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SACCHARIFICATION OF MELON (EGUSI) SEED SHELL TO YIELD REDUCIBLE SUGARS VIA ENZYMATIC HYDROLYSIS

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Abstract - In this work, proximate analysis of melon seed shell was carried out using the method of the Association of Organic and Applied Chemistry (AOAC). Enzymatic hydrolysis was experimented by using Aspergillus Niger as a crude enzyme isolated from soil at sawdust dump site and screened for cellulosic activities. Factors that could affect the hydrolysis of melon seed shell were screened using the Greco-Latin Square Design of experiment. Meanwhile, the result of the proximate analysis showed that the melon seed shell has 73.54% cellulose which can be converted into fermentable sugar. The small percentage lignin content (0.37%) of this shell is also a good indication that the substrate can be easily hydrolysed. At 2.5g/50ml of enzyme dosage, 40^0C, pH of 7 and 7 days, the yield of glucose gave 60.30% for enzymatic hydrolysis. The dosage of enzyme was not a significant factor and could be held constant. It was found that temperature, pH and time have significant effects on hydrolysis

Keywords: Melon (Egusi) seed shell, enzymatic hydrolysis, Screening of Factors.

1.1 INTRODUCTION

"Egusi" melon (Colocynthis citrullus lanatus) is a vegetable crop commonly cultivated in West Africa **(**Van et al., 2004**).** Egusi melon is one of the members of the group of crops considered to have been neglected, more than other members of its group that most botanical description of the crop are made with reference to other closely related members of the family such as the water melon (C. lanatus) which has received more attention of researchers (Ogbonna, 2013). Melon (egusi) is a member of the cucurbitaceae family, the seeds of egusi are very popular throughout Africa and it is available throughout the year. Egusi seeds have one side rounded and the other side has a pointed tip. Each seed is covered with hard outer shell. The seeds are generally white or creamy in colour (www.onlyfood.net/egusi.html). The hard outer shell of melon seed so far, has been more or less a waste. This source has widespread abundance and the procurement is relatively cost free. Hence, the bio-conversion of this waste (melon seed shell) to glucose). Ogbe and George (2012), carried out studies on the proximate analysis of melon Husk and reported that the husk contained appreciable amounts of crude protein (19.14% $\pm$ 0.46), Carbohydrates (61.01% $\pm$ 0.35), crude fibre (8.12%$\pm$0.85), ash (7.73%$\pm$0.12), crude fat (1.71$\pm$0.04) and fatty acid (1.37%$\pm$0.03). There is therefore, a need

to employ a systematic study on the production of glucose from melon (egusi) seed shell. This study however focused on: Enzymatic hydrolysis of melon (egusi) seed shell and the Screening of factor for the hydrolysis of egusi melon seed shell as the research gap or subject.

2. MATERIALS AND METHODS

2.1 Preparation of Raw Material

2.1.1 Melon (Egusi) seed shell Preparation

The shell of egusi melon seed obtained from New Market Ogui Enugu, Nigeria was washed to remove dirt. It was subsequently dried under the sun. The dried substrate was powdered with an electric grinder, packed in polyethylene bags and stored at room temperature prior to analysis. The chemicals used were purchased from Gerald Chemicals Ltd Ogbete Main Market, Enugu, Enugu State, Nigeria and they were of analytical grade.

