

## Production of Octenyl Succinic Anhydride-Modified Waxy Corn Starch and Its Characterization

ZHIQIANG LIU,<sup>\*,†,‡</sup> YIN LI,<sup>\*,§</sup> FENGJIE CUI,<sup>||</sup> LIFENG PING,<sup>⊥</sup> JIANGNING SONG,<sup>#</sup>  
 YANIV RAVEE,<sup>‡</sup> LIQUN JIN,<sup>†</sup> YAPING XUE,<sup>†</sup> JIANMIAO XU,<sup>†</sup> GENG LI,  
 YAJUN WANG,<sup>†</sup> AND YUGUO ZHENG<sup>\*,†</sup>

Institute of Bioengineering, Zhejiang University of Technology,  
 Hangzhou 310014, People's Republic of China, NSF I/UCRC for Biocatalysis and Bioprocessing of  
 Macromolecules, Polytechnic Institute of New York University, Six Metro Tech Center,  
 Brooklyn, New York 11201, Department of Plant Science, North Dakota State University, Fargo,  
 North Dakota 58105, School of Food Engineering and Biotechnology, Jiangsu University,  
 Zhenjiang 2120013, People's Republic of China, Institute of Quality and Standard for Agricultural  
 Products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, People's Republic of China,  
 and Department of Biochemistry and Molecular Biology, Monash University, Clayton, Melbourne,  
 VIC 3800, Australia

The objective of this work is to investigate the effects of reaction conditions on the synthesis of octenyl succinic anhydride (OSA)-modified starch from waxy corn starch and to study the characteristics of the OSA-modified starch as well as its applications. A mathematical model was developed to investigate the influences of various processing condition factors on the production of the OSA-modified waxy corn starch production and predict the optimum reaction conditions. The maximal degree of substitution (DS) of OSA-modified waxy corn starch (0.0204) was predicted to occur when the starch concentration was 31.2%, the pH was 8.6, the reaction temperature was 33.6 °C, and the reaction time was 18.7 h. Repeated reactions for producing OSA-modified waxy corn starch were carried out in a 5 m<sup>3</sup> reactor under the optimized conditions for verification of the model. The characteristics of modified waxy corn starch including infrared spectrum, scanning electron microscopy, and pasting property were tested and emulsification capacity of the OSA-modified starch were evaluated as well.

**KEYWORDS:** Octenyl succinic anhydride-modified waxy corn starch; optimization; degree of substitution; enzyme-treated OSA-modified starch; application

### INTRODUCTION

Introduction of substituents to the starch granule has been widely used to change the physical properties of starch for desirable functional properties such as solubility, texture, adhesion, dispersion, and heat tolerance, because the lack of pasting consistency and stability limits the use of native starch in foods and other fields including textiles, cosmetics, pharmaceuticals, construction and, paints industries (1). These changes for native starch depend on the types and the degree of substitution (DS) (1). In the modification of starches, hydrophilic substituents including acetyl, hydroxypropyl, and phosphate

groups usually cause reduced gelatinization temperature and increased pasting viscosity (2, 3). Especially, the starches modified by dicarboxylic acid anhydride, containing both hydrophilic and hydrophobic groups, are well-known to improve emulsification properties and can also be used as the emulsifier for encapsulation after hydrolysis in food systems.

Among all modifications with dicarboxylic acid anhydride, only starch modified with octenyl succinic anhydride (OSA) is permitted for food applications and the maximum addition level of OSA allowed is 3%. Unlike typical surfactants, starch alkenyl ester contains both hydrophobic and hydrophilic groups (4). The octenyl succinate is attached to the starch through an ester linkage, which makes OSA-modified starch form strong films at the oil–water interface, giving emulsions that are resistant to reagglomeration. Because the glucose moiety or residual of starch binds to water while the lipophilic, octenyl part binds to oil, complete separation of the oil and water phases is prevented (5), which has contributed to the extensive application of OSA-modified starch in food additives, clouding agents, salad dressings, creams, fragrances, emulsion paints, lattices, coatings,

\* Corresponding author. Tel.: +86-571-88320379; Fax: +86-571-88320630. E-mail addresses: microliu@gmail.com, yin.li@ndsu.edu, zhengyg@zjut.edu.cn.

<sup>†</sup> Zhejiang University of Technology.

<sup>‡</sup> Polytechnic Institute of New York University.

<sup>§</sup> North Dakota State University.

<sup>||</sup> Jiangsu University.

<sup>⊥</sup> Zhejiang Academy of Agricultural Sciences.

<sup>#</sup> Monash University.

and adhesives (5–7), as well as biodegradable plastics (8). OSA-modified starch may also serve as a carbohydrate source in a medical nutritional product developed for people suffering from diabetes (9).

Previous studies have indicated that the DS value is correlated with application properties of OSA-modified starch (4). However, there are few reports available on the variation in the optimization of preparing conditions and physical properties of the OSA-modified waxy corn starch except the original paper indicating that alkenyl succinates of waxy corn starch have higher viscosity in aqueous suspension, lower gelatinization temperature, and less tendency to retrograde than unmodified starch (5). Moreover, investigation of the physical properties of OSA-modified waxy corn starch may lead to improved quality and broadened applications in the food industry.

The objective of this study is to develop an optimized process for the synthesis of OSA-modified waxy corn starch using response surface methodology (RSM) and for evaluating DS. In terms of independent variables, four process parameters were starch concentration, pH, temperature, and time course while DS was chosen as response value (dependent variable). In order to improve the application properties, the OSA-modified waxy corn starch was treated by  $\alpha$ -amylase. Furthermore, the detailed characteristics of modified starch were also intensively investigated, and emulsification capacity of enzyme treated OSA-modified starch was evaluated.

## MATERIALS AND METHODS

**Materials.** Waxy corn starch was purchased from Dacheng Chemicals Company (Changchun, China). Octenyl succinic anhydride (OSA) was purchased from Sigma-Aldrich Chemicals (St. Louis, MO).  $\alpha$ -Amylase was purchased from Genencor Co. Ltd. (Wuxi, China). Refined olive oil with 0.918 g/mL of density was purchased from the local market. Other chemicals and reagents used were of analytical grade and commercially available.

**Methods.** *Preparation of OSA Modified Waxy Corn Starches.* Two hundred and fifty grams of waxy corn starch was added into distilled water in a glass reactor with agitation. The starch concentration was kept in a range from 25% to 50%. pH of the starch slurry was adjusted by 2% NaOH solution. To this mixture, 3.0% OSA (% starch) was added dropwise for 2 h. During the addition, pH and temperature were maintained at set points. The reaction was carried out for 6–24 h. At the end of reaction, the pH of the starch slurry was adjusted to 6.5 using 2% HCl. The resulting OSA-modified waxy corn starch was washed three times with water, filtered, and then dried in a vacuum oven at 45 °C. After drying, the samples were ground to 0.25 mm and kept in an airtight container for further analysis.

