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OPTIMIZATION OF PROCESS PARAMETERS FOR THE ALKALI TREATMENT OF ALPHA CELLULOSE FROM GROUNDNUT HUSK (ARACHIS HYPOGAEA)

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ABSTRACT: *Microcrystalline cellulose (MCC) is described as purified, partially depolymerised cellulose prepared by treating α-cellulose, obtained as a pulp from fibrous plant with mineral acids. Groundnut husk of 1.00mm particle size was selected for the alkali treatment using sodium hydroxide. Response Surface Methodology (RSM) was used to optimize the extraction of alpha cellulose from groundnut husk (Arachis hypogaea) a specie of Samnut 10, it was treated using alkali method using different concentration of NaOH. The independent variables include time, concentration and temperature were optimized using central composite design (CCD). The Analysis of variance (ANOVA) showed that temperature was the most influential factor for the extraction of alpha cellulose. Under optimal conditions; temperature at 80^0C, time at 1h and concentration ratio of 0.90, the percentage yield and percentage purity of the alpha cellulose obtained were 76.44% and 40.89% respectively. The theoretical values for the percentage yield of the extracted alpha cellulose were close to the experimental one, resulting in small error percentages of 2.73% and 0.187%, respectively. Thus, it can be concluded that the RSM technique based on CCD design is suitable for optimizing the variables influencing the extraction of alpha cellulose.*

KEY WORDS: Groundnut husk (GH), microcrystalline cellulose (MCC), groundnut husk (Arachis hypogaea)

INTRODUCTION

Microcrystalline cellulose is a purified, partially depolymerised non-fibrous form of cellulose. It is widely use in cosmetic, pharmaceutical and food industry as fat substitute, stabilizer, thickener, filler-binder, anticaking agent and adsorbent (*Matrosovich et al*, 2006) are used for counting viruses, as an alternative to carboxymethyl cellulose (Hindi, 2016). Microcrystalline cellulose are obtained from both softwood and hardwood which has different chemical composition (cellulose, hemicelluloses, and lignin) and structure. Sources of cellulose and its derivatives obtained from Agricultural waste are: cotton linters, orange peel, rice husk, corn cob, groundnut husk, sugar cane bagasse, calabash, bark of palm nut trees (Chukwuemeka, 2012).

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The chemical composition of groundnut husk is 14.7- 18.7% of hemicellulose, 37.5 - 40.5% cellulose, 26.4 - 30.2% lignin and 0.4 – 5.9% of ash contents (Lakshunmu, 2013).

The process of extraction of microcrystalline cellulose utilizes the removal of hemicellulose and lignin which constitutes the matrix for cellulose micro fibrils. The isolation and characterization of microcrystalline cellulose from different sources like corn stalk (Reddy et al. 2005), rice husk (Reddy and Yang, 2005; Hanani et al., 2017), wood (Orts, 2005), sugar cane bagasse (Sun, 2004), cotton (Ohwoavworhua et al. 2005; Li *et al.*, 2019), coconut husk (Rosa, 2010), rice husk, sugar cane bagasse, corn cob, and cotton (John et al. 2011), sugarcane bagasse, rice straw, durra stalk and groundnut shell (Mohammed et al., 2015), Bamboo fiber (Lalduhsanga et al., 2013), oil palm (Loo et al., 2016), groundnut husk (Ohwoavworhua et al., 2009; Chukwuemeka et al., 2012; Rani et al., 2016) etc. have been previously reported.

However, some of the researchers investigated the optimum processing conditions for obtaining the maximum yield of microcrystalline in some of the agricultural waste such as orange peel waste, corn cob using Response Surface Methodology (RSM) and selecting Central Composite Design (CCD). Sources like corn cob (SIrikalaya et al., 2013), palm tree trunk (Hamid et al., 2014), orange peel waste (Akhabue et al., 2017), coconut (Rahman et al., (2018),

However, the optimization of alpha cellulose from groundnut husk has not been reported. Therefore, this work aim to study the effect of process variables on the desired responses of the percentages yield and purity of MCC-GH using Response surface methodology (RSM) to develop polynomial mathematical model. Comprehensive experimental layout or design matrix for data assessment and model simulation studies with optimization of the process was applied. Statistical significance of the experimental observations shall be investigated in terms of Analysis of Variance (ANOVA). In this work, the optimal size of (1.00mm) that was established in the previous investigation by the same researcher was used for the RSM studies and incorporated into the DOE using a central composite design to input the variables within a low and upper limits. Hence, this research studies investigated the optimization of process parameters for the alkali treatment of alpha cellulose from groundnut husk (Arachis hypogaea)

