



Research article

Multi-response and multi-criteria optimization of acid hydrolyzate detoxification of cocoa pod husks: Effect on the content of phenolic compounds and fermentable sugars



Ouattara Leygnima Yaya^{a,*}, Kouassi Esaïe Kouadio Appiah^a, Soro Doudjo^a, Amadou Kiari Mahamane Nassirou^a, Fanou Guy Didier^a, Yao Kouassi Benjamin^a, Allali Patrick Drogui^b, Dayal Rajeshwar Tyagi^b

^a Laboratoire des Procédés Industriels de Synthèse, de l'Environnement et des Energies Nouvelles (LAPISEN), Unité Mixte de Recherche et d'Innovation en Sciences des Procédés Chimiques, Alimentaires et Environnementaux, Institut National Polytechnique Félix Houphouët-Boigny, Yamoussoukro, China

^b Institut National de la Recherche Scientifique (INRS Eau Terre et Environnement), Université du Québec, 490 Rue de la Couronne, Québec City, Canada

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ABSTRACT

Dilute acid hydrolysis is the most common and effective method for converting lignocellulosic substrates into fermentable sugars. However, this hydrolysis partially degrades the lignin into phenolic compounds (PC), inhibiting the fermentation medium by retaining it in the hydrolyzate. Response surface methodology is a modeling and optimization technique used to examine the effect of multiple factors on a given response. In this study, shows the removal of PC from cocoa pod husks hydrolyzate, while preserving a considerable level of reducing sugar (RS). An Alkalization from pH 11 with NaOH, then readjustment of pH to 6 with H₂SO₄ were first carried out, while eliminating 89.39% of PC and 13.41% of sugars. Then, an optimization of the activated carbon detoxification of the hydrolyzate was carried out by considering the contact time factors (X₁), carbon to hydrolyzate ratio (X₂) and the agitation speed (X₃) in a Box-Behnken plan. The optimal conditions were 60 min of contact, a carbon to hydrolyzate ratio of 1.984% (w/v), and a stirring speed of 180 revolutions per minute (rpm). 0.153 mg/mL of PC and 6.585 mg/mL of RS remained in the hydrolyzate, corresponding to 95.18% of PC and 28.88% of RS lost.

1. Introduction

Agro-industrial activities generate huge amounts of lignocellulosic biomass, the inappropriate disposal of which, leads to environmental pollution, phytosanitary problems and waste of valuable biomass resources [1]. This biomass consists essentially of cellulose, hemicelluloses, lignins and extractables. However, its use in bioprocesses is hampered by the recalcitrance of lignin [2] which represents a protective barrier for polysaccharides (cellulose and hemicellulose). Several strategies using alkalis (NaOH, Mg(OH)₂, KOH) [3] or strong acids (H₂SO₄, HCl, HNO₃) [4,5] have been used for its delignification. However, they have limitations; among others, the cost of the treatment, the toxicity of the reagents [6,7] and incomplete delignification. In addition, the subsequent acid

* Corresponding author.

E-mail address: leygnima.ouattara18@inphb.ci (O.L. Yaya).

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hydrolysis of the delignified substrate leads to the formation of sugar degradation compounds (furanic compounds and carboxylic acids) and residual lignin (phenolic compounds). These compounds are inhibitors of the fermentation process, and it has been reported that, PC exert a considerable inhibitory effect on the fermentation of lignocellulosic hydrolyzate resulting in slow kinetics and limited productivity and yields [8]. They are more toxic than other potent inhibitor molecules (furan aldehydes, weak acids and other degraded by-products), even at very low concentrations. Indeed, their low molecular weight allows them to penetrate cell membranes and damage internal structures, causing changes in cell morphology [9]. In this context, several strategies have been implemented [10, 11]. Among them, the overliming combined with activated carbon treatment has been reported as an effective method for the reduction of inhibitors [8]. Furthermore, during the neutralization process, recent reports have shown that NaOH, Ca(OH)₂ or NH₄OH, furfural and 5-HMF are significantly eliminated by precipitation [12]. However, these reports have failed to further investigate the optimal conditions for PC removal, while minimizing the loss of RS. This fact triggers the use of the response surface methodology in this study. The response surface methodology is a mathematical and statistical modeling technique used to analyze the effect of different factors and their interactions on a given response [13]. This methodology offers an interaction of several variables on the response in less time. According, in this study, a neutralization by NaOH then H₂SO₄ of the acid hydrolyzate from the substrate delignified by KOH, the cocoa pod husks were carried out. Then hydrolyzate was first alkalinized with NaOH, then neutralized with. The response surface methodology follows a multi-response optimization process of commercial activated carbon detoxification through a Box-Behnken design based on PC removal and RS loss minimization.

2. Materials and methods

2.1. Plant material

The plant material and the resulting powder (Fig. 1) consist of cocoa pod husks (CPH) from the cocoa harvest in Soubré (South-west area of Côte d'Ivoire). This cocoa belongs to the Amazonian *Forastero* type, which originates from the southern region of Panama. The main sub-varieties are *Amelonado* and *Arriba*, and the Ivorian cocoa is from the *Amelonado* sub-group. The cocoa pod husks were harvested from September 26, 2022, to October 2, 2022, in a high-productivity plantation, and then pre-treated mechanically.