*Optimization of DS Values for OSA-Modified Waxy Corn Starch Production by Box–Behnken Experimental Design.* Response surface methodology (RSM) was used to achieve the maximum DS value for optimization of the production process of OSA-modified waxy corn starches. In this study, the Box–Behnken experimental design with four factors, including starch concentration, temperature, pH, and reaction time, was used to fit a second-order response surface model (10). This methodology allows the modeling of a second-order equation that describes the process. DS values were analyzed by multiple regression through the least-squares method to fit the following equation:

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j \quad (1)$$

where  $Y$  is the predicted response variable (DS value);  $A_0$ ,  $A_i$ ,  $A_{ii}$ ,  $A_{ij}$  are constant regression coefficients of the model, and  $X_i$ ,  $X_j$  ( $i = 1, 4$ ;  $j = 1, 4$ ,  $i \neq j$ ) represent the four independent variables (starch concentration, temperature, pH, and reaction time). The accuracy and general ability of the above polynomial model could be evaluated by the coefficient of determination  $R^2$ .

*Determination of DS of OSA-Modified Waxy Corn Starches.* The DS is the average number of hydroxyl groups substituted per glucose

unit. It was determined by alkali saponification followed by back-titration of excess alkali. Octenyl succinylation level of the modified starch was determined using the titrimetric method of Gracza (11). Twenty-five milliliters of a 0.5 M NaOH was added to the suspension of the OSA-modified waxy corn starch (5 g of starch in 50 mL distilled water) and then shaken for 24 h. Excess of alkali was titrated with 0.5 M HCl, using phenolphthalein as an indicator. A blank was simultaneously titrated with native unmodified starch. DS was determined from % OSA substitution. The equations are as follows:

$$\text{OSA substitution (\%)} = (V_{\text{blank}} - V_{\text{sample}}) \times 0.1 \times M \times 100/W \quad (2)$$

where  $V_{\text{blank}}$ , volume of HCl required for blank titration;  $V_{\text{sample}}$ , volume of HCl required for sample titration;  $W$ , weight of sample taken (g);  $M$ , molarity of HCl solution.

$$\text{DS} = 162 \times \text{OSA substitution\%} / (21000 - 209 \times \text{substitution\%}) \quad (3)$$

where 162 is the molecular weight of glucose unit, 21 000 is 100  $\times$  molecular weight of octenyl succinyl group, and 209 is the molecular weight of octenyl succinyl group minus molecular weight of hydrogen atom.

*Determination of Free OSA.* The free OSA was extracted as described by Park and Goins (12). OSA-modified waxy corn starch (4.2% w/v) was prepared by stirring at 60 °C for 4 h. A 2.5 mL aliquot was transferred into a 15 mL conical centrifuge tube to which 2.5 mL of methanol was added to precipitate starch. After centrifugation at 2500 rpm for 10 min, the supernatant was transferred into a test tube. After the addition of pentadecanoic acid (0.15 mg) as the internal standard, the pH of the supernatant was adjusted to pH 2 with 150  $\mu$ L of 1 M HCl. The supernatant was extracted with 5 mL of ethyl ether and then with 2.5 mL of ethyl ether twice. The extracts were combined, dried over anhydrous sodium sulfate, and freed of solvent under a nitrogen stream. The dried extract was redissolved into 1 mL of methanol (12). A 50  $\mu$ L aliquot was mixed with 100  $\mu$ L of methanol, and 5  $\mu$ L was injected into a HPLC, free OSA was quantified on a  $\mu$ -Bondapack C<sub>18</sub> column (10  $\mu$ m, 4.6 mm  $\times$  250 mm, Millipore-Waters, Bedford, MA) with detection at 220 nm by a mobile phase of methanol: water (85:15, v/v) at 1 mL/min. The retention time for free OSA was about 5.2 min.

**Enzymatic Treatment of Modified Starch.** OSA-modified waxy corn starch (50 g, dry weight) was mixed with water to a 35% suspension by weight containing 1% of CaCl<sub>2</sub>. Then  $\alpha$ -amylase at 20 U per gram starch was added into the mixture. The suspension was heated in a 1 L beaker at 80 °C for different time with stirring. Then the sample was incubated at 100 °C for 20 min and transferred to a 55 °C water bath to get different dextrose equivalent (DE) values. Sodium acetate buffer (360 mL, 0.5 M, pH 5.0) was added and the mixture stirred for 10 min. Water was added to bring the total volume of the suspension to 1800 mL. The suspension was then carried out spray drying using a laboratory-scale unit (Shanghai, China) at a flow rate of 3 mL/min and inlet and outlet temperatures of 120 and 75 °C, respectively. The products were stored in the sealed bottles for further analysis. The extent of conversion is quantified by DE, and the release of reducing sugars was determined using the dinitrosalicylic acid method (13). The solid content in the supernatant was determined by Abbe-refractor (Shanghai, China). The values of DE were calculated by the following equation:

$$\text{DE} = \frac{\text{amount of reducing sugars (mg/g)}}{\text{content of solid in the supernatant (mg/g)}} \times 100\% \quad (4)$$

**Scanning Electron Microscopy (SEM).** Intact or residual starch granules were mounted on a specimen holder with carbon cement and coated with gold. The samples were then examined by a Cambridge S360 scanning electron microscope (Cambridge Scientific Instruments Ltd., Ely, Cambs, UK).

**Infrared Spectrometry.** A quantity of the sample with a specially purified KBr was finely ground to remove scattering effects from large

crystals. This powder mixture was then crushed in a mechanical die press to form a translucent pellet, through which the beam of the spectrometer can pass. IR spectra were obtained by using Bio-Rad FST IR spectrometer (BioRad Laboratories, Hercules, CA).

**Pasting Properties.** Pasting properties of starches were measured at 6% dry solid by Brabender viscometric analysis according to Approved Method 61-01 (AACC, 2000), which measures the change in viscosity of the starch as it is heated and then cooled under constant stirring. The viscosity measurements were carried out using a Brabender Viskograph E (Brabender OHG, Duisberg, Germany) equipped with a 700-cmg cartridge and operated at a speed of 125 rpm. The heating profile is as follows: from 30 to 90 °C at a rate of 1.5 °C/min; heating at 90 °C for 30 min; cooling from 90 to 50 °C at a rate of 1.5 °C/min; holding at 50 °C for 30 min; cooling from 50 to 30 °C at a same rate. A plot of the paste viscosity in arbitrary Brabender units (BU) was used to determine the pasting temperature at maximal (peak) viscosity and viscosity at the end of cooling.

**Application of Enzyme-Treated Modified Starch.** Enzymatic OSA-modified waxy corn starch and maltodextrins were dissolved into water at pH 8.0 and thoroughly mixed at 90 °C, and the mixture was referred to as the water phase. Refined olive oil and emulsion were added into a 500 mL flask and incubated at 65 °C, and the mixture was referred to as the oil phase. The water phase and oil phase were efficiently mixed by a high-speed emulsifier (Shanghai, China). The emulsified mixture was homogenized twice at 70 °C, 25 MPa, and then the solution was spray-dried for further use.

