

Characterisation of Agricultural and Agro-Industrial Residues as Raw Materials for Ethanol Production

C. Martín*, Y. López, Y. Plasencia, and E. Hernández

Bioresource Technology group, Department of Chemistry and Chemical Engineering, University of Matanzas, 44740 Matanzas, Cuba

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Sugarcane bagasse, rice hulls, peanut shells, and cassava stalks are abundant lignocellulosic residues that could be considered as raw materials for ethanol production in tropical countries. The knowledge on the composition of lignocellulosic materials is important for their effective utilisation. In this work, a systematic comparison of the chemical composition of sugarcane bagasse, peanut shells and two sorts of rice hulls and cassava stalks, was performed using analytical acid hydrolysis and HPLC analysis. The highest carbohydrate fraction, $w = 641 \text{ g kg}^{-1}$ of the dry matter was detected in bagasse, which also contained the highest amount of xylan ($w = 221 \text{ g kg}^{-1}$). Rice hulls obtained from an artisan mill had the highest glucan fraction, $w = 497 \text{ g kg}^{-1}$, whereas peanut shells displayed the highest lignin fraction ($w = 365 \text{ g kg}^{-1}$). The highest ethanol potential, around 163 L t^{-1} , was estimated for rice hulls and bagasse. However, the potential of bagasse would be higher than that of rice hulls if conversion of xylan fraction to ethanol would be considered.

Key words:

Lignocellulosic materials, agricultural residues, biomass analysis, ethanol

Introduction

Lignocellulosic materials are the world's most widely available low-cost renewable resources to be considered for production of organic fuels, chemicals, and new-generation materials. During the last years, the need for materials in an expanding world population has renewed the interest in the use of agro-based resources, especially in those countries where little wood resources are left or where legal restrictions on the use of wood are in place.¹ Residues from agriculture, forestry and food industry have become important raw materials for pulp and paper industry and other wood-based applications.² Due to their low cost and high availability, lignocellulosic materials are promising raw materials for production of ethanol, an environmentally friendly fuel that is already been used as substitute or additive to gasoline.³

A huge diversity of lignocellulosic wastes is available around the world.⁴ Sugarcane bagasse, rice hulls, peanut shells, and cassava stalks are agricultural and agro-industrial residues, that could be considered important for ethanol production in tropical countries. These materials are available on a renewable basis since they are generated by the harvest and processing of sugar cane (*Saccharum officinarum*), rice (*Oriza sativa*), peanut (*Arachis hypogaea*) and cassava (*Manihot dulcis*), which are

regularly cultivated crops with a very high social demand in many countries.

Potential applications for those residues include energy generation⁵ and production of activated charcoal,⁶ single-cell protein,⁷ and pulp and paper.⁸ However, except bagasse, which is largely used as solid fuel for energy generation to run sugar mills, as well as for pulp and paper production, and cattle feed manufacturing,^{9,10} the other materials are of low economic value and are mainly abandoned in the fields or burnt causing environmental problems. Therefore, they could be considered as potential raw materials for production of chemicals of economic and social interest, such as fuel ethanol, provided that they have a sufficiently high content of carbohydrates. Whereas bagasse and rice hulls have already been investigated for ethanol production,^{11–14} no reports on utilisation of hydrolysates of peanut shells and cassava stalks as substrates for ethanolic fermentation were found in the reviewed literature.

Different industries have their own specific requirements for the raw materials. For evaluating a material as a feedstock for a given production, it is important to know its chemical content. For forestalling the potential ethanol yield from the above-mentioned materials, it is necessary to gather information about their content of cellulose and hemicelluloses. However, the knowledge on the composition of these materials is still incomplete.

*Corresponding author (E-mail: carlos.martin@umcc.cu)

While the composition of sugarcane bagasse has been reported by several authors,^{15–17} and rice hulls have been investigated to some extent,^{14,18} the reports on the composition of peanut shells and cassava stalks are scarce. On the other hand, diverse reports reveal variations in the composition of different sorts of bagasse and rice hulls, and some important components, such as acetyl groups are frequently omitted. Furthermore, comparative information on chemical composition of different biomass materials is often based on reports from different laboratories.

