

Original Research Article

NOVEL LINEARIZED KINETIC MODELING OF STARCH HYDROLYSIS

ABSTRACT

The hydrolysis of starch into glucose by acid and enzyme techniques has recorded higher glucose recovery and optimization of processes, but lacks the fitting of the results into a kinetic linear model. The application of kinetic linearized model effect of temperature on pH and acid concentration during hydrolysis of starch into glucose was studied. The experiment was conducted at different durations using reported preparatory techniques and average values of triplicates were reported. The maximum glucose yield of 18.20 mg/ml was observed on 4 hours at 60 °C from acid hydrolysis and similarly observed at 4 hours at 100 °C for enzyme hydrolysis. The lowest glucose yield of 10.0 mg/ml and 11.1 mg/ml were both recorded at 30 minutes duration for acid and enzyme hydrolysis respectively. The correlation coefficient of acid hydrolysis when starch was hydrolyzed for 2 hours at 80 °C had a value of 1 (line of best fit) while the weakest linear relationship (0.715) was obtained was in enzymatic hydrolysis when starch was hydrolyzed at 4 hours for 80 °C. Hence, the highest glucose yield was not automatically the most efficient process. The linear model equations showed that acid hydrolysis of starch had a positive energetic interaction while enzymatic hydrolysis had a negative energetic interaction. The slope and intercept of acid hydrolysis were all positive and indicated a positive relationship with parameters. All enzymatic hydrolysis had negative slope and indicated inverse relationship with the parameters. Therefore, the model allows researchers to make well interpretations of their results using linearized kinetic model.

Keywords: Starch, Hydrolysis, Glucose, Linearized model.

1. INTRODUCTION

Cassava is a common occurring plant in Nigeria usually processed into different starch stables [1]. The starch precursors are usually subjected to various acid and enzymatic hydrolyzing techniques to optimize the production of starch or glucose [2]. Acid hydrolysis involves the conversion of lignocelluloses content to valuable products that are edible, while enzymatic hydrolysis has been presented as bio-friendly approach for both waste peel and fibres of cassava [3, 4]. However, challenges such as non-specific binding, low immobilization yield, and acute diffusion problem exist for large proteins during enzymatic hydrolysis [5, 6]. These occur because the rates of hydrolysis depend on the molecular size and structure of the substrates. Hence, researchers are continuously investigating production kinetics and improved approaches that will give higher recovery of glucose at cost effective process like use of microwave pre-treated hydrolysis [7]. However, studies on linear relationships with parameters are uncommon.

The sustained studies on acid catalysed hydrolysis of starch into reducing sugars have relatively provided information on physico-chemical and functional properties of starch and glucose [8, 9], but no linear model has been developed for such hydrolysis of starch into glucose. The Optimization of acid hydrolysis of starch for glucose production has also seen a progressive trend towards maximum glucose concentration and recovery [3, 7, 10, 11, 12]. Consequently, factors like temperature, pH, acid concentration, particle size and pre-treatment are known to affect yield of final product [11, 12, 13, 14] but lacks physical translation into a linear model.

Additionally, enzymatic hydrolysis has found useful applications with enzymes such as cellulase, β -glucanase, fungal glucoamylase and amyloglucosidase [15, 16, 17, 18] in conversion of starch to

glucose. Subsequently, the nature of the product formed depends on the enzyme, substrate and particular approach used [19, 20, 21]. On the other hand, the acid hydrolysis depends on factors such as acid concentration, particle size and pre-treatment to yield optimally [11, 12, 13, 14]. Thus the study will utilize the effect of temperature on acid concentration and pH of enzymes during hydrolysis in order to investigate for the first time, the application of linearized kinetic model interpretation of results obtained from cassava starch hydrolysis into glucose.

2.0 MATERIAL AND METHODS

2.1 Cassava Preparation

The cassava tuber was sampled from a small scale cassava farmland in Mbaize, Imo State of Nigeria. The harvested cassava was peeled and washed with water. The peeled tuber was chipped into small sizes and dried at 60 °C for 48 hours in a cabinet dryer. The dried chips were further dry-milled and screened to obtain the flour [6, 24]

2.2 Enzyme Preparation

α -amylase and amyloglucosidase were obtained from the culture collection unit of the Department of Biotechnology, National Root Crops Research Institute,(NRCRI) Umudike, Abia State Nigeria.

