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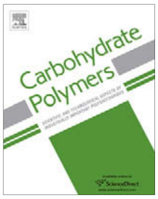

Effects of reaction conditions on the physicochemical properties of cationic starch studied by RSM

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ABSTRACT

Granular cationic starches were prepared in aqueous phase without the addition of swelling inhibiting salts. Response surface methodology (RSM) was performed to analyze the effects of reaction conditions on physicochemical properties of the products. As the reaction time was prolonged from 2 to 5 and to 24 h, the relative contribution of the temperature to degree of substitution (DS) turned from minor to prominent. Good correlations were observed between the DS and the pasting temperature of the 2, 5, and 24 h cationized starches. By contrast, variation in the correlations between DS and the other physicochemical properties, with respect to reaction durations, revealed the processing pattern of cationization in starch granules along extending reaction times. This deduced pattern was confirmed by the granular and molecular characterizations using confocal laser scanning microscopy and high performance anion exchange chromatography, respectively.

1. Introduction

Cationic starches are important industrial derivatives in which the starch is given a positive ionic charge by introducing ammonium, amino, imino, sulfonium, or phosphonium groups (Xie, Liu, & Cui, 2005). Among different cationic groups the tertiary amino or quaternary ammonium group brings out the cationic starch derivatives with the most commercial importance (Rutenberg & Solarek, 1984). The utilization of cationic starches is mainly located in the field of paper making, as they can improve mechanical strength and drainage of paper sheets, retention of fines, dyes and fillers, running of paper machine, and can decrease biological oxygen demand of paper mill effluent (Jensen & Winters, 1986; Nachtergaele, 1989; Wurzburg, 1986). Cationic starches are also applied as flocculants in waste water treatment, or as additives in the manufacturing of textile, cosmetics, detergents, and adhesives (Solarek, 1986). In addition, studies on the employment of cationic starches in drug delivery and oil recovery are also in progress (Mulhbacher, Ispas-Szabo, Lenaerts, & Mateescu, 2001; Zhang, Ju, Zhang, Ma, & Yang, 2007). The wide spread performance of cationic starches in industry is based on their relatively low price, excellent properties, and biodegradability (Zhang et al., 2007). Recently, cationic starches are manipulated more case by case in laboratory research. For instance, Sakai-Kato et al. (2006) made cationic starch dynamic coating additive in capillary electrophoresis for protein analysis; Li, Hou, and Zhu (2007) studied the thiotropic properties of suspensions consisting of cationic starch and aluminum magnesium hydrotalcite-like compound.

Conventional preparation methods of cationic starches can be categorized into extrusion, semi-dry, and wet process. In the extrusion process, reagent is added to the dry starch during extrusion (Gimmler & Meuser, 1994; Valle, Colonna, & Tayeb, 1991); in a semi-dry process, reagent is sprayed onto the starch then the mixtures are exposed to heat treatment (Hellwig, Bischoff, & Rubo, 1992); in wet process, reactions are processed either homogeneously in DMSO or heterogeneously in an aqueous or alcoholic suspension under alkaline conditions (Heinze, Haack, & Rensing, 2004; Kweon, Hanover, Sosulski, & Han, 1997). Both extrusion and semi-dry methods benefit from preparation convenience, but may be troubled with residual salts, reagents or by-products (Radosta et al., 2004). From this view point, the employment of wet process may promise better quality of the product, while homogeneous cationization could be troubled with the high viscosity of starch paste. Besides, the addition of swelling–inhibiting salts (e g, sodium sulphate) or alcohols into the reaction medium in heterogeneous process causes problematic waste treatment and unfavorable inhibition of granular swelling (Huber & BeMiller, 2003). Therefore, starch cationization through a more manageable route still asks for further investigations.

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The changes in physicochemical properties of cationic starches have been examined in many researches. Generally in the rapid-viscoanalysis (RVA), lower pasting temperature, and higher peak viscosity were observed from cationic starches derived from different botanical source; however, changes in setback of cationic starches varied from different study results (Kweon et al., 1997; Siau, Karim, Norziah, & Wan Rosli W.D., 2004). In the determination of thermal properties using the differential scanning calorimetry (DSC), lower transition temperature, and smaller gelatinization enthalpy of cationic starches compared to those of native starches were reported (Radosta et al., 2004; Yook, Sosulski, & Bhirud, 1994). Although most researches agreed that the cationic substitution by aqueous methods occurred preferentially at the amorphous region, as evidenced by the unchanged X-ray diffraction patterns (Aggarwal & Dollimore, 1998; Yook et al., 1994) and the remained birefringence under polarized light microscopy (Radosta et al., 2004), the conclusion from Manelius, Buleon, Nurmi, and Bertoft (2000), in which acid hydrolysates of cationic starches were fractionated and analyzed, deviated from this postulation. Since one of the possibilities for new, chemically modified starch products is the control of reaction sites within starch granules (BeMiller, 1997), it is worth investigating more profoundly the spatial distribution of cationization inside the starch granules.

It is possible to prepare cationic starch through salts-free aqueous cationization, if the temperature, pH, and other reaction conditions were appropriately controlled to avoid gelatinization of starch granules. However, until now only a few studies have worked on starch cationization through this method (Kuo & Lai, 2007; Manelius et al., 2000), and none of them have monitored the profiles of product properties under different levels of reaction parameters with the experimental design of response surface methodology (RSM). In this study, we intend to thoroughly investigate the changes, with extending reaction time, in degree of substitution (DS), reaction efficiency (RE), pasting, and thermal properties of cationic starches under varying reaction temperature and amount of cationizing agent. Morphology and molecular properties of cationic starches were characterized to verify the deduction according to the RSM analysis. Factors related with the alteration in structural property of cationic starches and hence their time-dependent variations in the changes of each physicochemical behavior were also discussed.

2. Experimental

2.1. Materials

Native corn starch was purchased from Gu-Tong Food Industry Ltd. (Chiayi, Taiwan). The cationizing agent CHPTAC ((3-chloro-2-hydroxypropyl) trimethyl ammonium chloride) was provided by Aldrich Chemical Co. (Milwaukee, WI, USA). NaOH, ethanol, and HCl were purchased from Nacalai Tesque Inc. (Kyodo, Japan), Taiwan Sugar Corporation (Tainan, Taiwan), and Sigma–Aldrich Co. (Taufkirchen, Germany), respectively.