In this work, the Klason analysis procedure, which is based on the sulfuric acid hydrolysis of the polysaccharides contained in the extractive-free material followed by HPLC quantification of the released sugars, is used for the systematic comparison of the chemical composition of sugarcane bagasse, peanut shells, and two sorts of rice hulls and cassava stalks. The potential ethanol yield from these materials is estimated.

Materials and methods

Raw material

Sugarcane bagasse from the 2004 harvest was generously donated by “Horacio Rodríguez” sugar mill (Matanzas, Cuba). Cassava stalks and peanut shells were acquired from local producers (Matanzas Delegation of Ministry of Agriculture, Cuba). A portion of cassava stalks was debarked manually. Two different sorts of rice hulls were utilised. One sort consisted of residues from an industrial mill (CAI arrocero Hoyo Colorado, Matanzas, Cuba). The second sort was obtained from a low-efficiency artisan rice mill (Pedro Betancourt, Matanzas, Cuba), and contained particles of grain that were not removed. All the materials were air-dried to a dry matter (DM) mass fraction of $w = 900\text{--}920\text{ g kg}^{-1}$, milled to pass a 1-mm screen and stored in plastic bags in a dark chamber at room temperature.

Analysis

The dry matter (DM) fraction was determined by drying 500 mg samples of the materials at 105 °C until constant mass. For determination of the ash fraction, the samples were incinerated at 550 °C during 3 h. Extractives were determined gravimetrically after a Soxhlet extraction with $\varphi = 96\%$ ethanol during 24 h. For determination of the carbohydrate and lignin fraction, 16 mg samples of extractive-free materials were hydrolysed first with 72% H_2SO_4 during 1 h at 30°C and then for another hour with 4% H_2SO_4 at 121 °C. The mixture was separated by vacuum filtration through previously

weighed filter crucibles and the lignin fraction was determined gravimetrically (Sartorius AG, Model BP 211D, Goettingen, Germany). Lignin fraction was corrected for ash after incineration of the hydrolysis residue using the procedure described above. The hydrolysates were treated with $\text{Ba}(\text{OH})_2$ for removing sulphate anions. The sulphate-free hydrolysates were analysed by HPLC (Perkin Elmer, Norwalk, CT, USA). Glucose, xylose, arabinose, and acetic acid were separated with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) using $c = 4\text{ mmol L}^{-1}\text{ H}_2\text{SO}_4$ as mobile phase at a volume flow rate of $Q = 0.6\text{ mL min}^{-1}$ at 45 °C, and detected using an IR detector (Bischoff Chromatography 8120, Leonberg, Germany). Four replicates, including two sugar standards for considering sugar degradation during hydrolysis, were run for each sample.

Results and discussion

A schematic representation of the analysis procedure, used for determining the composition of the six different lignocellulosic residues investigated in this work, is shown in Fig.1. The Klason procedure was chosen as the analysis method because it is a common analytical tool for analysis of biomass components, it is a relatively easy to perform and well reproducible method, and it has been applied to many different lignocellulosic materials in different laboratories.^{15,19} It is based on the H_2SO_4 hydrolysis of the polysaccharides contained in the extractive-free material, followed by chromatographic quantification of the released monomeric sugars. Based on the concentration of the sugars, the relative amounts of the polymers from which they are derived can be calculated stoichiometrically.

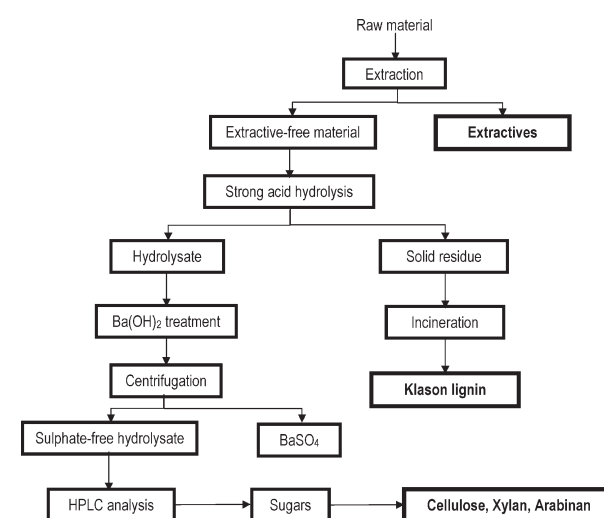


Fig. 1 – Scheme of the analysis procedure used for determination of the composition of the investigated materials

