Bioethanol from Jatropha Seed Cakes Produced by Acid Hydrolysis Followed by Fermentation with Baker's Yeast

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Abstract

Jatropha curcas seed cake is produced annually as a co-product of the biodiesel production chain. A significant percentage of this solid waste is composed of polymeric carbohydrates. In this work, the residual polysaccharides present in Jatropha seed cake are converted to bioethanol by sequential hydrolytic and fermentative processes. Starch, hemicellulose and cellulose contents were quantified and presented 4.80, 10.41 and 16.88 grams of each polysaccharide per 100 grams of seed cake, respectively. The 3^{k-p} fractional factorial design, whose factors were time, temperature and sulphuric acid concentration, resulted, under the best condition, in the conversion of 82% of the starch and hemicellulose present in the Jatropha seed cake to reducing sugars. Based on the results obtained in bench-scale alcoholic fermentation of the hydrolysate of Jatropha seed cake by Saccharomyces cerevisiae, the production of 88.5 litres of ethanol from the cake generated from the extraction of 1000 kg of oil can be predicted.

Keywords: physic nut; ethanol; acid hydrolysis; saccharification; biofuel

1. Introduction

The physic nut, *Jatropha curcas*, belonging to the *Euphorbiaceae* family, is a large and fast-growing shrub, whose average height is two to three metres that, under special conditions, it can reach five metres in height. The shrub has small yellow-green flowers, and the fruit is a capsule with three smooth, slightly flattened and elliptical black seeds, within which is found the white kernel, tender and rich in oil (Makkar et al. 1998a, Makkar et al. 1998b). The seeds of *J. curcas*, which weighs from 0.44 g to 0.72 g, is composed of approximately 29.9% to 31.9% husk and 68% to 70% kernel, depending on the variety and the agricultural practices utilized (Matínez-Herrera et al. 2006). The kernel contains 52.9 to 58.4% oil, 17.8 to 32.8% protein, 3.6 to 4.4% ash, 3.1 to 4.3% fibre and 5.5% total starch (Martínez-Herrera et al. 2006; Giibitz et al. 1999, Foidl et al. 1996).

The oil from *Jatropha curcas* seeds presents a potential for the production of biodiesel (Foidl et al. 1996, Mohibbe Azam et al. 2005) and biokerosene (Bailis and Baka 2010).

The use of *J. curcas* seeds as animal or human feed is restricted due to its toxicity and the presence of antinutritional factors, such as phorbol esters, phthalates, lectins, saponins and trypsin inhibitors (Matínez-Herrera et al. 2006, Chivandi et al. 2004), requiring a detoxifying treatment for this kind of application (Haas and Mittelbach 2000).

To foster the development of the biofuels industry, the Brazilian Ministry of Agriculture, Livestock and Supply released, in early 2008, the cultivation of *J. curcas* for biodiesel production. According to Singh and Singh (2010), the annual oil yield can reach 2.0 to 3.0 t/ha. Considering that the average amount of oil extracted from *Jatropha* seeds is 30% by weight, each ton of oil extracted generates about 2.3 tonnes of vegetal cake. Regarded as a crop to complement the production of biodiesel, the use of *J. curcas* will produce thousands of tons of seed cake. In this scenario, a major challenge is to guarantee methods or processes to increase the value of these residues, making the biodiesel industry consistent and competitive. One possible strategy is to leverage the significant percentage of carbohydrates present in this residual biomass, 30 to 38% (Inekwe et al. 2012), for the production of bioethanol through hydrolytic and fermentative processes, as has been suggested for other residues from the biodiesel industry (Macedo et al. 2011, Melo et al. 2008). The possibility of using the seed cake for the production of bioethanol would furnish, in theory, the ethanol demanded by the biodiesel plant for the process of transesterification.

