Carbohydrate Polymers 77 (2009) 25–31

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Optimization of the synthesis of 1-allyloxy-2-hydroxy-propyl-starch through statistical experimental design

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ARTICLE INFO

Article history:
Received 16 June 2008
Received in revised form 6 November 2008
Accepted 28 November 2008
Available online 10 December 2008

Keywords:
Biopolymers
Starch modification
Degree of substitution
Experimental design

ABSTRACT

The synthesis of 1-allyloxy-2-hydroxy-propyl starches was studied using a statistical experimental design approach. The etherification of two different granular maize starches with allyl glycidyl ether (AGE) in a heterogeneous alkaline suspension was investigated. The optimal reaction conditions were found via experimental design and the obtained response factor, e.g. the degree of substitution (DS) of the starch hydroxyl group, was statistically evaluated. The effects of six process factors on DS, namely the starch concentration, the reaction time, the temperature, and the amount of NaOH, Na_2SO_4 and AGE were investigated. The statistical analysis showed significant impact of the temperature, the amount of NaOH and the amount of AGE on the DS for both starches. Optimum conditions for the highest DS for waxy maize starch were: 0.0166% AGE (based on dry starch (ds)) and 1.0% NaOH (ds) at 34 °C in 4 h; on dent maize starch, these were 0.0099% AGE (ds) and 1.0% NaOH (ds) at 37 °C in 16 h.

1. Introduction

Starch is the most widely produced industrial polysaccharide. Starch production keeps on growing continuously because of the steadily increasing demand in food and non-food applications. In 2005, 58 million tons of starches were produced worldwide (Grüll, Jetzinger, Kozich, Wastyn, & Wittenberger, 2006). Because of its abundance and properties, starch has been of interest in pharmaceuticals, biodegradable plastics, paper etc. Chemical and enzymatic modification is a way to alter the structure of starch in a controllable manner, to enhance functional properties of starches and to extend the number of applications (Moser, 1986; Taggart, 2007). Etherification and esterification are the most common chemical derivatisations (Grüll et al., 2006; Rutenberg & Solarek, 1984). The properties of modified starches are depending upon the number, the distribution and the nature of the substituents (Richardson & Gorton, 2003; Taggart, 2007) and those with a degree of substitution (DS) up to 0.20 are of commercial importance (Taggart, 2007).

In our previous work, we have synthesized and characterized etherified granular starch derivatives containing allyl groups (Huijbrechts et al., 2007, 2008). We have shown that granular maize starch could be etherified easily by reacting allyl glycidyl ether (AGE) in heterogeneous suspension in the presence of Na_2SO_4 and NaOH as, respectively, starch granule stabilizer (Huijbrechts et al., 2007; Richardson & Gorton, 2003; Rutenberg & Solarek, 1984) and reaction enhancer (Burgt et al., 2000; Huijbrechts et al., 2007) leading to DS up to 0.20. The reaction temperature was kept below the gelatinization temperature of the substrate starches to facilitate the production of starch derivatives in granular form (Kesler & Hjermstad, 1950; Richardson & Gorton, 2003; Rutenberg & Solarek, 1984). The etherification of starch with AGE changed not only the chemical structure of granular maize starches, but also the physicochemical properties were altered (Huijbrechts et al., 2008). Waxy maize and dent maize starch derivatives show higher final and pasting viscosity, better swelling power and solubility than their native counterparts.

The modification of starch most likely depends on a large number of reaction conditions, including the temperature and the reagent concentration. Moreover, the condition of the starch granule, i.e. the product quality, cannot be predicted with a physical model. For the optimization of chemical processes, an experimental design approach, including statistical analysis, can be used (Guan & Hanna,

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doi:10.1016/j.carbpol.2008.11.037

2004; Kshirsagar & Singhal, 2007; Montgomery, 2005; Myers & Montgomery, 1995; Romo, Peñas, Sevillano, & Isasi, 2006). Mostly, the experimental design is used to investigate reaction conditions in order to determine the most important process factors and secondly to determine the optimum conditions for a chemical process.

In this study, the optimization of the etherification of granular starch with AGE to achieve a high degree of substitution is studied. The synthesis of the new material 1-allyloxy-2-hydroxy-propyl-starch derivatives of dent maize starch and waxy maize starch is investigated with the aim to find the optimum process conditions. A two step approach was followed: firstly the process parameters were identified (screening phase), and secondly the processes were optimized based on these parameters (improving phase). In the screening phase, the starch concentration, the reaction time, the temperature, the amount of NaOH, the amount of Na_2SO_4 and AGE were evaluated as reaction variables. To the best of our knowledge, this is the first time that such a study to optimize process parameters for starch etherification with allyl glycidyl ether using response surface analysis is reported.

