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Optimization of glucose syrup production process using sabrang potato starch (Coleus tuberosus Benth) by hydrolysis enzimatic

Yunianta, P.Widya Dwi Rukmi, Suburi Rahman*

Departemen of Agricultural Technology, Faculty of Agricultural Technology, Brawijaya University, Malang, (INDONESIA)

E-mail : suburi_rahman@yahoo.com

ABSTRACT

The enzymatic hydrolysis of starch is one of many options in the food and beverage industry in particular in the manufacture of glucose syrup. In this study the effect of time and the use of saccharification enzyme glucoamylase concentrations studied and optimized to produce glucose syrup from sabrang potato starch (Coleus tuberosus Benth). There are two variables used in this study with three levels in each variables. The first variable is saccharification time which are 12, 24 and 36 hours. The second variable is glucoamylase concentrations which are 3.00, 4.50 and 6.00 mg / ml. The results in the hydrolysis of starch potato sabrang enzymatically analyzed using Design Expert software version 7. Statistical significance of the model is validated by the F-test for analysis of variance ($p \leq 0.05$). The results showed that the optimum conditions for saccharification time of 27 hours 49 minutes, and glucoamylase concentration of 4.96 mg / ml. The maximum production at optimum conditions produced glucose levels by 22.69%, with the value of dextrose equivalent as much as 89.11% and produced 75.63% of glucose.

KEYWORDS

Hydrolysis;
Saccharification;
Glucose;
Dextrose equivalent.

INTRODUCTION

Glucose syrup production continues to grow along with the increasing development of the food and beverage industry. Sugar products are widely used as a sweetener, the fermentation process, and raw materials such as various derivatives sorbitol, maltitol, and so on[16]. Development of science and technology has successfully revealed the importance and potential materials containing starch as a raw material in the glucose syrup industry. One of the tuber starch has great potential to be developed into glucose syrup is sabrang potato starch, because it contains up to 20% carbohydrates (mainly starch)[11].

Glucose syrup, or glucose liquid is clear and viscous liquid, with a major component of glucose derived from starch hydrolysis[15], the sweetness intensity of 80[5]. Glucose syrup is the result of hydrolysis of starch that can be done either acid[2,7], or enzymatically[1,10]. Production of glucose syrup by enzymatic hydrolysis occurs through two important phases, the first phase of the liquefaction and the second phase is saccharification[17]. Liquefaction is the process of starch hydrolysis to convert it into smaller molecules than oligosaccharides using α-amylase enzyme to produce maltodextrin, while saccharification is a further hydrolysis process

Regular Paper

where maltodextrin as a result of the liquefaction stage is hydrolyzed and convert into glucose by glucoamylase[4,13].

Several factors may affect glucose results obtained on liquefaction and saccharification processes which are factors of temperature, pH, substrate concentration, enzyme concentration and hydrolysis time. This research aims to study the hydrolysis of sabrang potato starch into glucose syrup to get the maximum glucose syrup, the use of enzyme concentration and hydrolysis time were investigated as an important factor in the process of making glucose syrup from sabrang starch potato. The effect of enzyme concentration and hydrolysis time optimized to determine the condition of a good process in the manufacture of glucose syrup from sabrang starch potato. Response surface method is one of the techniques used to understand how far an optimum process is influenced by variables with experimental data and require little time and cost in a study, the ultimate goal of this method is to optimize the response[8].

MATERIALS AND METHODS

Materials used in this study is sabrang potato (Coleus tuberosus Benth), which were obtained from local farmers in Lombok West Nusa Tenggara Province, Indonesia. A-amylase enzyme Liquozyme Supra brand with activity of 19.07 U / ml and Novozyme A 9913 glucoamylase Cheme Germany with activity of 9.63 U / ml, which is obtained from the Laboratory of Biochemistry in Faculty of Agricultural Technology in Brawijaya University, Malang - Indonesia. Distilled water, $CaCO_3$, NaOH 1N, 0.1N HCl, $NaCO_3.H_2O$, H_2SO_4, rochelle salt, sodium bicarbonate, sodium sulfate anhydrous, $CuSO_4$, $CuSO_4.5H_2O$, ammonium molybdat, Na_2HASO_4 and dinitrocalycylic acid (DNS). The tools which used are the grinding machine, blender, sieve size 100 mesh, knives, water bath shaker, centrifuge, electric oven, and a pH meter.