2.2. Chemicals and solvents

Chemicals and solvents were of analytical quality and were used in this state.

2.3. Methods

2.3.1. Preparation of the acid hydrolyzate

The CPH powder, devoid of extractables, is delignified in the optimal conditions obtained in our previous study [14] (KOH 1% (Merck KGaA, Germany) for 4 h at room temperature and 15 min in the autoclave (Digital vertical, LX-C autoclave, HEFEI Huatai) at 128 °C under a biomass/solvent ratio of 9.143%). The delignified substrate is hydrolyzed in 250 mL SCOTT flacons, using sulfuric acid (H₂SO₄) (95–98%, Scharlab S.L, Spain), in 26.95 min in the autoclave, a biomass to hydrolyzate ratio of 9.53% (w/v), in 4.92% (w/v) H₂SO₄.

2.3.2. Adjusting the hydrolyzate pH

The concentrated acid hydrolyzate was made alkaline to pH 11, with NaOH crystals (Merck KGaA, made in Germany), and maintained at this pH for 1 h. It is centrifuged then filtered on Whatman paper. Then, the pH of the solution is adjusted to 5.5 by means of concentrated sulfuric acid (H₂SO₄), and maintained for 1 h, then filtered. The hydrolyzate is stored in a cold room at 4 °C, for 24 h, then analyzed after filtration, for its RS and PC content.

2.3.3. Optimization of activated carbon detoxification

The neutralized hydrolyzate was detoxified with activated carbon basing on a Box-Behnken design (BBD) three factor-tests forecast, contact time (15–60 min); activated carbon to hydrolyzate ratio (1–5% w/v); stirring speed (100–180 rpm) (Table 1).

This design perfectly fits the quadratic response surface and generates a second-order polynomial regression model. The factors and their field of variation were selected based relying on previous researches highlighting their impact on the activated carbon detoxification process of lignocellulose [15,16]. The 15 flacons were incubated in a water bath stirring (*Julabo SW1*). The liquor obtained



Fig. 1. Fresh cocoa pod husks, powder and its acid hydrolyzate.

Table 1
Coded variables and actual levels used in this Box-Behnken design.

Variables	Coded symbols	Coded levels		
		-1	0	1
Contact time (min)	X ₁	15	30	45
Charcoal to hydrolyzate ratio (% w/v)	X ₂	1	3	5
Stirring speed (rpm)	X ₃	100	140	180

after each test was collected after centrifugation, filtered and analyzed for its RS and PC content. The relationship between real and coded values was described by equation (1).

$$xi = \frac{Xi - X0}{\Delta Xi} \quad (1)$$

xi and Xi and X0 are respectively, the encoded, values and the real values of the independent variable at the core point of the independent variable and ΔXi is the change in X_i . The answers (PC and RS content) are calculated through equation (2), by *Design Expert* (DEMO version 11, Stat-Ease, Minneapolis, MN).

$$Y = b_0 + \sum b_i X_i + \sum \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 \quad (2)$$

b_0 is the medium effect, b_i the main effects, b_{ij} the second-order interaction effects, b_{ii} the quadratic effects and X_i and X_j the coded variables. This equation is effective in experimental models and has allowed the study of linear, quadratic and interaction effects of factors that influence CPH hydrolysis.

2.3.3.1. Model fitting and statistical analysis. Design Expert software was used to generate the experimental data and develop the regression model. The model modification quality was expressed by the regression coefficients (R²) and the lack of adjustment. Statistical significance was determined by Fisher's test, F-value, p-value, and t-test (ANOVA). The response zones were evaluated to state the optima which fit the second-order polynomial equation.

2.3.3.2. Model validation. To prove the validity of the model, a series of confirmation experiments were performed in optimal conditions. The model validation was based on the miscalculated analysis and on the coefficient of determination (R²) between the predicted results and those experimentally obtained.

2.3.4. Analytical methods

The analyzes were carried out at the analysis and expertise laboratory (LABEX). This laboratory is accredited and Meets the requirements of the standard ISO/IEC 17025 : 2017.

2.3.4.1. Lignocellulosic composition of the substrate. The insoluble quality lignin in the sulfuric acid was determined by gravimetry following the Laboratory Analysis Procedure (LAP) adopted by the National Renewable Energy Laboratory (NREL) [17]. The holocellulose was determined according to ASTM D 1104-56 standard. The cellulose is isolated from holocellulose after dissolution hemicelluloses in a dilute alkaline solution [18]. The hemicellulose content is obtained while making difference of the cellulose content from the one of holocellulose.

2.3.4.2. Estimation of reducing sugars (RS) and phenolic compounds (PC). The RS from acid hydrolysis were estimated through the use of the dinitrosalicylic acid (3,5-DNS) method described in Miller (1959) [19] using a UV-visible spectrophotometer (UV/VIS Jasco V-530) at a wavelength of 540 nm. The PC in the acid hydrolyzate were estimated by the Folin-Ciocalteu method [20].

Table 2
Chemical composition of cocoa pod husk.

