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RESPONSE SURFACE METHODOLOGY FOR OPTIMISING CROSS-LINKING CONDITIONS OF UNRIPE BANANA (*Musa balbisiana* VAR. Abu) STARCH

(Kaedah Tindak Balas Permukaan untuk Pengoptimuman Keadaan Penghubung Silang Kanji Pisang Belum Masak (*Musa balbisiana* Var. Abu))

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Abstract

Starch is the most abundant organic compound found in nature after cellulose. However, native starch has undesirable properties which could limit its application in food industries. Therefore, starch modification is necessary to enhance its positive attributes and to eliminate the limitations of its specific application in food manufacturing. This study aims to optimize the conditions of the cross-linking of unripe banana (*Musa balbisiana* var. Abu) starch using response surface methodology (RSM). The optimization was conducted using the central composite design (CCD) approach. The extracted unripe banana (*Musa balbisiana* var. Abu) starch was cross-linked with sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) mixture as the cross-linking agent. The two independent variables selected were temperature (40.0- 60.0 ℃) and pH (9.00-11.00). The maximum degree of cross-linking was obtained at the following optimum conditions: temperature 50.57 ℃ and pH 9.52. The degree of cross-linking obtained at the optimum conditions of RSM was verified, and the value obtained was 81.73%. There was no significant difference (*p*-value >0.05) between the predicted and verified degree of cross-linking values. Thus, it indicated that the predicted optimum condition by RSM can be accepted. These outcomes of this study are very important as they would assist the food industries in developing and designing the process of optimal cross-linking of starch from unripe bananas, which can be used in the future.

Keywords: response surface methodology, unripe banana, banana starch, starch modification, cross-linking

Abstrak

Kanji adalah sebatian organik yang paling banyak ditemui dalam alam semula jadi selepas selulosa. Walau bagaimanapun, kanji semula jadi yang belum diproses mempunyai sifat yang tidak diingini yang boleh mengehadkan penggunaannya dalam industri makanan. Oleh itu, pengubahsuaian kanji adalah perlu untuk meningkatkan kebaikan justeru dapat menghapuskan had penggunaan khususnya dalam pembuatan makanan. Matlamat kajian ini adalah untuk mengoptimumkan keadaan ikatan silang kanji pisang belum masak (*Musa balbisiana* var. Abu) menggunakan kaedah tindak balas permukaan (RSM). Pengoptimuman telah dijalankan dengan pendekatan reka bentuk komposit pusat (CCD). Kanji pisang belum masak (*Musa balbisiana* var. Abu) yang diekstrak telah diikat silang dengan campuran natrium trimetafosfat (STMP) dan natrium tripolifosfat (STPP) sebagai agen penghubung silang. Dua pembolehubah bebas yang dipilih ialah suhu (40.0-60.0 °C) dan pH (9.00-11.00). Darjah maksimum penghubung silang diperoleh pada keadaan optimum berikut: suhu 50.57 °C dan pH 9.52. Darjah silang yang diperoleh pada keadaan optimum RSM telah disahkan dan nilai yang diperoleh ialah 81.73%. Tidak terdapat perbezaan yang signifikan (nilai p >0.05) antara tahap ramalan dan disahkan nilai silang silang, oleh itu ia menunjukkan bahawa keadaan optimum yang diramalkan oleh RSM boleh diterima. Hasil kajian ini adalah sangat penting kerana ia akan membantu industri makanan untuk membangunkan dan mereka bentuk proses pengikatan silang kanji yang optimum daripada pisang yang belum masak, yang boleh digunakan pada masa hadapan.

Kata kunci: kaedah tindak balas permukaan, pisang belum masak, kanji pisang, pengubahsuaian kanji, penghubung silang

Introduction

Starch, a storage carbohydrate in plants, can be found abundantly in leaves, flowers, fruits, seeds, stems, and roots [1]. It has wide functions and applications in food industries and is commonly used as a thickener, binder, gelling agent, foam stabilizer and texture improver [2]. Starch can be basically manufactured by the combination of the following steps: grinding of the starch-rich crop and wet separation technique to sediment the starch granules in water. Starch has gained much attention due to its usefulness in food and non-food industries, wide availability, low cost of extraction and purification, edibility and total composability without toxic residues [3, 4]. Most of the starches are obtained from cereals such as corn, wheat and various rice, tubers, and roots such as potatoes and cassava. In this regard, unripe bananas have been studied as a potential source of starch [5-7].

