ORIGINAL PAPER

Optimizing Conditions for TEMPO/NaOCl-Mediated Chemoselective Oxidation of Primary Alcohols in Sweet Potato Residue

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Abstract Sweet potato residue (SPR), a by-product of the sweet potato-based beverage industry, contains health-beneficial dietary fiber, but it has been limited to secondary-use applications due to poor water solubility. The water solubility of polysaccharides in SPR increases following chemoselective oxidation of the primary alcohol group through 2,2,6,6-tetramethyl-1-piperidinyl oxoammonium ion (TEMPO)/sodium hypochlorite (NaOCl)-mediated oxidation. In the present study, a central composite experimental design was used to optimize conditions for chemoselective oxidation of SPR with three variables, including TEMPO concentration (0.05–0.25 mmol, X_1), NaOCl concentration (4.0–12.0 mmol, X_2), and pH of the reactants (9.5–11.5, X_3). The quadratic polynomial model equation describing this relationship was as follows: degree of oxidation(%) = $-871.929 + 975.405X_1 + 20.7181X_2 + 143.358X_3 - 120.682 X_1^2 - 0.590426X_2^2 - 5.78182X_3^2 - 7.72500X_1X_2 - 76.4000X_1 X_3 - 0.550000X_2X_3 (R^2 = 0.9633)$. The maximum degree of oxidation (98.3%) was consistent with the expected value (100%) and was obtained from a TEMPO concentration, NaOCl concentration, and reactant pH of 0.1980 mmol, 9.6236 mmol, and 10.6271, respectively. The introduction of a C6 carboxyl group without oxidation of the secondary alcohols was confirmed by ^{13}C nuclear magnetic resonance and Fourier transform infrared spectroscopy. The water solubility of the oxidized products was 99.95%, whereas that of native SPR was <26.45%. The initial velocity of the oxidation showed a strong linear relationship ($R^2=0.9239$) with the degree of oxidation, indicating that initial velocity could be another quantitative index for determining the degree of oxidation and productivity of oxidized SPR. These results suggest that oxidized SPR could be used as a new water-soluble dietary fiber in the food industry.

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Introduction

Sweet potato residue (SPR), which contains water-insoluble polysaccharides, is produced during sweet potato-based beverage and food manufacturing. The water-insoluble polysaccharides in SPR consist of cellulose, curdlan, and chitin, and they may function to increase food volume without increasing caloric content, promote satiety, and reduce low-density lipoprotein cholesterol (Brown et al. 1999). Therefore, these polysaccharides have

received attention as a new functional food component (Elleuch et al. 2011). However, they have limited secondary-use application due to poor solubility in water. To overcome this disadvantage, many researchers have attempted to develop alternative methods, such as chemical and enzymatic modification (Bragd et al. 2004). The chemical modification of polysaccharides is an interesting methodology to develop new polymers with specific properties (Yalpani 1985). Among the chemical methods, catalytic and chemoselective oxidation of the primary alcohol groups in polysaccharides using a stable radical such as 2,2,6,6-tetramethyl-1-piperidinyl oxoammonium ion (TEMPO) have opened a new field of research (de Nooy et al. 1995). Chang and Robyt (1996) reported increased water solubility of water-insoluble polysaccharides, such as cellulose and chitin, using TEMPO/NaOBr-mediated selective oxidation of primary alcohol groups. Additionally, Isogai and Kato (1998) studied TEMPO-mediated oxidation and the effects of oxidation conditions on the chemical structure and the degree of polymerization in cellulose. Bromide-free TEMPO-mediated oxidation of potato starch has also been reported (Bragd et al. 2000), as NaBr present in the waste stream should be avoided due to environmental and toxic concerns.

Response surface methodology (RSM), an experimental design technique to investigate optimum conditions in multivariable systems (Box et al. 1978), is able to generate the model that accurately describes the overall process by study relationships between responses and independent variables (Cochran and Cox 1957; Thompson 1982). Furthermore, RSM not only provides information with less cost and within a short time but also results in the rapid and efficient development of new products and processes (Myers et al. 2009). Statistical optimization using RSM is widely applied in biotechnology, such as culture medium preparation for bacteriocin production (Li et al. 2002), lipase-catalyzed synthesis of phytosteryl esters of oleic acid (Kim and Akoh 2007), and fermentation processes for pullulan production (Jiang 2010).

The objectives of this study were to determine the effects of TEMPO concentration, NaOCl concentration, and reactant pH on chemoselective oxidation and to identify the optimum conditions to attain a higher degree of oxidation. Furthermore, it was evaluated the industrial applicability of solubilized SPR produced from chemoselective oxidation as a source of novel soluble dietary fiber.

Materials and Methods

Materials

SPR was a gift from the National Crop Experiment Station, Rural Development Administration (Suwon, Republic of Korea). TEMPO was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium hypochlorite (NaOCl) and sodium hydroxide (NaOH) were obtained from Daejung Chemicals & Metals Co. Ltd. (Shihueng, Republic of Korea). Deuterium oxide (D_2O) and dimethyl sulfoxide, as the ^{13}C nuclear magnetic resonance (^{13}C-NMR) solvents, were purchased from Sigma-Aldrich Co. All other chemicals were of analytical reagent grade.

