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Effect of Process Parameters on the Viscosity and pH of Acetic Anhydride Modified Cassava Starch

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ABSTRACT

Design Expert was utilised for response surface modelling to optimize experimental conditions for the modification of cassava starch. A total of 13 runs were carried out and the results obtained were used to generate models. The significance and adequacy of the model generated were analysed using analysis of variance (ANOVA). The effects of three factors: composition (1-10 wt./vol. %), time (20-40mins) and temperature (25-40ºC) on two responses: viscosity(cp), and pH were studied and suitable and significant quadratic models were built to predict the responses and various graphs depicting favourable relationships were obtained. The modification using different reaction parameters gave a viscosity range of 142 to 275cp from that of 262cp of the native starch while the pH obtained ranges from 6.7 to 8.46 which is an improvement from that of 4.54 of native starch. The model that best explain the effect of acetic anhydride composition (A), temperature (B), and time(C) on pH and Viscosity were; (for viscosity) $Viscosity$ = +268.00 − 19.6A + 2.12B − 0.47C −32.88$A2$ − 35.87$B2$ + 2.12$C2$ + 3.63AB + 3.63AC − 0.62BC, (and pH) pH = +8.46 + 0.15A + 1.250E −003B + 0.13C − 0.48$A2$ − 0.64$B2$ − 0.071$C2$ + 0.061AB − 0.23AC − 0.049BC. These mathematical models generated gave good fits to the experimental results (R^2 greater than 0.99).

Keywords: pH, Viscosity, Modified starch, RSM, Process parameters, Design Expert

INTRODUCTION

Starch has been an unlimited subject of research for many decades and is an inexpensive, readily-available material with wide application in food and processing industry. There is a continuous need to improve its properties by different modification procedures and expand its application. Acetic anhydride is a very useful organic acid used for modifying starches for food and non-food application depending on the properties desired and obtained.

Cassava is a stable food eaten by many citizens of Nigeria as well as other tropical countries. It serves this function by being eaten in raw or processed form. The carbohydrate content of cassava is about 40% higher than rice and 25% more than maize. Also, it is the cheapest source of calories for both human and animal consumption (Muhammad-Lawal *et al.,* 2013). With these facts, cassava is considered a major player in the country's food security apart from the fact that it is a raw material for industrial processes and also a source of income for its producers and marketers. In food application, one of the advantages of cassava starch when compared to corn is the absence of the undesired "cereal flavour" (Demiate and Kotovicz, 2011).

Native starches irrespective of their source are undesirable for many industrial applications because of their inability to withstand processing conditions such as extreme temperature (has low thermal resistance), diverse pH and high shear rate (has low shear resistance) (Singh *et al.* 2007), high ability to retrograde, loss of viscosity, syneresis tendency and thickening power upon cooking and storage particular at low pH. Native starches also yield pastes of poor stability which decreases its shelf life (storage stability), causes shrinkage and the release of water. In order to improve on the desirable functional properties and overcome its limitations and defects, native starches are often modified. Starch is one of the most important but flexible food ingredients possessing value added attributes for innumerable industrial applications. The most common sources of food starch are corn, potato, wheat, cassava/tapioca and rice. Cassava is second only to sweet potato as the most important starchy root crop of the tropics (Grace, 1997). Nigeria is the world largest producer of cassava (Daramola and Osanyinlusi, 2006) and cassava is the most economic source of starch in the country as it is now being grown widely as food crop and for industrial purposes. In Nigeria, cassava is a staple food for both rural and urban areas and in recent years it has been transformed from being a subsistent crop to an industrial cash crop. Starch's use now extends far beyond its original design as a source of biological energy. Therefore, it is prudent that cassava starch should be focus for modification.

Modification, (alteration of the physical and chemical characteristics to improve structural properties) can be used to improve inherent poor physio-chemical properties of native starch thus tailor it to specific industrial applications (Miyazaki *et al.*, 2006). Starch modification can be broadly grouped into four classes namely: physical, chemical, enzymic and biological modifications (James *et al.*, 1997).