2.3 Acid Hydrolysis

50g cassava flour was weighed out and dispersed into a 150 ml H₂SO₄ with varied concentrations of 0.2 M to 1.0 M. Hydrolysis was initiated when cassava was dispatched into the hydrolyser and conducted at different acid concentration strength ranging from 0.2 M to 1.0 M. A water bath was set to maintain 60 °C to 100 °C within the selected time range of 30 minutes, 1 hours, 2 hours and 4 hours. 0.1 M of NaOH was used to bring the acidic slurry to neutral pH (clear sugar concentrate obtained; glucosyrup). The starch stock was prepared with 1gm/L concentration in sterile distilled water. The Blank was prepared using distilled water and DNS reagent. It was used as a substrate for optimization of acid treatment for starch hydrolysis. Sugar concentration was determined by DNS method (3, 5- Di Nitro Salicylic Acid) method [10, 22].

2.4 Enzymatic Hydrolysis

50g starch was dispersed into 150 ml of distilled water at a ratio of 1:3 to initiate enzymatic hydrolysis. The obtained white slurry was gradually heated up to 80 °C to break down the intermolecular bonds of starch for 30 minutes using water bath. Further heating was initiated in the water bath at 90 °C. A 2.0 ml of alpha amylase (Termanyl) was introduced into the slurry and allowed to equilibrate for 1 hour. The starch mixture was cooled at 60 °C and pH range maintained at 4.0 – 7.0. A 2.0 ml of glucoamylase enzyme was also added to the reaction mixture and then kept at selected temperatures of 60, 80, and 100 °C at varied time intervals of between 30 minutes and 4 hours. Incubation of substrates without enzyme addition was set as blank control. Glucose concentrations were determined by DNS method (3, 5- Di Nitro Salicylic Acid method), [22]. All reported experiments were done in triplicates. Average values were calculated and recorded.

3.0 RESULTS AND DISCUSSION

A plot of glucose yield against concentration of acid used was plotted as shown in figure 1 while the graph of glucose yield against pH of Enzymes were shown in figure 2. The tables 1 and 2 show tabular presentation of the linear models (equations) generated in their respective kinetic linear graphs.

3.1 Glucose Yield

The figure 1 below shows that as the acid concentration increases, the glucose yield also increases. The highest glucose yield of 18.20 mg/ml was observed at 4 hours of hydrolysis at 60 °C while the lowest glucose yield of 10.0 mg/ml was at 30 minutes for 60 °C during acid hydrolysis. Similarly, figure 2 below shows the highest glucose yield (20.1mg/ml) served for enzyme hydrolysis was at

pH of 4.0 for 4 hours at 100 °C while the lowest yield (11.1mg/ml) was obtained at pH of 6.0 at 30 minutes duration. The results showed that the reaction was favorable at longer duration and intermediate temperature. Albeit, there was a correlation of maxima and minima glucose yield at same duration of 4 hours and 30 minutes.

3.2 Model Equation

The linear equations (Y) consist of both the slope and the intercept in the equation of straight line graph. The linear model equations determined from the graph showed that acid hydrolysis of starch had a positive energetic interaction (exothermic reaction) while the enzymatic hydrolysis of starch, all had a negative energetic interaction (endothermic reaction). Figure 1 demonstrated that glucose concentration increases as acid concentration increases. Conversely, figure 2 below showed that glucose concentration decreases as the pH increases (reaction was favorable at higher temperature and lower pH). The highest enzymatic yield was 20.1 mg/ml while the lowest enzymatic yield was 11.0 mg/ml. 18.20mg/ml and 10.0mg/ml were the respective maximum and minimum acid hydrolysis yield. These demonstrated greater glucose yield from enzymatic hydrolyzing approach.