**Determination of Oil on the Surface of Capsules.** A 2 g sample (W) was dissolved into 20 mL methanol in a 50 mL beaker, mixed gently for 1 min, and then transferred to a ceramic funnel. The solution was filtered into an Erlenmeyer flask weighed previously as B, the original beaker was washed using 20 mL of ether, and the entire solution was transferred to the ceramic funnel, mixed gently for 40 s, and refiltered to the same Erlenmeyer flask. Then the Erlenmeyer flask containing the total filtrate was incubated at 65 °C to evaporate the filtrate. After evaporation, the Erlenmeyer flask was cooled to room temperature until no weight change was detected for periods longer than 1 h, and then the weight of the Erlenmeyer flask and its content was measured as C. The percent oil on the surface of the capsules was calculated by using the following equation:

$$\text{oil on the surface of capsules (\%)} = \frac{C - B}{W} \times 100\% \quad (5)$$

**Determination of Total Oil.** A 5 g sample was put into a bag made by weighted filter paper, immersed in petroleum ether for 1.5 h, and extracted by a solvent extraction system (Shanghai, China) at 80 °C for 3 h. The total oil content was determined, and the encapsulation efficiency and encapsulation productivity were calculated according to the contents of oil outside and inside of the capsules.

**Determination of Emulsifying Power and Emulsification Stability.** A 6 g sample of tested OSA-modified waxy corn starch and 56 mL of maltodextrins were dissolved into 438 mL of water and then heated to 50 °C. The mixture was emulsified at 10 000 rpm for 2 min. The emulsified solution was diluted 200 times by water, and then the optical density (OD) was determined by a spectrophotometer at 720 nm. OD was defined as the emulsifying power. The mixture was then transferred to a graduated centrifuge tube, and centrifuged at 1,000 rpm for 10 min.

The emulsion stability test was performed by dispersing 5 g of modified starch in 100 mL of a 1:1 mixture of water and refined olive oil, followed by heating at 75 °C for 30 min. The mixture was then transferred to a graduated cylinder, and the swollen volume was measured after 24 h. The stability of emulsification was calculated according to the rate of oil and water after centrifugation. The mixture of water and oil without tested samples was used as blank control.

**Statistical Analysis.** All measurements in this study were performed in triplicate if not specifically noted. Analysis of variance (ANOVA) was performed with the SAS program version 8.1 (SAS Institute Inc., Cary, NC). Least significant differences (LSD) for comparison of means were computed at  $P < 0.05$ .

**Table 1.** Maximum and Minimum Levels of Variables Used in the Central Composite Design

| variable                    | level |    |    |
|-----------------------------|-------|----|----|
|                             | −1    | 0  | 1  |
| starch concn (%) ( $X_1$ )  | 25    | 30 | 35 |
| temp (°C) ( $X_2$ )         | 30    | 35 | 40 |
| pH ( $X_3$ )                | 7     | 8  | 9  |
| reaction time (h) ( $X_4$ ) | 12    | 18 | 24 |

**Table 2.** Box–Behnken Experimental Design Matrix with Experimental DS Values

| trial | $X_1$ (starch concn), % | $X_2$ (temp) | $X_3$ (pH) | $X_4$ (time, h) | Y (DS) |
|-------|-------------------------|--------------|------------|-----------------|--------|
| 1     | −1                      | −1           | 0          | 0               | 0.0076 |
| 2     | −1                      | 1            | 0          | 0               | 0.0089 |
| 3     | 1                       | −1           | 0          | 0               | 0.0135 |
| 4     | 1                       | 1            | 0          | 0               | 0.0128 |
| 5     | 0                       | 0            | −1         | −1              | 0.0118 |
| 6     | 0                       | 0            | −1         | 1               | 0.0115 |
| 7     | 0                       | 0            | 1          | −1              | 0.0169 |
| 8     | 0                       | 0            | 1          | 1               | 0.0182 |
| 9     | −1                      | 0            | 0          | −1              | 0.0072 |
| 10    | −1                      | 0            | 0          | 1               | 0.0082 |
| 11    | 1                       | 0            | 0          | −1              | 0.0117 |
| 12    | 1                       | 0            | 0          | 1               | 0.0125 |
| 13    | 0                       | −1           | −1         | 0               | 0.0108 |
| 14    | 0                       | −1           | 1          | 0               | 0.0185 |
| 15    | 0                       | 1            | −1         | 0               | 0.0142 |
| 16    | 0                       | 1            | 1          | 0               | 0.0135 |
| 17    | −1                      | 0            | −1         | 0               | 0.0094 |
| 18    | −1                      | 0            | 1          | 0               | 0.0084 |
| 19    | 1                       | 0            | −1         | 0               | 0.0106 |
| 20    | 1                       | 0            | 1          | 0               | 0.0145 |
| 21    | 0                       | −1           | 0          | −1              | 0.0129 |
| 22    | 0                       | −1           | 0          | 1               | 0.0147 |
| 23    | 0                       | 1            | 0          | −1              | 0.0119 |
| 24    | 0                       | 1            | 0          | 1               | 0.0141 |
| 25    | 0                       | 0            | 0          | 0               | 0.0196 |
| 26    | 0                       | 0            | 0          | 0               | 0.0195 |
| 27    | 0                       | 0            | 0          | 0               | 0.0197 |

Regression analysis of the data and estimation of the coefficients of the regression equation were conducted by The Design-Expert software package (Version 7.0.2, 2006; Stat-Ease, Minneapolis, MN) (14). The statistical significance of the model was determined by the application of Fisher's *F*-test. The canonical analysis was carried out to predict the shape of the curve generated by the model and estimate the optimal condition. The two-dimensional response surface was used to describe the individual and cumulative effects of the variables as well as the mutual interactions between the independent and dependent variables.

## RESULTS

**Response Surface Model for Optimization of OSA-Modified Waxy Corn Starches Production.** Table 1 shows the coded levels and real values of four variables chosen for trials in the Box–Behnken experimental design. Four variables used for the factorial analysis, including starch concentration, temperature, pH, and reaction time, were designated as  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$ , respectively. In Table 2, 27 experimental runs with different combinations of four factors were carried out to optimize the DS values for the OSA-modified waxy corn starch production. In terms of the DS value, there was a considerable variation in the current study. The minimum DS value of 0.0072 was observed in run 9 under the conditions of 25% starch concentration, 35 °C, pH 8, and 12-h reaction, while the maximum DS value (0.0197) was achieved in run 27 (30% starch concentration, 35 °C, pH 8, and 18-h reaction).



