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COMPARATIVE ANALYSIS OF LIGNOCELLULOSIC COMPONENT IN STRAW AND HUSK OF MR219 AND MR263 BY RAPID CHEMICAL ANALYSIS

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ABSTRACT

Lignocellulosic materials comprise cellulose, hemicelluloses, lignin and others component such as pectins and protein. However, the content and composition of these components vary, depending on plant species, variety, plant growth and development condition, which are influenced by season and geographical location. In this study, the lignocellulosic component of straw and husk of MR219 and MR263, the main paddy varieties planted in Malaysia were chemically extracted and quantitatively evaluated via spectrophotometric method. The lignin content in the straw and husk of MR219 are 7.21% and 9.50%, respectively. While the the lignin within straw and husk of MR263 are 8.17% and 10.21%, respectively. The cellulose content in all samples is between 38.20% and 42.63%. On the other hand, the hemicellulose percentage in straw samples (7.14% – 9.57%) is lower as compared to husk (22.97% – 32.43%), for both MR219 and MR263. In conclusion, the lignocellulosic composition and content of paddy biomasses are able to be determined by rapid chemical extraction method and it is demonstrated that the paddy chemical lignocellulosic composition depends on paddy variety and parts.

Key words: lignocellulosic, cellulose, hemicellulose, lignin, MR219, MR263, acetyl bromide assay, anthrone assay, TFA hydrolysis, Seaman hydrolysis

INTRODUCTION

Plant biomass, such as rice straw and husk, are among the underexploited natural resources on the Earth (Remli *et al*., 2013; Bassyouni & Hasan, 2015; Mun, 2015). Rice straw and husk are made of lignocellulosic materials, which consist of energy-rich polymers such as lignin, hemicelluloses and cellulose (Vogel, 2008). These lignocellulosic materials have gained significant attention worldwide due to its potential as precursor for biofuel and bio composite production.

In Malaysia, rice cultivation covers 14% (622,500 ha) of the country agricultural areas, making it as one of the primary contributors to the country plant biomass after cocoa, palm and rubber tree (Hamid & Abidin, 2008; Ahmed *et al.*, 2012). The rice milling industry generates a huge amount of wasted rice straw and husk, which is estimated to be about 5,000,000 tonnes metric per year (Ministry of Agriculture and Agro-based Industry Malaysia, 2015). The common practice of straw and husk disposal (paddy biomass) adopted in many countries, including Malaysia is by open burning, that leads to environmental and health problems (Liu *et al.*, 2011; Kanokkanjana & Garivait, 2013; Rosmiza *et al*., 2014). It is hypothesised that an alternative practice to utilise the paddy biomass may provide a solution to reduce and further benefit the industry by the increase of annual income. This indirectly benefits the environment and community's health due to reduction of open burning and production of sustainable bioresource.

The challenge arises is in terms of quantity determination of the lignocellulosic materials in the paddy biomass, as the cell wall structural and

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chemical composition are different between paddy varieties or parts in a single plant. This diversity is due to the plant's genetics, tissue structure, growth and development condition influenced by the season and geographical location (Mäkelä *et al*., 2014; Tanger *et al*., 2015). The differences affect the biorefinery processes for cell wall depolymerisation of the rice straw and husk via chemical and/or enzymatic mechanism, underlining the needs for a thorough characterisation of plant lignocellulosic biomass and cell wall depolymerisation optimisation process.

The detail analysis of cell wall lignocellulosic composition from the local rice straw and rice husk biomass is limited. This study described a comparative analysis of the lignocellulosic composition of two main paddy varieties in Malaysia, which are the MR219 and MR263 varieties. These data are important for optimum utilisation of biomass from these varieties in the future.

MATERIALS AND METHODS

Preparation of lignocellulosic material

Rice straws and rice husks of MR219 and MR263 were collected from Yan, Kedah and Teluk Intan, Perak, respectively. Sample was ground into fine powder by using industrial food grinder and sieved to less than 0.05 cm × 0.05 cm size. Pre-cooled extractant (ethanol/pyridine/acetic acid/water with ratio 75:2:2:21) was added to the fine powder to prepare alcohol-insoluble residue (AIR) as described by Airianah *et al* (2016). The pellet of AIR was dewaxed to remove waxes, phenolics, pigments and oils, as described by Baird (2012) with some modification by adding toluene:ethanol (2:1, v/v) mixture in a Soxhlet apparatus for 12 hours, before being washed thoroughly with 75% ethanol and dried at 40°C.

