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Optimisation of reaction conditions of octenyl succinic anhydride (OSA) modified sago starch using response surface methodology (RSM)

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Abstract

Octenyl succinic anhydride (OSA) modified sago starch was prepared in order to improve the emulsification properties of native starch. In the present study, the major factors affecting esterification were investigated with respect to OSA concentration, pH and reaction time using response surface methodology (RSM) based on central composite rotatable design (CCRD) to obtain the highest value of degree of substitution (DS). Results shown that the optimum conditions for OSA concentration, pH and reaction time were 5.00%, pH 7.20 and 9.65 h, respectively. At optimum condition, the esterification of sago starch with OSA resulted in DS value of 0.0120. The DS increased linearly with the increase in amount of OSA, whilst pH and reaction time show a curvature trend on the value of DS. The value of DS was found to be significantly affected by all the three variables. The experimental values under optimum condition were in good consistent with the predicted values (0.0131), which suggested that the optimisation by RSM is more efficient process than conventional optimisation.

Introduction

Starch is a polysaccharide carbohydrate which made up by two types of polymers: amylose with α- (1→4) linear links and amylopectin made up by α-(1→6) branched links. Native starches are widely used in food industry. However in the native form, starches present several defects such as insoluble in cold water, rubbery pastes when heated, lack of clarity, easily retrograde during storage, absence of lipophilic groups and lack of emulsification properties, limit its use in industrial application (Shi and He, 2012). Native starches contain free hydroxyl groups at carbon 2, 3 and 6 of the glucose molecule, makes them highly reactive and suitable for modification (Segura Campos *et al*., 2008). In order to overcome the shortcomings of native starch and improved its functional properties, starch modification is done by alteration of the physical and chemical characteristics hence it can be tailored to specific food applications (Hui *et al*., 2009). One of these modifications includes the introduction of hydrophobicity to the native starch, by which the hydrogen bonding was disrupted during the process. These kinds of modified starch will acquire the amphipilic properties and find applications as emulsifiers in food systems.

The native starch is modified by treating with octenyl succinic anhydride (OSA), which includes the incorporation of hydrophobic and hydrophilic alkenyl group that gives the surface-active properties to the starch (Bhosale and Singhal, 2006). The esterification of starch with OSA depends on several factors such as reactant concentration, pH and reaction time. The successful modification with OSA depends on the control of reaction conditions to favour the substitution and difference in the degree of substitution (DS) may occur (Plate *et al*., 2012). Although esterification have been conducted across several botanical sources: Amaranth from *Amaranthus paniculatus* seeds (Bhosale and Singhal, 2006), *Indica* rice (He *et al*., 2006), *Phaseolus lunatus* (Segura-Campos *et al*., 2010), potato (Hui *et al*., 2009) and waxy corn (Liu *et al*., 2008), there are no esterification with OSA have been reported on the preparation conditions of OSA starch from sago.

Sago starch *(Metroxylon sagu)* is possibly the only example of a commercial starch derived from the stem of palm (sago palm) (Nor Nadiha *et al*., 2010). Sago starch is now finding increase attention in food industry in the production of vermicelli, bread, crackers, biscuits and many other traditional foods. Some physicochemical of sago starch has been reported to be similar with other type of starch; potato, in terms of moisture content and granular size also amylose content like those of corn starch (Maaruf *et al*., 2001). Apart from the similarity, sago starch has its unique properties by having smooth granule surface with some pitting and large granular size which range from 10μm to 50 μm (Abdorreza *et*

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al., 2012).

In the determination of DS, the response is affected by multiple independent variables, hence there is a need to use an optimization method that can determine the effect of all factor simultaneously. Moreover, in determination of optimal experimental conditions, the possibility of interactions between the independent variables should be considered (Samavati, 2013). To overcome the difficulty in finding the interaction of various parameters, response surface methodology (RSM) has been widely used (Kaushik *et al*., 2006). RSM is a collection of statistical and mathematical techniques useful in modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize the response (Anderson and Whitcomb, 2005). The objective of this study is to develop an optimum condition for the preparation of OSA sago starch using RSM by evaluating DS. Three independent variables were chosen include OSA concentration, pH and reaction time while DS was chosen as a dependent variable (response).

Materials and Methods

Materials

Sago starch (food grade) was purchased from Nee See Ngeng & Sons Sago Industries Sdn. Bhd (Sarawak, Malaysia). Octenyl succinic anhydride (OSA) (Lot# J1097009-A) was donated by Dixie Chemicals, USA. Other chemicals and reagents used were of analytical grade.