2. Experimental part

2.1. Materials and methods

Dent maize starch (27% amylose, MS), and waxy maize starch (0.9% amylose, WMS) were gifts from Tate & Lyle Food and Industrial Ingredients Europe (The Netherlands). Allyl glycidyl ether ($\geqslant$99% cat. A32608), sodium hydroxide standard solution (0.5065 *N*, cat. 319503) and dimethyl sulfoxide-d_6 (99.5%, cat. 175943) were purchased from Sigma-Aldrich Chemie B.V (The Netherlands). Sodium sulphate (99%, extra pure, anhydrous, cat. 19664) was obtained from Acros Organics (Belgium).

Parallel reactions of the experimental design were performed in Radley's carousel of 12 reactors equipped with 20-ml tube and magnetic stirrings bars (medium cross type).

^{1}H NMR spectra were recorded with a Bruker DPX300-MHz spectrometer at 45 °C. All ^{1}H NMR measurements were performed in DMSO-d_6 containing maleic acid as an internal standard. Prior to the experiment, the moisture content of WMS and MS was determined using a Karl Fisher titrator (Huijbrechts et al., 2007).

Design-Expert Version 7.1.3 (Stat-Ease Co. Minneapolis, MN, USA) was used to conduct the statistical analyses, surface plotting and optimization.

2.2. Experimental procedure

The synthesis of 1-allyloxy-2-hydroxy-propyl-starch (AHP-starch) proceeds via a one-step reaction as described in previous studies (Huijbrechts et al., 2007, 2008). As an example, experiment nr **1** of waxy maize starch is taken to explain the procedure. For the other experiments the same procedure was used with adjusted amounts of chemicals, time and temperature, as given in Table 2.

2.22 g of waxy maize starch (10.8% H_2O, 200 g/kg starch suspension (C_{starch}) and 0.1 g Na_2SO_4 (5% w/w dry starch (ds)) were added to 10 ml 0.02 M NaOH-solution (0.4% w/w ds) in a reaction tube. The reaction vessel (20-ml tube with a magnetic stirrer) was placed in the carousel thermostated at 20 °C. After heating to 20 °C, 0.34 ml allyl glycidyl ether (AGE, 0.0016% w/w ds) was added dropwise to the starch suspension and the mixture was stirred vigorously for 4 h. The reaction was stopped by cooling on ice-water. The reaction mixture was neutralized with 2.5 M HCl (pH = 7) and washed on a glass filter (G3) with H_2O (3 × 15 ml), ethanol (3 × 15 ml) and acetone (3 × 15 ml). The product yield (80%) was determined after drying in an oven at 60 °C for one night. The product yield was defined as the ratio of the weight of the dry product to the weight of the dry starch and the amount of AGE.

The moisture content of the products was determined by drying: 100 mg sample was added in a vial (1.5 ml and placed in a vacuum-oven at 60 °C for three days, until constant weight.

The degree of substitution (DS) of the product was determined using ^{1}H NMR and calculated using the equation described in the previous study (Huijbrechts et al., 2007). For this, a sample of AHP-starch (~8 mg) was dissolved in DMSO-d_6 (1 ml) and heated to 45 °C in order to obtain a higher resolution in the ^{1}H NMR spectra.

3. Results and discussion

The goal of the research described in this article was to determine the influence of different process conditions on the degree of substitution (DS) of 1-allyloxy-2-hydroxy-propyl-starch (AHP-starch) obtained during the modification with allyl glycidyl ether (AGE) (Huijbrechts et al., 2007). The influence of the process factors on the DS was investigated with the aid of an experimental design approach. In this approach several reaction parameters are varied between well chosen boundaries and the results are statistically analyzed.

Low DS etherified granular starch was synthesized in an aqueous slurry reaction, i.e. the starch modification was performed with high starch concentrations (20–40%) as usually applied in industrial processes (Funke & Lindhauer, 2001; Grüll et al., 2006; Moser, 1986; Radosta et al., 2004; Rutenberg & Solarek, 1984). As a catalyst, sodium hydroxide was added to enhance the AGE reaction with the primary hydroxyl group of starch (Huijbrechts et al., 2007; Kesler & Hjermstad, 1950; Radosta et al., 2004; Tsai & Meier, 1990; Warners, Stamhuis, & Beenackers, 1989). Consequently, to prevent swelling of starch under the alkaline reaction conditions, sodium sulphate was added as starch stabilizer (Moser, 1986; Richardson & Gorton, 2003; Rutenberg & Solarek, 1984). Furthermore, the etherification reaction was performed at temperature below 50 °C to avoid gelatinization of starch (Huijbrechts et al., 2007, 2008; Moser, 1986; Taggart, 2007).

A two stage approach was followed for experimental design: in the first stage the significant parameters were identified (screening phase) and in the second stage the process was optimized based on the significant parameters found in the screening phase (improving phase).

