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Optimization of process condition for laboratory scale enzymatic conversion of cassava starch to glucose syrup

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Abstract

Enzymatic hydrolysis of cassava starch for producing glucose syrup was evaluated using alpha-amylase (Novo's Termamyl 120L) and glucoamylase (Novo's AMG 300L). The cassava roots were detoxified, sliced and blended with deionized water forming a suspension of 35% (w/v) of the extracted cassava bringing the total weight of slurry to 120 g. The slurry was adjusted to different pH ranging from 5 to 8 (i.e. 5, 6, 7, 8) by the addition of varying drops of 1 N NaOH. The suspension was maintained under heating, increasing the temperature in 1 °C/minute, until the starch was completely dissolved. Calcium was added using calcium hydroxide (Calcium ions stabilizes the enzyme). After, it was cooled down, following stirring of the slurry for approximately 3 min, the slurry was subjected to enzymatic liquefaction carried out by addition of varying doses of 3.5ml, 7.0ml, 10.5ml and 14ml thermostable α-amylase per 120g of cassava slurry (venzyme/wfresh mash) and instantaneously heated to 100 ºC and held at this temperature for 10 min before it is cooled to 90 ºC and incubated in a water bath at this temperature for 2 hours to further hydrolyze the starch for total liquefaction and production of maltodextrin with a dextrose equivalent (DE) between 12 and 15. The resultant maltodextrin was further subjected to saccharification process in order to obtain a glucose syrup (GS) after adjusting its pH to varying levels (4.2, 4.4, 4.6, 4.8) for each saccharification experiment by adding varying proportions of 0.2 M of acetate buffer and the solution was cooled to 60 ºC. Varying concentration of previously produced and characterized glucoamylase solution (25, 50, 75, and 100ml) was added respectively and incubated at 60°C) for 48 hours under constant agitation. The glucose production was monitored using the glucose oxidase method. DE was achieved in varying degrees that ranged from 12.1±4.9 to 73.9±7.6. After this process, the resulting syrup was purified by ion exchanged chromatography using Amberlite Ira 120 (Cationic resin) and Amberlite Ira 410 (Anionic resin) and then concentrated by evaporation under reduced pressure. All experiments were carried out in duplicate and analysis of variance (ANOVA) using central composite experimental design with comparison test at $p \leq 0.05$ was used to measure the effect of changing variables among treatments. Correlation Pearson's test were applied to measure the strength of the interactions between the variables. Enzyme conditions for starch hydrolysis were optimized by a factorial experimental design (4x4x4=64) using various values of pH, reaction time and enzyme concentration. Analysis show that enzymes used in this investigation possess some remarkable properties which include quantitative conversion of starch to glucose. The starch extracted from cassava after complete liquefaction by alpha-amylase produced high starch conversion to maltodextrin syrup furnishing 105.2±1.3ml at enzyme concentration of 14ml, pH value of 7 and liquefaction time of 2 hours. Liquid glucose produced with the pure glucoamylase from Aspergillus niger revealed a dextrose equivalent of 73.9±7.6 at pH value of 4.6 and 150ml enzyme/105.2±1.3ml Maltodextrin at the end of the saccharification process. Cassava starch exhibited good potential as substrates for glucose syrup production.

Keywords: glucose syrup; enzymatic hydrolysis; alpha-amylase; starch slurry; glucoamylase, aspergillus Niger

Introduction

The high demands for sugars and the development of enzymatic technology have increased the production of sweeteners, especially for glucose and fructose syrups. Enzymatic processes permit natural raw materials to be upgraded and finished. Starch syrup is a purified and concentrated product obtained as a result of the processing of starch from cassava. Starch syrup is widely used in the food industry and cooking as a thickener and sweetener. The production of starch syrup occurs through enzymatic hydrolysis of cassava starch with subsequent purification. Dextrose syrups make the basis of production of all kinds of confectionery articles. Without application of glucose syrup it is impossible to produce fondant and icing, marshmallow and marshmallow sticks, fruit jelly and halvah, nougat and many other articles. Maltodextrins (MD) are enzymatic and/or acid hydrolysis products of starch, consisting of a-(1, 4) linked D-glucose oligomers and/or polymers, which are

normally defined as having a dextrose equivalent (DE) value < 20. They are commonly used as spray-drying aids for flavours and seasonings, carriers for synthetic sweeteners, texture providers, fat replacers, film formers, and bulking agents in the food industry (Chronakis, 1998).