Preparation of OSA starches

Preparation of OSA starches was based on the method described by Bhosale and Singal (2006) with some modification. The modifications include widening the range of OSA concentration and pH used, and also concentration of starch slurry was increased. One hundred and twenty five grams of sago starch were dispersed in 400 ml distilled water with agitation. The pH (5.0-10.0) of the slurry was adjusted by using 2% NaOH solution. To this mixture, OSA at 1.0- 6.0% (based on dry starch basis) was added dropwise for 2 hours. During the addition of OSA, the pH was maintained at the set point. The reaction was carried out at 6-24 hours. After the reaction time was ended, the pH of the starch slurry was adjusted to 6.5 using 2% HCl to halt the reaction. The resulted OSA starch was washed three times with distilled water and acetone and centrifuged at 3000 rpm. The modified starch was collected by filtration through Whatman filter paper No. 1 and dried in drying oven (Memmert, Germany) at 45°C for 24 h. After drying, the OSA starch was sieve with 0.35 mm siever and kept in airtight container.

Experimental design

The experimental design was generated using Design-Expert version 6.0.4 (Stat Ease Software). Response surface methodology (RSM) was used to estimate the effect of independent variable (OSA concentration, X_1; pH, X_2; reaction time, X_3) on the value of degree of substitution (DS). A central composite rotatable design (CCRD) was performed for designing the experimental data. Randomization of experiments is required to minimize the effects of unexplained variability in the observed responses due to extraneous factors (Samavati, 2013) . A set of 20 experiments was performed, each variable varied at five coded levels: -α, -1, 0, +1 and +α (α = 1.68). The independent variables and their levels are shown in Table 1. The factorial design consists of eight factorial points, six axial points and six repetitions at the central point. The quadratic equation for the three independent variables can be described as (Wang *et al*., 2011):

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i + \sum A_{ij} X_i X_i \quad (1)$$

where Y was the predicted response variable (DS value); A_o, A_i, A_{ii}, and A_{ij} were constant regression coefficient of the model; and X_i and X_j (i = 1,2,3; j = 1,2,3) represented the three independent variables (OSA concentration, pH, and reaction time).

Table 1. Independent variables and their levels used in the response surface design

Independent variables		Factor level				
		-α	-1	0	1	+α
Concentration OSA (%)	X_1	1.00	2.00	3.50	5.00	6.00
pH	X_2	5.00	6.00	7.50	9.00	10.0
Reaction time (h)	X_3	6.00	9.65	15.00	20.35	24.00

Determination of DS of OSA modified starch

The degree of substitution (DS) is the number of hydroxyl groups substituted per glucose unit with OSA (Yusoff and Murray, 2011). The DS was determined by alkali saponification followed by back titration of excess alkali using the titrimetric method of Bhosale & Singhal (2006). About 25 ml of 0.5 N NaOH solution was added to the suspension of the OSA starch (5 g of starch in 50 ml of distilled water) which was continuously shaken for 24 h at room temperature. The excess alkali was titrated with 0.5

N HCl, using phenolphthalein as indicator. A blank titration was also performed by using unmodified starch. The DS was calculated as follows:

$$\text{OSA substitution}(\%) = \frac{(V_{blank} - V_{sample}) \text{ x } 0.1 \text{ x } N \text{ x } 100}{W} \quad (2)$$

where V_{blank} = the volume of HCl required for blank titration; V_{sample} = volume of HCl required to titrate the sample; W = the weight (g) of sample; N = the normality of the HCl solution.

$$\text{DS} = \frac{162 \text{ x OSA substitution } (\%)}{21\,000 - [209 \text{ x OSA substitution}(\%)]} \quad (3)$$

where 162 = molecular weight of the glucose unit; 21 000 = 100 x molecular weight of octenyl succinyl group; 209 = molecular weight of octenyl succinyl group minus the molecular weight of hydrogen atom.

Results and Discussion

Data analysis and evaluation of the model

The effect of three independent variables, OSA concentration (X_1), pH (X_2), and reaction time (X_3) on DS were studied during experimentation according to the range selected as Table 1. Based on the created design, 20 experiments were performed and the obtained results are depicted in Table 2. Based on the observed DS obtained from experiments, the maximum DS value (0.0141) was observed at the 6.00% OSA, pH of 7.50 and reaction time of 15 h while minimum DS value (0.0054) was achieved either at the OSA concentration of 2.00%, pH of 9.00, and reaction time of 20.35 h or at 2.00% OSA, pH 9.00 and 9.65 h conditions. The experimental data were used to determine the regression coefficients and the following models that describe the DS value in terms of coded parameters and actual parameters were acquired.

