Optimization of Dilute Acid Pretreatment of Corn Pericarp by Response Surface Methodology

José Ángel Granados-Arvizu, Aldo Amaro-Reyes, Blanca Estela García-Almendárez, Jorge Noel Gracida-Rodríguez, and Carlos Regalado*

Corn pericarp (CP) is an inexpensive agroindustrial by-product that is available in large quantity in Mexico. This work aimed to optimize the use of a dilute sulfuric acid pretreatment for CP hydrolysis to maximize the reducing sugars yield. A 2-step experimental design was used, first a full factorial followed by a central composite design (CCD). The CCD involved sulfuric acid (1.5% to 5%, v/v), a treatment at 121 ºC (15 min to 40 min), and varying CP content (10% to 20%, w/v). The response variables were the reducing sugars, glucose, and solubilized solids. Maximal responses were achieved at 3.4% (v/v) sulfuric acid, 20% (w/v) CP, and 22.3 min. A significant (R^2 = 0.99) second-order model predicted maximal operational conditions that were experimentally validated: 80.1 g/L reducing sugars, 12.8 g/L glucose, and 69.2% solubilization of CP solids. The dilute sulfuric acid pretreatment solubilized all hemicellulose in CP.

Keywords: Corn pericarp; Response surface methodology; Acid pretreatment; Optimization

Contact information: Department of Food Research and Postgraduate Studies, Faculty of Chemistry, Universidad Autónoma de Querétaro. Queretaro, 76010 Qro., Mexico;
** Corresponding author: regcarlos@gmail.com*

INTRODUCTION

Hemicelluloses currently represent the largest polysaccharide fraction that is wasted in the course of lignocellulosic ethanol production around the world. The high level of wastage can be attributed to the heterogeneous polymeric nature and low fermentability of the hemicelluloses (Gírio *et al.* 2010). Diverse agroindustrial residues, such as by-products, contain large amounts of hemicellulose. In Mexico, corn pericarp (CP) is a highly available agroindustrial waste (approximately 0.1 million tons/year) with high hemicellulose content (Díaz-Malváez *et al*. 2013). The CP is a byproduct of the wet corn milling industries (Yoshida *et al*. 2010), and it is mostly mixed into low-value animal feed (Huang *et al*. 2008). However, compared to other materials it has potential to produce bioethanol and other valuable co-products despite its high hemicellulose content (Gírio *et al.* 2010).

Cost-effective production of bioethanol from lignocellulosic materials requires the high-efficiency utilization of both cellulose and hemicellulose (Kuhad *et al.* 2011). Bioethanol production from industrial lignocellulosic biomass is comprised of several processes, including pre-treatment, chemical and enzymatic hydrolysis, fermentation, and ethanol recovery by distillation (Koradiya *et al.* 2016). The pretreatment represents the major cost of the overall process (Sims *et al.* 2010), and therefore several reports have focused on the optimization of this process using statistical methods (Kim *et al*. 2014; Koradiya *et al.* 2016).

Due to the simplicity, low cost, and effectiveness, dilute acid pretreatment is considered the most likely alternative for commercial operations (Jung and Kim 2015), and lignocellulosic materials pretreated with diluted H_2SO_4 have achieved high hydrolysis yields (Alvira *et al.* 2010). This treatment enhances carbohydrate hydrolysis, especially those comprising the lignin-hemicellulose barrier that covers cellulose (Jung and Kim 2015). However, there are no reports showing optimization strategies to achieve high reducing sugars yield from corn pericarp employing dilute sulfuric acid pretreatment. Bura *et al.* (2003); Yoshida *et al.* (2010), and Myat and Ryu (2014) have optimized such yields using other pretreatments, achieving lower results than those reported here. In addition, Van Eylen *et al.* (2011) reported that acid pretreatment on CP may not achieve similar results as for other lignocellulosic materials, because of the distinct buffering capacity of corn cobs and corn stover. Therefore, this study aims to use dilute sulfuric acid as part of an optimized pretreatment strategy for CP hydrolysis to maximize reducing sugars yield by response surface methodology.

EXPERIMENTAL

Materials

The CP was obtained from Ingredion, San Juan del Rio, Queretaro, Mexico. It was dried at 55 ºC for 48 h to 10% (w/w) moisture in an oven (Memmert, Model UN110, Schwabach, Germany), and the biomass was then stored in plastic bags before use. The composition of CP was determined from neutral (Ankon 2011a) and acid detergent fibers (Ankon 2011b), and it was (% dry basis): 21.48 ± 0.64 hemicellulose, 8.41 ± 0.21 cellulose, 0.37 ± 0.06 lignin, and 69.74 ± 0.89 soluble solids. It was ground using a laboratory mill Tecator (Fargo, ND, USA) to a particle size between 0.46 mm and 0.84 mm.

