https://doi.org/10.17221/22/2021-CJFS

Optimised preparation and characterisation of lotus root starch oxidised with sodium hypochlorite (NaOCl) using response surface methodology

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Citation: Xu J.S., Yang H.Y., Zhang C.Y., Liu C.Y. (2022): Optimised preparation and characterisation of lotus root starch oxidised with sodium hypochlorite (NaOCl) using response surface methodology. Czech J. Food Sci., 40: 61–68.

Abstract: In this paper, response surface methodology (RSM) was used to study the optimised process conditions of lotus root starches modified by treatment with sodium hypochlorite (NaOCl). Based on the Box-Behnken design, quadratic models were developed to correlate the reaction variables. Analysis of variance (ANOVA) revealed that the active chlorine content was the most significant variable for the response. Under the experimental conditions in this paper, the calculated carboxyl content (CN_{COOH}) of obtained samples was approximately 0.98% ± 0.02% (n = 5). The spectra of Fourier transform infrared (FTIR) spectroscopy displayed that the formation of carboxyl groups successfully occurred on the oxidised lotus root starches. Scanning electron microscope (SEM) analysis showed that the oxidised lotus root starch (CN_{COOH}, 0.98%) granules were mostly spherical in shape and their surfaces were slightly rougher than those of native lotus root starch. This work may contribute to providing technical support and theoretical guidance for the production of oxidised lotus root starches using NaOCl as an oxidising agent.

Keywords: native starch; oxidised starch; response surface design

Oxidised starches are prepared by the reaction of starches with the designed amounts of oxidants; they can be used to produce oxidised starches, for instance, ammonium persulfate ($(NH_4)_2S_2O_8$), sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), etc. (Zhu 2017; Bustillos-Rodríguez et al. 2019; Zheng et al. 2019). Among different oxidant sources, the most popular commercial production of oxidised starches usually uses NaOCl as an oxidant, because it mainly oxidises the amorphous lamellae of starch granules, as well as its availability and well-known effects on starch properties (Wei et al. 2016; Vanier et al. 2017). In an alkaline medium, NaOCl oxidises the C-2, C-3, C-6 hydroxyl (OH) groups on amylose and amylopectin polymer molecules first to carbonyl (C=O) groups and then to carboxyl (COOH) groups (Wang and Wang 2003; Zhou et al. 2016). More importantly, further studies have shown that amylose and amylopectin polymer may also undergo partial depolymerisation depending on the degree of oxidation. In addition, the ratio of carbonyl and carboxyl groups in oxidised starches directly affects the physicochemical properties of starch, thus making oxidised starches suitable for a variety of industrial applications (Tomasik and Schilling 2004; Vanier et al. 2012). Consequently, depth research on oxidation conditions is required so that oxidised starches can suitably be utilised in various industrial applications. As far as we know, the reaction factors of oxidised starches,

Supported by the Natural Science Foundation of Guangdong Province (Project No. 2020A1515011042), the Science and Technology Projects of Guangdong Province (Project No. 2019ST115) and Guangdong University of Petrochemical Technology Research Funds (Project No. 2018rc37).

https://doi.org/10.17221/22/2021-CJFS

i.e. reaction pH, reaction temperature, reaction time, NaOCl concentration, starch molecular structure and starch source, have a great influence on the degree of NaOCl oxidation (Kuakpetoon and Wang 2008; Sangseethong et al. 2009). As a consequence, it is necessary to establish the optimised preparation process of oxidised starches for further research, mechanism and application.

For the optimisation of complex process conditions, there are two commonly used methods, namely the classical single factor test and the response surface methodology (RSM) (Xu et al. 2019). The former is the way of considering one-factor-at-a-time that the optimisation process uses only one variable at a time, leaving other factors unchanged under certain conditions. Although this method is tedious, time-consuming and lacks information about the interaction of various factors, it cannot reach the optimal value and ignore the interaction between variables. However, the latter, namely RSM facilitates the simultaneous study of interactions between two or more variables, and it is an effective mathematical and statistical tool for performing improvements and optimising the independent factors that influence the response in a given set of experiments. Because multi-factor studies at a single time can be done, RSM can largely reduce the research workloads and time consumption compared to the former method. Additionally, RSM also provides an appropriate model for the optimisation of the preparation process in comparison with conventional variable control approaches, because it defines the actions of independent variables as well as their interaction effects (Belwal et al. 2016). In that case, RSM has been employed for optimising an experimental design such as Box-Behnken or central composite ones to fit a second-order polynomial, and it was found successful in predicting the model of various process factors in a convenient manner such as food science and engineering, chemical engineering and technology, and environment fields, etc. (Belwal et al. 2016; Xu et al. 2019; Zhang et al. 2020).