Preparation of raw materials

Potato tubers sabrang sorted, cleaned with water, peeled, then washed with running water. Bulbs then milled and screened, after mixed with water in the ratio 1:5 (1 Kilogram tuber pulp mixed with 5 liters of water). Screening results deposited 10 hours. Starch deposition results rinsed again with water and precipitated for 3 hours. Dried starch deposition results using the electric oven at a temperature of 60 ° C for 5 hours, then on the rollers and on the 100 mesh sieve. Starch is stored in a sealed plastic to be used as raw material glucose syrup.

Sabrang potato starch hydrolysis enzymatically

7.5 grams of sabrang potato starch added into 25 mL volumetric flask, added distilled water to mark boundaries, homogenized and pH set to 5,3. Gelatinization of starch at 90 °C for 10 minutes, then liquefaction with α-amylase enzyme. After the saccharification, first should be cooled the result solution from liquefaction and adjusted pH to become 4,5. Saccharification process at a temperature of 60 °C with glucoamylase concentration (3.00; 4.50 and 6.00 mg / ml) and time (12; 24 and 36 hours). Glucose syrup results later in the centrifuge at 2,500 rpm for 15 min, then performed an analysis dextrose equivalent (Lane Eynon method in the International Starch Institute, 1999) and glucose levels[12]. Glucose yield calculations performed on the average percentage of the final results obtained by optimization.

Research design

This study uses the steepest slope with two factors as the initial step of determining the center of time and the use of saccharification enzyme concentration (unpublished data) and continued with CCD optimization using response surface method. Optimized variables consisted of two factors, namely saccharification time (hours) and glucoamylase concentration (mg / ml), the results of the response in the form of glucose (%) and dextrose equivalent (%). A highs and lows factors of real variables encoded by +1 and -1, whereas the midpoint coded with 0. Independent variable in this study is the time (x1) and enzyme concentration (x2) encoded by the level presented in TABLE 1. Saccharification process optimization phase of the program Design Expert computerized system has 13 treatment that is time and concentration glucoamylase saccharification. Response data dextrose equivalent glucose levels and is used to analyze the optimization process of making glucose syrup saccharification of sabrang potato starch based variable saccharification enzyme concentration and time.

TABLE 1 : Variable code and the actual value of the research variables

Variable	Parameter	Level Code		
		-1	0	+1
X_1	Saccharification time (hour)	12	24	36
X_2	Enzyme concentration (mg/ml)	3,00	4,50	6,00

RESULT AND DISCUSSION

This study investigated the saccharification process for obtaining the maximum glucose syrup. Responses were used in this study is the level of glucose and dextrose equivalent. Saccharification is an advanced stage of the liquefaction process, which is the process of bond breaking α - 1, 4 and α - 1, 6 glucoside to produce glucose from starch using glucoamylase. Hydrolysis saccharification process is the final stage in the manufacture of glucose syrup and is the most important stages in producing glucose. According Endah et al.[6], saccharification is a very important milestone for producing high glucose, so that the process conditions such as the use of enzymes for saccharification determine glucose results to be obtained. Optimization is performed to determine the optimum time points saccharification is needed and how much the use of the enzymes needed to produce the maximum glucose. Combination treatment saccharification process optimization results glucose response (Y1) and dextrose equivalent value (Y2) from the central composite design are presented in TABLE 2.

The data in TABLE 2 shows that the treatment center point, which is 24 hours of saccharification time and glucoamylase concentration 4.50 mg / ml glucose response and produce dextrose equivalent value is best compared with the response to other treatments. Response data and dextrose equivalent glucose obtained was used in the statistical analysis to optimize the process variables of time and concentration of glucoamylase saccharification required. Prediction equations appropriate optimization models obtained with the help of the program Design Expert Version 7.