Table 1 – Main components of the investigated materials, $w / g\ kg^{-1}$

Material	Total carbohydrate ¹	Extractives ¹	Klason lignin ²	Ash ¹	Acetyl groups ²	Total
Rice hulls (artisan)	603 (14.3)	59 (2.8)	135 (5.0)	157 (10.5)	8 (0.8)	962
Rice hulls (industrial)	548 (12.5)	41 (1.4)	208 (5.1)	176 (9.7)	11 (1.4)	984
Peanut shells	349 (4.1)	79 (3.5)	365 (11.3)	69 (4.0)	27 (1.8)	889
Cassava stalks (with bark)	453 (4.6)	72 (3.1)	253 (3.6)	75 (3.7)	28 (1.5)	881
Cassava stalks (debarked)	556 (4.2)	51 (0.7)	209 (4.2)	57 (0.4)	28 (1.0)	901
Sugarcane bagasse	641 (2.9)	53 (1.4)	181 (1.7)	23 (0.1)	33 (1.8)	924

Average of two (¹) and four (²) replicates. Standard deviation shown in parentheses.

The results shown in Table 1 revealed that sugarcane bagasse had the highest carbohydrate fraction among the investigated materials. The total mass fraction of carbohydrates in bagasse accounted for $w = 641\ g\ kg^{-1}$ of the dry material. Relatively high carbohydrate fraction, above $w = 500\ g\ kg^{-1}$, was also displayed by both sorts of rice hulls and by debarked cassava stalks.

Three different carbohydrate components, cellulose, xylan and arabinan, were quantified. Although, cellulose fraction was high in several materials, the highest fraction, $w = 497\ g\ kg^{-1}$, was found in artisan rice hulls (Fig. 2). That material is the residue of a low-efficiency artisan mill, which renders rice hulls containing grain remains. Since the method used in this work did not allow distinguishing between cellulose and starch, the content of cellulose in artisan rice hulls was probably overestimated due to the presence of starch contained in the grain remains. The presence of starch in artisan mill-rice hulls was demonstrated in an additional mild sulphuric acid hydrolysis experiment (data not shown). However, the quantification of starch mass fraction by amylase treatment deserves further attention for an accurate determination of cellulose contained in artisan mill-rice hulls. Cellulose frac-

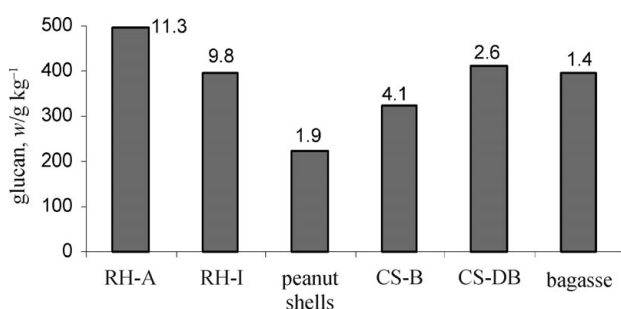


Fig. 2 – Glucan mass fraction of artisan mill-rice hulls (RH-A), industrial mill-rice hulls (RH-I), peanut shells, bark-containing cassava stalks (CS-B), debarked cassava stalks (CS-DB) and sugarcane bagasse, $w/g\ kg^{-1}$. Standard deviations are shown at the top of the bars

tions of around $w = 400\ g\ kg^{-1}$ were observed in debarked cassava stalks, industrial mill-rice hulls and sugarcane bagasse, whereas in peanut shells it was only $w = 225\ g\ kg^{-1}$.