The low-conversion glucose syrup is a product of a limited starch hydrolysis with the content of reducing substances (including dextrose equivalent) equal to 26-35%.

The low-conversion glucose syrup is to be characterized by a high viscosity, by a binding capability and anti-crystallization ability, by a low sweetness. The low-conversion glucose syrup can be applied in a confectionery production as an anti-crystallization agent, sweetness regulator, binding agent and foaming agent. Low glucose content allows reducing a hygroscopicity of confectionery articles and heightening their shelf life and that is especially important for boiled sweets. Appropriate binding properties of the low-conversion glucose syrup allow using it rather efficiently in a construction industry instead of phenol-formaldehyde resins by a forming of some types of construction materials. The starch high-conversion glucose syrup, with the content of reducing substances equal to 45% and that of glucose - more than 30%, has a heightened sweetness and reduced viscosity comparing with other types of glucose syrup. These properties are responsible for its application in the production of soft confectionery fillings, jams, toppings, sauces and ketchups. The conversion of starch to various sweeteners is achieved through a chemical (acid) or an enzymatic process. (Yankov *et al*., 1986) [10]. Glucose, an important industrial product of starch hydrolysis finds application as bulk sweetener in the food pharmaceutical (Aboje, 2007) and confectionary industry (Fox and Cameron, 1982 [4]. The production of glucose, maltose and dextrins from starch of maize, banana (Igoe, 1989; Bello-Perez *et al*., 2002) [6] cassava (Aboje, 2007) and sweet potato (Omemu *et al*., 2004) [7] has been well documented in many parts of the world. However, production of these important products of starch hydrolysis in Nigeria has been largely obtained from starch of tubers such as cassava whose cultivation is in large scale in the Southern part of the country (Aboje, 2007). Gelatinization of starch is necessary to increase the surface attack for the liquefying enzymes. In the conventional liquefaction process, cassava starch slurry is heated to 100 °C in a water bath for 10 min with heat stable α-amylase and then the mixture is cooled to 90°C and incubated at this temperature for 2hours. Even if as reported by Rickard *et al*. (1991) that cassava starch has the lowest gelatinization temperatures (66-73 °C) among tuber starches, cassava starch susceptibility to enzyme attack is influenced by several factors, such as amylose and amylopectin content, crystalline structure, particle size and the presence of enzyme inhibitors. Adejumo *et al*., (2011) reported that among these factors, granular structure is believed to be the most important: cassava starch granules are dispersed or gelatinized in aqueous solution during liquefaction and mildly exo-corroded under thermostable α-amylase treatment.

This study is aimed at the production of glucose syrup by the enzymatic hydrolysis of cassava starch and determining some optimum conditions for achieving the highest yield, which includes values of pH for liquefaction and saccharification processes, enzyme-substrate reaction temperature and enzyme concentration (alpha-amylase/cassava slurry ratio and glucoamylase/maltodextrin ratio).

Materials and Methods

Raw Materials

Cassava roots were collected directly from a rural market at Olosha, in Mushin Local Government area of Lagos State. The roots were peeled and cut with a knife. They were mixed together and grated into small sticks with Kenwood Chef Major Titanium KM020 and Vegetable Processor AT340 supplied by the Pilot Plant section of the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos.

Heat-stable alpha-amylase (Novo's Termamyl 120L) and glucoamylase from Aspergillus niger used in this research work were supplied by the Biotechnological Department of the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos-Nigeria.

Liquefaction Stage

A suspension of 35% (w/v) previously extracted cassava starch was prepared and the pH adjusted between 5.0 and 8.0 by the addition of varying drops of 1 N NaOH and stirred. The suspension was maintained under heating, increasing the temperature in 1 °C/minute, until the starch was completely dissolved. Calcium was added using calcium hydroxide (Calcium ions stabilizes the enzyme). After, it was cooled down, volume ranging from 3.5ml to 14ml of heat-stable commercial alpha-amylase (Novo's Termamyl 120L)/120g of fresh mash was mixed into the slurry and instantaneously heated to 100 ^{o}C and held at this temperature for 10 min before it is cooled to 90 oC and incubated in a water bath at this temperature for 2 hours to further hydrolyze the starch for total liquefaction. At the end of this step, the starch was converted to dextrins with a dextrose equivalent (DE) between 12 and 15. (DE is the total reducing sugar in the syrup expressed as dextrose on a dry weight basis).