TEMPO/NaOCl-Mediated Chemoselective Oxidation

Chemoselective oxidation of SPR was conducted using a procedure modified from a previous report (Chang and Robyt 1996). SPR (10 mmol of anhydroglucose units, 1.62 g) was suspended in 200 mL of distilled and deionized water (DDW). The pH of the suspended SPR solution was adjusted to the appropriate value with 4 N HCl or NaOH before starting the oxidation reaction. The oxidation reaction was initiated by adding 9–11% NaOCl and TEMPO to the SPR solution and conducted at 10 °C. The pH of the reactant was monitored and maintained to the desired value by adding 0.5 N NaOH using an autotitrator (pH Stat; Metrohm, Herisau, Switzerland). A schematic representation of the chemoselective oxidation reactor system is shown in Fig. 1. When 20 mL of 0.5 N NaOH (10 mmol) was consumed, the oxidation reaction was stopped by adjusting the pH to 7.0. The resulting solution was centrifuged at 8,800×g for 30 min to remove residual insoluble material, and the oxidized SPR in the supernatant was precipitated by adding three volumes of ethanol. The pellet was washed three times with 600 mL of ethanol, followed by centrifugation at 8,800×g for 30 min. The pellet was dried at 60 °C in a vacuum oven for 24 h and then stored at −20 °C.

Degree of Oxidation

The degree of oxidation (DO; in percent) of the primary alcohol groups in the polysaccharides was calculated from the following Eq. 1 by determining the amount of 0.5 N NaOH consumed to maintain the initial pH of the reactants:

$$\text{degree of oxidation(DO, \%)} = \frac{\text{mol of 0.5 N NaOH consumed to maintain the initial pH}}{\text{initial mol of the primary alcohol in polysaccharides}} \times 100 \qquad (1)$$
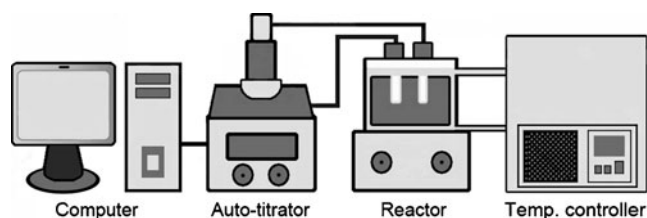
Fig. 1 Schematic illustration for TEMPO/NaOCl-mediated chemoselective oxidation

Experimental Design and Statistical Analysis

RSM was used to optimize the chemoselective oxidation conditions for a maximum DO. Central composite design was used to determine the effects of TEMPO concentration, NaOCl concentration, and pH of the reactant on the DO. The independent variables (X_i) and their levels are presented in Table 1, and the response (Y) was the degree of oxidation (in percent). The design scheme was composed of 20 experiments with 2^3 factorial points and six axial points ($\alpha = 2.0$), and the central point was replicated six times (Table 2). A quadratic polynomial model was assumed to predict the response Y (DO, in percent), using the following Eq. 2:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j, \qquad (2)$$

where β_0 is the model constant; β_i, β_{ii}, and β_{ij} are the model coefficients of the intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are independent variables. The experimental data were analyzed using version 15 MINITAB statistical software (Minitab, State College, PA, USA) for regression analysis and to evaluate the statistical significance of the equation. The quality of the polynomial model equation fit was expressed by the coefficient of determination (R^2).

Measurement of Water Solubility

The water solubilities (in percent) of native and oxidized products were determined according to the method of Chang and Robyt (1996). Sample (100 mg) was dispersed in 30 mL of DDW and stirred at 300 rpm for 12 h at 25 °C. Then, the dispersion was centrifuged at 8,800×g for 15 min at 4 °C, and the pellet was dried at 60 °C in a vacuum oven for 24 h. Water solubility (in percent) was calculated from the following Eq. 3:

$$\text{Water solubility}(\%) = \frac{\text{initial weight of SPR} - \text{weight of dried pellet}}{\text{initial weight of SPR}} \times 100. \qquad (3)$$

Structural Analysis of Chemoselectively Oxidized Polysaccharides

A liquid-state ^{13}C-NMR experiment was conducted using an AVANCE 600 instrument (Bruker, Ettlingen, Germany) to estimate the conversion of primary hydroxyl groups to carboxyl groups by TEMPO/NaOCl-mediated chemoselective oxidation. The samples were spun at a rate of 20 Hz at 25 °C with a spectral width of 42 kHz. The acquisition time was 0.773 s, and line broadening was 3 Hz. The native SPR (60 mg/mL) was dissolved in deuterated dimethyl sulfoxide, and the oxidized SPR (60 mg/mL) was dissolved in D_2O. Tetramethylsilane was used as an internal standard.

Fourier transform infrared (FT-IR) spectra were obtained using a Nicolet 6700 FT-IR spectrophotometer (Thermo Scientific, Waltham, MA, USA) equipped with an attenuated total reflectance accessory. The spectra were recorded in transmission mode from 650 to 4,500 cm^{-1} at a resolution of 8 cm^{-1} at 25 °C.