3.3 Correlation Coefficient (R²)

The correlation coefficient determines the extent to which changes in one variable affects the other. The correlation coefficient of both figure 1 and 2 can be seen more elaborate at tables 1 and 2 respectively. The results showed that there was no negative correlation coefficient in both acid and enzymatic hydrolyses. The strongest linear relationship was obtained in table 1 for acid hydrolysis when starch was hydrolyzed at 2 hours for 80 °C and had a positive correlation value of 1 (line of best fit), while the weakest linear relationship was obtained in table 2 for enzymatic hydrolysis when starch was hydrolyzed at 4 hours for 80 °C and had a positive value of 0.715. These demonstrated that the effect of experimental variables were unstable and erratic at 4 hours for 80 °C during enzymatic hydrolysis. However, it had greater yield of glucose (14.8mg/ml) than 11.1mg/ml (lowest yield) obtained at pH of 6.0 at 30 minutes which had a R² = 0.824. The tables also showed independent behaviors of correlation coefficient among graphs and the sensitivity of linearized regression application in explaining the hydrolysis of starch using acid and enzymes. However, detailed observation indicated that enzymatic hydrolysis gave a better correlation coefficient (0.920 at 30 minutes, 0.869 at 1 hour, 0.910 at 2 hours and 0.967 at 4 hours) for all time intervals of 100 °C. These tied a correlation that higher temperature and lower pH favors glucose production during enzymatic and acid hydrolysis of starch from *Manihot Esculenta*.

3.4 Slope and Intercept

The slope and intercept of the equations shown in tables 1 and 2 can also be seen from the equation of straight line graph as per generated from their graphs. All the graphs in acid hydrolysis gave a positive slope which meant that in all the acid hydrolysis, there were positive relationships with parameters. Therefore when the acid concentration increases, the yield also increases. Furthermore, the intercept was highest (15.24) when the starch was hydrolyzed at 60 °C for 2 hours; similarly, the lowest intercept was observed when the starch was hydrolyzed at 100 °C for 1hr. Thus averagely, the intercepts of acid hydrolyses at 100 °C for 30 minutes, 1 hour, 2 hours and 4 hours gave the lowest sum of intercept and had corresponding highest yield of glucose during hydrolysis. However, since the intercepts were greater than 9.622 and less 15.25; this confirms that even very dilute acid will cause hydrolyses of starch obtained from *Manihot esculenta*. The graphs in enzymatic hydrolysis each had a negative slope respectively. This indicated an inverse relationship with the parameters (when the pH increases, the glucose yield decreases). The highest intercept for enzymatic hydrolysis was (32.7) when starch was hydrolyzed for 4 hours at 100 °C. While the lowest intercept was observed when the starch was hydrolyzed at 60 °C for 30 minutes.

