Review

Structural analysis for lignin characteristics in biomass straw

Seyed Hamidreza Ghaffar, Mizi Fan*

School of Engineering and Design, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom

A R T I C L E   I N F O

Article history:
Received 31 March 2013
Received in revised form
15 July 2013
Accepted 19 July 2013
Available online xxx

Keywords:
Straw lignin
Quantitative and qualitative analysis
NMR
FT-IR
Structural characteristics
Thermal analyses

A B S T R A C T

Agricultural by-products are the most promising feedstock for the generation of renewable, carbon neutral substitutes for synthetic materials (e.g. biofuel, building materials). The demand for efficient utilisation of lignin biomass has induced detailed analyses of its fundamental chemical structures and development of analysing technologies. This paper reviews the structural analysis techniques for straw lignin together with the morphology of the lignin biomass and the study of the form and structure of organisms and their specific structural features. The review showed that the studies on lignin could be divided into the qualitative and quantitative analyses; different analytical methods could provide significantly different results that are even sometimes not directly comparable. Among many techniques reviewed, the magnetic resonance techniques have proved to be efficient analytical tools for the structural elucidation of these complex biopolymers. Quantitative and qualitative structural analysis of lignin indicated a great potential for industrial crops optimisation due to in-depth microstructure interpretation, and detailed and accurate chemical composition although the composition and structure of straw lignin have been discovered highly complex and varied considerably within and among plants. The structure of lignin has remained one of the most difficult biopolymers to characterise, however recent advances in analytical chemistry and spectroscopy have dramatically improved the understanding of this natural resource, and further value added utilisations are being expected for the lignin and its related biomass.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Research concerning lignin has significantly increased due to its renewability and availability and the value added production of lignin based products, such as biofuel and sustainable construction materials for replacing petrochemicals based products [1,2]. The structure of lignin is probably the single most important parameter for understanding and hence utilising lignin materials, and has been analysed by many research workers e.g. Refs. [3–5]. A number of chemical and physical methods used for characterising lignin’s structure are destructive. However there are non-destructive methods too, such as FT-IR which is believed to be one of the most informative methods of lignin investigation, ultraviolet (UV), carbon-13 nuclear magnetic resonance spectroscopies ($^{13}$C NMR) and gas permeation chromatography (GPC) [6].

Lignin is an extremely complex three-dimensional polymer (typically found in vascular plants) formed by radical

* Corresponding author. Department of Civil Engineering, Brunel University, London UB8 3PH, United Kingdom.
E-mail address: mizi.fan@brunel.ac.uk (M. Fan).
0961-9534/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biombioe.2013.07.015

Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
coupling polymerisation of p-hydroxycinnamyl, coniferyl and syringyl alcohols; these three lignin precursors monolignols give rise to the so-called p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units, which show different abundances in lignin from different groups of vascular plants, as well as in different plant tissues and cell-wall layers [7]. These aromatic building units are linked with a variety of ether and carbon–carbon bonds. The predominant linkage is the so-called β-O-4 linkage. About 40–60% of all inter-unit linkages in lignin are via this ether bond. Different types of linkage between phenyl propane units form three-dimensional net structures and make it difficult to completely degrade non-regular lignin macromolecules. Due to lignin’s structural complexity it is hard to come to an agreed model for their chemical and physical structure. Lignin is primarily a structural material to add strength and rigidity to cell walls and constitutes between 15 and 40% mass fraction of the dry matter of plants, whether wood, straw or other natural woody plants. Lignin is more resistant to most forms of biological attack than cellulose and other structural polysaccharides [8–10]. Lignin acts as a matrix together with hemicelulloses for the cellulose microfibrils which are formed by ordered polymer chains that contain tightly packed, crystalline regions (Fig. 1). Covalent bonds between lignin and the carbohydrates have been suggested to consist of benzyl esters, benzyl ethers and phenyl glycosides [11–13].

There is a little study of lignin native (in situ) which does not change the structure of lignin, such as, chemically unmodified lignin by using ionic liquid 1-ethyl-3-methylimidazolium acetate [Emim] (CH3COO) as a pre-treatment solvent [14]. However, there has been much interest for industrial lignin due to the existence of large scale manufacturing processes, i.e. wood pulping, one of the largest industries in the world in which the main objective is to separate individual fibres from wood and to do so lignin must firstly be removed, and lignin from various resources and industries were studied through elemental analysis in order to understand the fundamentals of lignin and their properties for value added applications [15]. Huge quantities of lignin are extracted by wood pulping and other industries annually, though the bulk of which is still either burned to recover energy or otherwise considered waste. Only about 1–2% of this lignin is used to make other products [16]. Despite lignin’s unique characteristics as a natural product with multiple chemical and biophysical functionalities, it is largely underutilised due to its image as low quality and low value added material. Current work of researchers has focused on expanding the frontiers of application of NMR to straw lignin analysis in order to explore lignin’s potential [4,17,18].

The main goal of this paper is to study in details the structure of straw lignin and to review the analytical techniques up to date for the analysis of lignin; this will then contribute to a better understanding of lignin and hence could lead to the optimisation of lignin for specific and value added industrial utilisations.

2. Composition and morphology of straw

Straw consists mainly of three groups of organic compounds: cellulose, hemicellulose and lignin which account for more than 80% of the dry matter of cereal straws such as oats, barley and wheat [19]. Straw also contains various other organic compounds including protein, small quantities of waxes which protect the epidermis of the straw, sugars, salts and insoluble ash including silica. Different fractions across the straw vary in chemical composition which is also affected by soil type and fertiliser treatment. Overall, there is a higher concentration of cellulose in internode, of ash (in which silica is the main constituent) in leaf and leaf base and of lignin in the node cored compared to the other parts of straw (Table 1) [20,21].

Similar to wood, straw is considered a natural composite material because of its composition of polysaccharides (celullose and hemicelulloses) and lignin. The former two components are hydrophilic and the latter is hydrophobic. However, they are practically insoluble in water due to hydrogen bonding and covalent bonding with lignins [6]. The ultrastructure of straw fibres, like wood tracheids, consists of middle lamella (ML), primary wall (P) and secondary walls (the outer S1, the middle S2, the inner S3). The percentage volume fraction (PVF) and thickness of various morphological layers of wheat straw can be different from those of wood (e.g. spruce) (Table 2) [22], especially the S1 layer of wheat straw fibre is thicker than that of the spruce tracheid and PVF of ML and cell corner (CC) in wheat straw fibre is greater than that of spruce tracheid.