Parameters	Proportion (%)
Ash	9.24 ± 0.63
Carbohydrates	67.07 ± 2.20
Extractables	13.26 ± 0.84
Lignin	28.54 ± 3.07
Holocellulose	48.65 ± 5.67
Cellulose	31.68 ± 2.07
Hemicelluloses	16.97 ± 2.07

3. Results and discussions

3.1. Chemical composition of raw cocoa pod husk

The chemical composition of the cocoa pod husk studied is shown in Table 2.

The ash content is $9.24 \pm 0.63\%$. This value is higher than those reported in several tables. This could be due to soil composition. In addition, high values of holocelluloses (hemicelluloses and celluloses) ($48.65 \pm 5.67\%$) are obtained and confirmed by the high values of carbohydrates ($67.07 \pm 2.20\%$), throwing light on strong consistency with those reported in the literature [21,22]. However, Nazir et al. (2016) [23], suggested levels close to 45% for the cellulose content. This rich composition could help to amplify the range of applications of this agro-resource, while increasing its importance on the global market. Furthermore, it represents a potential substrate for anaerobic and aerobic digestion, and its hydrolyzate could serve as an input for the fermentative production of bioactive compounds that can be used in various fields of industry. However, it contains high levels of lignins ($28.54 \pm 3.07\%$) and extractables (13.26 ± 0.84), the reduction of which is crucial in order to release carbohydrates. Nevertheless, this process generates fermentation inhibitors, such as PCs total.

3.2. Effect of neutralization on RS and PC contents

Fig. 2 shows the RS and PC contents of the acid hydrolyzate of CPH before (A) and after neutralization (B), with sodium hydroxide and sulfuric acid.

After the neutralization phase, a significant proportion of phenolic compounds (89.39%) was eliminated, as reported by Silva-Fernandesa et al. (2017) [10], who showed that increasing of pH of the middle, could act as a first detoxification step. In addition, this step has entailed the elimination of 13.41% of RS, according to the conclusions of Santana et al. (2018) [24], who observed no significant loss of sugars in the acid hydrolyzate of CPH. Therefore, greater losses of sugars after pH adjustment were reported [24,25]. The existence of electrostatic interactions creates between these sugars present in the hydrolyzate and the sodium (Na^+ or Ca^{2+}) and sulfate (SO_4^{2-}) ions, respectively released by NaOH, $\text{Ca}(\text{OH})_2$ and H_2SO_4 , could be one of the probable reasons. In addition, the presence of these cations in the hydrolyzate could improve the flocculation process. Indeed, they are auxiliary flocculation agents, which promote interactions among the anions (phosphate, sulfate, carbonate) in the hydrolyzate [10].

3.3. Optimization of activated charcoal detoxification

3.3.1. Box-Behnken design (BBD)

The results for the PC and RS contents are displayed in Table 3.

The maximum RS value (7.3 mg/mL) is obtained in test 1, after 30 min of stirring at 140 rpm and a charcoal/hydrolyzate ratio of 1%. In these conditions, the quantity of PC was at its maximum value (0.29 mg/mL). Similarly, the lowest PC content (0.05 mg/mL) was recorded in test 12, after 45 min, at a stirring speed of 180 rpm and a ratio of 5%, while the RS content had significantly decreased; from 7.3 mg/mL to 5.15 mg/mL, corresponding to the minimum sugar content. This suggests the importance of using a high charcoal/hydrolyzate ratio and a prolonged contact time, in order to eliminate significant proportions of PC. However, this option seems unfavorable to sugar collection. It therefore, from the analysis of this table, conditions that are too severe favor the elimination of PCs but lead to a considerable loss of RS. Hence the interest of optimizing the process conditions, based on minimizing the PC content and maximizing the recovery of RS.

3.3.2. Model modification and statistical analysis

A regression coefficient of $R^2 = 0.99$ was obtained for both responses (PC and RS content). This shows that 99% of the total variance is explained by the model. In addition, predicted R^2 values of 0.86 and 0.84 were obtained, respectively, for the PCs and RSs, showing a reasonable agreement with the adjusted R^2 which is 0.97 (Table 4); with differences less than 0.2. In addition, an adequate accuracy measures the signal-to-noise ratio by comparing the range of predicted values at the design points to the average prediction error [26]. The ratios obtained are 27.21 and 26.21 respectively for PC and RS.

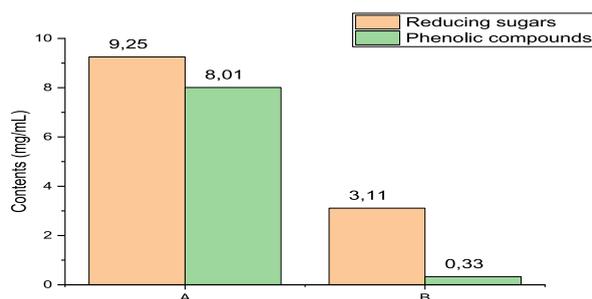


Fig. 2. Effect of CPH acid hydrolyzate neutralization before (A) and after neutralization (B) on the content of sugars and phenolic compounds.

Table 3
Experiment matrix and PC, RS content in CPH hydrolyzate.