Saccharification Stage

After liquefaction, the resulting maltodextrin syrup had its pH adjusted to values ranging between 4.2 and 4.8 with 0.2 M of acetate buffer and the solution was cooled to 60 ^{o}C. Varying concentration of previously produced and characterized Glucoamylase (Novo's AMG 300L) solution (25, 50, 75, and 100ml) was added respectively and incubated at 60 °C for 48 hours under constant agitation. Glucoamylase releases single glucose units from the ends of dextrin molecule so the glucose production was monitored using the glucose oxidase method. After this process, the resulting syrup was purified by ion exchanged chromatography using Amberlite Ira 120 (Cationic resin) and Amberlite Ira 410 (Anionic resin) and then concentrated by evaporation under reduced pressure.

The dextrose equivalent (DE) was calculated as described by Whitehurst and Law (2002) using the equation: DX = % glucose × 1. 0 + % maltose × 0.5 + % maltotriose × 0.33

The carbohydrates profile (fructose, glucose, maltose, maltotriose and polysaccharides) were determined by high performance liquid chromatography HPLC using a Waters HPLC, USA according to Abdel – Aal *et al*., 1993 [1].

Statistical analysis

All experiments were done in duplicate and analysis of variance (ANOVA) with central composite experimental design with comparison test at $p \leq 0.05$ was used to measure the effect of changing variables among treatments. Correlation Pearson's test were applied to measure the strength of the interactions between the variables. The software excel 2016 was also used.

Results and Discussion

Acid hydrolyzed cassava root slices used for the study showed a DE close to 80 and 37% glucose on dry weight (w/dw). The first part of the process aimed at obtaining MD with DE < 20 designed under 64 experimental treatments (4x4x4=64) using various values of pH, reaction time and enzyme concentration.

Alpha-amylase enzyme was not very effective in the conversion of starch syrup to maltodextrin at pH below 6 and above 7 respectively.

All in all, glucoamylase enzyme of Aspergillus niger did not perform excellently probably because it contains amyloglucosidase, which hydrolyses only the α–1,4 glucosidic linkages and not capable to hydrolyze the α–1,4 as well as α–1,6 linkages and hence less effective for saccharifying the liquefied starch. Therefore, Measuring the effective period and enzyme concentration for the liquefaction step the following experiments were carried out to find out the effective conditions for the liquefaction step i.e., the shorter period and the optimum enzyme concentration, which could be used.

Table 1: Parameters showing the lowest and highest Enzyme activity and Maltodextrin Production

Liquefaction Process							Volume of Maltodextrin syrup formed (ml)
Starch Slurry (Substrate) Conc.	Enzyme Performance	pH of Substrate (Starch Slurry)	Volume of Heat Stable α-Amylase Enzyme used (ml)	Substrate : Enzyme ratio	Liquefaction Temp. (°C)	Liquefaction Time (Hours)	
35% DS (120g of cassava starch)	Lowest Activity & Lowest Yield	5	3.5	400.00	60 °C	1	11.4±1.6
	Highest Activity & Highest Yield	7	14.0			2	105.2±1.3

Table 2: Liquefaction Parameters showing 20 outcomes selected from the 64 Experimental Runs Furnishing the Best Experimental values of Maltodextrin Syrup Production

pH of substrate (Starch Slurry)	Liquefaction Time (Hours)	Volume of Heat Stable α-Amylase Enzyme (ml)	Amount of Maltodextrin syrup Formed (ml)
5	4	10.5	77.9±4.1
6	4	10.5	78.8±3.2
7	4	10.5	79.8±5.4
8	4	10.5	76.4±7.6
5	1	14.0	70.6±2.6
6	1	14.0	72.2±7.7
7	1	14.0	73.4±6.4
8	1	14.0	71.0±1.6
5	2	14.0	101.3±7.6
6	2	14.0	104.1±2.1
7	2	14.0	105.2±1.3
8	2	14.0	103.9±3.3
5	3	14.0	101.7±1.2
6	3	14.0	104.3±7.5
7	3	14.0	105.1±8.6
8	3	14.0	103.6±1.2
5	4	14.0	100.9±4.1
6	4	14.0	103.0±2.2
7	4	14.0	104.9±7.6
8	4	14.0	102.7±6.2