Lignin Content Analysis

Lignin content in MR219 and MR263 sample was analysed by using acetyl bromide assay, as reported by Fry (2000) and Foster *et al* (2010a) with some modification. Freshly made acetyl bromide solution (25% v/v in glacial acetic acid) was added to a portion of dewaxed sample, heated at 50°C for 2 hours. Samples were then heated at 50°C for 1 hour, with 15 minutes mixing interval. Sample was then mixed with 2 M sodium hydroxide (NaOH) and 0.5 M hydroxylamine hydrochloride (NH_2OH•HCI), diluted with glacial acetic acid before absorbance was read at 280 nm. The percentage of acetyl bromide soluble lignin (%ABSL) was determined by using ratio as described by Fry (2000), where absorbance of 0.24 is equal to 10 µg/mL lignin.

Non-cellulosic Component Isolation

Dewaxed sample was hydrolysed to isolate the non-cellulosic component (hemicellulose and pectin), as described by Fry (2000) and Foster *et al* (2010b), with some modification. 0.1 g dewaxed sample was hydrolysed with 1 ml of 2 M trifluoroacetic acid (TFA) and incubated at 121°C for 2 hours, with regular mixing at every 15 minutes. Supernatant with non-cellulosic component was harvested and subjected to anthrone assay meanwhile pellet was subjected to cellulose isolation.

Cellulose Isolation

Cellulose isolation was done as described by Foster *et al* (2010b), with some modification. Pellet from the non-cellulosic isolation was washed twice with 75% ethanol, followed by acetone, air-dried before being treated with Updegraff reagent (acetic acid:nitric acid: water, 8:1:2, v/v) at 80°C for 1 hour. Supernatant was discarded and pellet was subjected to Saeman hydrolysis by using 72% sulphuric acid (H_2SO_4) and incubated for 1 hour at room temperature with continuous rotation. Supernatant was subjected to cellulose analysis by using anthrone assay.

Anthrone Assay

Anthrone assay was conducted as described by Fry (2000), with some modification. Anthrone solution, 0.2% (w/v), was added into the hydrolysate (non-cellulosic and cellulosic samples) and heated at 100°C for 5 minutes. The mixture was cooled to room temperature before being analysed with microplate reader (ChroMate, Awareness Technology, USA) at 630 nm absorption. Standard curve of hemicellulose and cellulose fraction was prepared by using commercial 0.1% xylose and 0.1% glucose stock solution respectively.

RESULTS AND DISCUSSION

MR219 is a local paddy variety that was introduced in 2001 while MR263 was introduced in 2010. MR219 is a crossbreed of MR137 and MR151, a high yield paddy variety (Talei *et al*., 2013). On the other hand, MR263 is a crossbreed of SPM156 and MR221, where it is more suitable for less fertile soil (Hussain *et al*., 2012; Agricultural Department Peninsular Malaysia, 2014). In 2013, it was reported that more than 50% of paddy planted in Malaysia were the MR219 and MR263 varieties (Agricultural Department Peninsular Malaysia, 2014), making them the ideal source for lignocellulosic materials analysis. The abundance of lignocellulosic materials has the potential to be utilised and manipulated for biorefinery industry (Binod *et al*.,

2010). Due to this factor and lack of detail information regarding paddy biomass composition, it is important to evaluate the lignocellulosic composition of these two varieties to provide detailed information for the efficient and maximum usage of local paddy biomass.

Acetyl Bromide-Lignin in Straw and Husk of Paddy MR219 and MR263

Lignin has the most complex structure with three subunits, namely guaiacylpropane (G), syringylpropane (S) and p-hydroxyphenylpropane (H) (Chen, 2014). The subunits present in a very small amount in the plant cell wall biomass, which act as a glue between cellulose and hemicellulose, as well as preventing the plant cell wall and fibre from chemical and physical damage (Reddy & Yang, 2005; Anwar *et al*., 2014).