Polynomial equations to model predictions glucose response of multiple regression analysis of experimental data response, the polynomial equation glucose response shaped quadratic model with the form of the equation Y1 = 21.97 + 1.43 x1 + 1.12 x2 - 0.81 X1X2 - 1, 86 x12 - 1.39 x22.... (1), while for response model predictions dextrose equivalent polynomial equation multiple regression analysis of experimental data response, the response polynomial equation dextrose equivalent quadratic model with the form of the equation Y2 = 88.10 3.45 + 2.95 x1 + 0.29 x2 + X1X2 - 5.16 x12 - 5.97 x22..... (2), where x1 and x2 is the value of variables tested code each time saccharification (hours) and glucoamylase concentration (mg / ml). Quality of the quadratic model response and dextrose equivalent glucose levels determined by analysis Sequential Model Sum of Squares, Lack of Fit Test and Model Summary Statistics.

The results of the three model selection process that has been done, the best model for the response surface glucose and dextrose equivalent is quadratic model and then performed analysis of variance for the quadratic model. Results of analysis of variance (ANOVA) of the program Design Expert for response to glucose levels can be seen in TABLE 3, while for dextrose equivalent responses are presented in TABLE 4.

TABLE 2 : Response data optimization saccharification process central composite design

Saccharification Variables		Variable Code		Respond	
Time (hour)	Enzyme Concentration (mg/ml)	x_1	x_2	Glucose Level (%)	Dextrose Equivalent (%)
36	3,00	1	-1	19,4524	75,8713
12	3,00	-1	-1	15,2017	70,6875
36	6,00	1	1	21,0375	86,1336
12	6,00	-1	1	20,0288	79,7749
24	4,50	0	0	20,8213	84,1120
24	4,50	0	0	21,4697	87,2820
24	4,50	0	0	21,0375	86,0448
24	2,38	0	-1,414	18,0836	73,5225
24	6,62	0	1,414	19,8847	76,5193
40,97	4,50	1,414	0	20,2450	82,3251
7,03	4,50	-1,414	0	15,8501	70,9482
24	4,50	0	0	22,6225	88,5274
24	4,50	0	0	22,8386	88,8223

Results of analysis of variance TABLE 3 shows the quadratic model treatment time and concentration of glucoamylase saccharification a significant influence on the response of glucose levels while the interaction was not significant. The F-test proving quadratic model saccharification time and concentration of glucoamylase has a high F value which is 21.95 times the value of the

Regular Paper

variable F and F value of variable concentrations is 12.27. Quadratic model has a smaller p-value of 0.0045 is less than 0.05, which means that the model is significant at the 95% confidence level. According to Baskar et al., (2008), the higher the level of significance of a model indicates that the model is very valid in determining the value of the response. Thus, in this study it can be concluded that the treatment time and concentration of glucoamylase saccharification has a high accuracy of the glucose response at the level of 95%. The model has a coefficient of determination R2 0.9085 (90.85%), this value is quite good value in describing the glucose response model. As expressed by Peatciyammal et al.[14], the R2 value for the model describing the response is the value of R2 is more than 80%. Adj R-Squared value of 0.8322, indicating that the model obtained in this study is quite good and from Adj R-Squared values obtained show that 83% of the total variation in glucose levels is determined by the independent variable and the remaining approximately 17% of total variation can not be explained by the model. Quadratic model to demonstrate appropriate response to glucose levels significantly. Second order polynomial equation of the glucose response Design Expert program code in the form of variables that equation (1). The actual equation is an equation that is needed to study the response of glucose levels that would be obtained if the value of the variable is treated differently. From the equation above shows the coefficient of x12 and x22 is negative, it indicates that the maximum stationary point of the response surface. Negative value of the coefficient of x12 and x22 squared variable indicates the maximum obtained quadratic pattern with form graph a parabola that opens downward. Saccharification time treatment, and different concentrations of glucoamylase give a very significant effect on the increased levels of glucose in which the amount of glucose levels ranged from 15.20% to 22.83%. Saccharification time of 24 hours and the use of glucoamylase concentration of 4.50 mg / ml is the optimum point saccharification process is done because it produces the highest glucose levels which amounted to 22.83%. Graphs the interaction effect between time and concentration glucoamylase saccharification to glucose response is presented in Figure 1.