A major drawback of the various studies reviewed is the investigation of one factor at a time in the preparation of oxidised starches (Sangseethong et al. 2009; Vanier et al. 2012; Fonseca et al. 2015). This is because it cannot resolve the interactions between the factors involved and it is difficult to determine the optimal value for predicting the best response. Therefore, this work is aimed at using native lotus root starch as raw material, NaOCl as an oxidising agent for preparing oxidised starches, and optimisation of active chlorine content, reaction pH and reaction time to develop models for the prediction of carboxyl group contents of oxidised starches using Box-Behnken experimental design method and RSM. The interactions of reaction factors and optimisation were studied using Design Expert Software 8.0.6.1. Oxidised lotus root starch with different carboxyl group contents based on the RSM results was further characterised to examine their structure and morphological behaviour.

MATERIAL AND METHODS

Material. Native lotus root starches (99.0% purity, food grade) used in this work were bought from Yangzhou Lianshun Food Co., Ltd (China). NaOCl [analytical reagent (AR)] was purchased from Nanjing Chemical Reagent Co., Ltd (China). All other reagents (AR) were of analytical grade and were provided by Xilong Chemical Co., Ltd (China). Deionised water was used for all experiments.

Oxidised starch preparation. According to the method reported by Costa De Conto et al. (2011) with a minor modification, oxidised lotus root starches were prepared. The typical procedure was as follows: firstly, 40 g (LOS-1; Yangzhou Lianshun Food Co., Ltd, China) of native lotus root starch (dry basis) was dispersed in 60 g of deionised water in a 250 mL three-necked flask equipped with a stirrer and a thermometer (WNG; Wuqiang County Will Instrument Factory, China) to obtain the starch slurry. The starch slurry was continuously stirred in a 35 °C water bath (HH-W; Changzhou Yate Experimental Instrument Co., Ltd., China) while its pH level was adjusted to the designed pH value (pH 9 ~ 11) with a standardised 1.0 M sodium hydroxide (NaOH) solution, and then NaOCl was added slowly after continuous stirring for 30 min (D2025W; Shanghai Mei Yingpu Instrument and Meter Manufacturing Co., Ltd., China) until reaching different final concentrations (4.0 ~ 5.0 g of active chlorine per 100 g of the native starch). Secondly, the reaction system was continued for an additional time (90 ~ 150 min) at the designed pH value. In the meantime, the standardised 1.0 M sulfuric acid (H_2SO_4) solution was used to maintain the pH value during the reaction. After the reaction, the pH value of the starch slurry was adjusted to 7.0 by adding the standardised 1.0 M H_2SO_4 solution. Finally, obtained slurry samples were filtered using a Buchner filter funnel (Hebei Dingsheng Longhua Experimental Instrument Co. Ltd, China) and washed twice with distilled water until being detected chlorine-free, then dried in an air circulation oven at 45 °C for about 48 h (DHG; Zhongxin Medical Instrument Co., Ltd., China) until the moisture con-

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tent of samples was about 10% w/w; then ground (YM; Yongkang Jince Industrial and Trade Strength Factory, China), screened (GS-1; Hebei Wandu Wire Mesh Manufacturing Co., Ltd., China) and stored in a dryer (PC-3; Jiangyan District Kangda Experimental Equipment Factory, China) for characterisation.

