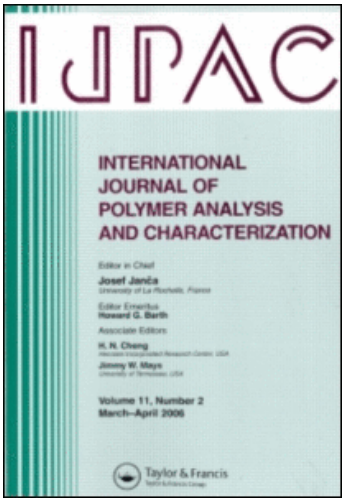

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Runcang Sun[a]; J. Mark Lawther[a]; W. B. Banks[b]
[a] BioComposites Centre, University of Wales, Bangor, Gwynedd, United Kingdom [b] School of Agricultural & Forest Sciences, University of Wales, Bangor, Gwynedd, United Kingdom

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Extraction and Characterization of Xylose-Rich Pectic Polysaccharide from Wheat Straw

RUNCANG SUN[a,*], J. MARK LAWTHER[a] and W. B. BANKS[b]

[a]*BioComposites Centre and* [b]*School of Agricultural & Forest Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, United Kingdom*

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Xylose-rich pectic polysaccharide (XRPP) was extracted from defatted and protein-free wheat straw with a pH 1.6 solution adjusted with 20% hydrochloric acid. The yield of XRPP was 1.1% of the dried wheat straw. The isolated XRPP contained 44.8% galacturonic acid released by pectolyase treatment and 32.1% released by 2 N trifluoroacetic acid hydrolysis. The XRPP also contained 17.1% neutral sugars released by pectinase treatment and 28.4% released by acid hydrolysis. The major sugar components were found to be xylose and galactose. The weight-average molecular weight (M_W) of XRPP was about 8000 g/mol. The XRPP had low viscosity and low optical rotation as compared to citrus pectin. A comparison of the FT-IR spectroscopic data of citrus pectin and XRPP showed that the extracted XRPP belongs to pectic substances and differs from hemicelluloses with an intensive absorption band at 1740 cm^{-1}. The content of phenolic acid and aldehydes associated in the isolated XRPP, determined by alkaline nitrobenzene oxidation, was 1.10%.

Keywords: Xylose-rich pectic polysaccharide, pectin, wheat straw, sugars, lignin, FT-IR, phenolic acids and aldehydes

INTRODUCTION

There is growing interest in the use of cereal straws, such as wheat straw for a foodstuff after upgrading its digestibility by various methods, or as a raw material for paper and board production. This is particularly important in areas with limited forest resources.[1] For these reasons, good physico-chemical characterization of cereal straw is necessary.

*Corresponding author.

The pectic polysaccharides are restricted to the primary cell wall and middle lamella of higher plant tissues and growth zones.[2] Their important physiological and nutritional functions, such as hypocholesterolemic effect, increased excretion of faecal sterols and lipids, binding of polyvalent cations and increased requirement for vitamin B_{12} have been reported in a number of publications.[3] They are most abundant in soft tissues, such as rinds of citrus fruit, sugar beet pulp, and apple peels, but are present in only small proportions in woody tissue.[4] These substances are widely used in various foods including jam, jellies and confectioneries as gelling agents, thickeners or emulsifiers.[5]

The pectic polysaccharides or pectins in plant tissues are most frequently branched heteropolysaccharides comprised of galacturonic acid, arabinose and galactose with small quantities of other sugars, including xylose and rhamnose. Traces of 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose are usually also present as constituents.[4,6] Rhamnose coexists with galacturonic acid in the main chain of pectin, and arabinose and galactose are found in the side chains. Depending upon sources, galacturonic acid residues of the pectin are esterified to various degrees, and the size and compositions of the neutral sugar side chains can vary.[7]

To date, the structural features of the pectic polysaccharides and the cell wall have been studied extensively by the techniques of chemical analysis and enzymatic degradation. In addition, research on isolation and physicochemical characterization of pectin from citrus peels, apple peels, sunflower head residues and sugar beet pulp has been reported.[7] However, the pectic polysaccharides extracted from wheat straw have been only reported by Przeszlakowska.[8] He extracted 0.44% pectic substances from wheat stem. In addition, information regarding wheat straw pectin has been also illustrated by Harbers and co-workers[9] with scanning electron microscopy. They showed that wheat straw cell walls possess a relatively small amount of pectin.

