Carbohydrate Polymers 82 (2010) 653–662

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Green starch conversions: Studies on starch acetylation in densified CO_2

Henky Muljana, Francesco Picchioni, Hero J. Heeres*, Leon P.B.M. Janssen

Department of Chemical Engineering, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 5 November 2009
Received in revised form 7 May 2010
Accepted 18 May 2010
Available online 25 May 2010

Keywords:
Acetylation
Potato starch
Starch acetate
Densified CO_2

ABSTRACT

The acetylation of potato starch with acetic anhydride (AAH) and sodium acetate (NaOAc) as catalyst in densified CO_2 was explored in a batch reactor setup. The effects of process variables such as pressure (6–9.8 MPa), temperature (40–90 °C), AAH to starch ratio (2–5 mol/mol AGU), NaOAc to starch ratio (0.1–0.8 mol/mol AGU) and water content (1–15.2%, w/w) on the degree of substitution (DS), the anhydride conversion (X_{AAH}) and the selectivity of the reaction (S_{SA}) were explored. At these conditions, acetylated potato starch with a range of DS values (0.01–0.46) is accessible in densified CO_2. The X_{AAH} varied between 10 and 80%, whereas the S_{SA} is relatively low (2–18%). Empirical models were developed to quantify the effects of the process variables on DS, X_{AAH} and S_{SA}. The temperature, water content and NaOAc to starch ratio have the highest effect on the DS, X_{AAH} and S_{SA} values. For comparison, a number of experiments were performed in water and the results confirm that densified CO_2 is a good solvent for the starch acetylation reaction.

1. Introduction

The properties of native starch sources are not always sufficient for the foreseen applications and often physical or chemical modifications are required. Esterification of starch is a well-known chemical modification reaction to improve the gelatinization temperature, thermal stability and to reduce the tendency for retrogradation (Betancur, Chel, & Canizares, 1997; Gonzalez & Perez, 2002; Jarowenko, 1986; Saartrat, Puttanlek, Rungsardthong, & Uttapap, 2005; Singh, Chawla, & Singh, 2004). Starch acetates are used in many applications. Examples are the use as thickening agents in a variety of food products, as sizing agents for textile (wrap sizing) and paper (surface sizing) and as gummed tape adhesives (de Graaf, Broekroelofs, & Janssen, 1998; Elomaa et al., 2004; Raina, Singh, Bawa, & Saxena, 2006; Shon & Yoo, 2006; Singh, Kaur, & Singh, 2004; Singh, Chawla, et al., 2004; Xu, Miladinov, & Hanna, 2004). For most applications, relatively low DS products are required (DS range from 0.01 to 0.2). Acetylated starch with DS values higher than 2 are of interest because of possible applications as packaging material. However, the poor mechanical properties of the acetylated starches, such as the pronounced brittleness, still constitute a major shortcoming for further application of the products as packaging materials (Junistia et al., 2008).

Starch acetates are commonly produced in water as the solvent, by using acetic anhydride (AAH) as reactant and an alkaline base like NaOAc as the catalyst (Scheme 1). The starch source is activated with the base to form a starch alkoxide (ST–O⁻) which is more reactive than native starch (Scheme 1.1) (Tijsen, Kolk, Stamhuis, & Beenackers, 2001). Subsequently, the starch alkoxide will react with AAH to form the acetylated product and NaOAc (Scheme 1.2). An undesired side reaction is the hydrolysis of AAH to form acetic acid (AA) (Scheme 1.3).

In commercial starch acetylation processes, starch is suspended in water at concentrations ranging between 35 and 42% (Jarowenko, 1986) and then the reagents are added with a given dose rate. A high water concentration is required to avoid mixing problems in the reactor. As a consequence, the selectivity of the reaction towards the desired starch acetate product (S_{SA}) is reduced due to the simultaneous hydrolysis of AAH to AA. To improve the selectivity, organic solvents such as pyridine and DMSO may be used. However, these solvents have a much higher environmental impact than water. Hence, there is a strong incentive to develop green, environmentally friendly solvents for starch esterifications.

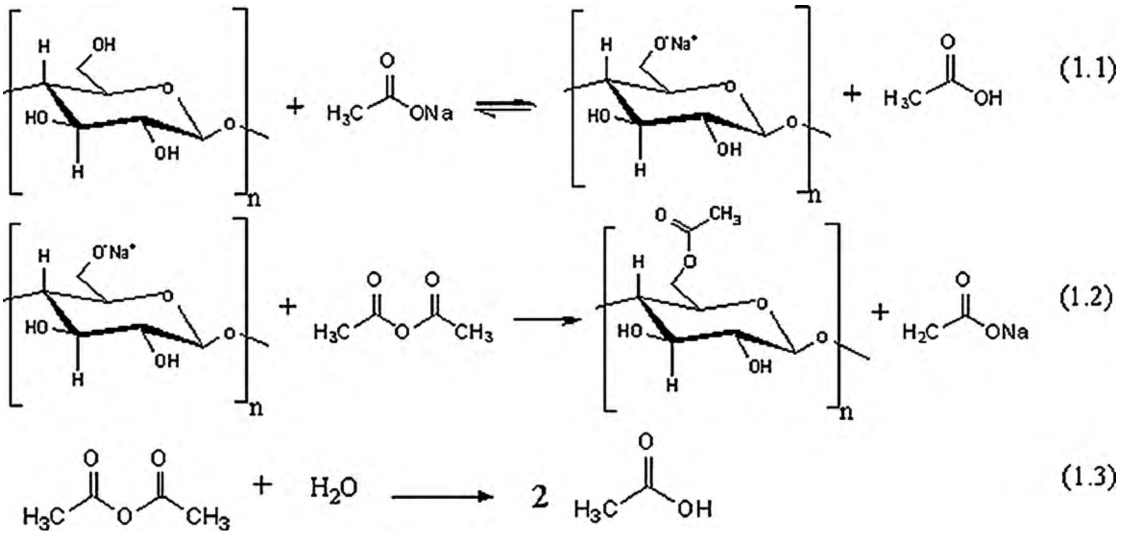
A possible solution is the use of densified CO_2. CO_2 is considered to be a 'green' solvent, it is non-flammable, relatively non-toxic and inert. In the supercritical state (critical temperature, T_c, of 31 °C and pressure, P_c, of 7.38 MPa), CO_2 has shown to be an excellent solvent and processing aid for polymer processing and is also an attractive medium for chemical reactions. Another advantage is the ease of separation of CO_2 from the reaction mixture by a simple reduction of the pressure (Beckman, 2004; Eckert, Knutson, & Debenedetti, 1996; Kemmere, 2005; Savage, Gopalan, Mizan, Martino, & Brock, 1995; Yalpani, 1993).

Recently, Harris, Jureller, Kerschner, Trzasko, and Humphreys (1999) showed that acetylated starch with DS values ranging from 0.2 to 2.4 could be synthesized in densified and supercritical CO_2

---

* Corresponding author. Tel.: +31 50 3634174; fax: +31 50 3634479.
E-mail address: H.J.Heeres@rug.nl (H.J. Heeres).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.05.032



Scheme 1. Representative scheme of the starch acetylation reaction using AAH as reagent and a basic catalyst (NaOAc).

(Harris et al., 1999). High amylose starch (Hylon RTM VII, 70% amylose content) was used and only a limited number of experiments were performed with other starch sources. Furthermore, systematic studies covering the effects of the process conditions were not reported. Here, we report our research activities on the synthesis of acetylated potato starch in densified CO_2. The effects of process variables on the reaction were determined. The experimental data were modelled using non-linear multivariable regression to quantify the effects of process variables on the DS, X_{AAH} and S_{SA}. These models will be valuable input for further process design and optimization purposes.