Currently, ethanol is produced industrially from raw materials with a high sugar content, such as the juice extracted from sugar cane (in Brazil) and sugar beets (in Europe), and starchy raw materials, such as corn (in the U.S.A.), cassava (in Thailand) and wheat (in Europe) (Fairbanks 2003). Current trends point to the development of technologies that allow the use of agro-industrial residues, such as lignocellulosic materials called biomasses, with the aim of reducing the costs associated with raw materials, which can reach two thirds of the total cost of production, as well as incrementing the productivity levels without the need to increase crop yields (Pereira Jr et al. 2008). Thus, the investigation of the use of biomasses, such as straw, bagasse, cakes, leaves and logging waste, among others, all of which contain carbohydrates that can be made available for fermentation through various physical or chemical processes, is a crucial step in the process of obtaining second generation alcohol.

This study sought to chemically characterise the *J. curcas* seed cake, produced from a variety grown in the state of Bahia, Brazil, as well as to improve the acid hydrolysis of susceptible carbohydrates present in the *Jatropha* seed cake and evaluate the production of ethanol from the released sugars.

2. Material and Methods

2.1 Acquisition of Fruits

The *Jatropha curcas* nuts were acquired from an experimental plant area in the state of Bahia, Brazil. The seeds were pre-processed in a screw press expeller (MPE 40 - Ecirtec) and then subjected to extraction of the residual oil with ethyl ether with a solid-liquid ratio of 1:5. After drying at 60 °C for 48 hours, the seed cake was crushed and sieved through a 0.5 mm mesh sieve.

2.2 Chemical Characterisation

The chemical characterisation of the *J. curcas* seed cake was accomplished by determining the percentage of moisture, ash, total protein and total lipids (AOAC 1992), total soluble sugars (Dubois et al. 1956), soluble fiber in neutral detergent (NDF), soluble fiber in acid detergent (ADF), lignin (Van Soest 1967), and starch (McCready at al. 1950). All the chemical analyses were performed in triplicate.

2.3 Chemical Saccharification Process

The conditions for hydrolysis with dilute acid were evaluated by a factorial experimental design using the 3^{k-p} model with three factors: hydrolysis time, temperature and H_2SO_4 concentration. The percent ratio of the cake mass to the amount of acid solution (% S/L) used was maintained at 20%. The response factor was the amount of reducing sugars released per 100 g of seed cake after the hydrolytic process. Data analysis was performed with the aid of the Statistica 7.0 (StatSoft, Inc) software. The following conditions were used for bench-scale hydrolysis: 50 g of Jatropha seed cake, 20% S/L ratio, 3% solution of H_2SO_4 at 119 °C for 20 minutes. After hydrolysis, the soluble fraction was separated by vacuum filtration through filter paper, and the pH was adjusted to 5.0 by the addition of $Ca(OH)_2$. The concentrations of reducing sugars (Southgate 1991) and glucose (GOD-POD method) were determined before and after a second filtration to remove the $CaSO_4$ that formed.

2.4 Fermentation

The hydrolysate was subjected to fermentation under anaerobic conditions using 3% *Saccharomyces cerevisiae* (dehydrated baker's yeast - Fleischmann®) as the fermentation agent at room temperature (25 °C ± 2). The development of the fermentation process, carried out in conical flasks coupled to fermentometers, was monitored by the evolution of CO_2 measured through the loss of mass by the fermentative system. At the end of the process, the ethanol content was analysed by colorimetric determination with K_2CrO_4 after sample distillation (Pilone 1985), and the concentration of residual reducing sugars was quantified.

3. Results and Discussion

3.1 Biomass Characterization

The extraction of lipids for the seed cake preparation was highly efficient, leaving only 1.15% residual lipids (Table 1). The seed cake presented a protein content of 31.8% and significant percentages of cellulose, hemicellulose and starch, 16.9%, 10.4% and 4.8%, respectively (Table 1). Liang and collaborators (Liang et al. 2010) encountered cellulose and hemicellulose contents of 13.5% and 26.8%, respectively, in Jatropha seed cakes from screw-pressed seeds using an Ankom fiber analyser. The differences in composition could be the result of different analytical procedures and inherent differences attributed to plant variety or growth conditions. The quantities of fibres and carbohydrates would allow Jatropha seed cake to be used in the composition of animal feed, if toxins and antinutritional factors were not present in this biomass (Matínez-Herrera et al. 2006). Its use for this purpose would require detoxifying treatment. The combined percentage of starch, cellulose and hemicellulose found (32.1%) suggests that, if completely utilised as a source of fermentable sugars, it would yield a quantity of ethanol equivalent to 16.4% of the cake´s dry weight. Mathematically, this would result in the production of about 207 litres of anhydrous ethanol per ton of seed cake.

