

1 Preparation of cationized starch from food industry waste biomass and its utilization in sulfate
2 removal from aqueous solution

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13 Sulfate removal;

14

15 Abstract

16

17 In this work, untreated starch-rich potato peel waste was used as a starting material in preparation of
18 cationized starch (CS)¹ in water solution with 2-chloro-3-hydroxypropyltrimethylammonium
19 chloride (CHPTAC) as the cationization reagent. The impact of various factors (activation time,
20 temperature, reaction time, the amount of CHPTAC and NaOH) on the degree of substitution (DS)
21 of CS was studied by using experimental design. The DS values were determined by ¹H NMR. The
22 highest DS (0.40) was obtained when the reaction time was 8 h, temperature 30 °C, the molar ratio
23 of CHPTAC and NaOH to AGU 3 and 3.75, respectively. The prepared CS was successfully used to
24 remove sulfate ions from an aqueous solution with ultrafiltration technique. Sulfate is a major
25 pollutant of water bodies so development of efficient techniques for its removal is detrimental. The
26 removal of sulfate in study was 74% at best.

27

28 1. Introduction

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¹ Abbreviations: CS, cationized starch; CHPTAC, 2-chloro-3-hydroxypropyltrimethylammonium chloride; DS, degree of substitution; PW, peel waste; ICP-OES, inductively coupled plasma-optical emission spectrometer; CCC, central composite circumscribed;

30 Harmful anions such as sulfate (SO_4^{2-}) are major pollutants of both natural waters and industrial
31 effluents (Mulinari & da Silva, 2008; Silva, Lima & Leao, 2012; Runtti et al., 2016). SO_4^{2-} ions are
32 less toxic than heavy metal ions, however excess SO_4^{2-} can cause an imbalance in the natural sulfur
33 cycle (Silva et al., 2012; Cao et al., 2011), have laxative effects for humans (Silva et al., 2012),
34 affect the taste of the water (Silva et al., 2012) and damage water pipes (MSAH, 2015). Therefore,
35 the removal of SO_4^{2-} is necessary to reduce the risks to the environment and human health.

36 The established methods for the removal of SO_4^{2-} ions include ion exchange and adsorption.
37 While they are rapid and effective processes, their downside is the utilization of costly materials. In
38 adsorption, for example, the typically used material is activated carbon, which is expensive. (Runtti
39 et al., 2016) Hence, it is important to develop cheap adsorption materials from alternative and
40 preferable renewable raw materials.

41 Potato peel waste (PW) is a non-edible waste stream generated considerable amounts by food
42 processing industry. Depending on the peeling process, i.e. abrasion, steam or lye peeling, the
43 amount of waste can range from 15 to 40% of the amount of processed potatoes (Arapoglou,
44 Varzakas, Vlyssides, & Israilides, 2010). Most of PW is discarded (Chen, Lawton, Thompson, &
45 Liu, 2012) and only a small amount of it is used as a supplementary animal feed, fertilizer or is
46 composted (Israilides et al., 2008). PW from the abrasion peeling process contains a large quantity
47 of starch, e.g. 52% (dry basis) (Camire, Violette, Dougherty, & McLaughlin, 1997). Starch is
48 globally the second most abundant carbohydrate. After some modification it is largely utilized in
49 industrial applications and it is a potential resource for bioenergy production. The utilization of PW
50 instead of native starch for the preparation of starch derivatives would promote the material
51 efficiency and the sustainable use of natural resources. Furthermore, the use of PW does not raise
52 ethical issues because it cannot be used as food. This makes it favourable compared to native starch.

53 In this work, we have studied the use of PW as a starting material for cationized starch (CS).
54 Cationized starches are important derivatives of starch and they are exploited extensively e.g. as
55 wet-end chemicals in paper industry and adsorbents in raw or wastewater purification (Haack,
56 Heinze, Oelmeyer, & Kulicke, 2002; Heinze, Haack, & Rensing, 2004; Krentz et al., 2006).
57 Typically, the commercial cationized starch derivatives have a low degree of substitution ($\text{DS} < 0.2$)
58 and they have been prepared by the reaction of native starch with 3-chloro-2-
59 hydroxypropyltrimethylammonium chloride (CHPTAC) or glycidyltrimethylammonium chloride
60 (2,3-epoxypropyltrimethylammonium chloride, GTAC) with a base like NaOH as a catalyst
61 (Heinze, Haack, & Rensing, 2004; Wang et al., 2009). In our previous work (Lappalainen,
62 Kärkkäinen, Joensuu & Lajunen, 2015) we studied the cationization of PW, which was dried,
63 ground and partly hydrolysed prior to the cationization step. The drying steps are energy consuming