With xylan and arabinan fraction of $w = 221$ and $25\ g\ kg^{-1}$, respectively, sugarcane bagasse was, by far, the material with the highest fraction of pentosans (Fig. 3). The fraction of pentosans was less relevant in the other materials, where xylan content ranged between 91 and $139\ g\ kg^{-1}$, and arabinan fraction ranged between 5 and $18\ g\ kg^{-1}$. The high pentosans fraction of bagasse is in accordance with previous reports,^{15,16,20} and makes it an attractive substrate for production of xylose derivatives such as xylitol and furfural. The high xylan mass fraction of bagasse is also an attractive feature for ethanol production provided that an efficient xylose-utilising organism is available.

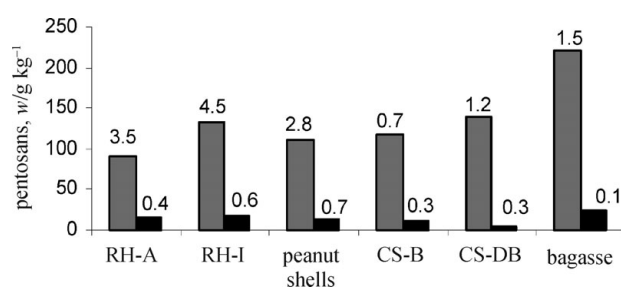


Fig. 3 – Mass fraction of pentosans in the materials, $w/g\ kg^{-1}$. Grey bars, xylan; black bars, arabinan. Same symbols as in Fig. 2.

Klason lignin is often determined as the solid residue of the acid hydrolysis. However, an exaggerated lignin fraction can be obtained if acid-insoluble minerals are not deducted.¹⁵ In this work, the hydrolysis residue was incinerated, and the obtained ash was subtracted from the mass of the residue giving a rather good idea about the lignin fraction in the materials (Fig. 1).

A very high lignin fraction, accounting for more than one third of the dry matter, was found in peanut shells (Table 1). This is in accordance with previous reports on analyses of different nut shells.²¹ The second highest lignin fraction was found in non-debarked cassava stalks. The lignin fraction of bagasse was lower than what has been reported previously.¹⁵ That is because in other reports the lignin content has been influenced by mineral components, whereas in this work the fraction of lignin was corrected for ash. The correction of lignin fraction for ash was especially important for rice hulls, which would give, otherwise, a two-times higher lignin fraction and an overall fraction exceeding 1000 g kg⁻¹.

Ash fraction was significantly higher in rice hulls than in the other materials. The relatively lower ash fraction of the hulls from the artisan mill, compared with those from the industrial mill, seems to be due to a dilution effect by the grain remains, which are mainly composed of starch and contain low amounts of mineral components. Ash may be a drawback for ethanol production, when mineral components have neutralising ability, which could increase the pH during acid hydrolysis or hydrothermal pretreatments, and higher temperatures or longer time would be required to achieve the desired hydrolysing effect.¹⁸ The high ash mass fraction of rice hulls is also a restriction for some applications such as solid fuel, but it could be beneficial for other uses. Although, the high silica fraction in rice hull ash is well acknowledged,^{22,23} the complete identification of individual mineral components is required for elucidating, whose applications the rice hulls ash is best suited for. An investigation on that issue is underway by this group.

Although acetyl groups are important components of hemicelluloses in hardwoods and graminaceous plants, they are seldom mentioned in reports on quantitative analysis of lignocellulosic materials. In this work, an approach to the quantification of the fraction of acetyl groups was made. The fraction of acetyl groups was calculated by multiplication of the acetic acid concentration in the hydrolysates by 0.72, a factor expressing the ratio between the molecular mass M of an acetyl group (43) and acetic acid molar mass M (60). The fraction of acetyl groups might have been underestimated, since hydrolysis was not performed in sealed tubes, and a part of acetic acid could have been volatilised. However, the results are useful for comparing the relative fractions of acetyl groups in the analysed materials. The highest fraction of acetyl groups was found in bagasse, and it was approximately three to four times higher than that of rice hulls, which was the material with the lowest fraction (Table 1). Peanut shells and cassava stalks had

a comparable acetyl fraction, which was considerably higher than that of rice hulls, but slightly lower than that of bagasse. For future work, it is advisable to perform the high temperature post-hydrolysis in sealed vessels, as recommended by *Kaar et al.* (1991),¹⁹ for retaining acetic acid and other volatile components, such as furfural, what would allow a more accurate determination of the constituents of the materials.