2. Materials and methods

2.1. Materials

Potato starch was kindly supplied by AVEBE (The Netherlands). The water content was 15.2% (w/w) as determined by drying the potato starch in a vacuum oven at 50 °C until constant weight. Analytical grade acetic anhydride (AAH) and sodium acetate (NaOAc) were purchased from Merck (Germany). High purity CO_2 ($\geq$99.7% volume) and N_2 ($\geq$99.9% volume) were used. All chemicals were used without further purification.

2.2. Reactor setup

The high-pressure setup consists of a double walled stirred reactor (Buchi, 500 ml), an oil bath with a temperature controller, a high-pressure pump unit and CO_2 and N_2 feeding bottles (Fig. 1). The maximum reactor temperature is 250 °C and the maximum pressure is 10 MPa. The reactor is equipped with an overhead stirrer and a Rushton type impeller. The high-pressure pump unit consists of a membrane pump (Lewa) with a capacity of 60 kg/h at a maximum pressure of 35 MPa. To prevent cavitation in the pump, the CO_2 is first cooled to 0 °C in a heat exchanger (Huber, The Netherlands). A heat exchanger with an oil bath is located after the pump and is used to heat the CO_2 to the desired temperature.

2.3. Experimental procedure for the acetylation of starch

2.3.1. Reactions in densified CO_2

Native starch (10 g, 0.062 mol AGU, dry basis), AAH (2–5 mol/mol AGU) and NaOAc (0.1–0.8 mol/mol AGU) were added to the reactor. The reactor was flushed with the N_2 to remove traces of air. The autoclave was pressurized with CO_2 and heated to the desired reaction temperature. If required, additional CO_2 was added to reach the pre-determined reaction pressure. After reaction, the liquid phase was separated from the solid product using a syringe. The solid product were further washed with water (1.5 l), filtrated and dried in a vacuum oven at 50 °C until constant weight. The dried product was used for further analyses. The amount of unreacted AAH in the liquid phase was determined using gas chromatography (GC).

2.3.2. Reactions in water

For the starch acetylation in water, native starch (10 g, dry basis), AAH (4.35 mol/mol AGU), and NaOAc (0.1 mol/mol AGU) were added to the reactor. Subsequently, water (purified by reverse osmosis, 14 ml) was added to obtain a 40% (w/w) starch–water suspension. After flushing with N_2, the autoclave was heated to the desired reaction temperature. After the pre-determined reaction time (typically 1 h), the reactor was cooled to room temperature. Product work-up was performed using the procedure described above for the densified CO_2 experiments.

2.4. Analytical equipment

The DS of the products was routinely determined using a hydrolysis method (Section 2.5). For comparison, the DS of some selected samples was also determined using ^1H NMR on an Oxford NMR AS 400 spectrometer operated at 400 MHz. The product (25 mg) was dissolved in DMSO–d_6 (2 g). ^1H NMR spectra were acquired at 60 °C (64 scans) using a relaxation time of 1.0 s.

The composition of the liquid phase was determined using GC. A GC-HP 5890 series II equipped with an FID detector and a HP 5 column (length = 30 m and I.D. = 0.25 mm) was applied. The GC was operated at the following conditions: injector temperature 280 °C, oven temperature 100 °C, detector temperature 300 °C and an inlet pressure of 86 kPa. Helium was used as the carrier gas with a flow rate of 2.2 ml/min.

Fourier transform infrared (FT-IR) spectra were acquired on a FT-IR (Spectrum 2000, Perkin-Elmer, UK) ATR (attenuated total reflectance) golden gate apparatus (Graseby-Specac Ltd., Orpington, UK). A total of 50 scans with a resolution of 4 cm^{-1} were measured. The morphology of native starch and acetylated products were determined using field emission scanning electron microscope (FESEM) on a JEOL 6320F.

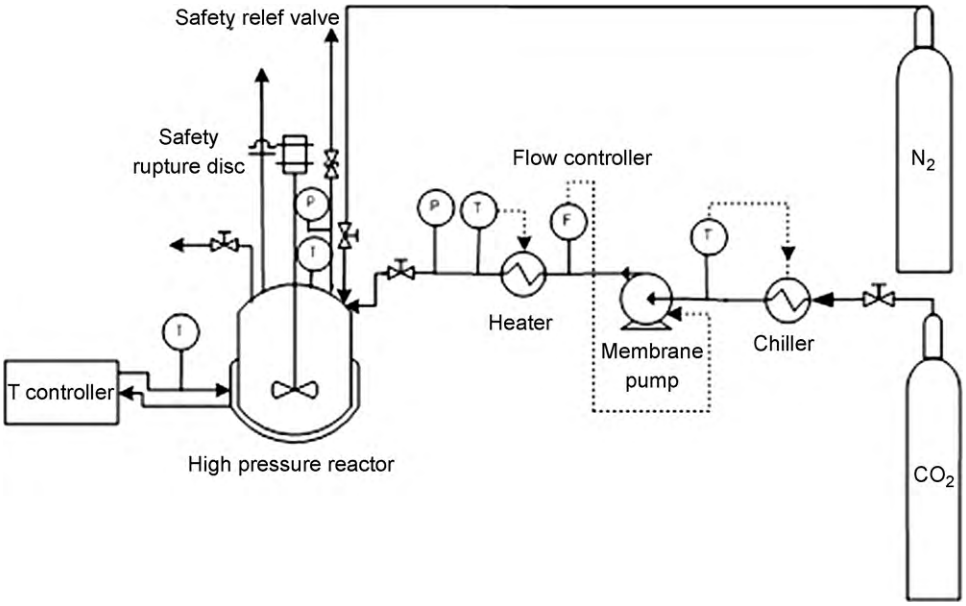


Fig. 1. High-pressure reactor setup.

2.5. Determination of the degree of substitution (DS)

2.5.1. Hydrolysis method

Determination of the DS by the hydrolysis method involves complete basic hydrolysis of the ester linkages and titration of the excess alkali with acid (Elomaa et al., 2004). A powdered sample (0.5 g) was weighed accurately and placed in a 250 ml Erlenmeyer flask with stopper. Reverse osmosis water (10 ml), 0.1N NaOH (25 ml) and a few drops of phenolphthalein were added. The mixture was stirred gently with a magnetic stirrer at room temperature for 2 h. Subsequently, the excess alkali was titrated with 0.1N (HCl) until the red color of phenolphthalein disappeared. The same procedure was carried out for the native starch. The DS was calculated using the following equation (Elomaa et al., 2004; Xu et al., 2004):

$$\mathrm{DS} = \frac{(V_o - V_1) \times N_{\mathrm{HCl}} \times 162}{(1000 \times m_{\mathrm{M}}) - ((V_o - V_1) \times N_{\mathrm{HCl}} \times 42)} \qquad (1)$$

where V_o is the titration volume of acid for the native starch (ml), V_1 the titration volume of acid for the acetylated sample (ml), N_{HCl} the normality of used HCl and m_{M} is the mass of the acetylated sample (g).

2.5.2. ^{1}H NMR method

The DS of the acetylated products was calculated by comparing the unit area of the acetate protons ($A_{\text{H-ace}}$, at δ 1.9–2.1 ppm) with the unit area of the starch protons ($A_{\text{H-agu}}$, at δ 3.6–5.6 ppm) using the following equation (Elomaa et al., 2004; Shogren, 2003; Xu, Miladinov, & Hanna, 2005):

$$\mathrm{DS} = \frac{7A_{\mathrm{ace}}}{3A_{\mathrm{agu}}} \qquad (2)$$

where $A_{\text{H-ace}}$ is the area of the methyl signals and $A_{\text{H-agu}}$ is the area of the proton signals of the anhydroglucose unit.

2.5.3. Comparison between the hydrolysis and ^{1}H NMR method

Fig. 2 depicts a parity plot of the DS values measured with the hydrolysis and ^{1}H NMR method for a number of samples. Evidently, agreement is good (R^2 of 0.98) which suggests that both methods may be applied for determination of the DS.