The objectives of this study, which is part of a research program on a multiuse approach to cereal straw fractionation using thermomechanical pulping, were to isolate the pectic polysaccharides from wheat straw and to study the physicochemical properties of the polymers.

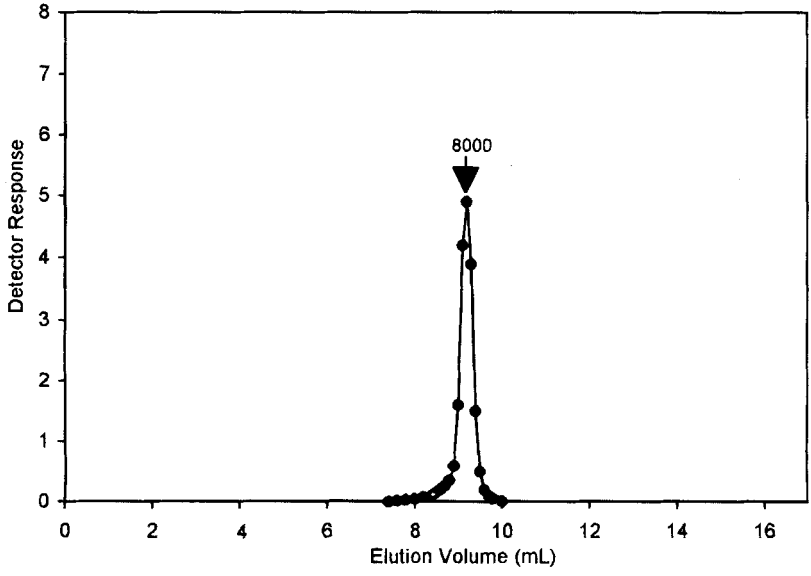
Experimental

Wheat straw was obtained from Silsoe Research Institute (Silsoe, Bedfordshire, UK), and was dried in a cabinet oven with air circulation at 60°C for 16 h. The dried wheat straw was then ground using a Christie

Laboratory mill to pass a 60-mesh size screen and stored at 5°C until use. The procedure for the preparation of wheat straw cell-wall material described by Carre *et al.*[10] and Düsterhöft and Voragen[11] was used in this study and with some modifications.[12]

The scheme for extraction and isolation of pectin from wheat straw is shown in Scheme 1. Xylose-rich pectic polysaccharide (XRPP) was extracted with pH 1.6 HCl solution at 85°C for 4 h from the defatted and protein-free cell-wall preparation. The extract was adjusted to pH 5.0 with ammonia, concentrated on a rotary evaporator under reduced pressure at 40°C against distilled water, and firstly precipitated with 5 volumes of 96% ethanol. After washing twice with 80% ethanol and drying in an air circulated oven at 60°C for 2 h, the pellet was redissolved with distilled water and then precipitated with 4 vols 96% ethanol. Before the pellet was gently ground, the precipitated pellet was washed twice with 70% ethanol and air-dried. The resultant white powder was labeled 'xylose-rich pectic polysaccharide' and kept in a refrigerator at 0°C until analysis.

For measurement of the neutral sugars and galacturonic acid in extracted XRPP, a comparison of acid hydrolysis with pectinase treatment on the



SCHEME 1 Scheme for extraction and isolation of xylose-rich pectic polysaccharide from wheat straw.

release of neutral sugars was used in this study. In the acid hydrolysis, The XRPP (60 mg) was hydrolyzed with 15 mL of 2 N trifluoroacetic acid at 120°C for 2 h in sealed pressure tubes.[13] The hydrolysate (10 mL) was dried by vacuum evaporation at 40°C. The dried materials were added to 10 mL of 4:1 mixture of ethanol: toluene and evaporated in a rotary-evaporator flask under reduced pressure to dryness again. 1.5 mL of anhydrous pyridine (stored over KOH) was added and solutions were transferred to glass vials. Sugars were converted to trimethylsilyl (TMS) ether derivatives by addition of 0.5 mL hexamethyldisilazane and 0.25 mL trimethylchlorosilane, with shaking in vials for 2 min, and analyzed by GC, using flame ionization detection, on a glass column (1.70 m × 2 mm i.d.) packed with 3% OV-17 on 80–100 Supelcoport (Supelco, Bellefonte, Penn.). The injector and detector temperatures were 180°C and 250°C, respectively, and the oven temperature programmed from 100° to 190°C at 2°C/min with a 5-min hold at 190°C. Nitrogen was used as a carrier gas, and its flow was maintained at 36 mL/min.