Carboxyl group content. According to the method reported by Lawal et al. (2005) with a slight modification, the carboxyl content (CN_{COOH}) of oxidised starches was determined. This procedure is as follows. About 5.0 g of samples (dry basis) was evenly dispersed in 25.0 mL of standardised 0.1 M hydrochloric acid (HCl) for 30 min (CL-1; Henan Juxing Chemical Co., Ltd, China). Afterwards, the obtained starch slurry was filtered (GS-1; Hebei Wandu Wire Mesh Manufacturing Co., Ltd., China) and washed with deionised water, and the resulting residue was washed to remove residual chlorine and transferred to an Erlenmeyer flask; then heated in a boiling water bath (HH-W; Changzhou Yate Experimental Instrument Co., Ltd., China) with continuous stirring for 15 min to ensure completed gelatinisation. The sample solution was titrated to the pH value of 8.3 with standardised 0.025 M NaOH when the sample solution was still hot. The CN_{COOH} of the sample was calculated according to the following Equation 1.

$$CN_{COOH} = \frac{\left(V_a - V_b\right) \times N \times 0.045}{W} \times 100 \quad (1)$$

where: CN_{COOH} – content of carboxyl groups (%); V_a – volume of NaOH used to titrate the sample (mL); V_b – volume of NaOH used to titrate the blank (mL); W – mass of sample (dry basis) (g); N – molar concentration of NaOH (mol).

Blank determination was run on the native starch in the same manner but being stirred in 25 mL of distilled water instead of a standardised 0.1 M HCl solution. All measurements were made in triplicate.

Fourier transform infrared (FTIR) analysis. The Fourier transform infrared (FTIR) spectra of samples were obtained using a Nicolet 380 spectrophotometer (American Digilab; US). The sample was applied to dry potassium bromide (KBr) pellets that were manually prepared using a hydraulic press (GHH; PerkinElmer, Spain). FTIR spectra were obtained in the region from 4 000 cm^{-1} to 400 cm^{-1}.

Scanning electron microscope (SEM) analysis. The morphology of samples was observed using scanning electron microscope (SEM) (Quanta 200F; FET, US). The sample was mounted on a sample holder by using silver paste and then coated with a thin layer of gold to prevent charging. Incident electron beam energies from 1.0 kiloelectronvolt (keV) to 30.0 keV were used. In all cases, the beam was at a normal incidence to the surface of the samples, and the measurement time was 100 s.

RESULTS AND DISCUSSION

Response surface analysis. The Box-Behnken experimental design of Design-Expert software 8.0.6.1 was used to optimise oxidation conditions of lotus root starches by RSM. Three variables, namely reactive chlorine content (A), reaction pH (B) and reaction time (C), each at three levels (4, 4.5, 5% w/w; 9, 10, 11; 90, 120, 150 min, respectively), were systematically investigated in this work, and the centre point was repeated 5 times. The CN_{COOH} (Y) of oxidised lotus root starches was taken as the response value, and the variable levels of the experiment are shown in Table 1. Experiments for 17 runs were conducted according to the designed combination in Table 1, and the resulting data were filled in the column Y. Multiple regression analysis was performed on the experimental data and the response variables were associated with the experimental variables by the following second-order polynomial Equation 2.

$$Y = 0.95 + 0.094A + 0.1B + 0.054C - \\ - 0.045AB + 0.048AC - 0.040BC - \\ - 0.19A^2 - 0.036B^2 - 0.12C^2 \quad (2)$$

where: Y – CN_{COOH} of oxidised lotus root starches; A, B, C – active chlorine content, reaction pH and reaction time, respectively.

The analysis of variance (ANOVA) results obtained from the response surface model (Table 2) showed that the derived model was sufficient to investigate the effects of three variables on the CN_{COOH} of oxidised lotus root starches.

In terms of evaluating the effectiveness and adaptability of the model, the calculated decision coefficient (R^2) was 0.95634, indicating that the model was effective and proficient within the experimental conditions. Obtained experimental data were analysed by ANOVA, and the results are listed in Table 2.