Table 1 Experimental range and levels of the independent variables used in RSM

Variables	Coded X_i	Coded levels					ΔX
		−2	−1	0	1	2	
TEMPO concentration (mmol)	X_1	0.05	0.10	0.15	0.20	0.25	0.05
NaOCl concentration (mmol)	X_2	4.0	6.0	8.0	10.0	12.0	2.00
Reactant pH	X_3	9.5	10.0	10.5	11.0	11.5	0.50

Table 2 Central composite design and the response for degree of oxidation

Run no.	Variables			DO (%)	
	X_1	X_2	X_3	Observed	Predicted
1	0.10 (−1)	6 (−1)	10.0 (−1)	69.91±0.58	68.823
2	0.20 (1)	6 (−1)	10.0 (−1)	80.64±0.51	81.708
3	0.10 (−1)	10 (1)	10.0 (−1)	87.62±1.60	88.818
4	0.20 (1)	10 (1)	10.0 (−1)	97.60±0.98	98.613
5	0.10 (−1)	6 (−1)	11.0 (1)	82.15±0.44	79.823
6	0.20 (1)	6 (−1)	11.0 (1)	87.58±0.63	85.068
7	0.10 (−1)	10 (1)	11.0 (1)	100.0±0.00	97.618
8	0.20 (1)	10 (1)	11.0 (1)	100.0±0.00	99.773
9	0.05 (−2)	8 (0)	10.5 (0)	81.27±3.82	82.912
10	0.25 (2)	8 (0)	10.5 (0)	98.28±0.86	97.952
11	0.10 (0)	4 (−2)	10.5 (0)	63.07±2.37	64.842
12	0.10 (0)	12 (2)	10.5 (0)	100.0±0.00	99.542
13	0.10 (0)	8 (0)	9.5 (−2)	81.53±2.60	79.777
14	0.10 (0)	8 (0)	11.5 (2)	88.87±2.58	91.937
15	0.10 (0)	8 (0)	10.5 (0)	93.90±0.20	91.639
16	0.10 (0)	8 (0)	10.5 (0)	93.14±1.24	91.639
17	0.10 (0)	8 (0)	10.5 (0)	91.81±0.85	91.639
18	0.10 (0)	8 (0)	10.5 (0)	92.42±1.60	91.639
19	0.10 (0)	8 (0)	10.5 (0)	87.95±0.23	91.639
20	0.10 (0)	8 (0)	10.5 (0)	89.30±2.90	91.639

For the identification of X_1, X_2, and X_3, refer to Table 1

Results and Discussion

Analysis of the Central Composition Experiment

The oxidation conditions including TEMPO concentration, NaOCl concentration, and pH of the reactant were optimized as independent variables to maximize the oxidation. The central composite experiment design and the corresponding response values are shown in Table 2. Using statistical analysis software (Design Expert; Stat-Ease, Inc., Minneapolis, MN, USA), a second-order polynomial model describing the correlation between the degree of oxidation and the three variables in this study was obtained in Eq. 4, as shown below:

$$Y = -871.929 + 975.405X_1 + 20.7181X_2$$
$$+ 143.358X_3 - 120.682X_1^2 - 0.590426X_2^2$$
$$- 5.78182X_3^2 - 7.72500X_1X_2 - 76.4000X_1X_3$$
$$- 0.550000X_2X_3. \tag{4}$$

Each of the observed values was compared with the predicted value, which was calculated from Eq. 4 (Fig. 2).

The statistical significance of Eq. 2 was confirmed by an analysis of variance (Table 3). The effects of variables as linear, quadratic, or interaction coefficients on the response were tested for adequacy. The coefficient of multiple determination of the polynomial model, termed R^2, was 0.9633, with no significant lack of fit at $P>0.05$. That indicated that the fitted model was able to explain 96.33% of the
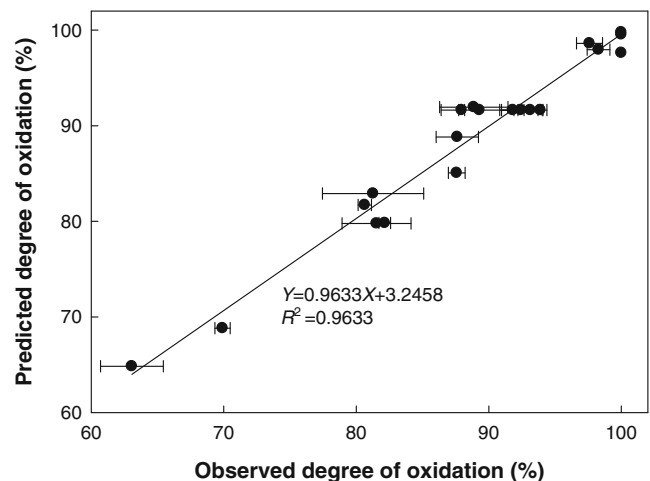

