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Optimization of sugar production from cassava starch using crude enzymes from maize, rice and sorghum malt extracts

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Abstract
Sugar syrup was produced by the hydrolysis of cassava starch with crude enzymes from maize, rice and sorghum malts. Cassava *(Manihot esculenta)* tubers were processed into starch. The cereals- paddy rice *(Oriza sativa)*, yellow maize *(Zea mays)* and sorghum (*Sorghuh bicolor)* were individually malted and their crude enzymes used for the production of sugar syrup. The diastatic power (DP) of the malted grains were determined. The malt extracts with highest diastatic power (best from each cereal- maize, rice and sorghum) were separately used to hydrolyze cassava starch at different temperatures, pH and time. The best three extracts (maize, rice and sorghum) were combined to hydrolyze cassava starch for sugar production. The result obtained were analyzed statistically and fitted into a polynomial regression model. The result showed that the production of reducing sugar was affected by the variation of the malt extract used for hydrolysis of cassava starch. The fit of the model was expressed by coefficient of determination (Adj.R^2) which was found to be (0.6804) for best maize malt extract (BMME+CASSAVA STARCH) and (0.5495) for best maize malt extract + best rice malt extract + Cassava (BMME+BRME+CASSAVA STARCH), respectively indicating that 68.04% and 54.95% of the variability in the responses can be explained by the model. Numeric optimization of (BMME+BRME+CASSAVA STARCH) yielded 250 g/l sugar at optimum temperature, pH and time values of 77.6 °C, 5.28 and 141.46 min, respectively. The interaction effect of these processing conditions hydrolyzed the cassava starch to produce the reducing sugars that could be beneficial in food systems.

Keywords: Cassava starch, hydrolysis, maize malt, rice malt, sorghum malt, sugar production, optimization.

Introduction
The high demand for sugar and the development of enzymatic technology have increased the need for the production of sweeteners especially glucose and fructose syrups. The need for sugars such as glucose has greatly increased due to the expansion in the food and pharmaceutical industries. Glucose which is a sweet and simple sugar is mainly added to the products like bakery and confectionery products in food industries (Kanlaya and Jirasak, 2004) [12]. Corn starch used to be the major raw material for glucose and fructose syrups production. However, good results have been obtained by using starch from cassava (*Manihot esculenta*). The problem still remains with the hydrolysis of cassava starch to obtain these sugars. Acid and enzyme hydrolyses which continued to be the most available methods of hydrolysis are still in use despite their attendant problems like formation of undesired products such as toxic compounds in the case of acid hydrolysis as well as costly nature of the modified enzymes. Cassava (*Manihot esculenta*) also called manioc is a root and tuber crop, cultivated as an annual crop in tropical and sub-tropical region for its edible starchy tuberous root. It is a major source of carbohydrate (starch) and is generally accepted and recognized as a good source of vital nutrients and energy for the body. Cassava is principally used in the food, textile and paper industries as well in the chemical industry for the production of alcohol among other uses (Henry *et al.*, 1998) [10]. Starch is a carbohydrate that consists of large number of glucose units which are joined together by glycosidic bonds (Vasquez *et al.*, 2009) [18]. Cassava is the newest addition to commercially available source of sweeteners and provides a fresh and guaranteed organic food ingredient source that is needed in the processing of a wide range of industrial products.
This work was therefore designed to explore the use of crude enzyme extracts from malted maize, rice and sorghum as alternative sources of enzymes with a view to optimizing the production of sugar from cassava starch.

The work was also intended to identify the best malt extract and the best combination of these malt extracts that have the capacity to give the highest yield of sugar for industrial use.

Materials and Methods
The fresh cassava tubers (*Manihot esculenta*), maize (*Zea mays*), rice (*Sativa oryza*) and sorghum (*Sorghum bicolor)* grains used for the production of sugar syrup were purchased from Agricultural Development Programme Unit of Ministry of Agriculture, Anambra State, Nigeria.

Preparation of Cassava Starch
The cassava starch was produced according to the method described by Abass (2016) [1]. Five kilogrammes (5 kg) of cassava tubers were peeled and washed with potable water, grated, pressed to remove water and dried under the hot air oven at 60 °C for 48 h. The dried mash was milled in a hammer mill, packaged in an air tight plastic container and kept in a refrigerator until needed for further use.

