

## Research Article

# Statistical Models for S.35 Sorghum Stems Conversion by Hydrothermal Treatment

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## Abstract

A three factor Doehlert design was adopted to build statistical models to optimize the action of temperature, time and liquid/solid ratio, on reducing sugars and total phenolic compounds content during sorghum stems hydrothermolysis. Therefore, the models revealed that increasing temperature and time allowed an increase of reducing sugars and total phenolic compounds content, while the impact of liquid/solid ratio was negatively significant. An augmentation of liquid/solid ratio was necessary for solubilization of hemicelluloses and lignin. Also, optimization of the concerted factors impacts for reducing sugars gave a combination of 210°C, 60 min, 10 mL/g with a maximal reducing sugar of 15.16 mg/mL, while optimization of total phenolic compounds content gave a combination of 210°C, 48 min, and 10 mL/g with a maximal total phenolic compound of 0.81mg/mL. Consequently, a compromise was found to obtain high sugar content ( $\geq 14$  mg/mL) and, to obtain at the same time high total phenolic compounds content ( $\geq 0.8$  mg/mL). It was also demonstrated that the best way of removing lignin from sorghum stem was to apply a lower temperature (150°C) and increase the pretreatment severity (meaning increase the reaction time).

## Keywords

- Sorghum stems
- Hydro thermolysis
- Experimental design
- Reducing sugars
- Total polyphenols

## ABBREVIATIONS

ANOVA: Analysis of Variance; AAD: Average Absolute Deviation; AAE: Ascorbic Acid Equivalent;  $A_r$ : Accuracy Factor;  $B_r$ : Bias Factor; Cal: Calculated or Theoretical Values Using the Models; Exp: Experimental Values Obtained; FAO: Food and Agriculture Organization of the United Nations; GHG: Greenhouse Gases; IRAD: Institute of Research and Agronomic Development; k: Number of variables; N: Number of Experiments; P: Probability Level;  $R^2$ : Coefficient of Determination; Res: Residue; RSM: Response Surface Methodology;  $x_j$ : Coded Variables given by the Doehlert Matrix;  $X_j$ : Real Variables;  $X_{0j}$ : Centre of Variable;  $X_j^{\max}$ : Upper Level of the Real Value a Factor;  $X_j^{\min}$ : Lower Level of the Real Value a Factor;  $\Delta X_j$ : Increment; Y: Response;  $Y_j$  cal: Theoretical Response;  $Y_j$  exp: Experimental Response;  $Y_{ThSR}$ : Mathematical Model for Reducing Sugars;  $Y_{ThCF}$ : Mathematical Model for Total Phenolic Compounds;  $\beta_{ij}$ : Interaction Terms Coefficient;  $\beta_{ii}$ : Quadratic Terms Coefficient;  $\beta_i$ : Linear Terms Coefficient;  $\beta_0$ : Constant

## INTRODUCTION

The biomass, which is universally considered as substitute energy to fossil fuels, can be turned into biogas, electricity, steam, hydrogen and biofuel [1]. The greenhouse gases (GHG) emissions

which are produced in quantity became too important to be controlled naturally by the forests and the oceans. It accumulates in the atmosphere and contributes to the warming of the planet [2]. Energy security and greenhouse gas emissions are placed on the global agenda and led to reflections on possible alternative energy [3]. Bioethanol has been identified as a viable transport fuel, and has been transformed into different chemicals [4]. Sugarcane, cereals or tubers are widely products which industrial techniques depend for its production. This is associated with easy processing of these substrates. Furthermore, significant stress was noted on prices and food security due to the use of sugarcane, cereals and tubers for the production of bioethanol [5]. Thus, lignocellulosic materials that are more abundant than the natural organic material origin, seem potentially economically viable. These materials comprise mainly paper mill waste, agricultural and forestry residues, wood, herbaceous crops, wood etc ... [6-8].

Sorghum (*Sorghum bicolor* (L.) Moench) is a major tropical cereal-bearing monocotyledonous plant found in the semi-arid areas of the world. It is a vital calorie- based food component in human nutrition in some parts of Africa [9]. Agricultural residues resulting from this culture are the stems which were not valorized. The transformation of lignocellulosic biomass to bioethanol comprises pretreatment step, enzymatic hydrolysis, fermentation and purification. If a valorization of sorghum stems

in bioethanol is theoretically possible, this conversion remains a challenge. Because of the porous aspect of lignocellulosic biomass and the crystallinity of the cellulose, hemicellulose and lignin may block access of the enzymes (cellulases) to cellulose. Pretreat becomes a necessary step to transform cellulose into ethanol [10]. One of the pretreatments highlights the use of hot liquid water (hydrothermolysis). This technique uses high pressures (above the saturation point), thereby maintaining water in the liquid state at very high temperature. This allows an ecological and sustainable way of using the lignocellulosic material [8,11,12]. Obtaining the solubilized lignin fraction depends on the operating conditions and the raw material [13-15].

The aim of the research was to determine the best process settings for the chosen operating factors such as, pretreatment temperature, pretreatment time and liquid/solid ratio for the maximum solubilization of hemicellulose (reducing sugars content) and lignin (total phenolic compounds content) using Doehlert experimental design.

## MATERIALS AND METHODS

### Biological material

S.35 sorghum stems cultivar was purchased from the Institute of Research and Agronomic Development (IRAD) in the far north (Maroua) part of Cameroon.

### Sample preparation

S.35 sorghum stems were washed using distilled water and dried (24 h, 105°C) using a Heraeus oven (Heraeus kendro laboratory products, type: T6, fabrication N°20001046, Germany) to remove the undesired particles. In addition, sorghum stem was milled into a powder form with a knife mill (Retsch GM 200 GmbH, Retsch-Allee 1-542781 Haan, Germany) which is particularly suitable for grinding and homogenizing soft to medium-hard, elastic, fibrous, dry or wet materials. It was assisted by a Retsch sieving shaker AS 300 which permitted to obtain particle size less than 1 mm and, milled stem material was kept in a secured plastic container at 25°C until uses.