Fig. 2 Relationship between the model predicted and observed degree of oxidation

Table 3 Analysis of variance of the response surface quadratic model

Source	Sum of square	Degree of freedom	Mean squares	F value	P value
Model	1,781.76	9	197.973	29.13	<0.0001
X_1	226.20	1	226.20	33.29	0.00002
X_2	1,204.09	1	1,204.09	177.19	<0.0001
X_3	147.87	1	147.87	21.76	0.0009
X_1^2	2.29	1	2.29	0.34	0.5745
X_2^2	140.24	1	140.24	20.64	0.0011
X_3^2	52.53	1	52.53	7.73	0.0194
X_1X_2	4.77	1	4.77	0.70	0.4215
X_1X_3	29.18	1	29.18	4.29	0.0650
X_2X_3	2.42	1	2.42	0.36	0.5639
Residual	67.96	10	6.80		
Lack of fit	41.46	5	8.23	1.54	0.3246
Pure error	26.80	5	5.36		
Corrected total	1,849.71	19			

$R^2=0.9633$, adj $R^2=0.9302$. For the identification of X_1, X_2, and X_3, refer to Table 1

variability in the response (Haaland 1989). The results in Table 3 indicated that the model used to fit response variables was significant ($P<0.0001$) and adequate to represent the relationship between the responses and the independent variables. As shown in Table 3, the TEMPO concentration had a significant linear effect ($P<0.01$) but had no significant quadratic effect; NaOCl concentration had a significant linear and quadratic effects ($P<0.01$); reactant pH had significant linear ($P<0.01$) and quadratic effects ($P<0.05$). However, none of the independent variables interacted significantly. Corresponding variables are significant if the P value becomes smaller (Box et al. 1978). A coefficient of variation (CV=2.95%) <5% indicates good precision and reliability of the experiments and that the model is reproducible.

Optimization of Chemoselective Oxidation Conditions and Model Validation

Figure 3 shows the effect of the independent variables on DO. In general, exploring the response surfaces indicated a complex interaction between the variables. Figure 3a shows the effect of TEMPO and NaOCl concentrations on the DO at a pH of 10.5. When the TEMPO concentration was constant, the DO increased with the increase in NaOCl concentration. At a fixed NaOCl concentration, the variation in DO was slight when the TEMPO concentration increased, indicating that the TEMPO concentration had a linear effect on the DO and that the NaOCl concentration had a quadratic effect on the DO. Figure 3b shows the effect of the TEMPO concentration and pH of the reactant on the DO at a NaOCl concentration of 8.0 mmol. At a high TEMPO concentration, the change in pH of the reactant had little effect on the

DO. However, at a low TEMPO concentration, the DO increased when the pH of the reactant increased. The effect of the NaOCl concentration and pH of the reactant on DO at a TEMPO concentration of 0.15 mmol is shown in Fig. 3c. An increase in both the NaOCl concentration and pH of the reactant increased the DO, indicating that both the NaOCl concentration and pH of the reactant had quadratic effects on the DO.

In this study, the conditions necessary for 100% of chemoselective oxidation of SPR are of interest. Based on the obtained model, the 100% chemoselective oxidation was achieved under optimal conditions at 0.1980 mmol of TEMPO, 9.6236 mmol of NaOCl, and 10.6271 of reactant pH, respectively. This experiment was performed under optimal conditions to examine the adequacy of the predicted model. The predicted DO was 100.0%, and the actual DO was 98.25±0.93. There was no significant difference between the predicted and the actual DO, indicating that the observed and predicted DO verified the validity of the model designed in this study.

