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Model study on dilute acid pretreatment of argan pulp for bioethanol production using response surface methodology

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Abstract: The present work describes comparative dilute acid pretreatment of the argan pulp (residue produced during the argan oil extraction) used as an economical source for bioethanol production. Response surface methodology was used to optimize the pretreatment process and to explore the effect of operational parameters (acid concentration, temperature, time and biomass loading), depending on the acid type (HCl, H2SO4) and pretreatment approach, on total and reducing sugars recovery, in addition to phenolic compounds rate as inhibitors produced during pretreatment process. Experimental results predict an optimal yield of total and reducing sugars of 171.46 mg/ml and 54.83 mg/ml, respectively, were achieved at an optimized time of 30 min with 7% of sulfuric acid at 160 °C using 40 % for biomass loading.

Keywords: Argan pulp; Dilute acid pretreatment; Optimization; Response surface methodology; Sugars recovery.

Introduction

Lignocellulosic biomass is as a potential and promising feedstock for biotechnological production of second-generation bioethanol [1, 2]. It is the most abundant carbohydrate source in nature. The use of this low-cost material avoids competition for the existing arable land and helps in reducing the greenhouse gas emissions [3-5], showing an important economic and environmental benefit [6].

Due to its complex structure, this biomass should be pretreated and modified to increase cellulose and hemicellulose accessibility and biodegradability [7,8]. Pretreatment aims to improve the extraction of fermentable sugars from biomass by altering the supramolecular structures of the lignocellulosic matrix and changing the crystalline structure of cellulose to make it more accessible to hydrolysis [9].

To date, a large number of pretreatment methods have been developed including chemical (alkali, acid, ionic liquid, etc.) [10-13], physicochemical (steam explosion, ammonia fiber explosion, microwave, etc) [14,15], physical and mechanical [16,17] pretreatments that are known to be effective for removing hemicelluloses and solubilize cellulose, Mustafa B. [1] reviewed detailed information on these pretreatment methods. Each type of these pretreatment techniques show advantages and disadvantages, but no one seems to be optimal for all applications using different types of lignocellulosic materials.

At present, dilute-acid (DA) pretreatment is the technology of choice for lignocellulosic ethanol production [18,19], it gives high reaction rates and improves cellulose hydrolysis [20]. There are two approaches of dilute acid pretreatment processes: low biomass loading (5–10% [w/w]) with high temperature (T >160°C) or high biomass loading (10–40% [w/w]) with lower temperature (T < 160°C) [21,22].

Dilute acid processes also lead to the generation of fermentation inhibitors such as acetic, levulinic acids, hydroxymethylfurfural (HMF), furfural and phenolic compounds [23-25] as a result of lignocellulose degradation. The nature and concentration of these inhibitors depend on the biomass composition, nature and pretreatment conditions, etc.

During pretreatment, phenolic inhibitors are mainly generated from the lignin degradation as well as the phenolic ester groups of hemicellulose including hydroxycinnamic acid, tannic acid and gallic acid [26-28] Larsson et al. [29] reported that phenolic compounds inhibit yeast growth by interfering with the cell membrane. Moreover, it has been found that phenols had strong toxicity to yeast growth even at lower molecular weight [30]. Therefore, it is essential to

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remove or to reduce the concentrations of these inhibitors via detoxification of the liquor obtained after pretreatment before fermentation [31].

Argania Spinosa (L.) Skeels (*A.spinosa*), is the lonely representative of *Sapotaceae* species in Morocco, with 800,000 ha of plantations and 500 kg/ha/ year of fruit productivity. This endemic tree is of monotypic genus represents an economical, social, environmental interest.

This strategic resource is internationally known for its famous nutritional and cosmetic oil. Besides, the process of argan oil production generates a considerable amount of by-products (43% of pulp, 52.6 % of shells and 2 % of oilcake) [32,33].

The pulp is the least valorized by-product; it mainly used as input in the diet of livestock. However, farmers use argan leaves (47-85%) as livestock and secondary they use argan pulp (7.2-32%). In fact, they distribute small amounts of argan pulp to weak animals (around 200g/animal/day), females in early lactation stage and young kids (25 to 30g/goat/day), and they use it (between October and December) as supplement to several components of the diet they give to goats or camels [34].

The present work is the first report on the pretreatment of Argane *(Argania Spinosa (L.) Skeels)* fruit pulp as a cheap source of second-generation bioethanol, it aims to apply the effective optimization tool for response surface methodology which combines mathematical and statistical techniques to optimize the dilute acid pretreatment of the biomass using hydrochloric acid and sulfuric acid to achieve the highest total sugars.

The concentration of sugars and phenolic inhibitors generated were determined to evaluate the effects of acid concentration, biomass loading, pretreatment temperature and duration using response surface methodology which is an effective optimization tool which combines mathematical and statistical techniques.

Material and Methods

Raw Material

Argane pulp fraction is collected from TIZNIT region (29°41′50″N 09°43′53″W) of southwestern Morocco. After that, the samples collected were supplied to the Biotechnology Laboratory of the National Institute of Agronomic Research (I.N.R.A.) in Rabat, Morocco. It was then dried, ground into a fine powder (size of *1*- 2mm) and stored until use.

