Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 28, No. 01, pp. 9 - 15, January - March, 2011

PEQUI CAKE COMPOSITION, HYDROLYSIS AND FERMENTATION TO BIOETHANOL

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(Submitted: October 13, 2009 ; Revised: May 3, 2010 ; Accepted: July 12, 2010)

Abstract - Pequizeiro (*Caryocar brasiliense* Camb) fruits have been evaluated as a potential raw material for the newly established biodiesel industry. This scenario demands applications using the solid co-product derived from the extraction of pequi oil, called cake or meal. This study analyses the acid hydrolysis of carbohydrates present in the pequi meal in order to obtain fermentable sugars and evaluates their conversion to bioethanol. There was 27% starch in the pequi meal. The use of a CCRD experimental design type to study the acid saccharification of pequi meal results in 61.6% conversion of its starch content to reducing sugars. Positive and significant linear effects were observed for H_2SO_4 concentration and temperature factors, while the quadratic effect of H_2SO_4 concentration and the linear effect of solid-liquid ratio were negative. Even, with non-optimized fermentative condition using 1% of dried baker's yeast in conical flasks, it was possible to obtain a value equivalent to 53 L of ethanol per ton of hydrolyzed pequi meal.
Keywords: *Caryocar brasiliense*; Pequi; Saccharification; Bioethanol; Biodiesel.

INTRODUCTION

Vegetal cakes derived from oil extraction for the biodiesel industry have been considered for the production of bioethanol (Melo et al., 2008; Macedo et al., 2009; Cerveró et al., 2010). This possibility is based on the presence of significant amounts of carbohydrates in this biomass, specifically starch and cellulose. Melo and collaborators (2008) evaluated the use of castor bean cake for this purpose by subjecting it to the processes of acid and enzymatic saccharification and they obtained hydrolyzates with 75 $g.L^{-1}$ of reducing sugars. Palm kernel cake, rich in mannans, has also been investigated for ethanol production by enzymatic saccharification of the lignocellulosic fraction (Cerveró et al., 2010). These researchers reported achieving 365g of mannose and glucose per Kg of palm kernel cake. Recently, the pequi fruit has been considered as a potential raw material for the newly established biodiesel industry (Beltrão and Oliveira, 2007; Demirbas, 2009). This scenario creates prospects for research on the use of cakes or meal derived from extraction of pequi oil. However, there is still a lack of knowledge regarding the chemical composition of pequi meal, mainly on the carbohydrate fraction and its potential for bioethanol production. The pequizeiro (*Caryocar brasiliense* Camb.) is a typical species of the Brazilian tropical Savanna biome and is protected by a federal law that prevents wood cutting and commercialization throughout the country (Ordinance No. 54 of March 03, 1987 - IBDF). The cultivation of the pequizeiro and the extraction, beneficiation, processing, consumption and commercialization of its fruits are standardized and stimulated in several Brazilian states in order to promote a sustainable management of the biome by the individuals who have traditionally explored the fruit . According to information on vegetal extraction and forestry from IBGE (2008), 5,531 tons of pequi

*To whom correspondence should be addressed
This is an extended version of the manuscript presented at the SINAFERM 2009 - 17th National Symposium on Bioprocesses 2009, Natal, Brazil.

kernel was extracted in Brazil in 2008, of which 49.9% in the state of Ceará and 28.6% in Minas Gerais state.

In this particular study, the amount of starch occurring in pequi meal was measured and the dilute acid hydrolysis process applied to obtain fermentable sugars, whose fermentability was evaluated with baker's yeast for bioethanol production.

MATERIAL AND METHODS

Pequi Meal Production

Pequi meal was prepared by preprocessing of the pulp and kernels of its fruits obtained in Diamantina, MG, in a continuous press for partial extraction of oil, followed by extraction of residual oil with diethyl ether. The resulting meal of the degreased fruit fraction was dried in an oven with forced air circulation and consisted of 17.1% kernel and 82.8% pulp, resulting in a pale yellow-toned product.

