ORIGINAL RESEARCH ARTICLE

Modifying cooking banana starch using octenyl succinic anhydride improves the amylose-amylopectin ratio of starch. A chemometrics approach.

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Abstract

The disadvantage posed by native starch during food application had led to starch modification using physical or chemical techniques. This research therefore, aimed at modelling and optimizing the amylose-amylopectin ratio of modified cooking banana starch using chemometrics approach (response surface methodology). This was done by varying different concentration of octenyl succinate anhydride concentration (3-5%), reaction time (30-60 mins) and pH (8-10) using Box-Behnken design. The result obtained revealed the significance and accuracy of the model in predicting the amylose-amylopectin ratio of the modified starch owing to its low p-value ($p < 0.001$) and high coefficient of determinant ($R^2 > 0.97$). The adequate precision value greater than 4 was an indication that the model can navigate within the design space. Finally, an optimal value of 3.32% octenyl succinate anhydride concentration, reaction time of 32.04 mins and substrate pH of 8 was obtained which resulted in predicted amylose-amylopectin ratio of 0.806.

Keywords*: Amylose-amylopectin ratio; modified starch; chemometrics; cooking banana*

1. Introduction

The increasing world population has compelled the need for food processors and manufacturers to explore underutilized plants that serve as a rich starch sources. Native starch has existing disadvantages in its functionality such as its disposition to retrogradation and less stability to thermal and mechanical treatment (Cahyana et al., 2019) which deters its use for varieties of applications in food. This, however, calls for an absolute requirement for the exploration of specific technologies to modify starch properties.

Starch functions as an important food reserve in plants and it is a principal component of the majority of storage organs in tubers, legumes, cereal grains. It is commonly utilized for various applications such as in food additives typically as thickeners together with stabilizers, in pharmaceutical industries as a source of energy in addition to its use in the production of ethanol as well as gel (Khawas & Deka, 2017). It forms a major dietary constituent in all human populace contributing to the structure (viscosity or texture) of a great range of foods produced on a large scale and for home use. Starch, a carbohydrate, comprises a considerable extensive number of units of glucose which are connected by glycosidic bond (Sonthalia & Sikdar, 2015). The functionality of starch is related to two (2) significant-high molecular weight constituents of carbohydrates; amylose (a soluble linear polymer) and amylopectin (an insoluble extremely branched polymer), in addition to the organization of these two large molecules.

Modification of starch functionality attributes using octenyl succinic anhydride (OSA) enhances characteristics such as solubility, its nutritional, stabilizing, swelling power, thermal, rheology, encapsulating, interfacial and pasting characteristics (subject to the ratio of amylose to amylopectin) (Sweedman et al., 2013). The amylose-amylopectin ratio plays a major role in the interaction of starch and water. Reddy et al. (2015) reported that peak viscosity among different banana cultivars was a function of leaching of amylose, granules swelling and amylose-amylopectin ratio. They also concluded that the thermal properties of banana starch may be subject to starch granule distribution, extraction procedure and the complexes of amylose-amylopectin.

The amylose-amylopectin ratio of starch has a significant outcome on the modification of starch physical and chemical properties such as solubility, syneresis, texture, retrogradation, gelatinization, viscosity, texture and water retention which are important standards of choice selection of suitable food products from starch (Karakelle et al., 2020). This limitations in solubility of native starches in water result in restrictions in their industrial usage, hence the need for their modifications to change their structure by hydrolyzing them into smaller molecules. OSA starch (the resulting starch after esterification with octenyl succinic anhydride) has had wide usage industrially especially as an additive in food and also as a replacement for diverse food substances such as emulsifiers, proteins, fats and gum arabic (Sweedman et al., 2013). Biopolymers, which can be employed as emulsion stabilizers in varieties of products are usually produced from this chemical modification.

Different modifications on banana, which is an efficacious starch source have been studied by several researchers. Industrially, banana starch in its native form has various setbacks such as reduced heat stability as well as the tendency towards retrogradation (Cahyana et al., 2019), hence, the need for physical, chemical or enzymatic modification. Banana starch modification can be achieved by modifying conditions of the environment such as atmosphere and temperature for shelf-life extension. Adiyanti and Subroto (2020) reported that OSA banana starch can serve as a stabilizing agent in emulsions increasing hydrophobic properties. Asides from its usefulness as a stabilizing agent in emulsions, banana starch that has been modified also possesses increased elastic characteristics in comparison to its native starch. These researchers compared the properties of native banana starch and modified starch and reported changes in the structure of amylose content, digestibility, starch granules and crystal structure as well as emulsifying properties of modified banana starch. Quintero-Castaño et al. (2020) also characterized the physical, chemical, structural, morphological and functional properties of starch modification from Gros Michel banana. They concluded an alteration in the ratio of amylose-amylopectin after modification.