Effect of Chemoselective Oxidation on Water Solubility

The water solubilities (in percent) of native and oxidized SPR produced by the TEMPO/NaOCl-mediated chemoselective oxidation were determined. After chemoselective oxidation, the water solubility of the oxidized SPR increased dramatically to almost 100%, whereas that of native SPR was less than 26% (Table 4). This dramatic elevation in water solubility after chemoselective oxidation could be due to the strong hydrophilicity exerted by the carboxyl group. This result agreed with those of Chang et al. (2004) and Ahn et al. (2009).
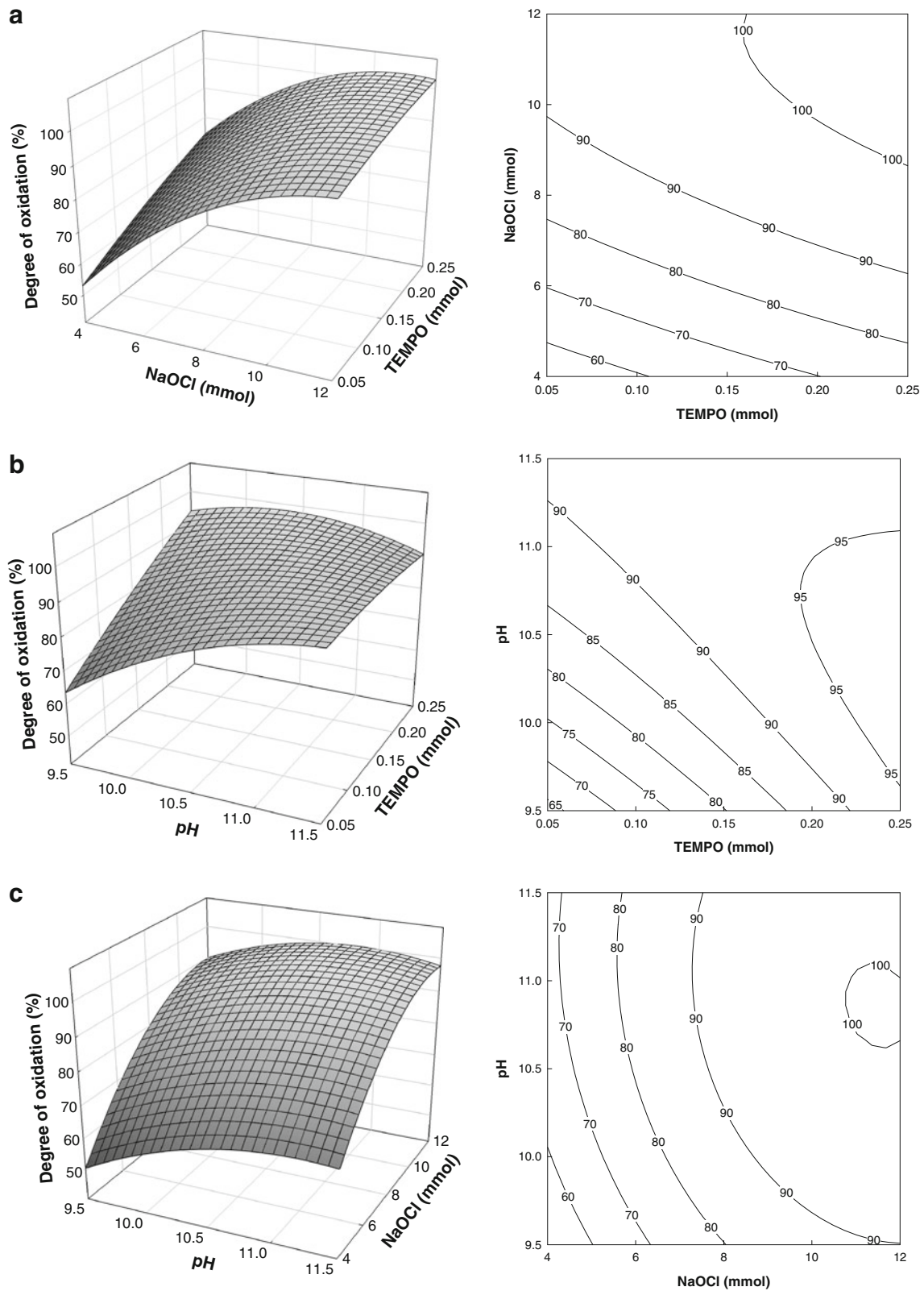
Fig. 3 Response surface and contour plots showing the effects of variables on the degree of oxidation. **a** Effect of TEMPO and NaOCl on the DO at a reactant pH of 10.5. **b** Effect of TEMPO and pH on the DO at a NaOCl concentration of 8.0 mmol. **c** Effect of NaOCl and pH on the DO at a TEMPO concentration of 0.15 mmol

Table 4 Water solubility (in percent) of native and oxidized SPR under the optimal condition

Sample	Water solubility
Native SPR	26.45±3.05
Oxidized SPR	99.95±0.05

^{13}C-NMR Spectrometric Analysis

^{13}C-NMR has been used for structural investigations of polysaccharides (Gidley and Bociek 1988; Matsuo 1984). Figure 4 shows the ^{13}C NMR spectra of the SPR before and
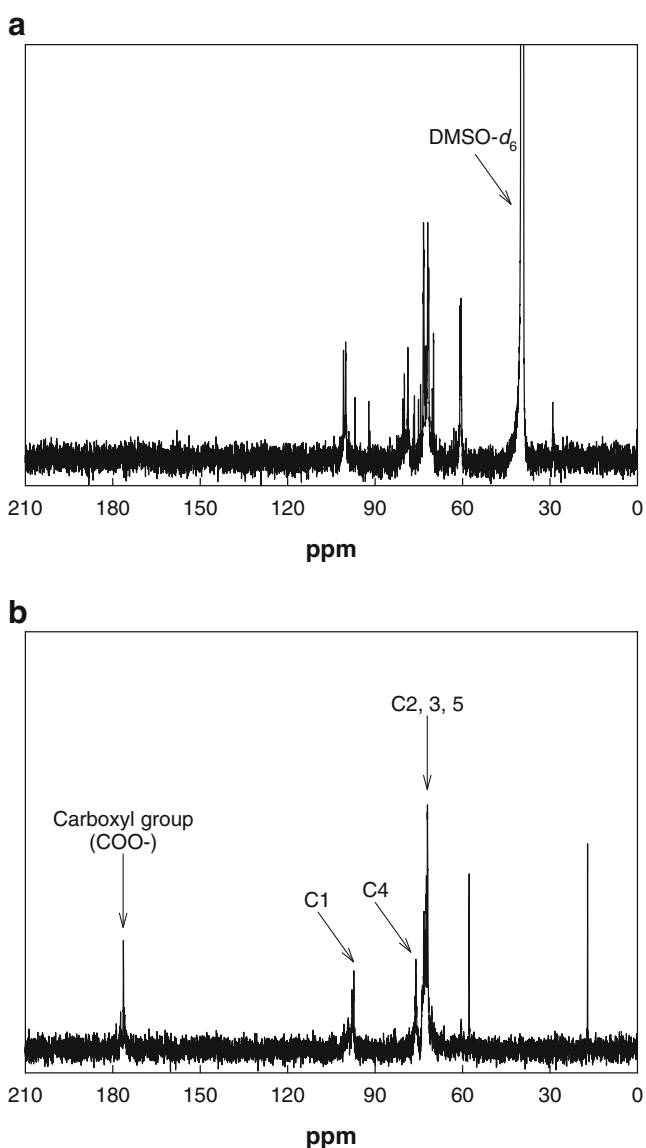
a