METHODOLOGY

Materials

Groundnut husk was obtained from Institute of Agricultural Research A.B.U Zaria, Nigeria and the specie and type of the groundnut husk was identified in Biological Sciences Department and Biochemical Laboratory as the raw material. The chemicals used were NaOH

(BDH England), Ethanol, Nitric Acid, NaCLO. The result is shown in Table 1, all chemicals used are of reagent.

Proximate Analysis:
1 g of the untreated groundnut husk was used to examine the percentages of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) to calculate percentages of cellulose, hemicellulose, lignin and ash content as shown in Table 2.

Production of Alpha Cellulose
Groundnut husk was washed with distilled water and dried for three days at room temperature. The dried groundnut husk was ball milled and sieved to obtain 1.00mm particle size. The groundnut husk were measured for 25g and treated with Sodium Hydroxide (750 ml distilled H_2O, 0.25- 0.90 g/ml of NaOH) for twenty runs. The factors obtained from the Central Composite Design (CCD) as presented in Table 3 were the temperature, time and concentration that were used to optimize the process. The dark slurry obtained was then filtered and washed several times with distilled water until pH 7 was recorded and then oven dried at 70^0C for 2hrs. Finally, the dried alpha cellulose was stored in plastic containers. The responses of the percentage yield of the extracted alpha cellulose and the percentage purity were recorded as shown in Table 3.

Percentage Yield
Percentage yield was calculated using the following equation:

Percent yield (%) = $\frac{W3}{W1}$ X 100 .....Eq (1)

Where:
W3 is the weight of oven dried lignin free (pure cellulose) sample.
W1 is the weight of oven dried raw sample.

Fourier Transform Infrared (FTIR) Spectroscopy
FTIR analysis was carried out using Shimadzu FTIR-8400S Japan spectrometer with scanning ranges of 4000.0 cm^{-1} to 500.0 cm^{-1} using Kbr pellet.

Bulk and Tap Densities
A 1.3 g quantity each of the powder samples was placed in a 50 ml clean dry measuring cylinder and the volume, V_o occupied by each of the samples without tapping was determined. After 100 taps occupied volumes, V_{100}, were determined. The bulk and tap densities were calculated as the ratio of weight to volume (V_0 and V_{100} respectively).

True Density

The true densities (Dt), of cellulose powders were determined by the liquid displacement method using xylene and pycometer. 0.5 g quantity of cellulose powder was placed in a dry pre weighed pycometer and the rest filled with 50 ml xylene (SG 0.86) as the immersion fluid, the weight of the pycometer filled with only liquid has previously been established and density of the powder was computed according to the following equation:

Dt = w [(a + w)-b] x SG.....Eq (2)

Where w is the weight of powder, SG is specific gravity of solvent, a is weight of bottle + solvent and b is weight of bottle + solvent + powder

The Carr's index and Hausner's ratio

These were determined from the values of the bulk and tapped densities results obtained above.

$$\text{Carr's Index} = \frac{Dtap - Dbulk}{Dtap} X\ 100 \quad Eq\ (3)$$

$$\text{Hausner's Ratio} = \frac{Dtap}{Dbulk} \quad Eq\ (4)$$

Powder Porosity

This was derived from the values of true and bulk densities when fitted into the equation:

$$e = 1 - \frac{Dtap}{Dtrue} X\ 100 \quad Eq\ (5)$$

Where D_{tap} is the tapped density, D_{true} is the true density and e is the Porosity. (chukwuemeka *et al.*, 2012)

pH determination:

1 g of the powder material was shaken with 50 ml of distilled water for 5 min and the pH of the supernatant liquid was determined using a pH meter.

Angle of repose

To obtain the angle of repose a long cylindrical tube, open at both ends, was used. The tube was perpendicularly placed on a clean cardboard paper and filled flat with the cellulose powder. The tube was then gradually lifted away, vertically, from the cardboard. The height *h*, and radius *r*, of the conical heap formed were measured, and then the angle of repose, θ,

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was calculated. Determination was done in triplicate and the average taken (Ohwoavworhua,*et al.*. 2004).

$$\theta = \tan^{-1}(h / r) \quad \text{Eq (6)}$$

SEM

Particle morphology of the MCC samples was studied by using China JSM 7500 field SEM.