However, for optimal response during the modification process, there is a need for an application of chemometrics tools such as response surface methodology, artificial neural network or other machine learning algorithms. Chemometrics is the application of statistic and mathematical modeling to chemical data. This approach especially optimization techniques had gain wide application in food processes. Akande et al. (2017) use the approach in optimizing extrusion process during the production of amaranth-based porridge. Fasuan et al. (2018) in their own research uses the approach in the modification of amaranth starch for its suitability as functional ingredient in mayonnaise production. Although, this approach had been widely used, it's application in production of starch from cooking banana with high amylose-amylopectin ratio is scanty hence the aim of this research.

2. Materials and methods

The cardaba banana used for this research was obtained from the teaching and research farm of Obafemi Awolowo University Ile-Ife, Nigeria. Chemicals used for the analysis were obtained from Sigma-Aldrich, U.S.A.

2.1. Starch extraction

The extraction of the cardaba banana starch was carried out using the method of Olawoye et al. (2020a). Briefly, the cardaba banana was de-bunched, washed and subsequently peeled underwater to avoid enzymatic browning of the banana. After peeling, the banana was cut into small sizes and wet-milled using a Stephan milling machine (Germany). Following milling, water was added to the banana mash obtained in the ratio of 1:10 (w/v) (is it weight : volume or volume: volume?) and was subsequently passed through 200 μm sieves to obtained a whitish starch slurry. The starch slurry was left to stay for 6 hours to allow the starch to settle down. This was followed by washing the starch three times with water and allowing it to settle down. After, the final washing, the starch slurry was made to stay overnight, after which the water was decanted and the starch obtained was dried at 45 ℃ for 8 hours. the dried starch was pulverized and packaged in an airtight container before modification and analysis.

2.2. Starch modification

The modification of cardaba banana starch using octenyl succinate anhydride was carried out following experimental design using the method described by Olawoye and Gbadamosi (2020).

2.3. Experimental design

The experimental design for the modification process was carried out using a three-factor Box-Behnken design. The three factors and levels considered for the modification process are succinate anhydride (2-5%), time of modification (30-60 mins) and pH of the substrate (8-10). The response measure after the modification process was the amylose-amylopectin ratio. In the experimental design, a 12-factorial design and 5 centre points were generated. After the experimental design, a second-order polynomial model (Eq. 1) was fitted to determine the relationship between the independent variables and experimental response. The goodness of fit of the experimental model was evaluated using analysis of variance (ANOVA) while the effects of the various model terms coupled with their interaction were evaluated using the Pareto chart. The Box-Behnken design of the response surface methodology was performed using Design Expert 13.0.1 (State-Ease Inc., Minneapolis, U.S.A.).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X^2{}_i + \sum_{i<j}^{k} b_{ij} X_i X_j + e \quad (1)$$

Where Y is the response variable (amylose-amylopectin ratio), b_0 is the intercept value, b_i (I = 1, 2, ….k) is the first-order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficient of X_i. X_i and X_j are the independent variables that affect the dependent (response) variables and e represents the random error.

2.4. Amylose content determination

The amylose content of the modified starches was determined following the method described by Nwokocha et al. (2011). Briefly, 1ml of 95% ethanol was dispersed into a test tube already containing 0.1 g (dry basis) of modified cardaba banana starch followed by the addition of 9ml of 1M NaOH solution. The mixture was vortexed and heated in a hot water bath at 45 ℃ for 10 mins for the solubilization and gelatinization of the starch. After heating, the gelatinized starch was transferred into a 100 ml volumetric flask and was filled to mark using distilled water. From the starch solution, 5 ml was taken and dispensed into a 100 ml conical flask and 1 ml of 1 M acetic

acid as well as 2 ml of iodine solution (0.2 g I_2/2 g KI). The solution made up to 100 ml using distilled water and was allowed to stay for 20 mins for colour development. The absorbance of the solution in a 1 cm cuvette was read using a UV-Vis spectrophotometer at 620 nm. Iodine solution devoid of starch was used in the reference cell. Potato amylose with the concentration range of 10 - 50 mg was used for the preparation of the calibration curve from whence the amylose content of the modified cardaba banana starch was obtained through extrapolation.