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Among these modification methods, chemical means is the most frequently used process due to its superiorities compared to other aforementioned methods (John and Raja, 1999). Practically every industry in existence uses starch or its derivatives in one form or another. In foods and pharmaceuticals starch is used to influence or control such characteristics as texture, moisture, consistency and shelf stability. It can be used to bind or to disintegrate; to expand or to densify; to clarify or to opacify; to attract moisture or to inhibit moisture; to produce smooth texture or pulpy texture, soft coatings or crisp coatings; in papermaking as wet-end additives for dry strength, surface sizes and coating binders, as adhesives (corrugating, bag, bottle labelling, laminating, cigarettes [tipping, side-seam], envelopes, tube-winding and wallpaper pastes), for warp sizing of textiles, and for glass fibre sizing. Various starch products are used to control fluid loss in subterranean drilling, work over and completion fluids (for oil, gas or water production). It can be used to stabilize emulsions or to form oil resistant films. Modified starches are also used in tableting and cosmetic formulations. Some starch is incorporated into plastics to enhance environmental fragmentation and degradation. Thermoplastic starch and starch–polymer composites can replace petroleum-based plastics in some applications. Newer applications include use of non-digestible starch as neutraceuticals. The future of starch may include a role in detergents (Chung-wai and Daniel, 2009). Starch truly serves as a multifunctional ingredient in the food industry (Miyazaki *et al.*, 2006).

The objective of this work was to utilised response surface modelling (Design Expert) to study and optimize the effects of some process parameters (composition, time and temperature) on the properties (viscosity and pH) of modified cassava starch.

MATERIALS AND METHOD

The materials used in this research work and where they were sourced are listed below

Cassava tubers: sourced locally from Kaduna Central Market, Kaduna State.

Reagents: Acetic anhydride, HCl and NaOH; sourced from Steve Moore Chemicals. Co, in Emanto along Kwangila axis, Sabo Gari, Zaria, Kaduna State and used without purification.

Distilled water from Chemical Engineering Department, ABU Zaria.

Extraction of Cassava Starch

Cassava starch was extracted following the methods of Akpa and Dagde (2012) and Adeoya *et al*. (2015) with modifications. Fresh cassava roots were peeled, washed and then pounded in a mortar to reduce particle size. The resulting cassava mesh was then milled and mixed with water. The mesh was filtered through with a single layer of nylon cloth and the resulting filtrate was filtered with a double layer nylon cloth to obtain starch solution. The starch was separated from most of the water by decantation. The resulting starch was then washed and dewatered by placing in a clean bag. It was then air dried for five days. The resulting lumps were then pounded and sieved (500µm) to obtain powdered native cassava starch.

RSM Design

The procedure of optimization has been represented in the form of a flowchart as shown in Figure 1. A set of 13 runs were designed to optimize cassava starch modification. Four independent variables (factors) composition, temperature and time were investigated and each variable in the design was studied at four different coded levels (responses) namely viscosity (R1) and pH (R2). The generated RSM design is shown in Table 1.

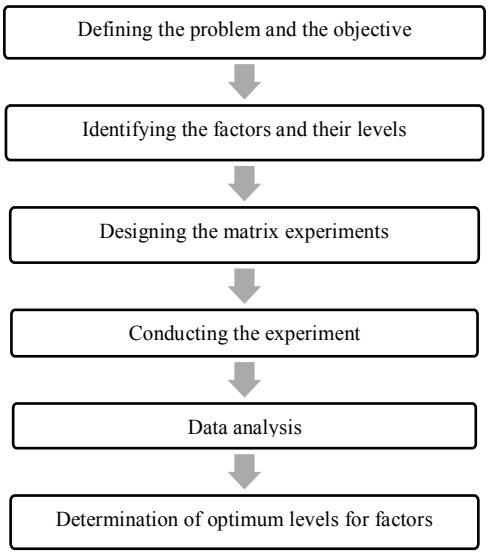

Figure 1: The Procedure of using RSM for Optimization

Table 1: RSM Experimental Design

RUN	Factor 1 A:Composition (wt/vol %)	Factor 2 B:Temperature (°C)	Factor 3 C:Time
1	5.50	32.50	30.00
2	5.50	32.50	13.18
3	10.00	40.00	40.00
4	5.50	32.50	46.82
5	10.00	40.00	20.00
6	1.00	25.00	40.00
7	13.07	32.50	30.00
8	10.00	25.00	40.00
9	5.50	32.50	30.00
10	1.00	40.00	40.00
11	1.00	25.00	20.00
12	1.00	40.00	20.00
13	10.00	25.00	20.00

The influence of individual factor from the experiments and their performance at optimum condition by using RSM approach were analysed by the software Design Expert (version 6.0.6).