Table 3: Correlation Pearson's chat showing the strength of the interactions between the variables

	Intercept	A-pH of substrate (Starch Slurry)	B-Liquefaction Time	C-Volume of Alpha-Amylase Emzyme	AB	AC	BC	A^2	B^2	C^2
Intercept					-inf		-inf	inf		
A-pH of substrate (Starch Slurry)		1.000	0.000	-0.000	0.249	0.956	-0.000	-0.000	0.000	-0.000
B-Liquefaction Time		0.000	1.000	-0.514	-0.000	-0.000	0.942	0.000	0.210	-0.514
C-Volume of Alpha-Amylase Emzyme		-0.000	-0.514	1.000	-0.000	0.000	-0.196	0.000	-0.408	1.000
AB	-inf	0.249	-0.000	-0.000	1.000	0.092	0.000	-0.000	0.000	-0.000
AC		0.956	-0.000	0.000	0.092	1.000	0.000	0.000	0.000	0.000
BC	-inf	-0.000	0.942	-0.196	0.000	0.000	1.000	-0.000	0.080	-0.196
A^2	inf	-0.000	0.000	0.000	-0.000	0.000	-0.000	1.000	0.000	0.000
B^2		0.000	0.210	-0.408	0.000	0.000	0.080	0.000	1.000	-0.408
C^2		-0.000	-0.514	1.000	-0.000	0.000	-0.196	0.000	-0.408	1.000