**Table 3.** Analysis of the Variance (ANOVA) for the Response of Xylanase Production<sup>a</sup>

| source        | degree of freedom | sum of squares | F-value | P > F  |
|---------------|-------------------|----------------|---------|--------|
| linear        | 4                 | 0.0000996      | 20.53   | <0.001 |
| quadratic     | 4                 | 0.000226       | 46.63   | <0.001 |
| cross-product | 6                 | 0.0000253      | 3.48    | 0.0313 |
| total model   | 14                | 0.000351       | 20.68   | <0.001 |
| total error   | 12                | 0.0000145      | —       | —      |

<sup>a</sup> Coefficient of variation (CV) = 8.42; determination coefficient ( $R^2$ ) = 0.96.**Table 4.** Least-Square Fit and Parameters (Significance of Regression Coefficient)

| model term | degree of freedom | estimate  | standard error | t value | P > t                |
|------------|-------------------|-----------|----------------|---------|----------------------|
| intercept  | 1                 | 0.0196    | 0.000636       | 30.83   | <0.0001 <sup>a</sup> |
| $X_1$      | 1                 | 0.00216   | 0.000318       | 6.79    | <0.0001 <sup>a</sup> |
| $X_2$      | 1                 | -0.000217 | 0.000318       | -0.68   | 0.5084               |
| $X_3$      | 1                 | 0.00181   | 0.000318       | 5.69    | 0.0001 <sup>a</sup>  |
| $X_4$      | 1                 | 0.000567  | 0.000318       | 1.78    | 0.0999               |
| $X_1^2$    | 1                 | -0.0064   | 0.000477       | -13.42  | <0.0001 <sup>a</sup> |
| $X_1X_2$   | 1                 | -0.0005   | 0.000551       | -0.91   | 0.3817               |
| $X_2^2$    | 1                 | -0.00289  | 0.000477       | -6.06   | <0.0001 <sup>a</sup> |
| $X_1X_3$   | 1                 | 0.00123   | 0.000551       | 2.23    | 0.0460 <sup>a</sup>  |
| $X_2X_3$   | 1                 | -0.0021   | 0.000551       | -3.81   | 0.0025 <sup>a</sup>  |
| $X_3^2$    | 1                 | -0.00228  | 0.000477       | -4.77   | 0.0005 <sup>a</sup>  |
| $X_1X_4$   | 1                 | -0.00005  | 0.000551       | -0.09   | 0.9291               |
| $X_2X_4$   | 1                 | 0.0001    | 0.000551       | 0.18    | 0.8589               |
| $X_3X_4$   | 1                 | 0.0004    | 0.000551       | 0.73    | 0.4814               |
| $X_4^2$    | 1                 | -0.00311  | 0.000477       | -6.53   | <0.0001 <sup>a</sup> |

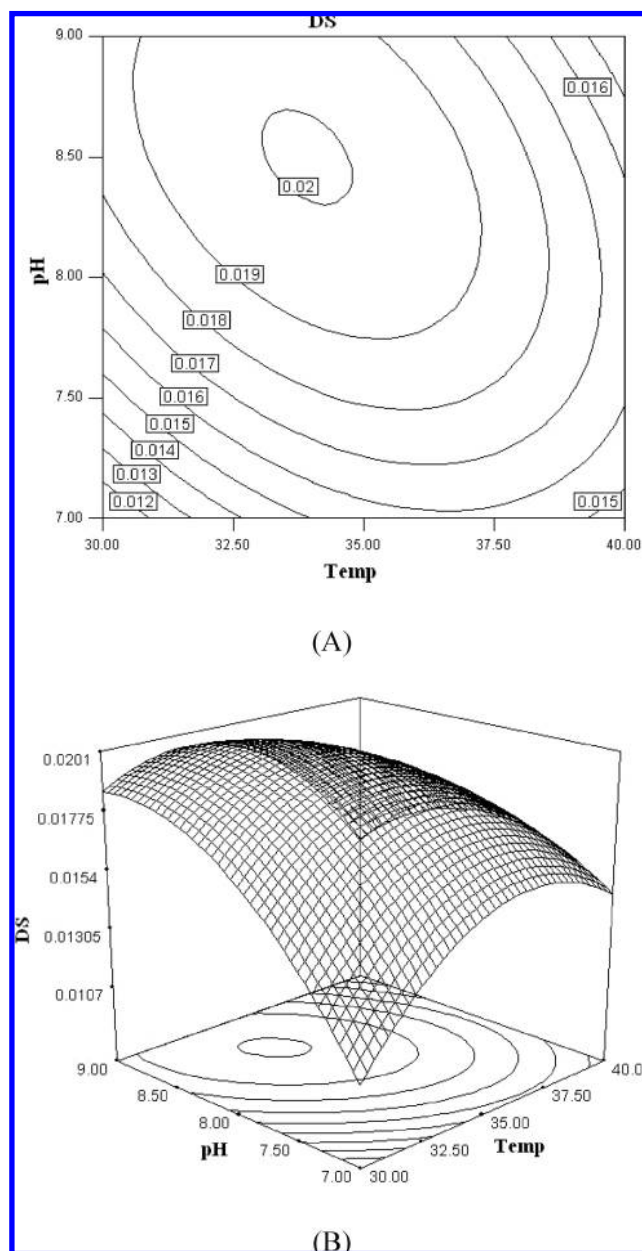
<sup>a</sup> Significant at 5% level ( $P < 0.05$ ).

Fisher's statistical test for the analysis of variance (ANOVA) was performed to test the accuracy of the model, and the results are presented in **Table 3**. The fitness of the model can be validated by the determination coefficient ( $R^2$ ) and correlation coefficient ( $R$ ). In the current study, the  $R^2$  was 0.96, which indicated the sample variation of 96% for DS was attributed to the independent variables, and only about 4% of the total variation cannot be explained by the model. A relative low coefficient of variation (CV) (8.42) in this study indicated better precision and reliability of the experiments. Linear, quadratic, and cross-product terms were all significant at  $P \leq 0.05$  level, showing that the second-order model will be employed in this optimization process. **Table 4** shows the Student  $t$ -distribution and the corresponding  $P$ -values, along with the parameter estimates. As to the first order, only starch concentration and pH value were found significant to the model. Positive coefficients for starch concentration and pH value indicated that DS value generally increased with an increase in the value of the variables. All the second orders of the four variables were significant to the model. The interactions between starch concentration and pH, and temperature and pH were found to be significant to the model. By applying multiple regression analysis on the experimental data, the following second-order polynomial equation was found to best explain the OSA-modified waxy corn starch production:

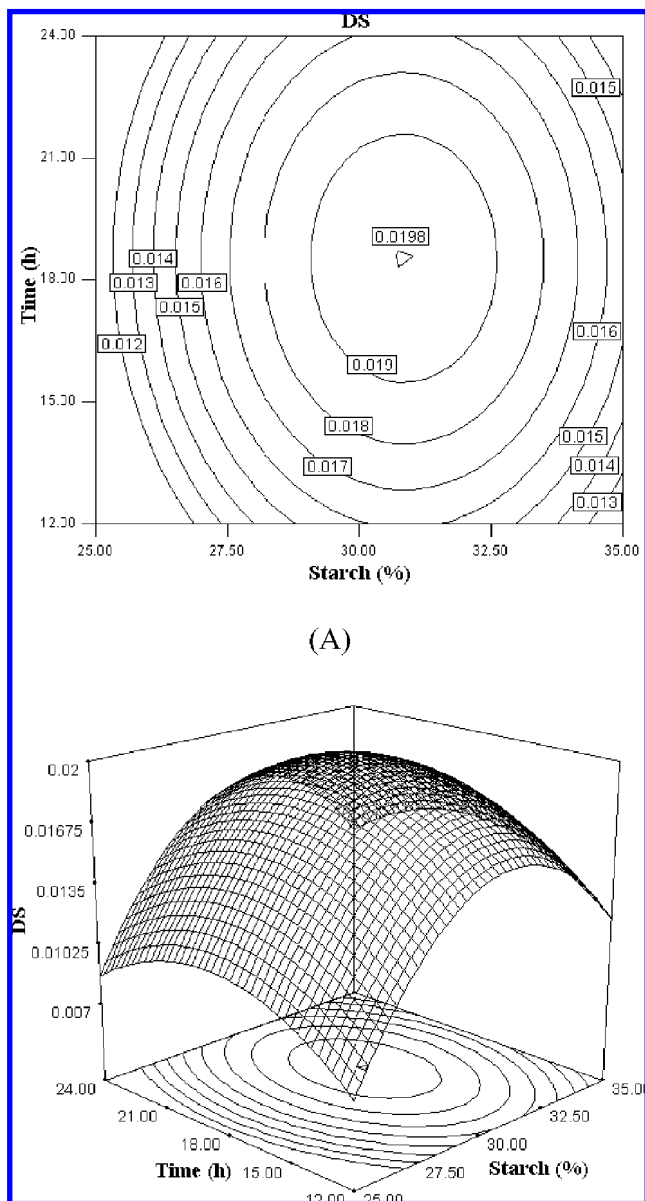
$$\begin{aligned} \text{DS} = & 0.0196 + 0.00216X_1 + 0.00181X_3 + \\ & 0.00123X_1X_3 - 0.0021X_2X_3 - 0.0064X_1^2 - \\ & 0.00289X_2^2 - 0.00228X_3^2 - 0.00311X_4^2 \quad (6) \end{aligned}$$

where  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  are the coded values of starch concentration, temperature, pH, and reaction time, respectively.

**Figure 1** depicts the contour plot (A) and its corresponding 3D plot (B), showing the effects of temperature and pH on the

**Figure 1.** Response surface plot and contour plot of the combined effects of temperature and pH on the DS values for the OSA-modified waxy corn starch production.

DS value, while the starch concentration and reaction time were fixed at their middle levels (30% and 18 h, respectively). **Figure 1A** indicates that the DS value gradually increased from the temperature of 30 °C to approximately 34 °C and then decreased with increase in temperature. It holds the same situation with pH. The DS response value generally increased when the pH was raised from 7 to 8.6 and then fell as the pH went above 8.6. From the analysis by Design-Expert software, it was suggested that the optimal temperature was 33–34 °C and the optimal pH was 8.4–8.6 for the production of OSA-modified waxy corn starch. **Figure 2** presents the contour plot (A) and its corresponding 3D plot (B) showing the effects of starch concentration and reaction time on DS value with the temperature and pH fixed at their middle levels (35 °C and 8, respectively). The analysis of **Figure 2** shows that the optimal ranges of starch concentration and reaction time for the DS value were 31.0% to 31.5% and 18 to 20 h, respectively, and the maximum predicted value was obtained at about 0.0200.



**Figure 2.** Response surface plot and contour plot of the combined effects of starch concentration and time on the DS values for the OSA-modified waxy corn starch production.

The canonical analysis was further used to predict the optimal conditions for the OSA-modified waxy corn starch production. The stationary point presenting a maximum DS value had the following critical values: starch concentration = 31.2%, temperature = 33.6 °C, pH = 8.6, reaction time = 18.7 h. The predicted DS value for these conditions was 0.0204. After obtaining the optimal reaction conditions using RSM, we tested the feasibility of scale-up using a 5-m<sup>3</sup> reactor. At this scale, the reaction conditions (starch concentration = 31.5%, pH = 8.6, temperature = 34 °C and reaction time = 19 h) that were set up based on the optimized conditions produced a mean DS of 0.0208.

**Hydrolysis of OSA.** Acid hydrolysis of starch has been widely used in the past. It is now largely replaced by enzymatic processes, as acid hydrolysis in the starch process required the use of corrosion resistant materials, gave rise to high color and saltash content (after neutralization), needed more energy for heating, and was relatively difficult to control. So  $\alpha$ -amylase was used in this study for hydrolysis of modified starch. After gelatinization, liquefaction, and saccharification, the tested

modified starch is readily liquefied by partial hydrolysis with enzymes and saccharified by further enzymatic hydrolysis. The level of starch hydrolysis is monitored by DE value that indicates the extent to which the starch has been cleaved. In this study, three hydrolyses of OSA-modified waxy corn starches with different DE values were achieved by controlling the hydrolysis time (11, 15, 16). After hydrolysis, the hydrolyzed samples were spray-dried for further use.

**Analysis of the Residual of OSA in Modified Starch.** The residual of OSA in modified starch was analyzed by HPLC, and the retention time for OSA is about 5.20 min. The residual of OSA was calculated according to the equation about peak area and content. The results showed that the residual of OSA in modified starch prepared in this study is about 0.1%, which is lower than the value set by the U.S. Food and Drug Administration (FDA). The result indicates that the modified starch prepared based on the optimized conditions in this study could be applied into food making process.

**Scanning Electron Microscopies.** The results of the scanning electron microscopy for native and modified waxy corn starch showed that the native starch granules were oval in shape with sizes around 50 nm (Figure 3). The OSA-modified waxy corn starch did not show any morphological changes with respect to the native starch granules, indicating that the condition for octenyl succinylation did not cause any detectable change in the morphological characteristics. These results are in good agreement with the previous reports (4, 16).

**Infrared Spectrum Analysis of Octenyl Succinyl Groups.** The introduction of octenyl groups could be confirmed by FST IR spectroscopy. The detailed infrared spectrums for native and modified waxy corn starch are shown in Figure 4. Compared with the infrared spectrum of native starch, there is an apparent stretch peak for ester carbonyl groups at 1730 cm<sup>-1</sup>, which suggested that OSA was successfully introduced into native starch and combined with ester bonds. In this study, the reactions were carried out in the alkaline system with Na<sup>+</sup>; thus, the OSA-modified waxy corn starches were synthesized in the form of starch sodium octenyl succinate derivatives. The peak occurred at 1570 cm<sup>-1</sup> related to the asymmetric stretch of vibration of carboxylate RCOO<sup>-</sup> group. Except 1570 cm<sup>-1</sup> and 1730 cm<sup>-1</sup>, the infrared spectrums for both native starch and modified starch are similar. From the analysis of the spectra, we deduced that after modification, the octenyl succinylation reaction happened and no other substance was introduced into OSA-modified waxy corn starch.