X-ray diffraction Analysis (XRD)

X-ray diffraction patterns of the cellulose samples were obtained using an X-ray diffractometer (Philips X-ray Analytical). Samples for analysis were prepared by pressing the powder into the cavity of a sample holder and smoothing with a glass slide. They were scanned from 5-400 2q with a Cu anode X-ray operated at 40 kV and 40 mA in combination with a Ni filter to give a monochromatic Cu-Kα radiation (l = 1.5418 Å). The X-ray beam is directed at a sample and measuring the scattered intensity as a function of the outgoing directions. Once the beam is separated, the scatter also called a diffraction pattern, indicates the sample's crystalline structure. The crystallinity index (CI) was calculated using Equation 3.7, as proposed by Rani et al (2016)

$$CI = \frac{I_{002} - I_{AM}}{I_{002}} X\ 10 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots Eq\ (7)$$

where:
I_{002} = is the intensity of the peak (002) lattice diffraction at ($2\theta = 16 - 18^0$)
I_{AM} = the Iam is the intensity diffraction at (2θ degrees = $20\text{-}26^0$)

RESULTS AND DISCUSSION

Groundnut Husk Identification

The groundnut husk was identified and Table present the result.

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Table 1: The Identification of the untreated Groundnut husk

Kingdom:	Plantae
Genus:	*Arachis*
Species:	*Samnut 10*
Binomial name	*Arachis hypogaea*

Proximate Analysis

The Chemical composition of the untreated groundnut husk was investigated using a gravimetric method to determine the proximate properties. Table 2.0 presents the results obtained.

Table 2: Proximate Properties of Untreated Groundnut Husk

S/No.	Parameter	Value (%)
1	Ash	6.1
2	Hemicellulose	9.48
3	Lignin	35.78
4	Alpha Cellulose	44.16
5	Moisture Content	2.3

Actual Design of the Experiment

Research Surface Methodology was employed to maximize the production of alpha cellulose from groundnut husk by the optimization of the process conditions. The interaction among process variables was determined by statistical techniques. Central composite design was employed to investigate the effect of critical parameters i.e; temperature, time, and concentration of the process on the groundnut husk. The three variables and their value range were selected based on preliminary studied. Three dimensional surface were applied to investigate and validate the influence on process variables on the treatment of alpha cellulose.

The reaction time (1-2h), temperature (80 - 98^{0}C) and sodium hydroxide concentration (0.25 - 0.90 g/ml) were the input variables as shown in Table 3.

Table 3: Summary of Factors input of the Upper and Lower Limits for Alpha Cellulose

Constraints						
		Lower	**Upper**	**Lower**	**Upper**	
Name	**Goal**	**Limit**	**Limit**	**Weight**	**Weight**	**Importance**
A:Time (h)	is in range	1	2	1	1	3
B:Tempt (^{0}C)	is in range	80	98	1	1	3
C:Conc (ratio)	is in range	0.25	0.9	1	1	3
Percentage yield (%)						
Percentage response (%)						

The alkali treatment of the groundnut husk was carried out using Central Composite Design (CCD) and the responses of percentage yield and purity of the alpha cellulose are presented in Table 4.

Table 4: The Actual Design of the Experiment and Results of Percentage Yield and Percentage Purity of the Alpha Cellulose

ACTUAL DESIGN						
Std	**Run**	**Factor 1 A:Time (hr)**	**Factor 2 B:Tempt (0C)**	**Factor 3 C: Conc (ml/750)**	**Response 1 Percentage MCC Yield (%)**	**Response 2 Percentage MCC Purity (%)**
9	1	0.66	89	0.575	73	45.45
16	2	1.5	89	0.575	70	45.66
18	3	1.5	89	0.575	72.19	37.65
19	4	1.5	89	0.575	74	50.93
3	5	1	98	0.25	73	47.80
10	6	2.34	89	0.575	68	47.86
17	7	1.5	89	0.575	72	46.60
7	8	1	98	0.9	70	35.85
6	9	2	80	0.9	75	44.98
2	10	2	80	0.25	70	43.57
8	11	2	98	0.9	75	53.20
4	12	2	98	0.25	74	44.65
5	13	1	80	0.9	80	43.30
12	14	1.5	104	0.575	76	38.08
13	15	1.5	89	0.284	74	49.32
14	16	1.5	89	1.122	76	43.35
1	17	1	80	0.25	78	45.68
11	18	1.5	73.86	0.575	75	39.30
15	19	1.5	89	0.575	73	43.03
20	20	1.5	89	0.575	73	41.07