### Pretreatment process

Pretreatments was carried out in a stainless-steel laboratory scale cylindrical vessel of 200 mL, using a ventilated oven Memmert type (854 Schwabach, Germany) while, pretreatment temperature, time and liquid/solid ratio ranges were 150-210°C, 15-60 min, 10-30 mL/g respectively. The quantities of sorghum stem and water needed to obtain a suitable liquid/solid ratio were introduced in the reactor, and put in an oven. Once, the mixture was heated (in the oven) at the chosen temperature at the indicated time (Table 1). After treatment, the reactor was rapidly cooled with cooling water in order to achieve 25°C. After that, the mixture was filtrated through filter paper and, the liquid phase was analyzed for reducing sugars and total phenolic compounds content.

### Experimental design, modeling, validation of the model and optimization

Response surface methodology (RSM) using Doehlert design was executed to realise trials, in order to model and optimize

**Table 1:** Physicochemical characteristic of sorghum stem.

	Percentage (%)
Dry matter	93.31 ± 0.55
Water content	6.56 ± 0.70
Ash	6.11 ± 0.07
Crude fiber	60.97 ± 1.06
Cellulose	36.00 ± 1.41
Lignin	24.50 ± 0.71

the solubilization of hemicellulose and lignin. The individual variables (factors) were the temperature ( $x_1$ ), time ( $x_2$ ), and liquid solid ratio ( $x_3$ ). The intervals of these factors were:  $X_1$ , 150-210°C;  $X_2$ , 15-60 min;  $X_3$ , 10-30 mL/g. The responses were reducing sugars and total phenolic compounds content.

From the coded variables, many equations were used to transform them into real values to realize experiments in the laboratory. Those equations were as follow:

$$X_j = X_j^0 + x_j \Delta X_j \quad (1)$$

$$X_j^0 = \frac{X_j^{\max} + X_j^{\min}}{2} \quad (2)$$

Where:  $X_j$ : Real variables,  $X_j^0$ : Centre of variable,  $\Delta X_j$ : Increment,  $x_j$ : coded variables,  $X_j^{\max}$ : upper level of the real value a factor,  $X_j^{\min}$ : lower level of the real value a factor

The 3 factors Doehlert design had given a total of 16 experiments (with 4 replicates at the central point) as shown in Table 1.

The conversion of the matrix of the coded value in the experimental matrix is shown in Table 1 with, as answers, reducing sugars and total phenolic content. The relationship between the dependent and independent variables was constructed in the literature by a second order equation [16]. Experimental data obtained from the experimental design were correlated with the following second-order equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \dots + \sum \beta_{ii} x_i^2 + \beta_{ij\dots z} x_i x_j \dots x_z + e \quad (3)$$

With: Y, the response,  $x_i$  and  $x_j$  were the variables,  $\beta_0$  were the constant,  $\beta_i$  was the linear terms coefficients,  $\beta_{ii}$  was the quadratic terms coefficients,  $\beta_{ij}$  was the interactions terms coefficients and 'e' was random error.

The model coefficients of the statistics were generated using the Stat graphics Centurion 16 version 16.1.11 software (StatPoint technologies, Inc). The curves were realized using Sigmaplot version 12.5 (WPCubed, GmbH, Germany).

The adjustment of the second order multivariate polynomial equations was obtained by calculating the coefficient of determination  $R^2$ . The models were validated using two distinct

methods. The first method was the average absolute deviation (AAD) [17], and the second method was to calculate the polarization factor (Bf) and the accuracy factor (Af) [18]. The applied formulas were as follows:

$$AAD = \frac{\left[ \sum_{i=1}^N \left( \frac{|Y_{i,exp} - Y_{i,cal}|}{Y_{i,exp}} \right) \right]}{N} \quad (4)$$

$$B_f = 10^{\frac{1}{N} \sum_{i=1}^N \log \left( \frac{Y_{i,cal}}{Y_{i,exp}} \right)} \quad (5)$$

$$A_{f1} = 10^{\frac{1}{N} \sum_{i=1}^N \left| \log \left( \frac{Y_{i,cal}}{Y_{i,exp}} \right) \right|} \quad (6)$$

With:  $Y_{i,exp}$  and  $Y_{i,cal}$  were respectively experimental and theoretical responses. While N was the number of experiments used in the calculation.

Each linear, interaction and quadratic contribution of each factor was obtained as follow:

For linear terms,

$$Contribution (\%) = \frac{|\hat{a}_i|}{\sum_{i=1}^k |\hat{a}_i| + \sum_{i=1}^k |\hat{a}_{ii}| + \sum_{i<j}^k |\hat{a}_{ij}|} \quad (7)$$

For quadratic terms,

$$Contribution (\%) = \frac{|\hat{a}_{ii}|}{\sum_{i=1}^k |\hat{a}_i| + \sum_{i=1}^k |\hat{a}_{ii}| + \sum_{i<j}^k |\hat{a}_{ij}|} \quad (8)$$

For interaction terms,

$$Contribution (\%) = \frac{|\hat{a}_{ij}|}{\sum_{i=1}^k |\hat{a}_i| + \sum_{i=1}^k |\hat{a}_{ii}| + \sum_{i<j}^k |\hat{a}_{ij}|} \quad (9)$$

Lastly, models were optimized as stated in the literature [19]. The intersection of the curves, representing the optimal zone, was highlighted.

### Dry matter content

The dry matter content of sorghum stems was fixed by thermogravimetry which is the standard method for determining the water content of total solids described by AFNOR [20]. It consists of measuring the loss of mass of the samples after stoving at  $105 \pm 2^\circ\text{C}$  until complete elimination of free water and volatiles.

### Determination of ash content

The ash content was obtained by the AFNOR method [20]. It consisted of an incineration of a test sample after drying it at

$105^\circ\text{C}$ , to constant weight in a muffle furnace at  $550^\circ\text{C}$ . Minerals are the ash after incineration.