Chemicals

Analytical grade sulfuric acid and sodium hydroxide were purchased from J.T. Baker (Pittsburgh, PA, USA). The dinitrosalicylic acid and sodium potassium tartrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). The glucose assay kit was acquired from R-Biopharm (Darmstadt, Germany).

Methods

To maximize the sugars yield by the dilute sulfuric acid pretreatment of CP, a 2-step experimental design was used. First, a full factorial followed by a central composite design was employed. The significance of the fitted models was tested by an analysis of variance (ANOVA) using Design Expert® (State-Ease, Version 9.0.4.1, Minneapolis, MN, USA), and adjusted determination coefficients (R^2).

Full factorial design

A 2^3 factorial design (FFD) (Design Expert® version 9.0.4.1, Minneapolis, MN, USA) was used to identify the factors significantly affecting sugars yield (Hsu *et al.* 2010). From preliminary experiments and previous reports (Kuhad *et al.* 2011; Avci *et al.* 2013), factors and ranges chosen were 1.5% to 5% (% v/v) sulfuric acid, 10 min to 40 min treatment time at 121 °C, and 10% to 20% (% w/v) CP solids content. This design comprises eight treatments and one center point, with two replicates (Table 1).

Table 1. Full Factorial Design 2^3 for Pretreatment of CP; Factors: Sulfuric Acid, Initial CP, and Treatment Time

Factor	Name	Units	Minimum	Maximum	Coded	Values	Mean
x_1	Time	min	15	40	-1.0 = 15	1.0 = 40	27.5
x_2	Sulfuric acid	% (v/v)	1.5	5	-1.0 = 1.5	1.0 = 5	3.25
x_3	Initial CP	% (w/v)	10	20	-1.0 = 10	1.0 = 20	15

Treatments were performed in 50-mL conical bottom tubes containing 35 mL of the reaction mixture according to Table 1. Heating was conducted in an autoclave (FELISA, FE-398, Jalisco, Mexico). After pretreatment, the samples were adjusted to pH = 5 with NaOH 5 M, followed by filtration and the solid fraction was dried at 90 ºC for 12 h. Acid pretreatment efficiency was estimated from the remaining solid fraction, and expressed as % solubilized solids. The total reducing sugars (Wood *et al.* 2012) and glucose (R-Biopharm, Darmstadt, Germany) were determined in the filtrate.

Central composite design

Considering the results of the FFD, a central composite design (CCD) (Design Expert ® version 9.0.4.1, Minneapolis, MN, USA) was performed to find the optimum conditions to maximize the total reducing sugars release, glucose, and % solubilized solids. The CCD was constructed using eight axial points, six star points ($\pm \alpha$), and six replicates at the central point (Table 2) (Montgomery 2005).

Table 2. Central Composite Design to Optimize Pretreatment of CP

Factor	Name	Units	Axial Point (-1.68)	Low Level (-1)	Central Level (0)	High Level (+1)	Axial Point (+1.68)
x_1	Time	Min	6	15	27	40	48
x_2	Sulfuric acid	% (v/v)	0.307	1.5	3.25	5	6.18
x_3	Initial CP	% (w/v)	6.59	10	15	20	23.4

Treatments were performed as depicted in the full factorial design section, and acid pretreatment efficiency, total reducing sugars yield, and glucose yield were determined as above-mentioned. The results were evaluated by ANOVA, and the predicted adjusted models that showed good fit to the data were used to estimate the optimal responses. The desirability function is useful to optimize multiple responses (Montgomery 2005), and it was employed to optimize the three responses. Cellulose, hemicellulose, and lignin were measured (Vogel *et al.* 1999) from the remaining solid fraction that resulted after application of the optimized operational conditions.

RESULTS AND DISCUSSION

Linear Model for Dilute Sulfuric Acid Pretreatment of Corn Pericarp

The ANOVA results of the FFD for the three responses are summarized in Table 3. A significant fit was found between the experimental and predicted data for all of the tested factors ($p < 0.05$). However, the low p value of the lack of fit indicates that linear

models did not explain some experimental data. Therefore, it was considered necessary to apply a quadratic model that might offer a better fit to experimental data.