Lack of Fit Test results obtained F value of 4.16 while the p-value 0.1055 which indicates that the inaccuracy of the model is not significant to the pure error and is considered as an appropriate model equations. The model has a coefficient of determination R2 0.9058 (90.58%) which is a pretty good value in describing a process response model. Baskar et al.[3] suggest that to describe a good response then the minimum value for R is 80%, the coefficient of determination is more than 80% is a good value for a response model describes the results of research[14], while Adj R-Squared values obtained at 0.8590, suggesting that the model is very good and from Adj R-Squared value indicated that 85% of the total variation of dextrose equivalent is determined by the independent variables and only about 15% of total variation that can not be explained by the model.

TABLE 3 : Analisa ragam (ANOVA) respon kadar glukosa

Source	Sum of Squares	df	Mean Squares	F Value	P-value prob>F	Description
Block	5,33	1	5,33			
Model	53,36	5	10,67	11,91	0,0045	significant
A-Waktu	16,46	1	16,46	18,37	0,0052	
B-Konsentrasi	10,03	1	10,03	11,20	0,0155	
AB	2,63	1	2,63	2,93	0,1377	
A^2	19,67	1	19,67	21,95	0,0034	
B^2	10,99	1	10,99	12,27	0,0128	
Residual	5,38	6	0,90			
Lack of Fit	1,98	2	0,99	1,17	0,3987	not significant
Pure Error	3,39	4	0,85			
Cor Total	64,06	2				

Description: A = variable x1 (saccharification time), B = x2 variables (enzyme concentration); AB, A^2, B^2 = interaction between variables

TABLE 4 : Analysis of variance (ANOVA) dextrose equivalent response

Source	Sum of Squares	df	Mean Squares	F Value	P-value prob>F	Description
Block	42,01	1	42,01			
Model	443,17	5	88,63	11,53	0,0049	significant
A-Waktu	95,44	1	95,44	12,42	0,0125	
B-Konsentrasi	69,55	1	69,55	9,05	0,0237	
AB	0,35	1	0,35	0,045	0,8392	
A^2	152,18	1	152,18	19,80	0,0043	
B^2	203,55	1	203,55	26,49	0,0021	
Residual	46,11	6	7,68			
Lack of Fit	31,13	2	15,56	4,16	0,1055	not significant
Pure Error	14,98	4	3,74			
Cor Total	531,28	12				

Description: A = variable x1 (saccharification time), B = x2 variables (enzyme concentration); AB, A2, B2 = interaction between variables

Regular Paper

TABLE 5 : Results of analysis and prediction of glucose and dextrose equivalent optimum solution point selection process optimization using design expert program

Saccharification Time (Jam)	Enzyme Concentration (mg/ml)	Prediction		Experimental Result	
		Glucose Level	Dextrose Equivalent	Glucose Level	Dextrose Equivalent
27, 49	4 96	22,36	89,05	22,69	89,11

Quadratic model fit to demonstrate equivalent dexrose response significantly. Second order polynomial equation dextrose equivalent of Design Expert program code in the form of variables that equation (2). The equation looks x12 and x22 coefficient is negative, it indicates that the maximum stationary point of the response surface. Negative value of the coefficient of x12 and x22 squared variable indicates the maximum obtained quadratic pattern with form graph a parabola that opens downward. Dextrose equivalent response best results obtained on treatment for 24 and saccharification enzyme concentration 4.50 mg / ml with dextrose equivalent value of 88.82%, this is an optimal point of treatment time and concentration glucoamylase saccharification. The relationship between time and concentration glucoamylase saccharification of the dextrose equivalent response is presented in Figure 2.

Optimization is based on the analysis of each response to treatment. Model analysis of the significant response, further optimized to obtain the optimum treatment. Optimization of saccharification process of treatment time and concentration of glucoamylase were statistically analyzed using Design-Expert software version 7. Variable time points saccharification and optimum concentration of glucoamylase that provide optimal results and dextrose equivalent glucose levels are determined on the response surface curves in Figure 3. Solution points saccharification optimum time and concentration glucoamylase Design Expert program computerized results and the predicted value and the response analysis results obtained from the treatment are presented in TABLE 5.