F-test and P-value were used to measure the significance of the model coefficients. In general, the model and its corresponding variables are more significant at high absolute F-values and low P-values. As shown in Table 2, the F-value of the model was 15.36 and the

https://doi.org/10.17221/22/2021-CJFS

Table 1. Experimental program and results for carboxyl group content obtained from the Box-Behnken design

Run	*A* (%)	*B*	*C* (min)	*Y* (%)
1	5.0	10	150	0.88
2	4.5	10	120	0.98
3	4.5	9	150	0.78
4	4.5	10	120	0.97
5	4.0	9	120	0.47
6	4.0	11	120	0.81
7	4.5	10	120	0.93
8	5.0	10	90	0.61
9	5.0	11	120	0.89
10	4.0	10	150	0.58
11	4.0	10	90	0.50
12	5.0	9	120	0.73
13	4.5	11	150	0.86
14	4.5	11	90	0.90
15	4.5	9	90	0.66
16	4.5	10	120	0.97
17	4.5	10	120	0.92

A – active chlorine content; *B* – reaction pH; *C* – reaction time; *Y* – carboxyl group content

P-value was 0.0008, indicating that the model was highly significant. The lack-of-fit *F*-value and *P*-value were 6.29 and 0.0539, respectively, which confirmed the applicability of the model to the predication of variations. Furthermore, the coefficients (A, B, A^2, C^2) were very significant because of displaying the very low *P*-values ($P < 0.01$); the coefficients (C, AB) were significant because of displaying the very low *P*-values ($P < 0.05$). But, because the *P*-values ($P > 0.05$) were large, the coefficients (AC, BC, B^2) were not significant.

The 3D response surface and contour diagrams were created by using Design-Expert 80.6.1 to predict the relationship between independent variables and dependent variables in the work. Obtained results are shown in Figure 1. These diagrams provided the visual interpretation of the interactions between two tested variables and the relationships between the response of each variable and the experimental level. The steep surface in the diagrams indicated the significance of the effects between the variables. As shown in Figures 1A and 1B, when the reaction time (C) was fixed at 120 min, the combined effects of the active chlorine content (A) and the reaction pH (B) on the CN_{COOH} of the oxidised starches were determined. Figure 1B shows a steep response surface, which indicates a significant interaction between the active chlorine content and the reaction pH. However, Figure 1C shows that the response surface was relatively flat, indicating that the interactions between reactive chlorine content and reaction time were not significant. Figures 1E and 1F show similar trends for reaction pH and reaction time. Based on the above response surface analysis, it was found that the active chlorine content and the reaction pH were of great significance for the preparation of oxidised lotus root starches.

Table 2. Estimated regression coefficients and analysis of variance (ANOVA) for CN_{COOH} of the oxidised lotus root starch ($R^2 = 0.95634$; $CV\% = 6.18$; Adeq precision = 12.555)

Source of variance	Sum of squares	Degree of freedom	Mean square	*F*-value	*P*-value	Significance difference
Model	0.440	9	0.049	20.49	0.0008	**
A	0.070	1	0.070	29.48	0.0008	**
B	0.084	1	0.084	35.24	0.0039	**
C	0.023	1	0.023	9.69	0.0446	*
AB	0.008	1	0.008	3.40	0.0123	*
AC	0.009	1	0.009	3.78	0.1016	–
BC	0.006	1	0.006	2.68	0.0639	–
A^2	0.160	1	0.160	65.93	0.0007	**
B^2	0.005	1	0.005	2.26	0.2214	–
C^2	0.059	1	0.059	24.69	0.0016	**
Residual	0.017	7	0.002	–	–	–
Lack of fit	0.014	3	0.005	6.29	0.0539	–
Pure error	0.003	4	0.0007	–	–	–
Total	0.460	16	–	–	–	–