When it comes to anatomical structure of straw, normally the sclerenchyma cells in the bundle itself have a small diameter and a thick fibre wall while the extra vascular fibres often have a much larger diameter and a very variable wall

![Fig. 1 – Cellulose strands surrounded by hemicelullose and lignin [1].](Image)

| Table 1 – Chemical components of wheat straw determined by gravimetric analysis [20]. |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
|                          | Leaf Internode Leaf base Node core |
| Hot-water soluble         | 14.6 7.2        | 18.9 13.2       |
| Lignin                    | 15.3 14.2       | 14.1 16.7       |
| Hemicellulose             | 32.4 33.8       | 34.2 32.7       |
| Cellulose                 | 37.7 44.8       | 32.7 37.5       |
| Ash                       | 11.5 4.7        | 12.4 6.3        |

Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
thickness. In wheat straw these fibres occur as coarse bast fibres just inside the epidermal layer (Fig. 2A). Unlike wheat, the cross-section of rice straw reveals a different type of ultrastructure, beside the tubular concentric ring structure, the centre of the rice straw internodes contains a core (Fig. 2B and C). Moreover, rice is an aquatic plant and has a different type of protecting layer, including substances composed of lignin, amorphous silica, and other inorganic substances.

### 3. Analytical techniques for structural analysis of straw lignin

The need of properly characterising and classifying lignin for the promotion of the new applications is clear. At the product development level, appropriate analysis will facilitate the definition of specifications for commercial purposes and also allow the reliable monitoring of physical and chemical modifications which eventually will promote the understanding of the relationship between properties and performance. The utilisation of lignin for a range of natural and industrial purposes is dependent on the analysis of lignin and the characteristics of this polymer. Also a comprehensive understanding of lignin is needed when considering processes such as various pre-treatment techniques, including physicochemical and biological pre-treatments. The complicated and heterogeneous structure of lignin has been traditionally revealed by a series of chemical analysis, such as thioacidolysis (TA) [26], nitrobenzene oxidation (NBO) [27] and derivatization followed by reductive cleavage (DFRC) [28]. Nevertheless recent developments in nuclear magnetic resonance (NMR) technology have made this technique the most widely used technique in the structural characterisation of lignin, the reason being its versatility in indicating structural features and structural transformation of lignin.

The studies on lignin are divided into two different sections: qualitative and quantitative analyses. Some researchers (e.g. Ref. [3]) divided the analytical methods of lignin

---

**Table 2 – The thickness and PVF of wheat straw [22].**

<table>
<thead>
<tr>
<th>Morphological layers</th>
<th>Wheat straw</th>
<th>Spruce tracheid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thick fibre</td>
<td>Thin fibre</td>
</tr>
<tr>
<td></td>
<td>Thickness (μm)</td>
<td>PVF (%)</td>
</tr>
<tr>
<td>ML + P</td>
<td>0.1–0.2</td>
<td>9.3</td>
</tr>
<tr>
<td>S1</td>
<td>0.1–0.3</td>
<td>0.2–0.3</td>
</tr>
<tr>
<td>S2</td>
<td>0.15–0.3</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>S3</td>
<td>–</td>
<td>5.4</td>
</tr>
<tr>
<td>CC</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Containing the CC region.

---

Fig. 2 – Micrograph of cross sections of (A) wheat straw under light microscope [23]; (B) wheat straw internode SEM (a) epidermis, (b) parenchyma, (c) lumen, (d) vascular bundles [24]; (C) rice straw internode SEM [25].
characterisation into two groups of destructive and non-destructive methods. Thioacidolysis (TA) [26], nitrobenzene oxidation (NBO) [27] and also derivatization followed by reductive cleavage (DFRC) [28] are amongst the most destructive methods used. The non-destructive methods include various spectroscopic methods (e.g. UV and Fourier transform infrared spectroscopy (FT-IR spectra)) and Nuclear magnetic resonance (NMR) techniques.

The chemical composition of agricultural biomass can also be analysed with near infrared (NIR) spectroscopy, e.g. Kelley et al. [29] tested the effectiveness of NIR for measuring the chemical composition of biomass that has been subjected to a wide variety of extraction and chemical treatments; Sanderson et al. used NIR for directly measuring the chemical composition of biomass [30].

Another technique to analyse lignin in the cell wall is based on mercurization which was firstly used in the early history of lignin chemistry to characterise its structure [31]. Mercurization is a reaction occurring under mild conditions; it seems to be less sensitive to the substitution pattern of the aromatic ring. The determination of lignin, thought apparently an easy matter, is one of the least satisfactory of the analysis commonly carried out on plant materials and woods. Almost all current methods contain the solution and hydrolysis of all other plant constituents and assume that the residue after such treatment is lignin. Various investigators have pointed out that this is not the case, but in spite of this vital objection, considerable reliance has been placed on figures so obtained.

3.1. Quantitative analysis

Quantitative measurement of lignin structures is an important aspect when it comes to investigating lignin; the measurement of various structures of lignin is possible when appropriate standards or pulse sequences are applied [32–34]. Quantitative analysis is based on gravimetry or UV- absorption [35] either to estimate lignin as an insoluble residue after strong sulphuric acid treatment (Klason lignin) [36] or to oxidise away the lignin from a holocellulose preparation (acidic chlorite lignin or permanganate lignin) [37]. The spectroscopic techniques, such as infrared (IR) and $^{13}$C nuclear magnetic resonance ($^{13}$C NMR) spectroscopy, are complementary to the above procedures since they provide information on the whole structure of the polymer and avoid the possibility of degradation artefacts [38]. This information is presented with quantitative values.

3.1.1. FT-IR

Among all the quantitative analysis techniques, FT-IR spectroscopy shows interesting characteristics, including high sensitivity and selectivity, high signal-to-noise ratio, accuracy, data handling facility, mechanical simplicity and short time and small amount of sample required for the analysis [39]. In addition, the spectrum of a lignin sample gives an overall view of its chemical structure [40]. FT-IR spectroscopy is non-destructive method of lignin investigation, which opens perspective to find structural discrepancies in lignin isolated by different methods.