b

Fig. 4 ^{13}C-NMR spectra of the native and selectively oxidized SPR by TEMPO/NaOCl-mediated oxidation. **a** Native SPR. **b** Selectively oxidized SPR

after chemoselective oxidation. Carbon chemical shifts have been previously identified in polysaccharides: 96–102 ppm for C1; 70–73 ppm for C2, C3, and C5; 77–83 ppm for C4; and 59–62 ppm for C6 (Singh et al. 1993). The signals of carbons C1, C2, C3, C4, and C5 were observed at 100.1, 73.4, 72.4, 78.8, and 71.6 ppm, respectively, in native SPR (Fig. 4a) and at 97.28, 73.28, 72.95, 76.03, and 72.38 ppm in oxidized SPR (Fig. 4b), respectively. The newly detected resonance at 177.3 ppm and the disappearance of the resonance at 60 ppm may have resulted from the formation of carboxylic acid converted from the C6 primary hydroxyl groups of the polysaccharides through the TEMPO/NaOCl-mediated chemoselective oxidation. Anelli et al. (1987) reported that secondary alcohols are oxidized to ketones during the oxidation of alcohols by oxoammonium. The absence of resonance in the 195–205-ppm region of the oxidized SPR showed that no ketone group was present, indicating that the secondary alcohols were not oxidized by TEMPO/NaOCl-mediated chemoselective oxidation.

FT-IR Spectrometric Analysis

The FT-IR spectra of native and oxidized SPR are shown in Fig. 5. FT-IR spectra of the oxidized SPR revealed a sharp peak at 1,600 cm^{-1} against the native SPR, indicating the presence of a C=O bond in oxidized SPR. Spectra showing a broad peak in the range 3,000–3,500 cm^{-1} of the native SPR were responsible for the presence of the OH group, and this peak was found in the oxidized SPR spectra. This result indicated that the other alcohol, not the primary alcohol in SPR, was not oxidized during the reaction. If the other alcohol groups were oxidized, the peak in the range of 3,000–3,500 cm^{-1} may have disappeared or its intensity would have decreased. Therefore, the FT-IR spectra provided a confirmative clue of the chemoselective oxidation of only the primary alcohol in SPR. This result agreed with that in a previous report by Ding et al. (2008).

Determination of Initial Velocity as a Quantitative Index for Determining Chemoselective Oxidation Degree and Productivity