2.2. Preparation of cationic starches

Cationic starches were prepared according to the method described by Kuo and Lai (2007). Native corn starch (90 g, on dry basis) was suspended in 100 mL of 1 mM NaOH and mixed with certain amount of CHPTAC (Table 1) which had been adjusted to pH 12 with 5 N NaOH. The mixture solution was adjusted to pH 11 with 2 N NaOH, and then diluted with 1 mM NaOH to 600 mL. The reaction was carried out at the specific temperature (Table 1) with continuously stirring (200 rpm). One-third amount by weight of the reaction mixture was taken away at 2, 5, and 24 h each time.

Table 1
Coded levels and corresponding values for the tests performed in RSM experimental design

Test	Block	Factor			
		X_1: temperature		X_2: molar ratio of CHPTAC/AGU	
		Coded level	Corresponding value (°C)	Coded level	Corresponding value
1	1	−1	30.0	−1	0.5
2	1	1	50.0	−1	0.5
3	1	−1	30.0	1	1.5
4	1	1	50.0	1	1.5
5	1	0	40.0	0	1.0
6	1	0	40.0	0	1.0
7	2	−1.414	25.9	0	1.0
8	2	1.414	54.1	0	1.0
9	2	0	40.0	−1.414	0.293
10	2	0	40.0	1.414	1.707
11	2	0	40.0	0	1.0
12	2	0	40.0	0	1.0

After diluting the reaction mixture at specific reaction time with an equal volume of 95% ethanol, the reaction mixture was centrifuged at 10000*g* for 10 min and the precipitate was collected. The collected precipitate was resuspended in 63% ethanol (300 mL) and neutralized with 4.8 N HCl. The cationic starch was washed with 63% ethanol several times till free of chlorides in washing solution. The obtained cationic starch was dried at 40 °C overnight. The nitrogen content of the cationic starches was determined following AACC Method 46-11A (Improved Kjeldahl Method, Copper Catalyst Modification). Degree of substitution of sample was calculated using the equation below (Heinze et al., 2004):

$$\mathrm{DS} = \frac{(162.15 \times \%\mathrm{nitrogen})}{1401 - (154.64 \times \%\mathrm{nitrogen})}$$

The DS determinations were run in duplicates.

According to Kweon, Bhirud, and Sosulski (1996) the reaction efficiency measured the percentage of added cationic reagent that had potentially reacted with the starch, and was calculated as follows:

$$\%\mathrm{RE} = \frac{\mathrm{DS}}{\mathrm{Added\ CHPTAC\ mol/mol\ starch}} \times 100$$

2.3. Pasting properties

The pasting properties of cationic starches were analyzed by using the Rapid Visco Analyser (RVA4, Newport Scientific Pty, Ltd., Warriewood, Australia) according to AACC Method 76-21 (AACC, 2000) with some modifications. Cationic starch suspension (8% (w/w), on dry basis) was held at 35 °C for 1 min, heated to 95 °C in 20 min, held at 95 °C for 10 min, cooled to 50 °C in 20 min, then held at 50 °C for 2 min. The paddle speed was 960 rpm for the first 10 s, and maintained at 160 rpm to the end of test. For 24 h cationized starches, 2.54% (w/w) starch suspension was used and the paddle speed was set at 160 rpm from the beginning to the end of test. Pasting temperature (PT, °C), peak viscosity (PV, cP), hot paste viscosity (HV), breakdown (BD = PV − HV), final viscosity (FV), and setback (SB = FV − HV) were obtained from the measurements. RVA measurements were run in duplicates; the CV values were between 0.6% and 10.1%.

2.4. Thermal properties

Thermal characteristics of the cationic starches were studied using a Modulated Differential Scanning Calorimetry (MDSC

2910, TA Instruments, New Castle, DE, USA). Samples for MDSC analysis were prepared according to Kuo and Lai (2007). The sample was heated in an MDSC from 10 to 120 °C at a heating rate of 5 °C/min, an amplification of ±1 °C, and a period of 40 s, with an empty pan as a reference. The results were calculated with TA Instruments analyzing software. The transition temperatures including onset (T_o), peak (T_p), and complete (T_c) temperatures (°C) were recorded from a plot of heat flow versus temperature. The width (WD) and the gelatinization enthalpy (ΔH, J/g) were derived from the range of the transition temperatures and the conversion from the area under the curve to Joules referred to the indium standard, respectively. MDSC measurements were run in duplicates; the CV values were between 0.0% and 14.8%.

2.5. Experimental design

Response surface methodology was performed with SAS 9.1 (SAS Institute Inc., 2004, Cary, NC, USA) to evaluate the effects of the two factors (reaction temperature and the amount of CHPTAC) on the physicochemical properties of cationic starches. The range set for two factors in the RSM design were predetermined to make cationic starches with the DS as high as possible while at the same time compromise with the lowest level of starch gelatinization. The corresponding values for the two factors varying at three levels (2^3, $\alpha = 1.414$) and their combinations for each test sample, determined using a central composite design (CCD), were displayed as Table 1. Each test sample was collected at 2, 5, and 24 h and the response variables (DS, RE, pasting, and thermal properties) were analyzed and modeled using the following equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

where Y is the predicted response; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the squared coefficient; and β_{ij} is the cross-product coefficient. The contour plot of each response variable was made using SAS 9.1 according to the predicted model equation.

2.6. Confocal laser scanning microscopy

Granular morphology of the cationic starches was observed by a confocal laser scanning microscopy (CLSM) (Leica TCS SP2 Confocal Spectral Microscope, Wetzlar, Germany) equipped with an argon laser. Starch granules were stained with aminofluorophore 8-amino-1,3,6-pyrenetrisulfonic acid (APTS, Molecular probes, Eugene, OR, USA) according to Kuo and Lai (2007). Prepared specimens were observed with Leica TCS SP2 (Wetzlar, Germany) with the objective len of 100× Plan apo/1.4 oil. The excitation wavelength was 488 nm (20% capacity) and the light was detected at the interval from 500 to 535 nm. The format of image was $30 \times 30\ \mu m^2$ and 512×512 pixels. Each line was scanned eight times and averaged to reduce noise during image acquisition.