Banana, a climacteric fruit that belongs to the *Musaceae* family and *Musa balbisiana,* is the most widely distributed banana in tropical and subtropical regions [8]. The banana fruits were usually harvested at the unripened stage for long handling and transportation because they are perishable fruits that could lead to serious economic losses during maturation [9-11]. The starch content from unripe bananas is between 70-80%, depending on their variety and maturity index, and its starch content is similar to other starches obtained from the endosperm of corn and potatoes [8, 12-14]. Apart from digestible starch, it also contains a high amount of resistant starch and amylose and amylopectin ratio that has potential in diabetes treatment since it contributes to low glycaemic index [7, 15]. Most of the starch obtained from different sources is in its native state. The native starches were found incompetent for industrial use, leading to the least suitability in processing [16] and posing many limitations with regards to their application and functionality that related to the processing conditions; for example- weak-bodied and they produce a cohesive and rubbery paste [16] and undesirable gel upon cooling, easily retrograded, having a lower solubility in cold water, lower viscosity and thickening characteristic after cooking and low shear stress and thermal resistance at higher temperature [17-20]. However, these limitations can be overcome and improved through starch modification treatments [20, 21].

There are several methods of starch modification treatments, which can be classified as physical and chemical methods. Chemical methods involve the use of chemical agents, whereas physical methods involve the application of several physical factors (temperature, pressure, shear, moisture, radiation, ultrasonic wave, and etc). The examples of physical methods include pre-gelatinization, extrusion, heat-moisturizing, annealing, and microwave treatments [22]. These methods are able to change particle size, surface properties, solubility index and functional properties such as water absorption [23]. The most common method for chemical modification is cross-linking treatments. Cross-linking treatment involves the introduction of functional groups to the native starch in the presence of cross-linking agents that are responsible for the formation of the intra or inter-ester or ether linkages between the hydroxyl (-OH) groups in the starch amorphous region [20, 21, 24-26]. The most commonly cross-linking agents used in food industries are phosphorus oxychloride or phosphoryl chloride ($POCl_3$), sodium trimetaphosphate (STMP), epichlorohydrin (EPCH) and sodium tripolyphosphate (STPP) [26-28]. STMP is the most widely used cross-linking agent as it is non-toxic and has a slow penetration rate [29]. According to Xie et al. [26], chemical modification is usually employed to enhance the mechanical strength of the starch. This method limits the starch swelling to a greater extent because the linkages formed restrict the mobility of the starch molecules in the amorphous region [30]. As a result, it reduces subsequent disintegration during hydrothermal processing [31]. In addition, it also increases resistance to high temperature, shear resistance and the stability of the swollen starch granules [32, 33]. Cross-linking not only alters the chemical and physical properties but also the thermal transition characteristic of the starch. However, its effect depends on the botanical sources of the starch and the cross-linking agent [21].

Cross-linking has been previously performed by many researchers using different temperatures and pH on different starch sources, including porous wheat starches, rice starches, tapioca starches and corn starches [20, 26, 34, 35]. Since the starch modification involves more than one factor to affect the desired response, therefore Response Surface Methodology (RSM) is the most suitable and effective tool to be used since it can provide relevant information in the shortest time with the least number of experimental runs [36]. According to Bambang et al. [37], optimization is a method used to identify and improve the best solution based on specific quality criteria such as process efficiency. It involves a few steps, including the selection of independent variables and responses, choosing of experimental design strategy, running of experiments and data collection, fitting of the model equation to experimental data, verifying the response graph and model (ANOVA), and lastly determining the optimal conditions for the design the possible approaches available in RSM is central composite design (CCD).