The time course of TEMPO/NaOCl-mediated chemoselective oxidation of SPR under the optimized condition is shown in Fig. 6. The oxidation rate decreased gradually as the oxidation reaction progressed. A gradual decrease in the oxidation rate may have been due to the reduced contents of native SPR or NaOCl to regenerate TEMPO from TEMP. In this study, the degree of oxidation was calculated using the amount of consumed 0.5 N NaOH to maintain the initial pH because carboxylate (–COO$^-$) resulting from the oxidized primary alcohol was neutralized through a one-on-one
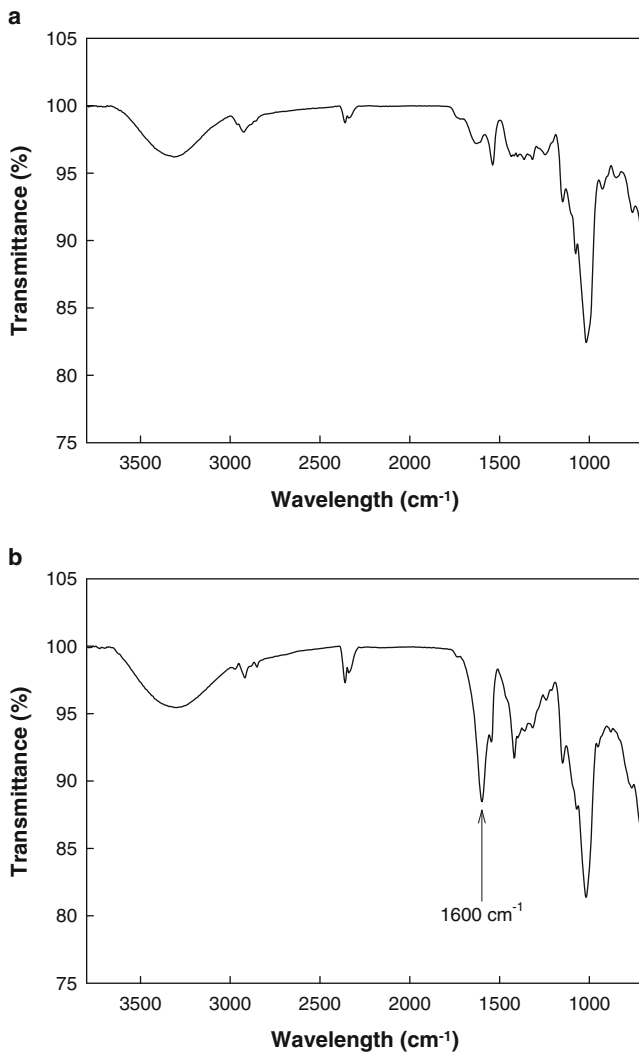
Fig. 5 FT-IR spectra of the native and selectively oxidized SPR by TEMPO/NaOCl-mediated oxidation. **a** Native SPR. **b** Selectively oxidized SPR
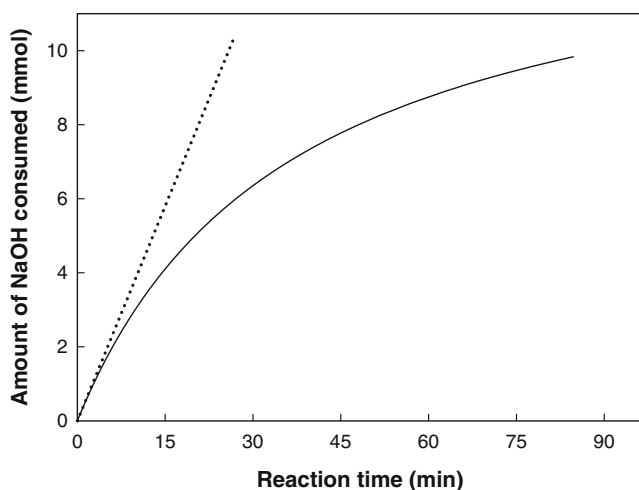

Fig. 6 Time course of TEMPO/NaOCl-mediated oxidation of SPR under the optimized conditions (*solid line*) and its initial velocity (*dotted line*)

reaction with NaOH. This result indicates that the oxidation rate depended on the initial SPR and the two catalysts (TEMPO and NaOCl) contents and that the oxidation rate influenced the degree of oxidation. Therefore, the initial velocity could be a quantitative index for chemoselective oxidation. The initial velocity (micromoles per minute) was calculated using the tangential slope of the fitted curve at $t=0$. The initial velocity of the oxidized SPR under the optimum condition was 373.2 ± 14.06 μmol of anhydroglucose units per minute. Ten preliminary experiments showed a relationship between the degree of oxidation and initial velocity ($R^2=0.9239$; Fig. 7). Because of the good correlation between the initial velocity and the degree of oxidation, the initial velocity could be used as a quantitative index for chemoselective oxidation. Based on the initial velocity, if a continuous steady-state operation is adopted to produce chemoselectively oxidized SPR in a future study, oxidized SPR would be attained at a maximum production rate of 97.7 kg of oxidized SPR (anhydroglucose units) per day.

Conclusion

The optimum condition for chemoselective oxidation was identified, and the degree of oxidation was significantly affected by TEMPO concentration, NaOCl concentration, and reactant pH. The optimum conditions for chemoselective oxidation by statistical analysis are as follows: 0.1980 mmol of TEMPO, 9.6236 mmol of NaOCl, and a 10.6271 of reactant pH. The coefficient of multiple determination (R^2) for the obtained model was 0.9633. Under optimal conditions stated above, the obtained model predicted 100% of the degree of oxidation, and the actual degree of oxidation was 98.25%. The water solubility of SPR dramatically increased from 26.5% to almost 100% through chemoselective oxidation. The structural analysis of oxidized
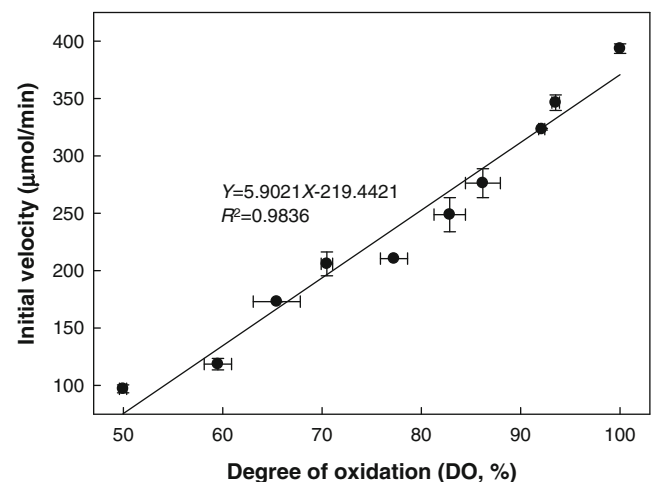

Fig. 7 Relationship between degree of oxidation and initial velocity

SPR using ^{13}C-NMR and FT-IR revealed the introduction of a carboxylate at C6 by chemoselective oxidation of the primary alcohol group. The initial velocity of oxidation could be useful to predict maximum oxidized SPR productivity (97.7 kg oxidized SPR/day) in a continuous steady-state operation. These results suggest that chemoselectively oxidized SPR is a suitable source of water-soluble dietary fiber for the beverage and food industry.