2.6. Determination of total AAH conversion (X_{AAH}) and product selectivity (S_{SA})

The X_{AAH} and S_{SA} were calculated on the basis of the chemical composition of the liquid phase after reaction (GC analyses). Here, X_{AAH} and S_{SA} are defined as:

$$X_{\mathrm{AAH}} = \frac{n_{\mathrm{AAH,o}} - n_{\mathrm{AAH}}}{n_{\mathrm{AAH,o}}} \times 100\% = \frac{n_{\mathrm{AAH,reacted}}}{n_{\mathrm{AAH,o}}} \times 100\% \qquad (3)$$

$$S_{\mathrm{SA}} = \frac{n_{\mathrm{AAH,p}}}{n_{\mathrm{AAH,reacted}}} \times 100\% \qquad (4)$$

In Eqs. (3) and (4), $n_{\mathrm{AAH,o}}$ is the initial moles of AAH, n_{AAH} is the moles of unreacted AAH, and $n_{\mathrm{AAH,p}}$ and $n_{\mathrm{AAH,reacted}}$ are the moles of acetate in the starch acetate product and the total moles of reacted AAH, respectively.

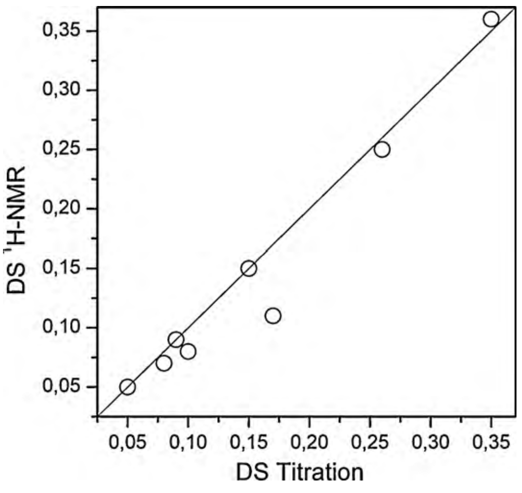


Fig. 2. Parity plot for the hydrolysis and ^{1}H NMR method for DS determinations.

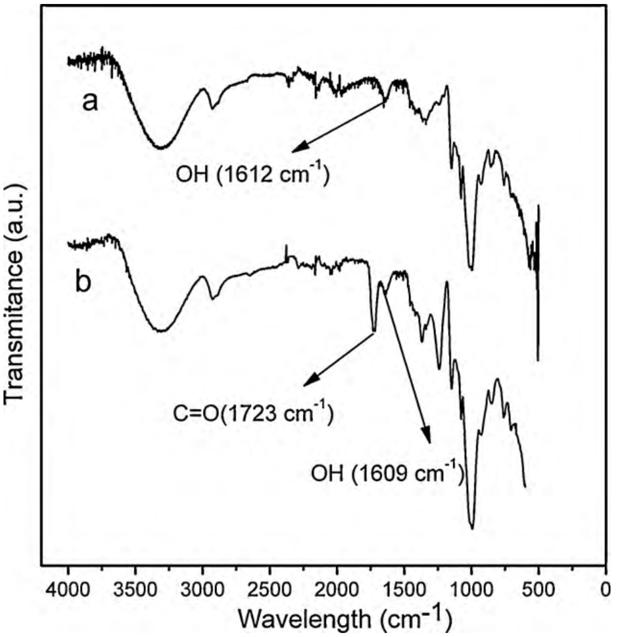


Fig. 3. FT-IR spectra of native potato starch (a) and acetylated products with DS of 0.1 (b).

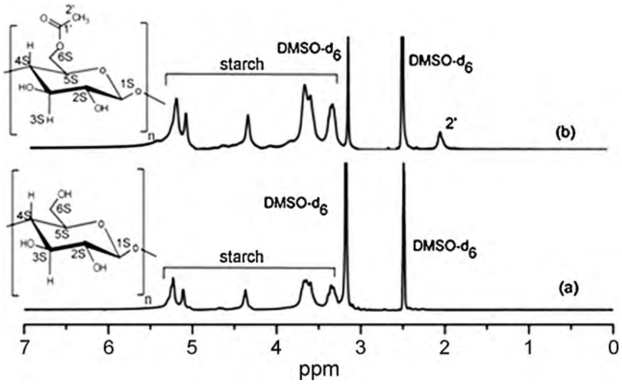


Fig. 4. ^{1}H NMR spectra of native potato starch (a) and acetylated product with DS of 0.1 (b).

3. Results and discussion

3.1. Screening experiments

Initially, a number of screening experiments were carried at a CO_2 pressure of 8 MPa, a temperature of 50 °C using NaOAc as the base (0.1 mol NaOAc/mol AGU) and acetic anhydride as the acetylating agent (4.35 mol AAH/mol AGU) for 1 h reaction time using native potato starch. After work-up, a white granular powder was obtained with a DS of 0.15 (hydrolysis method). The X_{AAH} and S_{SA} values were 32% and 11%, respectively. The latter value is relatively low, meaning that most of the acetic anhydride is converted to acetic acid by hydrolysis (see Scheme 1). This aspect is further discussed in Section 3.2. The product was characterized by FT-IR and ^{1}H NMR and SEM.

FT-IR spectra of native potato starch and an acetylated starch sample with a DS of 0.1 are given in Fig. 3. The major difference between both spectra is the strong absorption band at 1723 cm^{-1} arising from the acetyl group (C=O stretching) in the products (Fang, Fowler, Sayers, & Williams, 2004; Shogren, 2003; Singh, Chawla, et al., 2004; Xu et al., 2004). Other relevant peaks are those between 3000–3600 cm^{-1} (O–H stretching), 2950 cm^{-1} (C–H stretching), 1609–1612 cm^{-1} and 1420 cm^{-1} corresponding to OH and CH bending vibrations (Xu et al., 2004). The absorption bands in the range of 900–1300 cm^{-1} are mainly from highly coupled C–O and C–C vibrational modes (Rubens & Heremans, 2000; vanSoest, Tournois, deWit, & Vliegenthart, 1995).

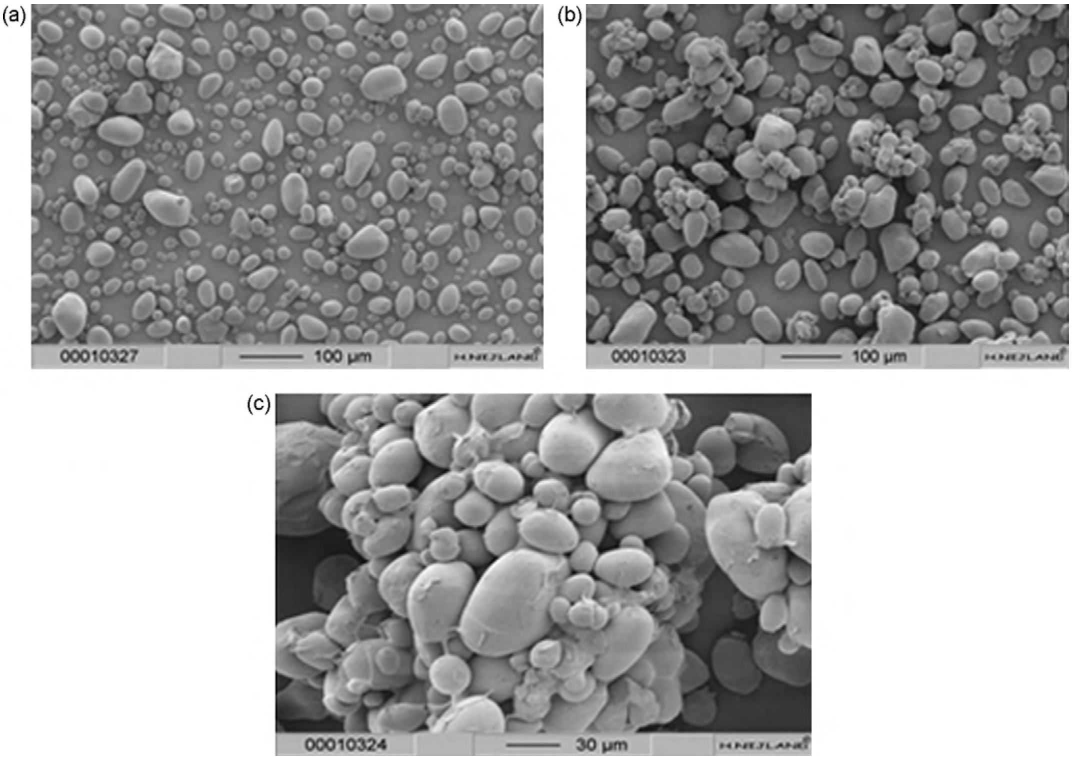


Fig. 5. SEM images of native potato starch (a) and the acetylated products with DS 0.15 (b and c).

