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Determination of Glucose Concentration by Fermentation Process of Cassava Peels Via Sonication

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Abstract: The large volume of cassava peels derived from gari processing activities are usually discarded as waste and allowed to decay in the open causing damage to the environment, thus resulting in several health related issues. In this study, glucose concentrations were determined from the fermentation of cassava waste peels using an ultrasound machine. Glucose concentration rates were estimated at 0.75 M, 1.00 M, and 1.25 M of acid hydrolysis. Moisture, protein, ash, fiber, lipid, and carbohydrates compositions were determined using proximate analysis. The results obtained showed that glucose concentration at 0.75 M increased with increase in time indicating a second order polynomial. The R-squared value calculated from the slope of the plot was 0.909. Glucose concentration at 1.00 M also increased with increase in time. However, as the time becomes large, glucose concentration at 1.25 M decreased progressively with time, and eventually leveled off to a constant value. The kinetic mechanism showed that the process followed pseudo-second order model equation with R-squared values of 0.996, 0.998, and 0.960 for 0.75 M, 1.00 M and 1.25 M, respectively. The cassava waste peels examined have appreciable levels of nutrients and can make useful contributions in animal nutrition and bio-ethanol production. It is evident that fermentation of cassava waste peels using ultrasound improved fermentation process and glucose concentration rates. Thus, the biotechnological approach is a veritable tool for economic utilization of agro-waste residues such as cassava peels waste.

Keywords: Cassava Waste Peels, Proximate Analysis, Glucose Evaluation, Sonication, Kinetics

1. Introduction

Cassava peels obtained from gari processing are usually discarded as waste into the environment, thus resulting in serious pollution problems and eventual distortion of the fragile ecosystem.

Literature has shown that the large volume of cassava processed is proportional to the volume of cassava peels wasted without proper use [1]. Hence, these cassava waste peels are left to ferment spontaneously which takes longer time to achieve and obviously they are exposed to unfavorable conditions such as bacterial attacks from pest and fluctuations in weather conditions.

Fermentation is one of the oldest biotechnological approaches, having been used in food processing and preservation as well as beverages production for over 6,000 years [2]. The fermentation process of staple foods serves as a succor to inhabitants in the rural and even urban areas and the resultant effects create room for preservation [3-5]. The process of fermentation improves the nutrient composition and consumption of foods through biosynthesis. The process adds value on the micronutrient bioavailability and supports degrading of anti-nutritional factors [6, 7].

Literature has revealed that the traditional processing of

cassava roots for gari production in Africa generates two major biological wastes namely, the cassava peels and the obnoxious fermented liquid that may cause damage to the environment [8]. Available data have shown that about 10 million tones of cassava roots are processed into gari annually in Nigeria alone [9]. However, since these peels could make up to 10% of the wet weight of the roots, it may serve as a potential resource for animal feeds if properly processed. Cassava peels is regarded as the skin of the cassava roots and as such after harvesting and processing constitute 25% of the whole plant [10].

With the advent of biotechnological techniques, there are great opportunities for economic utilization of agro-industrial wastes such as cassava peels.

Studies have largely focused on the benefits of sonication to fragment DNA samples to speed dissolution by breaking intermolecular interactions and other areas where sonication has been used in other industries.

This study however, will focus on the use of sonicator or ultrasonic energy as a useful biotechnological technique to advance the fermentation process of cassava peels to improve its nutritional value and hence, determine glucose concentration in the cassava waste peels.

2. Materials and Method

The cassava waste peels were obtained from some selected chop bars in Rumuekini main market. The cassava peels obtained were cut into smaller sizes and oven-dried for 3days to reduce moisture content to its minimal level. The dried cassava peels were milled to powder form using an electric blender and sieved with a sieve range of 150 μm to obtain homogenous powder.

