

Optimization of Reaction Conditions for Hydroxypropylation of Saba Banana Starch

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ABSTRACT

The aim of this study was to investigate the effects of three reaction variables on the hydroxypropylation of Saba banana (*Musa acuminata* x *Musa balbisiana*) starch. The variables were reaction pH (10, 11 and 12), amount of propylene oxide (5, 10 and 15% v/w) and reaction temperature (35, 40 and 45 °C). Response Surface Methodology (RSM) using Central Composite Design was employed to explore the effects of these three variables on the Molar Substitution (MS), pasting properties, freeze-thaw stability and thermal properties of the modified starch. Increasing the amount of propylene oxide, pH and temperature promoted higher level of substitution. All three factors were found significantly ($p < 0.05$) influenced the MS. These factors also affected the pasting temperature, peak viscosity, breakdown, setback and freeze-thaw stability of the starch pastes. The experimental factors only affected the onset temperature, peak temperature and gelatinization enthalpy of modified starches. In general, propylene oxide exerted the most pronounced effect on hydroxypropylation of Saba banana starch as compared to the reaction pH and temperature. The optimal reaction conditions for hydroxypropylation of Saba banana starch was successfully optimized and validated.

Keywords:

Banana starch; hydroxypropylation;
pasting properties; freeze-thaw stability;
thermal properties; RSM

1. Introduction

Bananas are the most produced fruit in Malaysia with the average self-sufficiency ratio of 103% between 2013 and 2017. In 2017, the planted areas cover 35,000 hectares (Ha) and the production was more than 350,000 metric tonnes and accounted for 24% of Malaysia's total fruit production in that year [1]. Among the states in Malaysia, Sabah which is located in the north of Borneo Island is one of the main banana producers. The most widely planted cultivar in Sabah is Saba banana (*Musa acuminata* x *Musa balbisiana*). Saba banana is a cooking banana and often eaten steamed, deep-fried (banana fritters) or made into other traditional snacks by the local community. Besides

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internally consumed, the fruit is also exported to other states of Malaysia. Currently, the industrial applications of Saba banana are limited to the small scale production of banana crisps and banana flour. In recent decades, the promotion of regional economies by different worldwide agencies and the search of sustainable agricultural activities have motivated the exploration of alternative starch botanical sources, despite the traditional cereals and tubers. Starches from tropical fruits like banana have attracted attention due to the high starch content [2]. Owing to banana's highly perishable nature and considerably large quantities of lost by postharvest mishandling, converting banana at early maturity stage into starch can be a feasible solution to reduce banana waste [3].

In food industry applications, the functionality of starch is largely related to its gelatinization, pasting and retrogradation characteristics, but it is well established that starch in its native form have many shortcomings that cannot fulfil the required functionalities. For successful industrial applications, the deficiencies of native starch ought to be overcome by starch modification that may be accomplished by genetic, physical, chemical or enzymatic methods. Hydroxypropylation is a chemical modification of starches that introduces hydroxypropyl groups onto the polymeric chain of starch using propylene oxide as the etherifying reagent in the presence of an alkaline catalyst to facilitate the formation of nucleophilic starch-O⁻ alkoxide [4]. The substitution mainly takes place at three hydroxyl groups adjacent to the carbon atoms at 2, 3 and 6 positions in each anhydroglucose unit (AGU) [5] as shown in Figure 1. Hydroxypropylation of starch is classified as stabilized starch because the substituted ether groups are completely stable in both acidic and alkaline environments. Hydroxypropylation also reduces the tendency of starch pastes and gels to retrograde, while maintaining the ability of the starch to provide bulk, body, and other functionalities to the food products. This modified starch pastes and gels have less opacity and more pleasant texture, undergo less syneresis, more tolerant to cold storage as well as more freeze-thaw stable [6].

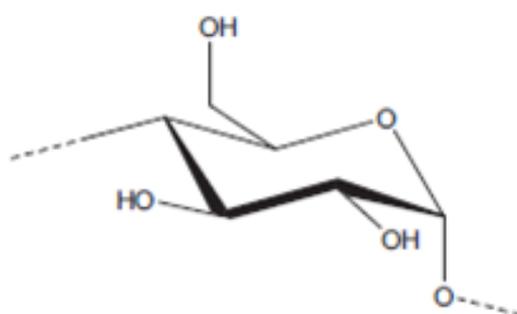


Fig. 1. Three hydroxyl groups adjacent to the carbon atoms at 2, 3 and 6 positions in an anhydroglucose unit of starch

In order to add value and enable wider applications of Saba banana, this study was aimed to optimize the reaction conditions for hydroxypropylation of Saba banana. Though extensive studies on hydroxypropylation of various kinds of starch are reported in the available literature, the reactivities and properties of resulted hydroxypropylated starches may vary by the botanical origin of the starch due to the variations in the molecular and granular structures and also the starch properties [6]. Hydroxypropylation conditions that involved the reaction pH, reaction temperature and quantity of propylene oxide were investigated. Our earlier work [7] reported degree of hydroxypropylation (molar substitution, MS) was significantly correlated to several important functionalities of modified Saba banana starch, such as freeze-thaw stability, pasting properties and

thermal behaviour; along with brief discussion on the influence of the above mentioned reaction conditions on the functionalities. Differ from previous report, this work elaborated the mathematical models obtained using Response Surface Methodology (RSM) for each of the investigated response; the optimization was also carried out to achieve more specific desirable functional properties. The validation of model was performed, follows by comparison of desirable functional properties between hydroxypropylated and native starch for verification purpose. Proper uses of design of experiments and statistical approaches have been reported effective and beneficial in optimizing various process parameters [8,9].

2. Methodology

2.1 Materials

Matured but unripe (total green peel colour) Saba banana (*Musa acuminata* x *Musa balbisiana*) was obtained from a local orchard in Keningau, Sabah, Malaysia. All chemicals used were of reagent grade or USP grade (as specified under respective sections) and were used as received without any further purification.