Preparation of Maize, Rice and Sorgum Malts
These were prepared according to the method described by Dziedzoave and Kom Laga (2006) [8]. One kilogramme (1kg) of each of these cereals (maize, rice and sorghum) was sorted to remove foreign materials and damaged or broken grains. The sorted grains were cleaned individually with two (2) litres of potable water and soaked separately in 5% sodium metabisulphite solution for 20 sec to prevent the growth of microorganisms. Residual sodium metabisulphite was washed off and the grains were steeped in two (2) litres of distilled water for 6, 12 and 18 h, respectively. The steep water was changed every 12 h to avoid microbial fermentation. The steeped grains were allowed to germinate separately for 72, 96 and 120 h at room temperature (28±2°C). During germination, the grains were individually stirred and sprinkled with potable water twice a day. Non-germinated grains were handpicked and discarded. The germinated grains were individually collected, spread on the trays and dried in a hot air oven (Model Gallenkamp 300 plus England) at 50°C for 18 h with occasional stirring of the grains at intervals of 25 min to ensure uniform drying. The dried grains were separately milled in a hammer mill and sieved through a 400 micron mesh sieve to obtain the grists. The grists produced were individually packaged in lidded plastic containers, labeled and kept in a cool dry place until needed for further use.

Production of Sugar Syrup From Maize, Rice and Sorghum Malts.
The sugar syrup was produced according to the method described by Ayernor *et al.* (2002) [5]. Approximately, 60, 70 and 80 grammes of each malt (maize, rice and sorghum) were added to one kilogramme (1kg) of cassava starch (Batch A). Another batch of 60, 70 and 80 grammes of maize, rice and sorghum malt extract were also added individually to one kilogramme of cassava starch (Batch B). Then, one litre (1L) of potable water was added to each batch of malt-cassava blend and the mixture was mixed thoroughly. The pH was adjusted to 4, 5 and 6 on each run of starch/malt extract mixture. Thirty grammes (30g) of calcium and 4.8 litres of boiling water were added to Batch A and the mixture was stirred for 10 min until no sign of whiteness was seen on each run. The same procedure was repeated for Batch B. The mixture was covered and cooled to 65 °C. The pH was adjusted and 160 g of maize and rice malt extracts were added again, stirred and left for 60, 120 and 180 min at 60, 70 and 80 °C, respectively. Thereafter, the mixture was boiled briefly, cooled and filtered. The filtrate was evaporated to half the volume of the original mixture and the reducing sugar produced in each case was collected, filtered and stored in small plastic bottles until needed for further use.

Experimental Design
The design was a two factor face centered central composite design (FCCCD) with 2 centre points and a total of 16 runs. The design was carried out using Design Expert (Version 8.0.7.1). The independent variables (for diastatic power) were soaking time (X_I) and germination time (X_2) as shown in Table 1, while the independent variables (for sugar yield) were temperature (X_3), pH (X_4) and Time (X_5) as shown in Table 2.

Table 1: Experimental Design for Diastatic Power with Actual Values

Soaking time(h)		Germination time (h)	Diastatic power (%)
1	12	96	
2	6	72	
3	12	96	
4	18	72	
5	12	120	
6	18	120	
7	12	72	
8	6	96	
9	18	0	
10	6	120	

Table 2: Experimental Design for Sugar Production with Actual Values

Samples	Temperature (X_3)	$p^H(X_4)$	Time Sugar yield (X_5)
1	60	6	60
2	80	6	180
3	70	5	120
4	70	4	120
5	70	6	120
6	60	4	60
7	70	4	120
8	70	5	120
9	80	4	60
10	80	6	60
11	80	4	180
12	60	6	180
13	60	5	120
14	70	4	120
15	70	4	180
16	80	5	120

Key:

	-1	0	+1
Soaking Time (h) X_1	6	12	18
Germination Time (h) X_2	72	96	120
Temperature (°C) X_3	60	70	80
pH X_4	4	5	6
Time (Min) X_5	60	120	180

NB: Each cereal had its design table. The tables were similar and the results were tabulated as follows:

Malt extract	Cassava starch	Reducing sugar yield%
BMME +	CS	
BRME +	CS	
BSME +	CS	
BMME +BRME +	CS	
BMME + BSME +	CS	
BRME + BSME +	CS	
BMME +BRME+BSME+	CS	

Key: CS = Cassava Starch**,** BMME = Best Maize Malt Extract, BRME= Best Rice Malt Extract
BSME = Best Sorghum Malt Extract

Statistical Analysis.