In the pectinase reaction, 60 mg XRPP was dissolved in the 15 mL of KH_2PO_4-NaOH buffer, pH 5.6 and 10 mg pectolyase (p-3026, 3.4 units/mg solid, Sigma) was added. The mixture was incubated for 7 h at 35°C in a reciprocal incubator. After filtration, 10 mL of filtrate was evaporated to dry at 40°C under reduced pressure. The sugars of arabinose, xylose, mannose, galactose and glucose were determined by GC after conversion to trimethylsilyl ether derivatives as method mentioned above. myo-Inositol was used as an internal standard. The values of rhamnose released by acid hydrolysis and pectinase reaction were determined by the quantitative colorimetric procedure of Dische and Shettles,[14] and Gibbons,[15] respectively.

The uronic acids released by acid hydrolysis and pectinase reaction were assayed colorimetrically as anhydrogalacturonic acid using 3-phenylphenol color reagent according to the procedure outlined by Blumenkrantz and Asboe-Hanson[16] with the modification of Wedig and co-workers.[17] A Hewlett-Packard diode array 8452A spectrophotometer was used to measure anhydrogalacturonic acid at a wavelength of 520 nm. A standard curve was constructed using 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL of galacturonic acid.

Methyl ester content was determined according to the method described by Wood and Siddiqui.[18] Acetic acid content was estimated using the transesterification method outlined by Browing.[19] The weight-average molecular weights of the XRPP were determined by gel permeation chromatography.[12]

Viscosity was measured using a Brookfield Synchro-Lectric Viscometer (Model LV). A citrus pectin was used as a reference. XRPP samples (2%, w/v) were prepared in 0.1 M sodium phosphate buffer, pH 7.0, allowed to hydrate at 4°C for 16 h.[20,21] Viscosity was then estimated at 25°C.

Optical rotation was determined on a polarimeter (Perkin Elmer, type 108) according to the methods described by Phatak *et al.*[20] and McCready *et al.*[22] XRPP samples (1.0%, w/v) were prepared in double distilled water, and solutions were centrifuged before measurement of optical rotation. A citrus pectin was again used as a reference.

Gelling property of XRPP samples was tested according to the procedures of Phatak *et al.*[20] and Chang and Miyamoto.[23] XRPP samples were prepared in distilled water at a concentration of 1.0% (w/v).

The method for alkaline nitrobenzene oxidation of lignin remaining attached to/associated with XRPP fraction is given in ref.[12] IR spectra were obtained on an IR spectrophotometer (Mattson Cygnus 100), using a KBr disc containing 1% finely ground samples. All chemicals were of analytical or regent grade. All experiments were performed in duplicate and yield is given on a dry wheat straw weight basis.

RESULTS AND DISCUSSION

The yield of XRPP and the contents of anhydrogalacturonic acid, methoxyl, acetyl and ash in XRPP are summarized in Table I. Extraction of wheat straw at 85°C for 4 h yielded XRPP value of 1.1% for the given extraction solution. The anhydrogalacturonic acid contents released by pectinase treatment and acid hydrolysis in the XRPP were found to be 44.8 and 32.1%, respectively. This result indicated that pectolyase p-3026 treatment XRPP under the conditions chosen is more effective for the release of galacturonic acid than 2 N trifluoroacetic acid hydrolysis at 120° for 2 h, which only 71.7% galacturonic acid was released. Further hydrolysis with 2 N trifluoroacetic acid at 120°C or increases of trifluoroacetic acid concentration/hydrolysis temperature are necessary for release of all of the galacturonic acid in extracted XRPP. The methoxy content was low, 5.8%, indicating that wheat straw XRPP is a low-methoxy XRPP. The data also indicates that the extracted XRPP possesses acetyl groups; the acetyl content of XRPP was 6.0%. Partial acid hydrolysis of the acetyl groups restored the gelation power of the pectin[24] because the presence of acetyl groups on a relatively

TABLE I The chemical composition of XRPP extracted with pH 1.6 HCl at 85°C for 4 h (1 g wheat straw/100 mL extractant) from defatted, protein-free and water-soluble polysaccharide-free wheat straw.