Percentage Yield of Alpha Cellulose

Table 5 shows the statistical analysis of the response of the percentage yield of alpha cellulose after hydrolysis treatment. The results indicated an extensive extraction of alpha cellulose using equation 1 and the response recorded. These values were feed into the design of experiment (DOE) using central composite design (CCD) to compute for the results and the model equation of the ANOVA shows that the model is significant having the probability value of 0.0027 which is less than 0.0500.The time represented by (A), concentration (B) and concentration (C) are the factors of interest for the model equation. However in this case A,

AB, B^2, C^2 are significant model terms. This implies that the proposed model fit the experimental data and the independent variables or parameters has considerable effects on the response as shown in Table 5.

Table 5: Statistical ANOVA Analysis of Response for the Percentage Yield of Alpha Cellulose

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	133.26	9	14.81	7.01	0.0027	Significant
A-Time	*17.39*	*1*	*17.39*	*8.23*	*0.0167*	
B-Tempt	*6.36*	*1*	*6.36*	*3.01*	*0.1135*	
C-Conc	*5.12*	*1*	*5.12*	*2.42*	*0.1506*	
AB	*45.13*	*1*	*45.13*	*21.35*	*0.0009*	
AC	*6.13*	*1*	*6.13*	*2.90*	*0.1195*	
BC	*10.12*	*1*	*10.12*	*4.79*	*0.0535*	
A^2	*4.00*	*1*	*4.00*	*1.89*	*0.1992*	
B^2	*22.20*	*1*	*22.20*	*10.50*	*0.0089*	
C^2	*16.33*	*1*	*16.33*	*7.73*	*0.0195*	
Residual	21.14	10	2.11			
Lack of Fit	*11.90*	*5*	*2.38*	*1.29*	*0.3939*	*not significant*
Pure Error	*9.24*	*5*	*1.85*			
Cor Total	154.40	19				

Std.Dev--------- 1.45 R-Square ---------- 0.8631
Mean --------- 73.56 Adj-Square ---------- 0.7399
C.V. % --------- 1.98 Pred R-Square --------- 0.3276
PRESS --------- 103.82 Adeq Precision -------- 10.196

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The Model Equation

Percentage Yield = 72.34
$-1.13A - 0.68B + 0.61C + 2.38AB + 0.88AC - 1.12BC - 0.53A^2 + 1.24B^2 + 1.06C^2$..... (8)

Percentage Purity of Alpha Cellulose

Proximate Analysis method was used to obtain the response for the percentage purity of alpha cellulose. The response was computed using ANOVA and presented in Table 6. The p-value is 0.2433 which is greater than 0.050 indicating a non-significant model. This means that the independent variables; time, concentration and temperature has no considerable effect on the response.

Table 6: Statistical ANOVA Analysis of Response for the Percentage Purity of Alpha Cellulose

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	221.81	9	24.65	1.58	0.2433	not significant
A-Time	*23.26*	*1*	*23.26*	*1.49*	*0.2503*	
B-Tempt	*0.27*	*1*	*0.27*	*0.017*	*0.8981*	
C-Conc	*15.21*	*1*	*15.21*	*0.97*	*0.3470*	
AB	*26.75*	*1*	*26.75*	*1.71*	*0.2198*	
AC	*73.75*	*1*	*73.75*	*4.72*	*0.0549*	
BC	*0.74*	*1*	*0.74*	*0.047*	*0.8323*	
A^2	*17.27*	*1*	*17.27*	*1.11*	*0.3177*	
B^2	*42.71*	*1*	*42.71*	*2.74*	*0.1292*	
C^2	*13.88*	*1*	*13.88*	*0.89*	*0.3679*	
Residual	156.14	10	15.61			
Lack of Fit	*48.90*	*5*	*9.78*	*0.46*	*0.7955*	*not significant*
Pure Error	*107.24*	*5*	*21.45*			
Cor Total	377.95	19				

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Std.Dev--------- 3.95 R-Square ---------- 0.5869
Mean --------- 44.37 Adj-Square ---------- 0.2151
C.V. % --------- 8.91 Pred R-Square --------- -0.5360
PRESS --------- 580.52 Adeq Precision -------- 4.417

The Model Equation

Percentage Purity = 44.12
$1.13A + 0.14B - 1.06C + 1.83AB + 3.04AC - 0.30BC + 1.09A^2 - 1.72B^2 + 0.98C^2$..... (9)

Plots on Percentage Yield and Percentage Purity of the Alpha Cellulose

The plots presents the interaction between the process parameters; Time, Temperature and Concentration on the percentage yield and percentage purity of the alpha cellulose.

