CHEMICAL AND PHYSICAL CHARACTERIZATION AND ACID HYDROLYSIS OF A MIXTURE OF *JATROPHA CURCAS* SHELLS AND HUSKS

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Received April 1, 2014

Jatropha curcas L. is a tropical plant with considerable potential for producing biodiesel and other products of high economic and social interest. During the biodiesel production process from *J. curcas* different residues, such as shells and husks are generated. In this work, the physical characterization of *J. curcas* fruits was performed, and the chemical composition of a mixture of shells and husks was determined. The physical characterization revealed that shells and husks account, respectively, for 25.0 and 27.8% of the fruit weight. The compositional analyses of the material showed a quite high content of glucans (32.8% w/w) and xylans (16.4% w/w), which indicates the potential of *J. curcas* shells and husks for production of ethanol, xylitol and other glucose- and xylose-derived products. Acid hydrolysis was applied to a mixture of shells and husks under different sulphuric acid concentrations (from 0.5 to 4.5%), temperatures (170-220 ºC) and time (10-20 min), and the hydrolytic conversion of xylan was evaluated. A zone of experimental conditions giving maximal xylan conversion was identified at around 4% H_2SO_4, 180 ºC and reaction time below 10 min.

Keywords: *Jatropha curcas* shells, ethanol, dilute-acid hydrolysis, pretreatment

INTRODUCTION

Jatropha curcas L., also known as physic nut, is an oil-bearing plant, which is considered an attractive feedstock for producing biodiesel to be used as a transportation fuel.[1] Biodiesel is produced by trans-esterification of vegetable oils or animal fats with short-chain length alcohols. While important amounts of biodiesel are nowadays produced from edible sources, such as soybean and sunflower oil, it is a challenge to use feedstocks that would not compete with human food.[2] *J. curcas* is a drought-resistant tree belonging to the *Euphorbiaceae* family,[3] whose seeds yield up to 49% of a non-edible oil[4] that can be used for fuel production without affecting the food sector.

J. curcas is cultivated in many countries in the tropical and subtropical areas.[5] In Cuba, it is present as a wild plant in the whole country, and dedicated plantations are currently been developed in several provinces.[6] *J. curcas* can be utilized in the agriculture, pharmaceutical industry and medicine, among many other applications,[7] but the oil is its most valuable derivative since it bears a high promise for biodiesel production. In a recent investigation including different plants, it was shown that the high oil yield of *J. curcas* and its fatty acid composition make it one of the most promising oil-bearing seeds for biodiesel production in Cuba.[4]

During biodiesel production from *J. curcas* several by-products, such as shells, husks and press cakes, are generated. Dry *J. curcas* fruit contains around 37.5% shell and 62.5% seed, and

the seed contains about 42% husk and 58% kernel.[8] Although the integral utilization of all the fractions generated in biodiesel production is a requirement for the sustainability of the process, not much attention has so far been devoted to developing uses for the residues generated during de-shelling of *J. curcas* fruits and decortication of their seeds for oil extraction.

In our opinion, ethanol production from *J. curcas* shells and husks is an energetic use that deserves being investigated. The *in situ* produced ethanol could be directed, together with the oil, to biodiesel production in an integrated configuration. The integration of ethanol and biodiesel production processes using a single source of biomass would allow considerable reduction of the energy costs compared with the autonomous production of each of them, and would lead to a decrease in the generation of solid wastes.[9] If *J. curcas* shells and husks are used for ethanol production, their polysaccharides – cellulose and hemicelluloses – have to be hydrolysed for rendering sugars, which could be fermented by ethanologenic organisms. Enzymatic saccharification is a promising approach for hydrolysing cellulose,[10] but it requires a pretreatment step for enhancing the accessibility of the substrate to enzymes. Recent reports[11-14] stressed the effectiveness of acid prehydrolysis as a pretreatment method for the enzymatic hydrolysis of the cellulose contained in *J. curcas* shells, but the pretreatment of mixtures of shells and seed husks has, to our knowledge, not yet been reported. In this work, the chemical composition of a mixture of *J. curcas* shells and husks is investigated and the dilute-acid hydrolysis of their xylan is evaluated.