### Determination of crude fiber content

Crude fiber content of the samples was calculated by the method Weende [21]. This method involved treating the sample by boiling it for 30 min in sulfuric acid (0.25 N), filtered and then boiling again for 30 min with sodium hydroxide (0.31 N). After filtration, the residue obtained was dried at  $105^\circ\text{C}$  for 8h, then incinerated at  $550^\circ\text{C}$  for 3h and weighed.

### Determination of cellulose content

The cellulose content was established by a standard method [22]. It consisted in treating gram rod into a solution composed of a mixture of 15 mL of acetic acid at 80 % and 1.5 mL of concentrated nitric acid. The whole was heated to reflux for 20 min. After heating, the mixture was filtered and the obtained residue was washed with hot water and then dried at  $105^\circ\text{C}$  for 14 h and, finally weighed. The dry residue was incinerated at  $550^\circ\text{C}$  for 4 hours and then weighed again. Cellulose content was expressed in percentage of dry matter.

### Determination of lignin content

Lignin content was established by a standard method [23]. It consisted in proceeding with an acid hydrolysis of polysaccharides (cellulose and hemicellulose). At the end of hydrolysis, the dark residue which was obtained was lignin.

One gram of material was weighed and mixed with 15 mL of sulfuric acid (72 %) in a 100 mL beaker. The mixture was then placed in a water bath at  $30^\circ\text{C}$  for 3 h. After that, the mixture was filtered. The residue obtained was then boiled with 15 mL of sulfuric acid (4 %) for 1 h so as to complete the hydrolysis. At the end of boiling, the solution was filtered. The residue from the hydrolysis was dried till constant mass. The dried residue was incinerated at  $550^\circ\text{C}$  and then weighed. The lignin content was expressed as a percentage of dry matter.

### Reducing sugar content

The estimation of reducing sugar content in the liquid phase was established by the colorimetric method, using 3, 5-dinitrosalicylic acid (DNS reagent) [24].

Ten grams of sodium hydroxide were weighed and added to distilled water (700 mL) and, the mixture was stirred. After that, sodium potassium tartrate was incorporated and, all the content was agitated again. Then, ten

grams of 3, 5-dinitrosalicylic acid (DNS) were incorporated after total dissolution, with a continuous stirring of the solution, till the mixture became homogenous. Furthermore,  $\text{Na}_2\text{SO}_3$  (0.5 g) and phenol (2 g) was added and dissolved. Finally, distilled water was in addition to adjust the volume to 1000 mL and, after stirring for about 30 seconds, the DNS solution was kept in a dark container, away from light.

For analysis, 0.5 mL of the sample and 0.5 mL of DNS solution, was mixed in test tubes and, boiled for 10 min in a Memmert boiling water bath. Then, test tubes were chilled immediately to  $25^\circ\text{C}$  and, 5 mL of distilled water was incorporated. After

mixing the test tubes, the absorbance was measured at 540 nm. Standard curve using glucose was realized to determine sugar concentration.

### Total phenolic compounds

Total phenolic compounds, which were released during pretreatment, were estimated using a modified Folin-Ciocalteu method [25].

One milliliter of 1/10th dilute extract, 1 mL of a 17% (m/v) Na<sub>2</sub>CO<sub>3</sub> and 5 ml of Folin-Ciocalteu reagent (0.5 N) was put in a test tube and mixed. After that the tube was left for 30 min at 37°C in a Memmert water bath. The absorbance was read against a blank at 760 nm. The content of phenolic compounds was calculated as equivalents of Gallic acid by using the calibration curve.

### Establishment of equation between reducing sugars content, pretreatment temperature and severity factor

The severity factor is an approximate indication of the conditions of lignocellulosic biomass processing and whose reaction simulates a pseudo-first-order that combines the temperature treatment and time. In order to calculate the severity factor, the follow equation was used:

$$R_0 = \int \exp\left(\frac{T(t)-100}{14.75}\right) dt = t \times \exp\left(\frac{T(t)-100}{14.75}\right) \quad (10)$$

Since the severity factor was defined by  $\text{Log}_{10}(R_0)$  then, equation [10] was rewritten to:

$$\text{Log}_{10}(R_0) = \text{Log}_{10}\left(t \times \exp\left(\frac{T(t)-100}{14.75}\right)\right) \quad (11)$$

Using the response surface methodology permitted to establish a relation between processing parameters and responses. That relation was presented as a multivariable (3 variables) polynomial equation. By fixing the third variable (Liquid/solid ratio) constant, the polynomial equation was simplified to 2 variables one (time and temperature). Since the severity factor was time and temperature dependent, a model was designed to link the responses (reducing sugars and total polyphenols) to the severity factor and temperature pretreatment. After obtaining the model, it was traced using Sigmaplot version 12.5 (WPCubed, GmbH, Germany) in order to respect the shape.

## RESULTS AND DISCUSSION

### Sorghum physicochemical characterization

The physicochemical characteristics of sorghum stem (dry matter, water content, ash, cellulose, hemicellulose and lignin) have been completed and the results are shown in Table 1.

The dry matter content of sorghum stalks was 93.31 %. They were a little hydrated as they contained only 6.56 % of water. All cellulose and lignin represented by the staple fiber was 60.97 % of the dry matter. Cellulose was the major component in sorghum stalks (36 %), followed lignin (24.50 %) and ash content 6.11 %.

Crude fiber content was higher than the value of 49.47 % [26] obtained in the literature. This would be because the soil type, the genetic characteristics of the plant, the type of fertilizer, environmental conditions and climate affect the composition of plants. Cellulose, lignin and ash content of the stem were in agreement with the literature where, it was found 35 %, 19 % and 7.02 % respectively for the cellulose content, lignin and ash [27].

### Modeling

A three factors Doehlert experimental design (16 experiments) was carried out in order to estimate the impact of temperature, time and liquid/solid ratio on the solubilisation of hemicelluloses and lignin fractions during hydrothermolysis. The result of the solubilisation of these two fractions being reducing sugars content and total phenolic compounds content. Thus, at the end of each experiment the hydrolysate was gathered and analyzed. The results of these analyzes are given in Table 2.