Variables				Responses	
Trials	Contact time (X ₁) (min)	Charcoal to hydrolyzate ratio (X ₂) (%)	Stirring speed (X ₃) (rpm)	Reducing sugars (mg/mL)	Phenolic compounds (mg/mL)
1	30	1	140	7.30	0.29
2	60	1	140	7.13	0.23
3	30	5	140	5.92	0.10
4	60	5	140	5.85	0.09
5	30	3	100	6.13	0.14
6	60	3	100	5.90	0.13
7	30	3	180	6.07	0.13
8	60	3	180	6.04	0.12
9	45	1	100	6.07	0.22
10	45	5	100	5.33	0.08
11	45	1	180	6.93	0.21
12	45	5	180	5.15	0.05
13	45	3	140	6.20	0.15
14	45	3	140	6.20	0.15
15	45	3	140	6.30	0.14

Table 4
Statistical analysis

Phenolic compounds					Reducing sugars				
Source					Source				
Linear	<0.0001	0,05	0,86	0,77	Linear	0,002	0,02	0,64	0,40
2FI	0,79	0,04	0,83	0,49	2FI	0,58	0,02	0,61	0,17
Quadratic	0,006	0,18	0,97	0,87	Quadratic	0,001	0,18	0,97	0,84
Cubic	0,18		0,99		Cubic	0,18		0,99	

3.4. Statistical analysis

Both of these values are greater than 4, indicating an adequate (desirable) signal. Therefore, the quadratic model can be used to navigate the design space chosen for this study.

3.4.1. Suggested model equations

The equation in terms of coded factors can be used to make predictions about the response for given levels relating to each factor. By default, the high levels of the factors are coded +1, and the low levels are coded -1. Plus Signs (+) represent a synergistic relationship, while minus signs (-) indicate antagonism between variables [27]. The quadratic equations obtained by regression analysis are presented in equations (3) and (4), for the content of PC and RS.

$$PC = 0.147-0.011 X_1-0.079 X_2-0.008 X_3+0.013 X_1X_2-0.005 \times 2 \times X_3+0.010 \times X_1^2 + 0.020 X_2^2-0.027 \times X_3^2 \tag{3}$$

$$RS = 6.23-0.063 X_1-0.648 X_2+ 0.095 X_3+0.025 X_1X_2-0.260 \times 2 \times X_3+0.241 \times X_1^2+0.076 X_2^2-0.439 \times X_3^2 \tag{4}$$

Table 5
Analysis of variance of regression model for the phenolic compounds content.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.057	9	0.006	58.29	0.0002	Significant
X ₁	0.001	1	0.001	9.35	0.028	
X ₂	0.049	1	0.049	457.96	<0.0001	
X ₃	0.001	1	0.0005	4.15	0.097	
X ₁ X ₂	0.001	1	0.0006	5.77	0.062	
X ₁ X ₃	0.000	1	0.0000	0.0000	1.000	
X ₂ X ₃	0.0001	1	0.0001	0.9231	0.381	
X ₁ ²	0.0004	1	0.0004	3.70	0.113	
X ₂ ²	0.002	1	0.0015	14.21	0.013	
X ₃ ²	0.003	1	0.0027	25.00	0.004	
Residual	0.0005	5	0.0001			
Lack of Fit	0.0005	3	0.0002	4.75	0.179	not significant
Pure Error	0.0001	2	0.0000			
Cor Total	0.057	14				

These equations were used to evaluate the impact of the independent variables (contact time (X_1), charcoal/hydrolyzate ratio (X_2) and stirring speed (X_3)) on the PC and RS contents, after activated carbon detoxification. The parameters of the equation were obtained by multiple regression analysis of the experimental data considering all the terms, including those that are not significant since they could also contribute to some extent to the variance of the suggested models.

3.4.2. Analysis of variance (ANOVA) for the suggested quadratic model

The statistical analysis of the PC and RS content regression model using the Box-Behnken design is presented in Tables 5–6. Table 5 presents the analysis of variance for the model quadratic suggested. The F-value of the model $58.29 > 0.05$ and its p-value $0.0002 < 0.05$ confirm the significance of the model. Indeed, there is only a 0.02% chance that such a high F value could occur due to noise. Also, the p-value of no fit of 0.179 implies its non-significance with respect to pure error. Indeed, there is a 17.90% chance that an F value of such a large misfit will occur due to noise. Consequently, there is a good agreement between adjusted and predicted R^2 and an adequate precision greater than 4, as shown in paragraph 3.3.2; then the model provides good predictions for the average results [15]. Moreover, the analysis of this table showed that the linear effects (X_1 , X_2) of the independent variables, contact time and charcoal/hydrolyzate ratio and the quadratic effects (X_2^2 , X_3^2) of the charcoal/hydrolyzate ratio and stirring speed had a significant effect ($p < 0.05$) on the PC content, in a decreasing order of significance ($X_2 > X_3^2 > X_2^2 > X_1$). Thus, the analysis of equation (3) makes it possible to understand that the carbon/hydrolyzate ratio had the highest negative effect and had the highest positive quadratic effect. Indeed, the increase in this ratio leads to greater adsorption of PC, followed by a slight desorption, when this ratio reaches a certain level. Saturation of the adsorption sites could be the cause of this finding. Similarly, the quadratic effect of stirring speed had a strong negative impact, leading to PC accumulation, followed by adsorption at high stirring speeds. Also, contact time had the smallest negative effect. Note that shaking speed had a non-significant effect ($p > 0.05$) and no interaction term was significant.