Infrared radiation is electromagnetic radiation which covers the wavenumbers between 13,300 cm$^{-1}$ and 3.3 cm$^{-1}$. The infrared region is usually divided into 3 regions: near infrared (13,300–4000 cm$^{-1}$), middle infrared (4000–200 cm$^{-1}$) and far infrared (200–3.3 cm$^{-1}$) regions. Organic compounds have fundamental vibration bands in the mid infrared region, which is why the region is widely used in infrared spectroscopy. An infrared spectrum is unique to each substance and can therefore, in principle, be used as an impartial characteristic to identify the sample. Every lignin IR spectrum has a strong wide band between 3500 and 3100 cm$^{-1}$ assigned to OH stretching vibrations. This band is caused by the presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds. The intensity of the band increases during demethylation and decreases during methylation since during demethylation the O–H bonds in methoxyl groups bonded to 3rd or 5th carbon atom of the aromatic ring are split and CH$_3$ is replaced by a hydrogen atom producing a new OH group. During methylation the O–H bonds are split and H is replaced by CH$_3$ group and the amount of OH group decreases hence the intensity of the band decreases [41]. Acetylation of the lignin causes a partial or full band loss since almost all of OH groups are replaced by CH$_3$COO $^-$ [42].

The assignments of FT-IR absorption bands for the lignin normally include the aromatic skeleton vibrations at 1606, 1507 and 1434 cm$^{-1}$, in which the aromatic semicircle vibration (a vibration involving both C=C stretching and a change of the H–C–C bond angle) is assigned at 1507 cm$^{-1}$ [43], the carbonyl and unconjugated ketone and carboxyl group stretching at 1732 cm$^{-1}$ and the small band of the conjugated carbonyl stretching such as detected in organosolv lignin at 1653 cm$^{-1}$. More detailed assignment are summarised in Table 3 [7,44,45].

3.1.2. Thermal analysis

Thermal analysis has recently become prominent, especially for fibres, plastics and other polymeric materials. Thermal
analysis is a set of techniques used to describe the physical or chemical changes associated with substances as a function of temperature. The thermal stability of the isolated hemicelluloses, cellulose and lignin preparations is determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA can be used to monitor the weight loss of the lignin as it is heated, cooled or held isothermally, while DSC is performed to determine the melting temperature and enthalpy of the lignin. DSC is the most widely accepted method for determining glass transition temperatures (Tg) of lignin or modified lignin samples [46]. Thermoanalytical techniques, in particular TGA and derivative thermogravimetric (DTG), allow the information about chemical composition of straw to be obtained in a simple and straightforward manner. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) has also been applied to examine reaction products of thermal degradation [47] and an effective tool to investigate fast pyrolysis of biomass and on-line analysis of the pyrolysis vapours [48,49].

Py–GC/MS provides a rapid and easy alternative to tedious chemical degradation procedures for analysing the monolignol composition of lignin samples [50]. It requires only a small amount of lignin (<1 mg) and samples do not have to be isolated from the associated plant polysaccharides to be effective. Compounds separated on a GC column can easily be identified from their mass spectra as being derived from p-hydroxyphenyl (H), syringyl (S), or guaiacyl (G) propane units. Integration of the chromatograph peaks can determine if the sample in question comes from a G, S/G, or H/S/G type lignin.

For TGA and DSC the heating rate is usually between 5 °C and 10 °C. The tests are undertaken in both nitrogen and air atmosphere between room temperature of 25 °C–30 °C up to 730 °C–800 °C [51]. Due to the complexity of lignin and the difficulty in extraction, the literature related to the pyrolysis behaviour of lignin is scarce. However Müller-Hagedorn and Bockhorn [52] obtained kinetic parameters for straw lignin in a TGA based on the Levenberg-Marquardt and improved Runge–Kutta laws. The activation energies and frequency factor for barley straw lignin varied from 92 to 102 kJ mol⁻¹ and from 10⁻³ to 10⁻² min⁻¹ respectively. Ghaly et al. [53] used TGA and differential thermal analysis (DTA) to study the behaviour of oat straw in an oxidising atmosphere (15% oxygen and 85% nitrogen); two distinct reaction zones were observed on the TGA and DTA curves. Higher thermal degradation rates were observed in the first reaction zone due to the rapid release of volatiles as compared to those in the second reaction zone. The activation energies were in the range of 83–102 and 58–75 kJ mol⁻¹ for the first and the second reaction zones, respectively. The thermogravimetric dynamics parameters of wheat straw enzymatic acidolysis lignin (EMAL) were calculated by the methods of Kissinger and Ozawa, respectively; the activation energy of wheat straw EMAL was 103.92 and 107.69 kJ mol⁻¹ respectively with the methods of Kissinger and Ozawa [54].

Carrier et al. [55] have also investigated the suitability of TGA as a new method to obtain lignin, hemicellulose and α-cellulose content in biomass. The study indicated comparable results to common methods used for α-cellulose content; however, this method cannot be extended to the lignin content because of important deviations in the correlation curves. Through this alternative method, which is faster, easier to implement and less cost effective than the existing wet chemical techniques, it is possible to determine the hemicelluloses and α-cellulose contents of biomass samples.

Thermal degradation of lignin is reviewed by Brebu and Vasile [56] where the information on the temperature range, kinetics and mechanism of thermal degradation is presented. Samples for the thermal analysis of straw such as TGA are usually the same as those for FTIR spectroscopy test.

### 3.1.3. NMR

Nuclear magnetic resonance spectroscopy (NMR) has been shown to be reliable and comprehensive among the various physical and chemical methods for the characterisation of lignin, because it not only gives quantitative data but also provides some qualitative information (Table 4). An inclusive review on NMR application for structural analysis of lignin was published in 1971 [57].

In the past, proton NMR (¹H NMR) was mainly used for lignin characterisation and the ¹H NMR spectrum of acetylated lignin is used to determine the quantity of different hydroxyl groups. Lundquist [58–60] has published works about NMR characterisation of lignin. With the development of NMR techniques, ¹³C NMR became popular in lignin characterisation as it is powerful and capable of revealing a large amount of lignin structural information including the presence of aryl ethers, condensed and uncondensed aromatic and aliphatic carbons. Since the 1980s, many studies on ¹³C NMR spectra of lignin have been conducted [61–64]. Many attempts have been made to increase the sensitivity and signal-to-noise ratios of the quantitative ¹³C NMR spectra, especially for understanding the structural changes of lignin polymer in pulping processes and other isolation processes [3]. ¹³C NMR has been used to aid in the elucidation of pulping or delignification mechanism (soda pulping, kraft pulping and oxygen/peracid treatments), as well as pre-treatments, which

<table>
<thead>
<tr>
<th>NMR techniques</th>
<th>Quantitative data</th>
<th>Qualitative data</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹³C NMR</td>
<td>Aliphatic/phenolic OH groups and methoxy groups</td>
<td>Inter-unit bonding patterns</td>
</tr>
<tr>
<td>HSQC</td>
<td>Inter-unit bonding patterns</td>
<td>Inter-unit bonding patterns</td>
</tr>
<tr>
<td>³¹P NMR</td>
<td>Aliphatic/phenolic OH groups, guaiacyl, siringyl, condensed OH groups, carboxylic acids</td>
<td>Assignments of guaiacyl, siringyl, condensed OH groups, carboxylic acids</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Hydrocarbon contaminant, aliphatic and aromatic acetate, protons in methoxyl groups, benzyllic protons in β-o structures</td>
<td>Improved spectral resolution of key lignin functionality</td>
</tr>
</tbody>
</table>

Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
are discussed in detail in a recent book by Ralph and Landucci [65].