The blue value of the modified starch was calculated using the equation below.

$$\boldsymbol{Blue\ value\ (BV)} = \frac{\boldsymbol{maximum\ absorbance \times 4}}{\boldsymbol{starch\ concentration\ (\frac{mg}{dl})}} \quad (2)$$

$$\%\boldsymbol{Amylose} = \boldsymbol{110.78 \times BV - 24.481} \quad (3)$$

$$\%\boldsymbol{Amylopectin} = \boldsymbol{100 - \%Amylose} \quad (4)$$

$$\boldsymbol{Amylose : Amylopectin} = \frac{\%\ \boldsymbol{Amylose}}{\%\ \boldsymbol{Amylopectin}} \quad (5)$$

3. Result and discussion

The result of the experimental design using Box-Behnken design (BBD) for the amylose-amylopectin ratio of the modified starch is shown in Table 1. As shown in the table, the amylose to amylopectin ratio of the starch varied between 0.26 and 0.80 for observed value while the predicted value range between 0.23 - 0.79.

Table 1. Experimental and predicted values of slowly digestible starch

	Independent variable			Amylose-amylopectin ratio	
EXP. Run	Succinate Concentration (%)	Time (min)	pH	Actual	Predicted
1	3	45	10	0.68	0.70
2	4	45	9	0.60	0.64
3	3	60	9	0.26	0.23
4	4	60	10	0.56	0.58
5	4	60	8	0.47	0.49
6	4	45	9	0.66	0.64
7	4	45	9	0.68	0.64
8	4	30	10	0.80	0.79
9	4	45	9	0.63	0.64
10	5	30	9	0.43	0.46
11	5	45	8	0.64	0.63
12	4	45	9	0.64	0.64
13	5	45	10	0.74	0.72
14	3	30	9	0.67	0.67
15	5	60	9	0.42	0.42
16	4	30	8	0.78	0.76
17	3	45	8	0.66	0.67

To describe the relationship between the experimental factors (succinate concentration, substrate pH and time) as well as the response (amylose-amylopectin ratio), the ratio of the amylose to

amylopectin was fitted using a quadratic regression model as shown in equation 6 below. As shown in equation 2, A, B, and C represent the moisture content, temperature and time, respectively.

$$\boldsymbol{AM: AMY = 0.64 + 0.006A - 0.12B + 0.03C + 0.10AB + 0.02AC + 0.002BC - 0.09A^2 - 0.11B^2 + 0.12C^2} \quad (6)$$

The ANOVA result of the model revealed that the statistical model for the resistant starch is significant owing to its low p-value (<0.0001) and its high Fisher test value (33.19). The result also revealed that among all the terms, the linear term of succinate concentration, the interaction term of succinate concentration and pH as well as interaction term of time and pH were not significant. Among the terms, it could be observed that the linear term of the reaction time was the most significant, evidence of its high F-value (112.01). The lack of fit which is the measure of the accuracy of the polynomial model is 0.3810. The non-significance of the lack of fit is an affirmation of the accuracy of the model. The quality of the model was examined and Table 2. shows the model quality parameter. As it could be seen from the result, the adequate precision which measures and compares the difference between experimental and predicted values was 30.17. According to Olawoye et al. (2020b), an adequate precision ratio greater than 4 is desirable and hence affirmed the accuracy of the model and it also indicates that the experimental model could be used to navigate the design space. The ability of the model to accurately fit the experimental data was determined by evaluating the coefficient of determinant (R^2) (Olawoye and Kadiri, 2016). According to the result presented in Table 2, the R^2 is 0.9771 which is an indication that 97.71% of the variation in the experimental data for the amylose-amylopectin ratio is acclaimed to the independent variables while 2.29% of the variation can't be explained by the experimental model.

The relationship between the experimental response (amylose-amylopectin ratio), as well as the model terms, is shown in the Pareto chart (Fig. 1) below. From the Pareto chart, it could be seen that among the model terms, the linear terms of succinate concentration and time as well as the quadratic term of pH had a negative value and hence, a negative synergistic effect of the model terms on the experimental response. Also, it could be seen that the Interaction terms AC and BC, as well as the linear term of succinate concentration, are below the reference red line which indicates their insignificance in predicting the experimental response.