2.7. Chain length distribution of amylopectin in cationic starches

The chain length distribution of isoamylase-debranched amylopectin from cationic starches was analyzed according to the method modified from Chung and Lai (2007). Forty-five milligrams cationic starch was dispersed in 0.5 mL 90% DMSO and the suspension was boiled for 10 min with continuous stirring. After cooling down, the solution was stirred at room temperature overnight, and then mixed with 3 mL 70 °C H_2O and 3 mL 40 mM sodium acetate buffer (pH 3.5). After cooling down to room temperature, the solution was added with 18 μL isoamylase (5.9 U/L) (Hayashibara Biochemical Laboratories Inc., Okayama, Japan), followed by incubation in a water bath at 37 °C for 3 h. The solution was finally boiled for 10 min to inactivate the enzyme, and diluted with equal volume of H_2O, and then filtered through a 0.45 μm PVDF membrane (Chrom Tech, Inc., Apple Valley, MN, USA). Twenty-five microliters of the filtrate was injected into the high performance anion exchange chromatography (HPAEC, Dionex Bio-LC system 300, Sunnyvale, CA, USA) system. The system apparatus included a Bio-LC gradient pump, a Dionex CarboPac PA1 column and a pulsed amperometric detector (PAD). The pulsed potentials and durations were: $E_1 = 0.05$ V ($t_1 = 420$ ms), $E_2 = 0.75$ V ($t_2 = 180$ ms), $E_3 = -0.15$ V ($t_3 = 360$ ms) at range 1 (sampling periods 16.67 ms). The eluent at a flow rate of 1 mL/ min was composed of 100 mM NaOH (eluent A) and 500 mM NaOAc in 100 mM NaOH (eluent B). The linear gradient of eluent B was: 0–50% during 0–70 min; 50–70% during 70–120 min. Glucose (Chem-Service, PA, USA), maltotriose, and maltohexaose (Fluka, Buchs, Switzerland) with known DP (degree of polymerization) were used as calibration standards. HPAEC determinations were run in duplicates.

3. Results and discussion

3.1. Effects of reaction conditions on the DS and RE of cationic starches

The products from the tests 4 and 8 (Table 1) of 24 h cationized starches were not collected owning to gelatinization and bursting out during the reaction process, therefore, the regression analysis for the response surfaces of 24 h cationized starches was performed based on the data of the other 10 test samples. The DS of the cationic starches prepared in this study fell between 0.003 and 0.121, and increased with increasing amount of cationizing agent and the reaction time (Fig. 1a–c). This result corresponded with previous researches (Heinze et al., 2004; Radosta et al., 2004; Siau et al., 2004). Compared with that of cationic starches reacting for 2 h (Fig. 1a), the response surface of 5 h cationized starches (Fig. 1b) had a steeper upward slope alone the direction of increasing temperature. This difference suggested that as the cationizing reaction time was prolonged from 2 to 5 h, the impact of temperature on the increase in DS became more pronounced than that of the amount of CHPTAC. The change in relative contribution from regression coefficients of these two reaction parameters on DS among 2 and 5 h cationized starches (Table 2) also identified with this transformation. The increase in temperature not only accelerated the cationic substitution, but also fastened the swelling of starch granules under pH 11, which in term facilitated the diffusion of cationizing agent toward the inner part of starch granules, and enlarged the effective area of cationization (Koide, 1998; Mentzer, 1984; Solarek, 1986). This phenomenon might serve as a critical factor for further enhancing in DS of the otherwise compact starch granules, since the cationic substitution was probably saturated on the outer surface or in the more accessible (i.e. the amorphous) part in starch granules after two hours of cationization. Siau et al. (2004) investigated the preparation of cationic sago starches using a central composite design with different levels of temperature, amount of cationizing agent, and NaOH. However, for their cationic starch reacted for 6 h, the influence of amount of cationizing agent on the rising in DS was still larger than that of the temperature. This disparity could be possibly resulted from the suppressed swelling of starch granules due to the utilization of Na_2SO_4 in their cationizing process (Kweon et al., 1997). For 2 h cationized starches under low amount of CHPTAC (molar ratio of CHPTAC/AGU $\leqq 0.65$), the RE remained approximately constant with the increasing temperature (Fig. 1d). By contrast, for 5 and 24 h cationized starches, RE increased with the raised temperature, but remained unchanged for 5 h and decreased for 24 h ones with the raised amount of CHPTAC (Fig. 1e and f). According to the equa-

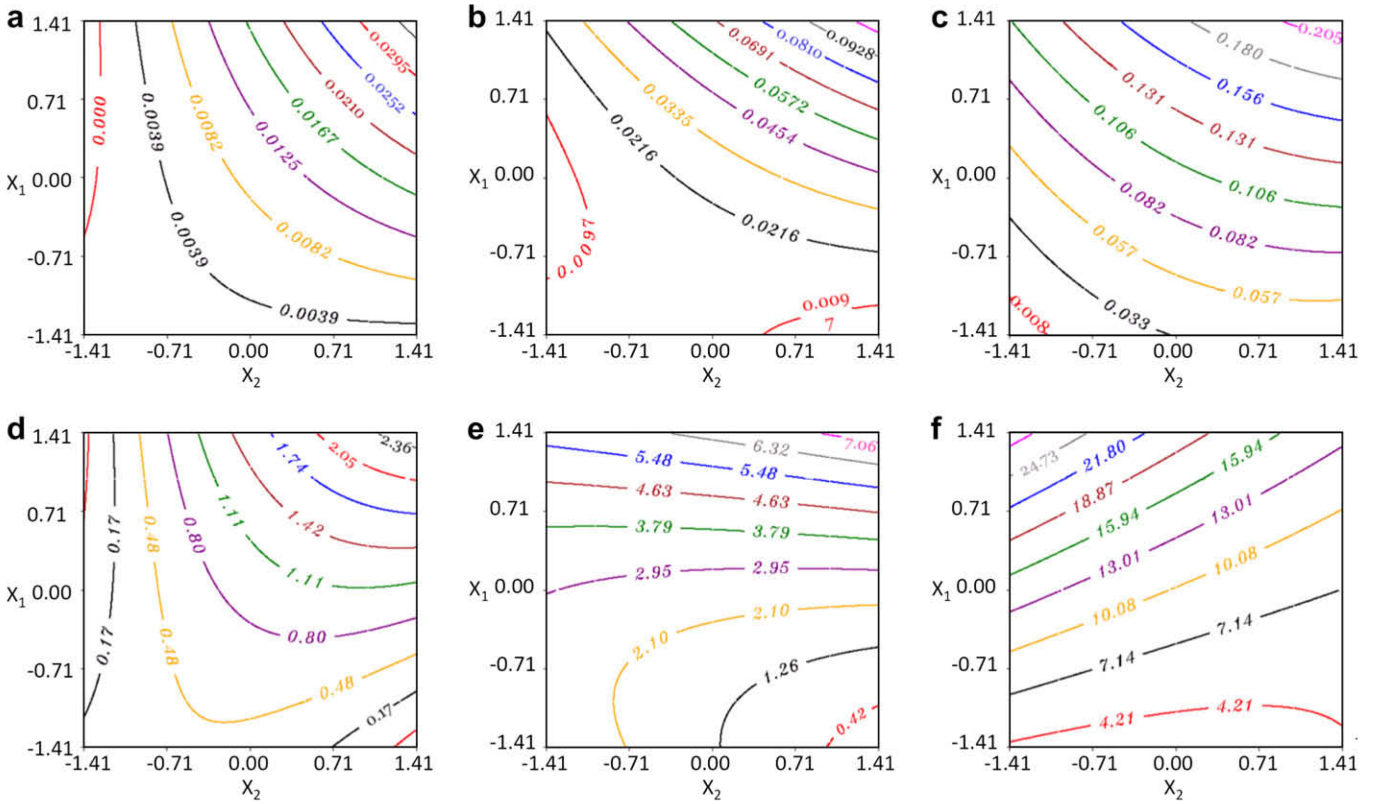

Fig. 1. Contour plots of the effects of temperature (X_1) and amount of CHPTAC (X_2) on the response surfaces of degree of substitution (DS) (a–c) and reaction efficiency (RE, %) (d–f) of cationic starches obtained after reacting for 2 (a and d), 5 (b and e), and 24 h (c and f).