2.1. Optimization Time and Hydrolysis Process

A 0.75 M HCl was dissolved in 150 ml of distilled water and 25g of the ground cassava peels was put into 250 ml beaker. Then, the prepared acid concentration were poured into the measured sample and stirred until a homogenous mixture was achieved. It was then placed inside the ultrasound machine and was removed for time intervals of 6, 15, 30, and 60 min. This process was repeated for different concentration of acid (1.0 M and 1.25 M) at different times as mentioned above.

2.2. Determination of Glucose

4 mL of anthrone reagent was added to supernatant and heated for 8 min in a boiling water bath. It was cooled rapidly and read when the green color changed to dark green color at 630 nm. From the plot of absorbance against concentration the amount of carbohydrates (as glucose) present in the sample tube was calculated.

2.3. Determination of Moisture Content

The crucible was dried in the oven at 105°C and cooled in desiccators. Then, the dried crucible was weighed (W_1). A 3 g of the sample was spread into the crucible and was accurately weighed (W_2). The crucible and its constant were transferred into an oven maintained at 105°C for 3 hrs and the crucible was then transferred into desiccators using a pair of tongs. It was allowed to cool for 30 min and weighed. The above steps were repeated until a constant mass (W_3) was recorded. The moisture content was calculated by:

$$\%\text{Moisture}=\left(\frac{W_3-W_2}{W_2-W_1}\right)\times 100 \quad (1)$$

2.4. Ash Determination

A clean dry crucible was ignited in the furnace and cooled in desiccators and weighed (W_1). The sample was introduced into the crucible and weighed (W_2). The crucible and content were charred over a low flame and was transferred into a muffle furnace. The furnace was then set to 500-550°C until sample was fully charred (grayish color). The crucible was then transferred into desiccators to cool and the cooled crucible was weighed (W_3). The total ash content was calculated as:

$$\%\text{Total Ash Content}=\left(\frac{W_3-W_1}{W_2-W_1}\right)\times 100 \quad (2)$$

2.5. Crude Fiber Determination

2 g of the sample was extracted with petroleum ether (W_1). The sample was boiled under reflux for 30 min with 200 ml of dilute hydrochloric acid and filtered. The residue was properly washed with water until it became acid free, then transferred into a beaker and boiled for another 30 min with 200 ml of dilute sodium hydroxide and filtered. It was transferred into ignited crucible. The residue was then washed 3 times with 20 ml ethanol and 2 times with 10 ml ether and it was finally dried in an oven. It was cooled and then weighed (W_2). The dried residue was transferred into a furnace and ignited, cooled and weighed (W_3). The crude fiber is calculated as:

$$\%\text{Crude Fibre}=\left(\frac{W_2-W_3}{W_1}\right)\times 100 \quad (3)$$

2.6. Determination of Fat

5 g of the sample was accurately weighed using a weighing balance (W) g. A flat bottomed flask was weighed empty and dry (W_1) g and the extractor was mounted on it. The thimble was held carefully halfway into the extractor and the weighed samples carefully transferred into the thimble. The weighing dish was rinsed with solvent and poured into the thimble which was then plugged with cotton wool and dropped into the extractor. The solvent was poured to reach about two-third of the volume of the flask and continuously extracted for 5 hrs. At the completion of extraction, the solvent was evaporated off on water bath. The flask was then dried, cooled and weighed (W_2) g. The fat content was calculated as:

$$\%\text{Fat}=\left(\frac{W_2-W_1}{W}\right)\times 100 \quad (4)$$

2.7. Determination of Protein Using Kjedahl Method

0.1 g sample was weighed into a clean 250 ml capacity conical flask and 3 g of digestion catalyst was added into the

flask and 20 ml concentrated sulphuric acid was also added and the sample was heated to digest. The content turned black to sky-blue coloration. The digest was cooled to room temperature and was diluted to 100 ml with distilled water. Thereafter, 20 ml of diluted digest was measured into a distillation flask and the flask was held in place on the electro thermal heater or hot plate. The distillation flask was attached to a Liebig condenser connected to a receiver containing 10 ml of 2% boric acid indicator. 40 ml of 41% sodium hydroxide was injected into the digest via a syringe attached to the mono-arm steelhead until the digest became strongly alkaline. The mixture was heated to boiling and the distilled ammonia gas through the condenser attached to the receiver beaker. The color of the boric acid changed from purple to greenish and ammonia distillate was introduced into the boric acid. The distillate was titrated with standard 0.1N hydrochloric acid solution back to purple from greenish. The volume of hydrochloric acid added to effect this change was recorded as titer value. The % organic nitrogen is calculated as:

$$\%\text{Organic nitrogen} = \frac{\text{Titer value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times 1} \quad (5)$$