**Pasting Properties.** The starch pasting properties measured with a Brabender viscograph are presented in Figure 5. The differences in the pasting properties among the starches tested in this study were distinct. It is speculated that the introduction of groups of OSA increases paste viscosities, and the temperatures that cause maximum paste viscosities are lower compared to those with native starch. However, after treatment with enzyme, the paste viscosities of modified starch gradually decreased. Moreover, the enzymatically modified starches showed a similar pasting temperature but lower hot and cool paste viscosities, whereas the modified starch without treatment exhibited a higher pasting temperature, less breakdown, and significantly higher hot and cool paste viscosities compared with the enzymatically modified starch (Table 5). This can be explained by the breakdown of  $\alpha$ -1,4 glucan bonds that not only reduced the molecules size and the molecular weight but also resulted in a lower position hinder and different relationship between the molecules compared with the starch before treat-

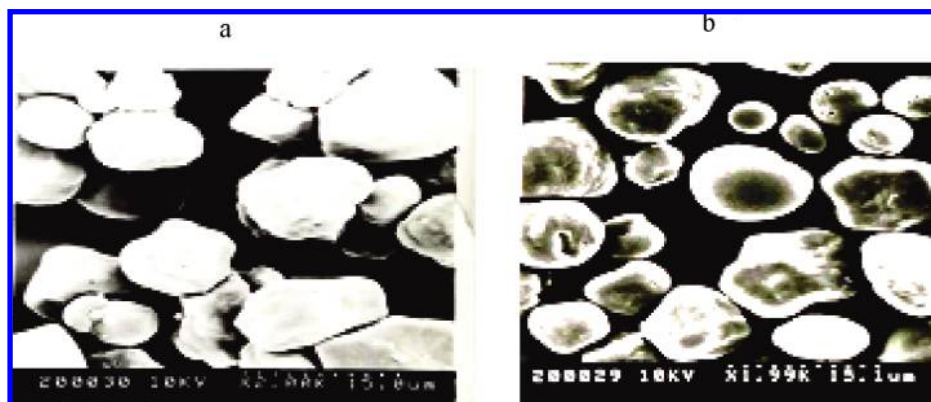


Figure 3. Scanning electron micrographs of waxy corn starch and modified starch granules: a, native starch; b, OSA-modified starch.

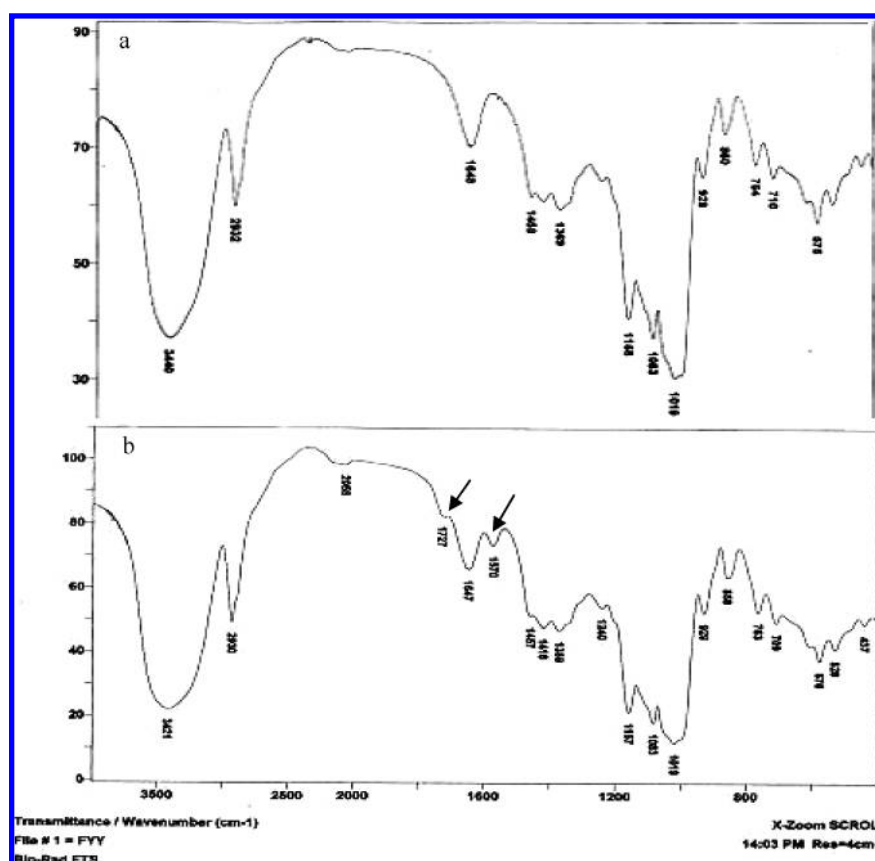


Figure 4. IR spectrum of native waxy corn starch (a) and OSA-modified starch (b).

ment. However, there is no significant difference found in pasting properties of these three enzyme-treated modified starches with different DE values.

**Emulsification Capacity and Oil Absorption Capacity.** In this test, the addition of the starch product can emulsify the water/oil mixture and result in complete mixing of the two immiscible liquids. The water/oil/starch dispersion was white in color and had a creamy appearance. The mixture was allowed to sit at room temperature for a period of at least 20 days, and no appreciable amount of water or oil was separated during this period.

Emulsification capacity and oil absorption capacity of OSA-modified waxy corn starches and different enzyme-treated OSA-modified waxy corn starches were determined. At the same time, the native starch was used as the control. When the enzyme-treated OSA-modified waxy corn starches were used as the reagent for emulsification, the ability of the materials to encapsulate the oil was accordingly improved, resulting in the

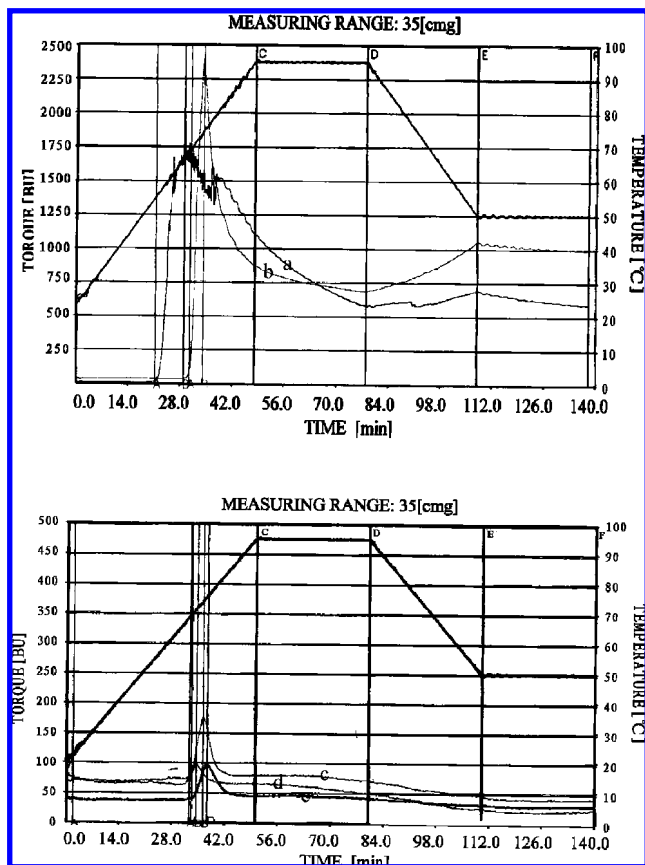
improvement of the rate and productivity of encapsulation.