3.1. Screening phase

Based on our experience and reported literature (Huijbrechts et al., 2007; Kesler & Hjermstad, 1950; Radosta et al., 2004; Tsai & Meier, 1990; Warners et al., 1989), six process factors, which were expected to have a significant influence on the DS, were selected for the screening phase. The effect of the following factors was investigated: the starch concentration (C_{starch}, g/kg starch suspension), the reaction time (h), the temperature (°C), the amount of NaOH (as weight percentage related to the amount of dry starch, %

Table 1
Experimental design: six variables at three levels[a].

Variables	Min–max levels		
	−1	0	1
C_{starch} (g/kg slurry)[b]	200	300	400
Reaction time (h)	4	10	16
Temperature (°C)	20	34	48
NaOH (% ds)[c]	0.4	0.7	1.0
Na_2SO_4 (% ds)[c]	5	20	35
AGE (% ds)[c,d]	0.0016	0.0091	0.0166

[a] The levels are indicated with 1, 0 and −1.
[b] C_{starch} = starch suspension.
[c] Variables are calculated as % (w/w) on dry starch (ds).
[d] AGE = allyl glycidyl ether.

ds), the amount of Na_2SO_4 (% ds) and the amount of AGE (% ds). The different reaction factors, inclusive boundary values, are presented in Table 1. All treatments were performed in random order and data were analyzed using a response surface regression procedure.

Table 2 gives an overview of the performed experiments. The experimental conditions were generated by the software package "Design-Expert 7.1.3". The experimental design scheme contained the first 35 experiments. The center points, which have mid-spec

Table 2
Experimental design and responses for the DS values of waxy maize starch (WMS) and dent maize starch (MS) (DS_{starch}).

Screening phase								
Entry[a]	C_{starch}[c] (g/kg)	Reaction Time (h)	Temperature (°C)	NaOH (% ds)[e]	Na_2SO_4 (% ds)[e]	AGE[d] (% ds)[e]	DS_{WMS}	DS_{MS}
1	200	4	20	0.4	5	0.0016	0.000	0.000
2	400	4	20	0.4	5	0.0166	0.035	0.000
3	200	16	20	0.4	5	0.0166	0.000	0.000
4	400	16	20	0.4	5	0.0016	0.000	0.000
5	200	4	48	0.4	5	0.0166	0.031	0.000
6	400	4	48	0.4	5	0.0016	0.019	0.000
7	200	16	48	0.4	5	0.0016	0.023	0.007
8	400	16	48	0.4	5	0.0166	0.067	0.059
9	200	4	20	1.0	5	0.0166	0.029	0.002
10	400	4	20	1.0	5	0.0016	0.000	0.002
11	200	16	20	1.0	5	0.0016	0.011	0.001
12	400	16	20	1.0	5	0.0166	0.026	0.012
13	200	4	48	1.0	5	0.0016	0.015	0.006
14	400	4	48	1.0	5	0.0166	0.075	0.034
15	200	16	48	1.0	5	0.0166	n.d.[e]	n.d.[e]
16	400	16	48	1.0	5	0.0016	0.063	0.107
17	200	4	20	0.4	35	0.0166	0.003	0.000
18	400	4	20	0.4	35	0.0016	0.000	0.000
19	200	16	20	0.4	35	0.0016	0.000	0.000
20	400	16	20	0.4	35	0.0166	0.006	0.000
21	200	4	48	0.4	35	0.0016	0.016	0.002
22	400	4	48	0.4	35	0.0166	0.007	0.000
23	200	16	48	0.4	35	0.0166	0.131	0.107
24	400	16	48	0.4	35	0.0016	0.044	0.081
25	200	4	20	1.0	35	0.0016	0.000	0.000
26	400	4	20	1.0	35	0.0166	0.040	0.007
27	200	16	20	1.0	35	0.0166	0.015	0.010
28	400	16	20	1.0	35	0.0016	0.010	0.006
29	200	4	48	1.0	35	0.0166	0.136	0.032
30	400	4	48	1.0	35	0.0016	0.189	0.028
31	200	16	48	1.0	35	0.0016	0.128	0.069
32	400	16	48	1.0	35	0.0166	0.042	0.185
33	300	10	34	0.7	20	0.0091	0.113	0.025
34	300	10	34	0.7	20	0.0091	0.085	0.053
35	300	10	34	0.7	20	0.0091	0.115	0.024
Improving phase								
36	300	4	45	0.4	20	0.0016	0.005	0.011
37	300	4	45	0.4	20	0.0016	0.010	0.012
38	300	4	45	0.4	20	0.0166	0.003	0.008
39	300	4	45	0.4	20	0.0166	0.004	0.003
40	300	18	45	0.4	20	0.0016	0.008	0.015
41	300	16	45	0.4	20	0.0016	0.012	0.005
42	300	16	45	0.4	20	0.0166	0.022	0.009
43	300	16	45	0.4	20	0.0166	0.012	0.023
44	300	4	45	1.0	20	0.0016	0.004	0.004
45	300	4	45	1.0	20	0.0016	0.014	0.011
46	300	4	45	1.0	20	0.0166	0.019	0.012
47	300	4	45	1.0	20	0.0166	0.011	0.024
48	300	16	45	1.0	20	0.0016	0.044	0.020
49	300	16	45	1.0	20	0.0016	0.036	0.026
50	300	16	45	1.0	20	0.0166	0.019	0.011
51	300	16	45	1.0	20	0.0166	0.021	0.017
52	300	10	45	0.7	20	0.0000	0.000	0.000
53	300	10	45	0.7	20	0.0182	0.004	0.019
54	300	2	45	0.7	20	0.0091	0.011	0.008
55	300	22	45	0.7	20	0.0091	0.010	0.050
56	300	10	45	0.1	20	0.0091	0.005	0.011
57	300	10	45	1.3	20	0.0091	0.030	0.035
58	300	10	45	0.7	20	0.0091	0.009	0.024
59	300	10	45	0.7	20	0.0091	0.009	0.017
60	300	10	45	0.7	20	0.0091	0.007	0.019