Previously, researchers have used RSM to optimize different starch modification processes in various studies. For example, the application of RSM for the pre-gelatinization process of sweet potato and walur starches [37, 38], to modify sago starch through octenyl succinic anhydride (OSA) process [39] and also the cross-linking of corn and wheat starches [40]. Hence, this study aims to optimize the conditions (temperature: 40-60 ℃ and pH value: 9.00-11.00) for unripe banana (*Musa balbisiana* var. Abu) starch modification using the cross-linking method to obtain cross-linked starch with improved physicochemical properties.

Materials and Methods

Materials

An unripe banana (*Musa balbisiana* var. Abu) at maturity index 2 (peel color: green with a trace of yellow) was purchased from a local seller/farmer in Kota Kemuning, Shah Alam. Sodium sulphate, sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium sulphate (Na_2SO_4) used were purchased from Sigma-Aldrich

Flour preparation

Banana flour was prepared by using the method from Ayo-Omogie et al. [41]. The banana was washed, peeled and sliced to 2-3mm thickness using a slicer and was frozen in a freezer (Sanyo, MDF-U5412, Japan) at the temperature of -40 ℃. The frozen samples were then transferred into the freeze dryer (Martin Christ, Alpha 1-4 LD plus), which operated at -50 ℃ and 0.1m Pa. After the drying was completed, dried samples were mashed using a pestle and mortar and kept in an air-tight container for further analysis.

Starch extraction

Agama-Acevedo et al. [42] used the starch extraction method with slight modifications. 100g of banana flour was suspended in 1 L of 1% sodium sulfate solution (pH 4.5), and the suspension was divided into several glass jars. All the glass jars were capped and kept in an incubator shaker at room temperature (27 ℃) for 12 hours. After that, the suspension was centrifuged at 3000 rpm for 15 minutes, and the supernatant was discarded. The sediment was then re-suspended with distilled water, filtered through 200 US mesh to remove the fibre in the solution and rinsed several times to ensure the maximum amount of starch could be collected. The residue was discarded, and lastly, the filtrate was centrifuged at 3000 rpm for 15 minutes. The white starch sediment obtained was collected and dried in a drying oven (Memmert, Germany) at 45 ℃ for 24 hours.

Experimental design

In this experiment, RSM was used to optimize the cross-linking of banana starch to achieve an optimal degree of cross-linking (%). The experiment was optimized using central composite design (CCD) and conducted in a randomized manner using the Design Expert 12 statistical package. The degree of cross-linking (%) was chosen as the response value, while the temperature (X_1) and pH (X_2) were selected as the independent variables. The ranges for both independent variables were temperature (40-60 ℃) and pH (9.00-11.00), as shown in Table 1.

Table 1. Coded and un-coded table for range and level of independent variables for cross-linking method

Independent Variables		**Factor Level**				
		-α	**-1**	**0**	**+1**	**+α**
Temperature, (℃)	X_1	30.00	40.00	50.00	60.00	70.00
pH	X_2	8.00	9.00	10.00	11.00	12.00

About 5g of unripe banana starch was dispersed in distilled water (75 mL) containing sodium sulphate (10%, w/w starch basis) and a 99:1 mixture of STMP/STPP (12%) as described by Park et al. [43]. The dispersion was adjusted to various pH (8.59-11.41) with 0.1 N NaOH solution and was kept at different temperatures (35.86-64.14 ℃) in a shaking water bath for 2 hours at 200 rpm. After cooling to ambient temperature, 0.1 N HCl solution was added until the dispersion reached pH 6.0. The starch was recovered by centrifuging at 3,600 rpm for 10 minutes (Kubota 5420, Japan), and the precipitate was washed with 100 mL distilled water (3 times). This sample was dried at 35 ˚C for 24 hours, ground in a mortar, sieved and later stored in a glass jar.