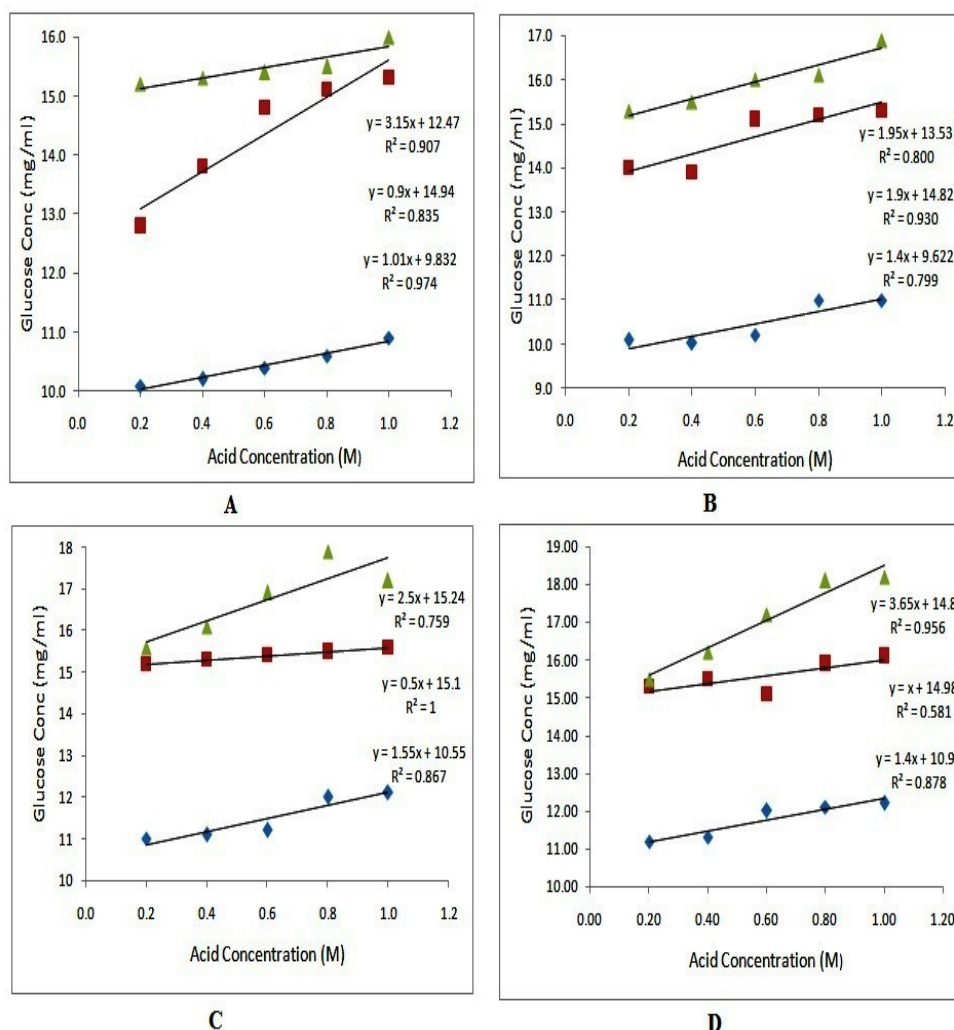


Fig 1: (L-R) Graph of glucose concentration against acid concentration at selected time intervals of (A) 30minutes, (B) 1hr, (C) 2hours, (D) 4hours

Table 1: Graphical equations table of acid hydrolysis at selected temperature and time

Acid Hydrolysis	30 minutes	1hr	2hours	4hours
60 °C	$Y = 3.15x + 12.47$ $R^2 = 0.907$	$Y = 1.95x + 13.53$ $R^2 = 0.800$	$Y = 2.5x + 15.24$ $R^2 = 0.759$	$Y = 3.65x + 14.85$ $R^2 = 0.956$
80 °C	$Y = 0.9x + 14.94$ $R^2 = 0.835$	$Y = 1.9x + 14.82$ $R^2 = 0.930$	$Y = 0.5x + 15.10$ $R^2 = 1$	$Y = 1x + 14.98$ $R^2 = 0.581$
100 °C	$Y = 1.01x + 9.832$ $R^2 = 0.974$	$Y = 1.4x + 9.622$ $R^2 = 0.799$	$Y = 1.55x + 10.55$ $R^2 = 0.867$	$Y = 1.4x + 10.92$ $R^2 = 0.878$