Acid Hydrolysis (Saccharification)

The optimization of acid saccharification was performed by using a central composite rotatable design (CCRD) as the experimental design with three variables, four central and six axial points. The evaluated variables were the temperature (111 – 123°C), the concentration of sulfuric acid (2 – 5 % w/w) and the solid-liquid ratio (S / L) (14 – 26 % w/w). Statistica software (StatSoft, Inc.), version 7, was used for statistical data analysis and construction of response surface plots.

The preparative hydrolysis of polysaccharides from pequi was performed in duplicate with 20g of meal in the best condition found in the results of the experimental design. The pH of the hydrolyzates was adjusted to 5.0 with calcium hydroxide and then filtered through paper under vacuum to remove the solid fraction of the saccharification process. Fifty milliliters of additional water was used to wash the solid residue of the filter paper in order to remove monosaccharides and disaccharides that were still present.

Fermentation

The tests of fermentability were performed in two distinct conical flasks equipped with a built-in glass accessory that allowed the escape of CO_2 without allowing the entry of air (fermentometer). Each 500 mL conical flask received about 100 mL of the pequi meal hydrolyzate soluble fraction with the reduced sugar content determined previously. No nutrients or antibiotics were added to the hydrolyzate. The yeast *Saccharomyces cerevisiae* (dried baker's yeast from Fleischmann) was used as inoculum at a concentration of 10 $g.L^{-1}$. After receiving the inoculum, the flasks were incubated at 30°C under orbital agitation of 100 rpm until the fermentation was done. The fermentative process was monitored through of release of CO_2 by weighing the reaction system at regular time intervals.

Chemical Analysis

Pequi fruit *in natura* and pequi defatted kernel and pulp were analyzed for moisture, total lipids, total protein, crude fiber and ashes according to methodology described by IAL (1985). The reduced sugar content was determined as described by Southgate (1991) after neutralization of the samples with $Ca(OH)_2$. Glucose concentration was determined by an enzymatic-colorimetric method (Lloyd and Whelan, 1969) using a commercial kit (Laborclin). Starch content was determined after extraction with perchloric acid (McCready et al., 1950) by the method of phenol-sulfuric acid (Dubois et al., 1956). The concentration of ethanol was measured by a spectrophotometric method based on ethanol oxidation with dichromate (Gibson and Blotner, 1938; Pilone, 1985), after the steam stripping of the samples.

RESULTS AND DISCUSSION

Chemical Characterization of Pequi Pyrenes and Pequi Meal

The composition and chemical analysis of the pulp and kernel of pequi, expressed on a wet weight basis, is summarized in Table 1. The oil content found in either kernel or pulp of pequi represents about 30% of the composition of each of these portions of the fruit. The high amount of oil is what defines pequi as a potential raw material for the production of fatty esters, used in the production of biodiesel. Lima et al. (2007), in their composition analysis, expressed on wet weight basis, found 33.4% of lipids in the pulp and 51.5% for kernel, but the moisture found in the pulp and kernel of pequi, by them was, respectively, only 41.5 % and 8.68 % against 51.3 % and 31.7 % of moisture found in the pulp and kernel of pequi in this research.

Table 1: Chemical characterization of pequi, expressed as a percentage on a wet basis.