*$P < 0.05$ is significant; **$P < 0.01$ is very significant; *CV* – coefficient of variation; *A* – active chlorine content; *B* – reaction pH; *C* – reaction time; Adeq precision – adequate precision

https://doi.org/10.17221/22/2021-CJFS

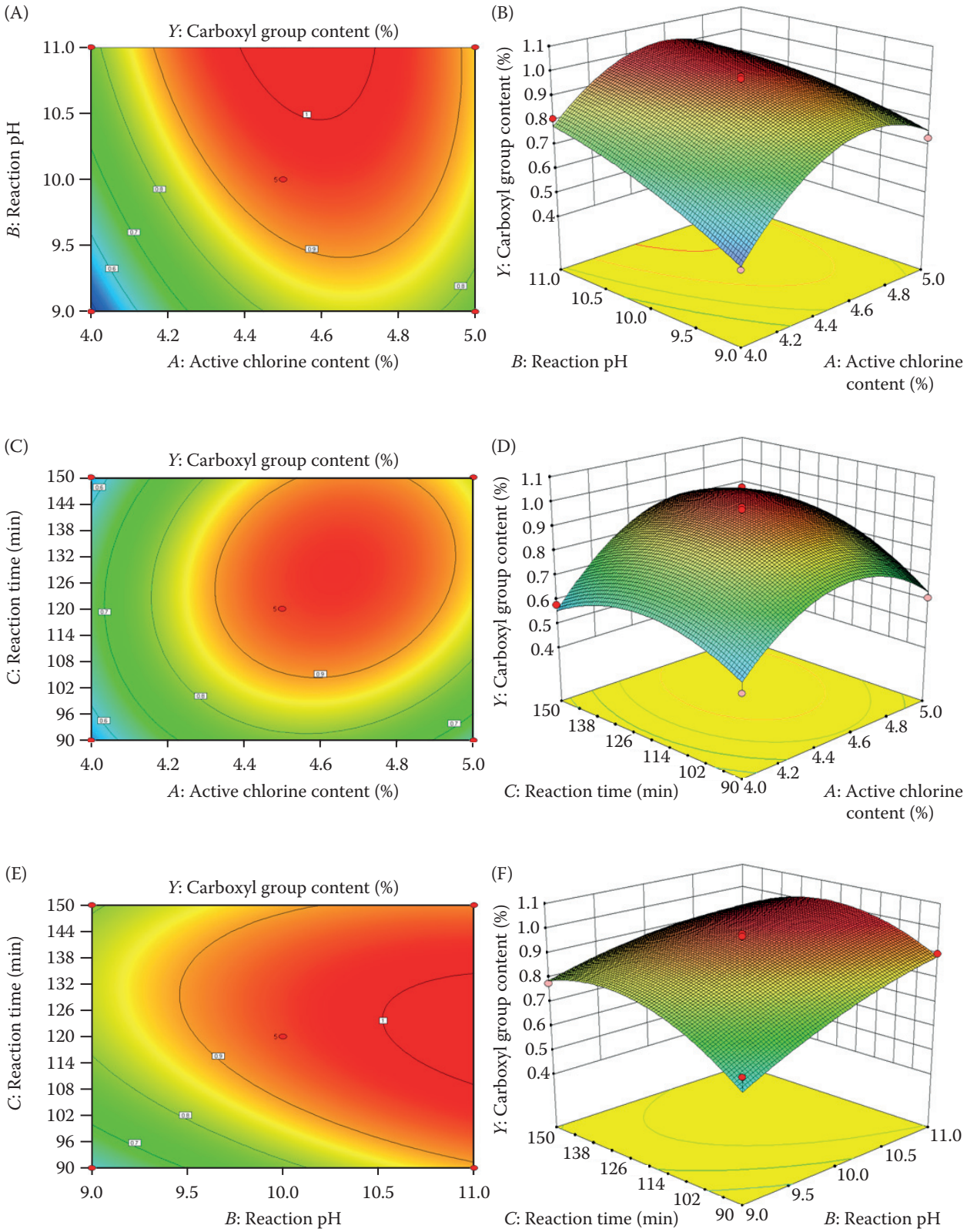

Figure 1. Effects of various factors on the preparation of oxidised lotus root starches: (A) 2D contour plot of *AB*, (B) 3D response surface plot of *AB*, (C) 2D contour plot of *AC*, (D) 3D response surface plot of *AC*, (E) 2D contour plot of *BC*, (F) 3D response surface plot of *BC*