2.2 Isolation of Banana Starch

Starch isolation was carried out according to Nimsung *et al.*, [10] with slight modification upon arrival. The pulp was peeled, sliced (~ 0.5 cm thick) and dried in a hot air oven at 50 °C for 24 h. The dried chips were milled and pass through a 100-mesh sieve to obtain the flour. After that, the flour was suspended in 0.05 N NaOH (System, SO460-70, AR) with 1:5 ratio (flour:solution) and homogenized using over-head stirrer (500 rpm). The slurry was centrifuged to obtain the sediment. The sediment was washed with distilled water (1:2) prior to centrifugation at 3000 rpm for 20 min at room temperature. The washing procedures were repeated three to four times. The pH of starch slurry was adjusted to 7.0 prior to the last round of centrifugation. The purified sediment was oven-dried at 50 °C overnight to obtain a white powder. The dried powder was ground to pass through 200 mesh sieve and stored in an air-tight container at room temperature until further use.

2.3 Experimental Design

A Central Composite Design (CCD) was employed to determine the relative contributions of three independent variables on hydroxypropylation of Saba banana starch. The three independent variables were the amount of propylene oxide (5%, 10% and 15%, v/w of starch dry weight, equivalent to 3, 6, 9 mL), reaction pH (10, 11 and 12) and reaction temperature (35, 40 and 45 °C). The responses measured were molar substitution, pasting properties, freeze-thaw stability and thermal behaviour. A total of 20 experimental units were generated from CCD as shown in Table 1.

2.4 Hydroxypropylation of Starch

Modification of starch was performed according to Lawal *et al.*, [4]. Starch (60 g, dry basis, d.b.) was added with 120 mL distilled water, followed by 12 g anhydrous sodium sulphate (Na₂SO₄, Sigma-Aldrich, 239313, ACS). The slurry was homogenized with magnetic stirrer for 30 min. The pH of the starch suspension was adjusted to the required value (Table 1) using 2 M NaOH (System, SO460-70, AR) and propylene oxide (Merck, 1576945, USP) was added as etherifying agent with continuous stirring. The reaction was carried out with continuous agitation using the required

temperature (Table 1) for 24 h. At the end of the reaction, the suspension was neutralized (1 M HCl, Sigma-Aldrich, 320331, ACS) and washed with distilled water. The hydroxypropylated starch was recovered by drying at 40 °C until the moisture content was approximately 10%. The powder was ground, sieved (100 mesh) and kept in air-tight container until further analysis.

Table 1

Independent variables and experimental design levels expressed in coded and actual values used for hydroxypropylation of Saba banana starch

Run	Coded Value			Actual Value		
	A (pH)	B (Propylene Oxide)	C (Temperature)	pH	Propylene Oxide* (% v/w)	Temperature (°C)
1	-1	-1	-1	10	5	35
2	1	-1	-1	12	5	35
3	-1	1	-1	10	15	35
4	1	1	-1	12	15	35
5	-1	-1	1	10	5	45
6	1	-1	1	12	5	45
7	-1	1	1	10	15	45
8	1	1	1	12	15	45
9	-1.682	0	0	9.32	10	40
10	1.682	0	0	12.68	10	40
11	0	-1.682	0	11	1.58	40
12	0	1.682	0	11	18.42	40
13	0	0	-1.682	11	10	31.6
14	0	0	1.682	11	10	48.4
15	0	0	0	11	10	40
16	0	0	0	11	10	40
17	0	0	0	11	10	40
18	0	0	0	11	10	40
19	0	0	0	11	10	40
20	0	0	0	11	10	40

* based on starch dry weight

2.5 Chemical Composition

A Halogen Moisture Analyzer (Mettler Toledo, HG53) was used to determine the moisture content of starch. 5 g of sample was weighed on the drying pan and the reading was taken after 5 minutes. Protein, fat and ash content of the starch was analysed according to AACC methods 08-01, 46-13 and 30-25, respectively [11].

2.6 Molar Substitution

Extent of hydroxypropylation was measured according to Ratnayake and Jackson [12] with slight modifications. 0.05 g of starch was added with 25 mL of H₂SO₄ (2 M, System, SU710-70, AR) and hydrolyzed in a boiling water bath until a clear solution was obtained. The solution was cooled at room temperature and diluted to 100 mL. After that, 1 mL of aliquot solution was pipetted into a 25 mL volumetric flask and immersed in an ice bath. 8 mL of concentration H₂SO₄ was added dropwise to the flask. After mixing, the flask was kept in a boiling water bath for 20 min, followed by immediate cooling in an ice bath for 10 min. 0.60 mL of ninhydrin reagent (Sigma-Aldrich, 151173, ACS) was added into the flask, followed by thorough shaking and incubation (25°C water bath for 100 min). The volume of the solution in the flask was made up to 25 mL with concentrated H₂SO₄

and mixed by inverting the flask several times. After 5 min, the absorbance was read (Lambda 35, Perkin Elmer) at 590 nm against a reagent blank. A standard curve was prepared with an aliquote (1 mL) of standard aqueous solutions containing 0, 10, 20, 30, 40 and 50 μg of propylene glycol (Merck, 1576708, USP) per mL. The degree of hydroxypropylation was calculated based on the molecular weight ratios of propylene glycol and hydroxypropyl content

$$\text{Hydroxypropyl group equivalents} = \text{Amount of glycol } (\mu\text{g}) \times 0.7763 \quad (1)$$

The molar substitution (MS) was calculated using

$$MS = \frac{(\text{weight of hydroxypropyl}/59)}{(\text{weight of starch}/162)} \quad (2)$$

where MS is defined as the level of substitution in terms of moles of monomeric unit (in the polymeric substituent) per mole of anhydroglucose unit.