Analysis	Pulp (% w/w)	Kernel (% w/w)
Moisture	51.32 ± 3.77	31.71 ± 0.64
Ash	0.55 ± 0.01	3.02 ± 0.04
Crude fiber	3.68 ± 0.46	1.05 ± 0.03
Total proteins	3.39 ± 0.06	20.79 ± 0.15
Total lipids	29.66 ± 0.57	32.53 ± 1.04
Soluble sugars	6.11 ± 0.81	1.82 ± 0.13
Starch	9.05 ± 0.47	6.19 ± 0.16
Total carbohydrates	11.40	10.90

The proximate composition (Table 1) determined for the pulp of pequi was close to that obtained by Sano and Almeida (1998), 56.77% moisture, 2.64% protein, 20.21% lipids, 0.72% ash, 11.60% fiber and 19.66% of total carbohydrates. The same was observed in reference to the values reported by Rodrigues et al. (2004) for pyrenes of pequi from south and north of Minas Gerais, namely 49.2% and 59.1% moisture, 4.2% and 2.2% protein, 20.5% and 25.1% fat, 0.4% and 0.5% ash, 6.8% and 4.9% fiber and 18.9% and 18.2% carbohydrates, respectively.

After extraction of fats for the preparation of the pequi meal, only 6.17% of the lipids remained (Table 2). The pequi meal presented 29.5% of total protein and significant percentages of fiber (16.63%) and starch (27.14%) (Table 2). If all of the starch content were converted to glucose and then fermented to ethanol, it would have produced about 14.6 g of ethanol per 100 g of pequi meal, based on a 95% fermentation yield.

Table 2: Chemical characterization of pequi meal, expressed as percentage on a wet basis.

Analysis	Pulp (% w/w)	Kernel (% w/w)	(Pulp + Kernel) (% w/w)
Moisture	16.97 ± 0.81	16.50 ± 0.52	19.25 ± 2.46
Ash	3.82 ± 0.23	8.76 ± 0.03	4.71 ± 0.23
Crude fiber	19.70 ± 3.11	6.72 ± 2.42	16.63 ± 0.94
Total proteins	21.38 ± 0.71	64.41 ± 0.80	29.51 ± 0.86
Total lipids	3.60 ± 0.91	6.01 ± 2.11	6.17 ± 1.81
Starch	27.70 ± 1.83	9.88 ± 1.08	27.14 ± 0.86

Saccharification of Pequi Meal

The statistical treatment of data derived from the CCRD experimental design (Table 3) resulted in two adjusted models with the equations $y_1 = 12.43 + 2.80x_1 + 5.12x_2 - 4.83x_2^2 - 3.58x_3$ ($R^2 = 0.96$) for the glucose response, and $y_2 = 5.79 + 0.83x_1 + 2.20x_2 - 1.29\ x_2^2 - 1.86x_3$ ($R^2 = 0.92$) for the reducing sugars response. The coefficient of determination values (R^2) for the adjusted models indicate that they are able to explain 96% and 92% of the total variation in the saccharification process, respectively. Only variables with a confidence level above 95% ($P < 0.05$) were considered to be meaningful and included in the model. The factor of major importance and significance for both response factors was the positive linear effect of concentration of H_2SO_4, followed by the negative quadratic effect of concentration of H_2SO_4, the negative linear effect of the S / L ratio and the positive linear effect of temperature (Table 4).

Table 3: CCRD 2^3 experimental design for starch hydrolysis and its response factors: concentration of reducing sugars (g per 100g of pequi meal) and the concentration of glucose (g per 100g of pequi meal).

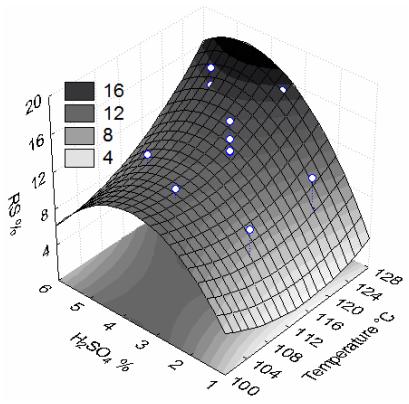
Independent variables			Dependent variables	
Temperature (°C) x_1	H_2SO_4 (% w/w) x_2	S/L(% w/w) x_3	RS (% w/w) y_1	Glucose (% w/w) y_2
111.0	2.0	14.0	10.20	5.04
111.0	2.0	26.0	4.47	2.80
111.0	5.0	14.0	12.66	7.62
111.0	5.0	26.0	10.99	4.87
123.0	2.0	14.0	11.12	6.08
123.0	2.0	26.0	7.50	4.28
123.0	5.0	14.0	17.67	6.98
123.0	5.0	26.0	15.86	5.19
106.9	3.5	20.0	13.30	4.87
127.1	3.5	20.0	16.44	6.95
117.0	1.0	20.0	3.47	2.32
117.0	6.0	20.0	10.06	7.42
117.0	3.5	9.9	16.73	7.63
117.0	3.5	30.1	9.83	5.19
117.0	3.5	20.0	12.10	7.06
117.0	3.5	20.0	14.79	6.26
117.0	3.5	20.0	13.43	6.20
117.0	3.5	20.0	13.53	6.51