Where, titer value=the volume of HCl used in titrating the ammonium distillate.

1.4=Nitrogen equivalent to the normality of HCl used in the titration 0.1 N.

100=the total volume of digest dilution.

100=percentage factor

1000=conversion factors from gram to milligram

20=integral volume of digits analyzed or distilled.

1=the weight of sample in gram digested.

However, the %protein was calculated as:

$$\%\text{Protein}=\%\text{Nitrogen} \times F \quad (6)$$

Where, F=Conversion factor

2.8. Determination of Carbohydrate

The carbohydrate content was calculated as:

$$\%\text{Carbohydrate}=100-(\%\text{ Moisture} + \%\text{Ash} + \%\text{Crude fiber} + \%\text{Fat} + \%\text{Protein}) \quad (7)$$

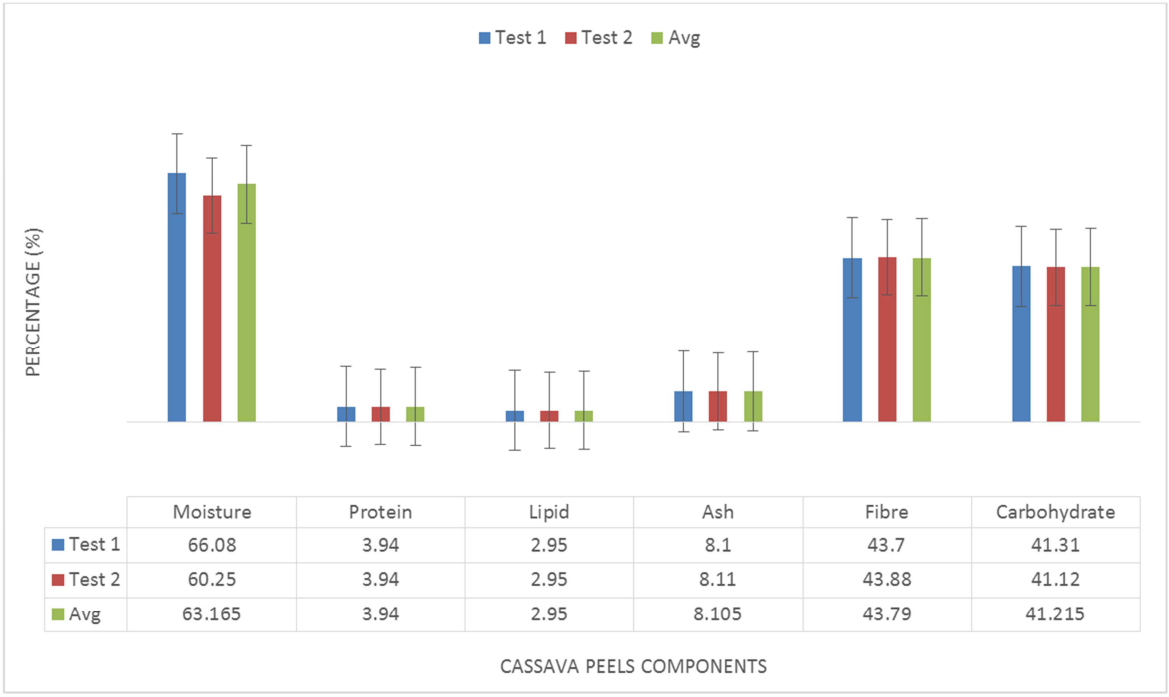
3. Results and Discussion

3.1. Proximate Analysis of Cassava Peels

Data in Table 1 shows the proximate parameters in percentages evaluated for the six cassava peels components. The proximate analysis showed that cassava peels protein percentage did not vary. Moisture content varied from 66.08 in test 1 to 60.25 in test 2 while ash content varied from 8.10 in test 1 to 8.11 in test 2. Fiber content varied from 43.70 in test 1 to 43.88 in test 2 while carbohydrate content varied from 41.31 in test 1 to 43.12 in test 2.