64 so in this work we studied if PW could be utilized as received from the peeling factory without any
65 pre-treatment. A central composite circumscribed (CCC) design was used to study the effect of
66 reaction temperature, time, activation time, the amount of the cationization reagent CHPTAC and
67 NaOH on the DS of CS. Microwave activation was used as a heating method. The goal was to find
68 the optimal reaction conditions for the cationization reaction of PW. Furthermore, the prepared CS
69 was used as an adsorbent material in SO_4^{2-} removal studies, which were performed by cationized
70 starch enhanced ultrafiltration method. Experimental design was used to find the optimal conditions
71 (pH, SO_4^{2-} concentration, the molar equivalent of CS to SO_4^{2-}) for the SO_4^{2-} removal. To our
72 knowledge the direct chemical modification of potato peel waste without any pre-treatment has not
73 been reported earlier. Also CS has not been used before for the removal of SO_4^{2-} ions.

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75 2. Materials and methods

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77 2.1 Reagents

78 Potato peel waste, produced by abrasion peeling process, was provided by Tervakankaan Peruna
79 Oy, Finland. The composition of PW was very heterogeneous and the colour was brown. The water
80 content of PW was 80% (determined by drying a sample of PW at 105 °C to constant weight). PW
81 was used as received from the peeling factory. All reactions were done using the same batch of the
82 PW. According to the PW supplier, Tervakankaan Peruna Oy, the starch content of the dry PW was
83 78% and the dry content of protein, ash, other polysaccharides, fat, potassium, phosphorus,
84 magnesium, calcium and sodium was 8.5%, 8%, 1.4%, 0.5%, 3.2%, 0.2%, 0.15%, 0.07% and
85 0.005%, respectively. The cationization reagent, Raisacat 188 [65% solution of N-(3-chloro-2-
86 hydroxypropyl)trimethylammonium chloride (CHPTAC)] was provided by Chemigate Oy (Lapua,
87 Finland). Other reagents were used as received from the suppliers.

88

89 2.2 Cationization of potato peel waste in microwave reactor

90 PW (3 g) was weighted into a microwave reactor vessel (size 10-20 ml) equipped with a magnetic
91 stirring bar. Elix water (11 ml) and NaOH (0.19 ml; 6.2 M aqueous solution) were added. In order
92 to enhance the reactivity of the peel waste, the mixture was heated in the microwave reactor
93 (Biotage Initiator with a single-mode microwave unit) at 60 °C for a time based on the experimental
94 design (0-3.6 h, Tables 1 and 3). Additional amount of NaOH (6.2 M aqueous solution) and
95 CHPTAC were added (at the levels required by the experimental design, Tables 1 and 3) and the
96 mixture was heated further in the microwave reactor at the temperature and for the reaction time
97 based on the experimental design (20-90 °C and 2-9 h, respectively, Tables 1 and 3). After the

98 reaction, the mixture was neutralized with 2 M HCl and the crude product was precipitated with
99 cold ethanol (50 ml). The precipitate was filtered with a sintered glass funnel (pore size 4) and
100 washed with 3*25 ml of cold ethanol. Finally, the precipitate was dried in an oven (35 °C) until
101 constant weight.

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103 2.3 Scale-up of cationization reactions in microwave oven

104 PW (7.5 g) was weighted into a Teflon microwave vessel (XP-1500 plus high-pressure Teflon®
105 TFM vessel, CEM Corp.) equipped with a magnetic stirring bar. Elix water (28 ml), NaOH (1.16,
106 2.33 or 3.49 ml; 6.2 M aqueous solution; Table 5) and CHPTAC (1.45, 2.91 or 4.36 ml,
107 respectively, Table 5) were added. The mixture was heated in the microwave oven (CEM Mars 5X,
108 CEM Corp.) at 80 °C for 3 h. After the reaction, the mixture was neutralized with 2 M HCl and the
109 crude product was precipitated with cold ethanol (150 ml). The precipitate was filtered with a
110 sintered glass funnel (pore size 4) and washed with 3*80 ml of cold ethanol. Finally, the precipitate
111 was dried in an oven (35 °C) until constant weight.