Quantitative $^{13}$C NMR is commonly used for the estimation of some specific component [65,66]. However, the most recent practices in the use of quantitative $^{13}$C NMR spectroscopy for the characterisation of lignin are confined to using the aromatic and methoxyl signals as internal standards in expressing the various functional groups per C$_6$ [65]. Such a practice is applicable to native lignin but inapplicable for industrial lignin or modified lignin [66]. To overcome the above defects, Xia et al. [66] suggested a novel protocol for acquiring quantitative $^{13}$C NMR spectra of lignin by using the internal reference compounds 1,3,5-trioxane and pentafluorobenzene. Trioxane offers a convenient internal standard for collecting inverse gated proton decoupled $^{13}$C NMR spectra for lignin. The internal reference compounds provide single and unoverlapped sharp signals in the middle of the spectral region, permitting superficial integration.

Detailed approaches for the quantification of different lignin structures in milled wood lignin (MWL) have also been reported by using quantitative $^{13}$C NMR techniques [67,68], including the amount of different linkages, various phenolic/etherified non-condensed/condensed guaiacyl and syringyl moieties. This approach is comparable to that reported from other wet chemistry techniques, but requiring only rather short experimental times. Much progress has been achieved on the $^{13}$C NMR spectra of lignin, but there still remain some issues to be explained, such as precise signal assignments and true quantification based on $^{13}$C NMR spectra of lignin, which are difficult due to signal overlap and other factors. Both solid-state and solution-state $^{13}$C NMR have been used for structural characterisation of lignin [69,70], however the solution-state NMR is more informative because of its better resolution, although soluble lignin preparations may not be representative of the whole lignin fraction in plants [71,72].

It is worth mentioning that structural characterisation of lignin could be directly investigated by NMR techniques via non-derivatization solvent systems, such as DMSO-d$_6$/NMI-d$_6$ [73], DMSO-d$_6$ [74] and DMSO-d$_6$/pyridine-d$_5$ (as gelling solvent) [75]. The rapid NMR characterisation method provides what appears to be the best tool for the detailed structural study of the complex cell wall polymers. The DMSO-d$_6$ (gel-state) method is used for lignin analysis, not polysaccharides analysis. Nevertheless, based on these non-acetylated solvent systems, lignin composition (notably, the syringyl: guaiacyl: p-hydroxyphenyl ratio) could be quantified without the need for lignin isolation [3].

In summary, with NMR techniques, the true quantification in lignin is difficult due to signal overlap and other factors. The best quantification method remains the relatively tedious inverse-gated technique, along with an internal standard substance. Fortunately, most of the NMR techniques are adequate when the researchers want to follow changes in structure of straw biomass over time during a particular treatment, provided that the desired precision is maintained [65].

3.1.4. Two-dimensional HSQC NMR

The interest to study the mixture of increasing complexity of lignin has created the demand to employ high magnetic field NMR spectrometers to improve resolution and sensitivity. The strong self-association of lignin chains and harsh treatment required for lignin-derived fragments solubilisation are major factors that make studying lignin primary structures difficult [76]. Quantification of complex samples with $^1$H NMR spectroscopy suffers from resonance overlap even at high magnetic fields, which can prevent an accurate quantification of sample compounds. Proton—carbon correlated two-dimensional (2D) NMR can exploit the wide chemical shift range of carbon, thus offering a significantly improved resolution. Therefore, the application of proton—carbon correlated 2D NMR in the determination of molar concentration of the sample compounds has gained interest in studies of natural products, biological samples and processes taking place in living organisms [77] and 2D NMR of both homo- and heteronuclear became popular and much powerful tools for lignin characterisation [78–80].

The most used and valuable 2D NMR is heteronuclear single-quantum-coherence (HSQC) that provides the correlation between directly bonded protons and carbons in two dimensions [67,81]. Overlapping protons that are attached to carbons with different shifts are separated by their carbon shift difference; while overlapping carbons may be separated by their attachment to protons with differing chemical shifts. Therefore, the apparent resolution of 2D spectra is much better than anything that can be achieved in 1D spectrum [12]. Thus, quantitative measurement of various structures of lignin is possible when appropriate standards or pulse sequences are applied [33,67]. The assignment for lignin correlations comes from the extensive database of lignin model compounds along with data from a long history of NMR of both isolated and synthetic lignin [32,82–87]. The 2D-HSQC experiments of non-acetylated lignin samples have been valuable in assigning major structures ($\beta$-O-4, $\beta$-5, etc.) in the lignin samples according to the previous studies and the previously mentioned database of lignin model compounds [80].

The HSQC approaches have also been valuable in assigning major structures of non-acetylated lignin from different origins in recent years, such as some non-woody plants [32], Jute fibres [82], bamboo [84,85,87], Triploid poplar [88–90] and wheat straw [83]. HSQC spectra have been crucial in recognising new and minor lignin structural units. The clear identification of dibenzodioxinocins (5–5 linkages) as major new structures in lignin has been a significant finding [91,92].

Evidence provided by 2D NMR is far more diagnostic than 1D data purely because of the simultaneous constraints that are revealed in the data. Consequently, the observation that there is a proton at 4.9 part per million (ppm) directly attached to a carbon at 84.4 ppm and a proton at 4.1 ppm attached to a carbon at 82.5 ppm is more revealing than just observing two new carbons at 84.4 and 82.5 ppm in the 1D spectrum [92]. Zhang and Gellerstedt [33] presented a new analytical method based on the 2D-HSQC NMR sequence and quantitative $^{13}$C NMR, which can be applied for quantitative structural determination of complicated polymers, such as lignin and cellulose derivatives. This method is a combination of HSQC and quantitative $^{13}$C NMR techniques, which gives more reliable data about the lignin linkages. A recent study showed that 2D-HSQC NMR spectra of enzymatic hydrolysis residue (EHR)
delivered very well resolved spectra that can be compared to that of MWL [93]. Based on the results obtained, it was found that in situ characterisation of pre-treated biomass by HSQC NMR analysis is a beneficial structural analysis methodology in the emerging biomass research field for the characterisation of EHR.

