 -1655	C=O stretching in conjugated p-subst. Aryl ketones	1670.4	1667.8
1593 -1605	Aromatic skeleton vibrations plus C=O stretching; S>G: G _{condensed} > G _{etherified}	1593.2	1604.7
1505 -1515	Aromatic skeleton vibrations (G>S)	1504.5	1512.9
1460 -1470	C-H deformations (asym in -CH ₃ and -CH ₂)	1462.0	1462.0
1422 -1430	Aromatic skeleton vibrations combined with C-H in plane deformations	1423.5	1423.5
1365 -1370	Aliphatic C-H stretching in CH ₃ and phen. OH	-	-
1325 -1330	Condensed S and G ring (G ring bound via position 5)	1327.0	1328.9
1266 -1270	G ring plus C+O stretching	-	-
1221 -1230	C-C + C-O + C=O stretching (G _{condensed} > G _{etherified})	1222.9	1219.2
1166	Typical for HGS lignins; C=O in ester groups (conj.)	-	-
1140	Aromatic C-H in -plane deformation (typical of G unit; G _{condensed} > G _{etherified})	-	-
1125 -1128	Typical of S unit; also secondary alcohol & C=O str.	1120.6	1116.8
1086	C-O deformation in sec. alcohol & aliphatic ether	-	-
1030 -1035	Aromatic C-H in - plane deformation (G>S) plus C-O deform. in primary alcohols plus C-H stretching (unconjugated) -HC=CH - out of plane deformation. (trans)	1028.6	1033.9
966 -990	C-H out of plane (aromatic ring)	-	-
915 -925	C-H out of plane in positions 2, 5 and 6 (G units)	-	918.0
853 -858	C-H out of plane in positions 2 and 6 of S units	-	-
834 -835	C-H out of plane in positions 2, 5 and 6 of G units	833.5	833.4
817 -832		-	-

of syringyl type structure than that of okra fibre's lignin, which is close to the methoxyl content in cotton stalk's lignin and the lignin present in dhaincha respectively (Jahan *et al.*, 2007a). Similar differences of lignin in jute stick and fibre was also found elsewhere (Islam and Sarkanen, 1993). The methoxyl content in okra fibre lignin and okra stick lignin was very similar to temperate hardwood (aspen) lignin and tropical hardwood (*T. orientalis*) lignin, respectively (Jahan and Mun, 2010).

band at 1600 cm^{-1} is assigned to the aromatic skeletal vibrations, 1510 cm^{-1} assigned to the aromatic skeletal vibrations coupled with C-H in plane deformations, 1460 cm^{-1} assigned to C-H deformations (asymmetric in methyl, methylene and methoxyl group). Both lignins showed stronger bands for syringyl unit than the guaiacyl units. The band intensity at 1327 cm^{-1} , 1122 cm^{-1} and 833 cm^{-1} for syringyl unit was higher in fiber lignin than in the okra stick lignin, while band at 1270 cm^{-1} , 1041 cm^{-1} and 918 cm^{-1} for guaiacyl units in okra stick lignin was stronger than fiber lignin. The results indicated the presence of both guaiacyl

Table IV. Assignments of signal and protons per C_9 structural unit in the ^1H NMR spectra of acetylated lignins of okra fibre and stick

Range ppm	Main assignments	Okra stick	Okra fibre
7.25 -6.80	Aromatic proton in guaiacyl units	0.45	0.31
6.80 -6.25	Aromatic proton in syringyl units	0.70	0.98
6.25 -5.75	H α of β -O-4 and β -1 structures	0.35	0.34
5.75 -5.24	H α of β -5 structure	0.56	0.30
5.20 -4.90	H of xylan residue		
4.90 -4.30	H α & H β of β -O-4 structures	1.53	1.20
4.30 -4.00	H α of β - β structures H of xylan residue	-	-
4.00 -3.48	H of methoxyl groups	3.72	4.47
2.50 -2.22	H of aromatic acetates	1.24	0.64
2.22 -1.60	H of aliphatic acetates	2.38	4.55
Total proton per C_9 structural unit		13.06	13.77

FTIR Spectra

To elucidate the structure of lignin, and investigate the differences in the structure of the dioxane lignin isolated from okra stick and fibre, FT-IR spectra were recorded and shown in Fig. 1. The assignment made by Faix (1991) is given in Table III. The ester carbonyl signal at 1714 cm^{-1} together and the band at 833 cm^{-1} suggests the presence of esterified *p*-coumaric units in okra fiber and stick lignin (Faix 1991), which are common in nonwood lignin (Seca *et al.*, 1998). The

and syringyl unit in the lignin molecule, but the syringyl unit in fibre lignin was higher than in the stick lignin. The result is consistent with the elemental analysis data.