Table 6 presents the analysis of variance for the quadratic model, on the RS content remaining after the activated carbon detoxification process.

The F value of 48.85 for this model implies that it is significant. That is, there is only a 0.02% chance that such a high F value could occur due to noise. Similarly, the F value of no fit the of 4.75 implies its non-significance with respect to pure error. This shows that there is a 17.88% chance that an F value of such a large misfit will occur due to noise. In addition, the analysis of Table 6 and Equation (4), show that the charcoal/hydrolyzate ratio (X_2) has the largest linear effect ($p < 0.05$). So that the stirring speed (X_3) has a weak positive effect, on the RS content, its quadratic term has the most important negative effect. However, a significant interaction effect (X_2X_3) between these two variables was found. Also, the contact time (X_1) presents the most important positive quadratic effect, even if its linear effect was not significant ($p > 0.05$).

3.4.3. Model fit diagnosis

Figs. 3 and 4 show the diagnostic plot for PC and RS content, respectively.

Figs. (3a, 4a) show that the predicted data (Y_{Pred}) agree with those observed (Y_{exp}). This is perceived through the arrangement of the points grouped around the regression line. In addition, since least-squares residuals are an important tool for judging the fit of the model [27], Figs. (3b, 4b) show that the percentage of the normal probability plot of the residuals was scattered along the straight line, which denotes a tolerable variance in variance.

Similarly, fig. (3c, 4c) reveals a very random distribution of the residual plots of the model. Indeed, this distribution does not respect any trend, and suggests good predictions of the maximum response as well as a constant variance. Therefore, the quadratic model is adequate and fitted [28] and the PC and RS contents of the CPH acid hydrolyzate can be predicted with the point prediction capability of the software.

3.4.4. Effect of independent variables on the content of phenolic compounds

The response surface methodology can be described as a graphical representation of data analysis equations [29]. It is very useful when one wants to evaluate the importance of several factors, in particular those which imply complex interactions [29]. In this

Table 6
Analysis of variance for the regression model of reducing sugars content.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.760	9	0.529	48.85	0.0002	Significant
X_1	0.031	1	0.031	2.88	0.1502	
X_2	3.350	1	3.350	309.60	<0.0001	
X_3	0.072	1	0.072	6.66	0.0493	
X_1X_2	0.003	1	0.003	0.2308	0.6512	
X_1X_3	0.010	1	0.010	0.9231	0.3808	
X_2X_3	0.270	1	0.270	24.96	0.0041	
X_1^2	0.214	1	0.214	19.77	0.0067	
X_2^2	0.021	1	0.021	1.96	0.2204	
X_3^2	0.712	1	0.712	65.73	0.0005	
Residual	0.054	5	0.011			
Lack of Fit	0.048	3	0.016	4.75	0.1788	not significant
Pure Error	0.007	2	0.003			
Cor Total	4.820	14				

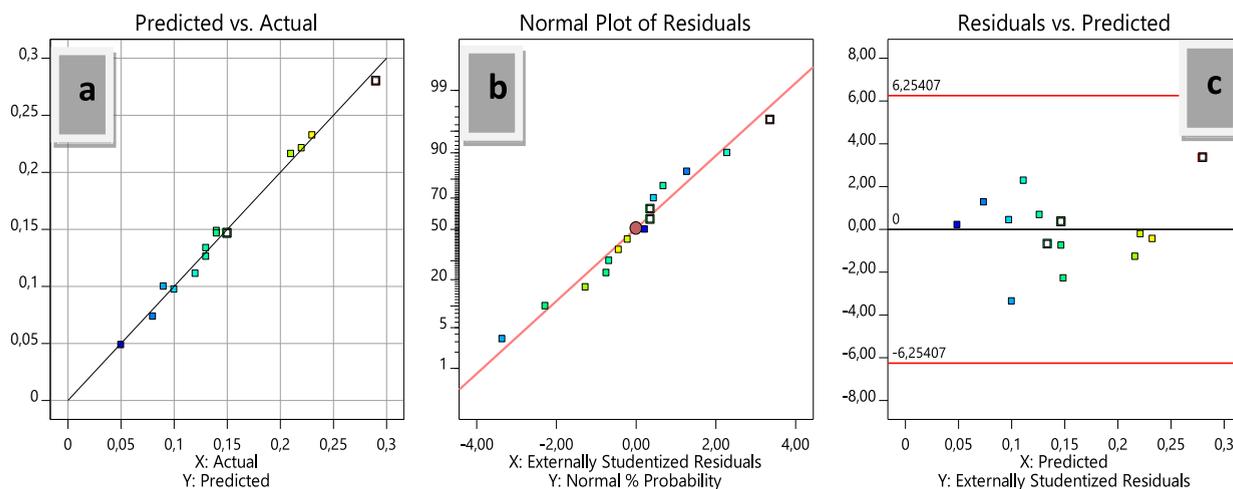


Fig. 3. Diagnostic plots for phenolic compounds content after detoxification a) observed concentration versus model predicted concentration, b) outwardly studentized residuals versus percent at normal probability, c) residual concentration versus that predicted by the model.