The results of the optimum point solutions saccharification process TABLE 5 showed that glucose levels by 22.36%, while the results of experiments by 22.69%. As for the prediction of maximum dextrose equivalent of 89.05%, while the experimental results by 89.11%. The correlation coefficient between the experimental values and the predicted maximum value of the program, namely: glucose levels have a correlation coefficient of 1.0147 and has a dextrose equivalent value of the correlation coefficient of 1.0006. The correlation

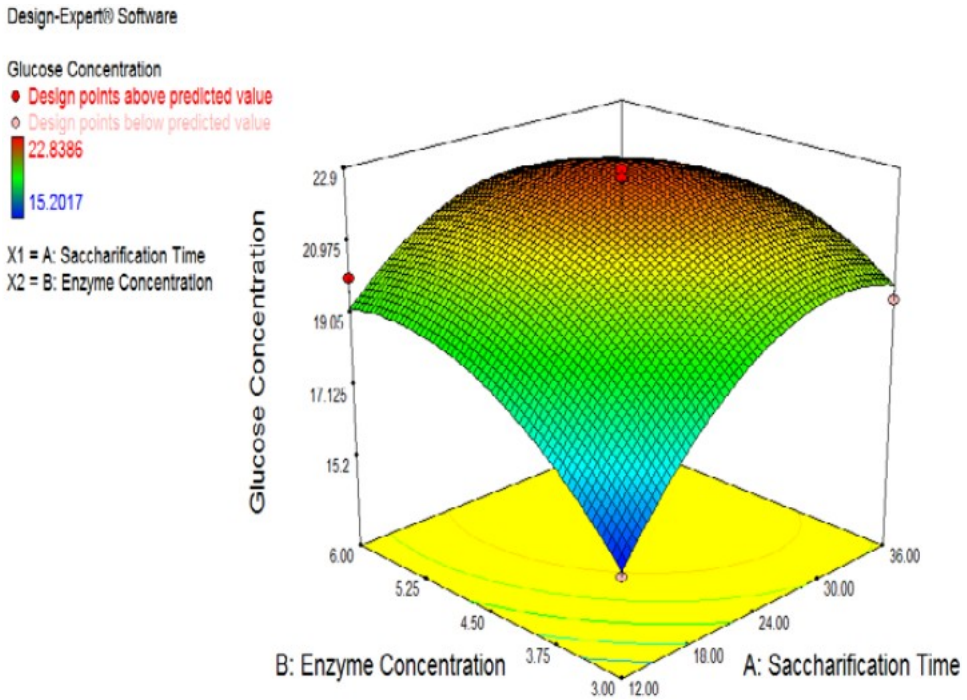

Figure 1: Response surface plot of the relationship between time and concentration glucoamylase saccharification to glucose response of enzymatic hydrolysis of sabrang potato starch