Heteronuclear multiple quantum coherence (HMQC) or HSQC spectra of lignin have been reviewed [80] and well reported by previous researchers [4,74,75,78,94–98]. The assignments in HMQC/HSQC spectra should be made with comprehensive knowledge of both carbon and proton chemical shifts for each structural type. 2D HSQC NMR technique is also useful in determination of the structural characteristics of oxygenated aliphatic region of lignin polymeric framework [32,99].

3.1.5. DFRC method

The derivatization followed by reductive cleavage method (DFRC) was developed by Lu and Ralph in 1997 as an alternative to thioacidolysis [28,100]. Because of the mild conditions used and its unique selectivity, the DFRC method has become widely used for characterising lignin from various origins [101–103]. One unique feature of the DFRC method is that esters on lignin remain fully intact during the DFRC procedure [104]; therefore, p-coumarates on lignin from grass plant materials can be detected and measured [105]. With a few modifications to the standard DFRC procedure by replacing the acetyl-containing reagents (acetic acid and AcBr) with the propionyl analogues, the modified DFRC method was applied to detect and quantify the naturally occurring acetates on lignin from some plants such as kenaf, aspen or other non-woody plants [106].

The DFRC procedure, as a basic component, has been modified or combined with other techniques to provide more precise and specific quantitative data about lignin structures [107]. The combination of DFRC with quantitative 31P NMR was shown to have significant potential for the determination of arylglycerol-β-aryl ether and other linkage. Coupled with the spread of advanced NMR techniques, during the past decade, lignin structural enquiries have been greatly facilitated by the development of various degradative protocols, such as DFRC which efficiently cleaves the β-aryl ethers in lignin. DFRC is a flexible method due to its three distinctly separated steps, allowing modifications designed to the quantification of different monomeric units and structures. On the basis of this flexibility, Tohmura and Argyropoulos [108] proposed the combination of DFRC with quantitative 31P NMR data.

3.1.6. H/S/G ratio

Qualitative or semi-qualitative indication of H/S/G ratio units are not too informative and on the other hand they are very laborious, lengthy and also prone to errors. Table 5 shows the p-hydroxyphenyl—syringyl—guaiacyl (H/S/G) ratio for different types of biomass which vary quite considerably amongst each other.

Thioacidolysis is the most used analytical method for lignin analysis to obtain information about uncondensed structures (H/S/G ratios) [12]. Thioacidolysis was developed by Lapierre, as an extension of acidolysis, to cleave β-aryl ethers in lignin so that the basic units linked by β-aryl ethers are released as monomers to be quantified by gas chromatography [26]. Therefore, the yields of monomers released by thioacidolysis reflect the proportion of uncondensed units in lignin. When thioacidolysis is used for grass lignin, esters on grass lignin may decrease the efficiency of β-ether cleavage by thioacidolysis [113,114]; hence this should be taken into consideration when interpreting thioacidolysis results for grasses.

Permanganate oxidation, nitrobenzene oxidation, GC–MS pyrolysis, thioacidolysis and DFRC are degradative methods that reveal the H/S/G composition of the lignin polymer. It must be mentioned that degradative analytical techniques are lengthy and complicated. All these methods release only a fraction of the lignin for analysis, they are based on the cleavage of lignin backbone and analyses of the fragments obtained, and provide partial data due to the specificity of the treatment.

The quantification of lignin H/S/G ratio has been reported by 13C NMR and HSQC NMR analysis [115,116].

3.2. Qualitative analysis

Qualitative lignin analysis relies rather much on studies of lignin degradation products; these methods only provide preliminary information which could then be useful for the quantification of lignin within straw.

3.2.1. SEM-EDX

Scanning electron microscopy (SEM) is used to examine the microstructure, morphology, crystalline structure and size of the straw. The microscope could be equipped with energy-dispersive X-ray (EDX) analyser, which provides useful element compositions and also information about the chemical composition of the straw.

Despite its importance as imaging technique, SEM is not capable of quantitatively evaluating the purity of typical lignin sample. Moreover, there is no published algorithm to convert such images into numerical data. SEM analysis also provides information on the particle size and the surface properties of straw. When the straw is being treated by means of chemical or mechanical treatments, the particle sizes might change and the surface of straw might become smoother or rougher depending on each step of the treatment.

Field emission scanning electron microscopy (FE-SEM) was used by Koo et al. [117] in order to observe the change in lignin distribution during pre-treatment; (Fig. 3), which is one of the key applications of SEM technique used for qualitative analysis of biomass.

3.2.2. TEM and optical microscopy

Transmission electron microscopy (TEM) has been proved to be an effective tool for determining cell wall ultrastructure.

<table>
<thead>
<tr>
<th>Type</th>
<th>Lignin (%)</th>
<th>H</th>
<th>S</th>
<th>G</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw</td>
<td>16–21</td>
<td>9</td>
<td>46</td>
<td>45</td>
<td>[109]</td>
</tr>
<tr>
<td>Rice straw</td>
<td>6</td>
<td>15</td>
<td>40</td>
<td>45</td>
<td>[109]</td>
</tr>
<tr>
<td>Rye straw</td>
<td>18</td>
<td>1</td>
<td>53</td>
<td>43</td>
<td>[110]</td>
</tr>
<tr>
<td>Hemp</td>
<td>8–13</td>
<td>9</td>
<td>40</td>
<td>51</td>
<td>[111]</td>
</tr>
<tr>
<td>Jute</td>
<td>15–26</td>
<td>2</td>
<td>62</td>
<td>36</td>
<td>[112]</td>
</tr>
<tr>
<td>Flax</td>
<td>21–34</td>
<td>4</td>
<td>29</td>
<td>67</td>
<td>[109]</td>
</tr>
</tbody>
</table>
TEM observations deliver further details of lignification and the distribution of lignin. Based on the intensity of the staining, the concentration of lignin in different morphological regions of straw could also be qualitatively examined by TEM micrograph, for example, Fig. 4 shows the distribution and concentration of lignin under TEM. The dark staining of the cell corner middle lamella (CCML) and compound middle lamella (CML) indicates that both of them are strongly lignified. Like SEM, TEM analysis will also be helpful in finding particle size and surface properties of straw.