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113 2.4 Scale-up of cationization reaction in an oil bath

114 PW (50 g) was weighted into a 500 ml round-bottom flask equipped with a magnetic stirring bar.
115 Elix water (160 ml), NaOH (23.3 ml; 6.2 M aqueous solution) and CHPTAC (28.8 ml) were added.
116 The mixture was heated in an oil bath at 80 °C for 3 h. After the reaction, the mixture was
117 neutralized with 2 M HCl and the crude product was precipitated with cold ethanol (500 ml). The
118 precipitate was filtered with a sintered glass funnel (pore size 4) and washed with 3*150 ml of cold
119 ethanol. Finally, the precipitate was dried in an oven (35 °C) until constant weight.

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121 2.5 Characterization of cationized products with ¹H NMR, FTIR and FESEM

122 A sample (10 mg) was taken from the product of each cationization reaction for ¹H NMR
123 measurement. D₂O (1 ml) was added and the mixture was carefully shaken. The mixture was
124 centrifuged (Eppendorf Minispinner) at 13 200 rpm for 5 min. The supernatant was decanted and its
125 ¹H NMR spectrum was recorded with a Bruker DPX 200 MHz spectrometer at ambient
126 temperature. The residual water peak (4.75 ppm) was used as a reference.

127 FTIR analysis was performed using a Perkin Elmer Precisely Spectrum One FT-IR spectrometer
128 equipped with an Universal ATR Sampling Accessory. The spectra were recorded with a resolution
129 of 4 cm⁻¹, 4 scans and a wave number range of 650-4000 cm⁻¹.

130 The morphologies of dried PW and cationized products were analysed with FEI Helios DualBeam
131 field emission scanning electron microscope. The samples were prepared by spreading them on a

132 carbon tape attached on the aluminum disks. Samples were coated with 30 nm of platinum and
133 observed at 1000x magnification.

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135 2.6 Removal of sulfate ions from aqueous solution with cationized starch enhanced ultrafiltration
136 Sulfate ion stock solution (100 mM) was prepared by dissolving magnesium sulfate heptahydrate,
137 $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, in Elix water and fixing the solution with conc. HNO_3 . In a typical experiment
138 SO_4^{2-} ion solution was freshly prepared by mixing a selected amount of stock solution (1, 2.5 or 4
139 ml, required by the experimental design, Table 2) with 180 ml of Elix water. The pH of the solution
140 was raised to 3 with standardized 0.1 M NaOH solution. Next CS (DS 0.44) was added into the
141 mixed solution. The amount of CS was calculated based on the molar ratio of CS: SO_4^{2-} (1:1, 5.5:1
142 or 10:1, based on the experimental design, Table 2). The molar mass ($M = 229 \text{ g/mol}$) of CS was
143 calculated by taking into account the DS of CS. The pH of the solution was slowly increased to 4, 6
144 or 8 (based on the experimental design) with 0.1 M NaOH while stirring gently. The volume of the
145 solution was then filled to 200 ml with Elix water. Finally the pH of the solution was checked and if
146 necessary adjusted again. After 15 minutes of stirring a 20 ml sample was then taken from the
147 solution. The SO_4^{2-} -CS solution was transferred to the Amicon 8400 ultrafiltration cell equipped
148 with a magnetic stirring bar and a regenerated cellulose membrane with a 3000 Da molecular cut-
149 off weight (Millipore, Billerica, Massachusetts, USA). The apparatus was placed on the magnetic
150 stirrer and connected to a nitrogen gas flow. The pressure was set to 3 bar with a mixing speed of c.
151 250 rpm. Three samples (20 ml) were taken from the retentate after which the filtration was stopped
152 and one 20 ml sample was taken from the concentrated solution inside the cell. All samples were
153 fixed with 0.5 ml of concentrated HNO_3 and analysed with ICP-OES.

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155 2.7 ICP-OES measurements

156 Sulfur contents of the aqueous samples taken during the CS enhanced ultrafiltration experiments
157 were determined with Perkin Elmer Optima 5300 DV inductively coupled plasma-optical emission
158 spectrometer (ICP-OES). The instrument was equipped with an AS-93plus auto sampler, a Ryton
159 double pass Scott-type spray chamber and the Gem Tip Gross-flow pneumatic nebulizer. The
160 instrumental parameters for ICP-OES were as follows: RF power 1.3 kW, nebulizer gas flow 0.8
161 L/min, auxiliary gas flow 0.2 L/min, plasma gas flow 15 L/min, and sample uptake 1.5 mL/min.
162 The normal resolution and axial mode of viewing were used in the measurements. All the samples
163 were analysed in triplicate.