Composition (%)*	*XRPP*
XRPP yield	1.1
anhydrogalacturonic acid	44.8†, 32.1‡
degree of methylation	35.5
methoxy content	5.8
degree of acetylation	32.4
acetyl content	6.0
ash (w/w)	6.9

*Data are expressed on a dry basis, and represent the mean of duplicate runs.
†released by pectinase reaction.
‡released by acid hydrolysis.

small amount in pectin might prevent pectin from gelling.[20] The HCl extracted XRPP contained low amount of ash, 6.9%. This result agrees with Phatak and co-workers study[20] of sugar-beet pulp pectin.

Summarized in Table II is the neutral sugar compositions of XRPP released by pectinase treatment and acid hydrolysis, respectively. In both cases, the XRPP was found to be rich in xylose and galactose content, but low in mannose content. The total neutral sugar content in XRPP released by acid hydrolysis was 28.4%, while it dropped to 17.1% by the pectinase

TABLE II The composition of neutral sugars (%) in XRPP extracted with pH 1.6 HCl at 85°C for 4 h (1 g wheat straw/100 mL extractant) from defatted, protein-free and water-soluble polysaccharide-free wheat straw.

Sugars	*(%)**	*(%)+*
rhamnose	1.2	1.1
arabinose	0.9	3.5
xylose	8.2	14.0
mannose	0.1	0.2
galactose	5.8	6.8
glucose	0.9	2.8
total	17.1	28.4

*released by pectinase treatment.
†released by acid hydrolysis.

treatment. In contrast to the slightly higher content of rhamnose in the XRPP released by the pectinase treatment than by acid hydrolysis, the arabinose, xylose, mannose, galactose and glucose contents were higher in the acid hydrolysis than in the pectinase treatment. This high contents of rhamnose and galacturonic acid released by pectolyase treatment illustrated that rhamnose coexists with galacturonic acid in the main chain of XRPP, and arabinose galactose and xylose are found in the side chains. Aspinall *et al.*,[25] in discussions about pectin in soybean cotyledons, suggest the possibility that most of the xylose residues occur as xylosyl short-side-chains branched on the rhamnogalacturonan backbones. Hence in wheat straw XRPP it is possible that a proportion of the xylose is present as an integral component of the acidic pectic fraction. In addition, as XRPP was degraded by pectinase, they must have some homogalacturonan regions in the molecules and, therefore, it will be concluded that XRPP belongs to a group of pectic substances. Although XRPP is a minor constituent of the polysaccharides in wheat straw, it probably has a distinct functional role in the cell walls.

Matsuura and Hatanaka[26] observed that xylose-rich acidic polysaccharide having high mannose content was present in appreciable amounts in Japanese radish. This contained large amounts of neutral sugars, the galacturonic acid contents being only 11–25%. Xylose, arabinose, and galactose were found to be the major constituents with xylose comprising more than 50% of the sugars in each sample with the exception of one isolated from the leaves. Ray and co-workers[27] have also isolated a kind of xylose-rich acidic polysaccharide, extracted with an aqueous 10% trichloroacetic acid, from the seeds of *Acacia auriculaeformis*. The composition of monosaccharides in this xylose-rich acidic polysaccharide were arabinose, 13.5; xylose, 18.0; galactose, 23.0; glucose, 10.5; and glucuronic acid, 35.0%, respectively. Besides, since pectin represents the material found in the primary cell wall of plants, it is probable that the qualitative nature, as well as the quantity of various pectic polysaccharides found in pectin, may vary with the degree of maturity/differentiation of the plant source.[13]

Due to presumably degradation during acid extraction, pH 1.6 HCl extract had low weight-average molecular weight, 8000 (Fig. 1). The viscosity (2%, w/v) was 3.10 cPs. This value was much lower than those observed for citrus pectin. The pH, molecular size, degree of methylation, and temperature significantly affect the viscosity of wheat straw pectin. However, this low viscosity of wheat straw pectin, which is similar to sugar beet pulp pectin, indicates a high potential for application in low-caloric,