The product yield of the screening phase and improving phase was, respectively, between [a]34–86% and [b] 34–90%.
[c] C_{starch} = starch concentration.
[d] The amount of NaOH, Na_2SO_4 and allyl glycidyl ether (AGE) are calculated as weight percentage related to the amount of dry starch (ds).
[e] Not determined.

factor settings, were repeated three times, to get an estimation of the reproducibility of the performed experiments. The 35 experiments of the design scheme were performed for each of the two maize starches used, waxy maize starch (WMS) and dent maize starch (MS). The obtained response, for the specification part of the screening, was the DS. The obtained DS values are also given in Table 2 (exp. 1–35).

As can be seen in Table 2 the DS values in the screening phase are between 0.000 and 0.189 showing that low substitution of granular starch was obtained under the performed reaction conditions. The product yield was found to be between 34–86%.

The DS value of condition number 15 has not been determined. The combination of the different factors resulted in gelatinized starches. This can be explained by the fact that the gelatinization temperature of starch in water is determined by several factors such as temperature, shear stress, amount of NaOH and amount of Na_2SO_4. It is common knowledge that the gelatinization temperature will decrease if the amount of NaOH and the shear stress are increasing, and the gelatinization temperature will increase with the increase of Na_2SO_4. Therefore, Na_2SO_4 helps to prevent gelatinization of starch during the reaction. Not gelatinized particles are favourable because gelatinized particles will give a lot of practical difficulties during purification, further modifications and processing. Although 5% (ds) of Na_2SO_4 was used in the experiment 15, this amount was not sufficient to compensate for the effect of the other factors on gelatinization of the starch. Since gelatinized starch is more accessible for AGE groups than granular starch, resulting in a higher DS, experiment 15 is not used in the model.

The responses of the different samples were analyzed using the statistical module of the experimental design program. The responses are statistically described with second-order interactions (2^m), which are characteristic for a Fractional Factorial Design. This approach allows to estimate the main effects and all interactions up to m (m is six variables in this study). The generalized regression model was used, as shown in Eq. (1).

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum \sum_{i<j} b_{ij} X_i X_j \quad \text{with} \quad i = 1{-}6 \text{ and } j = 2{-}6 \tag{1}$$

where Y = response, X_1 = starch concentration (C_{starch}), X_2 = reaction time, X_3 = temperature, X_4 = the amount of NaOH, X_5 = the amount of Na_2SO_4, X_6 = the amount of allyl glycidyl ether (AGE), b_0 = intercept and b_i = corresponding regression coefficients.

Significant factors were selected based on their F and p values in the statistical analysis. In this study, factors with a p value lower than 0.05 are significant. This means that, for example, when temperature appears to be a significant factor, the obtained DS is with a 95% certainty due to the increased temperature in the temperature range of 20–48 °C. This increase is higher than the standard error in DS. For both starches the model could not be optimized further.

Based on these statistical analyses, it was possible to determine the significant process factors. An overview of the effect of the critical process factor is shown in Table 3. In general, the temperature has the highest effect on AGE substitution for both starches.

WMS: The statistical reports show that only the effect of the temperature has a pronounced effect on the DS. The effect of other two process factors on the DS, the amount of AGE and NaOH concentration, is certainly present, although both main effects are non-significant. Furthermore, the starch concentration, the reaction time and the amount of Na_2SO_4 appear to have little or no effect. There are no interactions between the main factors which affect the substitution of WMS.