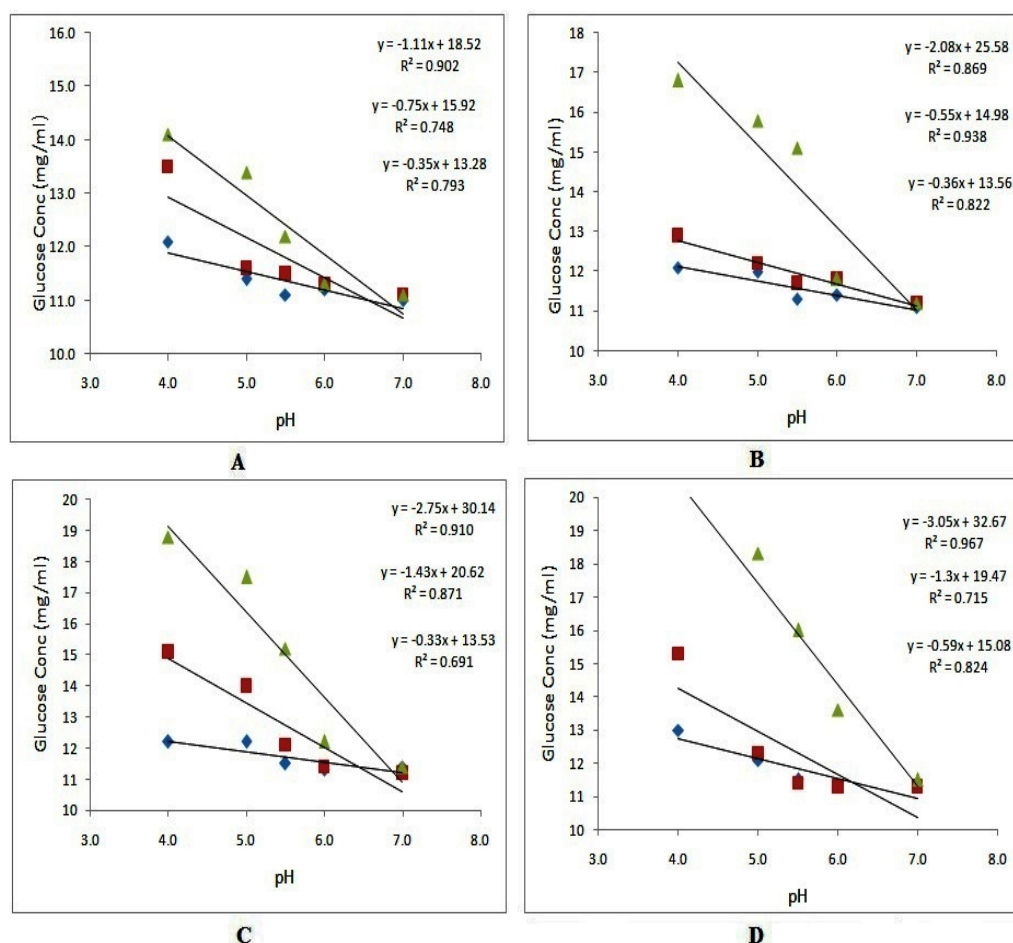


Fig 2: (L-R) Graph of glucose concentration against pH value at selected time intervals of (A) 30minutes, (B) 1hr, (C) 2hours, (D) 4hours

Table 2: Graphical equations table of enzymatic hydrolysis at selected temperature and time

Enzymatic Hydrolysis	30 minutes	1hr	2hours	4hours
60 ^o C	Y = -0.35x + 13.28 R ² = 0.793	Y = -0.36x + 13.56 R ² = 0.822	Y = -0.33x + 13.53 R ² = 0.691	Y = -0.59x + 15.08 R ² = 0.824
80 ^o C	Y = -0.75x + 15.92 R ² = 0.748	Y = -0.55x + 14.98 R ² = 0.938	Y = -1.43x + 20.62 R ² = 0.871	Y = -1.3x + 19.47 R ² = 0.715
100 ^o C	Y = -1.11x + 18.52 R ² = 0.902	Y = -2.08x + 25.58 R ² = 0.869	Y = -2.75x + 30.14 R ² = 0.910	Y = -3.05x + 32.67 R ² = 0.967

4.0 CONCLUSION

The results showed that as the acid concentration increases with higher temperature, the glucose yield also increases favoring longer duration and intermediate temperatures. The results showed that the reaction was favorable at longer duration and intermediate temperature. Furthermore, enzymatic hydrolysis gave a better correlation coefficient (0.920 at 30 minutes, 0.869 at 1 hour, 0.910 at 2 hours and 0.967 at 4 hours) for all time intervals of 100 °C. Additionally, all enzymatic hydrolysis had a negative slope respectively. This indicated inverse relationship with the parameters. The acid hydrolyses had a positive slope which meant that all the acid hydrolysis had a positive relationship with the parameters. Thus, the highest glucose yield was not the most efficient process when factors

such as temperature, pH and time are taken into consideration given that the highest glucose yield was not obtained when $R2 = 1$