2.7 Pasting Properties

Pasting properties of starch was investigated using a Rapid Visco Analyzer (RVA) (RVA-4, Newport Scientific) interfaced with a personal computer equipped with ThermoLine Windows software version 3 (TCW3). 3 g of starch (d.b.) was added with corrected volume of distilled water (total water = 25 mL) and subjected to the programmed heating and cooling cycle [13].

2.8 Freeze-Thaw Stability

Starch suspension (5% w/w) was heated at 95 °C under constant agitation for 30 min. Approximately 8 mL of starch suspension was weighed into a pre-weighed centrifuge tube and centrifuged at 3000 rpm for 10 min (Sorvall Biofuge Prime R, Thermo Scientific). After centrifugation, the supernatant was discarded and the sediment was subjected to successive freeze-thaw cycles. The alternate cycle started by freezing sample at -20 °C (24 h), followed by thawing at room temperature (2 h). A total of five freeze-thaw cycles were performed. The sample was centrifuged at the completion of each cycle (8000 rpm for 15 min), and the percentage of water separated was considered the extent of syneresis.

$$\text{Syneresis } (\%) = \frac{\text{Weight of separated liquid from starch gel (g)}}{\text{Total weight of starch gel before centrifugation (g)}} \times 100 \quad (3)$$

2.9 Thermal Properties

Differential Scanning Colorimeter (Diamond DSC, Perkin Elmer) equipped with a thermal analysis data system (Pyris Manager) was used to determine the gelatinization parameters (onset temperature, T_o ; peak temperature, T_p ; conclusion temperature, T_c ; enthalpy, ΔH) of the samples. Indium was used to calibrate the DSC. Measurement was made on starch:water (1:4) (w/w). The hermetically sealed pan was kept at room temperature for 24 h to achieve equilibrium prior to the measurement. The sample was scanned from 30 °C to 100 °C at 10 °C/min, and an empty pan was used as the reference for the measurement.

2.10 Statistical Analysis

Design Expert (Stat-Ease Corporation, Minneapolis) version 6.0.11 was used to analyze the data obtained. All the response variables were fitted by a second-order model in the form of quadratic polynomial equation as shown below

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \quad (4)$$

whereby Y is the predicted response; Y is a constant; β_o is the linear coefficient; β_i is the squared coefficient, β_{ij} is the crossproduct coefficient, and X_i is the factor.

The analysis of variance (ANOVA) was carried out to determine individual linear, quadratic and interaction regression coefficient. The significance of all the terms of polynomial equation was analyzed statistically by computing the F value at $p < 0.05$. The software was used to generate response surface and contour plots.

2.11 Optimization and Validation of Model

Optimization of hydroxypropylation of Saba banana starch reaction conditions was carried out using the Response Surface Methodology (RSM). The optimum values of the three independent variables were obtained by using the numerical method of the software. The contour plots of selected models were plotted and then overlapped to identify the desirable zone and to determine the optimum values. To validate the optimal reaction conditions, Saba banana starch was hydroxypropylated with the proposed optimal conditions and the relevant responses were measured to compare with the predicted values.

3. Results and Discussions

3.1 Molar Substitution

As shown in Table 1, the centre point (pH 11, 10% v/w propylene oxide, 40 °C) was replicated six times, thus 15 different reaction conditions were used to produce the hydroxypropylated banana starches. Native Saba banana starch contained 11.2% moisture, 1.03% protein, 0.17% fat and 0.48% ash. After modification, the moisture content of starch samples varied between 8.9 to 10.8%. The molar substitution (MS) of hydroxypropylated starches prepared in this study ranged between 0.059-0.148. The highest level of substitution obtained falls within the FDA guideline for maximum permissible level (MS not more than 0.2) for food applications [14]. Similar MS (0.07) was reported for hydroxypropylated plantain starch [4] modified under similar condition with run 15 (MS = 0.071), except the pH used was slightly higher (11.5) than this experiment. Table 2 illustrates the coefficients of the regression model for the determination of MS. Linear model was found best fit the experimental data obtained ($p < 0.01$) with 79.03% of the variations was explained by the model. All three variables significantly ($p < 0.05$) influenced the MS in a positive direction, and thus increase any of the factors may lead to higher level of substitution in the starch molecules. Comparison of the regression coefficient values reveals that propylene oxide amount and reaction temperature exerted more pronounced effect than the reaction pH in influencing the MS of the modified samples. However, different finding was found for hydroxypropylation of waxy maize starch where the reaction temperature showed less effect on the extent of reaction than pH [15].

Figure 2 shows increase of propylene oxide and pH during hydroxypropylation led to higher MS. Higher concentration of propylene oxide increased the availability of the etherifying agent in the proximity of starch granules caused, hence resulting greater rate of collision between starch alkoxide and propylene oxide [4,16]. By increasing the propylene oxide from 3 to 9%, the MS values for hydroxypropylated normal wheat starch, waxy wheat starch and waxy maize starch were reported to be 0.055-0.151, 0.043-0.133 and 0.049-0.139 respectively [17].