Optical microscopy is unable to provide the detailed features of the materials under examination but is a good tool to observe the lignin distribution within the straw. It is also a quick and easy method to observe the overall structure of straw and hence to observe the changes that may occur after any treatments used on straw. The evaluation of straw morphology for preliminary research can usually be carried out using optical microscopy or light microscopy. Basic parts, including epidermis, cortex, vascular bundle and pith of the straw, can be observed, with the epidermis cells arranging in parallel rows and being closely packed to protect the internal parts of the plant, the cortex being the zone between epidermis and the vascular bundles containing collenchyma and parenchyma cells, the pith being the central part of the stem which is composed of thin walled parenchyma cells, and the vascular bundles in the stem of the grass plants varying in number and size but having the same basic structure composed of xylem and phloem (Fig. 5).

4. Structure of straw lignin

Lignin monolignols are polymerised by a radical coupling process that links them by carbon--carbon or ether bonds. A linkage may occur at any of several different locations on each phenolic unit, causing many different linkage types to be
possible. The most common linkage types found in a lignin molecule are β-O-4, α-O-4, β-5, 5–5, 4-O-5, β-1 and β-β [119,120]. The ether type linkages dominate in native lignin, estimated to make up one half to two thirds of the total number of native plant lignin linkages. Monolignols form branch points within the polymer giving a network-like structure. Given the variety of linkages that occur, lignin molecules cannot be depicted as a series of regular, defined repeating units, as traditional polymers are. In contrast, lignin is a highly irregular complex polymer [119,120].

Lignin is one of major cell wall component of herbaceous crops. The composition and structure of lignin vary considerably within and among plants; Lignin has been classified into three groups: Gymnosperm (softwood), Angiosperm (hardwood) and Grass lignin. However, it was recently found that these categories are inadequate because of ignoring most of the herbaceous angiosperm lignin and some conifer families containing guaiacyl and syringyl types of lignin. It was suggested by Gibbes that lignin should be categorised into two groups: guaiacyl lignin and guaiacyl–syringyl lignin according to their overall chemical constitutions [12]. Each type also generally contains a small proportion of p-coumaryl units, though grass lignin generally contains a higher amount of p-coumaryl units than woody lignins [121]. Other phenolic monolignols have been identified, but they generally make up a much smaller portion of the lignin molecule [122]. Other phenolic compounds (alcohols, aldehydes, acids, esters and amides) have been reported to act as lignin precursors and illustrate the structural “plasticity” of the polymer and the adaptability of the lignification mechanisms in plants [123,124], but several of these compounds (such as p-hydroxyconiferaldehyde, ferulic acid or 5-hydroxyconiferyl alcohol) only provide a significant contribution to lignin in transgenic crops. Among these linkages, the possibilities of glycosidic, benzyl ether and ester linkages have been demonstrated with model compounds [13]. The association of lignin and carbohydrates in grass cell walls is largely through free radical coupling of ferulates or diferulates with monolignols or growing lignin oligomers. Due to the complexity of LCCs, the isolation of homogeneous preparation is the most important step for the following compositional analysis and structural characterisation. Many isolation methods for LCC have been tried, but the finely divided plant meal [131,132] and MWL [133] were preferred sources for LCC isolation because any chemical or biochemical treatment before LCC isolation would break possible linkages between lignin and carbohydrates.

The cross-linking of lignin and carbohydrates via ferulates/diferulates in grass cell walls has been demonstrated [12]; it is generally accepted that the cross-linking of carbohydrates and lignin by ferulates presents the greatest barrier to efficient utilisation of grass cell wall [113].

4.1. LCC linkages

Lignin is always associated with carbohydrates (in particular with hemicelluloses) via covalent bonds at two sites: α-carbon and C-4 in the benzene ring, and this association is called lignin carbohydrate complexes (LCC). The major inter-unit linkages within the lignin monomer (H, S, and G) are β-Ο-4, β-β, β-5, β-1 arrangements. The chemical linkages limit the efficient separation of lignin from plant cell wall. Thus, it is important to understand the LCC linkages of lignin samples.

Four types of linkages between lignin and carbohydrates have been suggested: glycosidic linkages, ester linkages, benzyl ether linkages and hemiacetal or acetal linkages [12]. Among these linkages, the possibilities of glycosidic, benzyl ether and ester linkages have been demonstrated with model compounds [13]. The association of lignin and carbohydrates in grass cell walls is largely through free radical coupling of ferulates or diferulates with monolignols or growing lignin oligomers. Due to the complexity of LCCs, the isolation of homogeneous preparation is the most important step for the following compositional analysis and structural characterisation. Many isolation methods for LCC have been tried, but the finely divided plant meal [131,132] and MWL [133] were preferred sources for LCC isolation because any chemical or biochemical treatment before LCC isolation would break possible linkages between lignin and carbohydrates.

The cross-linking of lignin and carbohydrates via ferulates/diferulates in grass cell walls has been demonstrated [12]; it is generally accepted that the cross-linking of carbohydrates and lignin by ferulates presents the greatest barrier to efficient utilisation of grass cell wall [113].

4.2. Physical properties

The physicochemical properties of straw lignin are known to be different from those of softwoods or hardwoods, with straw lignin possessing characteristic alkali solubility. The physicochemical state of lignin dictates how and where it can be utilised in the production of various products. The source from which lignin is obtained and the method of extraction has a strong bearing on its properties [16]. As lignin from different crops or treatment can be extremely diverse in

Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
structure and hence physical properties, it is necessary to determine these differences through analytical methods. The reactivity and physicochemical properties of lignin are dependent to certain extent on their molecular weight distribution (Table 6). The reactivity of lignin will impact on the attributes of the end products. For example, Muller and Glasser [134] found that kraft lignin-based phenol formaldehyde resins have superior properties to steam exploded lignin-based phenol formaldehyde resins. Methods for lignin molecular weight determinations are developed and described by previous researchers [135,136]; gel permeation chromatography (GPC) involves the chromatographic fractionation of macromolecules according to molecular size. The technique of fractionating macromolecules on Sephadex gel has been applied to lignin materials since early 1960s [137,138].

Another important parameter is the glass transition temperature, $T_g$, which is an indirect measure of crystallinity and degree of crosslinking and directly indicates the rubbery region of the material [139]. Lignin cross-linking degree will depend on the quantity of water and polysaccharides, as well as molecular weight and chemical functionalisation. Lignin cross-linking degree generally increases with increasing molecular weight.