Operational parameters of the pretreatment

In order to decrease the interaction between the main components of the cell wall and make them susceptible to both saccharification and fermentation [24], cellulosic materials need to be processed by pretreatment. In this work, comparative dilute acid pretreatment was conducted in the reflex apparatus using sulfuric acid and hydrochloric acid.

Besides, the comparative efficacy of the two types of dilute acid pretreatment approaches : (a) conditions : high temperature (T > 160 °C), with low solids loading (5–10% [w/w]) for the continuous-flow process, and (b) conditions : low temperature (T < 160 °C), with high solids loading (10–40% [w/w]) for the batch process was studied [1].

Response surface methodology (RSM) was used for the process optimization and to investigate the influence of the several independent variables or parameters and their interactions on the pretreatment efficiency (final response), the experimental design was created using the Nemrodw software.

To achieve the highest total sugars (mg/ml) (Y_1) and reducing sugars (mg/ml) (Y_2) yield, with lowest phenolic inhibitors (mg AGE/ml) (Y_3) generation from cellulosic structure of argan pulp; A central composite design was applied with four independent variables: (X_1) temperature (°C); (X_2) biomass loading (% w/v); (X_3) pretreatment time (min) and (X_4) concentration of acid (% w/v). Three levels (–1, 0, +1) was used for each factor (Table 1).

The following equation described the experimental design:

$$Yi = \beta 0 + \sum \beta iXi + \sum \beta ijXiXj + \sum \beta iiXi^2 + \varepsilon \qquad (1)$$

Where Yi is the predicted response by the response surface model; Xi and Xj are the coded value for each independent variables; β0 is the intercept coefficient; βi, βii, βij are the liner, quadratic and the interaction effects, respectively; and ε is experimental error.

Table 1. Levels of factors for the four variables used for the optimization of argan pulp pretreatment.

Level of factors	-1	0	1
Temperature (°C)	160.00	180.00	200.00
Biomass loading (% w/v)	2.00	6.00	10.00
Pretreatment time (min)	5.00	7.50	10.00
Acid concentration (% w/v)	0.20	3.60	7.00

(a) conditions: high temperature (T >160 °C), with low solids loading (5–10% [w/w]).

Level of factors	-1	0	1
Temperature (°C)	80.00	120.00	160.00
Biomass loading (% w/v)	10.00	25.00	40.00
Pretreatment time (min)	30.00	45.00	60.00
Acid concentration (% w/v)	0.20	3.60	7.00

(b) conditions: low temperature (T < 160 °C), with high solids loading (10–40% [w/w]).

Analytical methods

For the applied biomass (argan pulp) chemical characterization, dry matter and crude ash were determined according to the Association of Official Analytical Chemists [35] method. Protein was estimated by the Kjeldahl method with a conversion factor of 6.25. Crude fat was determined by the Soxhlet apparatus using petroleum ether, and the amount of cellulose was estimated according to the AOAC method. The results of each parameter were expressed in percentage (%) in proportion to the dry matter (MS).

The analysis of total sugars concentration of argan pulp before and after pretreatment (liquid fraction) was carried out using the colorimetric method of Dubois et al. [36], and reducing sugar were determined as glucose by using dinitrosalicylic acid (DNS) reagent at optical density 575 nm, by the method of Miller (1959) [37].

Total soluble phenolic compounds were determined in the liquid pretreated fraction of argan pulp using Folin-Ciocalteu reagent [38,] and the results were expressed as mg of gallic acid equivalents (GAE) per ml of the liquid pretreated fraction (mg GAE/ml) [39].

Results and discussion

Compositional data of argan pulp

The compositional data evaluated during our previous work was determined [40] as: (89.82 ± 0.01) % dry matter, (6.87 ± 0.14) % protein (Ntot 6.25), (5.97 ± 0.32) % fat, (16.53 ± 0.60) % cellulose, with a good amount of total extractable sugars (379.31 ± 0.59 (mg/g of dry matter)) and reducing sugars (254.35 ± 1.11 (mg/g of dry matter))

Dilute acid pretreatment

In the two parts of this study, a total of twenty-seven trails for the central composite design were developed to evaluate the effect of temperature, biomass loading, pretreatment time and concentration of acid and their interactive effect on argan pulp pretreatment. Total sugars and reducing sugars yield, in addition to phenolic compounds rate (in the liquid fraction of the pretreated argan pulp) were used as the response values (Table 3, Table 4).

Effect of dilute sulfuric acid pretreatment conditions

The purpose of this first part of work, was to evaluate the effectiveness of the two approaches of dilute sulfuric acid pretreatment : ((a) conditions : high temperature (T >160 °C), with low solids loading (5–10% [w/w]); and (b) conditions : low temperature (T < 160 °C), with high solids loading (10–40%)), in order to determine the propitious conditions to achieve the highest yield of sugars, using the response surface methodology. The results are depicted in Table 2.

Table 2. Experimental design using response surface methodology (RSM) for evaluating the effect of the dilute sulfuric acid pretreatment approach for argan pulp.