REFERENCES

1. Ogbon F.C and Okafor E.N. The resistant starch content of some cassava based Nigerian foods. Nigerian Food Journal. 2015, 33: (29-34). <http://dx.doi.org/10.1016/j.nifo.2015.04.007>.
2. Amenaghawon N.A, Osagie E.I and Ogbeide S.E. Optimisation of combined acid and enzymatic hydrolysis of cocoyam starch to produce fermentable hydrolysate. Pertanika journal of science and Technology. 2016, 24 (1), 123-136.
3. Onyelucheya O.E, Nwabanne J.T, Onyeluchaya C.M and Adeyemo O.E. Acid hydrolysis of cassava peel. International journal of scientific and technology research. 2016, 5 (1), 184-187.
4. Mohammed A, Onyeleke S.B, and Egwim E.C. Pre-treatment and hydrolysis of cassava peels for fermentable sugar production. Asian Journal of Biochemistry. 2013, 1-5. <http://dx.doi.org/10.3923/ajb.2013>.
5. Nuru A.E, Alawi S, and Siti N.A.R. Enzymatic saccharification of Tapioca processing wastes into biosugars through immobilization technology. Biofuel research journal. 2014, 2-6.
6. Bayitse R, Hou X, Bjerre A.B, and Saalia K.F. Optimization of enzymatic hydrolysis of cassava peel to produce fermentable sugars. AMBExpress. 2015, 5 (50). <http://dx.doi.org/10.1186/s13568-015-0146-z>.
7. Hermiatia E, Mangunwidjajab D, Sunarti, T.C, Suparnob O, and Prasetya B. Microwave-assisted acid hydrolysis of starch polymer in cassava pulp in the presence of activated carbon. Procedia Chemistry. 2012, 4: 238-244. <http://dx.doi.org/10.1016/j.proche.2012.06.033>.
8. Bej B, Basu R.K and Ash S.N. Kinetic studies on acid catalysed hydrolysis of starch. Journal of scientific and industrial research. 2008, 67: 295-298.
9. Wasinton S, Heri S and Nuru U. Production of reducing sugar from cassava solid waste by simultaneous ultrasonication and acid hydrolysis. Industrial journal of chemistry. 2014, 14(3): 233-238.
10. Chavan R, Saxena K and Tigote D. Optimization of acid hydrolysis process for free glucose recovery from starch. International journal of innovative science, Engineering and technology. 2015, 2(12).
11. Noorhalieza A, CheAfifi C.A and Onu H. Alkali pretreatment and acid hydrolysis of coconut pulp and empty fruit bunch to produce glucose. Journal Teknologi. 2015, 74(7): 7-11.
12. Echegi U.S.C, Ejikeme P.C.N, and Ejikeme E.M. Effects of process factors on the synthesis of Bio-ethanol from cassava tubers using H_2SO_4 as catalyst. The International journal of Engineering and Science. 2013. 2(5) 1-9.
13. Guerra Diasa, A.R, Zavareza E.R, Helbig E, Moura F.A., Vargas G.C, Ciacco C.F. Oxidation of fermented cassava starch using hydrogen peroxide. Carbohydrate polymers. 2011, 86: 185-191. <http://dx.doi.org/10.1016/j.carbpol.2011.04.026>.
14. Martin C, Wei M, Xiong S and Jonsson L.F. Enhancing saccharification of cassava stems by starch hydrolysis prior to pre-treatment. Industrial crops and Products. 2017. 97: 21-31. <http://dx.doi.org/10.1016/j.indcrop.2016.11.067>.
15. Bayitse R, Hou X, Bjerre A.B, and Saalia F.K. Optimization of enzymatic hydrolysis of cassava to produce fermentable sugars. AMBExpress. 2015, 5(60): 1-7. <http://dx.doi.org/10.1186/s13568-015-0146-z>.
16. Garba M.U, Mohammed A and Etim E.D. A kinetic study of the enzymatic hydrolysis of cassava starch. International journal of science and Engineering investigations. 2012. 1(11): 65-70.
17. Ayoola A.A, Adeeyo O.A, Efeovbokhan V.C and Ajileye O. Comparative study on glucose production from sorghum bicolor and *Manihot Esculenta* species in Nigeria. International Journal of Science and Technology. 2012. 2(6): 353-357.
18. Akinola S.O and Ayanleye T.A. The use of fungal glucoamylase enzyme for the production of glucose syrup from cassava starch. Acta SATECH journal of Lie abd Physical science. 2004. 1(2): 138-141.
19. Putra P.H, Sari Y.D, Riyanto and Awaluddin N. Time optimization and enzyme dosage in the process of hydrolysis of cassava peel waste. The 4th Asia-Oceania conference on green and sustainable chemistry. November 2013, 3-6. at New Taipei city hall, new taipei city, taiwan.

- 229 20. Ramachandran V, Pujari N, Matey T, and Kulkarni S. Enzymatic hydrolysis for glucose. A
230 Review. Internatioanl journal of science, Engineering and Technology research. 2013. 2(10):
231 1937-1942.
- 232 21. Aderibigbe F.A, Adejumo A.L, Owolabi R.U and Anozie A.N. Optimization of enzymatic
233 hydrolysis of *Manihot Esculenta* root starch by alpha -Amylase and Glucoamylase using
234 response surface methodology. Chemical and Process Engineering research. 2013. 9: 14-22.
- 235 22. Ayoola A.A, Adeeyo O, Efeovbokhan C.V, and Olasimbo A. Optimum hydrolysis conditions of
236 cassava starch for glucose production. International journal of advanced research in IT and
237 Engineering. 2013. 2(1): 93-101.