Table 2: Regression analysis of Amylose-amylopectin ratio

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.3119	9	0.0347	33.19	< 0.0001	significant
A-Succinate Concentration	0.0003	1	0.0003	0.2850	0.6100	
B-Time	0.1169	1	0.1169	112.01	< 0.0001	
C-Ph	0.0071	1	0.0071	6.80	0.0350	
AB	0.0412	1	0.0412	39.46	0.0004	
AC	0.0014	1	0.0014	1.29	0.2927	
BC	0.0011	1	0.0011	1.08	0.3330	
A^2	0.0320	1	0.0320	30.68	0.0009	
B^2	0.0525	1	0.0525	50.24	0.0002	
C^2	0.0656	1	0.0656	62.81	< 0.0001	
Residual	0.0073	7	0.0010			
Lack of Fit	0.0037	3	0.0012	1.33	0.3810	not significant
Adeq Precision	22.68					
C.V. %	5.33					
R^2	0.9771					
Adjusted R^2	0.9477					
Predicted R^2	0.7989					

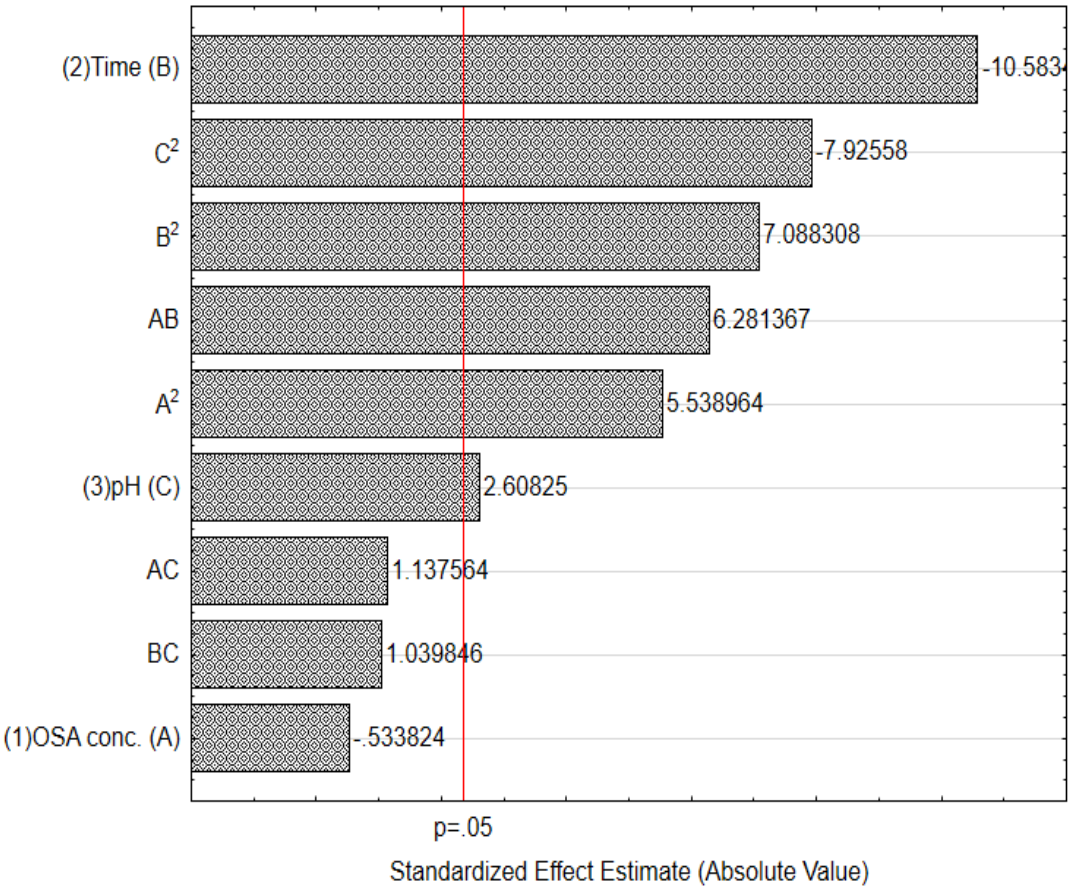

Figure 1: Pareto chart showing the effects of model terms on Amylose-amylopectin ratio