Regular Paper

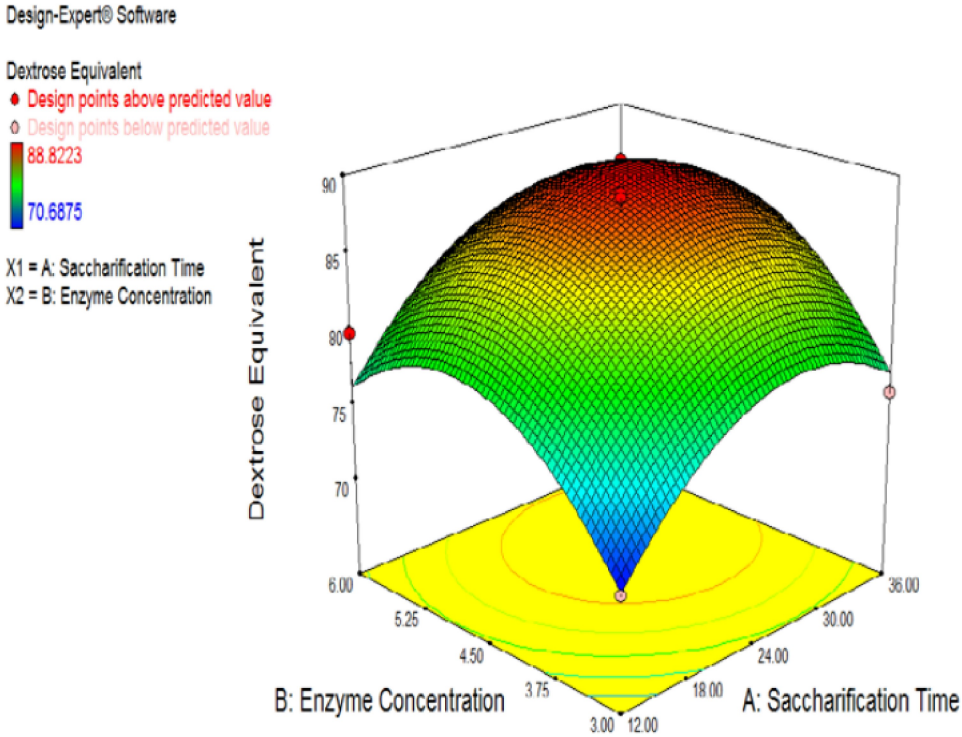

Figure 2 : Response surface plot of the relationship between time and concentration glucoamylase saccharification response to dextrose equivalent enzymatic hydrolysis of sabrang potato starch

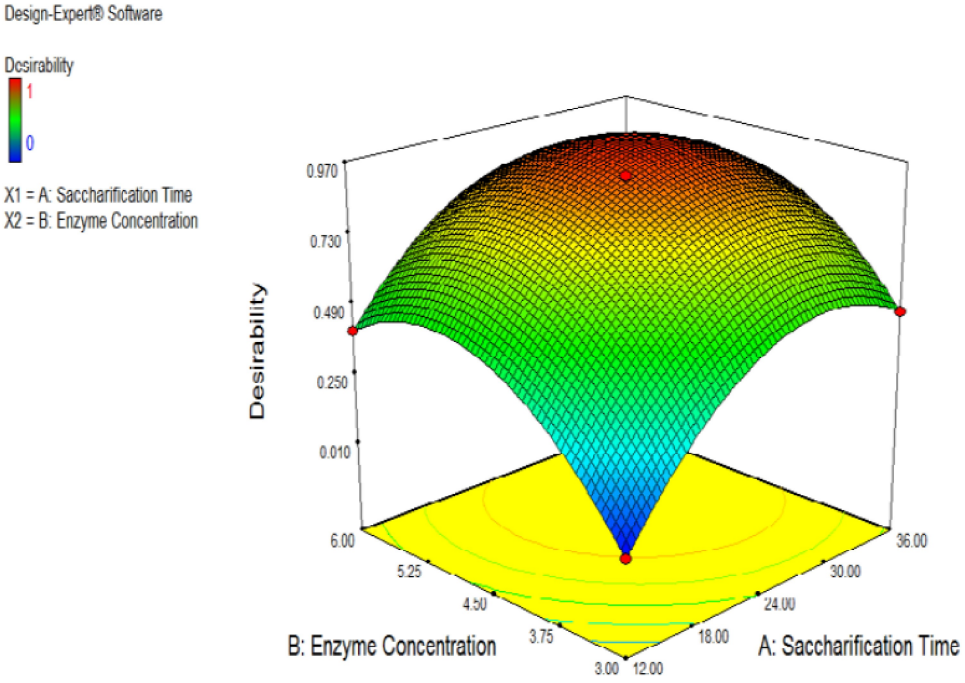

Figure 3 : Response surface plot of the optimization process and the relationship between time saccharification enzyme concentration on the response of dextrose equivalent glucoamylase enzymatic hydrolysis of sabrang potato starch

coefficient is not statistically significantly different at the 95% confidence interval between the experimental results with predicted values program. These results indicate that the relative value of the independent variable corresponding to yield an optimal response, in accordance with the experimental value of predictive value program, otherwise regression models accurate and suitable for the saccharification process.

Glucose yield value in this study was calculated based on the results of the validation process of saccharification after optimization. The results of calculations yield values of the process of producing glucose

syrup from sabrang potato starch is 75.63%, which is a fairly high value in the manufacture of glucose syrup.

CONCLUSION

Calculations using response surface method able to reveal an efficient saccharification process in the manufacture of glucose syrup from sabrang starch potato. Optimal conditions of saccharification process occurs in a time of 27 hours 49 minutes with as much glucoamylase concentration of 4.96 mg / ml glucose response with the result of 22.69%, 89.11% of dextrose equivalent, as well as the value of glucose level which is quite high at 75.63%.