Table 4: Estimated effects and *P* values for the reducing sugars concentration and glucose concentration in response to CCRD 2^3 experimental design, after exclusion of the non-significant terms at the level *P* = 0.05.

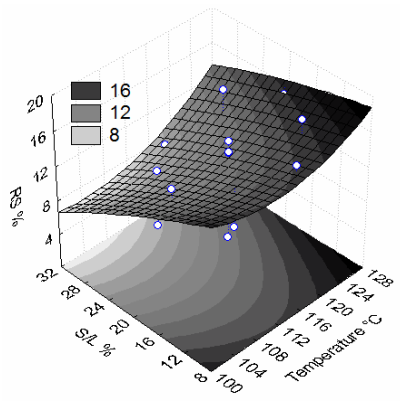
	Reducing Sugars			Glucose		
Factor	**Effect**	**Error**	***P* value**	**Effect**	**Error**	***P* value**
Mean	12.43	0.577	0.00000	5.79	0.321	0.00000
Temperature (L)	2.80	0.625	0.00206	0.83	0.348	0.04384
H_2SO_4 (L)	5.12	0.625	0.00004	2.20	0.348	0.00023
H_2SO_4 (Q)	-4.83	0.649	0.00007	-1.29	0.362	0.00713
S/L (L)	-3.58	0.625	0.00044	-1.86	0.348	0.00069

(L) – Linear effect; (Q) – Quadratic effect

The results from the experimental design were fitted to a 2nd order regression model expressing the interaction between the two independent variables, in which the 3rd variable was maintained at the central point. Figures 1 and 2 demonstrate the response surfaces generated by the proposed models. In the graphs presented in both figures, the negative effect of the S/L ratio on the saccharification process of the carbohydrate fraction present in the pequi meal is clear. Still, it is necessary to consider that a very low S/L ratio implies very dilute hydrolyzates that make the subsequent fermentation stage unfeasible or require an additional step of concentration of sugars. Further, the negative quadratic effect of the sulfuric acid concentration, predicted by statistical analysis (Table 4) and evident in surface plots (Figures 1 and 2), shows that values of H_2SO_4 greater than 4% start degradation of sugars, including glucose. Probably this effect is associated with the dehydration of glucose to hydroxy methyl furfural by the action of sulfuric acid, a known phenomenon observed by Melo et al. (2008) in their study of the acid saccharification of castor bean cake to produce ethanol.



(a)



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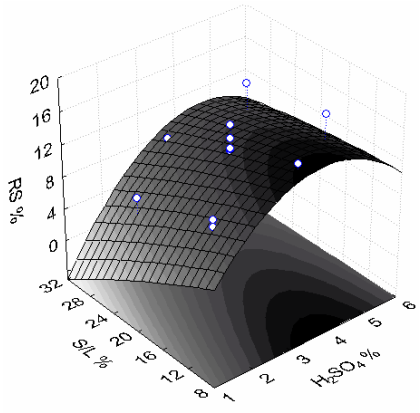

(c)

Figure 1: Response surface plots of the central composite design for the optimization of the acid saccharification of pequi meal as a function of reducing sugar (RS) concentration present in the hydrolyzate. Combined effect of H_2SO_4 concentration and temperature (A); S/L ratio and temperature (B); S/L ratio and H_2SO_4 concentration (C).