Table 2
Regression coefficients for the determinations of the degree of substitution (DS), reaction efficiency (RE), pasting, and thermal properties of cationic starches[a]

Reaction time (h)	Parameter	DS	RE	Pasting property						Thermal property				
				PT	PV	HV	BD	FV	SB	T_o	T_p	T_c	WD	ΔH
2	Intercept	0.0092^{**}	0.915^{**}	65.66^{**}	4316.85^{**}	853.23^{**}	3463.63^{**}	1706.72^{**}	853.49^{**}	58.76^{**}	65.24^{**}	73.35^{**}	14.59^{**}	11.65^{**}
	X_1[b]	0.0052^{**}	0.415^{*}	-1.91^{**}	1100.25^{**}	-36.24	1136.50^{**}	-115.70	-79.46^{**}	-1.97^{*}	-1.91^{*}	-1.86^{*}	0.11	-0.23
	X_2	0.0070^{**}	0.388^{*}	-4.36^{**}	1754.58^{**}	-66.00	1820.57^{**}	-189.29^{**}	-123.30^{**}	-2.61^{**}	-2.35^{**}	-1.86^{*}	0.75^{**}	-0.41
	X_{11}	0.0004	0.036	-0.30	472.30	26.65	445.65	31.97	5.32	0.43	0.45	0.32	-0.12	-0.42
	X_{12}	0.0046^{*}	0.405	-2.61^{**}	1096.75^{*}	-79.00	1175.75^{*}	-147.00	-68.00^{*}	-2.51^{*}	-2.39^{*}	-2.46^{*}	0.04	-0.18
	X_{22}	-0.0002	-0.212	0.08	-251.67	-4.36	-247.31	-13.30	-8.93	-0.19	-0.17	-0.09	0.10	-0.14
5	Intercept	0.0258^{**}	2.579^{**}	57.19^{**}	6811.82^{**}	690.00^{**}	6121.81^{**}	1337.50^{**}	647.49^{**}	50.87^{**}	57.93^{**}	68.26^{**}	17.39^{**}	9.64^{**}
	X_1	0.0199^{**}	1.849^{**}	-9.46^{**}	2174.53^{**}	-4.71	2179.24^{**}	71.45	76.16	-6.23^{**}	-5.95^{**}	-6.49^{**}	-0.26	-2.97^{**}
	X_2	0.0132^{**}	-0.189	-6.81^{**}	2267.83^{**}	-17.18	2285.01^{**}	-48.71	-31.53	-5.02^{**}	-4.72^{**}	-3.51^{*}	1.52^{*}	-1.99^{**}
	X_{11}	0.0071^{*}	0.681	-3.01^{*}	556.71^{*}	49.00^{*}	507.71^{*}	191.66^{**}	142.65^{**}	0.62	0.27	-1.61	-2.23^{**}	-2.19^{**}
	X_{12}	0.0124^{**}	0.575	-4.75^{**}	1554.50^{**}	50.50	1504.00^{**}	212.00^{**}	161.50^{*}	-3.56^{*}	-3.23^{*}	-3.89^{*}	-0.33	-0.92^{*}
	X_{22}	-0.0005	0.067	0.49	-139.50	57.51^{*}	-197.01	168.65^{**}	111.14^{*}	0.70	0.87	1.41	0.70^{*}	0.23
24	Intercept	0.0992^{**}	9.916^{**}	35.12^{**}	3111.72^{**}	211.50^{**}	2900.22^{**}	381.49^{**}	170.00^{**}	41.83^{-}	46.67^{**}	50.97	9.14^{*}	0.63
	X_1	0.0504^{**}	6.160^{**}	-21.91^{**}	136.46	-4.34	140.80	-109.31	-104.97	2.03	1.77	-2.43	-4.46	-3.42
	X_2	0.0271^{**}	-2.693^{**}	-18.69^{**}	444.61^{*}	37.05^{*}	407.56^{*}	15.25	-21.80	-1.34	-1.23_{*}	-3.43	-2.10	-2.32
	X_{11}	0.0027	0.867	-3.56^{**}	-770.21^{*}	-37.85	-732.36^{*}	-122.80^{*}	-84.95	6.42^{**}	7.19	6.19	-0.23	0.59
	X_{12}	0.0085_{*}	-1.928^{*}	-11.20^{**}	-292.40	4.12	-296.52	-84.66	-88.78	3.46^{*}	3.89	3.39	-0.07	0.58
	X_{22}	-0.0074	0.504	-4.21^{**}	-450.79^{*}	-22.02	-428.78^{*}	-36.62	-14.61	-0.04	1.09	4.23	4.28	1.41

[a] Values superscribed with ** and * indicated that the regression was significant at $p < 0.05$ and $p < 0.01$, respectively.
[b] X_1 = factor 1 (temperature); X_2 = factor 2 (molar ratio of CHPTAC/AGU); X_{11} = second order of factor 1; X_{12} = interaction of factors 1 and 2; X_{22} = second order of factor 2.

tion, RE is a function of DS and the amount of CHPTAC. Therefore, the insufficient multiplying in DS with respect to the increasing amount of CHPTAC would lead to the steady or even downward trend of RE, as observed in 5 and 24 h cationized starches. Khalil, Farag, and Hashem (1993) studied the starch cationization under different amount of NaOH, and found out the RE of cationic starches fell down when the ratio of hydroxide/amine was > 2.0. They explained that this was due to an unfavorable side reaction, in which the epoxide groups on cationizing agent hydrolyzed to form diols under the catalysis of hydroxyl radicals. However, the starch cationization in the present study was carried out at a constant pH of 11; the molar ratio of hydroxide/amine under this circumstance was considerably low compared to that of Khalil et al. (1993). Another suspected side reaction responsible for the decrease in RE was the de-etherification, which preferentially occurred at high temperature and long reaction duration. Still, whether the reduction in RE here was dominated by saturated cationization, by progressing side effects or both requires further validation. Although heterogeneous cationization, in which starches were kept in granular state, is inherently prone to result in lower RE than that of a homogeneous one (Heinze et al., 2004), the average RE acquired in this study is relatively low compared with those derived from similar cationization process (Bendoraitiene, Kavaliauskaite, Klimaviciute, & Zemaitaitis, 2006). Bendoraitiene et al. (2006) proposed that the RE of cationic starches might be attenuated if the amount of free water in the reaction medium far exceeds the one practically required. Accordingly the rather large bulk solution, which must contain respectable amount of free water, could be assigned to the low RE in the present study. Based on the response surfaces derived, cationic starches with the highest RE in this study could be obtained when high temperature (≧50 °C), low amount of CHPTAC (molar ratio of CHPTAC/AGU ≦ 0.65) and long reaction duration (24 h) were applied.