MS: The incorporation of AGE into MS is significantly affected by the reaction time, the temperature and the NaOH concentration. The other three process factors have a very small effect on the substitution. Additionally, some significant effects of interaction between the main factors on the DS were obtained. The combination of reaction time and temperature significantly affects the DS value of MS. Similarly, the combination of the NaOH concentration and the starch concentration has a significant effect on the DS value.

Table 3
Effect of the critical process factors in the screening phase for DS_{WMS} and DS_{MS}.

Factor	DS_{WMS}		DS_{MS}	
	F value	p value[a]	F value	p value[a]
C_{starch}	0.63	0.4340	0.36	0.5520
Reaction time	1.29	0.2674	77.81	**<0.0001**
Temperature	19.65	**0.0002**	120.02	**<0.0001**
NaOH[b]	4.07	0.0544	6.71	**0.0164**
Na_2SO_4[b]	0.81	0.3762	0.58	0.4559
AGE[b]	4.05	0.0550	1.05	0.3153
C_{starch} * C_{starch}	15.48	**0.0006**	–	–
C_{starch} * NaOH	–	–	4.35	**0.0483**
Reaction time * Temperature	–	–	68.65	**<0.0001**

[a] Factors in bold are significant ($p < 0.05$).
[b] Process factors are in amounts.

Based on this screening it was not possible to present a mathematical model (= equation) to describe the DS as a function of factor settings, since variables were only set at their min-max levels plus center points. However, this initial screening design allowed a primary selection of the reaction parameters that are significant for the DS. For the final optimization model the reproducibility of the model will be investigated in more detail. There will be even more replicates of the center points and duplicates of all selected star points.

3.2. Improving phase

Based on these screening results, our model was improved via a central composite experimental design (CCD). In this improving phase, four variables were evaluated, namely the reaction time, the temperature, the NaOH concentration and the amount of AGE. The levels for the other two process factors were constant at: 20% (w/w) Na_2SO_4 on dry starch (ds), and a starch concentration of 300 g/kg starch suspension. The experimental design scheme contained 25 additional experiments (exp. 36–60, see Table 2). The center points have mid-spec factor settings. Experiments at center point conditions have been repeated three times. Also, the influence of the factors at star point conditions has been checked. These star points have factor settings outside of the boundary ranges as defined in the screening phase. Extra samples, with factor settings within the boundaries, have been performed to improve the predictive power of the mathematical equations.

As can be seen all additional experiments could be performed on WMS and MS under the chosen conditions, since no gelatinization occurred. The DS values of the experiment 36–60 are up to DS of 0.050 which is lower than those of experiments 1–35 (up to DS of 0.189). This may be caused by the use of different starch batches. This difference is levelled off by using "blocks" in the statistical evaluation. Furthermore, the substitution of WMS and MS are different under the chosen experimental conditions in both phases. It seems that WMS is substituted to higher DS than MS. In the screening and improving phase, similar product yields were obtained.

Since reactions were performed in parallel, in a 12-tubes carousel, the error that might occur due to the position of each reaction tube on the circumference of the magnetic plate (i.e. variation in stirring speed, temperature, and turbulence). 24 Experiments were performed at center point conditions with maize starch, i.e. all reactions conditions were carried out at 45 °C for 10 h with 0.7%

Table 4
Regression equation coefficients[a] of a polynomial model[b] for DS on WMS and MS (DS_{starch}).

Coefficient	DS_{WMS}	DS_{MS}
b_0	-0.59044^{***}	-0.10711^{***}
Linear		
b_A	0.014647^{*}	$5.39\cdot10^{-4**}$
b_B	0.040043^{***}	$5.72\cdot10^{-3***}$
b_C	0.016657^{***}	0.015275^{***}
b_D	0.45841^{**}	3.41831
Cross product		
b_{AB}	$-1.11\cdot10^{-3***}$	–
Quadratic		
b_{BB}	$-5.91\cdot10^{-4***}$	$-7.72\cdot10^{-5**}$
b_{DD}	–	-172.717^{***}
Cubic		
b_{ABB}	$1.77\cdot10^{-5**}$	–
Probability of F	<0.0001	<0.0001
R^2 [c]	0.8294	0.6075
Adjusted R^2	0.8029	0.5540
Predicted R^2	0.7336	0.3824
Adeq.[d] precision	23.043	12.584

[a] *, ** and *** indicates significance at $p < 0.10$, 0.05 and 0.01, respectively.
[b] The model for degree of substitution (DS) as a function of the four process factors: A (reaction time), B (temperature), C (the amount of NaOH) and D (the amount of allyl glycidyl ether) were calculated as shown in Eq. (2).
[c] Coefficient of determination.
[d] Adequate.

ds NaOH and 0.0091% ds AGE. The influence of the position of the reaction tubes on DS was very small: average DS is 0.051 with a pooled standard deviation of 0.0032.