The sugar yields obtained from the hydrolysis of cassava starch with maize, rice and sorghum malt extracts were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS Version 20) software. The sugar yields obtained by the combination of the best maize malt extract (BMME), best rice malt extract (BRME) and best sorghum malt extract (BSME) were also subjected to one-way analysis of variance (ANOVA) using the same software. Significant means were separated using Turkey's Least Significance Difference (LSD) Test at $p<0.05$. Optimization of sugar production from cassava starch using maize, rice and sorghum malt extracts was done by the use of surface response methodology described by Awolu *et al.* (2016) [4].

Results and Discussion

Sugar Yields of The Malt Extracts After Hydrolysis With Cassava Starch. The sugar yields of each malt extracts after hydrolysis with cassava starch are shown in Table 3.

Table 3: Sugar yield from cassava starch hydrolysis using each malt extract with best diastatic power at different temperature, pH and time

Temp, X_3 (^{0}C)	P^H, X_4	Time X_{5}-(min)	BestMaize Malt (mg/l)	Best Rice Malt Extract (mg/l)	Best Sorghum Malt Extract mg/l
60	6	60	$43^a \pm 0.02$	$38^b \pm 0.02$	$14^c \pm 00.02$
80	6	180	$60^b \pm 0.02$	$82^a \pm 0.02$	$38^c \pm 0.02$
70	5	120	$60^a \pm 0.02$	$40^b \pm 0.02$	$28^c \pm 0.02$
70	5	120	$40^a \pm 0.02$	$37^b \pm 0.02$	$07^c \pm 0.02$
70	5	120	$35^c \pm 0.02$	$48^b \pm 0.02$	$57^a \pm 0.02$
60	4	60	$54^b \pm 0.02$	$61^a \pm 0.02$	$16^c \pm 0.02$
70	4	120	$64^a \pm 0.02$	$61^b \pm 0.02$	$30^c \pm 0.02$
70	5	120	$60^a \pm 0.02$	$40^b \pm 0.02$	$28^c \pm 0.02$
80	4	60	$43^b \pm 0.02$	$56^a \pm 0.02$	$07^c \pm 0.02$
80	5	60	$60^a \pm 0.50$	$53^b \pm 0.02$	$23^c \pm 0.02$
80	4	180	$38^a \pm 0.02$	$27^c \pm 0.02$	$36^b \pm 0.01$
60	6	180	$38^a \pm 0.02$	$31^b \pm 0.02$	$28^c \pm 0.02$
60	5	180	$45^b \pm 0.02$	$37^c \pm 0.02$	$57^a \pm 0.15$
60	4	180	$75^a \pm 0.02$	$59^b \pm 0.01$	$28^c \pm 0.02$
70	5	180	$50^a \pm 0.01$	$43^b \pm 0.01$	$28^c \pm 0.02$
80	5	120	$35^b \pm 0.03$	$72^a \pm 0.02$	$14^c \pm 0.02$

Data are means of triplicate determinations $\pm$ std deviation. Data in the same column bearing different superscripts differ significantly ($p< 0.05$).