4.3 Lignin distribution and concentration

Lignin’s properties are significantly different with carbohydrates such as activity and solubility, according to which the distribution of lignin in plant cell wall can be analysed. Many techniques have been applied to the study of distribution of lignin and organisation of cellulose components. One of the oldest procedures is selective staining which follows by the study under light microscope and another method to study lignin distribution is electron microscopy of lignin skeleton [140]. Although the mentioned methods are useful in studying lignin distribution, only qualitative or semi-quantitative data of lignin in various morphological regions can be provided. The studies that focused on quantitative data of lignin distribution in the secondary wall and compound middle lamella (CML) have been carried out by using UV microscopy [141]. UV absorption is a tool used for lignin identification because lignin absorption occurs at 280 nm. The weight concentration of lignin was estimated to be 16% and 73% for the secondary wall and CML of Norway spruce tracheids respectively [142]. Lange and Kjaer [143] proposed interference microscopy based on the pathways of rays which is another method for quantitative analyses of lignin distribution within the cell wall layers. With the development of electron dispersive X-ray analysis (EDXA) combined with SEM and TEM, the interference microscopy in conjunction with EDXA was applied to determine lignin distribution in wood and straw, in differentiating xylem and in chips during pulping [144,145]. The technique is fully quantitative and precise. Donaldson [146] used confocal laser scanning microscopy (CLSM) to study lignin distribution in agricultural fibres.

Although many chemical studies of grass lignin have been carried out, there is still little quantitative data on lignin distribution in grass species. Zhai and Lee [22] used SEM-EDXA technique to determine the Br-L X-ray counts of the different brominated wheat straw cell which are presented in Table 7. In fibre and non-fibre cells of wheat straw, the lignin concentration is the highest in the cell corners (CC) and the lowest in the secondary wall. In the morphological regions of all cells, the lignin concentration is the highest in parenchyma cells, followed by fibres, and is the lowest in epidermis cell [22]. Interference microscopy [144] and CLSM [147] were also used by researchers to examine the lignin concentration of wheat straw in the ML and in the secondary wall.

4.4 Lignin functional groups and their characteristics

The key chemical functional groups in lignin are the hydroxyl, methoxyl, carbonyl and carboxylic groups. The quantity of these groups depends on the genetic origin and isolation processes adapted. Functional group characterisation can be used to determine the lignin structure. Various analytical methods for determining functional groups in technical lignin have been studied, while the statistical comparison of the various analytical methods for hydroxyl groups have been found not fully comparable [148], the aminolysis and non-aqueous potentiometry are assumed to be the most reliable for phenolic hydroxyl and for determining the total carbonyl, and the oximating method was found the most reliable method which was first described by Faix et al. [149]. The

### Table 6 – Molecular weight and functional groups of lignin [1].

<table>
<thead>
<tr>
<th>Lignin type</th>
<th>M_w (g mol⁻¹)</th>
<th>COOH (%)</th>
<th>OH phenolic (%)</th>
<th>Methoxy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soda (bagasse)</td>
<td>2160</td>
<td>13.6</td>
<td>5.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Organosolv (bagasse)</td>
<td>2000</td>
<td>7.7</td>
<td>3.4</td>
<td>15.1</td>
</tr>
<tr>
<td>Soda (wheat straw)</td>
<td>1700</td>
<td>7.2</td>
<td>2.6</td>
<td>16</td>
</tr>
<tr>
<td>Organosolv (hardwood)</td>
<td>800</td>
<td>3.6</td>
<td>3.7</td>
<td>19</td>
</tr>
<tr>
<td>Kraft (softwood)</td>
<td>3000</td>
<td>4.1</td>
<td>2.6</td>
<td>14</td>
</tr>
</tbody>
</table>

### Table 7 – Lignin concentration and distribution in wheat straw [22].

<table>
<thead>
<tr>
<th>Cells</th>
<th>S</th>
<th>CML</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br-L X-ray counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick wall fibre</td>
<td>1620</td>
<td>2156</td>
<td>2992</td>
</tr>
<tr>
<td>Thin wall fibre</td>
<td>1340</td>
<td>2004</td>
<td>3336</td>
</tr>
<tr>
<td>Parenchyma cell</td>
<td>2005</td>
<td>2599</td>
<td>–</td>
</tr>
<tr>
<td>Epidermis cell</td>
<td>1022</td>
<td>1690</td>
<td>1743</td>
</tr>
<tr>
<td>Lignin concentration (g g⁻¹)</td>
<td>0.168</td>
<td>0.412</td>
<td>0.571</td>
</tr>
<tr>
<td>Thick wall fibre</td>
<td>0.154</td>
<td>0.339</td>
<td>0.664</td>
</tr>
<tr>
<td>Thin wall fibre</td>
<td>67.5</td>
<td>18.0</td>
<td>14.5</td>
</tr>
<tr>
<td>Thin wall fibre</td>
<td>57.5</td>
<td>20.3</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
phenolic hydroxyl group is a significant functionality affecting the physical and chemical properties of lignin polymer. Moreover, the chemical reactivity of lignin in various modification processes is influenced by its phenolic hydroxyl content which is significant in the reaction with formaldehyde for the production of lignin adhesive [150]. Lai described a procedure to determine free phenolic hydroxyl groups in lignin [151]. Common physical method used to estimate the phenolic hydroxyl content of lignin is UV spectroscopy, which is based on difference in absorption of phenolic units in neutral and alkaline solutions [152].

Methoxyl groups (–OCH₃) are found in lignin from all plants. The amount of the groups depends on plants species and on the method of isolation. The amount of the methoxyl groups is often used as a measure of the purity of lignin preparation, since the isolated lignin sometimes is contaminated with hydrocarbons. The classical method for methoxyl determination of lignin uses hydroiodic acid to promote demethylation and gas chromatography to determine the percentage methoxyl. Alternative method, which is less tedious, involves the use of proton nuclear magnetic resonance (¹H NMR) [153].

There are the primary aliphatic hydroxyl groups bonded to the γ-C-atom, secondary aliphatic hydroxyl groups bonded to the α-C-atom and phenolic hydroxyl groups bonded to the 4-C-atom of the aromatic ring in lignin. The accurate determination is difficult since they have very similar chemical properties and reactivity. The amount of total hydroxyl group in lignin was determined by potentiometry [154].