Determination of degree of cross-linking of the cross-linked unripe banana starch

The degree of cross-linking of the modified starch was determined using the method of Kaur et al. [44] with slight modification by using a rheometer (Anton Paar MCR 301). About 5 mL of distilled water was added into the rheometer cell containing 0.6 g of sample and was held at a temperature of 45℃ for 2 minutes. Next, it was heated up to 95℃ at the heating rate of 10℃/min and held for 3 minutes, then cooled to 45℃ at the cooling rate of 10℃/min. The peak viscosity values of the native and cross-linked starch were recorded, and the degree of cross-linking was calculated according to the following formula [45]:

$$\text{Degree of cross-linking, \%} = \frac{A-B}{A} \times 100 \qquad (1)$$

Where A is the peak viscosity of the native starch, and B is the peak viscosity of the modified starch.

Swelling power and solubility index of native and modified starches

The swelling power and solubility of native and modified starches were conducted according to the Kittipongpatana and Kittipongpatana methods [46]. About 0.4g (dry basis) of starch samples were mixed with 12.5 mL of distilled water in a 15.0 mL centrifuge tube (pre-weighed). The mixture was equilibrated for 5 minutes at 25 ℃ and heated to 60 ℃ in a shaking water bath for 30 minutes. Next, the samples were cooled in an ice water bath for 1 minute, kept equilibrated at 25 ℃ for another 5 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected, placed in an evaporating dish and dried at 130 ℃ for 4 hours. The swollen starch sediment was weighed. The swelling power and solubility of the sample was calculated as follows:

$$\text{Swelling power, (g/g):} \quad \frac{weight\ of\ sediment}{initial\ weight\ of\ dry\ sample} \tag{2}$$

$$\text{Solubility index, (\%)} \quad : \frac{weight\ of\ dried\ supernatant}{initial\ weight\ of\ dry\ sample} \times 100 \tag{3}$$

Results and Discussion

Optimization of cross-linking condition using response surface methodology

Cross-linking of starch is a method that involves the addition of functional groups into the starch molecules, which affects the physicochemical properties of this starch. Response Surface Methodology (RSM) was conducted at different temperatures (40-60 ℃) and pH values (9.00-11.00) (Table 2) to obtain the optimum condition for the cross-linking. Table 2 shows the experimental values (Y) and the predicted values (FITS) of the response generated by the software. The highest experimental and predicted response values were at run number 13, which were 79.70% and 79.27%, respectively, under the temperature of 50 ℃ and pH 10.00 (Table 2). On the other hand, the lowest experimental and predicted response values were at run number 4, which were 35.83% and 35.73%, respectively, at the temperature of 60℃ and pH 11.00. According to Almonaityte et al. [47], natural starches are unstable to changes in pH and temperature. Therefore, minor changes in pH and temperature could influence its degree of cross-linking. Furthermore, different cross-linking agent also have different optimal pH and temperature ranges [48, 49]. In certain conditions, some level of alkalinity can facilitate a reaction by transforming the hydroxyl groups within starch molecules into alkoxide anions through ionization [50]. The response obtained shows that the degree of cross-linking was in the range of 35.83% to 79.70% (Table 2).

Table 2. Full RSM experimental design and the comparison between experimental and predicted values of the degree of cross-linking (%)

Run	Factors		Response (Degree of cross-linking, %)	
	X_1	X_2	Y	FITS
1	40.00	9.00	62.40	62.65
2	60.00	9.00	60.08	60.33
3	40.00	11.00	42.75	42.66
4	60.00	11.00	35.83	35.73
5	35.86	10.00	48.50	48.42
6	64.14	10.00	41.95	41.88
7	50.00	8.59	71.62	71.30
8	50.00	11.41	39.61	39.77
9	50.00	10.00	79.56	79.27
10	50.00	10.00	79.37	79.27
11	50.00	10.00	79.16	79.27
12	50.00	10.00	78.54	79.27
13	50.00	10.00	79.70	79.27
R^2: 0.9997%, R^2 (adj): 0.9995%, CV: 0.65%				

Note: X_1: Temperature (℃), X_2: pH value, Y: Experimental value, FITS: Predicted value, CV: Coefficient of Variation

The goodness of fit of the regression model was defined by determining the R^2 and adjusted R^2, which provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. Theoretically, the R^2 values should be at least 0.8 to have a good fit model.