The mean values for reducing sugar obtained after using 60g/litre of best maize malt extract (BMME), 53g/litre of best rice malt extract (BRME) and 23g/litre of best sorghum malt extract (BSME) to hydrolyze cassava starch at a temperature of 80 ^{o}C, pH of 4 and holding of time 60 min, respectively showed that the 60g/litre of maize malt extract had significantly ($p<0.05$) higher sugar content at the same level of treatment than the 53g/litre of rice malt extract and 23 g/litre of sorghum malt extract as observed in sample 10. This observation is in line with the report of Cabello (1998) [21] which stated that maize malt is the major industrial raw material for glucose and fructose production in US and other parts of the world. The glucose and frutose syrups are largely used in pharmaceutical applications as well as in food industries (Voragen, 1998) [19]. The value obtained for 53g/litre, of best rice malt also revealed that the work done by Hammond and Ayenor (2001) [9] was true. They observed that when various types of cereal malts were used in hydrolysis of starch, rice malt competes favourably with maize and even higher at times than maize in terms of the amount of sugar produced. The value obtained using 82g/litre of rice was significantly ($p<0.05$) higher than the values obtained when 68g/litre of maize and 38g/litre of sorghum extracts were used compared to other samples. Mohammed *et al.* (2012) [14] observed that rice malt produced the highest yield of sugar during hydrolysis with different cereals. It has also been reported that malt produced from rice had higher average yield of sugar on hydrolysis when compared with maize and sorghum malts which are normally used in malting because of their high carbohydrate contents at maximum pH of 5, temperature of 80 ^{o}C and holding time of 60 min (Pomeranz, 1985; Adebowale *et al.*, 2010) [16, 2]. The value obtained when 57g/litre of sorghum malt extract was used was significantly higher ($p<0.05$) than the values obtained when 35g/litre of maize and 48g/litre of rice extracts were used as indicated in sample 13. This showed that some sorghum varieties exhibit good amylase activities especially alpha amylase activity, hence the reason for high yield of sugar by sorghum malt extract than the maize malt extract. Both sorghum and rice malt extracts generally produced high yields of reducing sugars and this observation is in agreement with reports of Adebowale *et al.* (2010) [2] and Aderigbe *et al.* (2012) [3]. The mean values of reducing sugar obtained from maize, rice and sorghum malt extracts (65, 61 and 30g/litre), respectively (sample 7) and those of 68, 82 and 38g/litre, respectively (sample 10) were higher than the other samples. Also, the 35, 48 and 57g/litre of maize, rice and sorghum malt extracts (sample 13) were significantly higher (p<0.05) than the other samples. These observations are in agreement with the findings of Mohammed *et al.* (2012) [14] who stated that the sugar yield increases linearly until it reaches the pH of 4 and pH of 5, respectively. This phenomenon is equally in agreement with the findings of Blandish (2002) [6] who reported that the amyloglucosidase (AMG) had a broad optimum pH of between 4.0 and 5.5. Rice malt has been said to be rich in many enzymes especially amylase, amyloglucosidase and dextrinase which help in increasing its sugar yield. However, the rate of decrease in the yield of sugar seemed to be faster beyond the pH of 5.5. Amyloglucosidase showed a very good stability at optimum pH of 5.5 and above the temperature of 50 ºC. If the pH is taken too far towards the acid or alkaline side, the changes may become irreversible because large changes in pH denature the enzymes (proteins) since most of their active sites contain acid or base groups. The mean values obtained in samples 5, 10 and 13 for maize, rice and sorghum malt extracts, respectively showed that the values obtained from 75, 59 and 28, 68, 82 and 38 and 35, 48 and 57g/litre were significantly higher (p<0.05) than the values obtained in the other samples. This finding is in agreement with the report of Mohammed *et al.* (2012) [14] who stated that at optimum temperature of 60-70 ºC, there is higher yield of sugar in enzyme catalyzed reaction. The increase in temperature beyond its optimum range tends to reduce reaction velocity by reducing the enzyme activity. In addition, the mean values obtained from maize, rice and sorghum malt extracts, respectively in sample 5 (75, 59 and 28g/litre), respectively were significantly p<0.05 higher than the values obtained for sample 9 (40, 33 and 07g/litre) after two hours. This showed that the time allowed for hydrolysis is also another factor that affected the reducing sugar produced (Mohammed *et al.*, 2012) [14]. Hence, the sugar yield is greater when the period was maintained up to two hours during hydrolysis.

Sugar Content from Combination of Extracts of Best Diastatic Power (Dp)

The sugar content of the combined malt extracts after hydrolysis with cassava starch at different temperatures, p^{H} and time are shown in Table 4

Table 4: Sugar content (mg/l) from combination of extracts with best diastatic power (Dp)

Temp, X_3 (^{0}C)	P^{H}, X_4	Time X_5(min)	BMME+ BRME (mg/l)	BMME+ BSME (mg/l)	BRME+ BSME (mg/l)	BMME + BRME + BSME (mg/l)
60	6	60	183^{b}±0.59	86^{c}±0.02	72^{d}± 00.02	214^{a}± 0.03
80	6	180	318^{b}±0.02	119^{c}±0.02	102^{d}± 0.02	325^{a}± 0.02
70	5	120	215^{b}±0.02	93^{c}±0.02	83^{d}±0.02	227^{a}±0.02
70	5	60	189^{b}±0.02	87^{c}±0.02	75^{d}±0.02	214^{a}±0.02
70	6	120	194^{b}±0.02	83^{c}±0.02	82^{d}±0.02	214^{a}±0.02
60	4	60	228^{b}±0.02	98^{c}±0.02	86^{d}±0.02	238^{a}±0.02
70	4	120	254^{b}±0.02	110^{c}±0.02	97^{d}±0.02	268^{a}±0.02
70	5	120	215^{b}±0.02	93^{c}±0.02	83^{d}±0.02	227^{a}±0.02
80	4	60	223^{b}±0.02	106^{c}±0.02	92^{d}±0.02	232^{a}±0.02
80	6	60	235^{a}±0.50	103^{c}±0.02	89^{d}±0.02	232^{b}±0.02
80	4	180	232^{a}±0.47	61^{d}±0.01	91^{c}±0.01	226^{b}±0.15
60	6	180	213^{b}±0.02	68^{d}±0.02	71^{c}±0.02	231^{a}±0.02
60	5	120	207^{b}±0.25	90^{c}±0.02	81^{d}±0.01	224^{a}±0.15
60	4	180	240^{b}±0.21	108^{c}±0.01	93^{d}±0.02	254^{a}±0.01
70	5	180	219^{b}±0.01	95^{c}±0.01	85^{d}±0.02	229^{a}±0.01
80	5	120	235^{b}±0.01	103^{c}±0.15	81^{d}±0.15	320^{a}±0.01