Experiment number	Coded variables				Response for (a) conditions			Response for (b) conditions		
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
1	-1	-1	-1	-1	19.35	4.00	3.53	5.36	40.57	15.39
2	1	-1	-1	-1	6.30	1.02	18.75	59.45	34.97	9.84
3	-1	1	-1	-1	89.75	38.71	11.09	126.03	47.97	22.36
4	1	1	-1	-1	53.33	26.07	12.16	156.06	35.99	8.26
5	-1	-1	1	-1	22.46	14.00	2.96	42.07	33.48	6.60
6	1	-1	1	-1	20.51	11.03	2.92	57.35	38.03	16.05
7	-1	1	1	-1	80.94	34.07	10.00	121.97	46.94	11.86
8	1	1	1	-1	79.23	29.95	3.00	171.46	54.83	19.48
9	-1	-1	-1	1	19.50	3.08	10.20	72.50	45.26	18.27
10	1	-1	-1	1	13.67	2.05	12.48	76.37	41.34	15.63
11	-1	1	-1	1	78.38	45.88	8.47	89.10	41.25	14.90
12	1	1	-1	1	60.49	33.45	7.52	89.38	51.27	6.80
13	-1	-1	1	1	10.44	1.02	2.06	123.82	35.53	16.52
14	1	-1	1	1	23.10	5.22	2.42	99.44	51.27	19.76
15	-1	1	1	1	71.12	34.27	5.99	147.91	53.36	16.35
16	1	1	1	1	77.98	47.83	5.66	198.15	50.45	13.40
17	-1	0	0	0	55.97	35.10	6.85	109.53	49.91	8.43
18	1	0	0	0	40.63	13.45	5.70	114.45	38.60	10.65
19	0	-1	0	0	24.53	10.02	6.33	58.42	24.69	9.52
20	0	1	0	0.	86.73	34.90	8.99	151.64	53.14	7.46
21	0	0	-1	0	50.11	17.55	5.72	110.57	50.67	15.70
22	0	0	1	0	65.81	35.73	7.48	114.17	51.53	12.26
23	0	0	0	-1	66.00	24.60	8.52	98.99	51.64	10.20
24	0	0	0	1	53.31	25.32	3.90	117.76	49.77	12.43
25	0	0	0	0	62.13	28.60	6.49	105.34	50.99	10.93
26	0	0	0	0	63.50	29.07	5.89	100.03	51.81	10.93
27	0	0	0	0	63.80	29.52	6.17	111.14	51.76	11.09

Four factors were used : (X_1) temperature (°C), (X_2) biomass loading (% w/v), (X_3) pretreatment time (min) and (X_4) concentration of acid (% w/v). Each factor was tested at high (+1), central (0) and low (-1) levels. Besides, two approaches were used ((a) conditions : high temperature (T >160 °C), with low solids loading (5–10% [w/w]) and (b) conditions : low temperature (T < 160 °C), with high solids loading (10–40% [w/w])) to achieve the highest total sugars (mg/ml) (Y_1) and reducing sugars (mg/ml) (Y_2) yield, with lowest phenolic inhibitors (mg AGE/ml) (Y_3).

Effect of pretreatment conditions on yield of sugar converted

Total sugars and reducing sugars yield were used as criterion to evaluate the efficiency of argane pulp pretreatment, as shown in the following quadratic polynomial models (equations : (2) (3) for total and reducing sugars, respectively, for (a) conditions; and (4) (5) for total and reducing sugars, respectively, for (b) conditions) obtained for the coded factors:

$$Y_{TS} = 60.517 - 4.037\ X_1 + 28.783\ X_2 + 3.373\ X_3 - 1.660\ X_4 - 10.904\ X_1^2 - 3.574\ X_2^2 - 1.244\ X_3^2 + 0.451\ X_4^2 - 2.562\ X_1X_2 + 5.566\ X_1X_3 + 0.602\ X_2X_3 + 3.058\ X_1X_4 - 0.836\ X_2X_4 - 1.488\ X_3X_4 \quad (2)$$

$$Y_{RS} = 27.170 - 2.226\ X_1 + 15.205\ X_2 + 2.295\ X_3 + 0.815\ X_4 - 1.949\ X_1^2 - 3.764\ X_2^2 + 0.416\ X_3^2 - 1.264\ X_4^2 - 0.803\ X_1X_2 + 2.484\ X_1X_3 - 1.194\ X_2X_3 + 1.688\ X_1X_4 + 3.207\ X_2X_4 - 0.961\ X_3X_4 \quad (3)$$

$$Y_{\ TS} = 108.623 + 7.379\ X_1 + 33.662\ X_2 + 13.362\ X_3 + 6.927\ X_4 + 1.808\ X_1^2 - 5.152\ X_2^2 + 2.188\ X_3^2 - 1.807\ X_4^2 + 8.261\ X_1X_2 + 3.335\ X_1X_3 + 7.558\ X_2X_3 - 4.242\ X_1X_4 - 12.993\ X_2X_4 + 15.439\ X_3X_4 \quad (4)$$

$$Y_{\ RS} = 48.855 + 0.138\ X_1 + 5.003\ X_2 + 1.452\ X_3 + 1.949\ X_4 - 3.267\ X_1^2 - 8.607\ X_2^2 + 3.578\ X_3^2 + 3.183\ X_4^2 - 0.484\ X_1X_2 + 2.297\ X_1X_3 + 2.058\ X_2X_3 + 1.504\ X_1X_4 - 0.984\ X_2X_4 - 0.143\ X_3X_4 \quad (5)$$