The value closer to 1.0 shows a better fitting of the model with the actual data and a good correlation between experimental and predicted values. The R^2 value obtained was 0.9997, which means 0.9997 of the variability in the observed response could be explained by the model, and the remaining 0.0003 of the variability cannot be explained by the model obtained due to other factors that are not included in the model (Table 2). It indicated a strong and reasonable fit of the models to the experimental data [51].

The adjusted R^2 is the corrected value for R^2 after the elimination of unnecessary model terms. The adjusted R^2 obtained should not be more than 0.2 difference from the R^2 value [52] and it was observed that the adjusted R^2 value was 0.9995. The smaller difference between the adjusted R^2 and the R^2 shows that there are many non-significant terms included in the model, and it signifies the adequacy of model accuracy [53]. The coefficient of variation (CV) is the ratio of the standard error of estimation to the mean value of the observed response [54] and expressed as percentage (%). The CV of the model was 0.65% (Table 2). Firatligil-Durmus and Evranuz [55] stated that a model can be considered reasonably reproducible if its CV is not more than 10%. Therefore, the obtained CV has good reproducibility for this model and can be employed for the optimization process. A response surface regression analysis was performed, and the result for both regression and coefficient for the optimization of cross-linking of unripe banana starch can be presented as a coded equation at a 5% level:

$$Y_1 = 79.27 - 2.31X_1 - 11.15X_2 - 1.15X_1X_2 - 17.06X_1^2 - 11.86X_2^2$$

Where, Y1 = Degree of cross-linking (%), X_1 = Temperature (°C), and X_2 = pH value

Analysis of variance for the optimization of cross-linking condition of unripe banana starch

The effects of experimental variables on the linear, square and interaction terms were tested for adequacy and fitness by analysis of variance (ANOVA). The summary of the results obtained is presented in Table 3. The p-value is the probability of the factors having a very little or insignificant effect on the response. The larger F-value signifies the better fit of the RSM model to the experimental data. The F-value should be larger, and the p-value should be lower than 0.05 in order for the model to be statistically significant [56]. Therefore, all the regression, linear, square, and interaction models found in this study are highly significant as they have p-values lower than 0.05.

The lack-of-fit test measures the variation of the data concerning the fitted model and is one of the important aspects of a reduced model. If the lack of fit is significant, it means that the model does not fit the data well. The data should be rejected if the results show any significance in the lack-of-fit test. Insignificant lack-of-fit indicates a good model, which means that the p-value must be higher than 0.05. The F-value of the lack-of-fit can be obtained by dividing the lack-of-fit mean square by its pure error mean square. In this study, the lack-of-fit of the selected model showed a non-significant result (*p*-value >0.05) with a *p*-value of 0.7270. Thus, the non-significant p-value indicates that the model is good and fits well with the experimental data, and the parameters have a significant effect on the response value [57].

Table 3. Analysis of variance (ANOVA) of overall effects of test variables on the optimization of cross-linking

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	Status
Model	3723.98	5	744.80	4712.63	< 0.0001	Significant.
X_1	42.80	1	42.80	270.79	< 0.0001	Significant
X_2	993.89	1	993.89	6288.73	< 0.0001	Significant.
X_1X_2	5.29	1	5.29	33.47	0.0007	Significant.
X_1X_1	2024.47	1	2024.47	12809.66	< 0.0001	Significant.
X_2X_2	979.20	1	979.20	6195.81	< 0.0001	Significant
Residual	1.11	7	0.1580			
Lack of Fit	0.2824	3	0.0941	0.4570	0.7270	Not Significant
Pure Error	0.8239	4	0.2060			
Total	3725.09	12				

Note: X_1: Temperature (°C), X_2: pH value, df: Degree of freedom

Contour and surface plot

The previously mentioned model was used for the optimization process, and the contour and surface plot was generated as the graphical representation of the response factor based on the regression equation above. The response variable was set to a maximum while both temperature and pH variables were kept in range all the time.