Table 1: Proximate Composition of Dry Jatropha Seed Cake

Analysis	Averages (% *w/w*)
Moisture	2.18 ± 0.17
Ash	6.24 ± 0.13
Lipids	1.15 ± 0.08
Protein	31.82 ± 0.78
Cellulose	16.88 ± 0.26
Hemicellulose	10.41 ± 0.33
Lignin	33.29 ± 1.86
Starch	4.80 ± 0.33
TSS	5.11 ± 0.15
NDF	60.58 ± 3.13
ADF	50.17 ± 2.1

TSS: total soluble sugars*;* NDF: neutral detergent fiber; ADF: acid detergent fiber

3.2 Biomass Hydrolysis

The amount of reducing sugars released in each treatment proposed by the factorial model is shown in Table 2. The quadratic model generated by the statistical analysis ($Y = 0.29X_1^2 + 0.65X_2^2 + 0.08X_3^2 - 2.62X_1 - 0.92X_2 + 4.21X_3$) presented a correlation coefficient of 0.88 and an adjust of 0.78. The hydrolytic condition with the best response resulted in 12.47 g of reducing sugar per 100 g of dry seed cake. Only 1.85 g of the reducing sugars released was glucose. Considering the combined percentage of starch and hemicellulose found in the seed cake (15.2%), it can be estimated that the hydrolytic condition that produced the best response was responsible for the conversion of 82% of the target polysaccharides to reducing sugars. The quantity of cellulose present in the Jatropha seed cake was not considered for calculation of the hydrolytic yield because the moderate concentration of sulphuric acid used in experimental design, conceptually, did not favour its hydrolysis. Ordinarily, dilute inorganic acids at temperatures above 121 to 160 ^oC are used for the pretreatment of lignocellulosic materials to remove the hemicellulosic fraction (Sun and Cheng 2002, Girio et al. 2010).

Table 2: Factors Used in 3^{k-P} Fractional Factorial Design, Respective Values of the Response Variable and Values Predicted by the Adjusted Mathematical Model

Time (min) X_1	Temperature (°C) X_2	H_2SO_4 (%) X_3	RS (%)	Predicted (%)	Residues
20	111	1	8.23 ± 0.70	8.13	0.09
20	119	3	12.47 ± 0.21	12.53	-0.06
20	127	2	9.37 ± 1.18	9.40	-0.03
40	111	3	11.30 ± 1.10	11.33	-0.03
40	119	2	10.22 ± 0.88	9.49	0.72
40	127	1	6.14 ± 0.66	6.20	-0.06
60	111	2	7.64 ± 0.17	7.70	-0.06
60	119	1	5.67 ± 0.23	5.70	-0.03
60	127	3	8.90 ± 0.65	8.81	0.09
40	119	2	7.71 ± 0.54	9.49	-1.78
40	119	2	9.90 ± 0.14	9.49	0.40
40	119	2	9.10 ± 1.13	9.49	-0.39
40	119	2	10.68 ± 0.32	9.49	1.19
40	119	2	9.44 ± 0.29	9.49	-0.05

RS: reducing sugars

The statistical analysis of the factorial design data showed that the sulphuric acid concentration was the factor of greatest significance to the *p*-level of 0.05 (Figure 1). The hydrolysis time had a significant and negative linear effect based on the model generated in the processing of the data. This effect is probably associated with the dehydration of monosaccharides to furfural and hydroxymethylfurfural by the action of sulphuric acid at high temperatures (Almeida et al. 2011). The analysis of the Pareto chart (Figure 1) also shows that the temperature did not have a significant effect on the hydrolysis process under the conditions evaluated.