3.1. Diagnostic effect of the experimental model

A diagnostic plot which determines the quality of the developed model on the residuals is presented in Figure 2 (a-d). Figure 2 (a) depicts the plot of a normal probability distribution of the residuals. According to Olawoye et al. (2020c), a studentized residual followed a normal distribution if a straight line is being formed, which commensurate with the findings of the research. In the case an S-shape is formed, the studentized residuals do not follow a normal distribution and hence there is a need for the transformation of the experimental data. Figure 2(b) denote the plot of the studentized residuals against the amylose-amylopectin ratio. As shown in the plot, there is random scattering of the data which is an indication that the response does not contribute to the variation of the experimental data and hence, the accuracy of the model in predicting the experimental process. The outlier t plot which is used to check if there is an outlier in the experimental process as a result of a large residual is shown in Figure 2(c). The plot ranged between a standard deviation of 3, any plot that falls above or below this limit is an indication of experimental error or an outlier in the experimental data. The findings in this study indicate that the plot is within the limit and hence, no studentized residuals variation due to outlier. Finally, the plot of the actual amylose-amylopectin ratio against the predicted value is shown in Figure 2(d). The alignment of the points (values) along the straight line is an indication of the goodness of fit of the model. Where the points are far away from the line or are scattered along the plot, then there exists an experimental error in the model used.

3.2. Effect of processing variables on the amylose-amylopectin ratio

The relationship between the processing parameters and the amylose-amylopectin was examined by plotting the 3-D response surface plot while keeping one independent factor at its centre point. Figure 3(a) shows the interactive effects of reaction time and succinate anhydride concentration while the pH is constant. The plot revealed that the maximum amylose-amylopectin ratio could be obtained within the space of the experimental design. As it could be seen from the surface plot, the succinate anhydride concentration had a linear effect on the response. An increase in the succinate concentration initially had an increasing effect on the amylose-amylopectin ratio, however, an increase in the Succinate anhydride concentration above 4.25% led to an insignificant decrease in the amylose-amylopectin ratio of the modified starch. The reaction time on the other hand had a quadratic effect on the response. Its increase from 25 - 40 mins resulted in a slight increase in the amylose-amylopectin ratio, an increase above 40 mins caused a significant decrease in the amylose-amylopectin ratio of the modified starch. The decrease in the amylose-pectin ratio observed as a result of an increase in the reaction time could be due to the leaching of amylose due to lengthening minutes. For maximum response, the reaction time was 37 mins at a succinate anhydride concentration of 4.1%. The effect of the pH of the substrate coupled with succinate anhydride concentration on the amylose-amylopectin ratio of the modified starch while keeping the reaction time constant is shown in Figure 3b. The result revealed that increasing the pH of the substrate up to 9.2 resulted in a decrease in the response. There exists a significant increase as the pH of the substrate progresses above 9.2. Increasing the succinate anhydride to a concentration of 4.2 caused an increase in the amylose-amylopectin reaction of the starch. Above 4.2 however, led to a significant decrease in the response. A combined effect of both modification parameters revealed that a succinate anhydride concentration of 4.1 coupled with maximum modification pH results in maximum amylose-amylopectin ratio. The simultaneous effect of the modification pH and time of modification on the amylose-amylopectin ratio is shown in Figure 3. (c). There exists a slight decrease in the response as the pH increases to 9.2 which further reduces as the pH increases above 9.2. The time of modification causes a slight but non-significant increase in the response when it increases from 25-41 mins, above this time, it was observed that the amylose-amylopectin ratio decreased significantly. As seen in the plot, the maximum amylose-amylopectin ratio was obtained when the time of modification was 40 min at a maximum pH.