A – active chlorine content; *B* – reaction pH; *C* – reaction time; *Y* – carboxyl group content

https://doi.org/10.17221/22/2021-CJFS

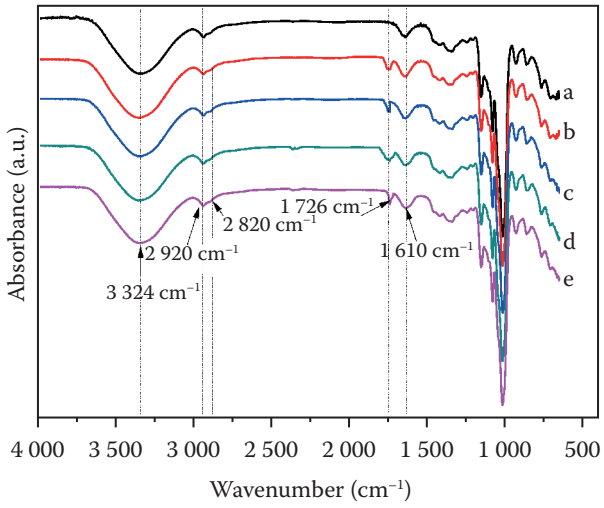

Figure 2. FTIR images of native lotus root starch and its oxidised starches with different CN_{COOH}

a – native starch; b – oxidised starch with CN_{COOH} (0.50%); c – oxidised starch with CN_{COOH} (0.65%); d – oxidised starch with CN_{COOH} (0.81%); e – oxidised starch with CN_{COOH} (0.98%); FTIR – Fourier transform infrared; a.u. – arbitrary unit

The response surface analysis showed that the following optimal conditions were obtained by using the model equation: active chlorine content 4.57% w/w, reaction pH value 10.83, reaction time 119.29 min. Under the optimal conditions, the maximum predicted value of the CN_{COOH} of the oxidised starches was 1.02%. In view of the practical preparation process of the oxidised starches, the optimised reaction conditions were as follows: active chlorine content 4.6% w/w, reaction pH value 10.8, reaction time 120 min. In order to further verify that there was no deviation between the predicted result and the experimental value, a series of verification experiments were carried out under the modified optimal conditions. Obtained results showed that the CN_{COOH} of the oxidised starches was 0.98% ± 0.02% (n = 5), which was no significant deviation from the predicted CN_{COOH} of 1.02%. Therefore, this model is suitable for predicting the reaction conditions of the oxidised starches.

FTIR analysis. The oxidised starches would lead to the conversion of hydroxyl groups on the molecule chains into carboxyl groups or aldehyde groups. The structure of the carbonyl group was detected by FTIR spectroscopy, as shown in Figure 2. The infrared spectrum peaks of native lotus root starch and its oxidised starches were assigned to their respective functional groups. The comparison between the native starch and its oxidised starches showed that all the characteristic peaks of the native starch and its oxidised starches were not obviously changed. Normally, the aldehyde groups in oxidised starches are easy to react with water in the air to form hemiacetals, and the absorption related to the aldehyde groups is difficult to detect by the infrared spectrum of samples using KBr pellets. As can be seen in Figure 2, the FTIR spectrum of oxidised starches showed new bands at 2 820, 1 726 and 1 610 cm^{-1} in comparison with that of the native starch. The former two peaks represent