**Table 6** shows the results for emulsification capacity and oil absorption capacity. The enzyme-treated OSA-modified waxy corn starches with DE 15 have the highest emulsification capacity and oil absorption capacity, as compared to other tested modified starches. The native starches did not show any emulsification capacity, and oil separated immediately after addition. Moreover, the encapsulation efficiency and encapsulation productivity of enzyme-treated OSA-modified waxy corn starches with DE 15 could reach 80.5% and 91.3%, respectively.

## DISCUSSION

**Starch Selection for OSA Modification Starch.** It is well understood that starch is composed of two distinct types of macromolecules: amylose and amylopectin with molecular weights of  $10^4$  to  $10^6$  and  $10^7$  to  $10^8$ , respectively. Amylose, a polymer of  $\alpha$ -D-glucopyranosyl units mainly combined by  $\alpha$ -1,4





**Figure 5.** Brabender viscoamylograph of samples: (a) waxy corn starch, (b) OSA-modified waxy corn starch, (c) DE = 10, (d) DE = 15, (e) DE = 20.

**Table 5.** Paste Properties of Samples with Different Treatments<sup>a</sup>

| sample           | A<br>(BU) | B<br>(BU) | C<br>(BU) | D<br>(BU) | E<br>(BU) | F<br>(BU) | B–D<br>(BU) |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|-------------|
| waxy corn starch | 17        | 480       | 175       | 137       | 210       | 199       | 343         |
| OSA-starch       | 17        | 2031      | 1113      | 579       | 693       | 590       | 1452        |
| DE = 10          | 81        | 193       | 87        | 80        | 59        | 56        | 113         |
| DE = 15          | 76        | 176       | 80        | 71        | 48        | 44        | 105         |
| DE = 20          | 73        | 97        | 65        | 50        | 27        | 26        | 47          |

<sup>a</sup> A, viscosity at onset temperature. B, viscosity at peak temperature. C, viscosity at 95 °C. D, viscosity after 30 min at 90 °C. E, viscosity at 50 °C. F, viscosity after 30 min at 50 °C. B–D, viscosity at setback temperature.

**Table 6.** Comparison of the Oil Absorption Capacity and Emulsification Capacity

| sample     | oil absorption capacity<br>(g of oil/g of sample) | emulsification<br>capacity (g) |
|------------|---|--------------------------------|
| blank      | 0   | 0                              |
| OSA-starch | 1.04  | 60.14                          |
| DE 10      | 1.36  | 80.62                          |
| DE 15      | 1.44  | 94.32                          |
| DE 20      | 1.38  | 86.72                          |

linkages, is defined as essentially a linear molecular chain. Amylopectin is a very large branched polymer consisting of linear  $\alpha$ -1,4-linked D-glucopyranosyl chains connected by  $\alpha$ -1,6 branch linkages, the latter comprising 4.0–5.5% of the total linkages (15). Waxy corn starch granules, composed of 99% amylopectin, were found to be composed of crystalline nanoplatelets around 5–7 nm thick with a length of 20–40 nm and a width of 15–30 nm (17). Because of its different content of amylopectin compared to conventional corn starch, waxy corn

starch is widely used as a stabilizer and thickener for food products, particularly those that undergo large temperature changes in processing and preparation. It is also used as an emulsifier for salad dressings. Other uses include remoistening adhesives in the manufacture of gummed tape, in adhesives, and in the paper industry. The use of waxy starch is gradually growing, especially because of increased demand for prepared foods (18–21). However, native waxy corn starch fails to meet the requirements in its application. Because of this reason, the properties of the starches need to be improved or modified, for example, by introduction of different groups with different functions by chemical or physical methods (22). Most of the earlier work on modification of starches by OSA was done with waxy corn starch, although starches from wheat, rice, and potato have been also similarly studied recently (23, 24). Therefore, waxy corn starch was used to carry out modification reactions in this study.

**Optimization of the DS Value for OSA-Modified Starch Production.** The production of OSA-modified waxy corn starch was influenced by reaction condition factors, such as pH, temperature, starch concentration, and reaction time, and especially by the interaction of these factors (16, 25). In this study, RSM was employed to optimize the conditions for preparing OSA-modified waxy corn starch. Response surface methodology (RSM), which has been extensively applied in the optimization of food manufacturing processes (26), is a collection of statistical techniques for experimental design, model development, factor evaluation, and optimum conditions searching. RSM could overcome the shortcomings of the classical or empirical methods such as the “one-factor-at-a-time-technique”, which is time-consuming process and incapable of searching for the global optimal condition especially when interactions between independent factors existed (27, 28). After optimization, it is found that the interactions between starch concentration and pH, and temperature and pH, were significant to the DS values for the production of OSA-modified starch. According to the optimized conditions, the reaction was upscaled and OSA-modified waxy corn starch with higher DS value (0.0208) was successfully obtained.

**Characteristics of OSA-Modified Starch.** On the basis of the esterification of starch with OSA under mild alkaline conditions, Cadwell and Wurzburg successfully modified starches with OSA, and the characteristics of modified starch were studied as compared to unmodified starch (29). Some recent work was also reported about the distribution of OSA within the modified starch granule and the effect of extent of modification on different physicochemical properties of starch (16, 30).

The physical properties of chemically modified starches are greatly affected by the properties of introduced groups and DS. Hydrophilic substitution such as acetyl, hydroxypropyl, and phosphate groups usually increases the gelatinization temperature and viscosity, but the magnitude of the change depends partially on the biological source of the parent starches. Hydrophobic substituents such as benzyl, allyl, and menthyl often result in more complicated changes (31). The incorporation of a bulky OSA grouping to hydrophilic starch molecules confers surface-active properties to the modified starch (32). As a result, OSA-modified waxy corn starch exhibits improved mixing characteristics and stability of elemental or natural emulsifying properties of oil (33).

In this study, the modified starches exhibit remarkable properties at significant levels greatly in excess of unmodified waxy corn starch. In addition, the starch products readily disperse in oil/water mixtures without extensive agitation to form

essentially homogeneous and stable emulsions which again resist separation for considerable periods at room temperature. These properties, along with the increased surface areas and internal void structure of the starch, render them highly suitable for use as thickening, stabilizing, and/or suspending agents, or as vectors for the delivery of biologically active ingredients. Moreover, dried powders of these starch products have a smooth, silky feel, making them highly desirable for use in diverse products such as cosmetics, pharmaceuticals, and deodorant sprays. For its excellent properties, hydrolyzed OSA-modified waxy corn starches have been widely used as gum arabic substitutes for encapsulation of sensitive food ingredients. This kind of modified starch can be considered as a good candidate for food additives.

In conclusion, in order to produce OSA-modified waxy corn starch with a higher DS value in a given addition of OSA, the process for the production of OSA-modified starch was investigated in detail in this study. Under the optimized conditions, the modified starch with DS = 0.0208 was obtained in a 5 m<sup>3</sup> upscale reactor. The characteristics of the modified starches with different DE values treated by  $\alpha$ -amylase were studied. After enzyme treatment, the OSA-modified waxy corn starches showed an excellent emulsification capacity and stability. This work will lay the foundation for the future studies and applications of OSA-modified starches.