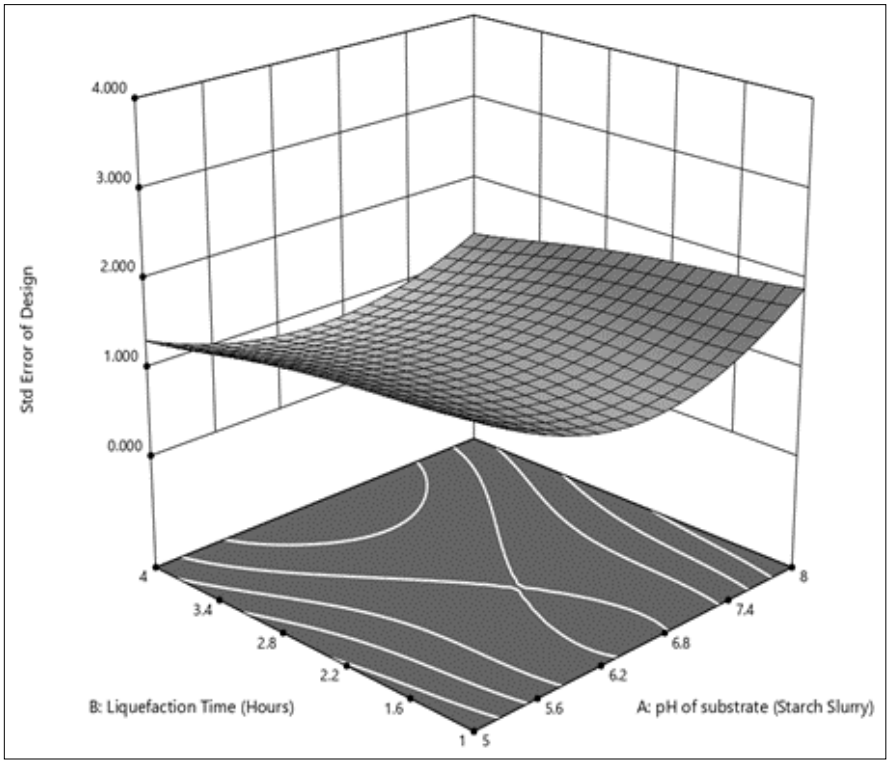

Fig 1: 3D Surface Model Graph Showing Multiple Interactions

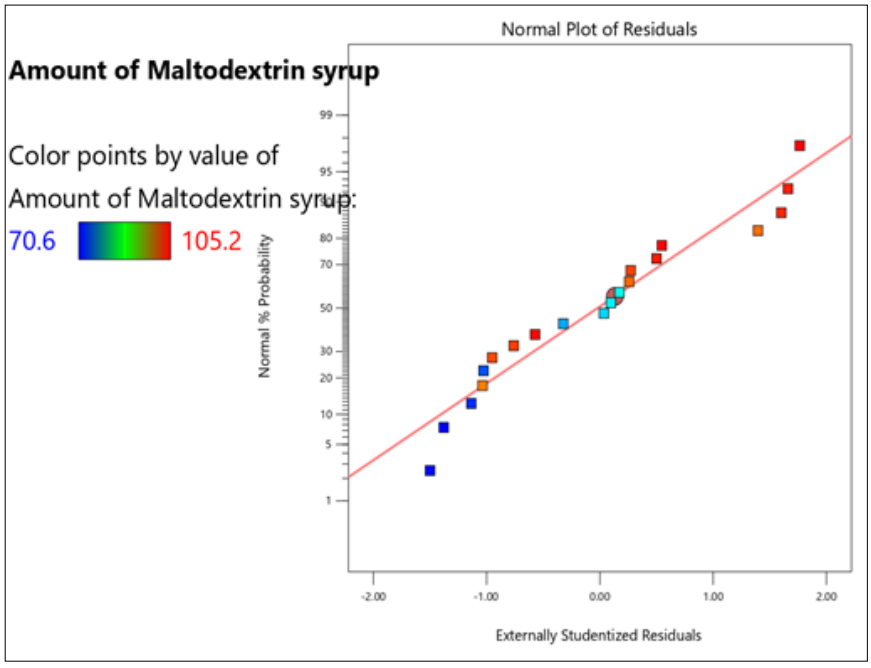

Fig 2: Normal Plot of Residuals to check for normality of residuals

Curve fitting, also known as regression analysis, is used to find the "best fit" line or curve for a series of data points. There are five (5) design points around the 3D surface model graph described by Figure 1 which presents two (2) sets of design points. Design points above predicted value and Design points below predicted value. The curve fitting on the 3D surface model graph examined the relationship between three predictors (independent variables i.e. pH of substrate, liquefaction time, and alpha-amylase enzyme concentration) and a response variable (dependent variable i.e. Malyodextrin syrup produced), with the goal of defining a "best fit" model of the relationship with the tip of the curves indicating on each factor the points of maximum yield. These are the design points of the optimum parameters of the independent variables furnishing the best conditions as substrate pH of 7, liquefaction time of 2hours and Volume of Heat Stable α-Amylase Enzyme of 14ml. The residuals are represented graphically by means of a residual plot as shown in figure 2. This normal probability plot indicates whether the residuals follow a normal distribution, thus follow the straight line. Here, the scatter had a definite pattern along the straight line which indicates that a transformation of the response may provide a better analysis.

Table 4: ANOVA for Linear model: Amount of Maltodextrin syrup

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2688.50	3	896.17	11.19	0.0003	significant
A-pH of substrate (Starch Slurry)	3.92	1	3.92	0.0489	0.8277	
B-Liquefaction Time	1734.45	1	1734.45	21.65	0.0003	
C-Volume of Alpha-Amylase Emzyme	2290.45	1	2290.45	28.60	< 0.0001	
Residual	1281.55	16	80.10			
Cor Total	3970.06	19				

Table 5: Fit Statistics

Std. Dev.	8.95	R^2	0.6772
Mean	92.01	Adjusted R^2	0.6167
C.V. %	9.73	Predicted R^2	0.5212
		Adeq Precision	8.0921

The Model F-value of 11.19 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. The Predicted R^2 of 0.5212 is in reasonable agreement with the Adjusted R^2 of 0.6167; i.e. the difference is less than 0.2. This model can be used to navigate the design space. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 6: Saccharification Parameters (using the Best Result in the Liquefaction Process 105.2±1.3ml ml Maltodextrin Syrup) showing the lowest and the highest Enzyme activity and Glucose Syrup Production