Preparation of Starch Acetate

Starch acetylation was carried out following the methods described by Sherry *et al.* (2005) and Adeoya *et al.* (2015) using the values generated in Table 2. A slurry of starch was prepared by dispersing fifty grams (50 g; dry weight basis) of native cassava starch in 100ml distilled water. The pH was adjusted to 8.0 with 3% NaOH and adjusted to a temperature of 32.50°C corresponding to Run 1 being prepared. 2.75ml acetic anhydride (5.5 wt/vol %) was then added slowly to the starch slurry while maintaining temperature of 32.50°C. After addition of reagents, the mixture is stirred for 30mins, and then adjusted to pH 7 using 10% HCl.

The starch was washed with distilled water and decanted to remove most of the water and then air dried for three days. The dried acetylated starch samples were then pounded and sieved (500 m) and packaged in plastic containers and kept in cool dry place for further analyses. The procedure above was repeated for the rest of the runs as predicted by the RSM model (Table 1)

pH Determination

To 10ml of distilled water in a beaker was added 1.5g of the modified starch and the mixture was thoroughly stirred. The pH meter was then inserted into the solution and the pH value was read and recorded.

Determination of Viscosity

The viscosities of the starch samples were obtained using a Faan viscometer. The gelatinized starch at a room temperature of 24°C was placed under the viscometer with the spindle immersed in the beaker. The spindle was set at a speed of 300rpm. The readings were recorded as soon as the starch stabilizes.

RESULTS AND DISCUSSION

The results of the responses observed from the experimental data in Table 1 of the RSM design model is shown in the Table 2:

Table 2: Observed results

RUN	Factor 1 A: Comp. (wt %)	Factor 2 B: Temp. (°C)	Factor 3 C:Time	R 1 Viscosity (cp)	R 2 pH
1	5.50	32.50	30.00	270	8.45
2	5.50	32.50	13.18	275	8.08
3	10.00	40.00	40.00	190	7.31
4	5.50	32.50	46.82	273	8.43
5	10.00	40.00	20.00	185	7.64
6	1.00	25.00	40.00	219	7.57
7	13.07	32.50	30.00	142	7.34
8	10.00	25.00	40.00	180	7.35
9	5.50	32.50	30.00	266	8.46
10	1.00	40.00	40.00	215	7.42
11	1.00	25.00	20.00	226	6.79
12	1.00	40.00	20.00	224	6.70
13	10.00	25.00	20.00	172	7.35

Where R is response

Response 1: Viscosity

Chemical Analysis

The viscosity of a substance is known as its resistance to flow and increases as temperature decreases or decreases as temperature increases (Coulson and Richardson, 2004). Starch paste viscosity can be increased or reduced by applying a suitable chemical modification (Agboola *et al.,* 1991). The viscosity of each sample was taken at a room temperature of 24°C and from the results obtained, the highest viscosity of 275cp was recorded at run 2 which has an acetic anhydride composition of 5.5 wt %, reaction time of 13.18mins and reaction temperature of 32.50°C while the lowest viscosity reading of 142cp was recorded at run 7. The viscosity of the native starch is 262cp and as it can be seen from Table 2, the viscosities of the samples ranges between 142-275cp indicating both decrease and increase from that of the native starch. This variation in viscosity as explained from the findings of Huang *et al.* (2007), who postulated that the viscosity of starch pastes could be influenced by the homogeneity of acetylation, i.e., whether it is limited to the outer lamellae of granules or located in their inner part. Saartrat *et al.* (2005) have demonstrated that the viscosity of acetylated starches is influenced by two factors: a weakening starch granule due to disruption of the inter- and intra-molecular bonds, and reduced bonding with water molecules due to the hydrophobicity of acetyl groups. Depending on the interplay between these two factors, the viscosity could be decreased or increased by acetylation.

Model Fitting and Statistical Analysis

The data were fitted with various models and their subsequent ANOVA showed that the effect of factors 1, 2, and 3 on Response 1 (viscosity) was most suitably described by quadratic polynomial model. The final model to predict the effect of process parameters (factors) is shown in equation 1 below:

$$\text{Viscosity} = +268.00 - 19.6*A + 2.12*B - 0.47*C - 32.88*A^2 - 35.87*B^2 + 2.12*C^2 + 3.63*A*B + 3.63*A*C - 0.62*B*C \quad \ldots\ldots\ldots\ldots 1$$

Where A is the coded value for composition of acetic anhydride (wt %),

B is the coded factor for temperature of reaction (°C) and C is the coded factor for time of reaction (mins)