Table 2

Regression coefficients for the determination of the molar substitution of hydroxypropylated *Saba* banana starch

Factor	Molar Substitution (MS)
Intercept	0.0810
Linear	
A (pH)	0.0097*
B (propylene oxide)	0.0130*
C (temperature)	0.0110*
CV %	9.58
R ²	0.8327
Adj R ²	0.7903

* Significant at $p < 0.05$

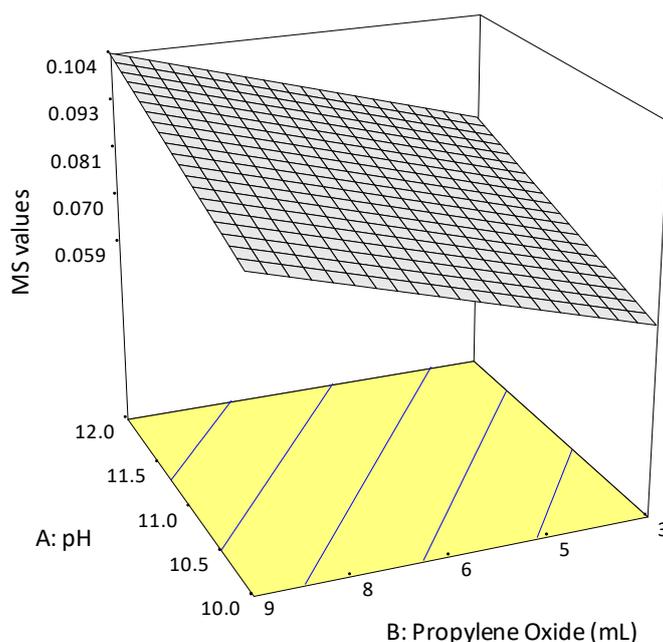


Fig. 2. Response surface plot of the effects of propylene oxide and pH on the molar substitution at 40°C

Increase of pH during reaction promoted the alkalization of banana starch whereby NaOH reacted with the hydroxyl groups of the starch to produce more starch alkoxide to participate in the subsequent etherification step [18]. More alkaline conditions might also reduce starch polymer associations and open up granules to facilitate the modification [19]. It is speculated that increasing reaction temperature accelerated the reaction of starch with chemical reagent to an extent that reaction sites are concentrated in more accessible granule areas [15]. At elevated temperature, diffusion of the alkaline catalyst and the penetration of propylene oxide are facilitated [16].

3.2 Pasting Properties

Pasting properties describe the rheological characteristics right before, during and after the gelatinization of starch under continuous stirring using a fix temperature profile. After multiple regression analysis, the empirical relationship between response variables and the independent variables are shown in Table 3. It is interesting to find that the influence of the experimental variables on the pasting temperature and breakdown can be fitted by linear model, whereas quadratic model fitted the experimental data obtained for peak viscosity and setback. Comparing among the four responses, setback was found to be the only one affected by all the three independent variables of this study. The changes in pasting temperature, peak viscosity and breakdown were mainly attributed to the amount of propylene oxide used ($p < 0.01$). The influences of the three independent variables on the pasting parameters were not identical to the MS, even though the variations in these parameters were highly dependent on the MS. Results obtained suggest that the pasting parameters were not proportionately related to the degree of hydroxypropylation (MS) that took place in the starch. In the present study, there was no significant model could fit the data obtained for peak time (data not shown).

Table 3

Regression coefficients for the determination of the pasting parameters of hydroxypropylated *Saba* banana starch

Factor	Pasting Parameters			
	Pasting Temperature (°C)	Peak Viscosity (cP)	Breakdown (cP)	Setback (cP)
Intercept	78.24*	4695.01	1077.37**	992.62**
Linear				
A (pH)	-0.76	-263.23	205.80	-339.04**
B (propylene oxide)	-1.77**	348.06*	523.89**	-484.13**
C (temperature)	-0.17	-165.48	-123.18	65.06
Quadratic				
A ²	–	-399.63*	–	89.81*
B ²	–	134.64	–	122.57**
C ²	–	-97.11	–	201.82**
Interaction				
AB	–	238.42	–	-5.67
AC	–	41.00	–	153.33**
BC	–	-50.33	–	-192.58**
CV %	1.79	9.27	8.74	10.02
R ²	0.8212	0.8812	0.8096	0.9703
Adj R ²	0.7557	0.7902	0.7781	0.9599

* Significant at $p < 0.05$ and ** significant at $p < 0.01$

Pasting temperature corresponds to the temperature at which gelatinization of starch begins [20]. Increase of propylene oxide had a negative linear effect (Figure 3) on the pasting temperature ($p < 0.01$), indicating starches with higher extent of modification required lower temperature for cooking. Hydroxypropylation altered the hydrophilicity of the starch polymer consequently changing the bonding of starch chains with water molecules. This led to an overall decline in bonding between starch chains, subsequently increasing the ease of hydration of starch chain [21].

Peak viscosity is related to the swelling power of starch granules, which is also an indicator of the extent of interaction between starch chains within the amorphous and crystalline region of starch granules [21]. This response was significantly affected by propylene oxide and negatively affected by the quadratic effect of pH ($p < 0.05$). The quadratic effect of pH appeared to be more

pronounced and this effect is apparently seen in Figure 3, in which the peak viscosity increased to a maximum and dropped drastically when the pH was increased from pH 11 to pH 12. Comparatively, the increase of peak viscosity happened more gradually with increment of propylene oxide from 3 to 9 mL (5 to 15 % w/v). After modification, the hydroxyl groups in the starch were substituted with hydroxypropyl groups through a nucleophilic substitution. The bulkier hydroxypropyl group can disrupt the inter- and intra-molecular forces in starch, consecutively weakened the starch granules, leading to increased rotational flexibility of starch amorphous regions, allowing in an increased water uptake and thus an increase in the swelling ability [22,23]. The introduction of hydrophilic hydroxypropyl groups might have exposed more hydrophilic OH groups already present in the starch granule thereby able to retain more amount of water [24]. Rice starches containing higher percent of hydroxypropyl groups were reported to have greater swelling power [21]. Hydroxypropylation also improved penetration of water into starch granules that enhanced amylose leaching, which also accounted for an increase in peak viscosity [16].