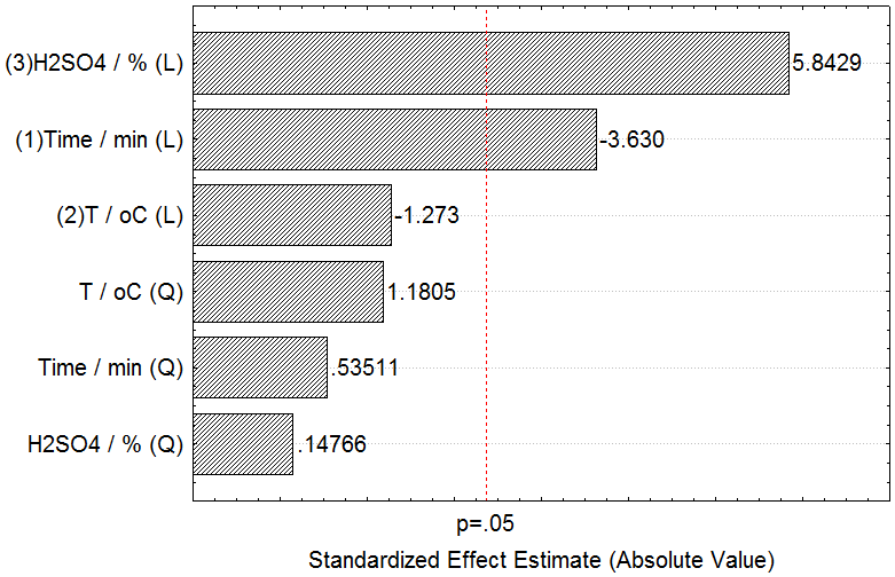

Figure 1: Pareto Chart with Linear and Quadratic Standardized Effects of the Factors Time, Temperature and H_2SO_4 Concentration

The combined effect of the factors based on the percentage of reducing sugars released (Figure 1) shows the negative effect of hydrolysis time and the positive action of the sulphuric acid concentration. Based on the analysis of Table 2, the combination of the hydrolysis time of 20 minutes, the temperature of 119 °C and the concentration of 3% H_2SO_4 was defined as the adequate condition for the hydrolysis of the Jatropha seed cake.

Under these conditions, 50 g of Jatropha seed cake was hydrolysed to obtain a soluble fraction that was used as must for fermentability analysis. The hydrolysate of Jatropha seed cake obtained under the best condition for the acid saccharification resulted in a volume of 200 mL of filtrate containing 17.93 g L^{-1} of reducing sugars, 19% of which consisted of glucose. Considering the solid-liquid ratio used, 7.17 grams of reducing sugars per 100 grams of dried seed cake were recovered after the hydrolytic process. This yield corresponds to approximately four times that found by Mohit et al. (2011) using the hydrolysis conditions of 5% H_2SO_4 during three days at 55 °C after pretreatment.

Considering only the starch and hemicellulose fractions contained in the seed cake, the yield of the hydrolytic process, indicated by the optimization of the experimental design and reproduced with 50 g of seed cake, was equal to 47%. Hypothetically, some of the reducing sugars released from the seed cake were adsorbed onto the insoluble fraction, a by-product of the hydrolytic process.

3.3 Fermentation

After adjusting the pH to 5.0, the soluble fraction of the hydrolytic process was subjected to a fermentability test by adding dehydrated baker's yeast, inoculated in the proportion of 3%. Two fermentations were performed, one with the undiluted hydrolysate and the other with the hydrolysate diluted with distilled water (1:1). The use of dilution was established as indirect way to investigate the possible inhibitory effect of the by-products of acid hydrolysis on the fermentative capacity of the yeast. In fact, there was an appreciable difference between the fermentative processes. The yield ($Y_{P/S}$) obtained with the diluted hydrolysate was 20% higher than that obtained with the undiluted hydrolysate (Table 3). It is likely that by-products known to inhibit the fermentation process, such as furfural and hydroxymethylfurfural, affected the fermentation (Kosaric and Vardar-Sukan 2001).