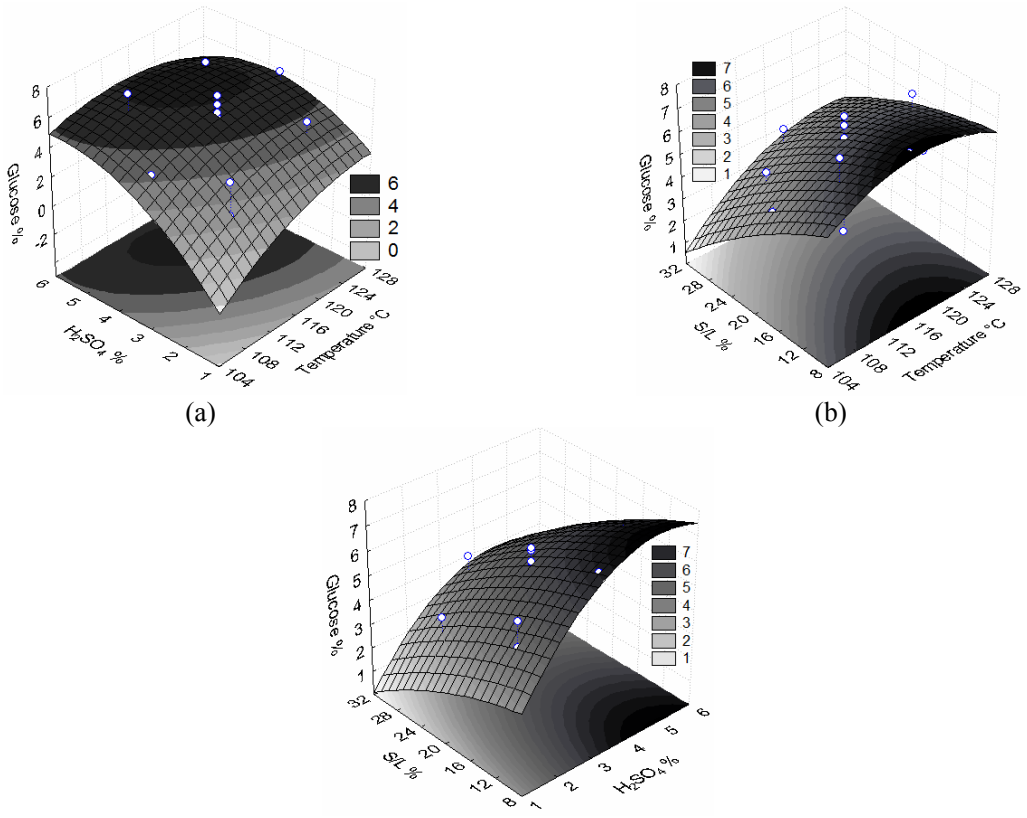

Figure 2: Response surface plots of the central composite design for the optimization of the acid saccharification of pequi meal as a function of glucose concentration present in the hydrolyzate. H_2SO_4 concentration and temperature (A); Effect of S/L ratio and temperature (B); S/L ratio and H_2SO_4 concentration (C).