Mathematical models obtained for reducing sugars and polyphenols after treatment were as follows:

$$y_{ThSR}(x_1, x_2, x_3) = 2.413 + 2.491x_1 + 1.525x_2 - 2.817x_3 + 0.889x_1x_2 - 1.069x_1x_3 - 2.579x_2x_3 + 1.252x_1^2 + 0.233x_2^2 + 3.583x_3^2 \quad (12)$$

$$y_{ThCP}(x_1, x_2, x_3) = 0.115 + 0.19x_1 + 0.033x_2 - 0.182x_3 + 0.04x_1x_2 - 0.082x_1x_3 - 0.116x_2x_3 + 0.08x_1^2 + 0.003x_2^2 + 0.194x_3^2 \quad (13)$$

With:  $y_{ThSR}(x_1, x_2, x_3)$  representing the mathematical model for reducing sugars;  $y_{ThCP}(x_1, x_2, x_3)$  representing the mathematical model for total phenolic compound;  $x_1$  temperature,  $x_2$  time and  $x_3$  liquid/solid ratio.

All the mathematical models were polynomial having several variables with determination coefficient of 0.975 and 0.990 (Table 3) respectively for reducing sugars and total polyphenols. These coefficients, linked to AAD value of 0.122 and 0.256 (Table 3) respectively (for reducing sugars and total polyphenols), obtained by calculation [17], allowed the validation of all models. Furthermore, bias factors of 1.018 and 1.045 (Table 3) linked to an accuracy factor of 1.124 and 1.244 (Table 3) respectively for reducing sugars and polyphenols, also permitted validation of models according to the literature [18]. The factors of the models appeared of first degree ( $x_1, x_2, x_3$ ), of second degree ( $x_1^2, x_2^2, x_3^2$ ) and of interaction form ( $x_1x_2, x_2x_3, x_1x_3$ ). Statistically, they were acknowledged significant or not when the probability (P) was  $\leq 0.05$  or  $\geq 0.05$  respectively.

### Singular and quadratic effects

**Effect of temperature:** The factor  $x_1$  corresponding to temperature, as sole factor, had significant impact on the reducing sugars and total phenolic compounds, with respective

**Table 2:** Doehlert experimental design: coded variables, real variables and responses.

N°	Factors						Responses					
	Coded variables			Real variables			Reducing sugars (mg/mL)			Total Phenolic compounds (mg/mL)		
	$x_1$	$x_2$	$x_3$	$X_1$	$X_2$	$X_3$	Exp	Cal	Res	Exp	Cal	Res
1	0	0	0	180	37.5	20	2.50 ± 0.10	2.41	0.09	0.10 ± 0.01	0.12	-0.02
2	1	0	0	210	37.5	20	6.38 ± 0.14	6.16	0.22	0.36 ± 0.05	0.39	-0.03
3	0.5	0.866	0	195	60	20	5.71 ± 0.13	5.85	-0.14	0.30 ± 0.03	0.28	0.02
4	-0.5	-0.866	0	165	15	20	0.86 ± 0.09	0.72	0.14	0.01 ± 0.00	0.03	-0.02
5	0.5	-0.866	0	195	15	20	1.74 ± 0.08	2.44	-0.70	0.20 ± 0.02	0.19	0.01
6	-0.5	0.866	0	165	60	20	3.29 ± 0.19	2.59	0.70	0.04 ± 0.00	0.05	-0.01
7	0.5	0.289	0.816	195	45	30	3.52 ± 0.16	3.60	-0.08	0.17 ± 0.02	0.17	0.00
8	-0.5	-0.289	-0.816	165	30	10	4.91 ± 0.08	4.83	0.08	0.25 ± 0.01	0.25	0.00
9	0.5	-0.289	-0.816	195	30	10	8.41 ± 0.29	7.93	0.48	0.51 ± 0.05	0.50	0.01
10	0	0.577	-0.816	180	52.5	10	8.71 ± 0.34	9.27	-0.56	0.46 ± 0.03	0.47	-0.01
11	-0.5	0.289	0.816	165	45	30	1.25 ± 0.07	1.73	-0.48	0.02 ± 0.00	0.03	-0.01
12	0	-0.577	0.816	180	22.5	30	3.47 ± 0.17	2.91	0.56	0.14 ± 0.01	0.13	0.01
13	0	0	0	180	37.5	20	2.48 ± 0.09	2.41	0.07	0.11 ± 0.01	0.12	-0.01
14	-1	0	0	150	37.5	20	0.95 ± 0.04	1.17	-0.22	0.03 ± 0.00	0.01	0.02
15	0	0	0	180	37.5	20	2.20 ± 0.00	2.41	-0.21	0.13 ± 0.01	0.12	0.01
16	0	0	0	180	37.5	20	2.47 ± 0.00	2.41	0.06	0.12 ± 0.01	0.12	0.00

**Abbreviations:** Res: Residue; Exp: Experimental; Cal: calculated (theoretical)

**Table 3:** Models validation data's.

Models	R <sup>2</sup>	AAD	B <sub>f</sub>	A <sub>f</sub>
$y_{ThSR} (x_1, x_2, x_3)$	0.975	0.122	1.018	1.124
$y_{ThCP} (x_1, x_2, x_3)$	0.990	0.256	1.045	1.244

**Table 4:** Estimated coefficients impact and contributions to the reducing sugars and total phenolic compounds.

Effects	Reducing sugars			Total phenolic compound		
	Coefficients	Probability	Contribution (%)	Coefficients	Probability	Contribution (%)
Constant	2.413	0.0000		0.115	0.0000	
$X_1$	2.491	0.0002	15	0.190	0.0000	21
$X_2$	1.525	0.0026	9	0.033	0.0320	3
$X_3$	-2.817	0.0001	17	-0.182	0.0000	20
$x_1^2$	1.252	0.0571	8	0.080	0.0083	9
$x_2^2$	0.233	0.6779	1	0.003	0.8773	0
$x_3^2$	3.583	0.0004	22	0.194	0.0001	21
$x_1x_2$	0.889	0.2574	5	0.040	0.1933	4
$x_2x_3$	-2.579	0.0176	16	-0.116	0.0096	13
$x_1x_3$	-1.069	0.2275	7	-0.082	0.0382	9

probabilities of 0.0002 and 0.0000 (Table 4). Its contributions were respectively 15 % and 21 % (Table 4). The impact of temperature on the reducing sugars and total phenolic content are shown in Figure 1. During the treatment, reducing sugars and polyphenols gradually increased from 2.78 mg/mL and 0.14 mg/mL respectively with increasing temperature to reach a maximum of 7.97 mg/mL for reducing sugars and 0.58 mg/mL for polyphenols at a temperature of 210°C.