Natural lignin contains a low concentration of COOH-groups. However, when native lignin is subjected to chemical or biological treatments, carboxyl groups are frequently detected in significant quantities, hence quantitative measurements of carboxyl groups may provide information regarding the degree to which the lignin has been degraded or modified as a result of treatment. Further carboxyl groups are produced during delignification as a result of oxidation of hydroxyl and carbonyl groups. Oxidation of the lignin structure will result in an increase of carboxyl absorption in the FT-IR spectra as it was observed by Xu et al. [7]. In work on lignin characterisation by Sahoo et al. [15], FT-IR analysis pointed out the differences in the bonding pattern and the functional groups. These different chemical functionalities are very important components when it comes to utilising lignin in biomass for composite applications, the final properties of the composites.

5. Wheat and rice straw lignin characteristics

Researchers have focused on the structural analysis of wheat and rice straw by using a combination of qualitative and quantitative techniques in order to either, optimise these renewable raw materials for industrial applications or to investigate the changes within the composition of straw biomass after a certain treatments. Most of the researches in this area are comparative studies between different types of biomass to establish the structural differences between them. Xu et al. [7] in a comparative study examined the chemical characteristics of lignin in order to get more information on chemical structures and relationship between physical properties and chemical structures. The analytical quantitative techniques such as FT-IR and ¹H and ¹³C NMR spectroscopy were used to investigate the changes occurring in lignin structure during the organosolv treatment processes. ¹H and ¹³C NMR spectra revealed that the lignin comprised guaiacyl, syringyl and a small amount of p-hydroxyphenyl units. The various covalent linkages and physicochemical interactions between lignin, cellulose, hemicelluloses, phenolic acids and other polyphenolic or proteinaceous or mineral extractives all contribute structural integrity to the wheat straw cell wall composite [155].

Recently, an in-situ quantitative 2D-HSQC NMR technique (the ball-milled plant cell wall was dissolved in DMSO-d₆) was used for characterising the changes in the cell wall during the hydrothermal pre-treatment (195 °C for 6 min) process of wheat straw for second-generation bioethanol production [156]. This study provides an effective quantitative method to reveal the structural changes of cell wall components based

---

Fig. 6 – ^31P NMR of wheat straw lignin phosphitylated with 2-chloro-4, 4, 5, 5-tetramethyl-1, 3, 2 dioxaphospholane. Please cite this article in press as: Ghaffar SH, Fan M, Structural analysis for lignin characteristics in biomass straw, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.07.015
on in situ 2D-HSQC NMR techniques. The results revealed substantial lignin β-aryl ether cleavage, deacetylation via cleavage of the natural acetates at the 2-O- and 3-O-positions of xylan, and uronic acid depletion via cleavage of the (1→2)-linked 4-O-methyl-α-D-glucuronic acid of xylan after hydrothermal treatment.

By combining mild alkaline hydrolysis with quantitative 31P NMR, Crestini and Argyropoulos [4] have been able to arrive at a protocol for determining the various ester linkages and their relative contributions to the overall structure of wheat straw lignin; their technique has been applied on samples of milled wheat straw (ML), dioxane acidolysis wheat straw lignin (AL), and a milled wood lignin from black spruce (BSL). A typical spectrum together with the detailed signal assignment can be obtained (based on earlier efforts of Granata and Argyropoulos [17] and Jiang et al. [18]) (Fig. 6).

Rice straw has high lignin and low nitrogen contents which in combination are responsible for its low nutritive value. Researchers in recent years have attempted to improve its nutritive value by chemical treatments [157]. Rice straw is highly heterogeneous and complex, and its fractionation and utilisation should be conducted on different levels according to structural properties. Dohnani et al. [158] evaluated the variability in chemical and morphological variables of European rice straw in their work with the aim to relate these variations to changes in “in sacco” degradation. In their work fifteen rice straw varieties are analysed for their chemical and morphological compositions, the results indicated that both chemical and morphological characteristics have great variability. For instance the ash content of rice straw samples ranged from 9.6 to 14.1% which are very low compared to the variability in chemical and morphological variables of European rice straw. The decomposition process of lignin covered a larger temperature range, between 250 and 600 °C, however at temperatures lower than 400 °C only 40% was decomposed, rendering charcoal as the residue product. This result implied that hemicelluloses degraded in first place, while lignin showed less degradation, and therefore, its structure was more stable. Cellulose, on the other hand, showed an important degradation process, mainly between 250 and 330 °C.

### Table 8 – Chemical composition of rice straw [162].

<table>
<thead>
<tr>
<th>Component</th>
<th>Range (%)</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>4.2–9.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>19.8–31.6</td>
<td>25.6</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30.3–38.2</td>
<td>33.9</td>
</tr>
<tr>
<td>Klason lignin</td>
<td>7.2–12.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>3.8–13.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Total ash</td>
<td>7.8–15.6</td>
<td>11.8</td>
</tr>
</tbody>
</table>

a Average of 34 samples.

6. Conclusions

The analytical techniques up to date for the structural analysis of straw lignin were reviewed and the resultant understandings of the structure of straw lignin were summarised.

The studies on lignin could be divided into the qualitative and quantitative analyses, the former includes SEM-EDX, TEM, optical microscopy and the latter includes FT-IR, TGA/DSC, NMR and DFRC. Different analytical methods applied to same lignin samples could provide significantly different results that are not directly comparable. This problem, coupled with the extreme heterogeneity of lignin and its chemical structure among different crop species, morphology and maturation degree has made to date the quantitative structural characterisation of lignin an open debate. More sensitive and reliable quantitative HSQC techniques are the most diagnostic and accurate analytical tools to investigate lignin structure, among them the magnetic resonance techniques when applied to lignin have proved to be efficient analytical tools for the structural elucidation of these complex biopolymers. These NMR techniques are capable of detecting and quantitatively determining all functional groups in lignin possessing labile hydroxyl groups, i.e. aliphatic OH, the various forms of phenolic OH and carboxylic acids.

The composition and structure of straw lignin varies considerably within and among plants. The nature of lignin carbohydrate linkages and occurrence is not completely clear and the isolation processes could affect the lignin structure. Because of the complexity of the chemical composition of lignin in straw, it is difficult to find a single technique to analyse its structure, hence the most accurate way to study the straw lignin could be the combination of several techniques, each providing partial but complimentary information.

### References


Aberu H, Freire M. Methoxyl content determination of lignins by 1H NMR. Ana Bras Ci 1995;67:379–82.


Lange P. The distribution of lignin in the cell wall of normal and reaction wood from spruce and a few hardwoods. Sven Papperstidn 1954;57:525–32.