Typical ^{1}H NMR spectra of native potato starch and the product are provided in Fig. 4. The broad and overlapped peaks in the δ 3.2–5.5 ppm range are assigned to starch protons (Elomaa et al., 2004; Junistia et al., 2008). The peak at δ 1.9–2.1 ppm corresponds to the methyl protons of the acetyl groups (Elomaa et al., 2004; Laignel, Bliard, Massiot, & Nuzillard, 1997; Xu et al., 2005). Thus, the ^{1}H NMR results confirm the presence of acetyl groups in the products.

The morphology of the product was studied using SEM. Typical SEM images of native starch and an acetylated product with a DS of 0.15 are given in Fig. 5. The morphology of the starch granules appears to be altered by the modification reaction. It seems that the starch granules agglomerate upon acetylation (Fig. 5(b) and (c)), a feature also commonly observed for acetylated starch products with a low DS value (0.1–0.2) prepared in water (Singh, Kaur, et al., 2004; Singh, Chawla, et al., 2004; Sodhi & Singh, 2005; Xu et al., 2004). According to Singh, Kaur, et al. (2004), the introduction of acetyl groups on the starch molecules results in an increase in intermolecular hydrogen bonding and leads to the fusion of starch granules.

3.2. *Systematic studies*

The effects of process variables like temperature, pressure, AAH and NaOAc intakes, and the water content of starch on the product DS, X_{AAH} and S_{SA} were studied in detail by varying one of the variables while keeping the other variables at a constant value. The CO_2 pressure was varied between 6 and 9.8 MPa. This pressure range is of particular interest as most changes in physical properties (such as density and viscosity) of solvents occur near the critical point. A detailed overview of the experimental conditions and results are given in Table 1. The effects of the individual process variables on product DS and reaction parameters will be discussed in the following sections. When relevant, the product DS and the X_{AAH} and S_{SA} will be compared with reactions carried out in water instead of densified CO_2. Similar

Table 1
Overview of experimental results on the effect of process conditions, reagent intake (AAH and NaOAc) and starch water content on the DS, X_{AAH} and S_{SA} in densified CO_2 [a].

No.	Pressure (MPa)	Temperature (°C)	AAH:AGU (mol/mol)[b]	NaOAc:AGU (mol/mol)[c]	Water content (% w/w)	DS	X_{AAH} (%)	S_{SA} (%)
1	8	40	4.35	0.1	15.2	0.08	15	11
2	8	50	4.35	0.1	15.2	0.15	32	11
3	8	60	4.35	0.1	15.2	0.2	44	11
4	8	70	4.35	0.1	15.2	0.27	53	12
5	8	90	4.35	0.1	15.2	0.41	57	16
6	9	40	4.35	0.1	15.2	0.07	18	9
7	9	50	4.35	0.1	15.2	0.15	33	11
8	9	60	4.35	0.1	15.2	0.22	44	12
9	9	70	4.35	0.1	15.2	0.29	53	13
10	9	90	4.35	0.1	15.2	0.39	63	14
11	9.8	40	4.35	0.1	15.2	0.04	13	8
12	9.8	50	4.35	0.1	15.2	0.15	32	11
13	9.8	60	4.35	0.1	15.2	0.23	42	13
14	9.8	70	4.35	0.1	15.2	0.24	49	11
15	9.8	90	4.35	0.1	15.2	0.43	62	16
16	6	50	4.35	0.1	15.2	0.14	36	9
17	7	50	4.35	0.1	15.2	0.15	37	9
18	8	50	4.35	0.1	15.2	0.15	32	11
19	9	50	4.35	0.1	15.2	0.15	33	11
20	9.8	50	4.35	0.1	15.2	0.15	32	11
21	6	70	4.35	0.1	15.2	0.29	53	13
22	7	70	4.35	0.1	15.2	0.29	48	14
23	8	70	4.35	0.1	15.2	0.27	53	12
24	9	70	4.35	0.1	15.2	0.29	53	13
25	9.8	70	4.35	0.1	15.2	0.24	49	11
26	6	90	4.35	0.1	15.2	0.43	59	17
27	7	90	4.35	0.1	15.2	0.46	58	18
28	8	90	4.35	0.1	15.2	0.39	57	16
29	9	90	4.35	0.1	15.2	0.39	63	14
30	9.8	90	4.35	0.1	15.2	0.43	62	16
31	8	50	4.35	0.1	2.5	0.01	6	4
32	8	50	4.35	0.1	6.3	0.02	12	4
33	8	50	4.35	0.1	9.1	0.02	17	3
34	8	50	4.35	0.1	15.2	0.15	32	11
35	8	50	2	0.1	15.2	0.16	80	10
36	8	50	3	0.1	15.2	0.15	50	10
37	8	50	4.35	0.1	15.2	0.15	32	11
38	8	50	5	0.1	15.2	0.12	28	9
39	8	50	4.35	0[d]	15.2	0.03	19	4
40	8	50	4.35	0.1	15.2	0.15	32	11
41	8	50	4.35	0.2	15.2	0.15	39	9
42	8	50	4.35	0.4	15.2	0.13	39	8
43	8	50	4.35	0.6	15.2	0.14	42	8
44	8	50	4.35	0.8	15.2	0.14	47	7

[a] All experiments were performed at 1 h reaction time.
[b] Mole ratio of acetic anhydride AAH to AGU units of starch.
[c] Mole ratio of sodium acetate (NaOAc) to AGU units of starch.
[d] No catalyst used in the experiment.

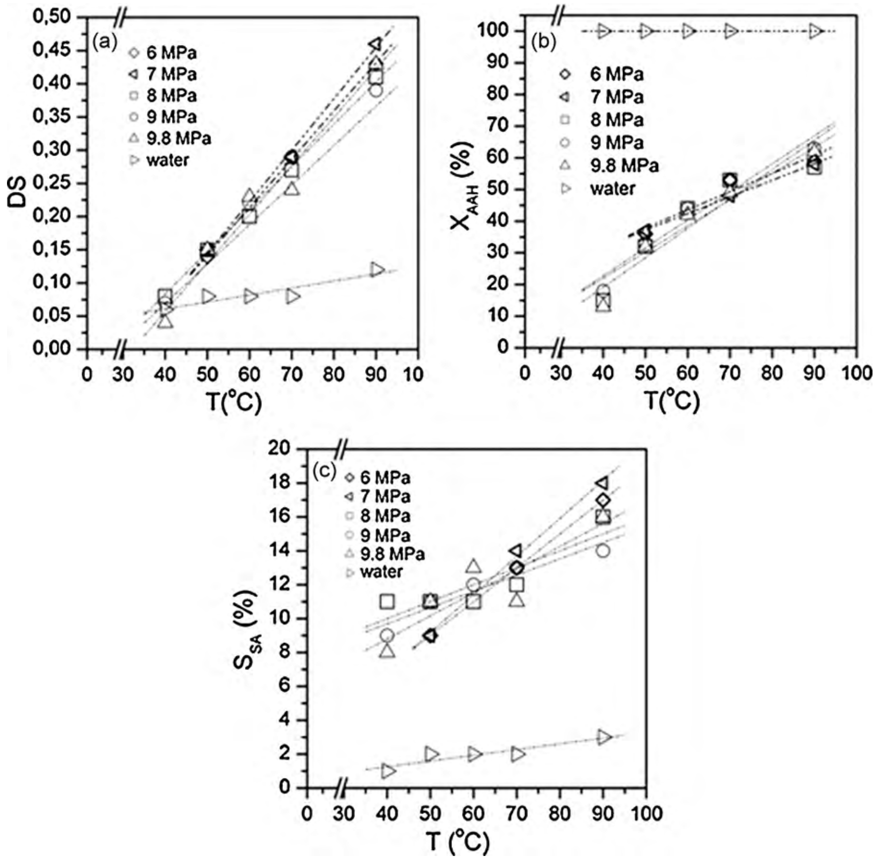


Fig. 6. The influence of temperature on DS (a), X_{AAH} (b), and S_{SA} (c) values at different pressures. Data for reactions in water are also included for comparison.

reagent intakes and temperatures were applied for these experiments.