Data are means of triplicate determinations ± std deviation. Data in the same column bearing different super scripts differ significantly (p<0.05).

The mean sugar values obtained from cassava starch hydrolysis using BMME + BRME which ranged from 183-318g/litre were significantly (p<0.05) higher than the values obtained with BMME + BSME (61-110g/litre) and BSME+BRME (71-103g/litre), respectively. This consolidated the earlier assertion made by Roberto *et al.* (2003) [17] that rice malt extract is rich in many enzymes especially amylase, amyloglucosidase and dextrinase. The amylase and dextrinase enzymes might be responsible for the production of maltose. The quantity of glucose present is a function of the type of enzyme used and the condition at which it was produced. The mean values of reducing sugar obtained while using individual extracts from maize, rice and sorghum malts (Table 4) under optimum conditions were significantly p<0.05 lower than the values obtained when combined extracts of BMME and BRME were used. This showed that the combined extracts of BMME and BRME had higher yield than the individual extracts of maize, rice and sorghum malts. However, cassava starch hydrolyzed with the combination of BMME, BRME and BSME extracts had the highest sugar yield (214-320g/litre) with dextrose equivalent not lower than 80. This is in agreement with the findings of Mohammed *et al.* (2012) [14] who reported that amyloglucosidase in combination with other enzymes yielded higher glucose and other sugars. The high values of sugar obtained from different malt extracts produced from maize, rice and sorghum when used either singly or in combined form showed that the three cereal grains can be used to hydrolyze cassava starch to produce sugar. However, the combination of the best malt extracts produced from the three cereal grains gave better results at a temperature of 60-70 ºC, pH of 5-6 and holding time of 120 min. This observation is in agreement with the findings of Adebowale (2010) [2] who reported that maize, rice and sorghum have been recommended for use in brewing industry in Nigeria due to their availability.

Model Fitting

Response surface methodology was applied to obtain the regression equation which represents the empirical relationship between the responses (sugar yield and the independent variables (temperature, pH and time). The significance of each coefficient was determined by using the 'F' values and p-values as shown in Table 5. It could be seen that the model F-value of 4.55 implies that the model is significant with p-value of 0.0395. The value of "Pro< F" less than 0.05 indicates that the model terms were significant. In this case, only A^2 (the quadratic effect of temperature is significant with p-value of 0.0194) and all other terms A, B, C, AB, AC, BC, B^2 and C^2, are statistically insignificant, given their P-values as 0.0913, 0.5128, 0.0999, 0.0999, 0.11126, 0.1611, 0.117 and 0.1651, respectively. The analysis of variance (ANOVA) for sugar yield from BSME + Cassava and the estimated regression coefficient for sugar yield from the BSME + Cassava are shown in Table 5 and 6, respectively.

Table 5: Analysis of variance (ANOVA) for sugar yield from BSME+CASSAVA. (Partial Sum of Square –Type III)

Source	Sum of squares	Df	Mean square	F-value	P-value Prov<F
Model significant	2741.80	9	304.64	4.55	0.0395
A-Temp	270.40	1	270.40	4.04	0.0913
B-P^H	32.40	1	32.40	0.48	0.5128
C-Time	435.60	1	435.60	6.50	0.0999
AB	253.13	1	253.13	3.78	0.0999
AC	231.13	1	231.13	3.45	0.1126
BC	171.1	1	171.12	2.55	0.1611
A^2	672.00	1	672.00	10.03	0.0194
B^2	857.46	1	857.46	12.80	0.117
C^2 R^2 0.8721 R^2Adj. 0.6804	167.28	1	167.28	2.50	0.1651

Final equation in terms of actual factors

Sugar BSME +CA=
+159.72500
+ 18.98422*Temp
+ 208.66983 *P^H
+0.399937*Time
+8.95833E-0.03 *Temp *P^H
-0.077083*Temp*Time
-0.15966 *$Temp^2$
+18.03448*P^{H2}
-2.21264E-0.03 *$Time^2$

Table 6: Estimated regression coefficient for sugar yield from BSME+CASSAVA.