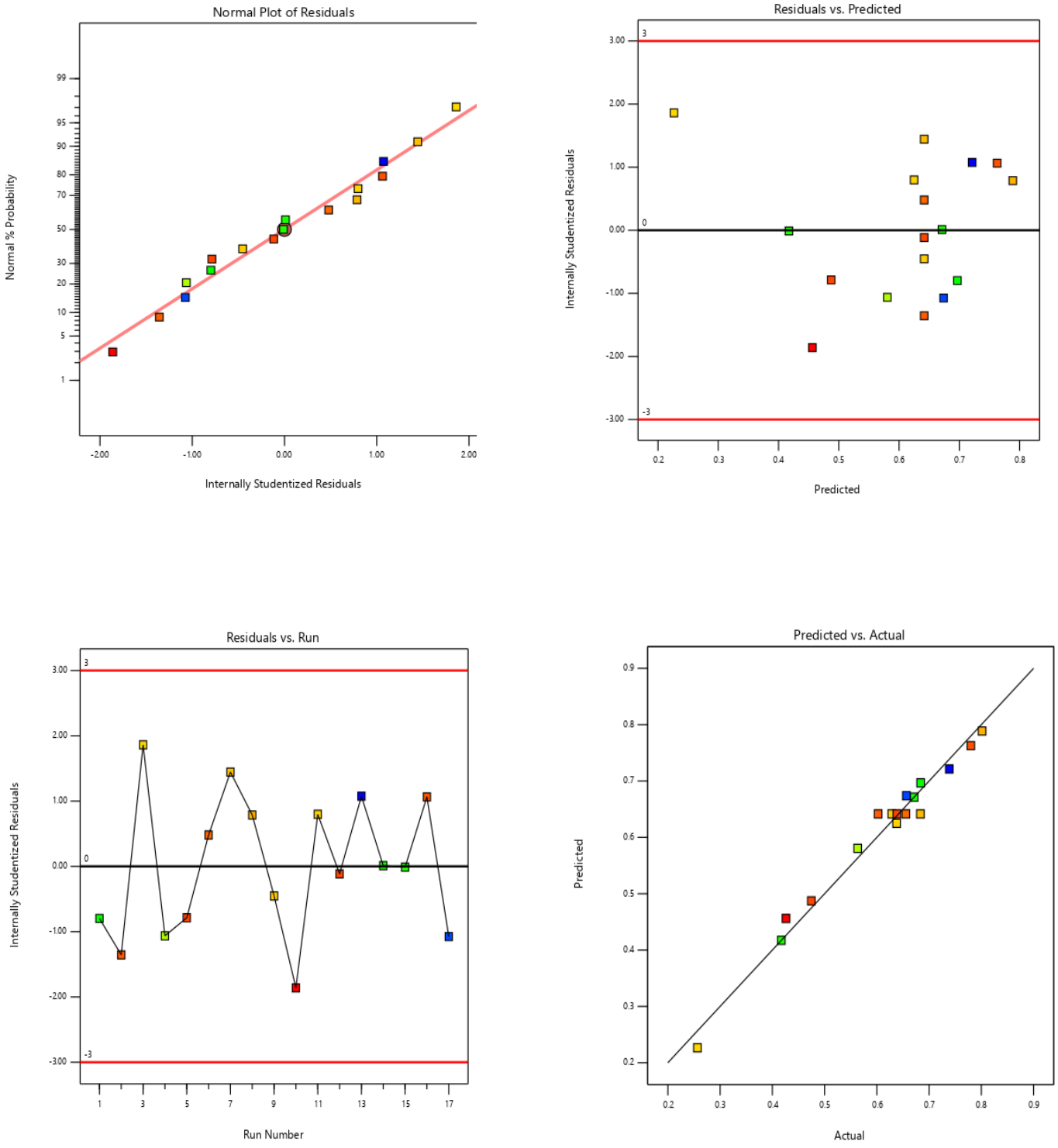

Figure 2: Diagnosis analysis of the model: (a) Normal Distribution Plot; (b) Studentized Residual and Predicted Amylose-amylopectin ratio Plot; (c) The Outlier t Plot; (d) Predicted vs actual value plot.