The system under study is a complex multiphase system involving at least a solid state (starch) and either a supercritical state (consisting of CO_2 and reagents) or a gas (CO_2) in combination with a liquid (reagents). During reaction, the starch particles do not dissolve, as was shown by separate experiments in a high-pressure view cell (Muljana, 2010). The reaction rates and selectivity in such a multiphase system are not solely determined by the intrinsic kinetics but also by mass transfer rates of reagents inside the starch particle. Recent work has shown that intra-particle mass transfer effects dominate acetylation reactions when the reactions are performed in densified CO_2 (Muljana, 2010). Thus, intrinsic kinetics including the conformation of the carbohydrates seem to be of less importance. The rate of intra-particular mass transfer is expected to be a function of the reaction solvent. We have shown earlier that $scCO_2$ dissolves to a certain extent in starch (Muljana, Picchioni, Heeres, & Janssen, in press) and leads to a loss of crystallinity (Muljana, Picchioni, Heeres, & Janssen, 2009), and both factors likely contribute to higher mass transfer rates in the starch particles and thus higher DS an conversion levels.

3.2.1. Temperature effects on reaction performance

With the dataset available, the effect of temperature on the DS, X_{AAH} and S_{SA} may be evaluated. The results are provided in Fig. 6(a)–(c). As expected, the highest reaction rates are observed at the highest temperatures, leading to the highest DS values in the range. At 90 °C, the DS was 0.43 at a pressure of 9.8 MPa. A similar temperature dependency was reported by Harris et al. (1999), though these experiments were performed at higher pressure (17.2 MPa). Here, the DS increased from 0.08 to 1.11 when the temperature was raised from 90 to 115 °C (Harris et al., 1999). As expected, the X_{AAH} also increases at higher temperatures. The highest conversion (63%) was observed at 90 °C. Thus the AAH conversion is not yet complete, indicating that higher DS values are possible at longer reaction times.

The S_{SA} values are all below 18%, indicating that the hydrolysis reaction is favored over the starch acetylation reaction. The S_{SA} increase at higher temperature (see Fig. 6(c)) suggest that the rate of the desired acetylation reaction is enhanced to a larger extent at higher temperatures than the rate of the hydrolysis reaction. This implies that the activation energy for the desired acetylation reaction in densified CO_2 is higher than for the hydrolysis reaction.

To gain insights in the potential of densified CO_2 for the acetylation reaction, a number of reactions were also performed in water as the solvent. Similar starch–water (40%, w/w), NaOAc (0.1 mol/mol AGU) and AAH intakes (4.35 mol/mol AGU) were applied and the reactions were conducted in the experimental setup used for the high-pressure experiments. The results are given in Table 2.

The DS values as well as the S_{SA} in water are always lower than for densified CO_2, see Fig. 6 for details. Furthermore, the X_{AAH} is always quantitative. Thus, the undesired hydrolysis reaction occurs to a considerably higher extent in water than in densified CO_2, leading to lower DS and S_{SA} values.

Table 2
The effect of temperature on the DS, X_{AAH}, and S_{SA} using water as the solvent.

T (°C)	DS	X_{AAH} (%)	S_{SA} (%)
40	0.06	100	1
50	0.08	100	2
60	0.08	100	2
70	0.08	100	2
90	0.12	100	3

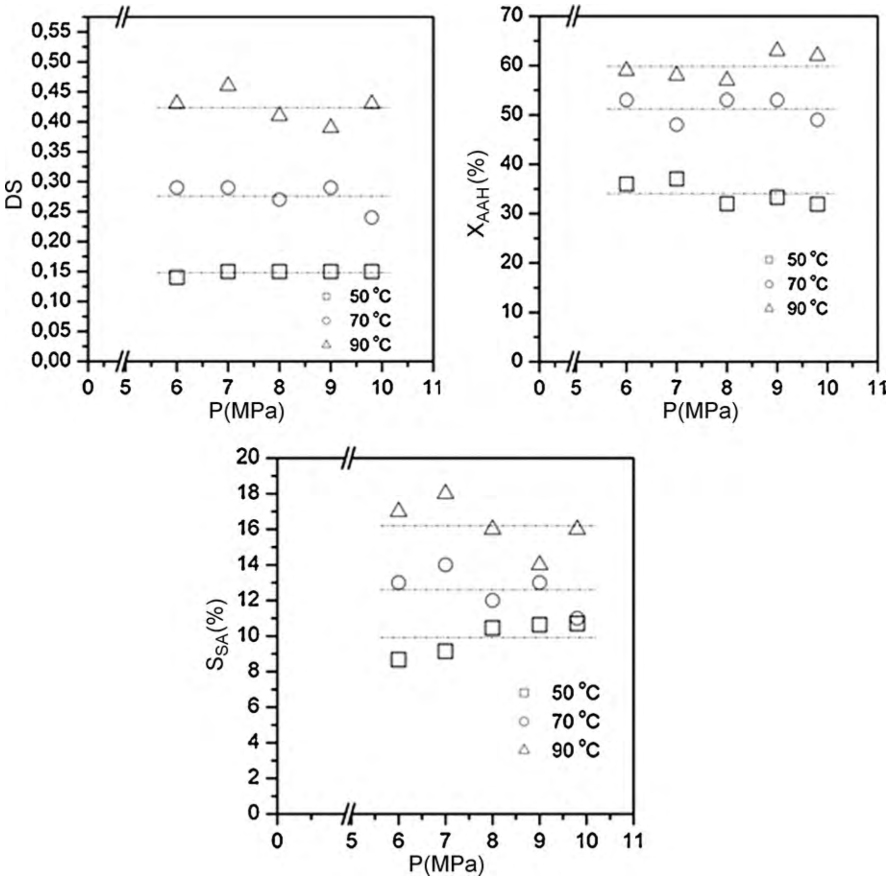


Fig. 7. The influence of pressure on DS (a), X_{AAH} (b), and S_{SA} (c) values at different temperatures.

3.2.2. Pressure effects on reaction performance

The pressure (6–9.8 MPa) has only a slight influence on DS, S_{SA}, and X_{AAH} values, see Fig. 7(a)–(c) for details. These findings are in contrast with the results reported by Harris et al. (1999). A decrease in the DS from 2.3 to 1.6 was found in these studies when increasing the pressure from 10.3 to 17.2 MPa (Harris et al., 1999). However, considerably higher temperatures were applied in this study (140 °C), making a proper comparison cumbersome.

When using supercritical solvents, large pressure effects on the reaction rates are expected near the critical point (Savage et al., 1995). For instance, Savage et al. (1995) reported a 2-fold increase in the *k*-values for the reaction between maleic anhydride and isoprene when going from just below the critical point of CO_2 to 9 MPa (Savage et al., 1995). These trends are not observed for the acetylation of starch (Fig. 7). This raises the question whether the supercritical state was achieved within the experimental ranges of this study. The reagents, particularly when present in higher amounts, can affect the critical properties of CO_2 considerably. For the system CO_2 and AAH, the critical point for an AAH mole fraction (x_{AAH-CO_2}) of 0.0972 was at 14.84 MPa and 87.12 °C (Calvo & de Loos, 2006), thus considerably higher than pure CO_2. The x_{AAH-CO_2} in our experiments is in the same range (0.11–0.14), and it is thus most likely that the reactions reported here were carried out at sub-critical instead of supercritical conditions.