2.1.2 Characterisation of Melon (Egusi) seed shell by Proximate Analysis

The Microkjeldahl Method was employed for the crude protein determination in addition to the Sohxlet method being applied for lipid measurement (Ighodaro,2012; Horwitz and Latima, 2005)**.** The lignocellulosic characterisation of melon (Egusi) seed shell was done by the method prescribed by Ayeni et al.,(2015) **:**

The Lignin content was determined by treating 205.51g of the de-fated sample with 500ml of 7.5% w/v aqueous hydrogen peroxide at 90^0C for 2 hours.

$$\% \text{ lignin} = \frac{Initial\ wt\ (120g) - final\ wt}{Initial\ wt} \times 100 \quad \textbf{(1.1)}$$

Meanwhile , the concentration of hemicelluloses in the Egusi Melon Seed Shell was analysed by treating 204.74g of the bleached sample with 500ml of 18% NaOH Solution at room temperature for 30 minutes and subsequently washed with 500ml of 20% acetic acid in hot water. The solution was washed again with hot distilled water to neutralise the residue and tested with pH meter to confirm the neutrality of the residue. The residue was allowed to dry. The percentage Cellulose and other extractives were given as:

$$\%\text{Hemicellulose} = \frac{Initial\ wt\ (100g) - final\ wt}{Initial\ wt} \times 100 \quad \textbf{(1.2)}$$

However, the % cellulose = 100% - (% lipid + lignin + hemicellulose + ash)

Table 2.1: The Proximate Analysis of melon (egusi) seed shell

Component	%W/W Composition
Lignin	0.37
Crude protein	0.79
Hemicellulose	21.18
Ash	3.00
Moisture	0.81
Fats and oil	1.92
Cellulose	73.54

2.2.1 Alkaline Peroxide Pretreatment Of Egusi Melon Seed Shell

Alkaline peroxide pretreatment of sample was done according to the method of Ana Diaz et al., 2013. 200g of powdered melon seed back was weighed into a 1000ml flat bottom flask. 500ml of 7.5% v/v hydrogen peroxide solution was introduced into the sample. A solution of 5M sodium hydroxide was used to adjust the pH of the media to 9 using a table top pH meter (Metler Toledo, Seven Compact Series). The flask containing the media was fixed to a reflux condenser with water circulating at the outer column. The whole set up was heated at 90^0C for two hours. It was allowed to cool and filtered using Buckner funnel connected to a pressure pump. The residue was washed several times with distilled water to neutralize the alkaline solution. The residue, which is the pretreated sample, was dried in hot air oven at 50^0C.

2.3. Isolation and Screening of *Aspergillus niger* (*A. niger*)

Aspergillus niger was isolated and screened for cellulase activities following the method described by (Ezeonu *et al.*, 2011). The isolated *A. niger* was thereafter multiplied by aseptically transferring a pinch of the fungus into different test tubes containing (Potato Dextrose Ager) PDA mounted in slant positions.

2.4. Inoculums for Hydrolysis

0.5% inoculum of the multiplied *A. niger* from a PDA slant was prepared by aseptically transferring 0.25 g of the pure and screened *A. niger* from the slant to a 50ml volumetric flask. Distilled water autoclaved at 121˚C for 15mins

was added to make the mark of the flask. The autoclaved water was allowed to cool before use. This inoculum was used for the whole hydrolysis experiment.

2.5. The Enzymatic Hydrolysis with *A. niger*

The enzymatic hydrolysis was carried out in 100ml amber bottle containing 2.0g of treated egusi melon seed shell in 50cm^3 of distilled water and incubated at 30^{o}C for 1day and at a pH of 3.Enzyme dosage was 0.5ml of 2.5g/50ml concentration. The mixture was filtered and the soluble sugar yield in the filtrate was measured using the refractometer (Model RF M960 available at PRODA, Enugu), while the reducible sugar yield was determined using the Dinitrosalicylic (DNS) method. The effect of time, temperature, enzyme dosage and pH were investigated following the Greco-Latin Square Design of experiment shown in table 2.2. The variation levels for Time were 1, 3, 5, 7 and 9days; 30, 40, 50, 60 and 70^{o}C for temperature; 0.5, 1.0, 1.5, 2.0 and 2.5ml per 50ml of hydrolysis solution for dosage; and 3, 5, 7, 9 and 11 for pH. The pH was adjusted using NaOH and H_2SO_4.