	Saccharification Process							Glucose syrup[Dextrose Equivalent (DE)%]
DE of Maltodextrin syrup used (%)	Volume of Maltodextrin syrup used	Enzyme Performance	pH of Substrate (Maltodextrin syrup formed)	Volume of Glucoamylase (AMG 200L, 0.15%) used (ml)	Substrate : Enzyme ratio	Saccharification Temp. (ºC)	Saccharification Time (Hours)	
15	105.2±1.3ml	Lowest Activity & Lowest Yield	4.2	25	400.00	60 °C	12	12.1±4.9
		Highest Activity & Highest Yield	4.6	100			48	83.9±7.6

Table 7: Saccharification Parameters showing 20 Best outcomes selected from the 64 Experimental Runs

pH of substrate (Maltodextrin Syrup formed)	Saccharification Period (Hours)	Glucoamylase (Vol. of Enzyme/Vol. of Maltodextrin) ml	Glucose Syrup (%) Dextrose Equivalent DE (%)
4.2	48	75	47.9±4.1
4.4	48	75	48.8±3.2
4.6	48	75	49.8±5.4
4.8	48	75	46.4±7.6
4.2	12	100	40.6±2.6
4.4	12	100	42.2±7.7
4.6	12	100	43.4±6.4
4.8	12	100	41.0±1.6
4.2	24	100	50.0±7.6
4.4	24	100	51.3±2.1
4.6	24	100	52.4±7.4
4.8	24	100	50.1±7.3
4.2	36	100	59.7±1.2
4.4	36	100	60.3±7.5
4.6	36	100	63.4±8.6
4.8	36	100	61.1±1.2
4.2	48	100	70.5±4.1
4.4	48	100	72.0±2.2
4.6	48	100	73.9±7.6
4.8	48	100	71.4±6.2

Curve fitting, also known as regression analysis, is used to find the "best fit" line or curve for a series of data points. There are four (4) design points around the 3D surface model graph described by Figure 5 which presents two (2) sets of design points described with red dots. Design points above predicted value and Design points below predicted value. The curve fitting on the 3D surface model graph examined the relationship between three predictors

(independent variables i.e. pH of substrate, 4.6, liquefaction time, 48 hours and Gluco-amylase enzyme concentration 100ml) and a response variable (dependent variable i.e. Malyodextrin syrup produced), with the goal of defining a "best fit" model of the relationship with the tip of the curves indicating on each factor the points of maximum yield. These are the design points of the optimum parameters of the independent variables furnishing the best conditions as substrate pH of 4.6, liquefaction time of 48hours and Volume of Heat Stable α-Amylase Enzyme of 100ml. The residuals are represented graphically by means of a residual plot as shown in figure 6. This normal probability plot indicates whether the residuals follow a normal distribution, thus follow the straight line. Here, the scatter had a definite pattern along the straight line which indicates that a transformation of the response may provide a better analysis.