Few methods are developed to determine the lignin amount in plant cell wall, namely acetyl bromide, thioglycolic acid and Klason method. The first two methods are based on lignin solubilisation whereas Klason is based on insoluble lignin fraction (Moreira-Vilar *et al*., 2014). However, acetyl bromide analysis is usually used for lignin content determination because of its rapidity, sensitivity and feasibility for small sample amount (Hatfield *et al*., 1999; Moreira-Vilar *et al*., 2014).

Figure 1 shows the acetyl bromide-lignin percentage in the rice straw and rice husk of MR219 and MR263. The lignin percentage in straw and husk of MR219 are 7.21% (±0.01) and 9.50% (±0.01), respectively, meanwhile lignin percentage in straw and husk of MR263 are 8.17% and 10.21% (±0.03 and ±0.02), respectively. As reported by Wannapeera *et al* (2008), lignin content in Thailand rice straw and rice husk are 24.4% and 22.3%, respectively, which is higher as compared to MR219 and MR263. In addition, lignin content in the local paddy varieties is much lower than the lignin content in other plant biomasses, such as 16.2% in bagasse and 21.3% in wheat straw (Pauly & Keegstra, 2008; Rani & Sari, 2012).

Lignin is used as an indicator for biorefinery conversion of plant waste in the biofuel or biocomposite production, in which the high lignin content causes biomass conversion into biofuel more difficult (Moreira-Vilar *et al*., 2014). Lignin layers enclosing the cellulose and hemicellulose complex protect them against enzymatic hydrolysis, a step in bioethanol conversion that hydrolyse monomeric sugar to form ethanol (Binod *et al*., 2010). In addition, biomass with low lignin content is more preferable because the conversion process is easier and more cost-effective due to less pretreatment process required to break the lignin layer for enzymatic action (Yu *et al*., 2014).

Quantitative Analysis of Lignocellulosic Cell Wall Component

Hemicellulose and cellulose fractions were isolated from the rice straw and rice husk of MR219 and MR263 and quantitatively analysed with anthrone assay. Cellulose that was isolated via Saeman hydrolysis by using 72% H_2SO_4 is also known as α-cellulose. Xylose is used as standard sugar in anthrone assay for hemicellulose analysis because it is the main compound of hemicellulose.

The cellulose content is higher in both paddy varieties as compared to the hemicellulose content (Figure 2). The cellulose content is 42.63% (±6.44), 42.46% (±2.4), 38.20% (±0.8) and 38.48% (±3.48)

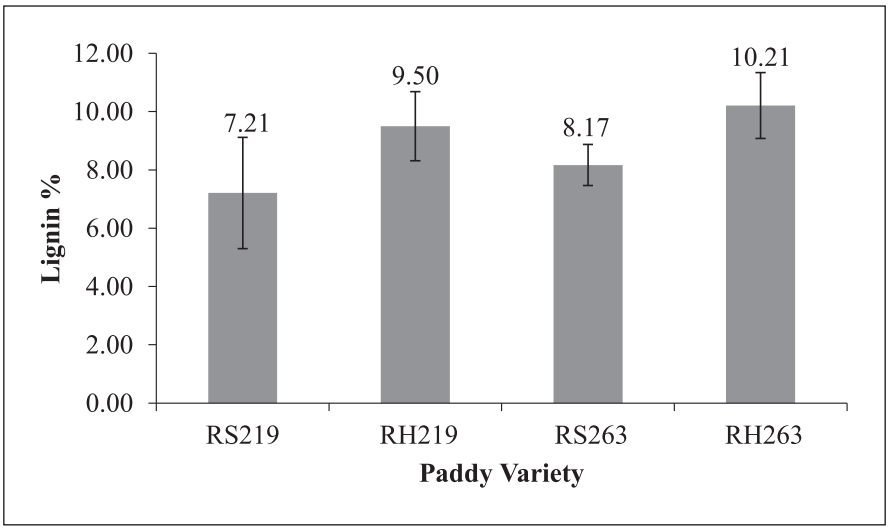

Fig. 1. Comparative quantitative analysis of acetyl bromide-lignin content in husk and straw sample. The percentage of lignin content in 0.1 g of RS219 (MR219 rice straw), RH219 (MR219 rice husk), RS263 (MR263 rice straw) and RH263 (MR263 rice husk) was determined by acetyl bromide assay. Values are means (n=3) ± s.e.