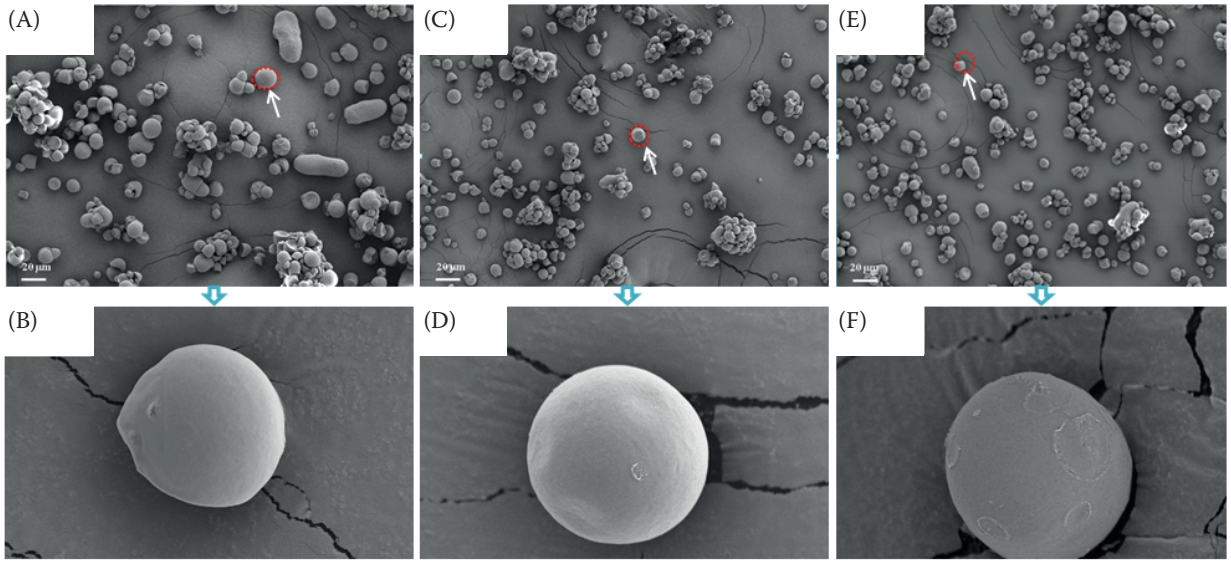

Figure 3. Scanning electron micrograph pictures at different magnifications: (A) native lotus root starch, (B) detail of native lotus root starch, (C) CN_{COOH} (0.50%) oxidised starch, (D) detail of CN_{COOH} (0.50%) oxidised starch, (E) CN_{COOH} (0.98%) oxidised starch, and (F) detail of CN_{COOH} (0.98%) oxidised starch

https://doi.org/10.17221/22/2021-CJFS

the formation of the aldehyde group, while the latter peak represents the absorption of -COONa, which is different from the absorption of the combined water at 1 610 cm^{-1}.

SEM analysis. SEM was used to scan the surface morphology of native lotus root starch and its oxidised starches prepared under the optimal processing conditions (Figure 3). Native lotus root starch granules were oval or elongated in shape and approximately 15 ~ 30 μm in diameter and had a smooth surface (Figures 3A and 3B). The oxidised starch (0.5% CN_{COOH}) granules were mostly spherical and had a smooth surface, indicating that the oxidisation treatments did not significantly damage the starch structure (Figure 3C and 3D). This phenomenon is because the oxidising agents preferentially act in the amorphous region, removing non-starch compounds such as fats and proteins. Normally, the morphologies of the oxidised starch prepared with NaOCl as an oxidising agent showed similar changes. As seen from Figure 3F, there were neither obvious changes nor damage to the microstructures of oxidised starch (CN_{COOH}, 0.98%) obtained under the following conditions: reaction time 120 min, reaction pH 10.8, and active chlorine content 4.6%; but their granule surfaces were slightly rougher than those of the native starch. Figure 3E shows that some fissures can be observed on surfaces of the oxidised starch granules, possibly due to localised extensive oxidation.

CONCLUSION

An RSM based on a three-level, three-factor Box-Behnken experimental design was conducted to study the effects of active chlorine content, reaction pH and reaction time on the CN_{COOH} of oxidised starches. The following optimum reaction conditions were obtained: fixed reaction temperature 35 °C; active chlorine content 4.6%; reaction pH 10.8; and reaction time 120 min. Under these conditions, the experimental CN_{COOH} of oxidised starches was about 0.98% ± 0.02%, which was close to the predicted CN_{COOH} (1.02%). Hence, RSM might be a valuable technique for the optimisation of efficient oxidisation of starches. SEM images showed that the surface of oxidised lotus root starches (CN_{COOH}, 0.98%) was slightly rougher than that of its native starch, but their morphologies were mostly spherical. And the FTIR results revealed that the NaOCl oxidation occurred in starch molecules in comparison with that of native lotus root starch. Further researches are planned in order to improve the application potential of oxidised lotus root starches.

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Received: January 20, 2021
Accepted: Janury 14, 2022
Published online: February 21, 2022