Again, the responses of the different samples were analyzed using the statistical module of Design-Expert 7.1.3. The responses are statistically described with second-order interactions and cubic terms, which are also characteristic for an optimization study as CCD. The generalized regression model is shown in Eq. (2).

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{iii} X_i^3 + \sum\sum_{i<j} b_{ij} X_i X_j + \sum\sum_{i<j<k}\sum b_{ijk} X_i X_j X_k$$
$$\text{with } i = A\text{–}D,\ j = B\text{–}D,\ k = C\text{–}D \qquad (2)$$

where Y = response, X_A = reaction time, X_B = temperature, X_C = the amount of NaOH, X_D = the amount of allyl glycidyl ether (AGE), b_0 = intercept and b_i = corresponding regression coefficients. Significant factors were selected based on their F and p values in the statistical analysis. In this study, factors with a p value lower than 0.05 are significant.

The results of the response surface model fitting in the statistical analysis are given in Table 4. The model is significant, as is evident from the probability for WMS and MS. The goodness of the fit of the model was checked by the determination coefficient (R^2). In this case, the value of the determination coefficient for WMS and MS (Table 4) indicates that less than 17.1% and 39.1% of the data, respectively, were not explained by the model. The value of adjusted determination coefficient (adj. R^2) is high for significances of the model. Consequently, the predicted determination coefficients (pred. R^2) are in reasonable agreement with adj. R^2 of both starches and the adequate precisions which measure the signal to noise ratio are greater than 4 (desirable). Based on the improving phase, it is possible to present a final optimization model to describe physical properties as a function of factor settings (Table 4). In addition, it was possible to determine the significant process factors.

The main effects of the process factors are shown in the perturbation plots (Fig. 1.). These plots represent the effects of all the factors at a particular point in the design, in this case the center point scaled at levels. Temperature has the highest effect on the AGE substitution for WMS and MS as obtained in the screening phase.

WMS: The temperature has a highly significant positive effect on the DS. The polynomial suggests that in the temperature range of 20–48 °C there is an optimum temperature for the incorporation of AGE (Fig. 1A). Less prominent, but significant is the NaOH concentration which has also a highly positive effect on DS for WMS. With an increasing amount of NaOH, more AGE is incorporated. Similarly, an increased amount of AGE induces an increased DS for WMS. The reaction time has the lowest effect and is not significant for the synthesis of AHP-WMS. However, a longer reaction decreases the AGE substitution. Furthermore, WMS is easier to substitute than MS, since a higher DS is obtained for WMS.

MS: The temperature has a highly significant positive effect on the synthesis of AHP-MS. Apparently, when the temperature is changed from 20 °C to 48 °C, there is an optimum temperature for the incorporation of AGE. Also, the NaOH concentration has a positive effect on the substitution. Although AGE is not significant as a main effect for substitution of MS, it is highly significant via the quadratic term as shown in the perturbation plot (Fig. 1B). Fi-

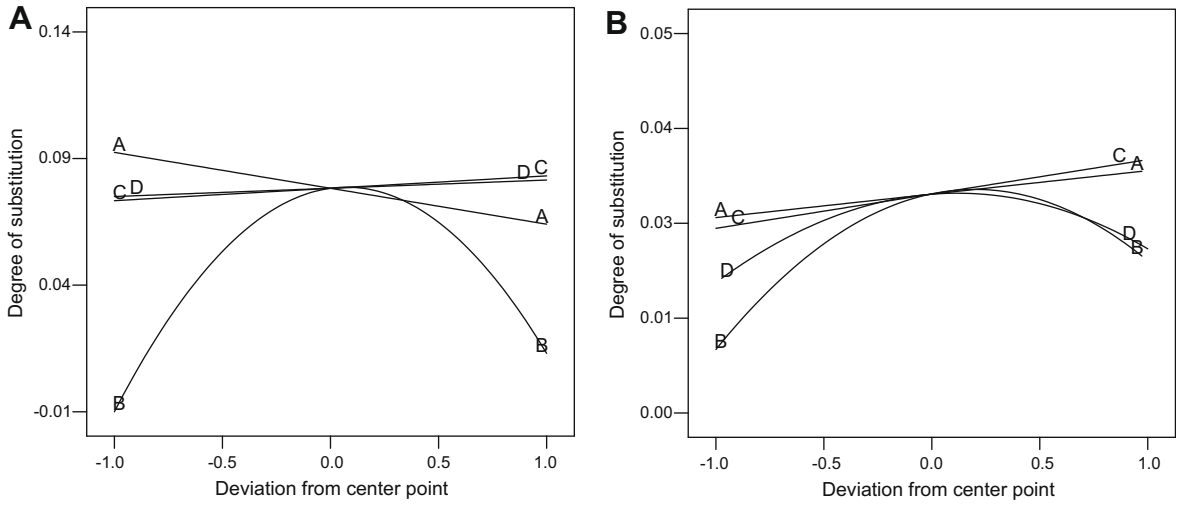

Fig. 1. Perturbation plots of modified WMS (A) and MS (B); *A*: reaction time, *B*: temperature, *C*: the amount of NaOH and *D*: the amount of allyl glycidyl ether generated at the center point (*A*: 10 h, *B*: 34 °C, *C*: 0.7% based on dry starch (ds) and *D*: 0.0091% ds).

nally, the reaction time is positively correlated to the incorporation of AGE in MS.