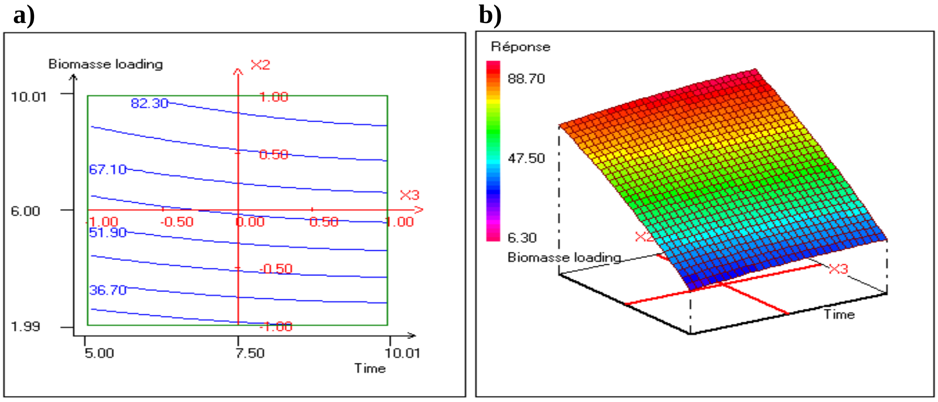
The results of experimental responses of the dilute sulfuric acid pretreatment attempted according to the two types of conditions ((a) and (b)) (Table 2, Fig.1), show that experiments performed at low temperature with high solids loading during a long time ((b) condition) led to the highest value of total and reducing sugars yield. Additionally, Table 2 shows that for (b) conditions, the experiment (16) performed at highest level of all factors (160 °C, 60 min, 40 % of biomass loading and 7% of acid concentration) allowed to obtain the highest total sugars yield (198.15 mg/ml) and 50.45 (mg/ml) of reducing sugars. However, the experiment (8) performed at the same factors level with modifying the acid concentration to the lowest level (from 7% to 0.2 %) decreases slightly the total sugars yield to 171.64 (mg/ml) but led to increase the reducing sugars to the highest level (54.83 mg/ml).

According to the previous models, equations (2) and (3) show, generally, that the biomass loading (X_2) had the most significant effect on total (TS) and reducing sugars (RS) recovery efficiency ($P<0.0001$), followed by the interactive terms of temperature and time (X_1X_3) for TS, biomass loading and acid concentration (X_2X_4) for RS ($P<0.0001$).

However, equations (4), (5) reveal that biomass loading (X_2) have high significant ($P<0.0001$) effect on TS and RS recovery efficiency, additionally, the interactive terms of time and acid concentration (X_3X_4) for TS, and quadratic effect of biomass loading (X_2^2) for RS impacted the recovery efficiency with high significance ($P<0.0001$).

Therefore, Figure 1 represent 2D (a, c, e, g) and 3D (b, d, f, h) response graphs of the biomass loading (X_2) effect during the reaction time on the total sugars and reducing sugars for the (a) and (b) conditions, respectively, that are drawn using NEMRODW software.

Figure 1 shows that the depolymerization of the lignocellulosic structure of the argan pulp to total (a, b for (a) conditions, and e, f for (b) conditions) and reducing (c, d for (a) conditions, and g, h for (b) conditions) sugars, using dilute sulfuric acid pretreatment, proceeded efficiently at the region located, in general, at highest level of biomass loading and increasing time reaction (exp 3, 8, 11, 16 and 20), which is in high agreement with the results given in Table 2.