References

Ahn, S. M., Lee, H. J., Kim, S. W., Lee, J., & Chang, P.-S. (2009). Physicochemical properties of selectively oxidized 1-monolaurin from 2,2,6,6-tetramethyl-1-piperidinyl oxoammonium ion/sodium hypochlorite-mediated reaction. *Journal of Agricultural and Food Chemistry, 57*(7), 2920–2924.

Anelli, P. L., Biffi, C., Montanari, F., & Quici, S. (1987). Fast and selective oxidation of primary alcohols to aldehydes or to carboxylic acids and of secondary alcohols to ketones mediated by oxoammonium salts under two-phase conditions. *Journal of Organic Chemistry, 52*(12), 2559–2562.

Box, G. E. P., Hunter, W. G., & Hunter, J. S. (1978). *Statistical for experimenters: An introduction to design, data analysis, and model building*. New York: Wiley.

Bragd, P. L., Besemer, A. C., & van Bekkum, H. (2000). Bromide-free TEMPO-mediated oxidation of primary alcohol groups in starch and methyl α-D-glucopyranoside. *Carbohydrate Research, 328* (3), 355–363.

Bragd, P. L., van Bekkum, H., & Besemer, A. C. (2004). TEMPO-mediated oxidation of polysaccharides: Survey of methods and applications. *Topics in Catalysis, 27*(1–4), 49–66.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: A meta-analysis. *American Journal of Clinical Nutrition, 69*(1), 30–42.

Chang, P.-S., & Robyt, J. F. (1996). Oxidation of primary alcohol groups of naturally occurring polysaccharide with 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion. *Journal of Carbohydrate Chemistry, 15*(7), 819–827.

Chang, P.-S., Seo, H.-M., Kwon, O.-T., Lee, H.-G., & Kim, Y.-S. (2004). Selective oxidation of primary alcohol group in 1-monostearoyl glycerol mediated by 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion. *Food Science and Biotechnology, 13*(2), 225–229.

Cochran, W. G., & Cox, G. M. (1957). Some methods for the study of response surfaces. In W. G. Cochran & G. M. Cox (Eds.), *Experimental designs* (2nd ed., pp. 335–375). New York: Wiley.

de Nooy, A. E. J., Besemer, A. C., & van Bekkum, H. (1995). Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans. *Carbohydrate Research, 269*, 89–98.

Ding, B., Ye, Y. Q., Cheng, J., Wang, K., Luo, J., & Jiang, B. (2008). TEMPO-mediated selective oxidation of substituted polysaccharides—an efficient approach for the determination of the degree of substitution at C-6. *Carbohydrate Research, 343*(8).

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry, 124*(2), 411–421.

Gidley, M. J., & Bociek, S. M. (1988). ^{13}C CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules: Relationships between glycosidic linkage conformation and solid-state ^{13}C chemical shifts. *Journal of the American Chemical Society, 110*(12), 3820–3829.

Haaland, P. D. (1989). Separating signals from the noise. In P. D. Haaland (Ed.), *Experimental design in biotechnology* (1st ed., pp. 61–83). New York: Marcel Dekker.

Isogai, A., & Kato, Y. (1998). Preparation of polyuronic acid from cellulose by TEMPO-mediated oxidation. *Cellulose, 5*(3), 153–164.

Jiang, L. (2010). Optimization of fermentation conditions for pullulan production by *Aureobasidium pullulan* using response surface methodology. *Carbohydrate Polymers, 79*(2), 414–417.

Kim, B. H., & Akoh, C. C. (2007). Modeling and optimization of lipase-catalyzed synthesis of phytosteryl esters of oleic acid by response surface methodology. *Food Chemistry, 102*(1), 336–342.

Li, C., Bai, J., Cai, Z., & Ouyang, F. (2002). Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. *Journal of Biotechnology, 93*(1), 27–34.

Matsuo, K. (1984). ^{13}C-NMR relaxation of polysaccharides: Dextran and amylose in dimethyl sulfoxide and water. *Macromolecules, 17*(3), 449–452.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response surface methodology: Process and product optimization using designed experiments* (3rd ed.). Hoboken: Wiley.

Singh, V., Zakiuddin, S., & Divakar, S. (1993). ^{13}C CP/MAS NMR spectroscopy of native and acid modified starches. *Starch-Starke, 45*, 59–62.

Thompson, D. (1982). Response surface experimentation. *Journal of Food Processing and Preservation, 6*(3), 155–188.

Yalpani, M. (1985). A survey of recent advances in selective chemical and enzymic polysaccharide modifications. *Tetrahedron, 41*(15), 2957–3020.