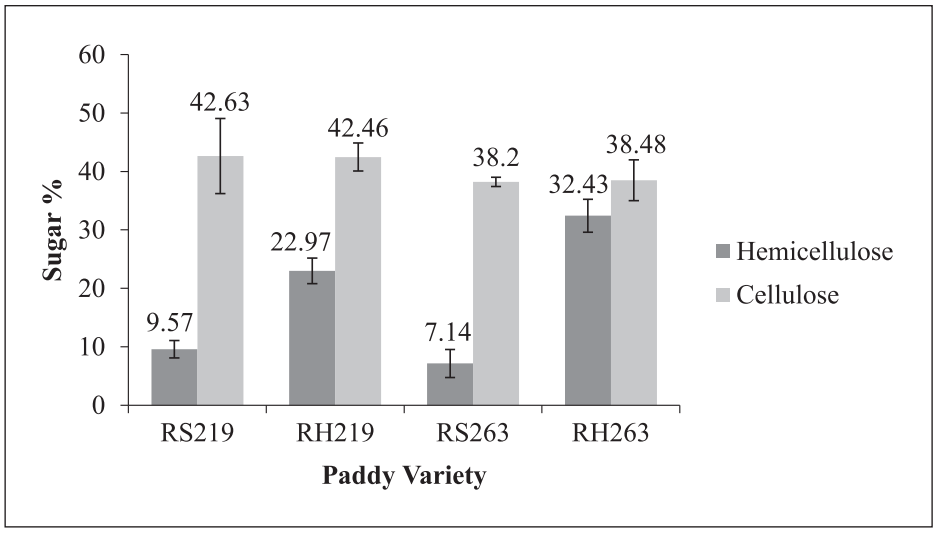

Fig. 2. Comparative quantitative analysis of major polysaccharides in lignocellulosic materials of MR219 and MR263. Chemical fractionation analysis of hemicellulose and cellulose content in husk and straw was determined by anthrone assay. Glucose and xylose are used as standard sugar for cellulose and hemicellulose, respectively. Values are means (n=3) ± s.e.

for MR219 rice straw, MR219 rice husk, MR263 rice straw and MR263 rice husk, respectively. Hemicellulose content is lower in straw samples of both varieties, with 9.57% (±1.49) in MR219 and 7.14% (±2.4) in MR263. Meanwhile, MR219 husk and MR263 husk show that hemicellulose content is two to four times higher than the straw samples.

Higher cellulose content and lower hemicellulose content in straw samples of both varieties indicate that rice straw of MR219 and MR263 are more suitable to be plant biomass material, especially for waste-to-energy application. Cellulose is the main component in plant that provides mechanical strength to natural fibre, that is enclosed by hemicellulose and lignin (Morán *et al*., 2008). The polymer is made up of a type of sugar as its backbone which is glucose, a hexose sugar. Plant biomasses with higher cellulose content are more frequently used as raw materials in biorefinery industry, as they are more preferred by microbes, because they utilise the monomeric sugar during fermentation better as compared to the five carbon sugar monomer, pentose sugar (Hendriks & Zeeman, 2009), a hemicellulose component. It is also easier to process and manipulate cellulose for other purposes as compared to lignin or hemicelluloses.

CONCLUSION

Analytical methods described in this experiment enable the determination of lignocellulosic component comprehensively and rapidly. As a conclusion, the chemical content and composition of lignocellulosic component in rice straw and rice husk of Malaysian local paddy, MR219 and MR263 are different. The difference is due to different paddy variety and parts. All the paddy biomass samples used in this experiment have low lignin and hemicellulose content but high cellulose content, making MR219 and MR263 as suitable candidates for bioethanol and biocomposite production in biorefinery industry, thus ensuring an easier and more cost-effective conversion process.

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