Semi-Preparative Saccharification of Pequi Meal

Based on the analysis of the response factors (Table 3) and the examination of plots of the response surfaces (Figures 1 and 2), 121ºC was chosen for the temperature, the concentration of H_2SO_4 was set at 4% and the solid-liquid ratio at 20% as a combined condition for acid saccharification. In this condition, 20 g of pequi meal were hydrolyzed in duplicate to obtain the broth used in the evaluation of fermentability. Hydrolyses performed under these predetermined conditions resulted in two volumes, one with 100 mL and the other with 92 mL, of filtered and diluted material, containing, respectively, 24.3 $g.L^{-1}$ and 27.5 $g.L^{-1}$ of reducing sugars. These values, expressed in grams of RS per 100 g of dry bran, represent, respectively, 12.2% and 12.6%. On average, this is 13.3% below the value (14.3% RS) predicted by the surface response model adjusted to the experimental design. Considering the percentage of starch in the pequi meal (27.1%), the average hydrolytic yield was only 45.7%. Higher yields would have been obtained by working with lower S / L ratios, as shown in Table 3. However, the concentrations of RS obtained in such conditions would be lower, resulting in fermented broths with very low concentrations of ethanol.

Fermentability of Pequi Meal Hydrolyzate

After adjusting the pH, the soluble fraction of the hydrolyzate was subjected to fermentation by *Saccharomyces cerevisiae* (dehydrated baker's yeast, from Fleischmann). The evolution of CO_2 stabilized by 60 hours of fermentation (Figure 3), indicating termination of the process, which, however, was continued for thirty hours more. The $Y_{P/S}$ yield obtained was 0.36 g / g (Table 5), representing a fermentation efficiency of 70.6%. This low value could be justified by the probable inhibitor effect of byproducts normally formed from acidic saccharification of plant biomass, represented mainly by furfural, hydroxymethyl furfural, levulinic and formic acids (Kosáric and Vardar-Sukan, 2001).

Table 5: Variables of the fermentation process conducted with the hydrolyzed pequi meal.

Fermentation	Time (hours)	RS ($g.L^{-1}$)		Glucose ($g.L^{-1}$)		Ethanol (g/L)	$Y_{P/S}$ (g/g)
		Start	End	Start	End		
Hydrolyzate 1*	89.7	24.3	1.96	11.25	0.13	7.95	0.36
Hydrolyzate 2*	89.7	27.5	2.28	11.46	0.34	9.43	0.37

* duplicate experiments

At the end of the fermentation, 8.69 g. L^{-1} of ethanol was produced on average. Considering the 20% S/L ratio used in the hydrolytic process, the amount of ethanol produced was equivalent to the production of 4.15 g of ethanol per 100 g of dry pequi meal, or 41.5 kg (52.6 L) of ethanol per ton of meal. Although lower than expected, the value obtained represents 75.1% of the amount of anhydrous ethanol (70 L) that is obtained from 1.0 t of sugarcane (Goldemberg and Guardabassi, 2010).

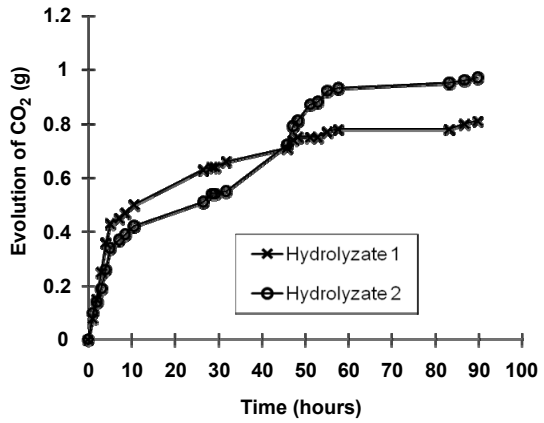

Figure 3: Progress of evolution of CO_2 during the fermentation of acid hydrolyzates of pequi meal.

CONCLUSIONS

The composition of pequi meal, particularly its high starch content, warrants the evaluation of its potential as a feedstock for ethanol production. The unspecific acid saccharification process to which the pequi meal was submitted proved to be limited. Nonetheless, pequi meal presents potential for bioethanol production, yielding 5.3 % (v/p) dry basis in fermentative conditions that were not optimized. Moreover, the solid residue coming from the fermentation process has a huge potential as a protein source for use in animal feed.

ACKNOWLEDGMENT

The authors would like to demonstrate their gratitude to FAPEMIG and CNPq agencies for their financial support.

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