This could be due for the reducing sugars by the fact that, at high temperatures (150°C-230°C) in water, the H bonds would weaken allowing the water auto ionisation in to  $H_3O^+$ , that would act then as catalysts. Another way would be the formation of hydronium ions using acetic acid mainly by uronic acid and acetyl groups. These acetyl groups that are present in the lignocellulose compounds are linked to hemicellulose. Hydration of these acetyl groups advantaged acidification of the medium and so the formation of these hydronium ions [13,28-31]. The consequence of acidification was the depolymerization of hemicellulose via selective hydrolysis of the glycosidic bonds of the oligosaccharides and monosaccharides.

For polyphenols, this is explained by the lignin that would be the aromatic molecule formed by the phenolic compounds. During hydrothermolysis, lignin-hemicellulose links would degrade through partial depolymerization and deep relocation [14, 32-36]. It was reported in the literature that the breakdown of lignin-hemicellulose bonds and partial depolymerization of lignin, produce some of the polyphenols present during

hydrothermolysis [14,37]. Using wood as raw material, the ether linkages would undergo a cleavage during hydrothermolysis process, due to an increase of the polyphenols content and a drop in molecular weight of lignin [38].

In its quadratic form ( $x_1^2$ ), temperature contributed to 8 % and 9 % (Table 4) respectively for reducing sugars and for polyphenols, the contributions were not significant for reducing sugar ( $P = 0.0571$ , Table 4) and was significant for polyphenols ( $P = 0.0083$ , Table 4).

**Effect of time:** The factor  $x_2$  corresponding to time, as sole factor, had significant impact on the reducing sugars and polyphenols, with respective probabilities of 0.0026 and 0.0320 (Table 4). Its contributions were respectively 9 % and 3 % (Table 4). That impact of time on the reducing sugars and polyphenols content are presented in Figure 2. During the treatment, reducing sugars and polyphenols increased from 2.78 mg/mL and 0.14 mg/mL respectively with increasing time to attain maximum level respectively of 7.53 mg/mL and 0.29 mg/mL at 60 min.

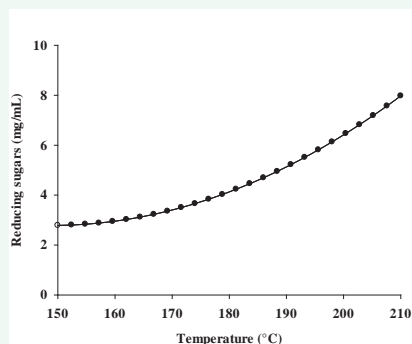
It could be explained for reducing sugars by the fact that, the impact of  $H_3O^+$  from acetic acid was higher than that from water autoionisation [39]. The effect of the autoionisation of water was limited at the initial stages of the reaction. Thus, the more time increased the more the hydronium ions produced from acetic acid would catalyze the hydrolysis of the hemicellulosic fraction, which would contribute to increase the reducing sugars content in the medium.

For the polyphenols, the observed phenomenon may be explained by the fact that the mechanisms of hydrothermal processes are similar to those of the dilute acid hydrolysis because they are both hydronium ions ( $H_3O^+$ ) catalyzed hydrolytic process. During the hydrothermolysis, water is the only chemical compound added to the substrate. The autoionisation of the water generated hydrolysis catalysts are hydrogen ions. These led to the depolymerization of lignin via selective hydrolysis of glycosidic, ether and ester linkages present in lignin-carbohydrates complexes. After, the hydronium ions produced by the acetic acid, would act as a catalyst, accelerating the reaction kinetics.

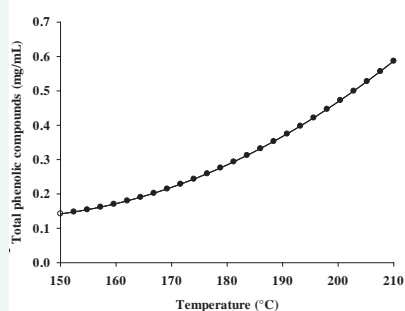
The time in his second degree ( $x_2^2$ ) had no significant impact for reducing sugars and polyphenols with respective probabilities of 0.6779 and 0.8773 (Table 4), its contribution was 1 % and 0 % (Table 4) respectively.

**Effect of liquid/solid ratio:** The factor  $x_3$  corresponding to liquid/solid ratio, as sole factor, had significant impact on reducing sugars and polyphenols, with respective probabilities of 0.0001 and 0.0000 (Table 4). Its contribution was respectively 17 % and 20 % (Table 4). The impact of liquid/solid ratio on reducing sugars and polyphenols content are shown in Figure 3. During the process, there was a progressive and respective decrease of reducing sugars and polyphenols from 2.78 mg/mL and 0.14 mg/mL to 1.39 mg/mL and 0.01 mg/mL at about 20 mL/g. This was followed by a small and steady increase up to 3.57 mg/mL and 0.14 mg/mL respectively at a liquid/solid ratio of 30 mL/g.

It could be due for reducing sugars, to a selective solubilisation of the external part of the stems, followed by a degradation of



**Figure 1a** Effect of temperature on reducing sugars content (time, 15 min and liquid/solid ratio, 10 mL/g).



**Figure 1b** Effect of temperature on total phenolic compounds content (time, 15 min and liquid/solid ratio, 10 mL/g).

reducing sugars to furfural and hydroxymethylfurfural [40,41]. The increase in reducing sugars content could be explained by the fact that increasing of liquid/solid ratio involved a uniform solubilisation of the hemicellulosic fraction thus producing reducing sugars. This production of reducing sugars would thus prevail on their degradation. An increase of liquid/solid ratio would be essential in order to efficiently solubilize hemicellulose.