The result of the proximate analysis indicates an increase in percentages for moisture, ash, and fiber while a decrease in carbohydrate content and no percentage variation for protein and lipid content of the cassava peels analyzed.

The average moisture content of the cassava peels during the experiment was 63.165% and the average ash was 8.105% (with standard deviation of ± 0.007071%).



	Moisture	Protein	Lipid	Ash	Fibre	Carbohydrate
Test 1	66.08	3.94	2.95	8.1	43.7	41.31
Test 2	60.25	3.94	2.95	8.11	43.88	41.12
Avg	63.165	3.94	2.95	8.105	43.79	41.215

Figure 1. *Comparing the percentages with their standard errors.*

Table 1. *Proximate analysis of cassava peels.*

Components	Test 1 (%)	Test 2 (%)	Average (%)	Standard Deviation (%)
Moisture	66.08	60.25	63.165	4.122433
Protein	3.94	3.94	3.94	0
Lipid	2.95	2.95	2.95	0
Ash	8.10	8.11	8.105	0.007071
Fibre	43.70	43.88	43.79	0.127279
Carbohydrate	41.31	41.12	41.215	0.13435

The result of the standard error for the proximate analysis as represented in Figure 1 clearly validates a considerable variation in protein, moisture, ash, carbohydrates, and fiber of cassava peels as reported by previous studies [11]. Thus, with respect to the proximate analysis and the composition profile of the cassava samples studied, the peels have appreciable high value of protein with the highest value found in sample (Test 1) at 3.94% and can make useful contributions in animal nutrition and bio-ethanol production.

The range of the moisture content (66.08 – 60.25%) was highly comparable to that of the work carried out by Christopher *et al*. [12] which gave moisture content of 66.08%. The range obtained from this study also compares favorably with the range of percentage carbohydrates level in cassava peels, though a higher range of 69.35 to 77.28% have been reported [11].

3.2. Standard Glucose Determination

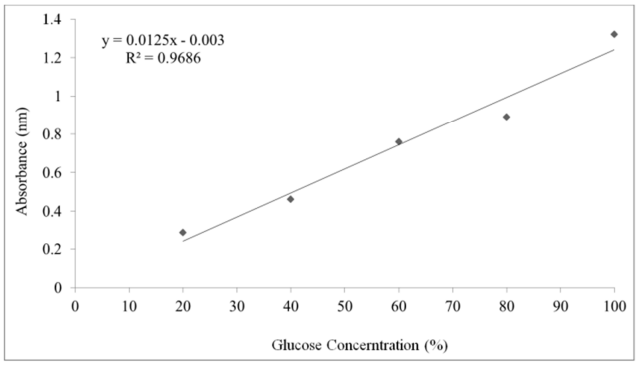


Figure 2. *Glucose concentration versus absorbance.*

3.3. Glucose Calculation

The amount of carbohydrate (as glucose) present in the sample can be calculated using a linear equation proposed by Mike [13].

$$y = mx + c \tag{8}$$

$$m = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1} \tag{9}$$

From Figure 2, the amount of carbohydrate (as glucose) present in the sample can be calculated for any given value of absorbance as:

$$y = 0.0125x + 0.003 \tag{10}$$

Making x subject of formula in equation (10), we have that:

$$x = \frac{y - c}{m} \tag{11}$$

Substituting values form and c from the plot, we have:

$$x = \frac{y - 0.003}{0.0125} \tag{12}$$

Where:
x = Glucose concerntartion in percentage
y = Absorbance in nm
0.0125=slope and 0.003=straight line constant.