Table 3: Variables for the Fermentation Process of *J. Curcas* Seed Cake Hydrolysate

Fermentation	Time (h)	RS (g/L)		Ethanol (g/L)	$Y_{P/S}$
		Start	End		
Undiluted must	76.3	14.11 ± 1.51	0.07 ± 0.01	6.01 ± 0.52	0.43
Diluted must (1:1)	76.3	7.22 ± 0,32	0.14 ± 0.01	3.80 ± 0,15	0.54

RS: reducing sugars

The fermentation process was terminated at 76 hours of fermentation, after cessation of CO_2 evolution (Figure 2). The ethanol concentration reached 6.01 g L^{-1} for the undiluted must and 3.80 g L^{-1} for the diluted must (Table 3). On the basis of the relative mass of ethanol produced, the ratio of 2.40 g of ethanol per 100 g of hydrolysed cake was established to evaluate the undiluted hydrolysate, and 3.04 g of ethanol per 100 g of hydrolysed cake for the diluted hydrolysate. Taking into account the latter fermentative condition, the projection for ethanol production per ton of Jatropha seed cake was 38.5 L of absolute ethanol.

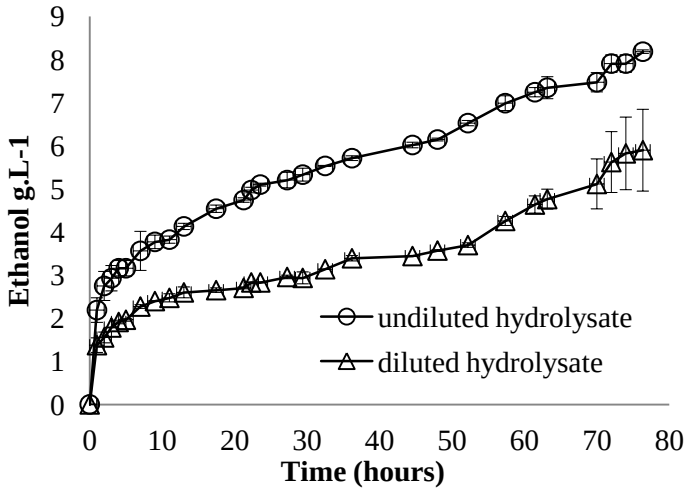

Figure 2: Progress Curves of the Alcoholic Fermentation of Acid Hydrolysate of Jatropha Seed Cake. The Calculated Ethanol Concentration Was Based on the Equivalence of Released CO2. The Results Represent the Average of Two Experiments

4. Conclusions

The overall yield of the saccharification and fermentation processes was 39.3% of the theorical yield of ethanol from the mass of starch and hemicelluloses present in Jatropha seed cake. Considering the proportion of 2.3 tons of cake for each ton of *J. curcas* seed oil extracted, it would be possible to produce approximately 88.5 L of ethanol from the residual cake obtained from 1000 kg of oil under the hydrolytic conditions described in this paper.

5. Acknowledgements

The authors thank the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) and the National Council for Scientific and Technological Development of Brazil (CNPq) for the financial support for the research.

6. References

Makkar HPS, Aderibigbe AO, Becker K. (1998). Comparative evaluation of nontoxic and toxic varieties of Jatropha curcas for chemical composition, digestibility, protein degradability and toxic factors. Food Chem. 62: 207-215.

Makkar HPS, Becker K, Schmook B. (1998). Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. Qual. Plant. - Plant Foods Hum. Nutr. 52: 31-36.

Martínez-Herrera J, Siddhuraju P, Francis G, Dávila-Ortíz G, Becker K. (2006). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chem. 96: 80-89.

Giibitz G M, Mittelbach M, Trabi M. (1999). Exploitation of the tropical oil seed plant Jatropha curcas L. Bioresour Technol. 67: 73-82.

Foidl N, Foidl G, Sanchez M, Mittelbach M, Hackel S. (1996). *Jatropha curcas* L as a source for the production of biofuel in Nicaragua. Bioresour Technol. 58: 77-82.