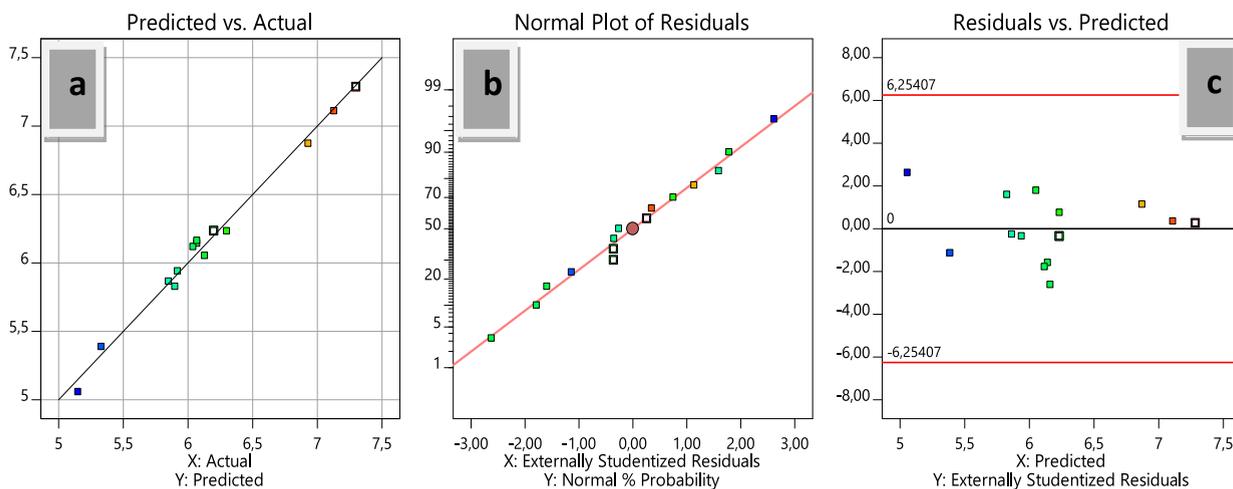


Fig. 4. Diagnostic plots for reducing sugars content remaining after detoxification a) observed concentration versus model predicted concentration, b) outwardly studentized residuals versus percentage at normal probability, c) residual concentration versus predicted.

context, the optimum of the variables was determined by plotting the three-dimensional surface graphs (Figs. 5 and 6), based on equations (3) and (4). Plots were generated by keeping one variable constant at the midpoint and varying the others over the experimental range. Fig. 3 presents the effect of the three factors studied on the PC content. The effects of contact time and charcoal/hydrolyzate ratio on PC content are shown in Fig. 5a. A slight decrease in the PC content is observed when the contact time increased, varying from 0.29 mg/mL at 30 min to 0.23 mg/mL for 60 min. However, an increase in the charcoal/hydrolyzate ratio led to a significant linear reduction of this inhibitor down to 0.1 mg/mL, for a ratio of 5%, as suggested in a recent report [15].

The negative effect of the term X_2 in equation (3), confirms this antagonism and could be due to a greater availability of the adsorbent in the hydrolyzate, which allows more bonds with toxic compounds, and consequently, a higher elimination rate. A similar observation of the dependence of the PC content, on the charcoal/hydrolyzate ratio has been reported [10]. These authors have shown that increasing the concentration of the adsorbent in the detoxification step improves the elimination of PCs. This would be due to the formation of hydrogen bridges between the oxygen carbonyl groups on these molecules and the phenolic hydroxy groups on the surface of the activated carbon.

Fig. 5b shows the interactive effect of the stirring speed and charcoal/hydrolyzate ratio on PC concentration. This figure shows a slight quadratic dependence of stirring speed on PC content. Indeed, the PC content increases up to 0.223 mg/mL, for a stirring speed of about 150 rpm, and begins to drop beyond that.

It emerges from the analysis of Fig. 5a and b, that the effect of the charcoal/hydrolyzate ratio is greater than the effect of the contact time and of the stirring speed. Fig. 5c confirms the weak linear and quadratic effects, respectively, of contact time and stirring speed on

Design-Expert® Software

Factor Coding: Actual

phenolic compounds (mg/mL)