Factor	Coeff estimate	Df	Std Error	95%CI	95% CI
				Low	High
Intercept	26.31	1	3.87	16.83	35.79
A-Temp	5.20	1	2.59	-1.13	11.53
B- pH	1.8	1	2.59	-4.53	8.13
C- Time	6.60	1	2.59	0.27	12.93
AB	5.62	1	2.89	-1.46	12.71
AC	5.38	1	2.89	-1.71	12.46
BC	-4.62	1	2.89	-11.71	2.46
A^2	-15.97	1	5.04	-28.30	-3.63
B^2	18.03	1	5.04	5.70	30.37
C^2	-7.97	1	5.04	-20.30	4.37

The Analysis of variance (ANOVA) for the sugar yields from the mixture of best sorghum malt extract and cassava starch (BSME+CASSAVA) are presented in Table 5. It was shown that the model is insignificant and as well exhibited high R^2adj value (68.04%). This showed that 68.04% of the response variable (sugar yield) was due to experimental variables, while 31.96% was due extraneous variables. Also, lack of fit is not good for a model. Only $X^2{}_5$, $X^2{}_3$ and $X^2{}_4$ (quadratic effects of temperature, pH and time) were significant. The regression equation or sugar yield obtained from the mixture of BSME+CASSAVA starch are shown in the equation 1. Therefore,

$y = bo + b_5X_5+b_3X^2{}_3+b_4X^2{}_4$………………..(1a)

$y=159.73-0.15966X^2{}_3+18.03448X^2{}_4+18.98X_3-208.67X^2{}_4$…………(1b)

From the ANOVA, only Time (X_5), $Temp^2$($X^2{}_3$) and pH^2 ($X^2{}_4$) exhibited significant difference ($p<0.05$) or F less than 0.01000 indicating that only these terms were significant and should be used to fit the regression model for the effect of independent variables. From the regression equation, the increase in the time of hydrolysis (X_5) and temperature (X_3) resulted in increase in the sugar yield. However, the increase in the pH (X_4) caused the decreased in the sugar yield. Figure 1 shows the 3-D surface plot which explain the effect of temperature and p^H on the mixture of BSME and Cassava starch. It should be noted that y = the responses (BMME, BRME, BSME and their sugar yields).

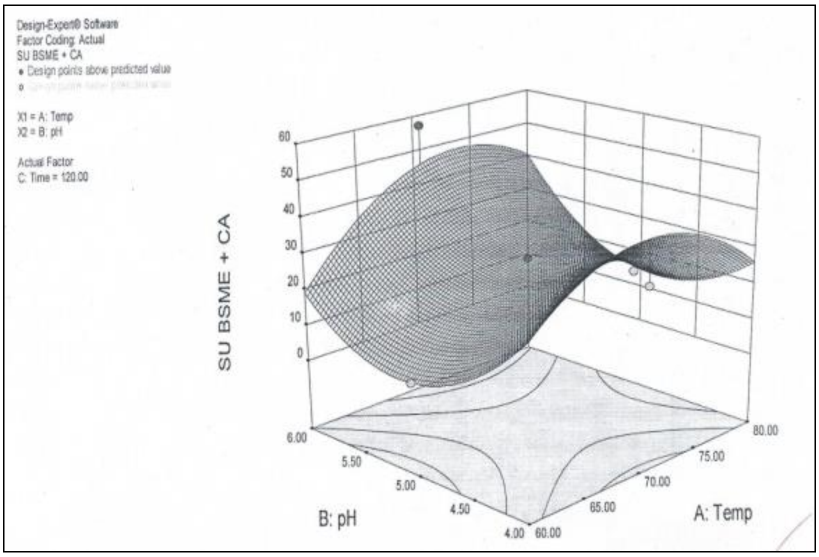

Fig 1: Surface plot for temperature versus pH on sugar yield from BSME +Cassava starch.

In addition, the analysis of variance (ANOVA) for response surface 2F1 model of sugar yield from BMME + BRME + Cassava and the estimated regression coefficient for BMME + BRME are shown in Table 7 and 8, respectively.