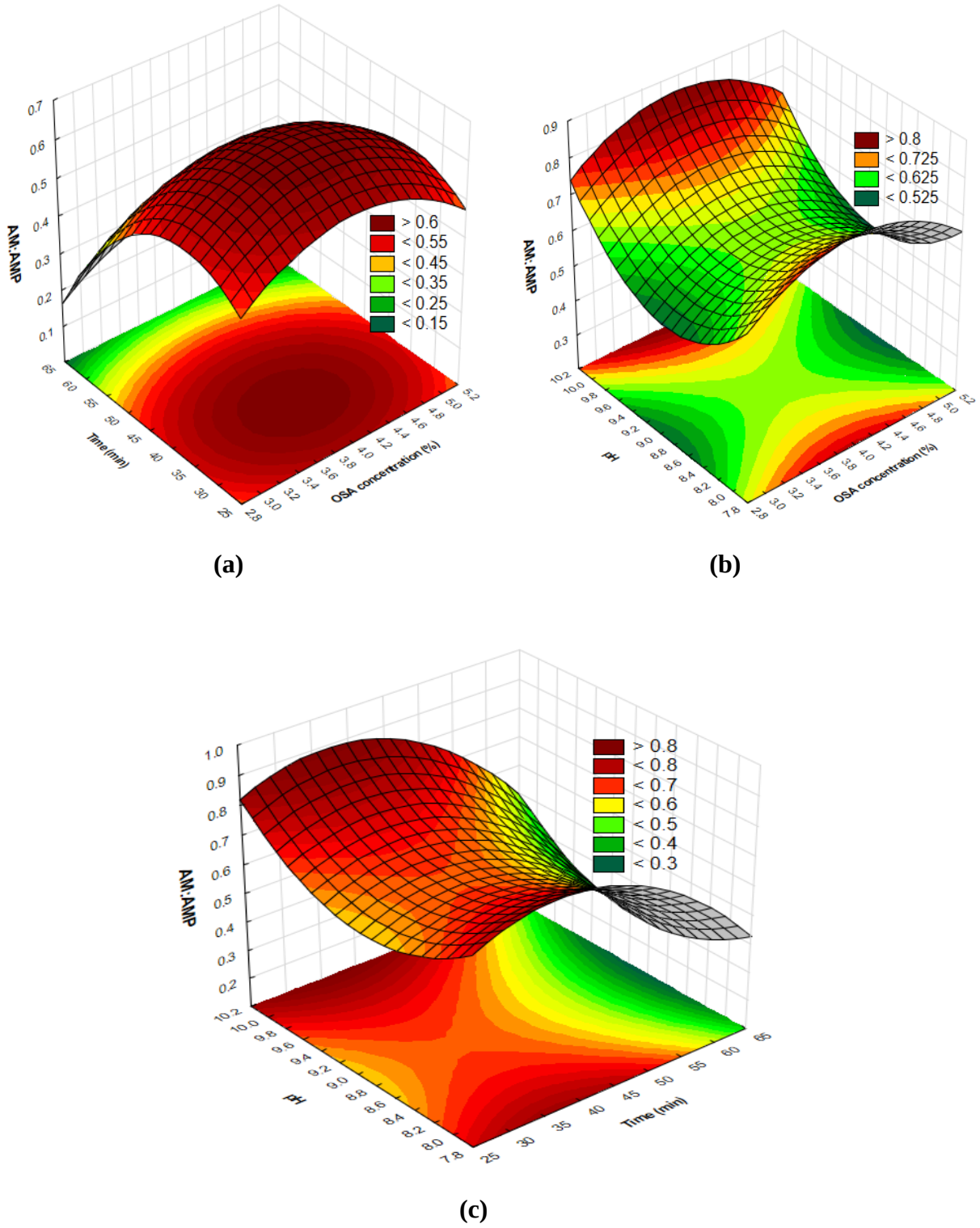

Figure 3: Effects of succinate anhydride concentration, modification time and pH on amylose-amylopectin ratio

3.3. Optimization of the process parameters

The processing variables which were succinate anhydride concentration, reaction time and pH of modification were optimized to obtain modified starch with maximum amylose-amylopectin ratio using response surface methodology. The processing parameter's goal was set in range while the amylose-amylopectin ratio was set at maximum. The optimal conditions obtained were succinate

anhydride concentration of 3.32%, the reaction time of 32.04 mins and substrate pH of 8. The predicted amylose-amylopectin ratio at the optimal conditions was 0.806. The predicted response by the modelling techniques was further validated by carrying out an experiment in triplicate based on the optimal condition. The result of the amylose-amylopectin ratio obtained from the experimentation was 0.812 which affirmed the accuracy of the optimization process.

4. Conclusion

In this study, octenyl succinate anhydride was used in the modification of cooking banana starch. For the maximization of the amylose-amylopectin ratio of the starch, the process variables such as the succinate anhydride concentration, modification time as well as the pH were optimized using RSM (a chemometric approach). The experimental model used (second-order polynomial model) accurately predict the amylose-amylopectin ratio owing to its high coefficient of determinant (R^2) as well as high adequate precision. For amylose-amylopectin maximization, the optimal process variables conditions were succinate anhydride concentration of 3.32%, reaction time of 32.04 mins and substrate pH of 8. The predicted amylose-amylopectin ratio at the optimal conditions was 0.806. The revealed the suitability of the chemometric approach in predicting and optimizing the amylose-amylopectin ratio.

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