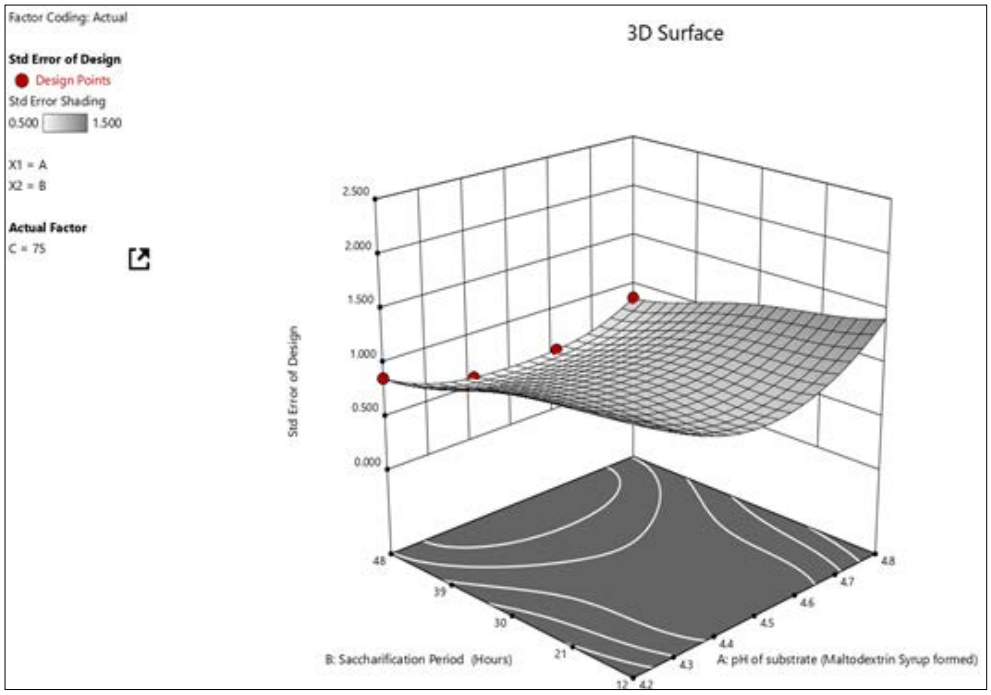

Fig 3: 3D Surface Model Graph Showing Multiple Interactions of Independent factors

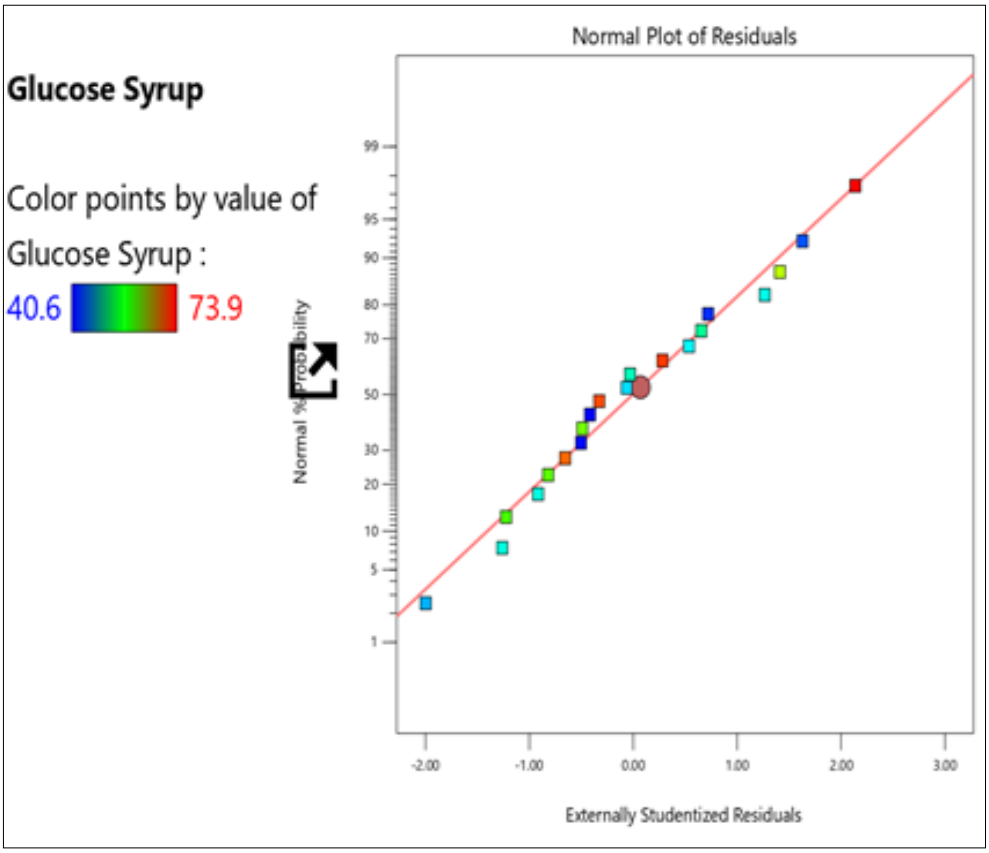

Fig 4: Normal Plot of Residuals to check

Table 8: ANOVA for Linear model: Response 1: Glucose Syrup

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2242.59	3	747.53	384.30	< 0.0001	significant
A-pH of substrate (Maltodextrin Syrup formed)	0.7056	1	0.7056	0.3627	0.5554	
B-Saccharification Period	2025.08	1	2025.08	1041.07	< 0.0001	
C-Glucoamylase (Vol. of Enzyme/Vol. of Maltodextrin)	1280.13	1	1280.13	658.10	< 0.0001	
Residual	31.12	16	1.95			
Cor Total	2273.72	19				

The Model F-value of 384.30 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

A range of solutions and choices were presented which were compared to determine which might be "best" in terms of considering all possible advantage/benefit (minimal cost, maximal profit, minimal error and optimal design) with the goal of understanding and resolving the modelling issue (ensuring the model fit and the data fit perfectly).