Table 7: Analysis of variance (ANOVA) for response surface 2F1 model of sugar yield from BMME+BRME +CASSAVA. (Partial Sum of Squares- Type III)

Source	Sum of squares	Df	Mean square	F-Value	P-value Prob>F
Model significant	10678.20	6	177970	4.05	0.032
A-Temp	-0.00	1	2890.00	6.58	0.0305
B-p^H	115.60	1	2890.00	6.58	0.0305
C-Time	2689.60	1	2689.60	6.12	0.0354
AB	3612.5	1	3612.50	8.22	0.0186
AC	312.50	1	312.50	0.71	0.4209
BC	1058.00	1	1958.00	2.41	0.1552
Residual	3955.55	9	439.51		
Lack of fit	3955.55	8	494.44		
R^2	0.7297				
R^2 Adj.	0.5495				

Table 8: Estimated regression coefficient for BMME +BRME

Factor	Coeff Estimate	df	Std Error	95% CI Low	95% CI High
Intercept	225.13	1	5.24	213.27	236.98
A-Temp	17.00	1	6.63	2.00	32
B- pH	-3.40	1	6.63	-18.40	11.60
C- Time	16.40	1	6.63	1.40	31.40
AB	21.25	1	7.41	4.48	38.02
AC	6.25	1	7.41	-10.52	23.02
BC	11.50	1	7.41	-5.27	28.27

The “Model F-value” of 4.05 implies that the model is significant. This has only 3.02% chance which is an indication that the “Model F-value which is large could occur due to noise. The value of “Pro> F” less than 0.0500 indicates that the model terms were significant. In this case, A, C and AB were significant model terms. A negative “Pred R-squared” implies that the overall mean has a better prediction of the response than the current model.

Final equation in terms of actual factors

Sugar BMME + BRME + CA =
+1036.57500
+ 10.17500 *Temp
+ 17.15000 *p^H
- 1.41417 *Time
+ 2.12500 *Temp *p^H
- 0.010417 *Temp*Time
- 0.19167 *p^H *Time

The ANOVA of sugar yield from the mixture of best maize malt extract, best rice malt extract and cassava (BMME+BRME+CASSAVA) are presented in Table 7. It was shown that the model was significant and as well exhibited a high R^2 adj. value (54.95%). This showed that the 54.95% of the response variable (sugar yield) was due to experimental variables, while the 45.05% was due to extraneous variables. Lack of fit is not good for a model. The regression equation for sugar yield obtained from the mixtures of BMME and BRME + CASSAVA are shown in equation 2.

$y = bo+b_3X_3+ b_4X_4.+b_5X_5$...............(2a)

$y = 1036.58+10.18X_{3+}175.15X_4-1.41X_5$………………(2b)

From the ANOVA, only Temp X_3 and Time X_5 could be used to fit the regression model for the effect of independent variables since they exhibited statistical significance ($p<0.05$). From the regression equation, it was found that the increase in the time of hydrolysis X_5 and temperature X_3 resulted in increased in the sugar yield. Figure 2 shows the 3-D surface plot for the effect of temperature and pH on the mixture of BMME+BRME+CASSAVA starch, while figure 3 shows the contour plot temperature versus pH on sugar yield from BSME + Cassava.

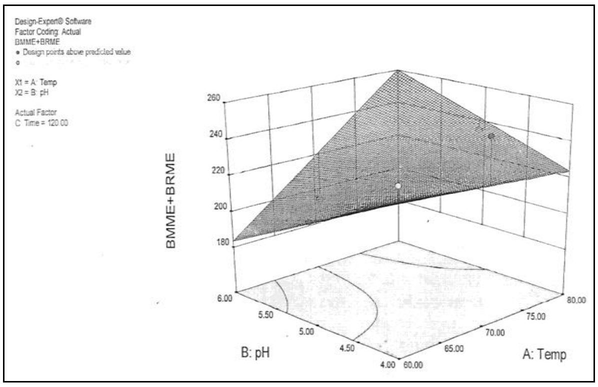

Fig 2: Surface plot for temperature versus pH on sugar yield from BMME+BRME+CASSAVA.