EXPERIMENTAL

Raw material

Freshly harvested *J. curcas* fruits were supplied by Sancti Spíritus Experimental Station of Pastures and Forages (Sancti Spíritus, Cuba). The fruits were sun-dried, de-shelled and decorticated in order to separate the kernel from the seed husk and shell. The resulting shells and husks were mixed together, air-dried and milled to 2-mm size. The milled material was dried at 50 ºC for 24 h and stored in plastic bags in a dark chamber at room temperature. A portion of the milled material was further screened, and the fraction passing the 80-mm screen but retained by the 20-mm one was used for compositional analysis.

Physical characterization

Three 1-kg samples of *J. curcas* fruits were peeled. The seeds were counted and then decorticated. The average number of seeds per fruit, and the weight percentage of shells, seeds, husks and kernels were calculated.

Analytical methods

The amounts of moisture, ethanol extractives, ash, glucans, xylans and Klason lignin in the raw material were determined using the NREL standard biomass analytical methods.[15-18] Moisture content was determined gravimetrically after drying duplicate samples at 105 ºC until constant mass. Ash was determined by incineration of aliquots at 550 ºC. The content of extractive compounds was determined after Soxhlet extraction with 96% (v/v) ethanol during 24 h. For determination of the polysaccharides, quantitative acid hydrolysis with 72% sulphuric acid was carried out. Glucose and xylose concentrations were determined by HPLC of the hydrolyzates. The content of cellulose and xylan was calculated from the concentration of glucose and xylose. Klason lignin was determined as the solid residue obtained after hydrolysis. The sugars were separated on an Aminex HPX 87H column (Bio-Rad, Hercules, CA, USA). A solution of 5 mM H_2SO_4 was used as mobile phase at a flow of 0.6 mL min^{-1} at 60 ºC. An RI detector (Shimadzu Corp., Kyoto, Japan) was used for detecting the sugars, and a UV detector (Waters 486) at the wavelength of 210 nm was used for furfural and HMF detection. The experimental results were analyzed using MINITAB, Statgraphics Plus 5.0 and Design Expert version 7.0.0, as statistical software.

Dilute-acid hydrolysis

Ten grams (dry matter) of the mixture of *J. curcas* shells and husks were suspended in 100 mL sulphuric acid in 140-mL stainless steel reaction vessels (Swagelok®, Solon, Ohio, USA). Since there were no reports about the acid hydrolysis of this mixed raw material, it was decided to use a quite broad range of sulphuric acid concentrations. The used concentrations were 0.5, 1, 2, 3, 3.5, 4 and 4.5%. The vessels with the reaction mixtures were immersed in an oil bath and heated to 170-220 ºC for 10-20 minutes. After finishing the hydrolysis, 10-mL samples of the hydrolyzates were taken and stored frozen until analysis. Triplicate experiments were carried out for each condition. The mean of the results and the standard deviations are shown in the tables. Xylose concentration (g/L) and xylan conversion (%) were the main response variables, but the formation of furfural and hydroxymethylfurfural (HMF) were also considered.

RESULTS AND DISCUSSION

Prior to this work, the characterization of seed husks of Cuban *J. curcas* was performed,[4] and the dilute-acid pretreatment of its shells was

investigated.[11,14] The interest in this study was to investigate mixtures of these materials, both of which are important by-products of *J. curcas* processing, and have potential for being used as raw materials for production of cellulosic ethanol, xylitol, furfural and other chemicals and fuels. With this aim, a mixture of shells and husks was prepared, and it was first physically and chemically characterized, and then submitted to dilute-acid hydrolysis.

Physical characterization of the raw material

The *J. curcas* fruits used in this work contained, on average 2.76 seeds, whose weight represented about 75% of the fruit dry weight, while the shells represented the rest (Table 1). The seeds were composed of 37.2% of husk and 62.8% of kernel. That is in agreement with a previous report on Cuban *J. curcas*,[4] but the percentage of kernel in the seed is a little bit higher than that of *J. curcas* from India.[8] Shells and husks account for 52.8% of the weight of *J. curcas* fruit. That is an important share taking into account that it is considerably higher than that of the oil (37%), the most valuable component of *J. curcas*.[19] That significant amount of material should not be ignored if *J. curcas* is going to be used integrally following a biorefinery strategy. Upgrading the husk and shells to valuable products would improve the economic feasibility of biodiesel production from *J. curcas*.