Concerning the total polyphenols, the liquid / solid ratio would have strongly impacted on the heat and hydronium ions ( $H_3O^+$ ) capacities to attack the lignocellulosic materials, thus inducing a heterogeneous material processing. This treatment would produce the preferential degradation of the outer side of lignocellulosic material, minimizing the impact of the degradation in the interior [42]. In acidic medium, the intermediate carbonium ion would have been produced with high affinity for the nucleophiles in the lignin complex [43]. Hydrolysis would have taken advantage on the depolymerization, even though reactions between the ions and carbonium nucleophilic would have taken the advantage on repolymerisation or condensation [44,45]. Proof of depolymerization while pretreating would be the loss of  $\beta$ -O-4 linkages [38,45] and a reduction in the molecular weight of the lignin during prolonged pretreatment [46,47]. Bigger cleavage of  $\beta$ -O-4 bonds without great amount of lignin monomers would suggest depolymerization followed by repolymerisation [48].

The impact of liquid/solid ratio in its quadratic form ( $X_3^2$ ), was statistically significant on both reducing sugars and polyphenols with respective probabilities of 0.0004 and 0.0001 (Table 4), with respective contribution of 22% and 21% (Table 4).

The impact of liquid/solid ratio in its quadratic form ( $X_3^2$ ), was statistically significant on both reducing sugars and polyphenols with respective probabilities of 0.0004 and 0.0001 (Table 4), with respective contribution of 22% and 21% (Table 4).

### Effect of interactions

Models were further exploited to predict the impacts of interactions ( $X_1X_2, X_2X_3, X_1X_3$ ) on reducing sugars and total polyphenols.

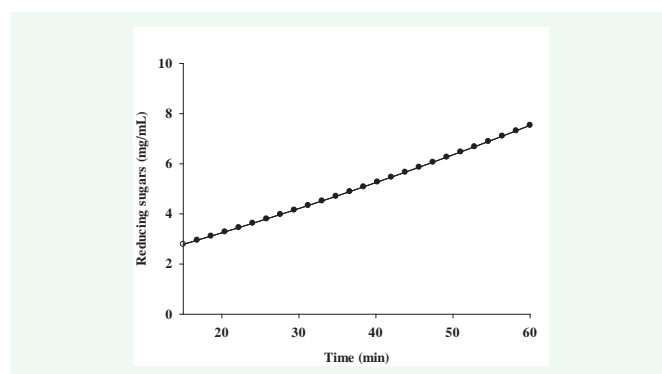
The interaction  $X_2X_3$  (Time/Liquid/solid ratio) had significant impact on reducing sugars content, with a probability of 0.0176 (Table 4) and a contribution of 16 % (Table 4). While the interactions  $X_2X_3$  and  $X_1X_3$  (Time/Liquid/solid ratio and Temperature/Liquid/solid ratio respectively) had significant impact on the total phenolic compounds content, with respective probabilities of 0.0096 and 0.0382 (Table 4). The contributions were 13 and 9% (Table 4) respectively.

**On reducing sugars:** Figure 4 showed the impact of the interaction  $X_2X_3$  (Time/Liquid/solid ratio) on the reducing sugars content at the temperature of 150°C. At the minimum time value (15min) and when varying the ratio from 10 mL/g to 30 mL/g, there was a decrease of the sugars content from 2.78 mg/mL to 0.79 mg/mL at about 20 mL/g and subsequently an increase in reducing sugars content from 0.79 mg/mL to 3.57 mg/

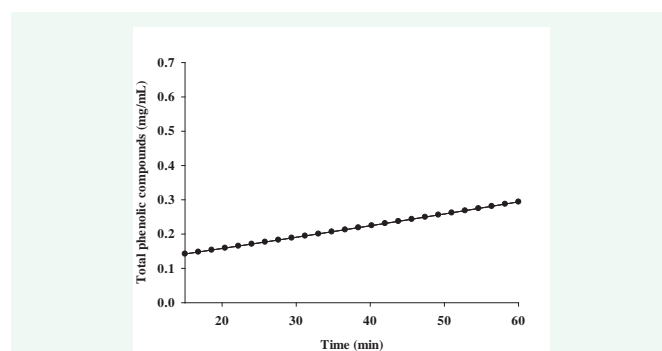
mL. This result could be interpreted by the fact that the liquid/solid ratio influenced the water absorption capacity, which led to the swelling of the fibres and increased the surface area by increasing the volume of the rods, improving the accessibility of acids generated during treatment. For low liquid/solid ratios, this accessibility of acidic catalysts would be unevenly distributed by causing a partial hydrolysis of the hemicellulose fraction followed by a breakdown of sugars. In addition, at the maximum value time (60 min), and when varied the ratio from 10 mL/g to 30 mL/g, there was a decrease in the reducing sugars content of 7.53 than 1.03 mg/mL. This could be because the acid catalyst generated during the treatment would cause sugars degradation.

**On total polyphenol content:** Figure 5a showed the impact of the interaction  $X_1X_3$  (Temperature/Liquid/solid ratio) on the total polyphenol content at 15 min. At the minimum temperature value (150°C), a variation of the ratio from 10 mL/g to 30 mL/g, induced a decrease in the content of total phenolic compounds from 0.14 mg/mL to 0.01 mg/mL was obtained, at about 20 mL/g. Thereafter an increase in the content of total phenolic compounds was observed from 0.01 mg/mL to 0.14 mg/mL. In addition, at the maximum temperature value (210°C), and when varying the ratio from 10 mL/g to 30 mL/g, there was a decrease in the content of total phenolic compounds from 0.58 mg/mL to 0.31 mg/mL.