3.2.3. The effect of water intake on reaction performance

The effect of water in the reaction mixture was studied by varying the water content of the native starch feed. As given in Table 1 (no. 31–34), the DS, X_{AAH}, and S_{SA} values drop significantly at lower water content. The DS trend is in line with literature data from Harris et al. (1999), where a significant decrease in DS values (from 2.47 to 0.08) was found when lowering the water content from 15 to 2.5% (Harris et al., 1999). Apparently, water plays a major role in the reactions and has a positive effect on the rate of the desired starch acetylation reaction.

To gain insights in the role of water in the reaction, the water intake was compared with the AAH intake and the AAH consumed in both the desired starch acetylation reaction and the undesired hydrolysis reaction. The data are given in Table 3. In all cases, AAH is present in excess and water is the limiting reactant. It is particularly evident that most of the AAH is consumed by the hydrolysis reaction and that the water conversion by this reaction is in all cases higher than 80%. This implies that the hydrolysis reaction is much faster than the reaction of AAH with starch. Thus, on average less AAH is available for the reaction with starch leading to lower reaction rates for the desired starch acetylation reaction. In addition, the hydrolysis reaction leads to the formation of acetic acid (Scheme 1) which has a negative effect on the starch acetylation reaction by driving the first equilibrium step (activation of starch by the base, see Scheme 1) to the left side.

These arguments to explain the effects of water on the reaction rate of the two parallel reactions occurring in the system (starch acetylation and anhydride hydrolysis) are based on single-phase kinetic arguments only. As mentioned above, the system under study is by far more complex and should either be regarded as a solid/scCO_2 system or maybe also a solid/liquid/liquid system or a combination thereof. The actual state will be depending on whether the CO_2/anhydride/acetic acid/water combination is actually in the supercritical state or present as separate liquid phases. Furthermore, there is indication that the reaction does not occur in scCO_2 but in densified CO_2 (*vide supra*). In such a multiphase system, not only the intrinsic kinetics of the reactions determine the selectiv-

Table 3
Reactivity of AAH for the starch acetylation and hydrolysis reaction.

No.	Water content in starch source (% w/w)	Water intake ($\times 10^{-3}$ mol)	AAH intake ($\times 10^{-3}$ mol)	AAH reacted with water ($\times 10^{-3}$ mol)	AAH reacted with starch ($\times 10^{-3}$ mol)
31	2.5	16.33	270	15.58	0.62
32	6.3	37.31	270	31.17	1.23
33	9.1	55.61	270	44.67	1.23
34	15.2	99.31	270	77.14	9.26

Table 4
Regression coefficients of DS, X_{AAH}, and S_{SA} models.

		DS		X_{AAH}		S_{SA}	
		Coefficients	t_o	Coefficients	t_o	Coefficients	t_o
Variable (x_i)	Intercept (b_o)	−0.298	−2.064	8.072	10.189	−1.467	1.304
Pressure (x_1)	b_1	-3.068×10^{-4}	−0.847	−0.034	−0.762	−0.013	−0.786
Temperature (x_2)	b_2	6.806×10^{-3}	28.702	3.134	10.741	0.132	11.712
AAH intake (x_3)	b_3	−0.01	−1.133	−43.946	−4.546	0.397	0.946
NaOAc intake (x_4)	b_4	−0.011	−0.547	53.167	4.386	−15.763	−3.343
Water content (x_5)	b_5	0.011	9.184	2.032	14.004	−0.627	−1.669
x_2^2	b_{22}	–	–	−0.017	−8.101	–	–
x_3^3	b_{33}	–	–	3.668	2.59	–	–
x_4^4	b_{44}	–	–	−44.206	−3.569	11.971	2.483
x_5^5	b_{55}	–	–	–	–	0.067	3.269
	R^2	0.97		0.98		0.91	
	$R^2_{adjusted}$	0.96		0.97		0.90	
	R^2_{PRESS}	0.96		0.94		0.84	

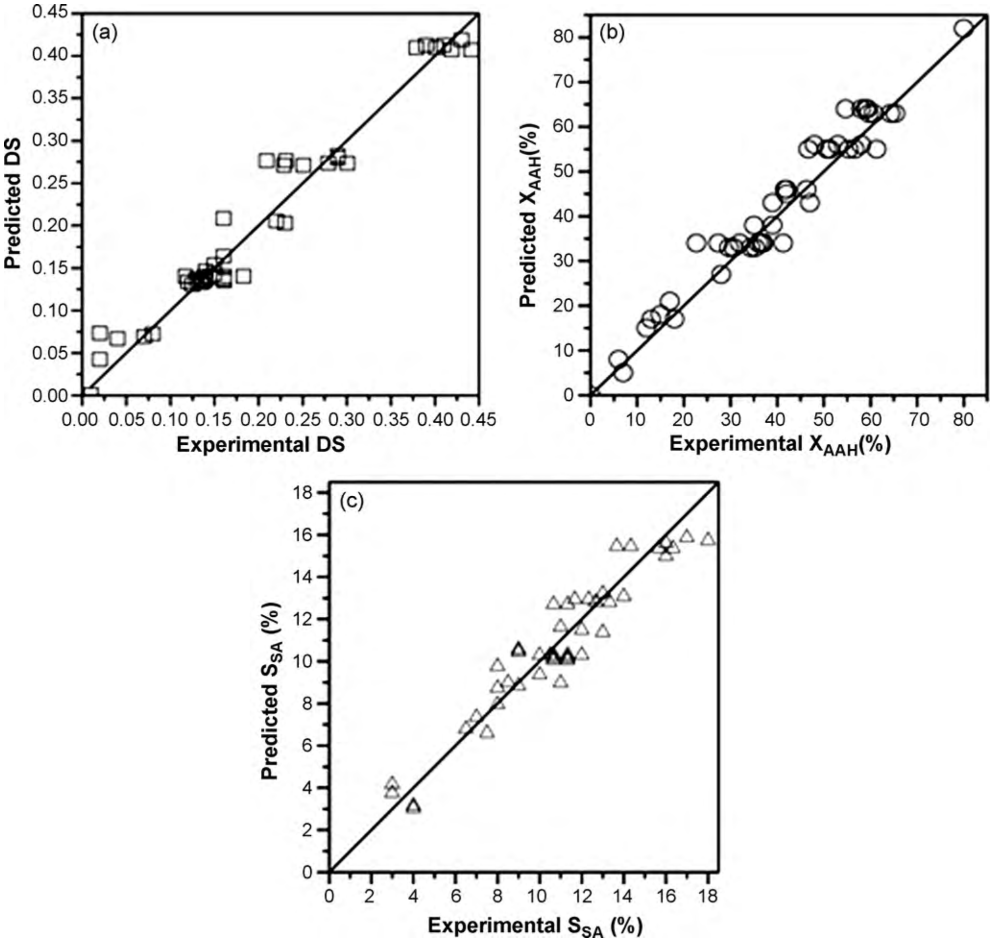


Fig. 8. Parity plot between predicted and experimental values of DS (a), X_{AAH} (b), and S_{SA} (c).