Chemical composition of the raw material

The analyses revealed that the mixture of *J. curcas* shells and husks contained 49.2% of polysaccharides, including both glucans and xylans (Table 2). This relatively high carbohydrate content is a positive feature of *J. curcas* shells and husks for being considered as a raw material for ethanol production either from cellulose alone or from cellulose and xylan if a xylose-utilizing micro-organism is used. Although in general the content of the main components of this material is in good agreement with the results of previous references, there are several specific issues that are worth highlighting.

Table 1
Physical characterization of *J. curcas* fruits

	Content, %		
	This work	Singh *et al.*[8]	Martín *et al.*[4]
Shells	25.0[1] ± 1.2	35-40[1]	-
Seeds	75.0[1] ± 1.2	65-60[1]	-
Husks	37.2[2] (27.8[1]) ± 0.9	40-42[2]	36.6[2]
Kernels	62.8[2] (47.2[1]) ± 1.7	60-58[2]	63.4[2]

[1]Percentage of fruit weight; [2]Percentage of seed weight

Table 2
Chemical characterization of the raw material (based on dry weight)

	Content, %		
	Shells and husks (This work)	Shells (Singh *et al.*[8])	Husks (Martín *et al.*[4])
Glucans	32.8 ± 1.8	33.8	16.9
Xylans	16.4 ± 0.7	9.7[1]	16.2
Lignin	25.1 ± 1.6	11.7	45.5
Extractives	5.8[2] ± 0.4	6.0[3]	5.5[4]
Ash	12.8 ± 0.3	14.9	4.7

Mean of three replicate determinations. [1]Reported as hemicelluloses, [2]Reported as ethanol extractives, [3]Reported as ether extractives, [4]Determined as the non-identified fraction

The glucan content of the mixture of *J. curcas* shells and husks was comparable with that detected in individual shells,[8] and it was almost two-fold higher than the one detected in husks alone.[4] On the other hand, the content of xylan in this work was higher than that of the shells and

comparable with that of the husks. The lignin content found in this work is considerably higher than that reported for shells and lower than the one of the husks, and it is quite close to the average of the lignin content of both components. The content of extractives also ranged between the contents reported for shells and husks.

The ash content of the mixture also ranged between the values reported for the shells and husks, but it was closer to that of the shells. In general, most of the values were very logical. The most surprising ones were regarding the carbohydrate composition, namely the contents of glucan and xylan, which were higher than it could be expected. That might be explained by the different origin of the samples.

Acid hydrolysis of xylan

The xylan contained in *J. curcas* shells and husks can be a source of xylitol, furfural, ethanol and other products. To benefit from its potential, xylan should be hydrolyzed to xylose by means of acids or enzymes. When acid hydrolysis of xylan is performed, the lignocellulosic material undergoes transformations that improve the enzymatic conversion of cellulose. Recently, we presented results about the enhancement of cellulose enzymatic hydrolysis upon dilute-acid pretreatment of *J. curcas* shells,[11] and the current work is focused on the hydrolytic conversion of xylan.

Initially, the hydrolysis of xylan with 2% sulphuric acid was investigated. The experiment included three different temperatures (180, 200 and 220 ºC) and times (10, 15 and 20 min). The temperature and the hydrolysis time were combined in such a way that the difference of the severity was maximal for the three experimental runs. In the experiment performed at 180 ºC and 10 min, whose severity factor was 3.4, xylose concentration (8.0%) and xylan conversion (47.8%) were the highest, glucose formation was low and the concentrations of the furan aldehydes, furfural and hydroxymethylfurfural, were minimal (Table 3). The severity factor was calculated by the equation proposed by Overend and Chornet:[20]

$$Ro = t \times e^{\frac{T-100}{14.75}} \quad \text{(Eq. 1)}$$

where 't' and 'T' are the time (s) and the operating temperature (K), respectively.