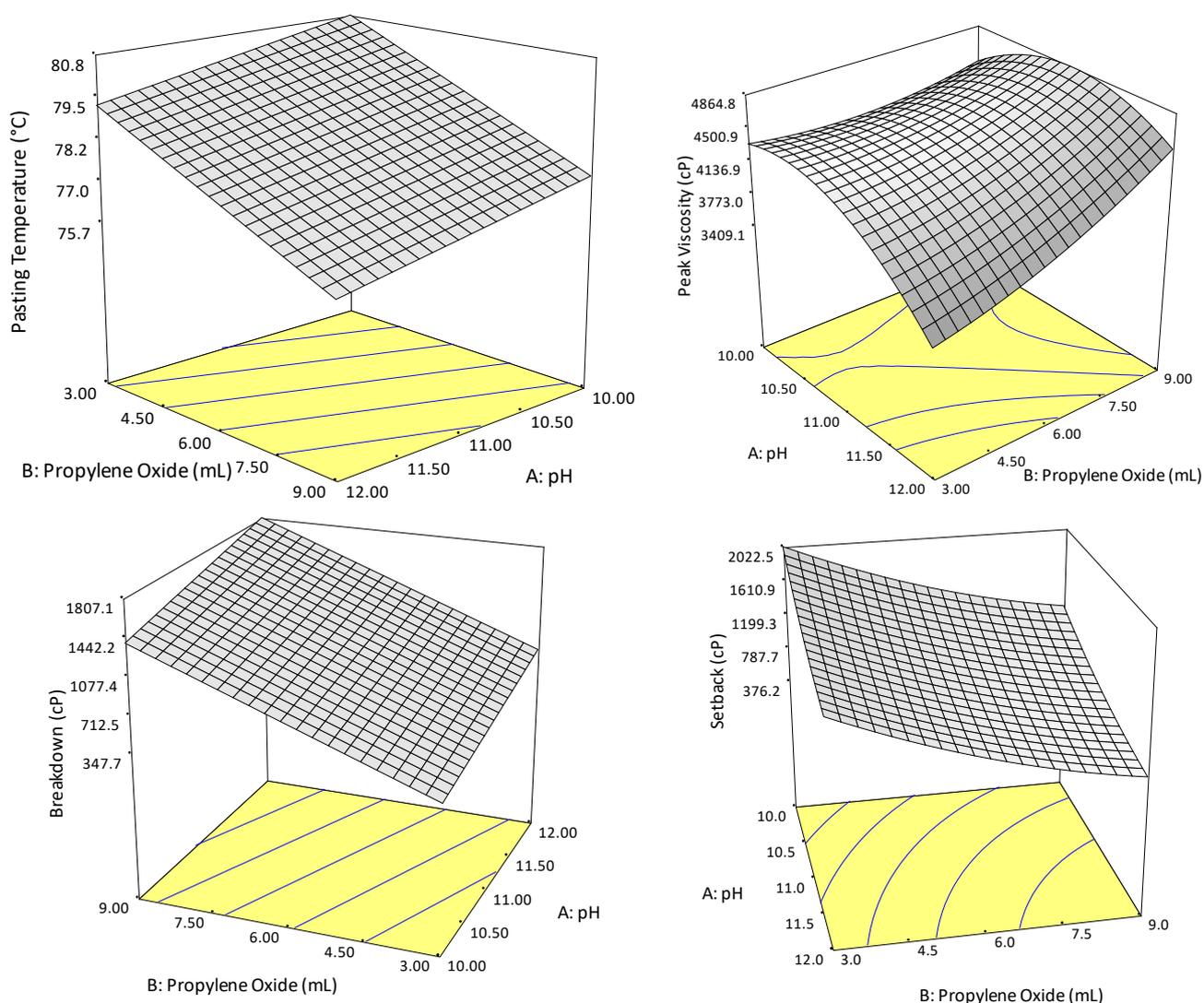


Fig. 3. Response surface plots of the effects of reaction pH and amount of propylene oxide (mL) on the pasting temperature (reaction temperature of 40 °C); peak viscosity (reaction temperature of 45 °C); breakdown (reaction temperature of 40 °C), and setback (reaction temperature of 40 °C) for hydroxypropylated Saba banana starch

Propylene oxide had major effect on breakdown ($p < 0.01$), in which breakdown increased linearly with increasing propylene oxide used in the treatment as shown in Figure 3. Breakdown measures the resistance of starch paste against shearing force at high temperature. Industrial applications favour starch with low breakdown as this implies less viscosity lost during heating and agitation in the processing. Results obtained indicate that higher level of hydroxypropylation may cause the swollen starch granules to be more fragile and tends to rupture. This is because starch with higher MS swelled to a relatively bigger volume, thus prompted the starch paste to greater breakdown. Setback implies the retrogradation tendency of a starch paste upon cooling. This response was significantly influenced by the negative effects of pH and propylene oxide ($p < 0.01$); quadratic effects of all the three factors ($p < 0.05$); interactive effect of pH and temperature; as well as negative interactive effect of propylene oxide and temperature ($p < 0.01$). Among these factors, pH and propylene oxide appeared to be more prominent by displaying relatively higher regression coefficients (Table 3). The bulky hydroxypropyl group reduced the internal hydrogen bond strength and quantity in starch. This substantially decreased the ability of starch to recrystallize thus lowered the retrogradation of starch paste [25].

3.3 Freeze-Thaw Stability

Freeze-thaw stability of the starch was measured as the amount of water expelled (syneresis) from the thawed semi-solid starch gel under the action of centrifugal force. Syneresis from freeze-thawed gels is attributed to the increase of molecular association between starch chains at lower or freezing temperature which exudes water from the gel structure [16]. The empirical relationships between reaction factors and the freeze-thaw stability of hydroxypropylated banana starches were related by quadratic models (Table 4).