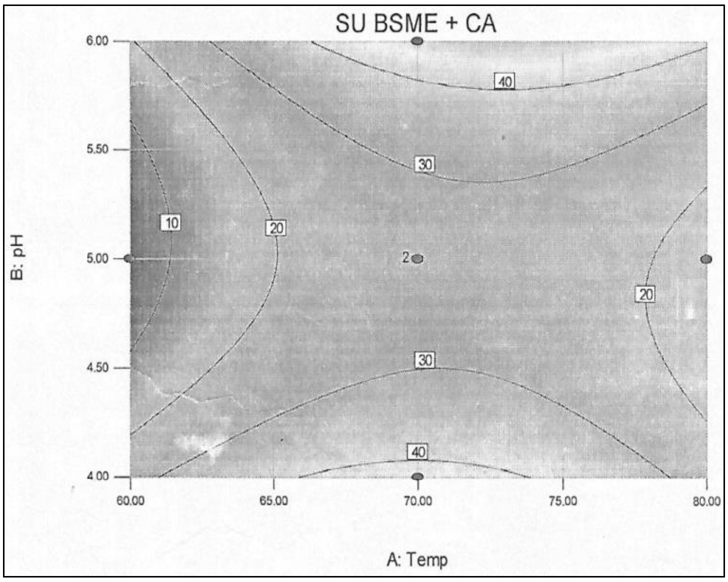

Fig 3: Contour plot for temperature versus pH on sugar yield from BSME+CASSAVA

Optimization

Response optimization of the parameters were performed using numerical optimization. The Design Expert Software was used to search for a combination of factor levels that would simultaneously satisfy the expected requirements placed on each of the responses and the factors. Optimization requires that goals (minimum and maximum targets) be set for the independent variables and responses where all the goals achieved could be combined into one desirability function (Meyers *et al.*, 2002) [13]. To find a good set of conditions that will meet all the goals, the three variables temperature (60-80 ^oC), pH (4-6) and time (1-3 h) were set within the range, while the responses were set at target. Desirability ranges from zero to one for a given response. To maximize a response, the closer the desirability to 1, the better the response values and to minimize (smaller is better), the closer the desirability to zero, the better the response values. After setting the goals for each response, the design expert software generated the optimum levels of temperature, p^H and time, respectively with the predicted responses as shown in Table 9. Below were the numerical optimization for the targeted responses. The values in Table 9 were the numerical optimization of some selected responses namely the BMME + BRME + CASSAVA, BRME + BSME + CASSAVA and BMME + BRME + BSME + CASSAVA, respectively. From the BMME + BRME + CASSAVA optimization, the process conditions, temperature (77.6 ^oC), pH (5.28) and time (141.46 h) were derived as the closest process parameters that could give the targeted value for BMME + BRME + CASSAVA with the optimum sugar desirability of 1.0. For BRME + BSME + CASSAVA, the response optimizer generated 74.6 ^oC for temperature, 5.67 for pH and 152 h for time as the process conditions that could give the targeted value of 86 with the optimum sugar desirability of 1.0. For BMME + BRME + BSME + CASSAVA, the following optimization results were obtained as stated below: target value (260), temperature (73.46 ^oC), pH (5.78) and time (170.5 h) with the optimum sugar desirability of 1.0. These were the optimum process conditions that yield the target results.

Table 9: Numeric Optimization

S/N	Responses	Lower unit	Upper unit	Target value	Temp.	p^H	Time	Sugar desirability
1	BMME+BRME+ CASSAVA	180	318	250	77.6	5.28	141.46	1.000
2	BRME+BSME +CASSAVA	71	102	86	74.6	5.67	152.83	1.000
3	BMME+BRME+BSME +CASSAVA	214	320	260	73.46	5.78	170.50	1.000

Conclusion

Enzymes from maize, rice and sorghum malt extracts were able to hydrolyze cassava starch and produce optimum yield of sugar without the addition of exogenous enzymes. The result showed that the production of reducing sugar was affected by variation of the malt extract used for the hydrolysis of cassava starch and process conditions. The highest yield of sugar was produced by combining the three cereal malts than when used individually. The fit of the model was expressed by coefficient of determination (adj.R^2) and it showed that equations 5 and 6 were suitable models for the description of the responses of the experiment in relation to the reducing sugar production. Numeric optimization of BMME + BRME + CASSAVA starch resulted in optimum values at various degrees of temperature, pH and time. The interaction effect of these processing conditions used for the hydrolysis of cassava starch produced desirable yields of reducing sugars. The reducing sugars produced from the hydrolysis of cassava starch with the use of maize, rice and sorghum malt extracts could be of great importance in both brewing and confectionary industries in Nigeria and other developing countries of the world.

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