The highest volume fraction of ethanol extractives was detected in peanut shells, followed by raw cassava stalks. The other materials had a rather uniform fraction of extractives. Although, using more than one solvent improves the accuracy of the determination of the extractives, we used only ethanol, because it is a cheap solvent that can solubilise a high number of compounds, and the extraction procedure is simpler than when multiple solvents are used.

As indicated in Table 1, debarked cassava stalks contained less extractives, ash, lignin and arabinan, but more glucan and xylan than undebarked stalks. This is an expected result, since it is known that barks of many species are rich in mineral components, arabinans and extractives, especially phenolic compounds.²⁴

The lack in mass closure to 1000 g kg⁻¹, which was evident for all the materials (Table 1), is due to the missing analyses of the uronic acid content and the acid-soluble lignin,¹⁵ as well as for the incomplete accounting of acetyl groups. Including more sugars in the analysis of the hemicellulose fraction could also render a better mass closure. In a recent work by this group, considerable contents of mannose and galactose were detected in dilute-acid hydrolysates of peanut shells and cassava stalks (*Martín et al.*, unpublished).

In order to give an approximate assessment of the suitability of the investigated materials for ethanol production, the potential ethanol yield from sugarcane bagasse, industrial mill-rice hulls, peanut shells and undebarked cassava stalks was calculated. Assuming that $w = 750$ g kg⁻¹ of cellulose is hydrolysed upon enzymatic or acid hydrolysis, and that an ethanol yield of $w = 410$ g kg⁻¹ of glucose is obtained by a conventional Baker's yeast fermentation, the ethanol yield was calculated for each material. As can be seen (Fig. 4), sugarcane bagasse and rice hulls are the most promising materials for ethanol production, due to their high potential ethanol yield from cellulose. From a techno-economic point of view, these materials are also the most appropriate for industrialisation since they are produced in very high amounts and are usually available in central collection sites, such as sugar and rice mills. In a scenario, where xylose could efficiently be con-

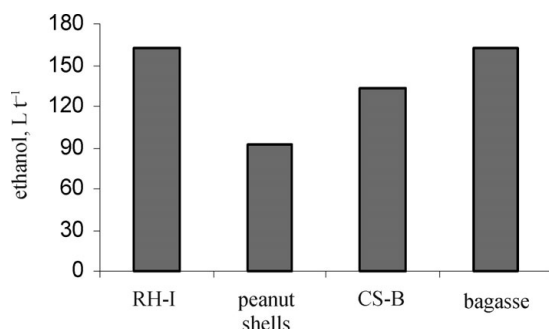


Fig. 4 – Ethanol potential of industrial rice hulls (RH-I), peanut shells, debarked cassava stalks (CS-B) and sugarcane bagasse, L t⁻¹

verted to ethanol, the potential of sugarcane bagasse would significantly increase due to its high xylan content (Fig. 3).

Conclusions

This investigation gives a systematic comparison of the chemical composition of different lignocellulosic residues and helps to elucidate their potential for ethanol production. The reproducibility of the obtained results was good, as can be deduced from the low standard deviations. However, further studies with a higher refinement degree, including triple extraction, amylase treatment for starch determination, quantification of pectin and acid-soluble lignin, as well as a more accurate determination of acetyl groups, are worthwhile. Due to the high diversity of lignocellulose wastes, it is recommendable to extend this work to other materials.

List of symbols

- c – concentration, mmol L⁻¹
 m – mass, g
 M – molar mass, g mol⁻¹
 n – amount of substance, mol
 Q – volume flow rate, mL min⁻¹
 w – mass fraction, g kg⁻¹
 φ – volume fraction, %

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