Increasing the severity factor to 4.17, by setting the temperature and the reaction time at 200 ºC and 15 min, respectively, led to a relatively lower xylose concentration and xylan conversion. This is the result of degradation reactions with formation of furfural, whose concentration increased dramatically in comparison with the previous experiment. At the same time, the concentration of glucose increased, but its degradation also was higher than previously, as shown by the increased HMF formation. These results are in agreement with the literature reporting that in high-temperature acidic media, pentoses are dehydrated to furfural and hexoses to hydroxymethylfurfural.[21]

A further increase of the severity (Log R_o = 4.9) led to a sharp decrease of xylose concentration combined with a new increase of furfural formation (Table 3). Glucose concentration also decreased, but that was not reflected by an increase in HMF formation. Instead, HMF concentration dropped as a result of degradation reactions. This can be explained by the high reactivity of furan aldehydes, which are rather instable and follow a typical growth-and-decay curve.[21]

Another experiment included sulphuric acid concentrations of 1, 2, 3 and 4%. For each acid concentration, hydrolyses were run at either 180, 200 or 220 ºC for 10, 15 or 20 min (Table 4). In the experimental set using 1% H_2SO_4 (Fig. 1-A), xylose concentration was rather low (5.7 g/L) at the mildest severity factor (180 ºC, 20 min, Log R_o = 3.7), it rose slightly when the severity increased to 4.17, but it decreased with a further increment of the harshness of the hydrolysis. Furfural formation was low at the lowest severity, it increased to some extension at Log R_o = 4.17, but still remained relatively low. When the hydrolysis conditions were set at Log R_o = 4.59, a two-fold increase of furfural concentration, compared with the experiment at intermediate severity, was observed. The increase of furfural formation was in good correspondence with the trend observed for xylose concentration and xylan hydrolytic conversion, whose maximum (31.5%) for the lowest sulphuric acid concentration was achieved at intermediate severity (experimental run 2, Table 4).

When 2% H_2SO_4 was used (Fig. 1-B), xylose concentration (8 g/L) was relatively high at the lowest severity, it dropped slightly at intermediate severity and sharply under the harshest hydrolysis conditions. Xylan conversion was higher than in the experiment with 1% H_2SO_4, and the highest value was reached at the lowest severity (experimental run 4, Table 4). The conversion could have been higher if xylose degradation had

been avoided or minimized. Xylose degradation occurred at a higher extent at this sulphuric acid concentration, as indicated by the furfural formation, which was already relatively high at the lowest severity. Furfural concentration, increased at intermediate severity, but then decreased. So, although the low xylose concentration found in the most severe experiment indicates that sugar degradation should have occurred, the detected amounts of furfural do not correspond to that expectation. That is because under the severe experimental conditions used, the furfural resulting from xylose dehydration has rapidly degraded. In fact, furfural is known to be very unstable and to degrade under the same conditions under which it is formed,[22] resulting in humins and formic acid.[23] In the hydrolyses performed with 3 and 4% H_2SO_4 (Fig. 1-C and 1-D), the trends of xylose and furfural formation and degradation were the same as in the set of experiments with 2% H_2SO_4. The main differences were that xylose concentration under the less severe conditions was considerably higher (9.8 g/L for 3% H_2SO_4 and 11.6 g/L for 4% H_2SO_4), and that its decrease with increasing the severity was sharper. The highest xylan conversion in all the experiments was achieved with the less severe hydrolysis for the highest sulphuric acid concentration (experimental run 10, Table 4). It is also noteworthy that the maximal xylose and furfural degradation occurred in the hydrolysis performed at the highest severity with 4% H_2SO_4.

Since the highest xylan conversions were found at 180 ºC, the lowest investigated temperatures, a new experiment was performed at 170-190 ºC. A 2^3-factorial design augmented with three center points was applied (Table 5). The highest xylose concentration, giving a 59.2% xylan conversion, was reached at 4% H_2SO_4, 180 ºC and 10 min, which is in agreement with the results of the previous experiment. The analysis of the contour plot of the response surface indicates that the maximal xylose concentration working with 4% H_2SO_4 and 180 ºC would be achieved at reaction times shorter than 10 min (Fig. 2).