Mohibbe Azam M, Waris A, Nahar NM. (2005). Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenergy. 29: 293-302.

Bailis RE, Baka JE. (2010). Greenhouse Gas Emissions and Land Use Change from *Jatropha Curcas*-Based Jet Fuel in Brazil. Environ Sci Technol. 44: 8684-8691.

Chivandi E, Mtimuni JP, Read JS, Makuza SM. (2004). Effect of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the zimbabwem *Jatropha curcas* provenace: a potential livestock feed. Pak J Biol Sci. 7: 1001-1005.

Haas W, Mittelbach M. (2000). Detoxification experiments with the seed oil from Jatropha curcas L. Ind Crops Prod. 12: 111-118.

Singh SP, Singh D. (2010). Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review. Renewable Sustainable Energy Rev. 14: 200-216.

Inekwe UV, Odey MO, Gauje B, Dakare AM, Nkeonye O, Bala S, Mohammed G. (2012). Comparative proximate composition of partially de-oiled Jatropha curcas seed from india, kaduna and edo. TASE. 4: 151-154.

Macedo AL, Santos RS, Pantoja L, Santos AS. (2011). Pequi cake composition, hydrolysis and fermentation to bioethanol. Braz J Chem Eng. 28: 9-15.

Melo WC, Silva DB, Santa Anna LMM, Santos AS, PEREIRA JR N. (2008) Produção de etanol a partir de torta de mamona (*Ricinus communis* L.) e avaliação da letalidade da torta hidrolisada para camundongo. Química Nova. 3: 1104-1106.

Fairbanks M. (2003). Álcool: Tecnologia amplia oferta e reduz custo do álcool. Química e Derivados. 417.

Pereira Jr N, Couto MAPG, Santa Anna LMM. (2008). Series on biotechnology: Biomass of lignocellulosic composition for fuel ethanol production within the context of biorefinery. 1th ed. Rio de Janeiro: Pereira Jr N. ed.

AOAC. (1992). Official methods of analysis of the Association of Official Analytical Chemistry. 12th. ed.; AOAC: Washington.

Dubois M, Gilles KA, Hamilton KK, Hebers PA, Smith F. (1956). Colorimetric method for determination of sugar and related substances. Anal Chem. 28: 350-356.

Van Soest PJ. (1967). Development of a comprehensive system of feed analysis and its applications to forage. J Am Sci. 26: 119-128.

McCready RM, Guggolz JM, Silveira V, Owens HS. (1950). Determination of starch and amylose in vegetables: aplication to pear. Anal Chem. 22: 1156-1158.

Southgate DAT. (1991). Determination of food carbohydrates. London: Applied Science Publishers LTD.

Pilone GJ. (1985). Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study. J Assoc Off Anal Chem. 68: 188-190.

Liang Y, Siddaramu T, Yesuf J, Sarkany N. (2010). Fermentable sugar release from Jatropha seed cakes following lime pretreatment and enzymatic hydrolysis. Bioresour Technol. 101: 6417-6424.

Sun Y, Cheng J. (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol. 83: 1-11.

Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Łukasik R. (2010). Hemicelluloses for fuel ethanol: A review. Bioresour Technol. 101: 4775-4800.

Almeida JR, Runquist D, Sànchez i Nogué V, Lidén G, Gorwa-Grauslund MF. (2011). Stress-related challenges in pentose fermentation to ethanol by the yeast *Saccharomyces cerevisiae*. Biotechnol. J. 6: 286-299.

Mohit SM, Chandrashekhar B, Tanushree C, Kanwal S. (2011). Production of bio-ethanol from jatropha oilseed cakes via dilute acid hydrolysis and fermentation by *Saccharomyces cerevisiae*. Int J Biotechnol Appl. 3: 41-47.

Kosaric N, Vardar-Sukan F. (2001). Potential source of energy and chemical products. In: Roehr M., ed. The biotechnology of ethanol: classical and future applications. Weinheim: Wiley – VCH Verlag GmbH.