REFERENCES

[1] A.F.Aderibigbe, A.N.Anozie, L.A.Adejumo, R.U.Owolabi; Optimization of Cassava Starch Hydrolysis by Sorghum Malt. New Clues in Sciences, **2**, 50-58 **(2012)**.

[2] A.N.Anozie, A.F.Aderibigbe; Optimization studies of Cassava starch hydrolysis using respon surface method, New Clues Science, **1**, 37-43 **(2011)**.

[3] G.Baskar, C.Muthukumaran, S.Renganathan; Optimization of Enzymatic Hydrolysis of Manihot Esculenta Root Starch by Immobilized α-Amylase Using Response Surface Methodology. International Journal of Chemical and Biological Engineering, **1(3)**, **(2008)**.

[4] F.Bastian; Starch and Sugar Technology. Textbook Writing Grant For Academic Staff Hasanuddin University. Departement of Food Science and Technology. Faculty of Agriculture. Hasanuddin University. Makasar, **(2011)**.

[5] M.A.Clarke; Sugar in Food Processing. International Sugar Journal, **99(1178)**, 114-118 **(1997)**.

[6] R.D.Endah, K.A.Enny, Fadilah; Preliminary Study of Simultaneous Saccharification and Fermentation reactions Flour Sorghum (Sorghum Bicolor L Moench) With Catalysts Enzymes glucoamylase and yeast (Saccharomyces cerevisiae). Equilibrium, **8(2)**, 7-11, July **(2009)**.

[7] S.Fairus, Haryono, A.Miranthi, A.dan Aprianto; Effect of HCl concentration and hydrolysis time to the acquisition of glucose that generated From Jackfruit seeds starch. Proceedings of the National Seminar on Chemical Engineering "Kejuangan" Technology Development for the Chemical Processing of Natural Resources Indonesia, 2010, ISSN, 1693 – 4393 **(2010)**.

[8] V.Gasperz; Engineering Analysis in Research. Publisher Tarsito Bandung, **(1992)**.

[9] ISI 27-1e Determination of Reducing Power and DE by Lane and Eynon's method. International Starch Institute, Science Park Aarhus, Denmark, **(1999)**.

[10] A.Kunamneni, S.Singh; Response Surface Optimization of Enzymatic Hydrolysis of Maize Starch for Higher Glucose Production. Biochemical Engineering Journal, **27(2)**, 179-190 **(2005)**.

[11] A.Leksonowati, Witjaksono; Morphogenesis on leaf, petiole, and stem segments black potato (Solenostemon rotundifolius (POIR) MORTON JK) by in vitro. Sustainable biological research: Research Center for Biology, Indonesian Institute of Sciences, **16**, 161–167 **(2011)**.

[12] G.L.Miller; Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, ISSN 0003-2700, **31(3)**, 426-428 **(1959)**.

[13] A.Nahri; Effect of Enzyme Loading and Time on Production of Sugar from Tapioka Starch Using Enzymeatic Hydrolysys: A Statistical Approach. A dissertation submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology) Faculty of Chemical & Natural Resources Engineering University Malaysia Pahang, **(2009)**.

[14] N.Peatciyammal, B.Balachandar, M.D.Kumar, K.Tamilarasan, C.Muthukumaran; Statistical Optimization of Enzymatic Hydrolysis of Potato (Solanum tuberosum) Starch by Immobilized α-amylase. International Journal of Chemical and Biological Engineering, **3(3)**, **(2010)**.

[15] SNI 01-2978-1992, Quality standards of glucose syrup Indonesia, **(1992)**.

[16] I.N.Widiase, Wisjnuprapto, I.G.Wenten; Kinetics bioreactor membrane enzymatic saccharification starch result for liquefaction. Proceeding of the National Seminar Chemical Engineering and Process. ISSN : 2004, 1411–4216 **(2004)**.

[17] D.W.S.Wong, G.H.Robertson, C.C.Lee, K.Wagschal; Synergistic Action of Recombinant a-Amylase and Glucoamylase on the Hydrolysis of Starch Granules. The Protein Journal, **26(3)**, **(2006)**.