Statistical significance of the model

The adequacy and significance of the model was justified by the analysis of variance (ANOVA). Analysis of the acquired data shows that the Model F-value is 908.97 which implies that the model is significant and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The F-value of the model has P-value 0.0001 < 0.05 (significance level) which indicates that the overall model has significant capacity to explain variation in response variable 1 (viscosity). At 5 % significance level, a model is considered significant if the P value (significance probability value) is less than 0.05 (Ahmed *et al*, 2016). In this case A, B, A^2, B^2, C^2, AB, AC are significant model terms. Thus, both linear (effects of A and B) and quadratic effects (effects of A^2, $B^2$and C^2) and interaction effect of AB and AC are also

significant. But interaction effect (effect of BC) is not significant.

The "Lack of Fit F-value" obtained from the analysis is 0.02 which implies that the Lack of Fit is not significant relative to the pure error. There is a 98.27% chance that a "Lack of Fit F-value" this large could occur due to noise. Insignificant lack of fit also reflects the model adequacy.

Coefficient of determination, R^2, always lies between 0 and 1 (Priya and Kanmani, 2011). However, Masoumi, *et al.* (2013) stated that if R^2 value lies between 0.90 and 1.00, the fitted regression equation is considered as a model having a high correlation. Thus, the R^2 = 0.9996 implies that the model can explain 99.96 % variation in R1. In addition, the value of adjusted determination coefficient Adj R^2 = 0.9985 was also satisfactory, confirming the significance of the model.
The value of coefficient of variation (CV = 0.76) implied that there is good validity in the experimental data and the model. The model can be considered reasonably reproducible if its CV is not greater than 10%. A lower value of CV clearly showed a high degree of precision. Adequate precision is the measurement of signal to noise ratio and the model also showed a high ratio of 91.104. The ratio greater than 4 is desirable. Thus, a ratio of 91.104 indicates an adequate signal (Masoumi *et al.,* 2013). The figures below show various graphical relationships between viscosity and the factors.

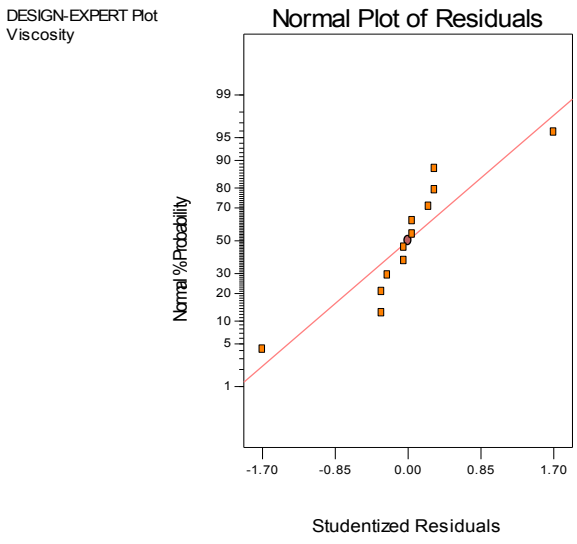

Figure 2: Normal plot of Residues

Figure 2, Figure 3, and Figure 4 shows the normal curve for the distribution, while Figure 5 depicts the relationship between viscosity and temperature and viscosity with composition at reaction time of 30mins.
It can be seen that viscosity is maximum at the intermediate temperature and composition.

Response 4: pH

Chemical Analysis

The pH of a solution is an indicator of the degree of alkalinity or acidity of the solution. The results obtained for pH as contained in Table 1 shows that the run with the highest pH is Run 9 while that of the smallest is Run 12. The pH of the native starch is 4.54.