Table 3. Analysis of Variance for the Full Factorial Design

Response	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Total Reducing sugars	Model	13111.30	6	2185.22	56.20	< 0.0001
	Lack of fit	773.39	2	386.7	1634.64	***< 0.0001***
	R^2 adj.	0.92				
Glucose	Model	418.25	6	69.71	22.10	< 0.0001
	Lack of fit	58.31	2	29.15	109.99	***< 0.0001***
	R^2 adj.	0.83				
%Solubilized solids	Model	789.95	6	131.66	22.87	< 0.0001
	Lack of fit	114.30	2	57.15	1257.04	***< 0.0001***
	R^2 adj.	0.83				

Optimization of Dilute Sulfuric Acid Pretreatment Conditions

A statistical analysis of the CCD (Table 4) showed that the models of the three experimental responses were significant ($p < 0.05$). From the ANOVA, it was observed that the determination coefficients (R^2) were > 0.95, which indicated a good agreement of the models with experimental data, and additionally $p > 0.05$ for the lack of fit.

Table 4. Analysis of Variance (ANOVA) for the Central Composite Design

Response	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Total Reducing sugars	Model	9371.60	9	1041.20	3186.9	<0.0001
	Residual	3.27	10	0.33		
	Lack of fit	2.59	5	0.52	3.80	0.08
	Pure error	0.68	5	0.14		
	Total	9374.80	19			
	R^2 adj.	0.99				
Glucose	Model	382.98	9	42.55	590.30	<0.0001
	Residual	0.72	10	0.07		
	Lack of fit	0.35	5	0.06	0.93	0.53
	Pure error	0.37	5	0.08		
	Total	383.7	19			
	R^2 adj.	0.99				
% Solubilized solids	Model	863.90	9	95.99	35333.20	<0.0001
	Residual	0.02	10	0.002		
	Lack of fit	0.01	5	0.002	0.79	0.60
	Pure error	0.02	5	0.0003		
	Total	863.90	19			
	R^2 adj.	0.99				

Three quadratics models were adjusted to the experimental data, as described by Eq. 2,

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x^2_1 + a_8x^2_2 + a_9x^2_3 \quad (2)$$

where x_1 is pretreatment time (min), x_2 is sulfuric acid concentration (% v/v), x_3 is CP solids concentration (w/v), and the term a_i refers to the model coefficients (Table 5).

Table 5. Coefficients of the Adjusted Model Corresponding to Responses to CCD

	Total Reducing Sugars	Glucose	Solubilized Solids
a_0	66.67	14.64	71.16
a_1	5.53	1.56	0.99
a_2	13.68	3.19	5.35
a_3	15.19	1.77	-1.88
a_4	-5.17	1.34	-3.15
a_5	2.27	1.59	0.69
a_6	8.92	1.29	NS
a_7	-7.99	-1.28	-0.59
a_8	-10.52	-1.22	-4.76
a_9	NS	-2.54	-0.17
R^2_{adj}	0.99	0.99	0.99

All coefficients are significant ($p < 0.05$), except where stated; NS: Not significant

The variable Y is the response, which for total reducing sugars and glucose was in g/L, while for solubilized solids it was weight, expressed in %. The use of coded coefficients allowed the influence of the three factors on each response to be identified. For total reducing sugars, the three linear factors showed a positive effect, where the CP solids concentration was the factor with the highest influence (15.19). The quadratic influence of time (-7.99) and sulfuric acid concentration (-10.52) is shown in Fig. 1, while the CP solids concentration showed no significance. Sulfuric acid exerted a greater influence on glucose than the pretreatment time and CP solids, when the linear coefficients of the model were considered.