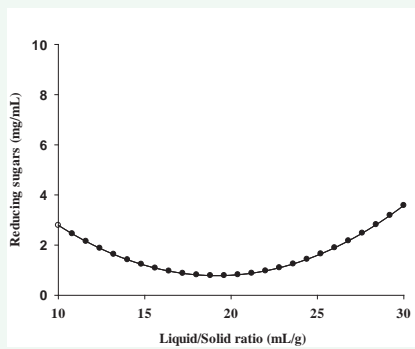
Figure 5b showed the effect of the interaction  $X_2X_3$  (Time/Liquid/solid ratio) on the total polyphenol content at 210°C. At



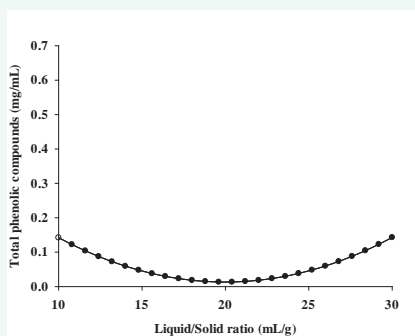
**Figure 2a** Effect of time on reducing sugars content (temperature, 150°C and liquid/solid ratio, 10 mL/g).



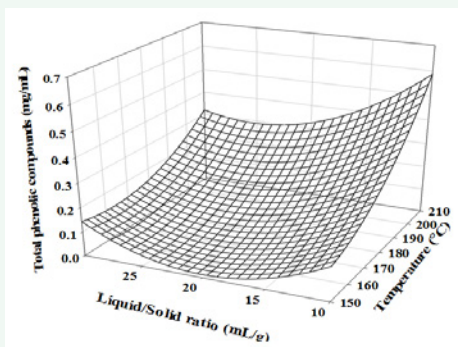
**Figure 2b** Effect of time on total phenolic compounds content (temperature, 150°C and liquid/solid ratio, 10 mL/g).



**Figure 3a** Effect of liquid/solid ratio on reducing sugars content (temperature, 150°C and time, 15 min).



**Figure 3b** Effect of liquid/solid ratio on total phenolic compounds content (temperature, 150°C and time, 15 min).



**Figure 4** Effect of interaction  $x_2x_3$  (Time/Liquid/solid ratio) on the reducing sugars content (temperature, 150°C).

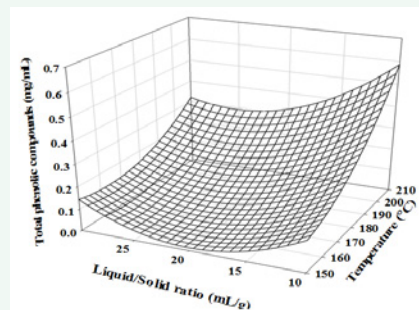
the minimum time value of 15 min and when varying the ratio from 10 mL/g to 30 mL/g, a decrease in the total polyphenol content from 0.58 mg/mL to 0.29 mg/mL was observed at about 25 mL/g, and subsequently increasing the total polyphenol content from 0.29 mg/mL to 0.32 mg/mL. In addition, at the maximum time value (60 min), and when varying the ratio from 10 mL/g to 30 mL/g, there was a decrease in the total polyphenol content from 0.88 mg/mL to 0.28 mg/mL.

The combined action of  $x_2x_3$  (temperature/liquid/solid ratio) and  $x_1x_3$  (time/liquid/solid ratio) generally resulted in a drop in total phenolic content. These observations were due to

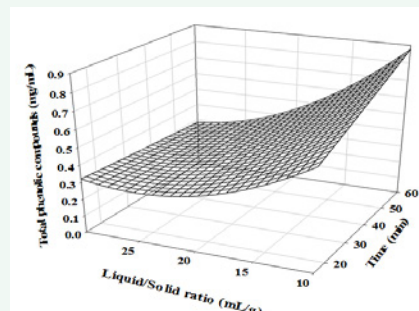
concomitant reactions of depolymerisation and repolymerisation of lignin [49,50].

### Optimization

After modeling and understanding the impact of the factors on the reducing sugars and total phenolic compound content. Optimization was done to define satisfactory domains of compromise for depolymerization of hemicellulose and lignin that result in the maximum reducing sugars and phenolic compounds. These domains were obtained for a reducing sugars content  $\geq 14$  mg/mL and total phenolic compounds content  $\geq 0.8$  mg/mL. The theoretical optimal combination of factors gave the following triplet for reducing sugars of 210°C, 60 min and 11 mL/g for temperature, time and liquid/solid ratio respectively. This triplet allowed for a maximal reducing sugar of 15.16 mg/mL. The theoretical optimal combination of factors for maximal contribution of total phenolic compounds gave as triplet of 210°C, 48 min, and 10 mL/g for temperature, time and liquid/solid ratio respectively. This triplet allowed for a maximal total phenolic compound of 0.81mg/mL. Taking in to account the theoretical combinations, the value of liquid/solid ratio was fixed at -0.816 meaning 10 mL/g. The contours plots were superimposed and the domain (reducing sugars content  $\geq 14$  mg/mL and total phenolic compounds content  $\geq 0.8$  mg/mL) was obtained as shown in Figure 6. Every combination falling in the highlighted zone respected the constraints.



**Figure 5a** Effect of the interaction  $x_2x_3$  (Temperature/Liquid/solid ratio) on the total phenolic compounds (time, 15 min).



**Figure 5b** Effect of the interaction  $x_2x_3$  (Time/Liquid/solid ratio) on the total phenolic compounds (temperature, 210°C).