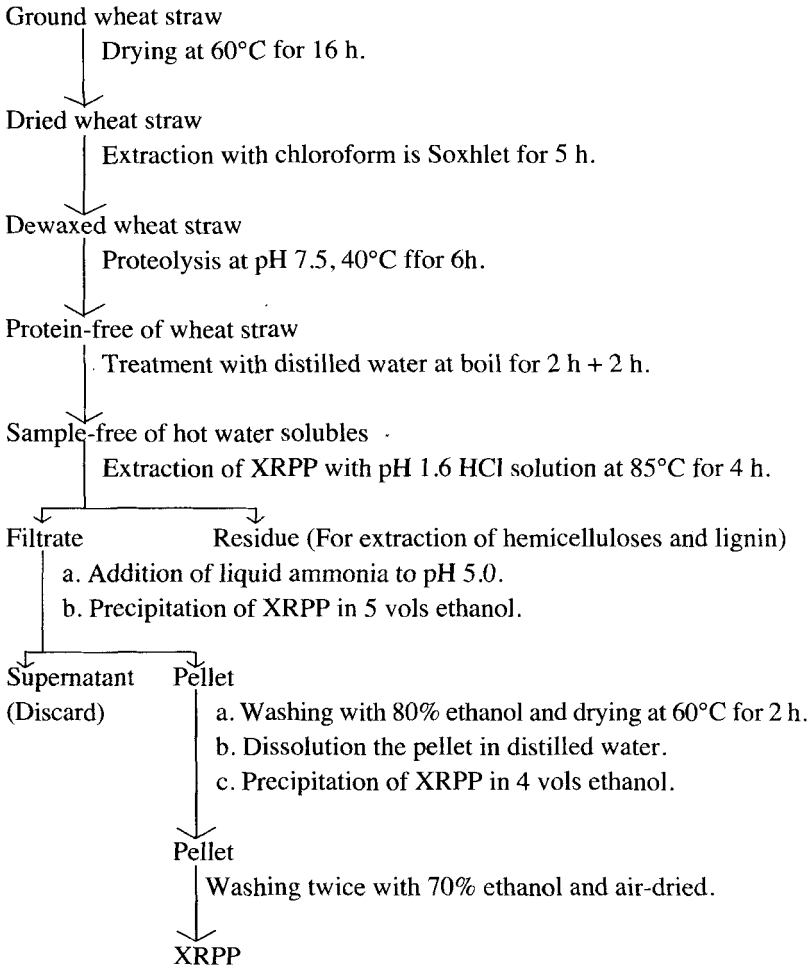


FIGURE 1 GPC molecular weight distribution of wheat straw xylose-rich pectic polysaccharide.

high fiber beverages.[20] The optical rotation of XRPP (1.0%, w/v) was 60°, which was also low compared to that exhibited by citrus pectin, as shown in Table III.

Because of the presence of acetyl groups, and the low viscosity and low molecular weight of the extracted wheat straw XRPP, no gel formation was

TABLE III Functional properties of isolated wheat straw XRPP

Sample	*Viscosity** (cPs, 25°C)	*Optical rotation*† $[\alpha]_D 25°$	*Weight-average molecular weight*
Citrus	93.50	+162	–
XRPP	3.10	+60	8000

*Viscosity determination: all the samples were in 2% concentration and measured at 25°C.
†The samples (1.0%, w/v) were prepared in distilled water and centrifuged before measuring optical rotation.

observed at 1% levels of addition to water. For comparison, citrus pectin at 1.0% formed a firm gel.

The FTIR spectra of citrus pectin and wheat straw XRPP (Fig. 2) appeared to be similar. Both of the spectra have absorptions at 1740, 1608, 1430, 1360, 1244, 1080, 1060, 1035, 890 and 524 cm^{-1}. The pectic substances belong to a class of carboxypolysaccharides which differ from neu-