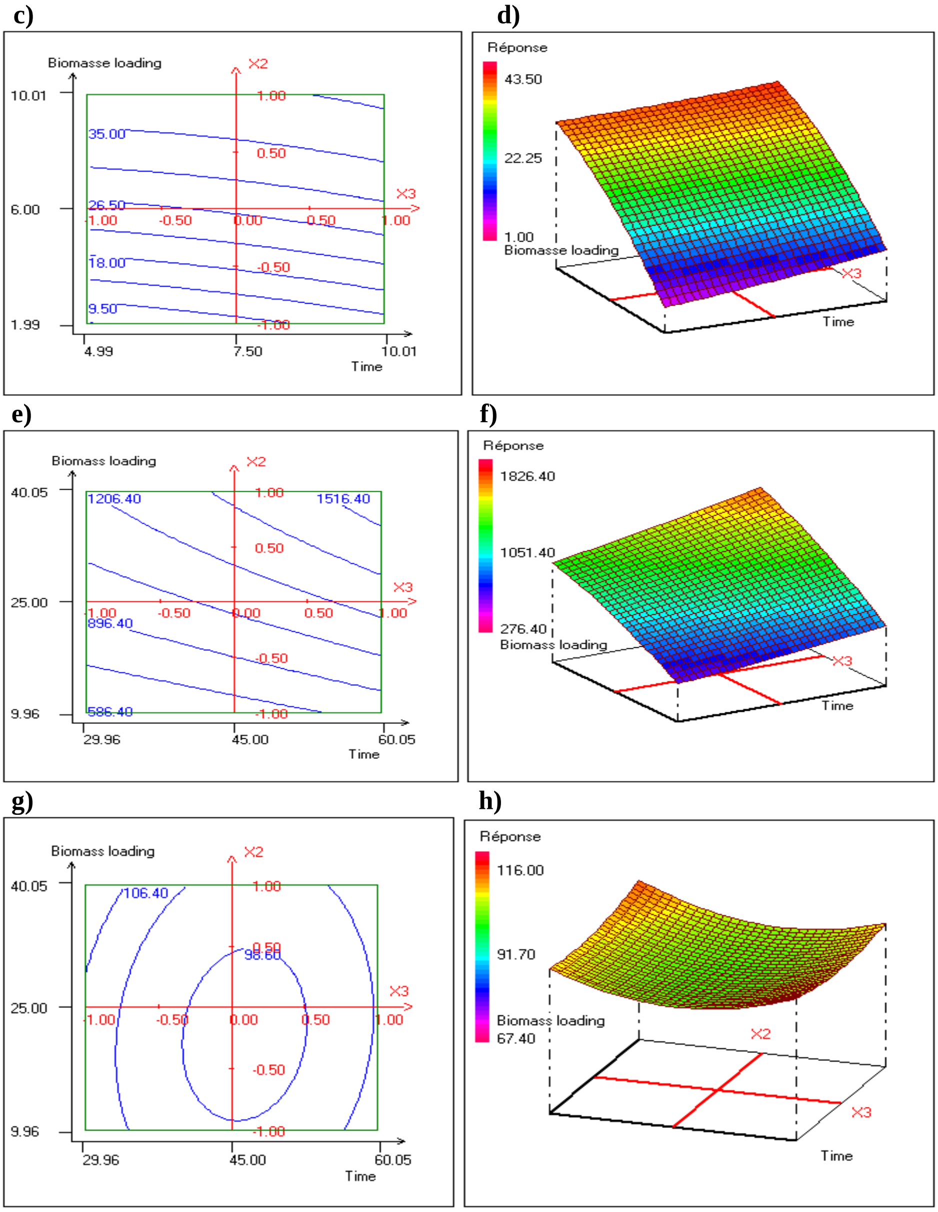


Figure 1. The 3D **(b, d, f, h)** response and their corresponding 2D **(a, c, e, g)** contour plot of the total sugars yield (**a, b** for (a) conditions, and **e, f** for (b) conditions) and reducing sugars yield (**c, d** for (a) conditions, and **g, h** for (b) conditions) in relation to biomass loading (X_2) during the reaction time (X_3), when temperature (X_1) and acid concentration (X_4) are constant at zero levels.

Effect of pretreatment conditions on phenolic compounds generated

Unfortunately, the liquid fraction obtained after dilute acid pretreatment contains various inhibitors of microbial growth [41,29] and cellulolytic enzymes [42], Kim et al. [25] reported that the most inhibitory molecules were lower molecular weight phenolic compounds, that have a strong toxic effect on the bacteria or yeast growth during fermentation process, in addition to the deactivating effect on cellulase and β-glucosidase enzymes [26,27, 30, 42]. Thus, the optimum conditions for this work were to generate lower values of phenolic compounds.

In our study, quadratic regression models for phenolic compounds generation were developed by response surface methodology, the following equations (equations : (6) for (a) conditions; and (7) for (b) conditions) express the significant effects of the variables and their interactions on phenolic compounds generated after pretreatment:

$$Y_{PC} = 6.330 + 0.526\ X_1 + 0.624\ X_2 - 2.635\ X_3 - 0.791\ X_4 - 0.129\ X_1^2 + 1.256\ X_2^2 + 0.196\ X_3^2 - 0.194\ X_4^2 - 1.564 X_1X_2 - 1.539\ X_1X_3 + 1.251\ X_2X_3 - 0.493\ X_1X_4 - 0.476\ X_2X_4 + 0.257\ X_3X_4 \quad (6)$$

$$Y_{PC} = 10.175 - 0.601\ X_1 - 0.373\ X_2 + 0.285\ X_3 + 0.779\ X_4 - 0.231\ X_1^2 - 1.281 X_2^2 + 4.209\ X_3^2 + 1.544\ X_4^2 - 1.377\ X_1X_2 + 2.984\ X_1X_3 + 0.561\ X_2X_3 - 0.492\ X_1X_4 - 2.051\ X_2X_4 + 0.768\ X_3X_4 \quad (7)$$

Equation (6) indicates that the linear term of time (X_3) had the most significant effect on phenolic compounds (PC) release efficiency ($P<0.0001$), while, biomass loading (X_2), acid concentration (X_4) and the interactive terms of temperature and biomass loading (X_1X_2), temperature and time(X_1X_3), biomass loading and time (X_2X_3) significantly ($P<0.05$) affected PC release efficiency.

Concerning the relative impact of terms on PC for (b) conditions, and comparing the factors coefficients equation (7) shows the high significant ($P<0.0001$) effect of the quadratic term of time (X_3^2), followed by the interactive terms of temperature and time (X_1X_3), biomass loading and acid concentration (X_2X_4), quadratic term of time (X_4^2), the interactive terms of temperature and biomass loading (X_1X_2), time and acid concentration (X_3X_4), linear terms of temperature (X_1) and time (X_4) with $P<0.0001$.

The experimental responses of PC for the two types of conditions ((a) and (b)) (Table 2), show that the liquid fraction obtained after pretreatment contains high levels of phenolic compounds with varying amounts (2.06 - 18.75 mg AGE/ml for (a) conditions, and 6.60 - 22.36 mg AGE/ml for (b) conditions).