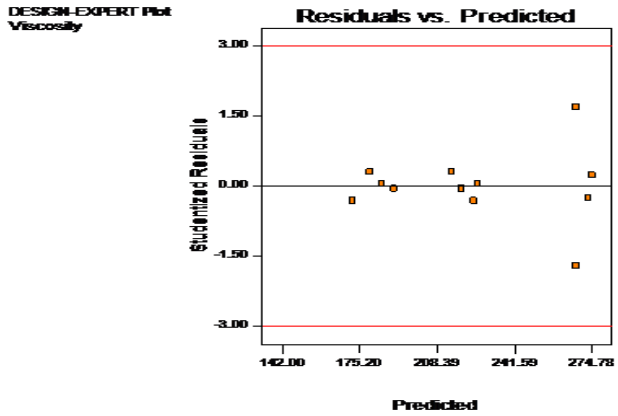

Figure 3: Residue vs. Predicted

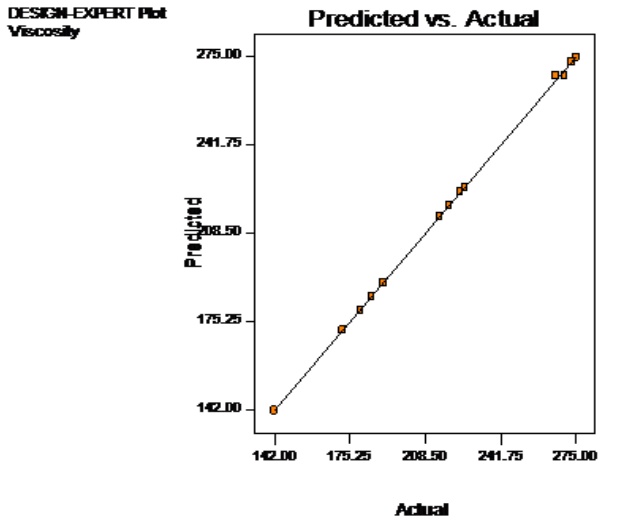

Figure 4: Predicted vs. Actual

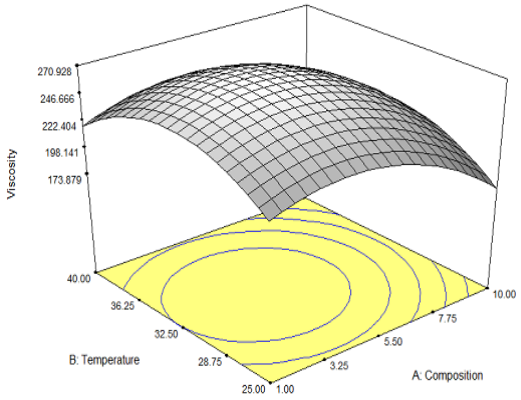

Figure 5: Response surface plot

In general, the pH of all the samples are within desirable limits of neutrality and since according to Akpa and Dagde (2012) it is necessary that the pH of starch tends towards neutrality so that it can be used in industries where a change in the pH of products is not desired thus the modified starches fit this application since the modification improved the pH of the native starch.

Model Fitting and Statistical Analysis

The data were fitted with various models and their subsequent ANOVA showed that the effect of factors 1, 2, and 3 on Response 4 (pH) was most suitably described by quadratic polynomial model. The final model to predict the effect of process parameters (factors) is shown in equation 1:

$$pH=+8.46 +0.15*A +1.250E-003*B +0.13*C-0.48*A^2 - 0.64*B^2 -0.071*C^2 +0.061*A* B -0.23*A*C -0.049*B*C \quad (1)$$

Where A is the coded value for composition of acetic anhydride (wt %),
B is the coded factor for temperature of reaction (°C) and C is the coded factor for time of reaction (mins)

Statistical significance of the model

The adequacy and significance of the model was justified by the analysis of variance (ANOVA). Analysis of the acquired data shows that the Model F-value is 91.70 which implies that the model is significant and there is only a 0.17% chance that a "Model F-Value" this large could occur due to noise. The F-value of the model has P-value 0.0017 < 0.05 (significance level) which indicates that the overall model has significant capacity to explain variation in response variable 4 (pH). At 5 % significance level, a model is considered significant if the P value (significance probability value) is less than 0.05 (Ahmed *et al*, 2016).

In this case A, C, A^2, B^2, AC are significant model terms. Thus, only linear (effects of A and C) and quadratic effects (effects of A^2 and B^2) and interaction effect (AC) are significant.

The "Lack of Fit F-value" obtained from the analysis is 150.12 which implies that the Lack of Fit is not significant relative to the pure error. There is a 5.76% chance that a "Lack of Fit F-value" this large could occur due to noise. Insignificant lack of fit also reflects the model adequacy.

Coefficient of determination, R^2, always lies between 0 and 1 (Priya and Kanmani, 2011). However, if R^2 value lies between 0.90 and 1.00, the fitted regression equation is considered as a model having a high correlation. Thus, the R^2 = 0.9964 implies that the model can explain 99.64 % variation in R^2. In addition, the value of adjusted determination coefficient Adj R^2 = 0.9855 was also satisfactory, confirming the significance of the model.