The desirability value was used to determine the optimum combination of the variables for the cross-linking conditions of the starch that leads to the maximized response while satisfying the restriction of the model [58,59]. The value for desirability is between 0.000 and 1.000 for a set of responses [51], and usually, the factor setting/combination that maximized the desirability for the optimization was chosen. The ideal optimum desirability in the RSM model is 1.000, with a value between 0.6 and 0.8 is considered acceptable. The highest desirability value of 1.000 (Figure 1) was obtained at a temperature of 50.57°C with pH 9.52 **(**these values have been rounded to the nearest integer**)** and was identified as the optimum condition for the cross-linking of the unripe banana starch in this study. A high desirability value signified that the optimized model had high potential in producing the desired outcome aimed for the model.

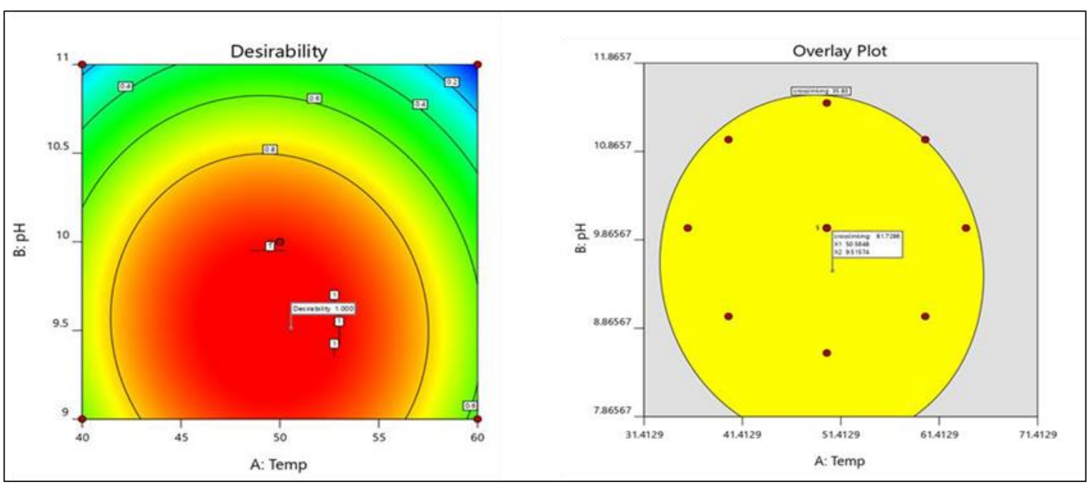

Figure 1. Desirability and overlaid contour plot for the degree of cross-linking (%) at the predicted optimum conditions

The overlaid contour plot is a technique used to combine the variables and response in a similar graph, and the conclusion can be made according to the interaction between the variables and response [60]. The overlaid contour plot is presented in Figure 1 in order to identify the feasibility of the response value at the optimum condition of the variables. The overlaid contour plot showed that the suggested optimum conditions for the temperature (50.57 ℃) and pH (9.52) fell in the yellow region, with the predicted value of the response (degree of cross-linking) of 81.73%. The yellow region means the entire range of all the intervals meets the specified criteria, which indicates that the conditions are feasible to be carried out. On the contrary, the grey region represents the area in which the conditions are not fit to be experimentally done.

The two-dimensional (2D) contour plot and three-dimensional (3D) surface plot (Figure 2) affirmed the effect of both temperature and pH as independent variables towards the degree of cross-linking. The red zone in both 2D and 3D plots represents the higher degree of cross-linking, while the blue zone indicates the lowest. The temperature and pH have greater interaction and also highly contribute to the response, as indicated by the steeper and more curved surface [61] (Figure 2). The 3D plot explained that the increase in temperature and time led to a substantial increase in the response until it achieved the optimal conditions at a temperature of 50.57 ℃ and pH 9.52. However, the response decreased significantly at the point higher than the optimal conditions, which means the variables have a negative impact on the degree of cross-linking. Relatively, the optimal conditions for this experiment correspond to the maximum degree of cross-linking of 81.73%.