$$\text{DS}=(9.990\text{x}10^{-3})+(2.119\text{x}10^{-3*}X_1)-(5.285\text{x}10^{-4*}X_2)-(5.632\text{x}10^{-4*}X_3)+(1.576\text{x}10^{-4*}X_1{}^{*}X_1)-(1.310\text{x}10^{-3*}X_2{}^{*}X_2)-(5.319 \text{ x } 10^{-4}\,{}^{*}X_3{}^{*}X_3) + (5.000 \text{ x } 10^{-5}\,{}^{*}X_1{}^{*}X_2) - (7.250\text{x}10^{-4*}X_1{}^{*}X_3)+(5.000\text{x}10^{-5*}X_2{}^{*}X_3) \quad (4)$$

$$\text{DS} = (-0.0309) + (2.1238 \text{ x } 10^{-3}\,{}^{*}\text{OSA concentration}) + (8.3613 \text{ x } 10^{-3}\,{}^{*}\text{pH}) + (7.2376 \text{ x } 10^{-4}\,{}^{*}\text{Time}) + (7.1309 \text{ x } 10^{-5}\,{}^{*}\text{OSA concentration}^2) - (5.9269 \text{ x } 10^{-4}\,{}^{*}\text{pH}^2) - (1.8572 \text{ x } 10^{-5}\,{}^{*}\text{Time}^2) + (2.2627 \text{ x } 10^{-5}\,{}^{*} \text{ OSA concentration}\,{}^{*}\text{pH}) - (9.1138 \text{ x } 10^{-5}\,{}^{*}\text{OSA concentration}\,{}^{*}\text{Time}) + (6.2854 \text{ x } 10^{-6}\,{}^{*}\text{pH}^{*}\text{ Time}) \quad (5)$$

The results obtained from CCD were then analyzed by analysis of variance (ANOVA). ANOVA was performed to test the accuracy of the model and the results are summarized in Table 3.

Table 2. The CCD matrix and the experimental data for the responses

Factor 1: **X_1**	**Factor 2:** **X_2**	**Factor 3:** **X_3**	**Response:** **Degree of Substitution**
2.00	9.00	20.35	0.0054
2.00	9.00	9.65	0.0054
3.50	7.50	24.00	0.0078
5.00	6.00	9.65	0.0125
3.50	7.50	6.00	0.0094
6.00	7.50	15.00	0.0141
2.00	6.00	9.65	0.0066
3.50	7.50	15.00	0.0109
3.50	7.50	15.00	0.0094
2.00	6.00	20.35	0.0070
5.00	9.00	9.65	0.0109
5.00	6.00	20.35	0.0094
3.50	7.50	15.00	0.0102
3.50	7.50	15.00	0.0098
5.00	9.00	20.35	0.0086
3.50	7.50	15.00	0.0098
1.00	7.50	15.00	0.0070
3.50	5.00	15.00	.0070
3.50	10.00	15.00	.0058
3.50	7.50	15.00	.0098

X_1:Concentration OSA, (%) X_2: pH X_3: Reaction time, (h)

The response variable could be said significant if there is a large *F*-value and a small *P*-value for each terms in the models (Yolmeh *et al*., 2014). The *P*-values and *F*-values were used to check the significance of each linear, quadratic and interaction term on the response. The smaller the P value, the more significant the term will be. The ANOVA result showed that the model is significant, as evidenced by very low probability value (P < 0.0001). This value indicates that only less than 0.01% chance that a 'Model F-value' could occur because of noise and the model equation adequately describes the data satisfactorily. From the Table 3, among all the variables, X_1, X_2, X_3, $X_2{}^{*}X_2$, $X_3{}^{*}X_3$, and $X_1{}^{*}X_3$ are the model terms that have significant effect on the DS value. The other term was found to be not significant. The less significant term cannot be excluded from the model in order to maintain hierarchy of the model. OSA concentration (P < 0.0001) gives the major effect on the DS value, followed by reaction time (P = 0.0009) and pH (P = 0.0014).