● Design points above predicted value

○ Design points below predicted value

0,05  0,29

X1 = A: Contact time

X2 = B: charcoal/hydrolysate ratio

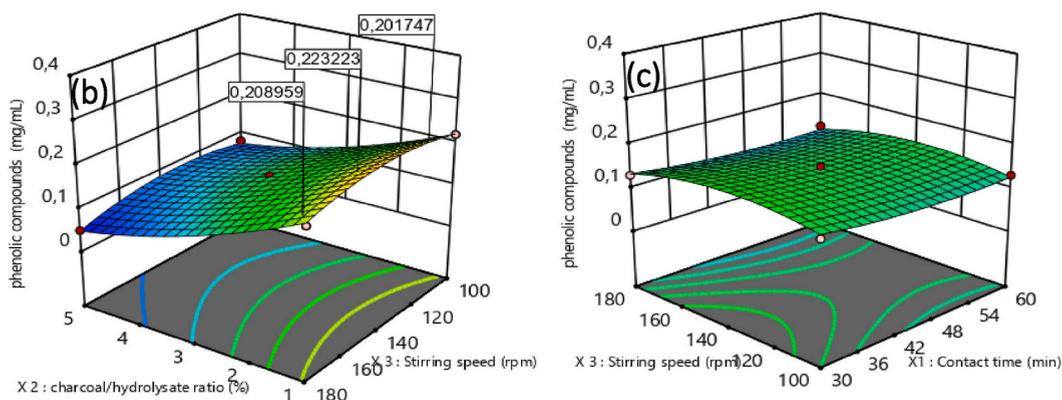
Actual Factor

Fig. 5. Surface graph (3D) showing the effect of contact time and carbon/hydrolysate ratio (a), carbon/hydrolysate ratio and stirring speed (b), contact time and stirring speed (c) on the content of phenolic compounds.

PC content. However, an increase in these factors leads to a small reduction in the PC content.

3.4.5. Effect of independent variables on RS content

The RS content was also analyzed in the detoxified CPH hydrolysate, and the effect of the independent variables is explained by Equation (3). Fig. 6 shows the effect of these factors on the RS content. The effects of contact time and charcoal/hydrolysate ratio are illustrated in Fig. 6a. This figure shows a weak linear effect of the contact time on the RS content. However, a positive quadratic dependence of the RS content is observed with this factor. Indeed, the increase in contact time led to a decrease in the sugar content up to 5.74% after 46 min of contact, then a gradual increase was observed.

The positive coefficient (+0.241) of the term X_1^2 , in equation (4) confirms our remarks. It can therefore be deduced that prolonged contact of the hydrolysate with the adsorbent could promote the recovery of sugars. Nevertheless, the charcoal/hydrolysate ratio has a very weak quadratic effect on the sugar content and a significant continuous decrease in sugars is observed when this ratio increases, with regard to the negative coefficient of the term X_2 in equation (4) and confirmed by the p-value in Table 6 ($p < 0.05$). A charcoal/hydrolysate ratio that is too high does not seem to be beneficial for the recovery of sugars. Fig. 6b illustrates the effect of the stirring speed and charcoal/hydrolysate ratio on retention of sugar. These factors had a significant linear effect on the RS content, but a significant negative quadratic dependence of sugar content with stirring speed was observed. Indeed, an increase in sugars is observed up to 6.48 mg/mL at a speed around 142 rpm, then a decrease was observed beyond that. In addition, a negative interaction effect (-0.260), in equation (4) was found between these two factors. This shows that these variables act in synergy to decrease the RS content. Fig. 6c illustrates, respectively, the positive and negative quadratic effect of the contact time and of the stirring speed on the content of fermentable sugars. In this study, the most suitable experimental set was chosen among the solutions by considering the desirability function, which was at the maximum level. On this basis, the optimal detoxification conditions were, 60 min for the contact time, a charcoal/hydrolysate ratio of 1.98%; a stirring speed of 180 rpm. A minimum total PC content of 0.153 mg/mL and a sugar content of 6.59 mg/mL still remained in the hydrolysate. This corresponded to an elimination of 95.18% of PC and a loss of 28.88% of fermentable sugars. Furthermore, detoxification of the acid hydrolysate of pomegranate peel waste was carried out using 2.5%

Design-Expert® Software

Factor Coding: Actual

Reducing sugars

- Design points above predicted value
 - Design points below predicted value
- 5,15  7,3

X1 = A: Contact time

X2 = B: charcoal/hydrolysate ratio

Actual Factor

Right-click to export full text of legend

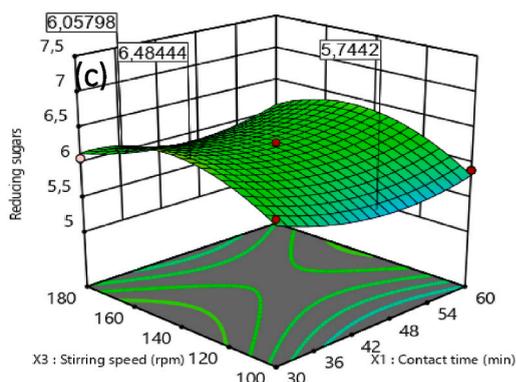
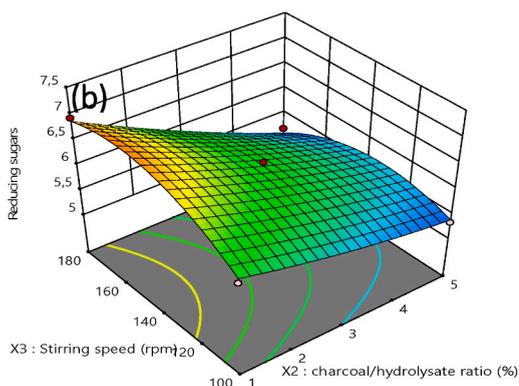
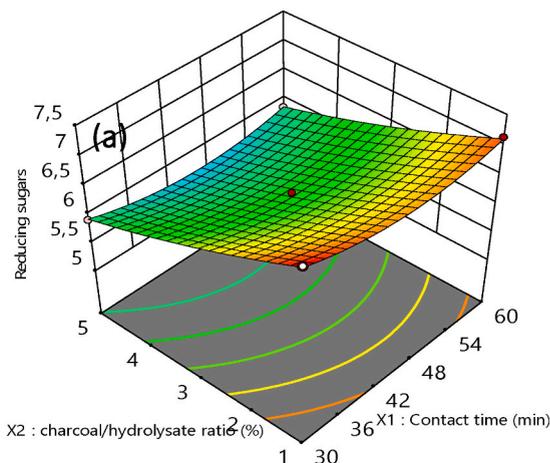