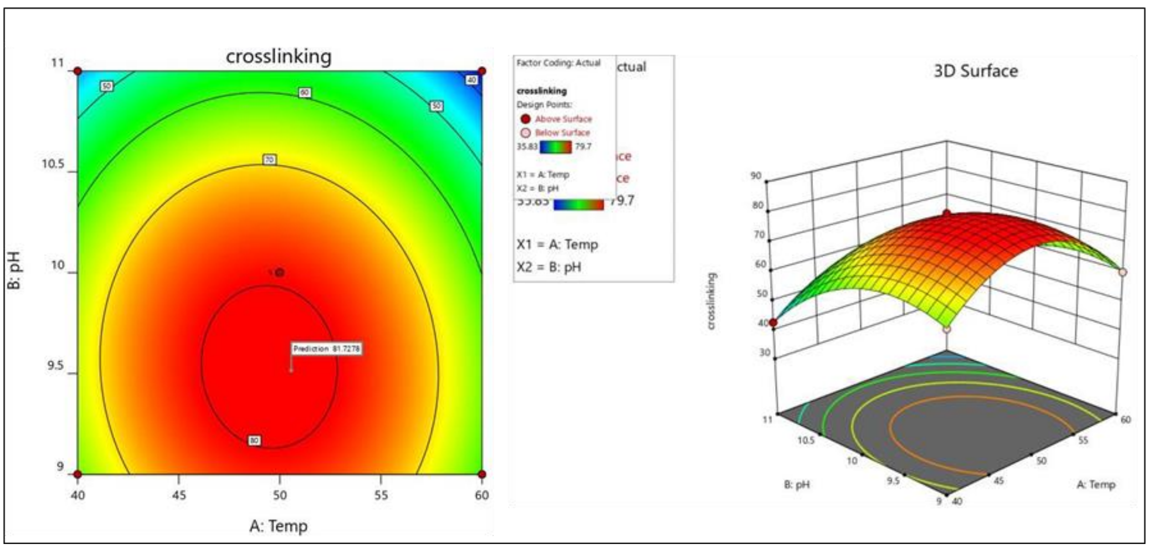

Figure 2. Two-dimensional (2D) contour plot and three-dimensional (3D) surface plot of the degree of cross-linking (%) at the predicted optimum conditions

Verification of the feasible optimum condition

In order to achieve a precise estimation of the optimum condition of all the variables and a prediction of the model fitness, both numerical and graphical validation plays a definite role in deciding the optimum variable combination for a maximum output [62]. The suitability of the model equation for predicting the optimum response values was further evaluated and tested using an independent sample t-test by SPSS between the experimental and predicted values. The result in Table 4 shows that there is no significant difference (p-value>0.05**)** between the experimental values (82.38%) and predicted values (81.73%) for all dependent variables. Therefore, the validation of the model was confirmed, and the mentioned model can be applied for the optimization of the cross-linking of unripe banana starch.

Table 4. Verification of the experimental and predicted value of the degree of cross-linking (%) at the optimum condition

Optimum Condition		Degree of cross-linking (%)	
X_1	**X_2**	**Y**	**FITS**
50.57	9.52	82.38[a]	81.73[a]

Note: X_1: Temperature (℃), X_2: pH Value, Y: Experimental Value, FITS: Predicted Value. Means with same letters within a column are not significantly different (p >0.05) between experimental and predicted values.

Degree of cross-linking and its effects on swelling and solubility

The degree of cross-linking of the cross-linked starch was measured and calculated using its peak viscosity value. Yin et al. [63] have mentioned that peak viscosity is the most useful property to measure the degree of cross-linking. As shown in Table 5, the peak viscosities obtained are between 8.40 and 18.99 cP, at 35 to 75% degree of cross-linking. It was observed that the peak viscosities of this modified starch decreased significantly as the degree of cross-linking was above 75%, with peak viscosity ranging between 6.01 and 6.35 cP. The highest degree of cross-linking obtained from this study was 79.70%, with a peak viscosity of 6.01 cP. Almonaityre et al. [47] explained that the degree of cross-linking affects the peak viscosity of the cross-linked starch paste, where starch paste with a higher level of cross-linking will show a lower peak viscosity [21]. The findings of this study agree with Babu et al. [64] (Table 5). The presence of an STMP/STPP mixture in an alkaline condition caused a large number of hydrophilic phosphorous groups to react with hydroxyl moieties of starch chains, enabling di-starch phosphate interaction in the inner of the granules [62, 63]. Kou et al. [65] also stated that a higher amount of phosphorous will produce a higher degree of cross-linking, and an increase in the alkalinity of the cross-linking medium will accelerate this reaction [48]. The introduction of the covalent bonds or the cross-links in the granules, therefore, decreased the mobility of the molecules and strengthened the glucose chain [47]. This outcome will inhibit the granular breakdown, producing more stable starch toward thermal and mechanical energy and resulting in a lower peak viscosity value.