2.6 Design of Experiment for factor screening

The following factors were screened for their statistical significance on the yield of simple sugar from melon seed coat by enzymatic hydrolysis:

1. Temperature of the hydrolysis
2. The pH of the process
3. The duration of the hydrolysis process
4. The dosage of the enzyme

Since there are four possible factors that could affect the yield of simple sugars, the screening was done using the Graeco Latin square design of experiment at 5 levels of each of the factors. The model for a Graeco Latin Square with one observation in the cell is:

$$Y_{ijkl}=\mu + \alpha_i + \beta j + \tau k + \lambda l + \varepsilon_{ijkl} \quad \textbf{(1.3)}$$

i,j,k,l =1,2,…m;

where $\Sigma \alpha_i = \Sigma \beta j = \Sigma \tau k = \Sigma \lambda l = 0$

variables α_i , βj ,λl, τk are the actual effect of I rows, j columns, k factor and l factor levels. The Graeco Latin Square Design is shown in Table 2.2 while the ANOVA Table of the Graeco Latin Square is as shown in Table 2.6. The significance of the factors was determined by comparing the F-statistic which is the ratio of the factor mean square to the error mean square with the critical F-statistic at the given degree of freedom (Murray & Larry, 2011). The operational matrix of the GLSD is shown in Table 2.3. The levels were chosen based on the results of similar experiment.

2.5.1 Enzymatic Hydrolysis

Table 2.2: Greco Latin Square design for screening of factors in enzymatic hydrolysis of melon (egusi) seed husks

	M1	**M2**	**M3**	**M4**	**M5**
T1	**1A**	**3B**	**5C**	**2D**	**4E**
T2	**2B**	**4C**	**1D**	**3E**	**5A**
T3	**3C**	**5D**	**2E**	**4A**	**1B**
T4	**4D**	**1E**	**3A**	**5B**	**2C**
T5	**5E**	**2A**	**4B**	**1C**	**3D**

Table 2.3: Design matrix (Greco Latin Square) for factors screening in enzymatic hydrolysis

Runs	**Time(days) M1-M5**	**temp (^{o}c.)** T1-T5	**E.Dosage (ml/50ml) A-E**	**pH 1-5**
1	1	30	0.5	3
2	3	30	1.0	7
3	5	30	1.5	11
4	7	30	2.0	5
5	9	30	2.5	9
6	1	40	1.0	5
7	3	40	1.5	9
8	5	40	2.0	3
9	7	40	2.5	7
10	9	40	0.5	11
11	1	50	1.5	7
12	3	50	2.0	11
13	5	50	2.5	5
14	7	50	0.5	9
15	9	50	1.0	3
16	1	60	2.0	9
17	3	60	2.5	3
18	5	60	0.5	7
19	7	60	1.0	11
20	9	60	1.5	5
21	1	70	2.5	11
22	3	70	0.5	5
23	5	70	1.0	9
24	7	70	1.5	3
25	9	70	2.0	7

Table 2.4: ANOVA Table of Greco Latin Square Design

Source of variation	F	SS	MS
Row	m-1	$SS_R = \frac{\sum_i Y_{i\circ\circ\circ}^2}{m} - \frac{Y_{\circ\circ\circ\circ}^2}{m^2}$	$MS_R = \frac{SS_R}{m-1}$
Column	m-1	$SS_C = \frac{\sum_j Y_{\circ j\circ\circ}^2}{m} - \frac{Y_{\circ\circ\circ\circ}^2}{m^2}$	$MS_C = \frac{SS_C}{m-1}$
Factor N	m-1	$SS_N = \frac{\sum_k Y_{\circ\circ k\circ}^2}{m} - \frac{Y_{\circ\circ\circ\circ}^2}{m^2}$	$MS_N = \frac{SS_N}{m-1}$
Factor P	m-1	$SS_P = \frac{\sum_l Y_{\circ\circ\circ l}^2}{m} - \frac{Y_{\circ\circ\circ\circ}^2}{m^2}$	$MS_P = \frac{SS_P}{m-1}$
Residual	(m-1)(m-3)	$SS_E = \sum\sum\sum\sum Y_{ijkl}^2 - \frac{Y_{\circ\circ\circ\circ}^2}{m^2} - SS_R - SS_C - SS_N - SS_P$	$MS_E = \frac{SS_E}{(m-1)(m-}$
Total	m^2	$\sum\sum\sum\sum Y_{ijkl}^2 - \frac{Y_{\circ\circ\circ\circ}^2}{m^2}$	