Fig. 2 shows the effect of the interactions between the process factors on the DS of WMS and MS.

WMS: As shown in the response surface plots for WMS (Fig. 2A), if the temperature is increased to around 37 °C, the highest substitution is obtained. A higher temperature reduces the DS value for the used amounts of AGE or the used reaction time. Furthermore, when the NaOH concentration or the amount of AGE is increased, the DS increases in the temperature range of 20–48 °C (not shown).

MS: The response surface plots for MS (Fig. 2B and C) show that when the NaOH concentration is increased at the optimum temperature of 37 °C, the highest DS value is obtained. Similarly, the amount of AGE induces the best AGE substitution at 0.010%, and with an increasing NaOH concentration, an increasing DS value is generated (not shown).

3.3. Optimization

In the improving phase the most pronounced process factors were obtained. Based on these parameters, the optimized conditions were generated using the optimization module of the experimental design program (Table 5). The aim was to have either a maximum DS, or maximum DS at low cost (i.e. lowest amount of AGE) or shortest reaction time for both starches.

As can be observed from Table 5, the optimal conditions for the best conversion of WMS and MS are different for all optimizations.

The optimized conditions generated with a maximum DS with no constraints of the other parameters show that the maximum DS that can be achieved for WMS and MS differs significantly, i.e. 0.102 for WMS and 0.039 for MS. It suggests that WMS is easier to substitute than MS with the generated process conditions. The most important difference in the process conditions between WMS and MS are the temperature and AGE concentration, 34.2 °C and 0.0166% ds and 37.0 °C and 0.0098% ds, respectively. However, even at these most optimal conditions the conversion efficiency is very low.

Subsequently, reaction conditions were generated which would give the maximum DS at the lowest amount of AGE and no constraints of the other conditions. The best substitution of WMS and MS is again at different temperature, respectively at 34.2 °C for WMS and at 37.0 °C in case of MS. The maximum DS of AHP-WMS is generated with lowest possible amount of AGE (0.0016% ds) with a remarkably efficient conversion. Oppositely, the synthesis of AHP-MS needs a higher amount of AGE (0.0031% ds) to generate the maximum DS. A slight decrease in substitution of both starches is revealed at these optimized conditions.

In the third optimization, the conditions were optimized for a maximum DS at the lowest amount of AGE in the shortest reaction time and no constraints for the temperature and the amount of NaOH. The best results for both starches are obtained after four hours. Even no differences in process conditions are obtained for a maximum DS of WMS because the shortest reaction time has already been achieved in the previous optimization. Consequently, the maximum DS for WMS is the same as in the optimized analysis. The maximum DS for MS (0.027) is lower than the DS value of the previous optimization. The optimum temperature for the synthesis of AHP-MS is 37.0 °C. Clearly, in all optimized analyses the best substitution of WMS is at a moderate temperature, around 34 °C in all cases. Furthermore, the highest NaOH concentration is needed for the best conversion of WMS and MS in the generated optimizations. The AHP-MS synthesis needs a higher amount of AGE (0.0038%) than AHP-WMS synthesis in this optimized analysis. Additionally, the conversion efficiency of WMS is similar to the previous optimization, although the conversion for MS is less efficient due to high amount of AGE and very low DS.