The value of coefficient of variation (CV = 0.93) implied that there is good validity in the experimental data and the model. The model can be considered reasonably reproducible if its CV is not greater than 10%. A lower value of CV clearly showed a high degree of precision. Adequate precision is the measurement of signal to noise ratio and the model also showed a high ratio of 27.682. The ratio greater than 4 is desirable. Thus, a ratio of 27.682 indicates an adequate signal (Masoumi *et al.,* 2013).

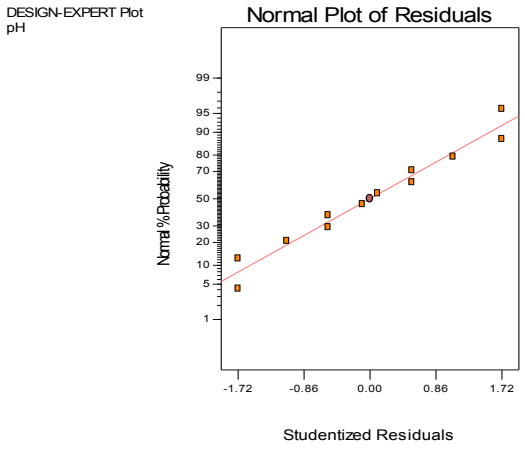

Figure 6: Normal Plot of Residue

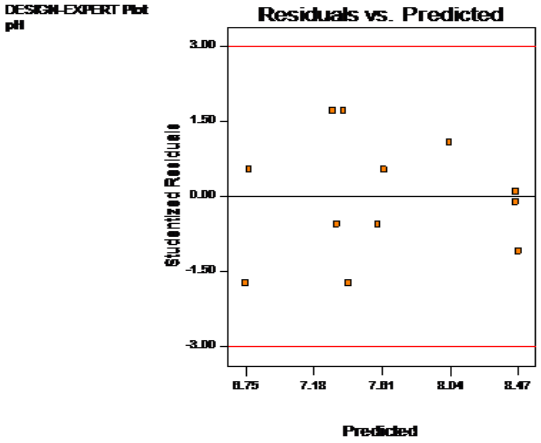

Figure 7: Residual vs. Predicted

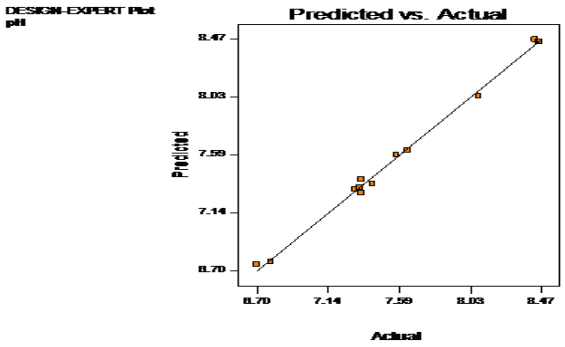

Figure 8: Predicted vs. Actual

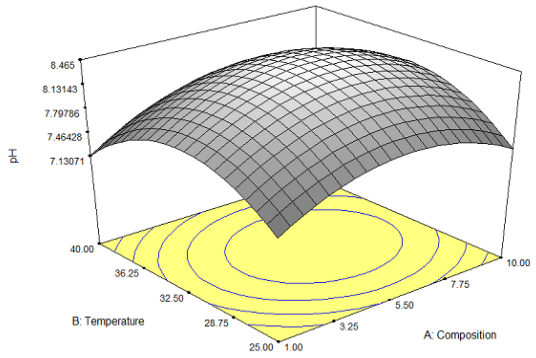

Figure 9: Response Surface Plot

Figure 6, Figure 7, and Figure 8 shows the normal curve for the distribution, while Figure 9 shows the relationship between pH and temperature and composition at reaction time of 30mins. It indicates lowest pH values at extreme points.

CONCLUSION

Modification of cassava starch to improve on its desirable properties was done using acetic anhydride and the optimal conditions for this process was analysed using RSM. The results obtained showed an improvement in some of the properties tested. The modification at different reaction parameters gave a viscosity range of 142 to 275cp from that of 262cp of the native starch indicating variations which can be attributed to reasons stated earlier. The pH obtained ranges from 6.7 to 8.46 which is an improvement from that of 4.54 of the native starch and thus makes the modified starches suitable for application in industries requiring neutral pH. The mathematical models generated gave good fits to the experimental results (R^2 greater than 0.99).

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