Table 5. Peak viscosity and degree of cross-linking (%) of each RSM run order

Run	Factor		Peak viscosity (cP)	Degree of Cross-Linking (%)
	X_1	**X_2**		
1	40.00	9.00	11.13	62.40
2	60.00	9.00	11.82	60.08
3	40.00	11.00	16.95	42.75
4	60.00	11.00	18.99	35.83
5	35.86	10.00	15.24	48.5
6	64.14	10.00	17.18	41.95
7	50.00	8.59	8.40	71.62
8	50.00	11.41	17.88	39.61
9	50.00	10.00	6.05	79.56
10	50.00	10.00	6.11	79.37
11	50.00	10.00	6.17	79.16
12	50.00	10.00	6.35	78.54
13	50.00	10.00	6.01	79.70

Note: X_1: Temperature (℃), X_2: pH, cP: Centipoise

Table 6 shows the swelling and solubility results for native and cross-linked starches at the optimum conditions suggested by RSM. Swelling is the hydration capacity of starch and the extent of starch granules interaction within both amorphous and crystalline regions [66]. In contrast, solubility is associated with the leaching of amylose from the starch granules during swelling [67]. The results revealed that both swelling, and solubility of native starch were reduced from 2.0528 g/g and 13.17% to 1.9011 g/g and 8.56%, respectively, after the cross-linking treatment at the optimum conditions (temperature: 50.57℃, pH: 9.52) with 81.73% degree of cross-linking. According to Carmona-Garcia et al. [68], a higher degree of cross-linking resulted in a decrease of hydration properties and inhibition of water penetration for swelling, thus lowering swelling of the starch [48, 65] and restricting the starch molecules (mainly amylose) to leach out from the starch granule [66, 67] and reduce the starch solubility [69].

Table 6. Swelling power and solubility index of native and cross-linked starch

Starch Sample	Native	Cross-Linked
Swelling power (g/g)	2.0528^{a} ± 0.0008	1.9011^{b} ± 0.0011
Solubility index (%)	13.17^{a} ± 0.0200	8.56^{b} ± 0.0200

Note: Means with different letters within the row are significantly different (p <0.05) between native and cross-linked samples

Conclusion

The optimisation of starch modification through cross-linking by using RSM was successfully investigated, and the data was systematically interpreted. The optimisation of the present work through CCD revealed that the best optimum conditions for a maximum degree of cross-linking (81.73%) were temperature 50.57 ℃ and pH 9.52. Both variables (temperature and pH) had a significant effect on the response (degree of cross-linking). It was also observed that the degree of cross-linking affects the peak viscosity of the modified starch in the RSM run. The peak viscosity decreased significantly when the degree of cross-linking was higher than 75. It was also observed that cross-linking has significant effects on the swelling power and solubility of the starch, whereby both the swelling power and solubility index of cross-linked starch were significantly decreased. Swelling power decreased from 2.0528 g/g to 1.9011, and the solubility index decreased from 13/17% to 8.56%. This was due to the new linkages created during the cross-linking process that strengthened the starch intermolecular interaction and integrity. Thus, this prevents chain disruption and breakage and reduces swelling power and solubility index by limiting swelling and amylose leaching. As additional covalent bonds were introduced through the cross-linking process, cross-linked starch exhibits higher tolerance to acid, heat, and shear forces and improves its viscosity properties. Therefore, cross-linked starch can be used as a thickening, texturizing and stabilizing agent in foods such as yoghurt, sauces and dairy products.

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