3. Results and Discussion

3.1. The Composition of Melon (Egusi) Seed Shell

Table 2.1 shows the result of the proximate analysis of melon seed shell. The result confirmed that the shell of melon seed is reach in carbohydrate which can be converted to glucose. Meanwhile, Ogbe and George, carried out studies on the proximate analysis of melon Husks and reported that the husk contained appreciable amount of Carbohydrates (61.01%±0.35) among others (Ogbe and George, 2012).The small percentage lignin content of this shell is also a good indication that the substrate can be easily hydrolysed.

3.2 The effect of temperature, time and pH on hydrolysis

The results of the preliminary experiment conducted to determine the effects of temperature, time and pH on enzymatic hydrolysis are shown in figures 3.1 –3.3

Figure 3.1 shows that the highest glucose yield of 37% was obtained around 1 to 3 days of incubation compared to other days. The fact that glucose inhibits the enzymatic hydrolysis could be responsible for the drop in glucose yield as time exceeds 3days. Figure 3.2 shows that the highest yield of simple sugar with pH falls around 5 to 7. While Figure 3.3 shows that, the highest yield of simple sugar occurred at 40^{o}C for enzymatic hydrolysis. It also confirmed that there is loss of enzyme activities as the temperature increases as reported by Zakpaa *et el.,(2009)*.