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168 2.8 Experimental design

169 2.8.1 Cationization of peel waste

170 The central composite circumscribed (CCC) design was chosen as the experimental design in order
 171 to study the effect of the reaction conditions on the preparation of CS from PW. The factors and
 172 their levels used in the experiments are given in Table 1. The CCC design consisted of five factors
 173 with two levels (high and low), including three centre points and star points. The levels for the
 174 factors were chosen based on the literature (Heinze et al., 2004; Pal, Sen, Karmakar, Mal & Singh,
 175 2008) and some preliminary experiments. They were also selected to be moderate but different
 176 enough from each other. Twenty-nine experiments were carried out including three replications
 177 determined at the centre point of the design in order to obtain the estimate for the experiment
 178 uncertainty. The star points (at the distance of ± 1.414 from the centre point) were fixed at 0 and 3.6
 179 h for the PW activation time (-0.6 h given by the CCC design was rounded up to 0), 20 and 90 °C
 180 for the reaction temperature, 2 and 9 h for the reaction time, 1:0.76 and 1:4.23 for the molar ratio of
 181 AGU:NaOH and 1:0.6 and 1:3.4 for the molar ratio of AGU:CHPTAC. The reaction temperature
 182 for the PW activation was set at 60 °C. All experiments were carried out in a random order and DS
 183 was used as the response. An empirical model was fitted to the results using MODDE 9.1
 184 (Umetrics) computer software. The statistical validation was determined using the ANOVA test at a
 185 95% confidence level.

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187 Table 1. The factors and their levels used in the central composite circumscribed design.

Factor	Low	Centre	High	Star point
Activation time (h)	0	1.5	3	-0.6; 3.6
Reaction temperature (°C)	30	55	80	20; 90
Reaction time (h)	3	5.5	8	2; 9
Molar equiv. of NaOH to AGU ^a	1.25	2.5	3.75	0.76; 4.23
Molar equiv. of CHPTAC to AGU ^a	1	2	3	0.59; 3.41

188 ^a AGU = anhydroglucose unit

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190 2.8.2 Removal of sulfate with cationized starch enhanced ultrafiltration

191 Preliminary SO₄²⁻ removal experiments in aqueous solutions were conducted in order to study the
 192 removal of SO₄²⁻ ions bound with CS by using the ultrafiltration technique. The binding
 193 experiments were conducted utilizing full 2³ factorial design. Eleven experiments were carried out

194 including three replications determined at the centre point of the design in order to obtain the
195 estimate for the experiment uncertainty. The concentration of SO_4^{2-} ions, the molar ratio of CS to
196 SO_4^{2-} ions and pH were selected as the factors for the experimental design. The levels for the
197 factors were selected based on the literature (Table 2) (Runtti et al., 2016; Cao et al., 2011). The DS
198 of the CS used in the experiments was 0.44.

199

200 Table 2. The factors and their levels used in the full 2^3 design.

Factor	Low	Center	High
SO_4^{2-} -S concentration (mg/l)	17	42	67
Molar equiv. of CS to SO_4^{2-}	1	5.5	10
pH	4	6	8

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202 3. Results and discussion

203

204 3.1 Optimization of reaction conditions

205 In this study cationized potato starch was prepared from potato peel waste. The central composite
206 circumscribed design was used to study the effect of the reaction conditions on the DS of the
207 products, which was used as the response in the experimental design. The DS values of the prepared
208 products as well as the reaction conditions for each experiment are presented in table 3.

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Table 3. Reaction conditions used in the central composite circumscribed design experiments, the run order of the reactions and the DS of the products of the cationization experiments.