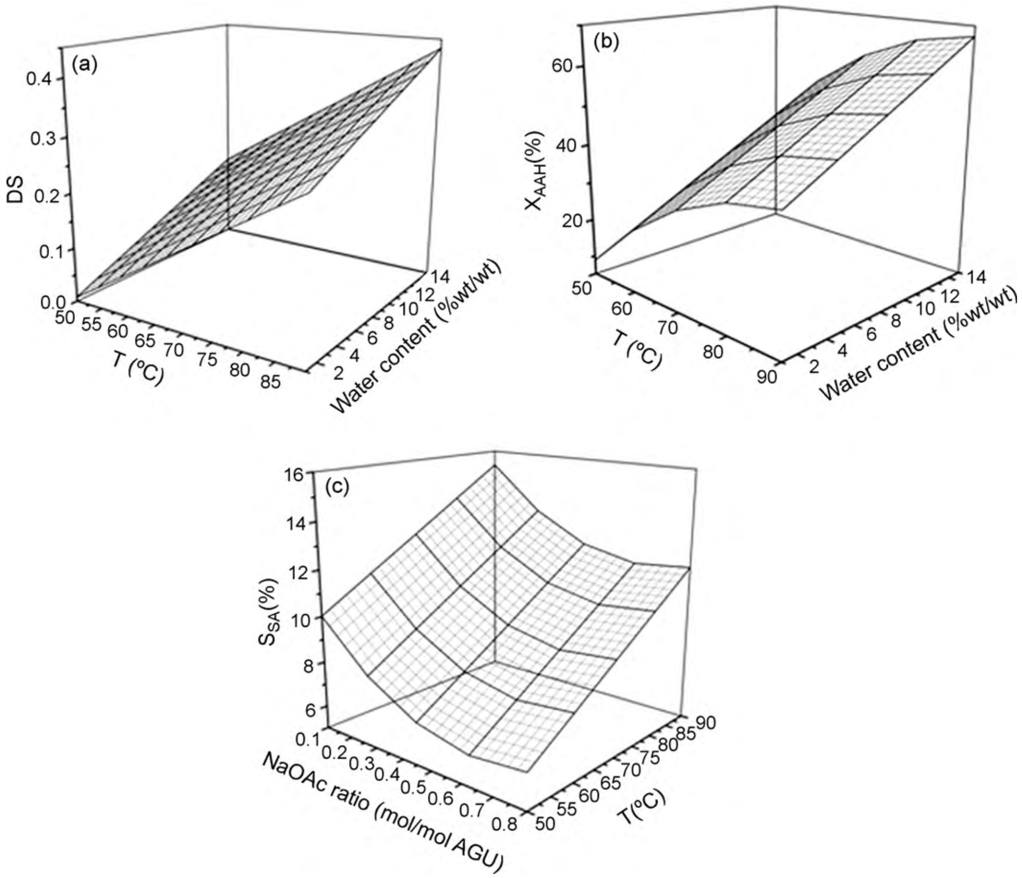


Fig. 9. Optimization studies using the empirical model on DS (a), X_{AAH} (b), and S_{SA} (c). (Data for DS and X_{AAH}: 8 MPa, 0.1 mol NaOAc/mol AGU, and 4.35 mol/molAGU. For S_{SA} 8 MPa, 4.35 mol AAH/mol AGU and 15.2% water intake.)

ity but also mass transfer and solubility effects may play a major and even decisive role. The latter also determines the locus of reaction (surface or also inside particle). It is clear that the locus of the desired acetylation reaction of starch will take place in or at the surface of the starch particle, whereas the hydrolysis reaction may also take place in the liquid phase outside a starch particle and this further complicates the analyses.

3.2.4. The effect of acetic anhydride (AAH) intake on reaction performance

The effect of the AAH intake on reaction performance was probed for 4 different AAH to starch ratios. The experimental results are given in Table 1 (no. 35–38). Clearly, the DS and X_{AAH} values decrease at a higher AAH ratio while the S_{SA} values were almost constant for all cases. Apparently, a higher AAH intake does not lead to a higher reaction rate for the desired reaction with starch. The results can be rationalized by considering the higher reaction rate of the undesired hydrolysis reaction compared to the starch acetylation reaction in combination with the formation of acetic acid which further lowers the rate of the starch acetylation reaction. Again, other effects related to the multiphase nature of the system cannot be excluded.

3.2.5. The effect of catalyst intake on reaction performance

The effect of the NaOAc intakes on DS, X_{AAH} and S_{SA} are shown in Table 1 (no. 39–44). The DS values are relatively constant while the S_{SA} values tend to decrease with higher catalyst intake. In contrast with the DS and S_{SA} trends, X_{AAH} increases with higher catalyst intake. Apparently, the AAH hydrolysis reaction is favored at higher NaOAc ratio. The opposite trend is reported in literature. Harris et al. (1999) showed that the DS increases from 0.2 to 2.4 when the catalyst ratio increases from 0.05 to 0.2 (mol/mol AGU) (Harris et al., 1999). These differences in the trends may be due to the higher catalyst intake in our experiments (up to 0.8 mol/mol AGU). Another possible explanation is established observation that the rate of the AAH hydrolysis reaction is a function of the NaOAc intake, with higher NaOAc concentrations leading to higher reaction rates (Bunton, Pitman, Fuller, & Perry, 1962; Butler & Gold, 1961; Oakenful, 1971; Oakenful, 1974). Thus, the basic catalyst not only affects the rate of the desired starch acetylation reaction but also the undesired hydrolysis reaction.

The acetylation reaction is also occurring without the NaOAc catalyst (Table 1). However, the DS value is much lower than in the presence of NaOAc, indicating that the addition of a catalyst is crucial to improve the starch reactivity. These findings are in agreement with the work of Dicke (2004) on the acetylation of starch using acetic anhydride and vinyl acetate in DMSO (Dicke, 2004). Here acetylation was also observed in the absence of a catalyst, though the reactivity was lower than in the presence of the catalyst.

3.2.6. Quantification of the effects of process conditions on reaction performance using empirical models

To quantify the effects of the process variables (temperature, pressure, AAH intake, NaOAC intake, and starch water content) on the DS, X_{AAH} and S_{SA}, the experimental data were modelled using an expression given in Eq. (5). All regression coefficients (b_i and b_{ij}) and the intercept (b_o) were calculated using *Mathcad*® software package.

$$y_i = b_o + \sum_{i=1}^{5} b_i x_i + \sum_{i=1}^{5}\sum_{j=1}^{5} b_{ij} x_i x_j \qquad (5)$$

A *t*-test (for statistical significance) was performed for regression coefficient and the non-significant terms were deleted from the model (Montgomery, 2001). The calculated regression coefficients are given in Table 4. Empirical models with a good R^2 and R^2_{adjusted} values (0.9–0.98) were obtained which imply that these models correctly describe and fit the experimental data. The parity plots of the three models are given in Fig. 8 and confirm these observations. Internal validation was performed using PRESS calculations (Montgomery, 2001) and relatively high R^2_{PRESS} values (0.84–0.96) were observed, indicative for good predictive capability of the models.

Temperature and water content have the most significant effect on the DS and X_{AAH} values of the product (based on the t_o values), while the NaOAc ratio and temperature have the highest influence on the S_{SA}. It is clearly shown by all models that pressure only has a limited effect on DS, X_{AAH} and S_{SA} values. Fig. 9(a)–(c) illustrates the model predictions of the effects of various process parameters on the DS, X_{AAH} and S_{SA} using the empirical models. The highest DS (0.43) and X_{AAH} (67%) values are obtained at the highest temperature in the range (90 °C) and highest initial water content of the starch (15.2%). The highest S_{SA} value (15%) is obtained at a low NaOAc ratio (0.1 mol/mol AGU) and highest temperature (90 °C).

These models may be applied to determine the process conditions to obtain a product with a pre-defined DS value at the highest X_{AAH} values.

4. Conclusions

In this work, the starch acetylation reaction with AAH as the reagent and NaOAc as the base catalyst using densified CO_2 as the solvent was investigated. Acetylated potato starches with a range of DS (0.01–0.46) values could be obtained. The effects of process variables on the acetylation reaction was examined and quantified with empirical models. Temperature, water content and NaOAc ratio were shown to have the largest influence on the DS, X_{AAH} and S_{SA} values while the influence of pressure within the observed pressure window (6–9.8 MPa) is rather limited. This study shows the potential of densified CO_2 as the solvent for starch modifications and particularly for starch acetate synthesis. We are currently conducting an in-depth study with higher CO_2 pressures (above 150 MPa) on the starch acetylation reaction. These results will be provided in the upcoming papers.