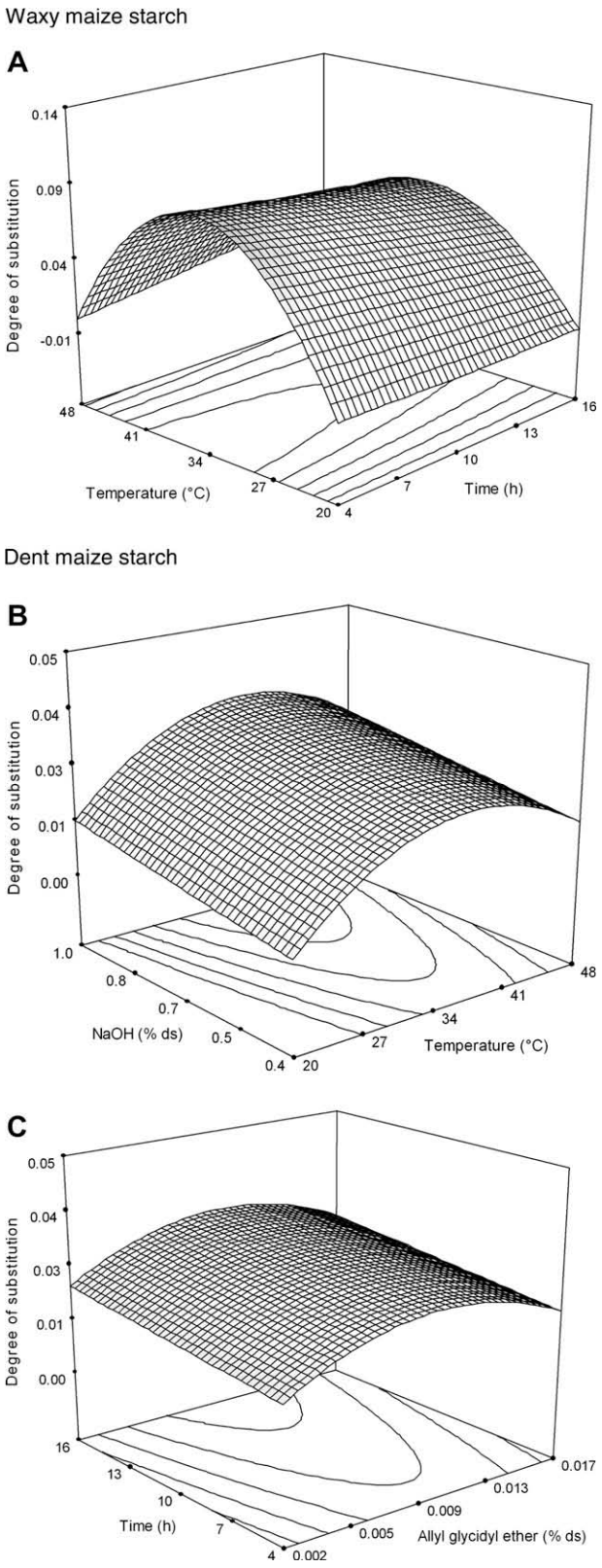

Fig. 2. Effects of temperature, the amount of allyl glycidyl ether, the amount of NaOH and reaction time on degree of substitution for waxy maize starch (A) and dent maize starch (B and C).

4. Conclusions

The influence of the different process conditions on the DS of AHP-WMS and AHP-MS is investigated via an experimental design

Table 5
Optimum conditions for the best conversion waxy maize starch (WMS) and dent maize starch (MS) with a maximum degree of substitution (DS), the lowest amount of allyl glycidyl ether (AGE) and the shortest reaction time.

Process factors	WMS			MS		
	Max. DS[a]	Lowest amount of AGE[b]	Shortest reaction time[c]	Max. DS[a]	Lowest amount of AGE[b]	Shortest reaction time[c]
Reaction time (h)	4.0	4.0	4.0	16.0	16.0	4.0
Temperature (°C)	34.2	34.2	34.3	37.0	37.0	37.0
NaOH (% ds)[d]	0.99	0.99	0.99	0.99	0.99	0.99
AGE (% ds)[d]	0.0166	0.0016	0.0016	0.0099	0.0031	0.0038
DS	0.102	0.096	0.096	0.039	0.031	0.027
Conversion efficiency (%)	4.3	42.1	42.0	2.8	7.1	4.9

[a] No constraints of the process factors.
[b] Maximum DS and no constraints of the other factors.
[c] Maximum DS and no constraints for temperature and the amount of NaOH.
[d] Process factors are in weight percentage related to the amount of dry starch (ds).

approach. In a two stage approach, a screening phase and an improving phase, the optimized process conditions have been investigated. The statistical analysis shows that the temperature has the largest effect on the modification of both starches. The NaOH concentration and the amount of AGE also have a large but less pronounced effect on the synthesis of AHP-starch.

The optimized analysis shows that WMS can be substituted up to DS = 0.102 using 0.0166% ds AGE, 1.0% ds NaOH at 34 °C in 4.0 h. Similarly, best conversion of MS can be up to DS = 0.039 using 0.0099% ds AGE, 1.0% ds NaOH at 37 °C in 16.0 h. The optimal generated conditions for the synthesis of AHP-WMS and AHP-MS differ extremely in the maximum DS and the conversion efficiency due to differences in the amount of AGE needed for the synthesis and the accessibility of starch for AGE.

Acknowledgments

We thank Mr. Barend van Lagen for ^{1}H NMR measurements. This research was conducted within the framework of the Carbohydrate Research Centre Wageningen, and partly financed by the Ministry of Agriculture, Nature and Food Quality of the Netherlands and the Dutch Ministry of Economic Affairs (project WSC. 6972).

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