Fig. 6. Surface graph (3D) showing the effect of contact time and carbon/hydrolyzate ratio (a), carbon/hydrolyzate ratio and stirring speed (b), contact time and speed stirring (c) on the reducing sugars content.

activated carbon, which led to a 62% reduction in PC [26]. Corn cob hydrolyzate could be effectively detoxified to remove furfurals (93%) and phenolics (94%) using activated carbon treatment with minimum loss of xylose (<5%) [30]. Another one study showed that activated charcoal can reduce the amount of acids and phenolic compounds in hydrolyzate [31]. Recent work [32] reported that overliming removed 75.6% of furan derivatives and 68.1% of aromatic monomers. In comparison, AC (5.0% w/v) removed 77.9% of furan derivatives and 98.6% of aromatic monomers. Santana et al. (2018) [24] applied activated carbon (DETOX), to the acid hydrolyzate of CPH, and obtained a 78% decrease in PC, i.e., 0.15 g L⁻¹ remaining, showing that this step is fundamental in the CPH detoxification process of the CPH. In addition, enzymes have been used with less efficiency for the detoxification of acid Hydrolyzates. Thus, the laccase enzyme from *Trametes maxima* IIPLC-32 was used to detoxify sugarcane bagasse hydrolyzate. The authors obtained 66% elimination of lignin-derived PCs in 55 h [33]. This treatment required high cost and sterile conditions.

Table 7
Proof test results.

Responses	Contact time (X ₁) (min)	Charcoal to hydrolyzate ratio (X ₂) (%)	Stirring speed (X ₃) (rpm)	Experimental value (mg/mL)	Predicted value (mg/mL)	Percentage error (%)
RS (mg/mL)	60,00	2.00	179.99	6,78	6.58	2,95
	60,00	2.52	161.30	6,67	6.55	1,80
	45,00	3.00	140.00	6,45	6.30	2,33
PC (mg/mL)	60,00	2.00	179.99	0,14	0.15	1,0%
	60,00	2.52	161.30	0,113	0.15	3,7%
	45,00	3.00	140.00	0.125	0.15	2,5%

3.4.6. Model validation

In order to confirm the accuracy of the responses predicted by the model, tests were carried out under optimal conditions, and compared to the values predicted by the model (Table 7).

An excellent correlation was observed between these values. Moreover, the percentage of error varied from 1% to 3.7% for the PCs, and from 1.80% to 2.33% for the RSs. These percentages were all below 5%. Therefore, the suggested model is accurate, and the analysis of response surface methodology can be considered as a useful technique to predict and optimize the PC removal and RS content remaining in the acid hydrolyzate of CPH after detoxification.

4. Conclusion

Detoxification of cocoa pod husk hydrolyzate with dilute sulfuric acid using activated carbon was optimized using response surface methodology. This detoxification was preceded by neutralization with sodium hydroxide and sulfuric acid. This study aimed to reduce the PC content, while minimizing the loss of RS. The optimal detoxification conditions were, 60 min of contact, a charcoal/hydrolyzate ratio of 1.98 %; a stirring speed of 180 rpm and a minimum PC content of 0.153 mg/mL and a sugar content of 6.59 mg/mL still remained in the hydrolyzate. This corresponded to an elimination of 95.18 % of PC and a loss of 28.88% of fermentable sugars. This strategy is effective in detoxifying the acid hydrolyzate of this biomass. Conclusions from the present study relate to the future use of CPH, whose enhancement of the delignified substrate involves low-cost acid hydrolysis and appropriate detoxification. The fermentation of detoxified Hydrolyzates into products with high added values appears to be an alternative use capable of reducing the impact generated by poor elimination of this biomass. However, the neutralization process, like detoxification with activated carbon, results in a significant loss of fermentable sugars. Additionally, detoxification does not completely remove phenolic compounds from the hydrolyzate. In the rest of this study, it would be interesting to evaluate the content of other inhibitory compounds such as furan compounds and organic acids.

Authors' contributions

Ouattara Leygnima Yaya: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kouassi Esaïe Kouadio Appiaha: Performed the experiments; Analyzed and interpreted the data.

Soro Doudjo, Yao Kouassi Benjamin, Allali Patrick Drogui, Dayal Rajeshwar Tyagi: Contributed reagents, materials, analysis tools or data.

Amadou Kiari Nassirou Mahamane Nassirou; Fanou Guy Didier: Conceived and designed the experiments.

Data availability statement

Data included in article/supp. material/referenced in article.

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Ethical approval

This article does not contain studies with human participants or animals.

Informed consent

Informed consent was obtained from all the individual participants included in this work.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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