Acid effect on argan pulp pretreatment

During this step of the study, the influence of the acid type on pretreatment of argan pulp has examined by comparing the effects of sulfuric acid and hydrochloric acid on the total recovery and reduction of sugars, as well as on the phenolic compound. Moreover, according to the results obtained in the previous part of work, this part of optimization was attempted using (b) conditions (low temperature (T < 160 °C), with high solids loading (10–40%)) as the optimum approach to obtain the maximum recovery of total and reducing sugars. The results are shown in Table 3, using the response surface methodology.

Table 3. Experimental design using response surface methodology (RSM) for evaluating the effect of acid type on dilute acid pretreatment of argan pulp.

Experiment number	Coded variables				Response for DSAP			Response for DHAP		
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
1	-1	-1	-1	-1	56.36	40.57	15.39	18.16	14.25	9.67
2	1	-1	-1	-1	59.45	34.97	9.84	18.26	17.25	12.47
3	-1	1	-1	-1	126.03	47.97	22.36	44.47	16.25	17.90
4	1	1	-1	-1	156.06	35.99	8.26	41.05	31.82	27.09
5	-1	-1	1	-1	42.07	33.48	6.60	11.11	5.00	11.31
6	1	-1	1	-1	57.35	38.03	16.05	32.73	15.25	13.85
7	-1	1	1	-1	121.97	46.94	11.86	43.07	22.05	20.16
8	1	1	1	-1	171.46	54.83	19.48	54.32	32.00	24.01
9	-1	-1	-1	1	72.50	45.26	18.27	92.49	35.25	12.88
10	1	-1	-1	1	76.37	41.34	15.63	50.05	26.25	18.50
11	-1	1	-1	1	89.10	41.25	14.90	83.37	30.00	10.52
12	1	1	-1	1	89.38	51.27	6.80	90.02	60.25	13.00
13	-1	-1	1	1	123.82	35.53	16.52	53.89	37.75	11.64
14	1	-1	1	1	99.44	51.27	19.76	56.25	20.52	19.09
15	-1	1	1	1	147.91	53.36	16.35	92.76	25.75	18.00
16	1	1	1	1	198.15	50.45	13.40	97.03	31.25	18.85
17	-1	0	0	0	109.53	49.91	8.43	80.26	24.25	17.85
18	1	0	0	0	114.45	38.60	10.65	80.47	25.75	12.62

19	0	-1	0	0	58.42	24.69	9.52	49.83	13.00	21.97
20	0	1	0	0.	151.64	53.14	7.46	98.01	24.00	19.74
21	0	0	-1	0	110.57	50.67	15.70	87.25	20.63	18.35
22	0	0	1	0	114.17	51.53	12.26	93.00	38.40	19.97
23	0	0	0	-1	98.99	51.64	10.20	89.31	30.90	17.54
24	0	0	0	1	117.76	49.77	12.43	84.50	50.83	18.97
25	0	0	0	0	105.34	50.99	10.93	91.14	25.24	18.81
26	0	0	0	0	100.03	51.81	10.93	90.20	24.50	18.90
27	0	0	0	0	111.14	51.76	11.09	90.18	24.90	18.85

Dilute sulfuric acid pretreatment (DSAP) and dilute hydrochloric acid pretreatment (DHAP) were used to illustrate the effect of acid on total sugars (mg/ml) (Y_1), reducing sugars (mg/ml) (Y_2) and phenolic inhibitors (mg AGE/ml) (Y_3) yield. Four factors were used : (X_1) temperature (°C), (X_2) biomass loading (% w/v), (X_3) pretreatment time (min) and (X_4) concentration of acid (% w/v). Each factor was tested at high (+1), central (0) and low (-1) levels.

Effect of acid type on sugars recovery

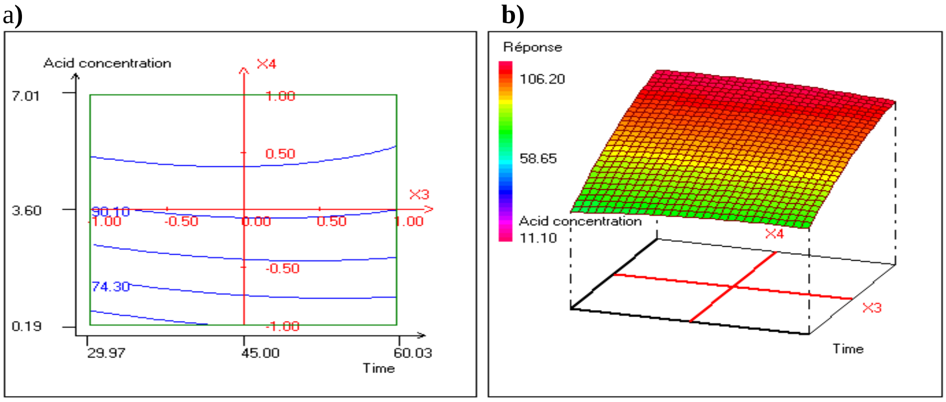
Based on the experimental measured data listed in Table 3, the dilute sulfuric acid pretreatment (DSAP) shown to be more efficient on the argan pulp depolymerization than dilute hydrochloric acid pretreatment (DHAP), whereas, both the higher total sugars (TS) (198.15 mg/ml, Exps. 16) and the higher reducing sugars (RS) (54.83 mg/ml, Exps. 8) yield were obtained using H_2SO_4 compared to HCl (98.01 mg/ml of TS, Exps. 20; and 60.25 mg/ml of RS, Exps. 12).