The Perturbation plot in figure 1a shows that at Point A (time) decreases while points B (temperature) and C (concentration) increases. However, at deviation from reference point 0.00 (coded units) both Point A, B and C coincided to give optimal percentage yield of 72.6% of alpha cellulose shown in Figure 1a. Similarly, the optimal percentage purity of the alpha cellulose is 44% as shown in figure 1b.

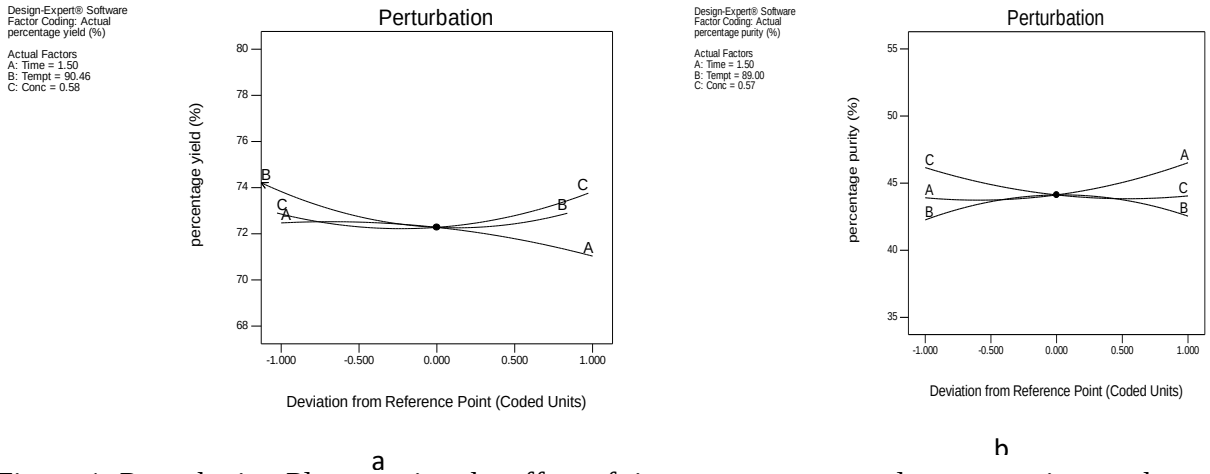

Figure 1: Perturbation Plot showing the effect of time, temperature and concentration on the percentage yield and the percentage purity of alpha cellulose

The Contour Plot Predicted versus Actual

The predicted against actual plot shows the effect of the model and compares against the null model. For a good fit the points should be close to the fitted line, with narrow confidence bands. The points on the left and right of the plot furthest from the mean have the most leverage and effectively pull the fitted lines toward the point. The outliers points affect the fit (*Garvasio et al.* 2008). Figure 2 shows the interaction between temperature, concentration and time for the percentage yield and purity of the alpha cellulose. The plot in figure 2a (percentage of alpha cellulose) shows how closely the points are to the line of regression having an R- Square value of 0.8631 (86.31%) and 73.99% adjusted R- Square value. However the R- Square value for the percentage purity of the alpha cellulose is further away from the line of regression as shown in figure 2b. The R- Square value for the percentage purity is 0.5869 (58.69%) and a low adjusted R- Square value of 21.51% respectively