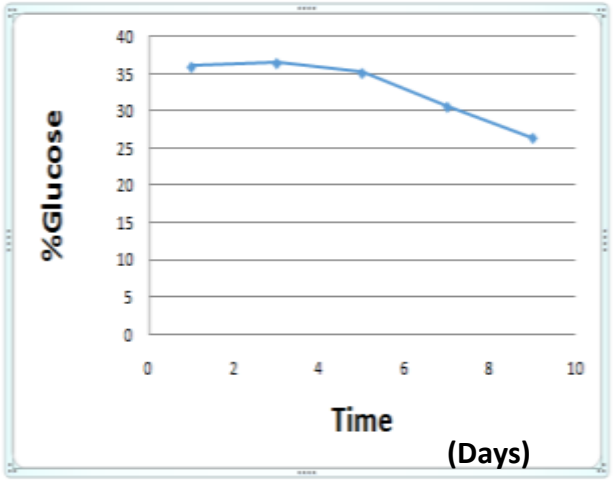

Fig. 3.1: The effect of time on enzymatic hydrolysis

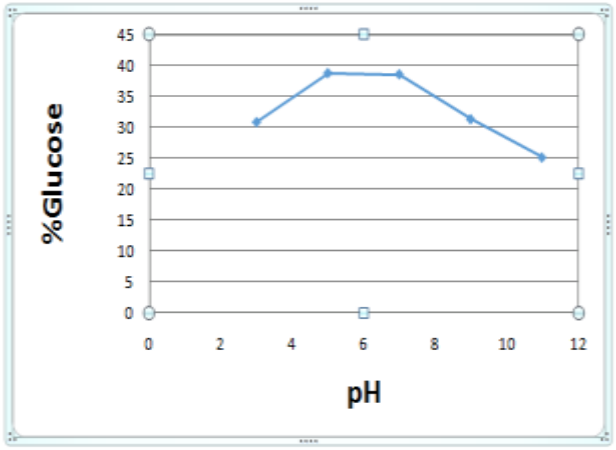

Fig.3.2: The effect of pH on enzymatic hydrolysis

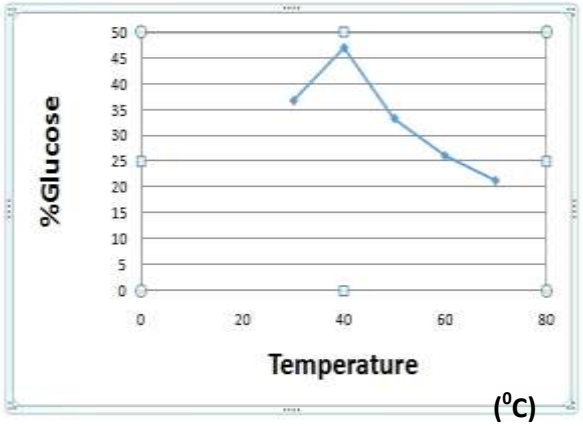

Fig. 3.3: The effect of temperature on enzymatic hydrolysis

3.3: Screening of factors for the hydrolysis of egusi melon seed shell

3.3.1 Enzymatic hydrolysis

Various factors were screened for their significance in enzymatic hydrolysis using the Greco-Latin Square Design of experiment. The results of the screening of factors for enzymatic hydrolysis of melon seed shell are shown in tables 3.1 and 3.2.

Table 3.1: The Greco Latin Square Design Matrix with response for Enzymatic Hydrolysis

Runs	Enzyme Dosage (g/50ml) (A-E)	Temp(°C) (T1-T5)	pH (1-5)	Time(days) (M1-M5)	Glucose yield (%)
1	0.5	30	3	1	30.42
2	1.0	30	7	3	29.16
3	1.5	30	11	5	7.56
4	2.0	30	5	7	31.86
5	2.5	30	9	9	31.50
6	1.0	40	5	1	59.22
7	1.5	40	9	3	41.58
8	2.0	40	3	5	41.40
9	2.5	40	7	7	60.30
10	0.5	40	11	9	32.58
11	1.5	50	7	1	40.86
12	2.0	50	11	3	32.76
13	2.5	50	5	5	30.78
14	0.5	50	9	7	30.78
15	1.0	50	3	9	31.50
16	2.0	60	9	1	32.76
17	2.5	60	3	3	31.32
18	0.5	60	7	5	32.40
19	1.0	60	11	7	33.30
20	1.5	60	5	9	54.00
21	2.5	70	11	1	19.08
22	0.5	70	5	3	18.00
23	1.0	70	9	5	19.80
24	1.5	70	3	7	19.44
25	2.0	70	7	9	30.24

Table 3.2: The ANOVA Table for enzymatic hydrolysis (factor screening)

Source of variation	Variation	Degree of freedom	Mean Square	f-statistics
Time	374.91	4	93.73	0.91
pH	676.81	4	169.2	1.65
Dosage	115.66	4	28.91	0.28
Temperature		4		4.81
	1974.21		493.55	
Residual	401.24	4	102.56	
Total	3551.82	20		

The ANOVA table shows that the f-statistics of pH and temperature were more than 1.0 and could be considered as significant factors for further studies according to Zivorad (2011). The f-statistics for time and enzyme dosage were less than 1.0. The f-value for time could however be approximated to 1.0. The high glucose yield was obtained within 3days of incubation compared to other days. The fact that glucose inhibits the enzymatic hydrolysis could be responsible for the drop in glucose yield as time exceeds 3days. The concentration of enzyme is always required in small quantity for many enzymatic actions. The dosage of enzyme was not a significant factor and could be held constant.

5. Conclusion

This study has shown that melon (egusi) seed shell has potential for glucose production. *Aspergillus niger* was discovered to be very effective in hydrolyzing melon seed shell when it is subjected to the right condition.

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