Table 4
 Results of analysis of variance (ANOVA) of response surface quadratic model for syneresis of hydroxypropylated Saba banana starch at two cycles of freeze-thaw treatment

Factor	Syneresis	
	Cycle 1	Cycle 5
Intercept	8.14**	20.47**
Linear		
A (pH)	-3.71**	-4.49**
B (propylene oxide)	-7.67**	-6.59**
C (temperature)	-0.23	0.05*
Quadratic		
A ²	0.74	-0.58
B ²	1.01*	-1.69*
C ²	4.16**	1.92*
Interaction		
AB	-0.71	0.16
AC	1.65*	2.35*
BC	-3.11**	-2.23*
CV %	11.18	10.03
R ²	0.8979	0.9408
Adj R ²	0.8691	0.9237

* Significant at $p < 0.05$ and ** significant at $p < 0.01$

Two polynomial equations were generated for the response at the first and the fifth freeze-thaw cycles for comparison. These two models were fitted to the spatial influence of the factors to the response with good prediction ($\text{Adj } R^2 = 0.97$ and 0.92). Though with slight different in the magnitude of influence (values of the coefficients), the ANOVA results for these two cycles indicate that the experimental factors influenced the response by similar model terms which involved the single terms of pH and propylene oxide ($p < 0.01$); quadratic terms of propylene oxide and temperature ($p < 0.05$); the interactive terms of pH and temperature ($p < 0.05$) as well as propylene oxide and temperature ($p < 0.01$). Data obtained evident that all three independent variables under study play significant role in improving the freeze-thaw stability of Saba banana starch. The negative effect of propylene oxide was more profound as indicated by the highest coefficient (Table 5). The reduction of syneresis with increasing propylene oxide is apparently seen in Figure 4.

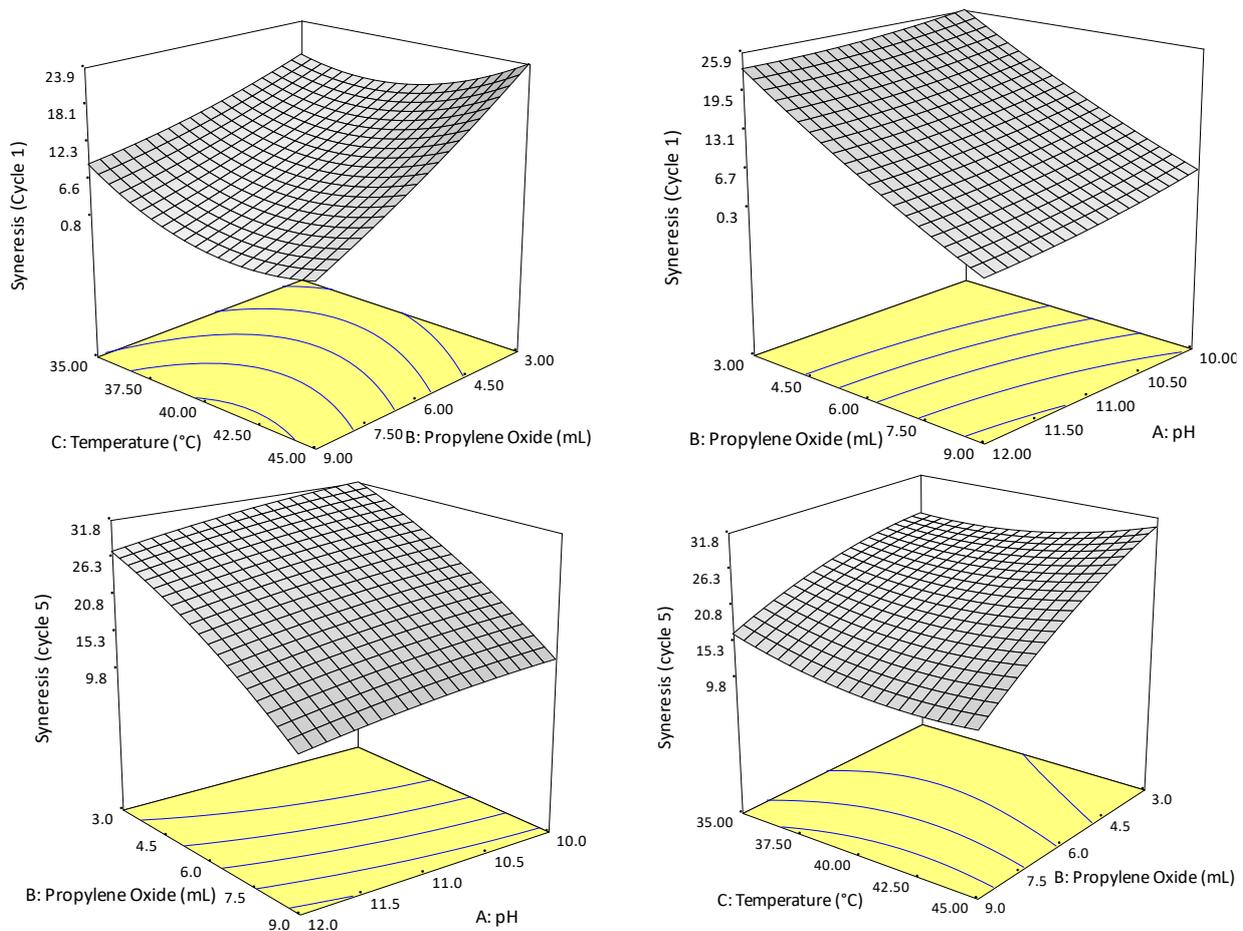


Fig. 4. Response surface plots of syneresis (%) of hydroxypropylate Saba banana starch at first freeze-thaw cycle (cycle 1) (top left at pH 11 and top right at 45 °C) , and fifth freeze-thaw cycle (cycle 5) (bottom left at 45 °C and bottom right at pH 10)

Significant reduction of syneresis of banana starch by hydroxypropylation was observed in which two modified starch with highest MS (0.148 and 0.116) did not expelled any substantial amount of water after first and second freeze-thaw cycles. Similar findings was reported for freeze-thawed barley starch, whereby the % syneresis at 10% hydroxypropylation was significantly lower than that at 5% hydroxypropylation due to higher amount of substitution of hydroxypropyl groups [24]. The retrogradation was also found reduced as molar substitution of hydroxypropyl groups increased in normal wheat starch, waxy wheat starch and waxy maize starch [17]. The steric effects imposed by the bulky hydroxypropyl groups inhibited proper alignment of starch chains during

chain aggregation and crystallization [26] thus minimized the water expelled. In general, the percentage of syneresis was observed to increase progressively as the number of freeze-thaw cycles increased. This was due to reorientation of the starch molecules after repeated freeze-thaw cycles and was therefore unable to hold the same amount of water as in the first cycle [24]. Freeze-thaw stability is a very vital property of starch that may widen the application in starch-based food products that require chilling or freezing storage. The reduction of syneresis implies better storage stability. It was reported that no water loss in custard made of hydroxypropylated pearl millet starch after seven days of cold storage [27].