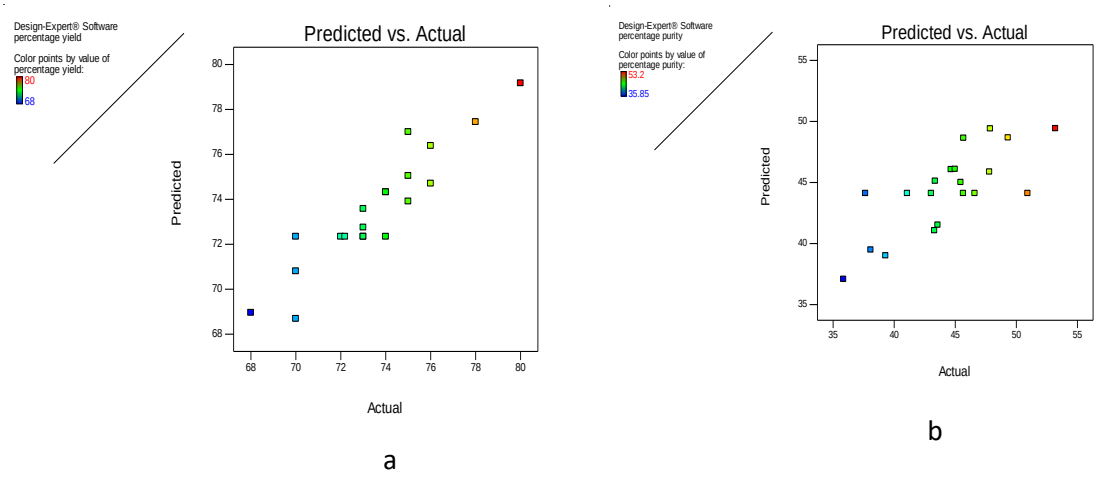

Figure 2: Plot of Predicted verses Actual on the percentage yield (a) and percentage purity of the alpha cellulose (b)

The 3-Dimensional Design

The 3-Dimensional plot combines the three process parameters; temperature, time and concentration. This plot depicts the relationship between them as regarding the percentage yield and percentage purity of the alpha cellulose. The 3D shows that temperature at 81^0C and concentration at 0.87 gives about 76.78% percentage yield, at 82^0C and 0.84 concentration the percentage yield is 75.96%, at 83^0C and 0.74 concentration the percentage yield is 73.90%.

But at high temperature of 97^0C and 0.25 low concentration the percentage yield is 74.31%. Therefore, high temperature with low concentration increases the percentage yield of the alpha cellulose as shown in Figure 3a.

However, in figure 3b, temperature at 80^0C at time 1.2, 1.4 and 1.6 hrs respectively gives 42.99%, 42.43% and 42.31% purity, at 89^0C the percentage purity at time 1.2, 1.4 and 1.6 hrs were 43.81%, 44.22% and 44.96% at 98^0C the percentage purity at time 1.2, 1.4 and 1.6 hrs were 41.62%, 43.01% and 44.22%. Therefore the independent variables does not affect the purity of the alpha cellulose.

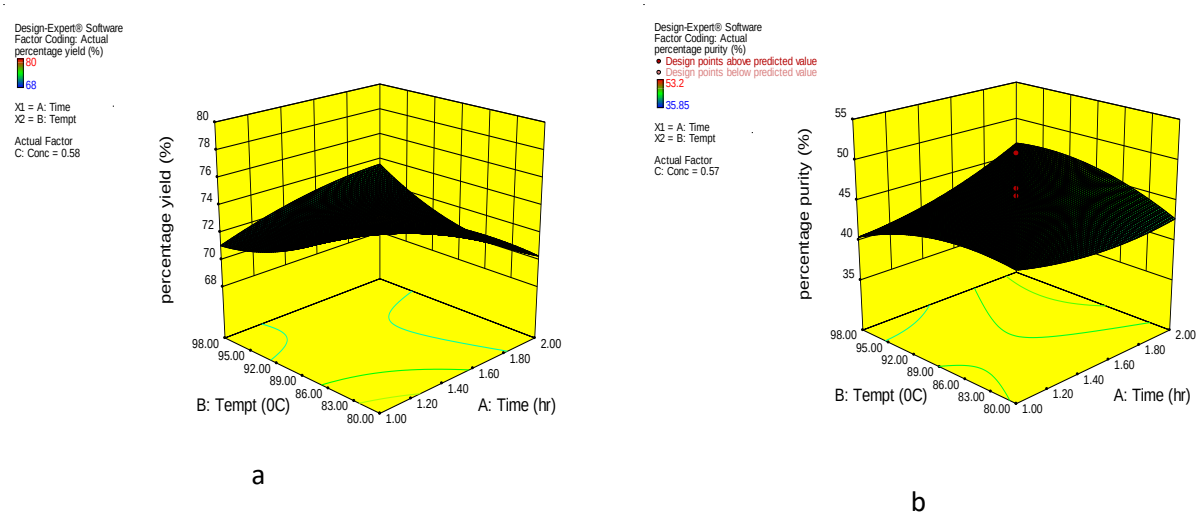

Figure 7: 3D plot of percentage yield (a) and percentage purity (b) of alpha cellulose