3.2. Effects of reaction conditions on the pasting properties of cationic starches

Due to extremely high viscosity in cold water, the 24 h cationized starches had concentration reduced from 8% to 2.5% for the RVA measurements. Furthermore, the PT and PV of the sample derived from test 10 in 24 h cationized starches could not be determined due to a sharp drop of viscosity at the beginning of the test, therefore, the RSM calculation of PT and PV of 24 h cationized starches were performed based on the data from the other nine samples. The pasting properties of starch were significantly altered after the cationization (Fig. 2). When cationic groups were introduced onto the starch molecules in native starch granules, the electrostatic repulsion (the Columbic force) and the stereo hindrance between each cationic group prohibited the inter-chain associations (the hydrogen bonding) among each starch molecular chain. This resulted in a weakened granular structure that, when being heated in water, permitted faster and greater water uptake in cationized starches than in native one. Consequently, the low PT, and high PV were observed in cationic starches. Also, the loosened structure in starch granules caused the rapid loss of granular integrity, which was evidenced by the large BD (Kweon et al., 1997; Liu, Corke, & Ramsden, 1999; Siau et al., 2004). Similar to the change in DS, the decrease in PT of cationic starches was initially more dependent on the increase in temperature but thereafter became primarily affected by the amount of CHPTAC when reaction time was extended from 2 to 5 h or more (Table 2 and Fig. 3a–c). This concurrence, as confirmed by the significant correlations between PT and DS ($R = -0.965$ and -0.995 for the 2 and 5 h cationic starches, respectively), implied a facile control of the gelatinization properties of cationic starches through appropriate regulation on their DS. The PT values below room temperature shown on the response surface of 24 h cationized starches (Fig. 3c) suggested that the enhance in DS of cationic starches could promote their cold water solubility and dispersibility, hence their paste clarity as well as stability against retrogradation (Solarek, 1986). For 2 and 5 h cationized starches, the profiles of PV (Fig. 3d and e) are very similar to those of their DS; that is, the higher the DS, the larger the PV. The significant correlations between PV and DS ($R = 0.971$ and 0.968 for the 2 and 5 h cationic starches, respectively) also identified with this phenomenon. By contrast, PV of 24 h cationized starches (Fig. 3f) reached a maximum near the center of the contour map and then went downward with even higher DS, leading to low correlation coefficient between PV and DS ($R = 0.038$). This might be due to an overall decay of granular integrity, which fastened the collapse of the crystalline region in starch granules, beyond a certain level of DS (Kuo & Lai, 2007). With increasing DS, the SB of 2 h cationized starches (Fig. 3g) generally fell down, while that of 5 and 24 h ones (Fig. 3h and i) displayed a maximum followed by a downward trend. The change in SB of cationic starches could be governed by a number of factors and by the interactions of these factors. First of all, the repulsion originating from the stereo effect and Columbic force between cationic groups prevented the reassociation of starch molecules after being gelatinized, and thus reduced the SB of cationic starches (Siau et al., 2004). On the other hand, however, as the compactness of starch granules was weakened by the introduced cationizing agent, the amount of released amylose could be consequently promoted during starch pasting, and thus contributed to an increase of SB (Liu et al., 1999). Besides, it has been reported that complete dispersibility of amylose and amylopectin from the starch granules could be attained after the cationization (Yook et al., 1994). Granules of cationic starch with high DS were thought to burst more easily and completely, resulting in a more homogeneous starch solution, in which the molecules and fragments of starch rearranged to develop a more organized gel structure with higher SB. Therefore, the change in SB of cationic starches should be ascribed to a combined result of several effects, including the molecular repulsion, the amount of amylose released during heating, and the final homogeneity of the starch solution. For cationic starches reacted for long duration (24 h), it is hypothesized that the outcome of increased amylose leaching and the better dispersibility of starch solution overwhelmed the influence of molecular repulsion, and thus resulted in a net increase in SB.

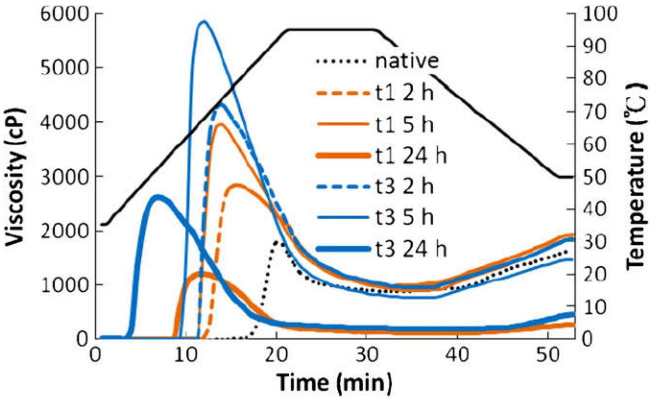

Fig. 2. Viscograms of native and cationic starches test 1 (*t*1 2 h, *t*1 5 h, and *t*1 24 h) and test 3 (*t*3 2 h, *t*3 5 h, and *t*3 24 h).