The fitness of the model was checked by lack-of-fit test (P < 0.05) which show the suitability of models to accurately predict the variation (Yolmeh *et al*., 2014). The ANOVA showed that the lack of fit (LOF) test was not significant and the model is adequate as evidenced by the value of LOF to be

Table 3. Analysis of variance (ANOVA) for the response surface quadratic model

Source	Sum of squares	Df	Mean square	F-value	P value
Model	1.023×10^{-4}	9	$1.1.37 \times 10^{-5}$	56.88	<0.0001*
X_1	6.133×10^{-5}	1	6.133×10^{-5}	306.79	<0.0001*
X_2	3.815×10^{-6}	1	3.815×10^{-6}	19.08	0.0014*
X_3	4.331×10^{-6}	1	4.331×10^{-6}	21.67	0.0009*
$X_1{*}X_1$	3.578×10^{-7}	1	3.578×10^{-7}	1.79	0.2106
$X_2{*}X_2$	2.472×10^{-5}	1	2.472×10^{-5}	123.65	<0.0001*
$X_3{*}X_3$	4.077×10^{-6}	1	4.077×10^{-6}	20.39	0.0011*
$X_1{*}X_2$	2.000×10^{-8}	1	2.000×10^{-8}	0.10	0.7583
$X_1{*}X_3$	4.205×10^{-6}	1	4.205×10^{-6}	21.04	0.0010*
$X_2{*}X_3$	2.000×10^{-8}	1	2.000×10^{-8}	0.10	0.7583
Residual	1.999×10^{-6}	10	1.999×10^{-7}		
Lack of Fit	6.707×10^{-7}	5	1.341×10^{-7}	0.50	0.7644
Pure Error	1.328×10^{-6}	5	2.657×10^{-7}		
Cor Total	19	1.043×10^{-4}			

Standard deviation $= 4.471 \times 10^{-4}$; $R^2 = 0.9808$; Adj $(R^2) = 0.9636$

X_1:Concentration OSA, (%) X_2: pH X_3: Reaction time, (h)
*significant at 5% level ($P<0.05$)

0.7644. The regression coefficient *(R^2)* and adjusted regression coefficient *(Adj R^2)* values were 0.9808 and 0.9636 respectively. The R^2 was used to check the correlation between observed and predicted values. From the values of R^2, only 1.92% variability in the observed response cannot be explained by the experimental factors and their interactions. The R^2 and *Adj* R^2 values for this model is almost close to each other, indicate that non-significant term have not been excluded in the model. Based on all the statistical tests, the developed model have shown to be good in representing the data and able to describe the relationship between the independent variables and response.

Effect of independent factors on the value of DS

Effect of OSA concentration and pH on DS

The effect of OSA concentration and pH on the value of DS with a constant reaction time at 15 h is illustrated in Figure 1(a). The reaction time was fixed at 15 h. It can be seen that as concentration of OSA increased, the value of DS will also increase. This trend can be explained as increasing in concentration of OSA, the possibility of esterification between starch and OSA molecule will also increase. OSA has low solubility in water, as a result only dissolved and extremely dispersed OSA could penetrate the entire granule (Zhang *et al.*, 2011). It is also well known that starch hydroxyl is immobile and their reaction depend on the presence of OSA molecules around the hydroxyl groups (Bhosale and Singhal, 2006). In the reaction mechanism, hydrogen bond breaking is the rate determining step when there is larger reagents used. The reagent needs to adopt to a specific configuration according to OH group that requires hydrogen bond breaking in order for the reaction to occur, and this configurational change is slow compared to the rate of reaction of the anhydride (Hill and Mallon, 1998). There is a need to use larger amount of OSA in order to favours molecule collision, thus it will make DS increase correspondingly. However for the effect of pH, it showed the curvature effect. It is supported by the *P*-value of quadratic term of pH ($P < 0.0001$) is more significant than linear term ($P = 0.0014$). This indicates that pH has a curvature (quadratic) response with DS. That is why initially increase in pH, the DS will increase and after the maximum DS, it will start decreasing with increasing pH. Therefore, we can conclude that the concentration of OSA and pH are significant factors influencing the value of DS.

Effect of OSA concentration and reaction time on DS

Figure 1(b) shows that effect of OSA concentration in the range of 2.00 – 5.00% and reaction time in the range of 9.65 – 20.35 h. From the figure, the maximum DS can be seen to be at OSA concentration of 5.00% and reaction time of 9.65 h. The effect of concentration OSA on DS can be clearly seen on this figure as the DS will increase with increasing OSA