3.4. Effect of Glucose Concentration with Time

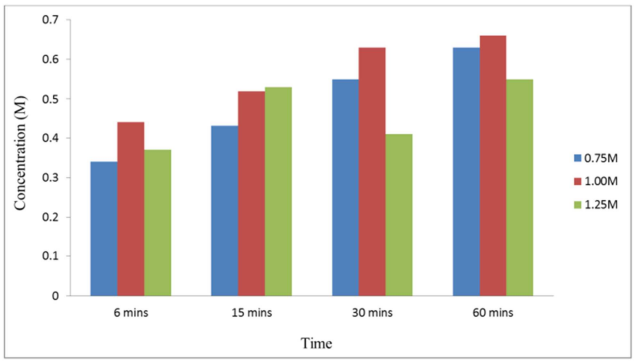


Figure 3. *Comparison of glucose concentration at different time intervals.*

The glucose concentrations, q_e , were determined at different time intervals but at constant molarity of 0.75 M. The glucose concentrations, q_e increased with increase in time and the second-order rate of concentrations, x^2, were estimated at different times. The plot of q_e versus time best-fit was second-order polynomial and the R-squared value, calculated from the slope of the plot was 0.909. The glucose concentration, q_e for any given time, t can be calculated as:

$$q_e = -0.0001t^2 + 0.0128t + 0.266 \tag{13}$$

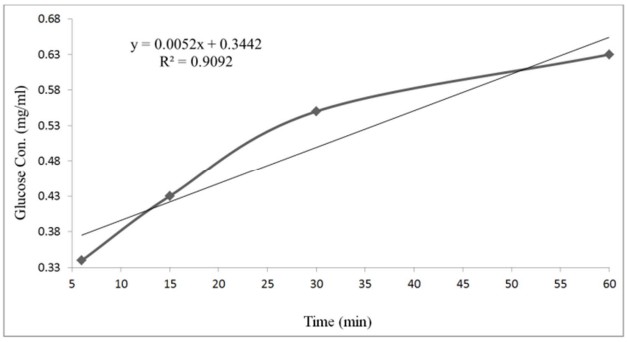


Figure 4. *Glucose concentrations against time at 0.75 M.*

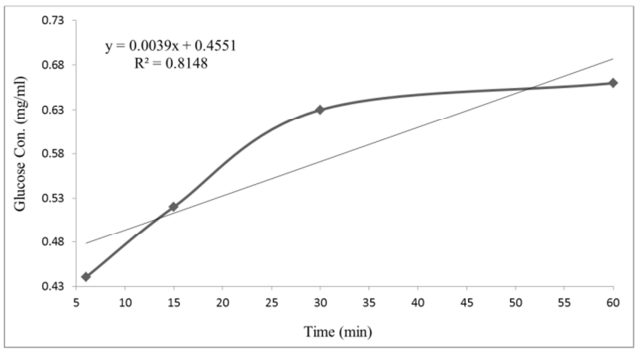


Figure 5. *Glucose concentrations versus time at 1.00 M.*

More so, the glucose concentrations at 1.00 M showed that the concentration increased as the time of the run increased. The plot of glucose concentration (q_e) against the time in Figure 5 showed regions of concentration dependence on time. In the low concentration region q_e increased almost linearly with concentration.

However, in the high glucose concentration region, the rate of increase in q_e decreased progressively with time and it eventually leveled off to a constant value that is dependent of further increase in run time.

Furthermore, the glucose concentrations at 1.25 M, q_e, showed that the concentration rate increased with increase in time of the run but as the time becomes large the concentrations started to decrease and showed a zero slope at time of 15 min. A fermentation study at different time values has established that as the time of the run becomes large there is always a decrease in catalytic activity of glucose concentration [14]. However, the presence of sonicator increased the efficiency of glucose at the various concentrations but the optimum was obtained for 1.00 M at 60 min [15].