3.3. Effects of reaction conditions on the thermal properties of cationic starches

As in the case of pasting properties, the thermal properties of native starch were also modified after the cationization. Generally the lower transition temperatures (T_o, T_p, and T_c), smaller ΔH and broadened WD of the gelatinization endotherm were obtained. However, the 24 h cationized starches showed no significant peak of heat flow in the DSC thermograms, indicating their complete gelatinization during the 6 h storage at room temperature prior to the heating program. This fact, together with the low pasting temperature appeared on the response surface, pointed out that the highly altered structure of these 24 h cationized starches could be readily disintegrated upon moisture treatment. Hence the regression analysis for response surfaces of thermal properties was only performed on cationic starches reacted for 2 and 5 h. The single narrow gelatinization endotherm in the plot of heat flow versus temperature recorded by DSC is ascribed to the water-mediated melting of starch crystallites, initiated by the stripping of starch chains in the swollen amorphous regions (Aggarwal &

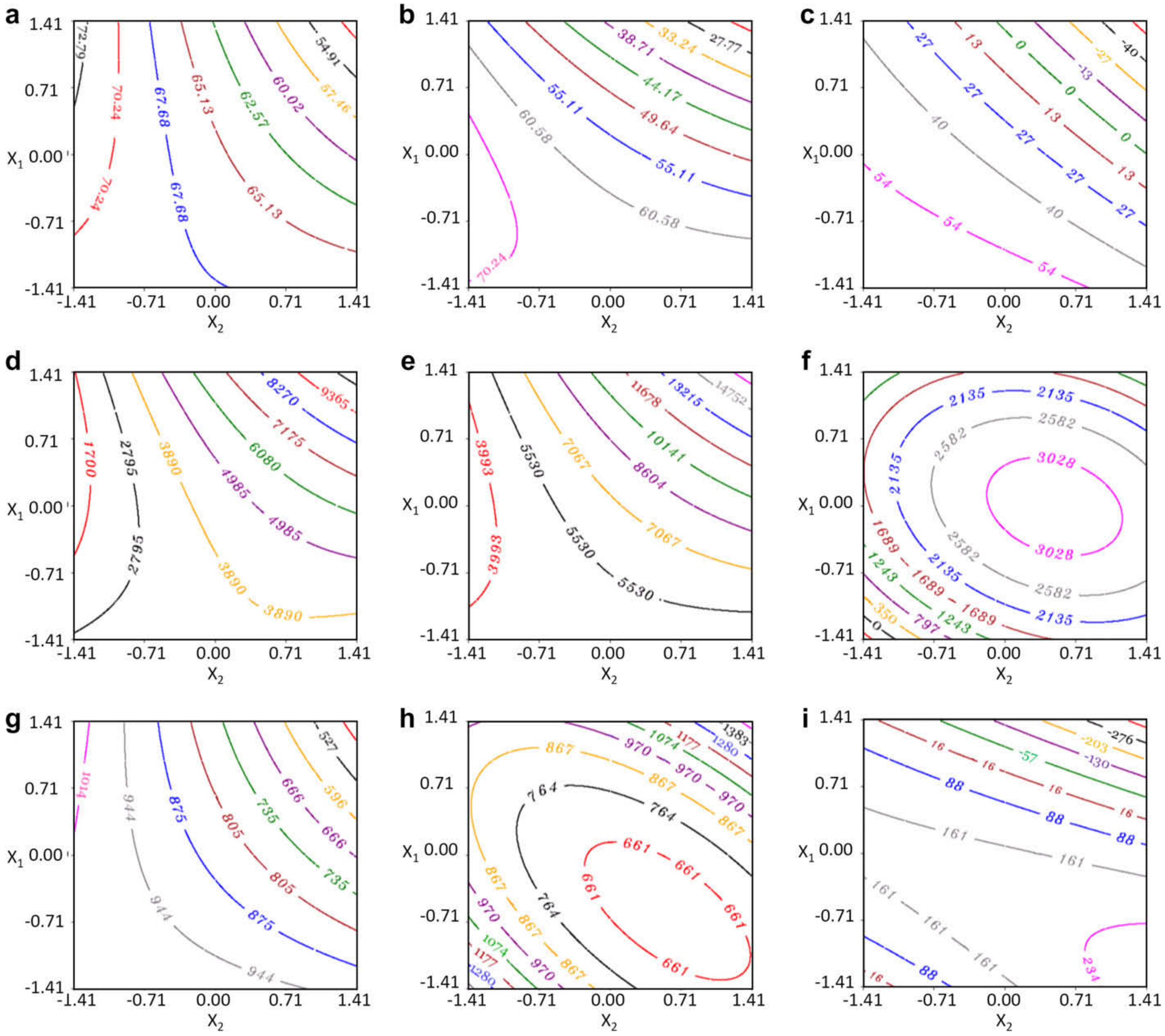

Fig. 3. Contour plots of the effects of temperature (X_1) and amount of CHPTAC (X_2) on the response surfaces of pasting temperature (PT, °C) (a–c), peak viscosity (PV, cP) (d–f), and setback (SB, cP) (g–i) of cationic starches obtained after reacting for 2 (a, d, and g), 5 (b, e, and h) and 24 h (c, f, and i).

Dollimore, 1998). It has been already known that the melting of amylopectin crystallites of starch granules could be accelerated through certain chemical modifications (Biliaderis, 1982; Biliaderis, Maurice, & Vose, 1980). After the cationization, the hindered chain–chain interaction of starch molecules due to the presence of cationic groups resulted in a loosened, more hydrophilic structure compared to that of native starch (Siau et al., 2004). During heating, the increase in hydration of starch molecules, starting from the amorphous region, provided more water for the crystal melting, thus resulted in a lower transition temperature and smaller gelatinization enthalpy than that of native starch (Kweon et al., 1997). As shown in Fig. 4a and b, the T_p of the 2 and 5 h cationized starches were highly correlated with the DS of their own ($R = -0.937$ and -0.948 for 2 and 5 h ones, respectively), suggesting a feasible modification of the thermal properties of cationic starches through regulation on the DS. With increasing amount of CHPTAC, the WD of the gelatinization endotherm of 2 h cationized starches increased correspondingly (Fig. 4c), while that of the 5 h cationized ones was more complicated (Fig. 4d), probably because of a more intense alteration on the prime structure of the starch granules. Contrary to the significant correlation between ΔH and the DS of 5 h cationized starches, the counterpart of 2 h cationized starches was insignificant. A detailed survey of the response surface (Fig. 4e) interpreted that the lack of correlation between ΔH and DS of 2 h cationic starches occurred only in the realm of low DS. Therefore, it could be postulated that in 2 h cationized starches, the cationic substitution occurred preferentially at the outer surface and in the amorphous region of starch granules, with low DS that was ineffective in inducing the melting of crystalline region, and thus on the reduction of ΔH value. Fig. 5 shows the CLSM optical sections of methanol–APTS-stained native and cationic starches test 7 after 2, 5, and 24 h of cationization. The diffusion pathway of methanolic APTS toward the granules of water phase cationized starches had been previously monitored with a confocal microscopy (Kuo & Lai, 2007). The migration of APTS in cationic starches was found to be the same as in native