The variations of the experimental responses are represented by the full quadratic polynomial models (Equation (4) and (5) for DSAP ; (8) and (9) for DHAP), showing the significance of linear terms, interactive terms, and quadratic terms on TS and RS recovery efficiency.

$$Y_{TS} = 91.466 + 0.033 X_1 + 14.518 X_2 + 0.502 X_3 + 19.327 X_4 - 11.580 X_1^2 - 18.025 X_2^2 - 1.820 X_3^2 - 5.040 X_4^2 + 2.319 X_1X_2 + 4.913 X_1X_3 + 3.328 X_2X_3 - 3.669 X_1X_4 + 0.491 X_2X_4 - 2.206 X_3X_4 \quad (8)$$

$$Y_{RS} = 27.435 + 2.766 X_1 + 4.936 X_2 - 1.332 X_3 + 7.393 X_4 - 3.712 X_1^2 - 10.212 X_2^2 + 0.803 X_3^2 + 12.153 X_4^2 + 4.641 X_1X_2 - 1.959 X_1X_3 - 0.799 X_2X_3 - 1.828 X_1X_4 - 1.431 X_2X_4 - 1.951 X_3X_4 \quad (9)$$

a) b)



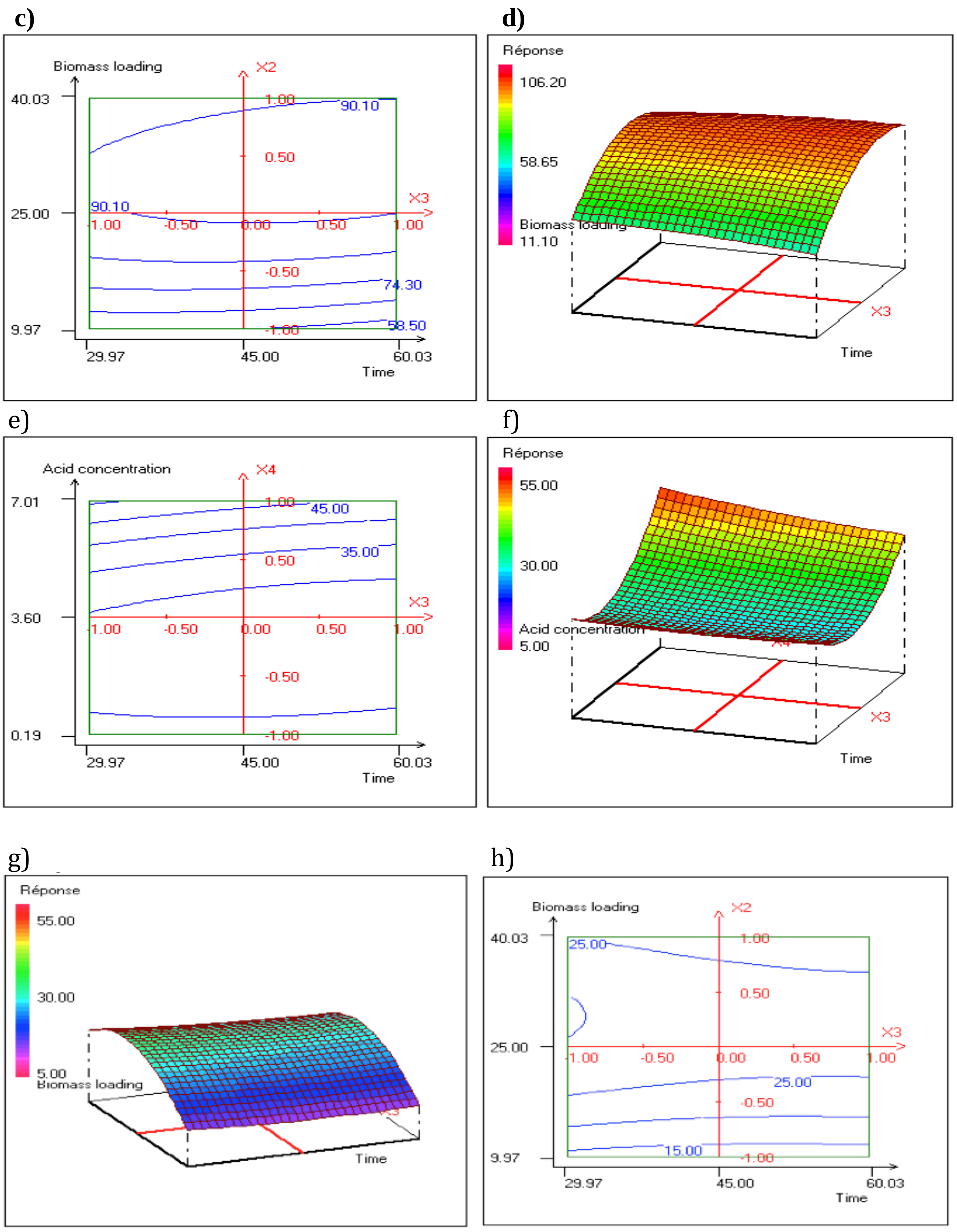


Figure 2. The 3D **(b, d,f , h)** response and their corresponding 2D **(a, c, e, g)** contour plot of the total and reducing sugars yield, for DHAP, in relation to linear terms of biomass loading (X_2) and acid concentration (X_4) effect during the reaction time (X_3), when temperature (X_1) and acid concentration (X_4) are constant at 120.00°C, 3.60 % (« 0 » levels).