Table 3
Experimental conditions and results of dilute-acid hydrolysis using 2% H_2SO_4

Experimental conditions				Concentration, g/L				Xylan conversion, %
Temperature, ºC	Time, min	c(H_2SO_4), %	Log R_o	Xylose	Glucose	Furfural	HMF	
180	10	2.0	3.40	8.0 ± 0.6	1.6 ± 0.2	0.4 ± 0.0	0.2 ± 0.0	47.8
200	15	2.0	4.17	7.4 ± 0.2	5.8 ± 0.5	3.4 ± 0.1	2.0 ± 0.0	44.3
220	20	2.0	4.90	0.5 ± 0.0	3.7 ± 0.1	5.6 ± 0.2	1.8 ± 0.0	3.0

Table 4
Experimental conditions and xylan conversion achieved in dilute-acid hydrolysis at different sulphuric acid concentrations

Experimental run	Experimental conditions				Xylan conversion, %
	Temperature, ºC	Time, min	H_2SO_4 conc, %	Log R_o	
1	180	20	1.0	3.70	30.4
2	200	15	1.0	4.17	31.5
3	220	10	1.0	4.59	9.3
4	180	10	2.0	3.40	43.2
5	200	15	2.0	4.17	39.9
6	220	20	2.0	4.90	2.7
7	180	20	3.0	3.70	47.1
8	200	15	3.0	4.17	6.3
9	220	10	3.0	4.59	4.3
10	180	10	4.0	3.40	62.2
11	200	15	4.0	4.17	7.2
12	220	20	4.0	4.90	0.5

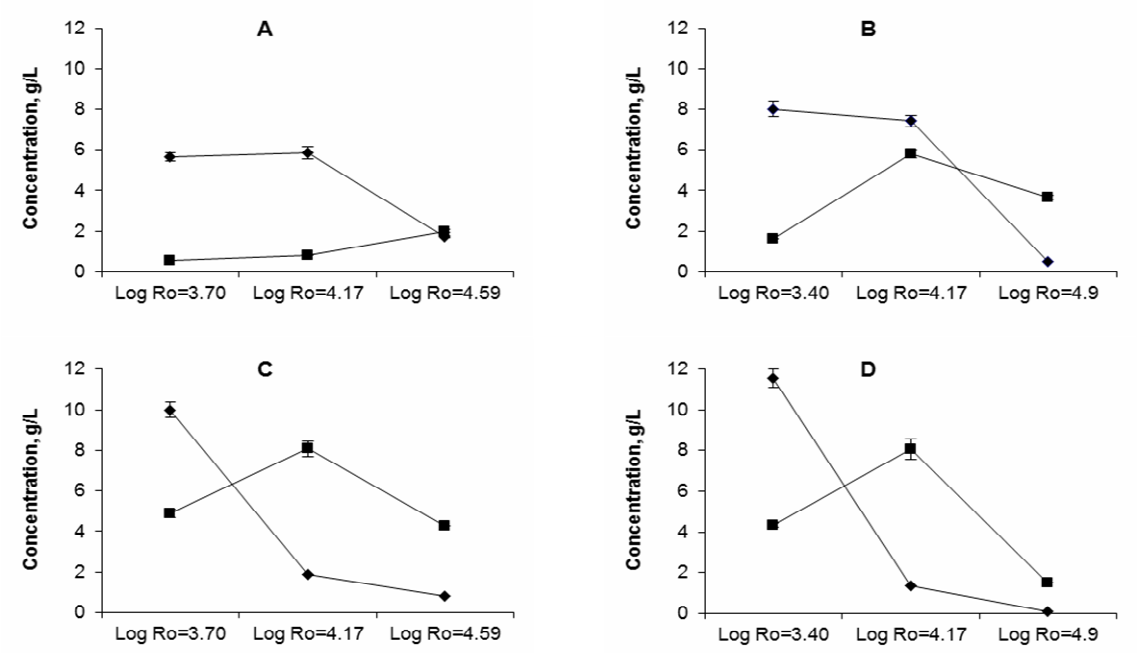

Figure 1: Concentration of xylose (rhombs) and furfural (squares) formed under different severity factors and different sulphuric acid concentrations: A, 1% H_2SO_4; B, 2% H_2SO_4; C, 3% H_2SO_4 and D, 4% H_2SO_4

Therefore, a zone with maximal xylose yields is identified at around 4% H_2SO_4, 180 ºC and hydrolysis time below 10 min. However, since xylan conversion was still rather low and furfural formation was detected even under the mildest conditions, for future experiments it is desirable to investigate lower acid concentrations. That would also be favorable for decreasing the corrosion problems, and all the costs related to the high acid concentration. For compensating the lower acid concentrations, longer reaction times are recommended. A study in this direction is now underway in our laboratory. The identification of the optimal conditions for the enzymatic hydrolysis of cellulose is another issue that is being considered in a new investigation.