^1H NMR

The integrated NMR spectrum obtained for acetylated okra fibre and okra stick lignin is shown in Fig. 2. Table IV lists the position of signal assigned by Lundquist (1992). Hydroxyl group: The numbers of free aliphatic and

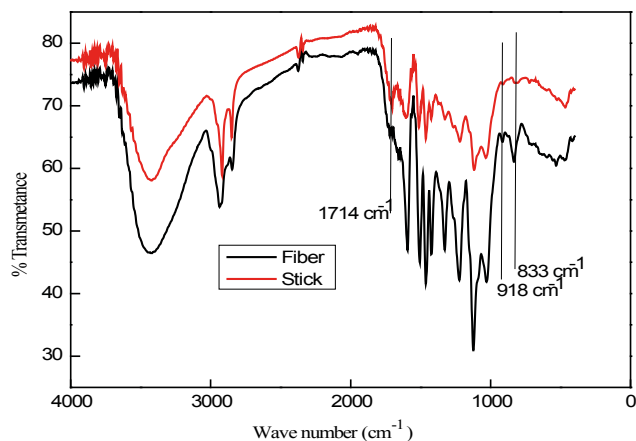


Fig. 1. FT-IR spectra of lignin isolated from okra fibre and okra stick

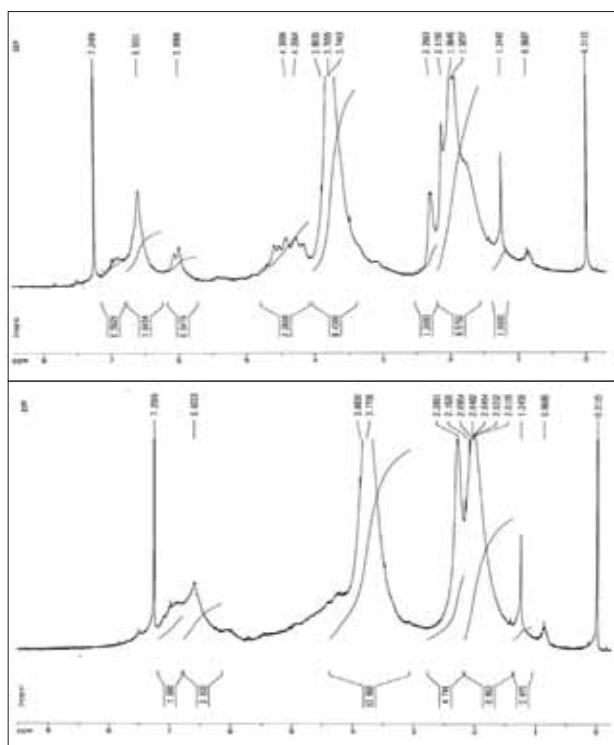


Fig. 2. FT-IR spectra of lignin isolated from okra fibre (top) and okra stick (bottom).

phenolic hydroxyl groups per C_9 unit were determined from the corresponding acetate signals. The numbers of proton per C_9 unit of phenolic hydroxyl group of okra stick and okra fibre lignin were 1.24 and 0.64, respectively. The proton of aliphatic hydroxyl group per C_9 unit was 2.38 and 4.55 for okra stick and fibre, respectively. It would expect that less aromatic and aliphatic OH for high molecular weight lignin.

Aromatic protons: Okra stick and okra fibre lignin spectra show two peaks in the aromatic proton region, which correspond to guaiacyl units (δ 6.9) and syringyl units (δ 6.6). Both lignins show stronger peak in syringyl units region (δ 6.6) than that of guaiacyl units region (δ 6.9) (Fig. 3). But stick lignin has higher guaiacyl peak than the corresponding fibre lignin. NMR integration shows that okra stick lignin contains 0.45 guaiacyl proton and 0.70 syringyl proton per C_9 units, while these are 0.31 and 0.98 for okra fibre lignin, respectively (Table IV), which suggests highly condensed lignins. Presence of lower amount of aromatic proton suggests the presence of *p*-coumaryl units. The result is in good agreement with the results of empirical formula and FTIR spectra (Table II).

β -O-4 structure: The aryl glycerol β -O-4 aryl ether linkage constitute the main intermonomeric connection in lignin (Lundquist 1992). NMR spectra of lignin of cotton stalks, jute stick and dhancha show that the structural element may contain both erythro and threo configurations due to the presence of proton at the C- α position of the side chain. The erythro protons (H_α) give stronger peak at δ 6.01 than the peak for threo form at δ 6.09 in okra fibre lignin. Akiyama *et al.*, (2003) reported that the angiosperms lignin contains higher erythro form in β -O-4 units than threo form. The average numbers of protons per C_9 (H_α & H_β) of β -O-4 in okra stick and fibre lignins were 1.53 and 1.20, respectively. The broad signal around 7.6 ppm was assigned to H-2/H-6 aromatic protons and H_α in *p*-coumaric acid structures (Ralph *et al.*, 1996), confirming their significant amounts in okra fibre and stick lignin.

Conclusion

The lignins isolated from the okra fibre and sticks are mainly composed with syringyl unit and guaiacyl unit. Okra fibre lignin contains higher syringyl unit compared with stick lignin. The OCH_3/C_9 is 1.49 for okra fibre lignin and 1.24 for stick lignin. From the 1H NMR spectra, the number of proton of phenolic and aliphatic hydroxyl group were estimated to be 0.41 and 0.79 for okra stick lignin and 0.21 and 1.51 for okra fibre per C_9 unit, respectively. 1H NMR study showed that the β -O-4 structural unit is higher for lignin of stick. Both lignins contain both erythro and threo configuration, but erythro proportion was higher. Overall the results of this investigation indicate that the okra plant lignin is of guaiacyl-syringyl and significant amount of *p*-coumaryl type.

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