### Relationship between responses, severity factor and pretreatment temperature

The model linking the response to the factors was defined by the equation 3. By fixing the third parameter (Liquid/Solid ratio), that equation (3) can be transformed and rewritten as follow:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3 X_1X_2 + a_4 X_1^2 + a_5 X_2^2 \quad (14)$$

The severity factor equation can be rewritten,

$$\text{Log}_{10}(R_0) = \text{Log}_{10}\left(X_2 \exp\left(\frac{X_1 - 100}{14.75}\right)\right) \quad (15)$$

If  $\text{Log}_{10}(R_0) = R$ , then equation (15) can be rewritten,

$$R = \text{Log}_{10}\left(X_2 \exp\left(\frac{X_1 - 100}{14.75}\right)\right) \quad (16)$$

From the equation (16),  $X_2 = \frac{10^R}{\exp\left(\frac{X_1 - 100}{14.75}\right)}$  (17)

That expression ( $X_2$ ) introduced in equation (14) gave equation (18) as follows:

$$Y = a_0 + a_1X_1 + a_2\left(\frac{10^R}{\exp\left(\frac{X_1 - 100}{14.75}\right)}\right) + a_{12}X_1 \left(\frac{10^R}{\exp\left(\frac{X_1 - 100}{14.75}\right)}\right) + a_{11}X_1^2 + a_{22}\left(\frac{10^R}{\exp\left(\frac{X_1 - 100}{14.75}\right)}\right)^2 \quad (18)$$

After transforming the equations (12) and (13) into real variables models (equation (14)), and after fixing the liquid/solid ratio to the minimum, the followings were obtained:

$$Y_{ThSR} = 1.39 \times 10^{-3} X_1^2 - 80.52 \frac{10^R}{e^{0.0678X_1}} - 0.43X_1 + 267.33 \frac{10^{2R}}{e^{0.1356X_1}} + X_1 \frac{10^R}{e^{0.0678X_1}} + 34.93 \quad (19)$$

$$Y_{ThCP} = 9 \times 10^{-5} \times X_1^2 - 4.09 \frac{10^R}{e^{0.0678X_1}} - 0.0253 \times X_1 + 3.44 \frac{10^{2R}}{e^{0.1356X_1}} + 0.045 \times X_1 \frac{10^R}{e^{0.0678X_1}} + 1.89 \quad (20)$$

After obtaining the equations, curves were plotted using Sigmaplot v12.5 and the curves were presented on Figure 7a and Figure 7b, where  $X_1$  is the temperature and R the severity factor

In Figure 7a and Figure 7b, it was observed that, at a fixed

temperature and, when increasing the severity factor, there was an exponential increase of reducing sugars and polyphenols in the liquor. That exponential shape stopped when increasing the temperature for reducing sugar but, for polyphenols, at higher severity factor, it was observed a decrease when increasing the temperature. While, for low severity factor it is noted with an increase of temperature, an exponential increase of both reducing sugars and polyphenols. The same increase was observed in the literature when pretreating corn stover and poplar wood [51-53]. It was finally observed from Figure 7b that, the best way of remove lignin during pretreatment of sorghum stem was, to execute lower temperature with long reaction time (higher severity).

### CONCLUSION

The study proposed to assess the impact of the hydrothermolysis treatment on the hemicellulosic and lignin fractions solubilization of S.35 sorghum stems in order to allow a better enzymatic hydrolysis of the cellulose fraction. The effects of three factors (temperature, time and liquid/solid ratio) on reducing sugars and phenolic compounds were studied during hydrothermolysis using Doehlert experimental design. Reducing sugars and phenolic compounds content during hydrothermolysis of S.35 sorghum stems was influenced by temperature, time

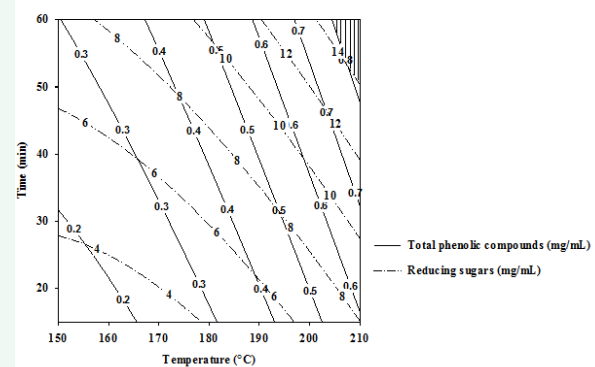


Figure 6 Response surface curves for the factor combinations providing for optimal results of reducing sugars and total phenolic compounds (Liquid/Solid ratio, 10 mL/g).

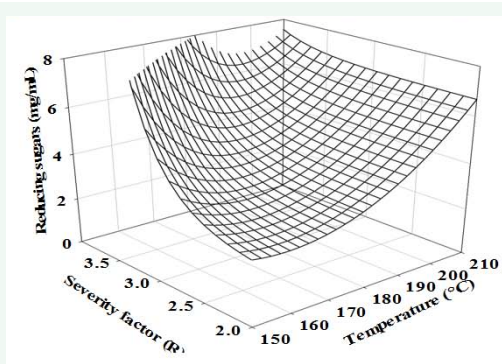
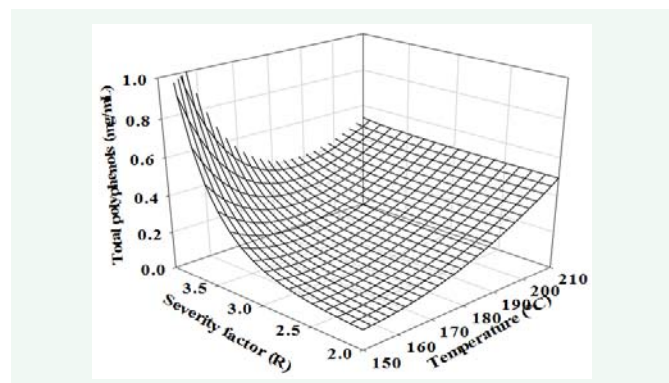


Figure 7a Impact of severity factor and temperature on reducing sugars production.



**Figure 7b** Impact of severity factor and temperature on total polyphenols production.

and liquid/solid ratio. The best treatment case that developed the maximum reducing sugars and phenolic compounds were determined. The lignin removal was studied and was much more time dependent than temperature dependent.

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## CONFLICT OF INTEREST

Authors are members of the University of Ngaoundere as student and as lecturers and they have a financial relationship with National School of Agro-Industrial Sciences (ENSAI) of the University of Ngaoundere. The authors declare that they have no conflict of interest.

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