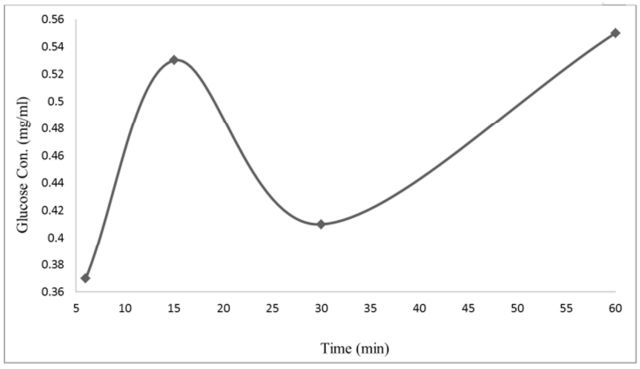


Figure 6. *Glucose concentrations versus time at 1.25 M*

3.5. Kinetic Evaluation

In order to propose the mechanism of fermentation of cassava peels via sonication, pseudo-second order model equation was adopted. The linearized form of the equation is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\,2}} + \frac{1}{q_e} t \qquad (14)$$

Where, k_2 is the equilibrium rate constant of pseudo-second order fermentation process in $gmg^{-1}min^{-1}$, t is the time of sonication in min, q_e and q_t are the amounts of glucose recovered at equilibrium and at a given time in mgg^{-1}.

The plot of $\frac{t}{q_t}$ versus t as presented in Figure 7 showed the R-squared values obtained from the kinetic model equation were 0.996, 0.998, and 0.960 for 0.75 M, 1.00 M and 1.25 M respectively.

The results obtained showed that the mechanism of glucose recovery followed pseudo-second order kinetic model equation. The kinetic model equation parameters for pseudo-second order are presented in Table 2.

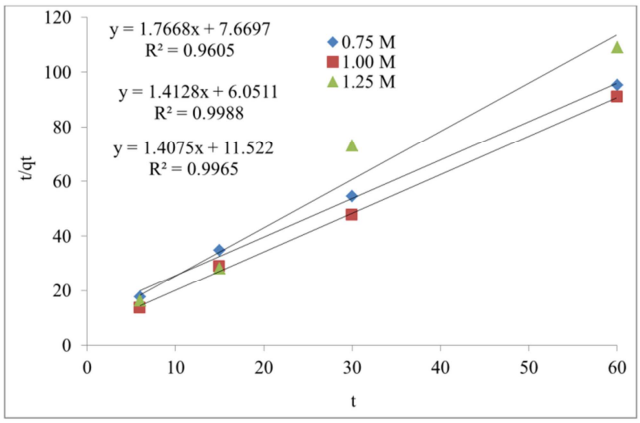


Figure 7. *Pseudo-second order plots for glucose determination via sonication.*

Table 2. *Pseudo-second order parameters for glucose recovery at 0.75 M, 1.00 M, and 1.25 M.*

Concentration (M)	q_e (mg/g)	k_2 (g/mg/min)	R^2
0.75	0.71	0.17	0.996
1.00	0.71	0.33	0.998
1.25	0.57	0.40	0.960

4. Conclusion

The determination of glucose concentration by fermentation process involving cassava waste peels has been studied by means of an ultrasound instrument. The glucose concentrations increased with increase in the time of the run, as well as increase in temperature of the solution.

For 1.25 M mixture, the glucose concentration initially increased with increase in time, reaching a zero slope at time 15 min and then decreased with further increase in time. The slope of the sample at 0.75 M acid hydrolysis gave glucose concentration which was about 3 twice as large as that at 1.00 M. The concentration rate exhibited pseudo first and second-order curve and with R-squared value of 0.902. The mechanism of glucose recovery followed pseudo-second order kinetic model equation with high R-squared values of 0.996, 0.998, and 0.960 for 0.75 M, 1.00 M, and 1.25 M mixtures respectively. Sonication as seen in this study aided and speeds up the fermentation rate of cassava waste peels.

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