Table 5
Conditions for acid hydrolysis of *J. curcas* shells and husks used in the last experiment

Experimental run	Temperature, ºC	Time, min	H_2SO_4 concentration, %
1	170	10	3.5
2	170	10	4.5
3	170	20	3.5
4	170	20	4.5
5	190	10	3.5
6	190	10	4.5
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8	190	20	4.5
9	180	15	4
10	180	15	4
11	180	15	4

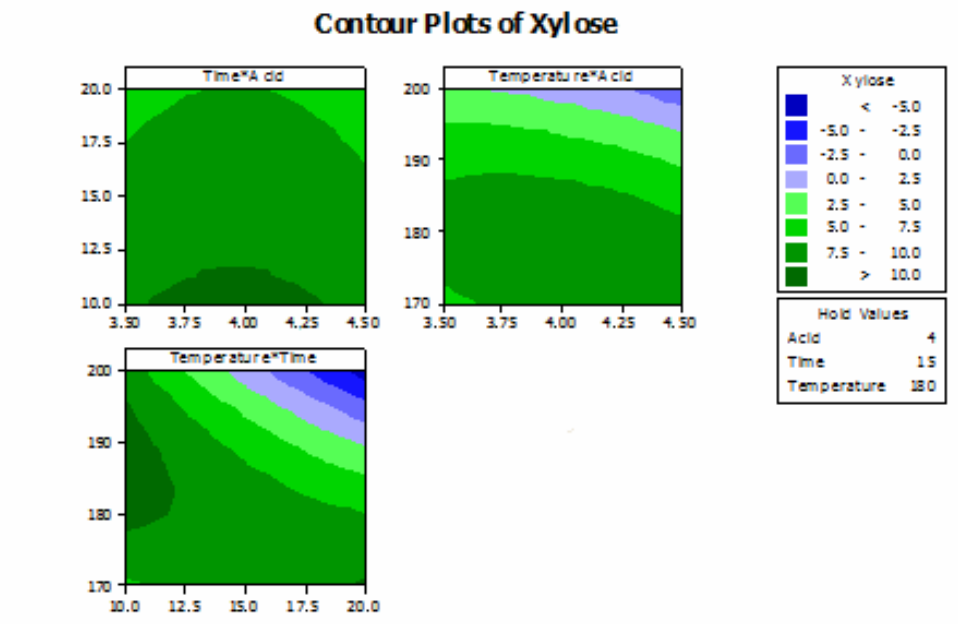

Figure 2: Contour plots of the response surface for xylose yield during acid hydrolysis of *J. curcas* shells and husks under different conditions

CONCLUSION

The shells and husks account for a major part (52.8%) of *J. curcas* fruit weight, and their relatively high glucan and xylan content makes them interesting raw materials for production of ethanol and xylose-derived products. The generated ethanol could be directed to the trans-esterification of the oil extracted from *J. curcas* kernel, in such a way that both the alcohol and the oil used in biodiesel production would come from the same raw material. The acid hydrolysis trials of *J. curcas* shells and husks revealed that maximal xylan conversion can be achieved at around 4% H_2SO_4, 180 ºC and reaction time below 10 min. Lower acid concentrations lead to lower hydrolytic conversion, while more severe conditions lead to xylose degradation.

ACKNOWLEDGMENTS: The financing from the Swedish International Program Office, supporting the collaboration between the University of Matanzas and the University of Borås, is gratefully acknowledged. The financial support given by the Alexander von Humboldt Foundation the research environment Bio4Energy (www.bio4energy.se) and by the Swiss Agency for Development and Cooperation through the BIOMAS-Cuba project is also gratefully acknowledged.

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