According to equation (8) and (9), total and reducing sugars, generally, are impacted with highest significance (P<0.0001) by the linear terms of biomass loading (X_2), acid concentration (X_4), and the quadratic terms of biomass loading ($X_2{}^2$), in addition to quadratic terms of time ($X_1{}^2$) for TS, and acid concentration ($X_4{}^2$) for RS.

Hence, 2D (a, c, e, g) and 3D (b, d, f, h) response graphs of the biomass loading (X_2) and acid concentration (X_4) effect during the reaction time are illustrated in Figure 2, showing their impacts on total sugars and reducing sugars recovery efficiency for DHAP, using NEMRODW software.

The design space illustrates that during dilute hydrochloric acid pretreatment (DHAP), the increasing biomass loading (X_2) and acid concentration (X_4) influence positively the yield of total and reducing sugars (Exps. 12). Generally, the maximum yield of total and reducing sugars could be obtained with 40 % (w/v) of biomass loading pretreated at 160 °C for 30 min with 7 % (w/v) (Figure 2 : b, d, f, h). The interactive effect of acid concentration and time (Figure 2b, f) on total sugars extraction (from 98.01mg/ml with level 0, Exps. 20 to 90.02 mg/ml with level 1; Exps.12) was not as distinct as that on reducing sugars (from 24 mg/ml with level 0, Exps. 20 to 60.25 mg/ml with level 1 ; Exps.12).

Effect of acid type on phenolic compounds generation

The experimental design for the phenolic compounds released during dilute hydrochloric acid pretreatment was described by the following equation (Eqs.10), expressing the significant effects of the variables and their interactions:

$$Y_{PC} = 19.004 + 1.642\ X_1 + 2.105\ X_2 + 0.917\ X_3 - 0.697\ X_4 - 3.844\ X_1^2 + 1.776\ X_2^2 + 0.081\ X_3^2 - 0.824\ X_4^2 - 0.127\ X_1X_2 - 0.338\ X_1X_3 + 0.634\ X_2X_3 - 0.124\ X_1X_4 - 2.725\ X_2X_4 + 0.655\ X_3X_4 \quad (10)$$

According to the developed model (Eqs.10) for phenolic compounds generation efficiency, biomass loading term (X_2) shows the most significant positive effect ($P<0.0001$), followed by the linear term of temperature (X_1) ($P<0.0001$), however, the quadratic term of temperature (X_1^2) had the most negative effect on phenolics generation followed by the interactive term biomass loading and acid concentration (X_2X_4), with $P<0.0001$.

Compared to dilute sulfuric acid pretreatment (DSAP), the biomass pretreated using hydrochloric acid (DHAP) generated more phenolics concentrations (6.60 - 22.36 mg AGE/ml for DSAP, and 9.67 - 27.09 mg AGE/ml).

Argane pulp optimum pretreatment conditions

The optimal conditions for argan pulp pretreatment were investigated for the four factors and their interactions, using Response surface methodology by Nemrodw software.

According to the responses predicted for this study, the first part of this work revealed that the maximum value of total and reducing sugars yield obtained when dilute acid pretreatment performed at low temperature (T < 160 °C) with high solids loading (10–40%) during a long time ((b) condition) using sulfuric acid. Meanwhile, during the second part, the dilute sulfuric acid pretreatment shown to be more efficient for the argan pulp pretreatment than dilute hydrochloric acid pretreatment, achieving both higher total sugars (198.15 mg/ml, Exps. 16, Table 3) and higher reducing sugars (54.83 mg/ml, Exps. 8, Table 3).

Since the aim of this work was to depolymerize the argan pulp and produce the highest recovery of reducing sugars (54.83 mg/ml). The optimal conditions adopted were listed as follows (Exps. 8, Table 3): pretreatment time of 30 min with 7% of sulfuric acid at 160 °C using 40 % for biomass loading, taking into account to perform at low pretreatment time that leads to a slight decrease in total sugars yield (198.15 mg/ml with level « 1 » Exps. 16, Table 3, and 171.46 mg/ml with level « -1 » Exps. 8, Table 3) with a value of phenolic inhibitors 19.48 mg AGE/ml that will be removed using lime method.

The validation experiments were performed in triplicate under the optimal conditions in order to confirm the reliability of the predicted values of the response. The results were well comparable to the predicted value, total and reducing sugars yield were 187.10 ± 1.2% and 48.87 ± 1.8%, respectively.

Conclusions

Comparative dilute acid pretreatment study was investigated to improve the saccharification of the argan pulp, using a composite value of total sugars, reducing sugars and phenolic compounds as the response value.
The results showed that dilute sulfuric acid at (b) conditions (low temperature (T < 160 °C), with high solids loading (10–40%)) leads to the highest total and reducing sugars yield. However, the quadratic polynomial models illustrate that biomass loading and acid concentration were, generally, the significant variables during the pretreatment process.

Overall, a technical-economic study is required to evaluate the viability of the optimized process, and its use as a special pretreatment method to enhance bioethanol production from argan pulp, which could used as new and cheap biomass for the bioethanol production.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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