Acknowledgements

The authors thank Harry Nijland (Polymer Department, University of Groningen) for the SEM analyses. We also acknowledge AVEBE for providing the potato starch and stimulating discussions. Henky Muljana acknowledges the University of Groningen for providing an Ubbo Emmius scholarship to carry out this research.

References

Beckman, E. J. (2004). Supercritical and near-critical CO_2 in green chemical synthesis and processing. *Journal of Supercritical Fluids*, *28*, 121–191.

Betancur, A. D., Chel, G. L., & Canizares, H. E. (1997). Acetylation and characterization of *Canavalia ensiformis* starch. *Journal of Agricultural and Food Chemistry*, *45*, 378–382.

Bunton, C. A., Pitman, I. H., Fuller, N. A., & Perry, S. G. (1962). Hydrolysis of carboxylic anhydrides. 2. Effects of salts of strong acids on hydrolysis of acetic anhydride. *Journal of the Chemical Society*, 4478.

Butler, A. R., & Gold, V. (1961). Hydrolysis of acetic anhydride. 6. Kinetics in deuterium oxide solution. *Journal of the Chemical Society*, 2305.

Calvo, L., & de Loos, T. W. (2006). High pressure vapour–liquid equilibria of the binary and some of the ternary and multicomponent mixtures of the carbon dioxide + acetic anhydride + alpha-methylbenzyl alcohol + acetic acid + alpha-methylbenzyl acetate system experimental and modelling results. *Fluid Phase Equilibria*, *244*, 179–187.

de Graaf, R. A., Broekroelofs, A., & Janssen, L. (1998). The acetylation of starch by reactive extrusion. *Starch/Starke*, *50*, 198–205.

Dicke, R. E. (2004). A straight way to regioselectively functionalized polysaccharide esters. *Cellulose*, *11*, 255–263.

Eckert, C. A., Knutson, B. L., & Debenedetti, P. G. (1996). Supercritical fluids as solvents for chemical and materials processing. *Nature*, *383*, 313–318.

Elomaa, M., Asplund, T., Soininen, P., Laatikainen, R., Peltonen, S., Hyvarinen, S., et al. (2004). Determination of the degree of substitution of acetylated starch by hydrolysis, ^{1}H NMR and TGA/IR. *Carbohydrate Polymers*, *57*, 261–267.

Fang, J. M., Fowler, P. A., Sayers, C., & Williams, P. A. (2004). The chemical modification of a range of starches under aqueous reaction conditions. *Carbohydrate Polymers*, *55*, 283–289.

Gonzalez, Z., & Perez, E. (2002). Effect of acetylation on some properties of rice starch. *Starch/Starke*, *54*, 148–154.

Harris, R., Jureller, S. H., Kerschner, J. L., Trzasko, P. T., & Humphreys, R. W. R. (1999). *Polysaccharide modification in densified fluid*. US Patent Office, Pat. No. 5977348.

Jarowenko, W. (1986). Acetylated starch and miscellaneous organic esters. In O. B. Wurzburg (Ed.), *Modified starches: Properties and uses* (pp. 55–77). Boca Raton, FL: CRC Press, Inc.

Junistia, L., Sugih, A. K., Manurung, R., Picchioni, F., Janssen, L., & Heeres, H. J. (2008). Synthesis of higher fatty acid starch esters using vinyl laurate and stearate as reactants. *Starch/Starke*, *60*, 667–675.

Kemmere, M. F. (2005). Supercritical carbon dioxide for sustainable polymer processes. In M. F. Kemmere, & T. Meyer (Eds.), *Supercritical carbon dioxide: In polymer reaction engineering* (pp. 1–14). Weinheim: Wiley-VCH.

Laignel, B., Bliard, C., Massiot, G., & Nuzillard, J. M. (1997). Proton NMR spectroscopy assignment of D-glucose residues in highly acetylated starch. *Carbohydrate Research*, *298*, 251–260.

Montgomery, D. C. (2001). *Design and analysis of experiments*. New York: John Wiley and Sons, Inc.

Muljana, H. (2010). *Modification of starch using supercritical carbon dioxide*. PhD Thesis. The Netherlands: University of Groningen.

Muljana, H., Picchioni, F., Heeres, H. J., & Janssen, L. P. B. M. (2009). Supercritical carbon dioxide ($scCO_2$) induced gelatinization of potato starch. *Carbohydrate Polymers*, *78*, 511–519.

Muljana, H., Picchioni, F., Heeres, H. J., & Janssen, L. P. B. M. (in press). Experimental and modeling studies on the solubility of sub- and supercritical carbon dioxide (scCO2) in potato starch and derivatives. *Polymer Engineering and Science*, doi:10.1002/pen.21786.

Oakenful, Dg. (1971). Kinetics of hydrolysis of acetic anhydride in concentrated salt solutions. *Australian Journal of Chemistry*, *24*, 2547.

Oakenful, Dg. (1974). Kinetics of hydrolysis of acetic-anhydride and reaction of 4-nitrophenyl acetate with imidazole in aqueous-organic mixed solvents. *Australian Journal of Chemistry*, *27*, 1423–1431.

Raina, C. S., Singh, S., Bawa, A. S., & Saxena, D. C. (2006). Some characteristics of acetylated, cross-linked and dual modified Indian rice starches. *European Food Research and Technology*, *223*, 561–570.

Rubens, P., & Heremans, K. (2000). Stability diagram of rice starch as determined with FTIR. *High Pressure Research*, *19*, 551–556.

Saartrat, S., Puttanlek, C., Rungsardthong, V., & Uttapap, D. (2005). Paste and gel properties of low-substituted acetylated canna starches. *Carbohydrate Polymers*, *61*, 211–221.

Savage, P. E., Gopalan, S., Mizan, T. I., Martino, C. J., & Brock, E. E. (1995). Reactions at supercritical conditions—Applications and fundamentals. *AIChE Journal*, *41*, 1723–1778.

Shogren, R. L. (2003). Rapid preparation of starch esters by high temperature/pressure reaction. *Carbohydrate Polymers*, *52*, 319–326.

Shon, K. J., & Yoo, B. (2006). Effect of acetylation on rheological properties of rice starch. *Starch/Starke*, *58*, 177–185.

Singh, J., Kaur, L., & Singh, N. (2004). Effect of acetylation on some properties of corn and potato starches. *Starch/Starke*, *56*, 586–601.

Singh, N., Chawla, D., & Singh, J. (2004). Influence of acetic anhydride on physicochemical, morphological and thermal properties of corn and potato starch. *Food Chemistry*, *86*, 601–608.

Sodhi, N. S., & Singh, N. (2005). Characteristics of acetylated starches prepared using starches separated from different rice cultivars. *Journal of Food Engineering*, *70*, 117–127.

Tijsen, C. J., Kolk, H. J., Stamhuis, E. J., & Beenackers, A. (2001). An experimental study on the carboxymethylation of granular potato starch in non-aqueous media. *Carbohydrate Polymers*, *45*, 219–226.

vanSoest, J. J. G., Tournois, H., deWit, D., & Vliegenthart, J. F. G. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research*, *279*, 201–214.

Xu, Y. X., Miladinov, V., & Hanna, M. A. (2004). Synthesis and characterization of starch acetates with high substitution. *Cereal Chemistry*, *81*, 735–740.

Xu, Y. X., Miladinov, V., & Hanna, M. A. (2005). Starch acetate–maleate mixed ester synthesis and characterization. *Cereal Chemistry*, *82*, 336–340.

Yalpani, M. (1993). Supercritical fluids—Puissant media for the modification of polymers and biopolymers. *Polymer*, *34*, 1102–1105.