Table 9: Correlation Pearson's chat showing the strength of the interactions between the variables

	Intercept	A-pH of substrate (Maltodextrin Syrup formed)	B-Saccharification Period	C-Glucoamylase (Vol. of Enzyme/Vol. of Maltodextrin)	AB	AC	BC	A^2	B^2	C^2
Intercept					-inf		inf	-inf		
A-pH of substrate (Maltodextrin Syrup formed)		1.000	0.000	0.000	0.249	0.894	-0.000	0.000	0.000	0.000
B-Saccharification Period		0.000	1.000	-0.514	0.000	-0.000	0.857	-0.000	0.210	-0.514
C-Glucoamylase (Vol. of Enzyme/Vol. of Maltodextrin)		0.000	-0.514	1.000	-0.000	0.000	0.000	-0.000	-0.408	1.000
AB	-inf	0.249	0.000	-0.000	1.000	0.000	-0.000	0.000	0.000	-0.000
AC		0.894	-0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
BC	inf	-0.000	0.857	0.000	-0.000	0.000	1.000	0.000	-0.000	0.000
A^2	-inf	0.000	-0.000	-0.000	0.000	0.000	0.000	1.000	-0.000	-0.000
B^2		0.000	0.210	-0.408	0.000	0.000	-0.000	-0.000	1.000	-0.408
C^2		0.000	-0.514	1.000	-0.000	0.000	0.000	-0.000	-0.408	1.000

In practice, it was necessary to add the amount substrate (starch slurry) to the reaction medium before the gradual addition of enzyme, especially at the higher concentrations of enzyme. It was noticed that as the enzyme concentration increased from 3.5ml to 14ml a remarkable increase in the amount of maltose was noticed through the 120 min of the liquefaction. When the reaction period prolonged beyond 120 min no increase was observed. So, the liquefaction period by Termamyl S must not exceed 120 min under the conditions used. In order to define the suitable pH within this period, the velocity of Termamyl S was calculated (ml maltose formed / min). It was found that the reaction velocity increased as the pH increased from 6 to 7 and it decreased when the pH was below 6 and above 7. So, the best pH to liquefy 35% starch slurry is between 6 and 7 and 2 hours liquefication time. No noticeable increase was observed when the liquefaction period extended beyond 120 min followed by saccharification for 48 hours.

Analysis show that enzymes used in this investigation possess some remarkable properties which include quantitative conversion of starch to glucose. The starch extracted from cassava after complete liquefaction by alpha-amylase produced the highest starch conversion to maltodextrin syrup furnishing 105.2±1.3ml at enzyme concentration of 14ml and pH value of 7. Liquid glucose produced with the pure glucoamylase from Aspergillus niger revealed a dextrose equivalent of 73.9±7.6 at pH value of 4.6 and 100ml enzyme concentration at the end of the saccharification process. Cassava starch exhibited good potential as substrates for glucose syrup production.

Finally, *Pearson's* test produced significant inverse correlations between cassava dilution amount and liquefaction time ($p \leq 0.05$), and between cassava dilution amount and enzyme dose ($p \leq 0.01$), highlighting the fact that together with starch gelatinization modality these are the key factors affecting the reducing sugar yield after liquefaction.

Conclusion and Recommendation

The aforementioned results are in agreement with other authors who reported that the liquefying step is very important because it prepares the starch molecules or "liquefying" it and increasing its susceptibility to the saccharifying enzymes (Guzmán- Maldonado and Paredes-López, 1995; Van der Maarel *et al*., 2002). The lower liquefaction time of 2hours and temperature of 90ºC should lead to decrease process costs and elimination of Millard reaction products, resulting higher product quality. The amount of glucose formed is more important than the percentage of hydrolysis. Therefore, the treatment, which has higher glucose content, in the same volume, is commercially viable due to the decrease in the processing cost.

Thus, the information gained from this research should contribute to improving our ability to advance biologically based processes by providing efficient and economical ways of enhancing the activity and stability as well as reusability of biocatalysts for use in bioprocessing applications.

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