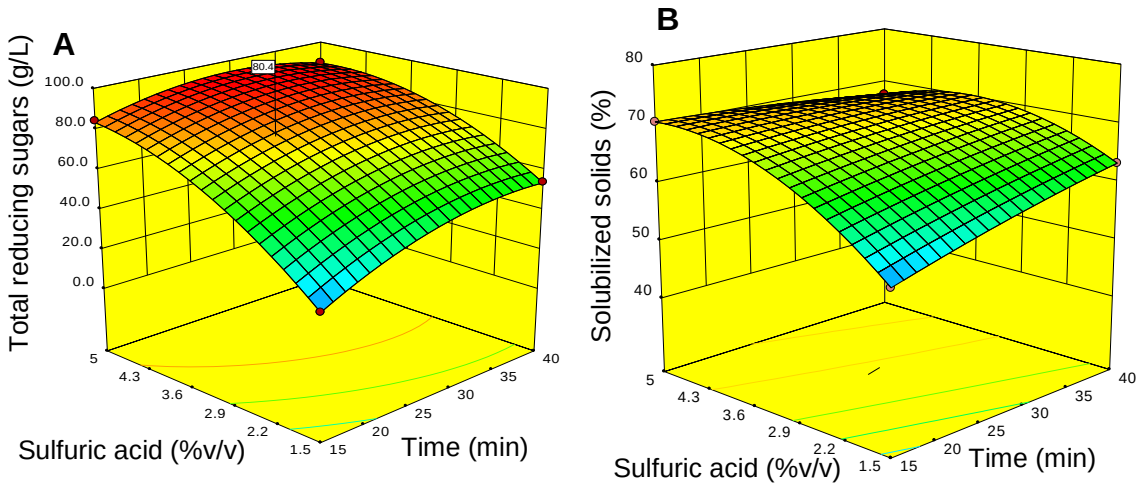

Fig. 1. A) Response surface plots of the effects of the time and H_2SO_4 concentration on total reducing sugars; B) Response surface plots of the effects of time, H_2SO_4 concentration, and CP solids content on solubilized solids (%); CP solids were fixed at 20% (w/v)

The quadratic term showing the highest effect on glucose was CP solids (-2.54) followed by pretreatment time (-1.28) (Fig. 2A). The quadratic interaction between CP solids and sulfuric acid is shown in Fig. 2B, as indicated by coefficients a_7 to a_9 (Table 5).

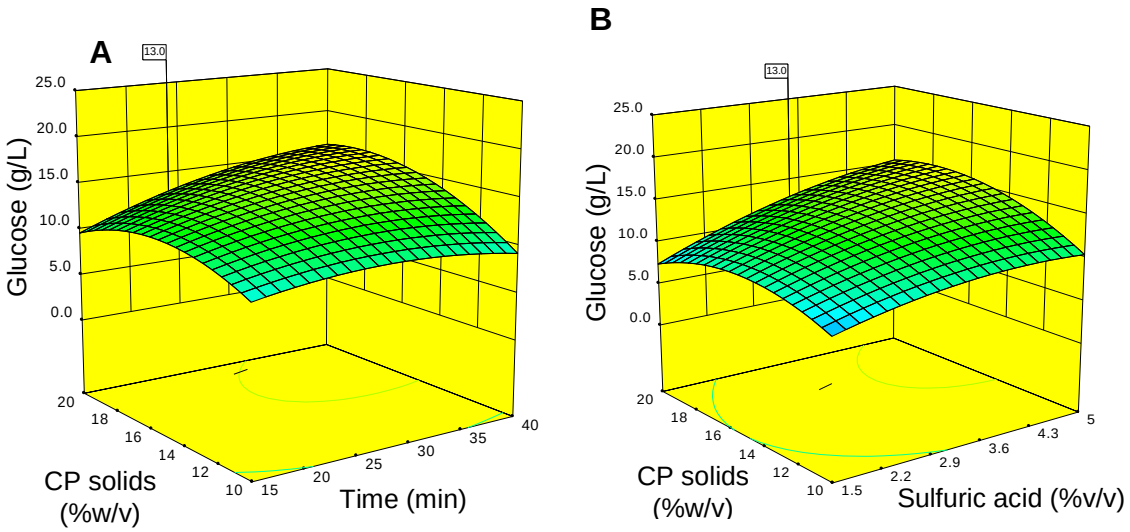

Fig. 2. Response surface plots of the effects of the time, H_2SO_4 concentration, and CP solids content on glucose: A) fixed sulfuric acid at 3.37% (v/v) and B) fixed time at 26.1 min

Thus, changes in the factors levels resulted in a minimal effect on glucose recovery. Concerning solubilized solids, sulfuric acid showed the highest influence on linear and quadratic parameters of the model (linear 5.35, quadratic -4.76), while the CP concentration exerted a negative linear influence (-1.88).

An increase in sulfuric acid produced more CP hydrolysis, but at 5% (v/v) the response reached a stationary point, while increased contact time produced almost linear increases of solubilized solids (Fig. 1B).

Response Optimization

The optimal conditions derived from the quadratic models were 3.37% (v/v) sulfuric acid and 20% (w/v) CP solids with a desirability of 0.68 (Fig. 3), and the results predicted by the models were 80.4 g/L of total reducing sugars, 13 g/L of glucose, and 68.9% of solubilized solids. Four replicates were performed using the optimal conditions, and the experimental results were 78.9 g/L ± 1.9 g/L total reducing sugars, 11.7 g/L ± 0.8 g/L glucose, and 68.8% ± 0.4% solubilized solids.