## LITERATURE CITED

- Rutenberg, M. W.; Solarek, D. Starch derivatives: Production and uses. In *Starch: Chemistry and technology*; Whistler, R. L., Ed.; Academic Press: New York, 1984; pp 312–388.
- Takahashi, S.; Maningat, C. C.; Seib, P. A. Acetylated and hydroxypropylated wheat starch. *Cereal Chem.* **1989**, *6*, 499–506.
- He, J.; Liu, J.; Zhang, G. Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat-moisture treatment: glycemic response and mechanism. *Biomacromolecules* **2008**, *9*, 175–84.
- Bhosale, R.; Singhal, R. Process optimization for the synthesis of octenyl succinyl derivative of waxy corn and amaranth starches. *Carbohydr. Polym.* **2006**, *66*, 521–527.
- Murphy, P. Starch. In *Handbook of hydrocolloids*; Phillips, G. O., Williams, P. A., Eds.; CRC Press: Boca Raton, FL, 2000; pp 41–65.
- Wurzburg, O. B. In *Food Polysaccharides and Their Applications*; Stephen, A. M., Ed.; Marcel Dekker, Inc.: New York, 1995; pp 67–97.
- Thomas, D. J.; Atwell, W. A. *Starches*; The American Association of Cereal Chemists, Inc.: St. Paul, MN, 1999.
- Jane, J. L.; Robert, R. J.; Nidolov, Z.; Roque, R. L. Degradable plastics from octenyl succinate starch. U.S. Patent 5,059,642, 1991.
- Wolf, B. W.; Wolever, T. M.; Bolognesi, C.; Zinker, B. A.; Garleb, K. A.; Firkins, J. L. Glycemic response to a food starch esterified by 1-octenyl succinic anhydride in humans. *J. Agric. Food Chem.* **2001**, *49*, 2674–2678.
- Li, Y.; Liu, Z. O.; Cui, F. J.; Xu, Y. Y.; Zhao, H. Application of statistical experimental design to optimize culture requirements of a novel *Aspergillus* sp. ZH-26 producing endoxylanase from agricultural waste material and evaluation of its performance on the degradation of arabinoxylans in mashing. *J. Food Sci.* **2007**, *72*, 320–329.
- Gracza, B. Minor constituents of starch. In *Starch: Chemistry and technology*; Whistler, R.; Paschall, E.; BeMiller, J.; Roberts, H., Eds.; Academic Press: New York, 1965; pp 105–107.
- Park, P. W.; Goins, R. E. Determination of 2-(2'-Octenyl)succinic Acid in Lipophilic Modified Starch by Gas Chromatography-Mass Spectrometry/Selected Ion Monitoring. *J. Agric. Food Chem.* **1995**, *43*, 2580–2584.
- Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428.
- Li, Y.; Liu, Z.; Cui, F.; Liu, Z.; Zhao, H. Application of Plackett-Burman experimental design and Doehlert design to evaluate nutritional requirements for xylanase production by *Alternaria mali* ND-16. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 285–291.
- Hizukuri, S. Starch: analytical aspects. In *Carbohydrates in Food*; Eliasson, A.-C., Ed.; Marcel Dekker, Inc.: New York, 1996; pp 347–429.
- Shorgen, R. L.; Vishwanathan, A.; Felker, F.; Gross, R. A. Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch. *Starch/Stärke* **2000**, *52*, 196–204.
- Putaux, J. L.; Molina-Boisseau, S.; Momauro, T.; Dufresne, A. Platelet nanocrystals resulting from the disruption of waxy maize starch granules by acid hydrolysis. *Biomacromolecules* **2003**, *4*, 1198–202.
- Billmers, R. L.; Mackewicz, V. L. Process for the preparation of hydrophobic starch derivatives. European Patent. EP 0761691, 1997.
- Eisses, J. F.; Lafoe, D.; Scott, L. A.; Weil, C. F. Novel, developmentally specific control of Ds transposition in maize. *Mol. Gen. Genet.* **1997**, *256*, 158–168.
- Kelder, J. D.; Ptasinski, K. J.; Kerkhof, P. J. Starch gelatinization in coiled heaters. *Biotechnol. Prog.* **2004**, *20*, 921–929.
- Najgebauer, D.; Grega, T.; Sady, M.; Tomasik, P. Polymeric complexes of cornstarch and waxy cornstarch phosphates with milk casein and their performance as biodegradable materials. *Molecules* **2004**, *9*, 550–567.
- Lu, Y.; Tighzert, L.; Dole, P.; Erre, D. Preparation and properties of starch thermoplastics modified with waterborne polyurethane from renewable resources. *Polymer* **2005**, *46*, 9863–9870.
- Tessler, M. M.; Billmers, R. L. Preparation of starch esters. *J. Environ. Polym. Degrad.* **1996**, *4*, 85–89.
- Shih, F. F.; Daigle, K. W. Gelatinization and pasting properties of rice starch modified with 2-octen-1-ylsuccinic anhydride. *Nahrung* **2003**, *47*, 64–67.
- Song, X. Y.; He, G. Q.; Ruan, H.; Chen, Q. H. Preparation and Properties of Octenyl Succinic Anhydride Modified Early *Indica* Rice Starch. *Starch/Stärke* **2006**, *58*, 109–117.
- Wang, Y. X.; Lu, Z. X. Optimization of processing parameters for the mycelial growth and extracellular polysaccharide production by *Boletus* spp. ACCC50328. *Process Biochem.* **2005**, *40*, 1043–1051.
- Cui, F. J.; Li, Y.; Xu, Z. H.; Xu, H. Y.; Sun, K.; Tao, W. Y. Optimization of the medium composition for production of mycelial biomass and exo-polymer by *Grifola frondosa* GF9801 using response surface methodology. *Bioresour. Technol.* **2006**, *97*, 1209–1216.
- Liu, Z. Q.; Hu, Z. C.; Zheng, Y. G.; Shen, Y. C. Optimization of cultivation condition for producing the 1,3-dihydroxyacetone from newly isolated *Pichia membranifaciens* using response surface methodology. *Biochem. Eng. J.* **2008**, *38*, 285–291.
- Cadwell, C. G.; Wurzburg, O. B. Polysaccharide derivatives of substituted dicarboxylic acids. U.S. Patent 2661349, 1953.
- Bao, J.; Xing, J.; Phillips, D. L.; Corke, H. Physical properties of octenyl succinic anhydride modified rice, wheat, and potato starches. *J. Agric. Food Chem.* **2003**, *51*, 2283–2287.
- Cho, K. Y.; Lim, S. T. Preparation and properties of benzyl corn starches. *Starch/Stärke* **1998**, *50*, 250–257.
- Trubiano, P. C. Succinate and substituted succinate derivatives of starch. In *Starch: Chemistry and Technology*; Whistler, R. L.; BeMiller, J. N.; Paschall, E. F., Eds.; Academic Press: Orlando, FL, 1986; pp 131–147.
- Mahmoud, M. I. Enteral nutritional hypoallergenic formula. U.S. Patent 4670268, 1987.

Received for review April 22, 2008. Revised manuscript received October 6, 2008. Accepted October 7, 2008.

JF802317Q