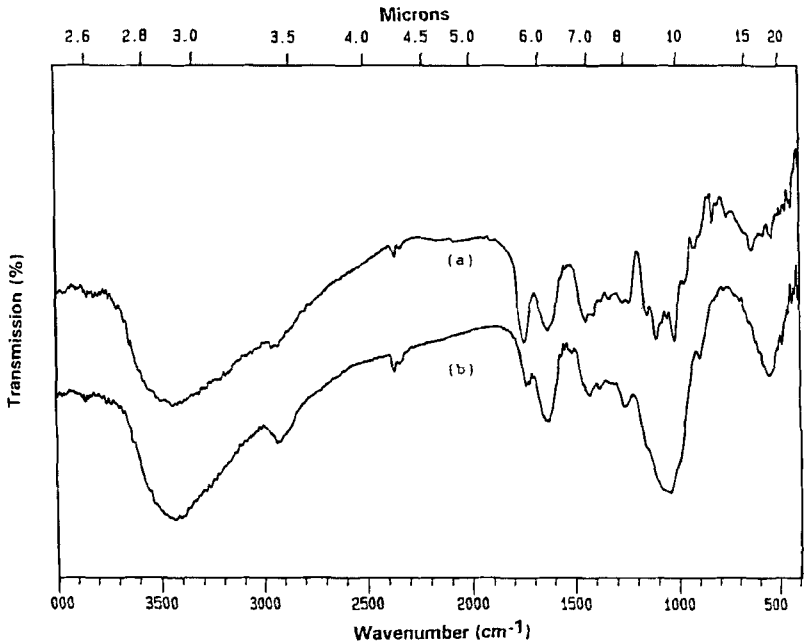


FIGURE 2 FT-IR spectra of citrus pectin (a) and wheat straw xylose-rich pectic polysaccharide (b).

tral polysaccharides, with an intense band in the region of 1740 cm^{-1} (for salts 1608 cm^{-1}) related to vibrations of the carboxyl group.[28] The extracted wheat straw hemicellulose and cellulose FT-IR spectra did not appear at this band (spectra not shown). From this point, the extracted wheat straw XRPP is assigned to pectic substances. The intensity ratio of the bands *vas*(COO^-) at 1608 cm^{-1} and $\nu(C{:}O)_{ester}$ at 1740 cm^{-1} corresponds to fully deesterified pectin and Me pectate.[29] Because of a much stronger absorptions at 1608 cm^{-1} than that at 1740 cm^{-1} in wheat straw XRPP, it is also clear that wheat straw XRPP is a low-methoxy XRPP, which is in accordance with the results obtained by colorimetry. However, on closer examination of the spectrum of the citrus pectin, it can be seen that there is a specific feature in the 950–1140 cm^{-1} region, where a group of six bands is observed at 950, 1008, 1035, 1060, 1080 and 1140 cm^{-1}. XRPP in this region has two weak absorption at 1008 and 1140 cm^{-1} and two very strong absorptions around 1060 and 1035 cm^{-1}, which can be ascribed to the neutral polysaccharides present in the extracted wheat straw XRPP. The very weak absorption at 1510 cm^{-1} in isolated wheat straw XRPP is due to aromatic skeleton vibrations in wheat straw lignin. These data indicated that the isolated XRPP fraction contained some amount of neutral polysaccharides and residual lignin.

The phenolic composition of the alkaline nitrobenzene oxidation of associated lignin in XRPP is summarized in Table IV. The total phenolic content in XRPP was 1.10%. The major components were found to be p-hydroxybenzoic acid, vanillin, syringic acid, and syringaldehyde. Gallic acid, protocatechuic acid and cinnamic acid appeared at trace amounts.

CONCLUSIONS

Our results indicated that wheat straw XRPP contained 5.8% methoxyl ester content and 6.0% acetyl ester groups. The XRPP also contained 28.4% neutral sugars, particularly rich in xylose. The viscosity of XRPP was very low. The isolated wheat straw XRPP did not form gels under the experimental conditions. The XRPP extracted under acidic conditions, such as pH 1.6 HCl, gave low molecular weight. In this work, wheat straw XRPP can be assigned to a group of polysaccharides termed as xylose-rich pectic polysaccharide. The fractional and structural characterisation of wheat straw XRPP is currently the subject of detailed further study in our laboratory.

TABLE IV The content (%) of phenolic acids and aldehydes of alkaline nitrobenzene oxidation of associated lignin in isolated XRPP from wheat straw.

*Phenolic acids and aldehydes**	*Content(%)*
gallic acid	0.00080
protocatechuic acid	0.0084
p-hydroxybenzoic acid	0.44
p-hydroxybenzaldehyde	0.032
vanillic acid	0.015
vanillin	0.19
syringic acid	0.13
syringaldehyde	0.13
ferulic acid	0.020
cinnamic acid	0.0050
unknown	0.11
total	1.10

*Determined by HPLC after alkaline nitrobenzene oxidation at 170°C for 2.5 h in steel autoclaves.

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