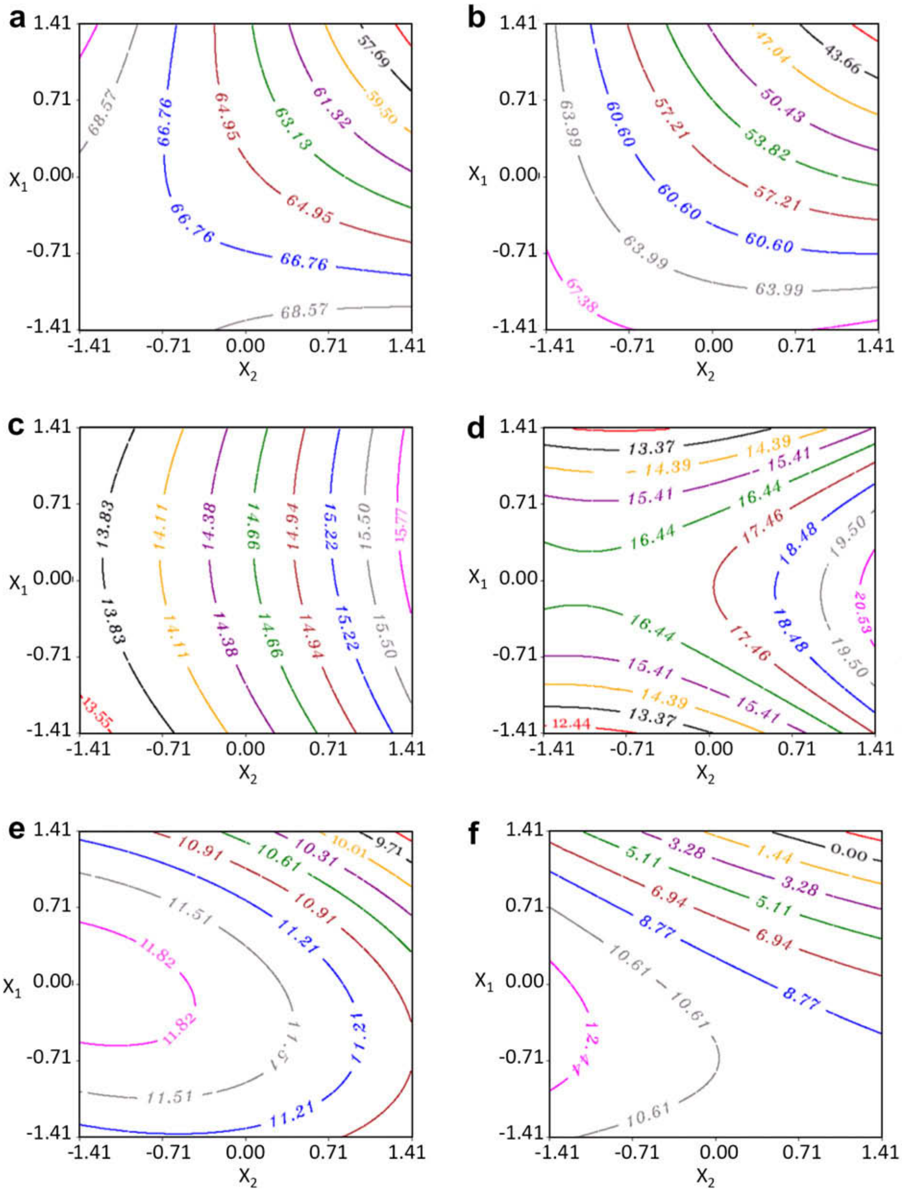

Fig. 4. Contour plots of the effects of temperature (X_1) and amount of CHPTAC (X_2) on the response surfaces of peak temperature (T_p, °C) (a and b), peak width (WD, °C) (c and d) and gelatinization enthalpy (ΔH, J/g) (e and f) of cationic starches obtained after reacting for 2 (a, c, and e) and 5 h (b, d, and f).

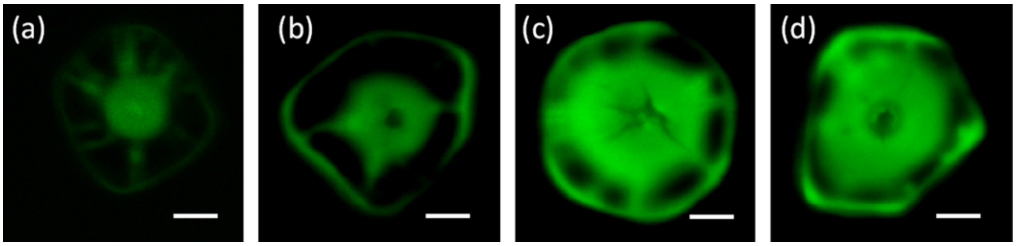

Fig. 5. CLSM optical sections of methanol–APTS-stained native (a) and cationic starches test 7 after reacting for 2 (b), 5 (c), and 24 h (d), scale bars correspond to 4 μm.

corn starch, where the APTS preferentially accumulated in the outer layer, the channels, as well as in the central cavity of starch granules, where the molecules arrange in a less ordered manner (Buleon, Colonna, Planchot, & Ball, 1998; Chung & Lai, 2006). The internal layer between the outer surface and the central matrix, believed to be primarily composed of semicrystalline starch, was the hardest area to be dyed. The APTS in 2 h cationized starches (Fig. 5a) centered in the interior region and on the outermost layer of granules, while in cationic starches reacted for more than 5 h much more APTS had penetrated outward into the internal layer (Fig. 5b and c). As cationic substitution weakened the structural integrity of starch granules, exposing more reducing ends on starch molecules to APTS, the change in APTS intensity in cationic starch could indicate the shift in regions of cationization inside the granules. The difference of dyeing pattern between 2, 5 and 24 h cationized starches thus could testify the deduction that the cationization occurred mainly in amorphous region in 2 h cationized starches, while began to modify the semicrystalline region in 5 and 24 h cationized starches.