SOLUTIONS OF THE OPTIMISATION OF THE ALPHA CELLULOSE

In Table 7.0, the responses were computed and analyzed which present the optimized solutions of the alpha cellulose. However, these conditions were used to validate the optimized data and recorded in Table 8.0. Therefore, under optimal conditions, the percentage yield and percentage purity of the alpha cellulose obtained were 76.44 % and 40.89 % as seen in Table 4.12 of number 1. This optimal value as seen present the most favourable result and therefore would be used for the multi stage pulping method. The desirability was 0.93 and selected because it presented the highest percentage yield and purity under optimal process conditions.

Table 7.0: Solutions of the optimization of the extraction of Alpha Cellulose

OPTIMIZE SOLUTION OF ALPHA CELLULOSE							
Number	**Time (h)**	**Tempt (^{0}C)**	**Conc (g/ml)**	**Percentage yield (%)**	**Percentage purity (%)**	**Desirability**	
1	**1.00**	**80.00**	**0.90**	**79.17**	**41.07**	**0.93**	**Selected**
2	1.01	80.00	0.90	79.15	41.07	0.92	
3	1.00	80.00	0.89	79.13	41.09	0.92	
4	1.02	80.00	0.90	79.11	41.08	0.92	
5	1.00	80.08	0.90	79.10	41.08	0.92	
6	1.00	80.00	0.89	79.10	41.11	0.92	
7	1.02	80.00	0.90	79.09	41.09	0.92	
8	1.00	80.14	0.90	79.06	41.09	0.92	
9	1.04	80.00	0.90	79.04	41.10	0.92	
10	1.00	80.00	0.88	79.04	41.15	0.92	
11	1.00	80.00	0.88	79.01	41.17	0.91	
12	1.05	80.00	0.90	79.01	41.11	0.91	
13	1.00	80.24	0.90	78.98	41.11	0.91	
14	1.00	80.00	0.87	78.94	41.22	0.91	
15	1.08	80.00	0.90	78.90	41.15	0.90	
16	1.00	80.44	0.90	78.84	41.14	0.90	
17	1.10	80.00	0.90	78.83	41.18	0.90	
18	1.10	80.00	0.90	78.81	41.19	0.90	
19	1.00	80.00	0.85	78.80	41.32	0.90	
20	1.00	80.63	0.90	78.71	41.16	0.89	

The validated results as presented in Table 8.0 shows that sample ID no.1 gives the favourable result as the optimum with percentage yield and purity of 76.44% and 40.89% at the temperature of 80^{0}C, time at 1h and ethanol to nitric ratio of 0.90 g/ml. The percentage error for the percentage yield and purity of the alpha cellulose were 2.73% and 0.18% respectively. Therefore, the optimal conditions would be used in the optimization and production of cellulose in the multistage pulping method.

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Table 4.12: The validated Solutions of the optimized Alpha Cellulose from Groundnut Husk

VALIDATED SOLUTION OF ALPHA CELLULOSE

Number	Time (h)	Tempt (^{0}C)	Conc (g/ml)	Percentage yield (%)	Percentage purity (%)	Desirability	
1	1.00	80.00	0.90	76.44	40.89	0.93	Selected
2	1.01	80.00	0.90	76.40	39.75	0.92	
3	1.00	80.00	0.89	77.16	38.28	0.92	
4	1.02	80.00	0.90	78.60	36.56	0.92	
5	1.00	80.08	0.90	76.16	39.13	0.92	

CONCLUSION

The Analysis of variance (ANOVA) showed that temperature is the most influential factor for alkali treatment and extraction of alpha cellulose. Under optimal conditions, the percentage yield and percentage purity of the alpha cellulose obtained were 76.44% and 40.89% respectively. The theoretical values for the percentage yield of the extracted alpha cellulose were close to the experimental results having an error difference of 2.73% percentage yield and 0.187% percentage purity respectively. Therefore, RSM technique based on CCD design is suitable for optimizing the variables influencing the extraction of alpha cellulose.

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