Table 5

Results of analysis of variance (ANOVA) for gelatinization of hydroxypropylated Saba banana starch

Factor	Gelatinization Properties			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Intercept	69.64*	72.93*	77.60	1.28
Linear				
A (pH)	-0.26	-0.40	-0.46	-0.29
B (propylene oxide)	-1.73**	-1.72**	-1.70	-0.32*
C (temperature)	0.29	0.007	-0.26	0.33
Quadratic				
A ²	1.08*	0.94*	–	0.002
B ²	-0.06	-0.46	–	0.09
C ²	0.37	0.46	–	0.73**
Interaction				
AB	-0.74	-0.75	–	0.26
AC	0.59	0.40	–	0.14
BC	-0.39	-0.43	–	0.21
CV %	2.20	1.96	2.14	11.39
R ²	0.8623	0.8694	0.4946	0.8394
Adj R ²	0.8434	0.8619	0.3999	0.8275

T_o – Onset temperature; T_p – Peak temperature; T_c – Conclusion temperature; ΔH – Gelatinization enthalpy

* Significant at $p < 0.05$ and ** significant at $p < 0.01$

3.4 Thermal Properties

The heating of starch suspensions in excess water and above a certain temperature causes an irreversible transition called gelatinization, which can be characterized by an endotherm obtained by differential scanning calorimeter (DSC). Quadratic models could be used to relate the factors to the onset temperature (T_o), peak temperature (T_p) and melting enthalpy (ΔH) of modified starches. However, no significant model was proposed for the conclusion temperature (T_c). Hydroxypropylation of finger millet starches also reported to lower predominantly the T_o and T_p as compared to T_c [16]. Propylene oxide was found to lower the gelatinization temperatures, whilst the quadratic effect of pH on the other hand increased the temperatures. The higher coefficient values suggested etherifying agent exerted more dominant effect, as seen in the 3-D plots (Figure 5). Reduction of T_o and T_p with increasing propylene oxide happens to be more remarkable than the changes of the temperature parameters along with the increment of pH. Hydroxypropylation is known to reduce the gelatinization temperature of starch. Two factors were proposed for the decrease in gelatinization temperatures of hydroxypropylated starches: (i) the addition of sodium hydroxide caused the defects to the starch crystallites during the reaction, and (ii) substituted hydroxypropyl groups led to the disruption of hydrogen bonds between starch chains, which

improved the mobility of the starch chains and indirectly decreased the melting temperature of the starch crystallites [17]. Hydroxypropylation was also proposed as internal plasticization that promoted gelatinization and hence lowering melting temperature of starch [23].

The linear effect of propylene oxide and quadratic effect of reaction temperature significantly affected the enthalpy of hydroxypropylation Saba banana starch. The reduction of enthalpy is seen more apparent at reaction temperature around 37.5 and 41°C, further increase of temperature raised the enthalpy. Increase of propylene oxide on the other hand decreased the enthalpy (Figure 5). The substituted hydroxypropyl groups disrupted the double helices because of the rotation of these flexible groups within the amorphous regions thus lowered the gelatinization enthalpy [28]. Generally, hydroxypropylation reduced the crystallinity of the starch granules and hence the enthalpy [17].