From this, the quadratic models developed from the experimental results are able to predict the operational conditions that should be used in the pretreatment of CP. The remaining solid fraction that resulted from the optimum condition showed the following composition (w/w): 23.76% ± 2.43% cellulose, 18.65% ± 1.93% lignin, 65.82% ± 2.05% soluble solids, and 0% hemicellulose. This was attributed to the dilute acid pretreatment at 121 °C, which was capable to completely hydrolyze hemicellulose (Saha 2003). Moreover, cellulose is more available to other processes such as enzymatic saccharification, from which 90% of the glucose yield can be potentially obtained (Jung and Kim 2015). Therefore, the dilute acid pretreatment at a relatively low temperature is

an alternative to minimize the formation of inhibitory compounds and is an option to produce fermentable sugars from corn pericarp (Saha 2003).

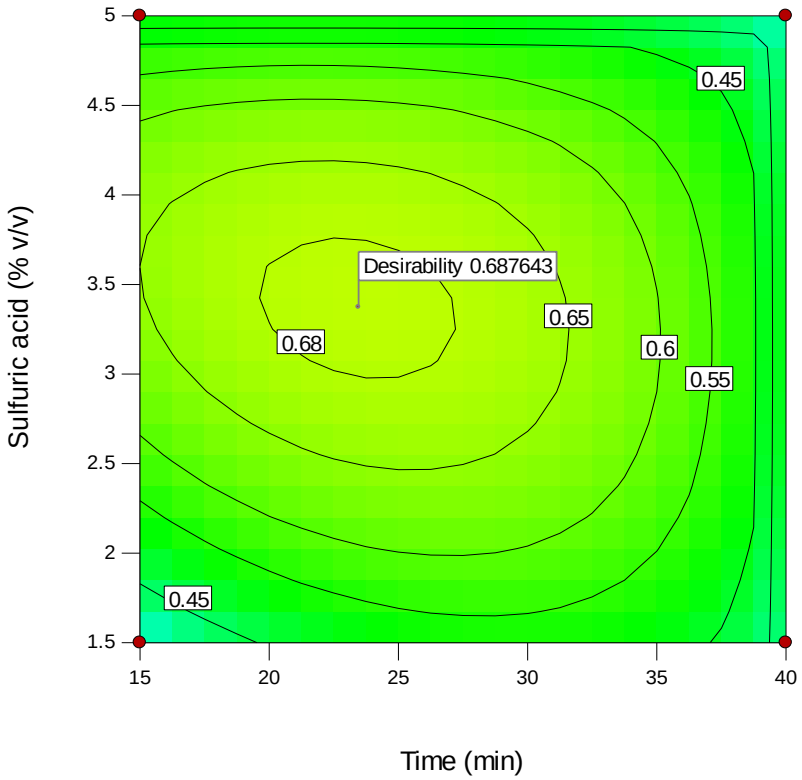

Fig. 3. Desirability graph for CP pretreatment optimization; optimal position for the three responses is marked (0.688), and the coordinates are: time = 23.45 min, sulfuric acid = 3.37 (% v/v); CP solids are fixed at 20% (w/v)

There have been few works dealing with by-products from corn wet milling, using conditions similar to those used here. Saha and Bothast (1999) tested dilute acid pretreatment (0.5 to 1% H_2SO_4 v/v, 121 ºC, 15 -60 min) on corn fiber, but it was not optimized. They found that longer time and higher acid levels increased reducing sugars yield, which is similar to our findings, but the present yields were 50% higher. Yoshida *et al*. (2010) reported an optimization strategy from microwave-assisted extraction of carbohydrates from corn fiber, and found an optimal solubilization rate of 70.8%, 2% more than our result at 121ºC, whereas carbohydrates yield was 53.8 g/100g, about 31% lower than that found in this work.

CONCLUSIONS

1. From RSM, optimized hydrolysis conditions that led to the maximum reducing sugars, glucose, and solubilized solids production were 3.43% (w/v) sulfuric acid and 20% (w/v) of CP content at 121 °C for 22.3 min.
2. A second-order empirical model was developed that successfully predicted the maximum yields of the three responses.

3. The dilute sulfuric acid pretreatment was capable of solubilizing all hemicellulose in CP.

ACKNOWLEDGMENTS

The financial support for the MSc and PhD grants from CONACYT is appreciated. This publication was financed using PFCE 2016 resources.

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Article submitted: April 26, 2017; Peer review completed: August 3, 2017; Revised version received and accepted: August 29, 2017; Published: September 12, 2017.
DOI: 10.15376/biores.12.4.7955-7963