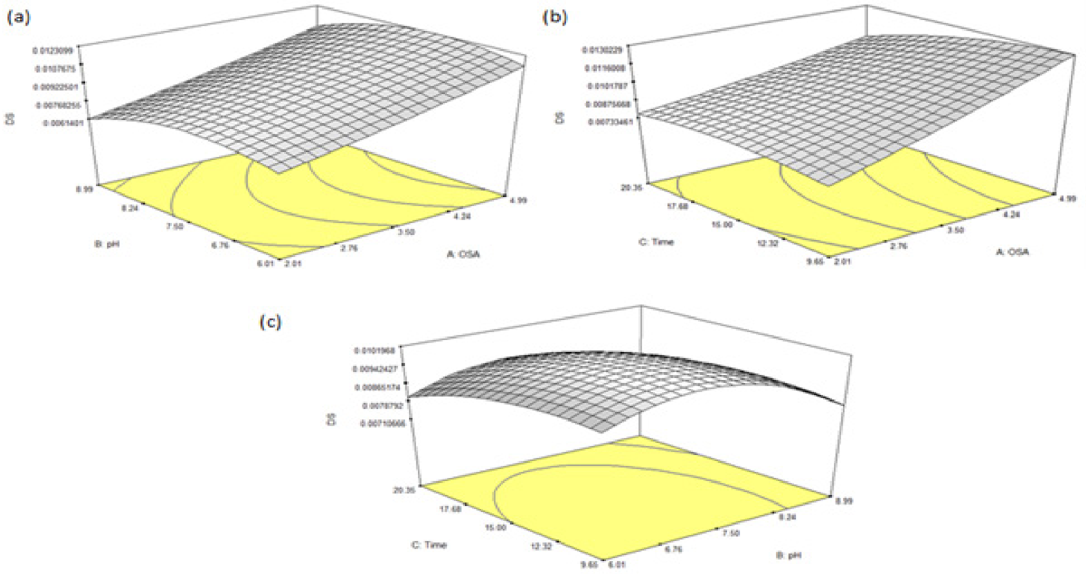

Figure 1. Response surface curve for the effects of (a) OSA concentration (%) and pH, (b) OSA concentration (%) and time (h), and (c) pH and time (h) on the value of DS

concentration. Nevertheless, there is only little effect of reaction time on DS. This agreed with Segura-Campos *et al*., (2008) and Song *et al*., (2006), who stated that long reaction times does not necessarily produce high DS value because anhydride needed for esterification is exhausted, thus hydrolysis will take place. Even though dissolution and diffusion of OSA into starch increase with increasing reaction time, at one point, side reaction will competes with esterification in the production of OSA starch. Finally, the side reactions become dominant as prolonging the reaction time and concentration of OSA was depleted due to esterification and hydrolysis (Han *et al*., 2011).

Effect of pH and reaction time on DS

In esterification, controlling the pH of aqueous slurry is important for the reaction to occur. In the formation of OSA starch, NaOH act as catalyst and start the reaction by the formation of alcoholate ions along the starch polymer (Hui *et al*., 2009). From Figure 1(c), the highest DS was achieved at pH around 7.00 – 8.00. The DS increased from 7.00 to 8.00, but decreased from pH 8.00 to 9.00. At pH lower than 7.00 and higher than 8.00, the DS will gradually decrease. These results was similar with Segura-Campos *et al.*, (2008) which explained that low pH values promoted esterification of sago starch with OSA in the present study and strongly alkaline condition promote de-esterification. At pH below 7.00, the hydroxyl groups are not sufficiently activated for nucleophilic attack of the anhydride moieties whereas at pH higher than 8.00, it will favours anhydride hydrolysis as the hydroxyl groups in the anhydro-glucose units of starch will be activated under basic condition (Han *et al*., 2011). Hence, relatively low pH (7.00 - 8.00) is more suitable for substitution in order to reduce undesirable secondary reactions.

Optimisation and validation of experiments

In this starch modification, the aim of experiments is to achieve a high DS as possible. The effect of each variable was predicted by Design Expert software and the best value was predicted as optimum condition. The optimum OSA concentration, pH and reaction time were found to be 5.00%, pH 7.20 and 9.65 h respectively with predicted DS of 0.0131 and a desirability value of 0.882. The adequacy of the model equations, Eq (4), can be validating by carried out verification experiment under optimal conditions. To validate the optimum conditions, three replicate confirmation experiments were conducted and analysed using T-test. A mean value of 0.0120 ± 0.00, obtained from the actual experiments, proved the validation of RSM model. The result shows that there is no significant difference between actual and predicted value, indicate that the actual values obtained is in good agreement with the predicted value calculated from the software. Thus, the model is suitable for predicting DS and also optimisation of the experimental conditions.

Conclusion

Sago starch was successfully esterified with OSA in aqueous slurry systems and the main effect affecting the modification was investigated. All variables studied shown to influence the value of DS. Based on the results, the optimum conditions for the DS were 5.00% OSA concentration, pH 7.20 and 9.65 h of reaction time. The information in the current paper is useful for further studies to understand the effect of modification on the physicochemical properties of sago starch and its emulsifying activity.

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