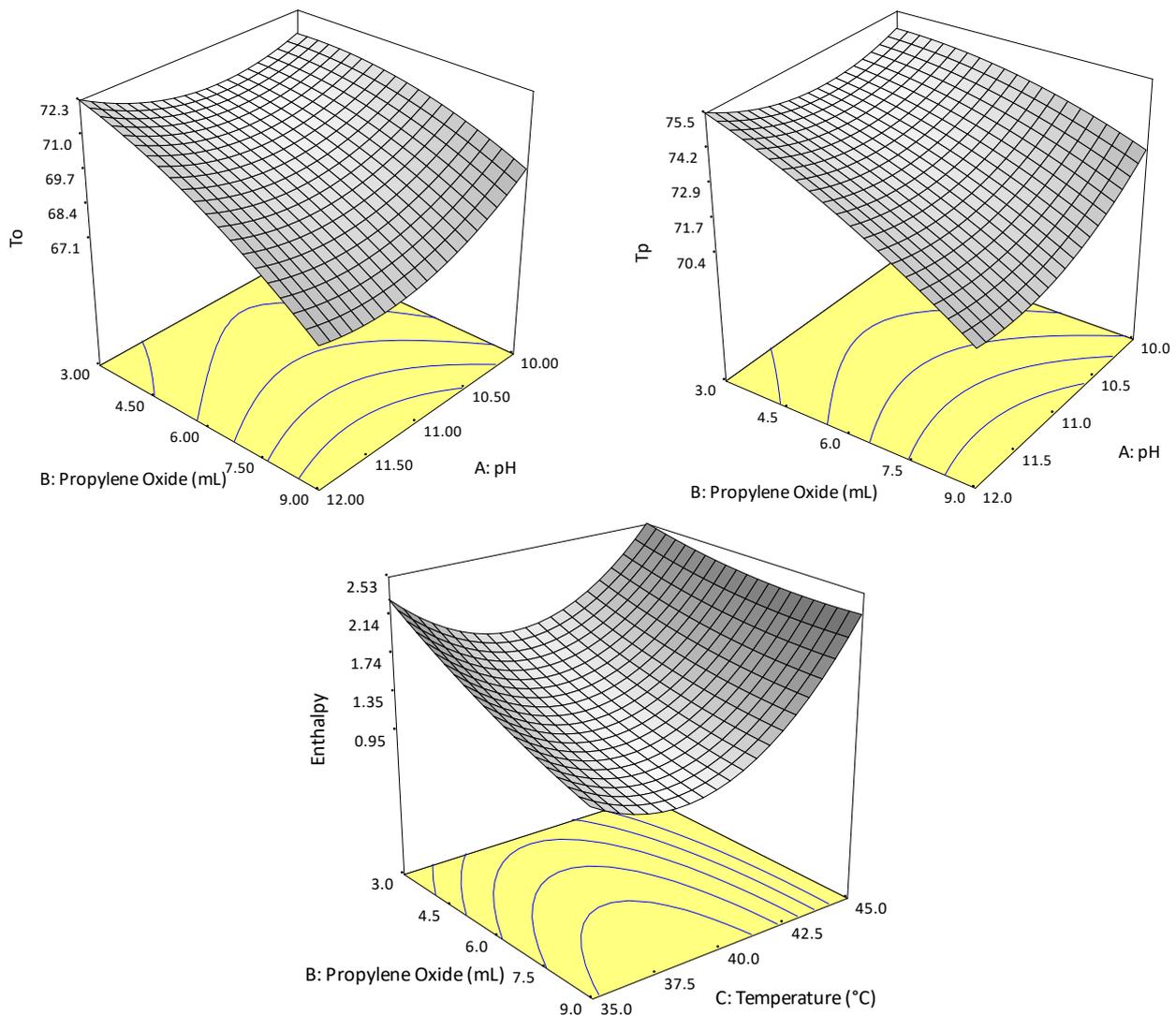


Fig. 5. Response surface plots of onset temperature, T_o (at 40 °C); peak temperature, T_p (40 °C); and gelatinization enthalpy (at pH 11)

3.5 Optimization and Verification

Results obtained indicated 73.3% of the hydroxypropylated Saba banana starch produced (using 11 combinations of reaction condition) had lower peak viscosity than the native starch (data not shown). As peak viscosity is an indicator for the thickening ability of a starch, maximizing this technology functionality could provide wider scope of application for the modified starch. In order to produce modified banana starch with high thickening ability and high stability against retrogradation, MS, peak viscosity, setback and syneresis (both cycles) were chosen to optimize the reaction condition for hydroxypropylation of Saba banana starch. Results obtained also indicated that the range of the levels used for three investigated factors in this study could produce hydroxypropylated starch with lower thermal properties as compared to the native counter-part (data not shown); and because of thermal properties was not the main targeted functionality, this response was therefore not chosen for optimization. The criteria for the responses were fixed: MS was fixed to achieve the highest possible degree of substitution; peak viscosity was fixed to achieve the maximum possible viscosity; whereas setback and syneresis were fixed to attain the lowest possible values. The optimum conditions proposed was reaction pH at 12, 15 % v/w (9 mL) of propylene oxide, and reaction temperature at 41.49 °C. The desirability for this optimal condition was 0.812. To confirm the optimal reaction conditions, Saba banana starch prepared under the optimal conditions was subjected to determination of MS, pasting properties, freeze-thaw stability (1 cycle) and thermal properties. Results obtained was comparable to the predicted values with the CV (coefficient of variation) ranged from 0.32 to 5.43 % [29] (Table 6). The hydroxypropylated Saba banana starch prepared with the optimal conditions was also found to have improved properties as compared to its native counterpart. Besides apparently low in retrogradation tendency, it also exhibited lower gelatinization temperatures and lower enthalpy. The peak viscosity for these two samples was comparable.

Table 6

Experimental data of the validation of predicted values at optimal hydroxypropylation conditions and properties of native Saba banana starch

Response	Predicted value	Experimental value	CV %	Native starch
Molar substitution (MS)	0.158	0.153	3.16	-
Pasting properties:				
Peak viscosity (cP)	4692.36	4637.33 ± 40.5	1.17	4796.00 ± 37.21
Setback (cP)	401.89	412.38 ± 20.11	2.61	1774.91 ± 41.08
Pasting temperature (°C)	75.66	75.90 ± 13.86	0.32	80.84 ± 0.92
Breakdown (cP)	1770.25	1690.74 ± 51.43	4.49	808.26 ± 30.37
Freeze –thaw stability:				
% Syneresis (C1)	-2.33	N.D	-	21.3 ± 2.1
Thermal properties:				
Onset temperature (°C)	67.56	66.98 ± 0.25	0.86	73.43 ± 0.77
Peak temperature (°C)	70.57	69.87 ± 0.29	0.99	77.11 ± 0.04
Conclusion temperature (°C)	75.36	74.51 ± 0.52	1.13	81.41 ± 2.56
Enthalpy (J/g)	1.29	1.36 ± 0.03	5.43	3.19 ± 0.20

4. Conclusions

Optimization of reaction conditions for hydroxypropylation of Saba banana was successfully carried out and the optimal reaction pH, reaction temperature and the amount of propylene oxide required had been identified. The optimal condition was validated and found to be fitted with the experimental values. In general, the amount of propylene oxide used had more pronounced

influence than reaction pH and reaction temperature in affecting the MS and other functional properties of hydroxypropylated Saba banana starch. Saba banana starch prepared under the optimal conditions shown improved functional properties than native Saba banana whereby it exhibited ease of cooking, low retrogradation and higher freeze-thaw stability. These positive properties could definitely turn Saba banana starch into a useful technological functional ingredient and hence widen its application in food industry.

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