3.4. Chain length distribution of amylopectin of cationic starches

Fig. 6 shows the anion exchange chromatograms of the isoamylase-debranched amylopectins from native and cationic starches tests 5 and 10 after reacting for 2, 5, and 24 h. To the best of our understanding, this is the first time for cationic starches the clear chain length distributions of amylopectins were obtained. The number on top of each peak depicted the DP of the certain side chain. The chain length of isoamylase-debranched amylopectin in native starch (Fig. 6b) distributed continuously from DP 6 to as high as DP 37, with all the peaks clearly separated from each other. In the chromatograms of cationic starches (Fig. 6c–h) by contrast, the highest DP with identifiable peaks decreased with increasing in the reaction time and the amount of CHPTAC. Besides, the signal intensities in the chromatograms of the 24 h cationized starches (Fig. 6g and h) were especially low compared to those of others. Furthermore, in the chromatograms of all of the cationic starches, the peak at the position corresponding to DP 7 seemly 'branched' into 2 or 3 peaks. The decrease in signal intensities in cationic starches could be simply due to lessened starch concentration of the injected samples, as change in physicochemical properties might affect the amount of cationic starches obtained after sample preparation for HPAEC analyzing. Manelius, Nurmi, and Bertoft (2005) reported that cationization on starch at higher substitution concentration could block the isoamylase action, leading to insufficient hydrolysis at the branch sites. This may reduce the amount of starch molecules available after filtration. However, when the concentrations of the to-be-injected samples from native and the 24 h cationized starches were determined and adjusted with the buffer solution to achieve equal value, the intensities of the 24 h cationized starches (data not shown) were still significantly lower than that of native one. The single peak appeared within 3 min in the elusion profile of native starch corresponded to the solvent in the sample solution. Since the density of cationic groups on starch molecules should grow higher with rising DS, the diminishing intensity, together with the abnormally high solvent peak of the 24 h cationized starches, suggested that a considerable number of cationic molecules in these samples were eluted out directly, instead of being exchanged in the column. The substitution of CHPTAC onto a starch molecular chains adds to their molecular

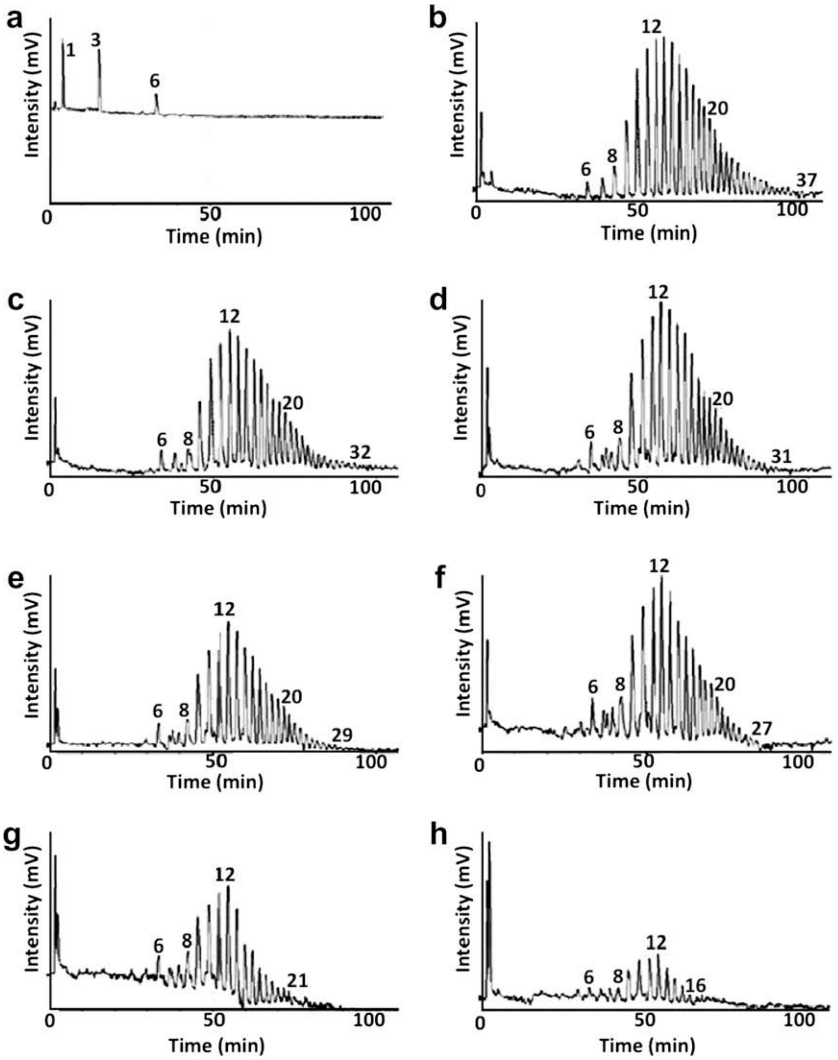

Fig. 6. Anion exchange chromatograms of glucose, maltotriose, and maltohexaose standards (a), native corn starch (b), cationic starches test 5 after reacting for 2, 5, and 24 h (c, e, and g), test 10 after reacting for 2, 5, and 24 h (d, f, and h). The number on the top of each peak indicated the degree of polymerization (DP) of the chain.

weight, hence may delayed their elution from the AEC column. On the other hand, however, due to the electrostatic repulsion of cationic charges between the cationic starches and the column resins, the cationic starches were also possible to elute out in advance. The 'branching' signals occurred at DP 7 were thus reasonable considering the two effects described above. Bertoft (2004) analyzed the chain length distribution of amylopectin from native starch after different degree of lintnerization and found out that the amylopectin side chain corresponding to DP 6–8 distributed predominantly in the amorphous region of starch granules. Therefore, the increasing significance of the 'branching' signals at DP 7 with raised DS of cationic starches would confirm the hypothesis about the preferential amorphous substitution based on the results from the MDSC and CLSM observations. The amorphous growth rings were believed to be responsible for this concentrated substitution (Manelius, Maaheimo, Nurmi, & Bertoft, 2002). Although previous studies combining enzyme hydrolysis and HPAEC have pointed out that the substitution of cationic groups in starch granules occurred preferentially in the amorphous regions (Manelius et al., 2000), the chain length distributions in the present work allowed us to further pinned down the mostly substituted side chain of amylopectins to be of DP 7. Furthermore, it is worth noting that for the 5 h cationized starch of test 10 the peak corresponding to DP 10 contained characteristic 'shoulders' attached to the main peak. This indicated not only the progressing cationization toward the crystalline regions but also the concentrated substitution on certain amylopectin side chains during this period.

4. Conclusions

In this study, cationic starches with DS ranging from 0.003 to 0.121 were prepared from the reaction of native corn starch with (3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) for 2, 5, and 24 h at pH 11 in aqueous phase without the addition of swelling–inhibiting salts. According to the RSM investigations, the impact of temperature on the increase in DS and RE, as compared with that of the amount of CHPAC, grew stronger as the cationizing time was prolonged from 2 to 5 h. The significant correlations between the DS and many of the pasting as well as the thermal properties of the 2 and 5 h cationized starches suggested a feasible managing of their gelatinization characteristics through control of the DS. The low PT and undetectable gelatinization endotherm of the 24 h cationized starches indicated their cold water solubility. For cationic starches reacting for 2 and 5 h, the various correlations of DS with SB, ΔH, and WD revealed their disparity in pattern of granular cationization. This difference was testified with the results of CLSM and HPAEC characterizations.

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