Exp.	Run order	CHPTAC ^a	NaOH ^b	Time (h)	Pre-treatment (h)	T (°C)	DS
1	12	1	1.25	3	-	80	0.11
2	27	3	1.25	3	-	30	0
3	11	1	3.75	3	-	30	0.25
4	23	3	3.75	3	-	80	0.33
5	20	1	1.25	8	-	30	0.10
6	4	3	1.25	8	-	80	0
7	25	1	3.75	8	-	80	0.11
8	2	3	3.75	8	-	30	0.40
9	3	1	1.25	3	3	30	0
10	10	3	1.25	3	3	80	0
11	6	1	3.75	3	3	80	0.25
12	18	3	3.75	3	3	30	0.30
13	5	1	1.25	8	3	80	0
14	9	3	1.25	8	3	30	0
15	21	1	3.75	8	3	30	0.19
16	22	3	3.75	8	3	80	0.38
17	8	0.6	2.5	5.5	1.5	55	0.25
18	14	3.4	2.5	5.5	1.5	55	0
19	15	2	0.76	5.5	1.5	55	0
20	24	2	4.23	5.5	1.5	55	0.28
21	13	2	2.5	2	1.5	55	0.24
22	29	2	2.5	9	1.5	55	0.33
23	7	2	2.5	5.5	-	55	0.36
24	19	2	2.5	5.5	3.6	55	0.32
25	16	2	2.5	5.5	1.5	20	0.16
26	28	2	2.5	5.5	1.5	90	0.23
27	26	2	2.5	5.5	1.5	55	0.27
28	17	2	2.5	5.5	1.5	55	0.33
29	1	2	2.5	5.5	1.5	55	0.37

228 ^a Molar equivalent of CHPTAC compared to AGU

229 ^b Molar equivalent of NaOH compared to AGU

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231 The DS values of the cationized starches were determined by ¹H NMR (see for chapter 3.3). Once

232 the DS values were gained the model was fitted to the data using the multiple linear regression

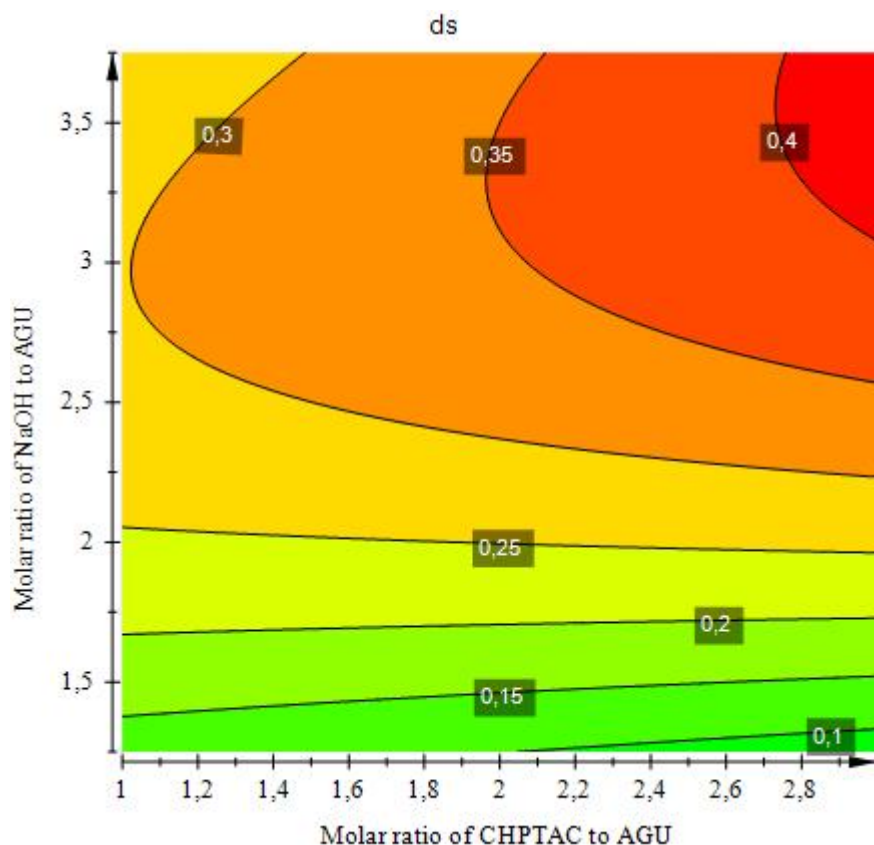
233 method. During modelling one data point (exp. 18) was excluded to improve the model. The
234 decision was based on the normal probability plot of residuals according to which the point was an
235 outlier. According to the analysed data, NaOH and CHPTAC were the only factors that had a
236 statistically significant effect on the DS of the CS (Supplementary material, Tables S1 and S2).
237 Also the squared terms of NaOH and temperature and the interaction term between the amount of
238 NaOH and the amount of CHPTAC had a significant effect (S1). Since temperature had an effect
239 through the squared term, it was left in the model (S1). The reaction time and the activation time
240 and their squared and interaction terms did not have a significant effect on the DS value of the CS.

241 Based on the obtained model, it was concluded that the amount of NaOH had the greatest impact
242 on the DS value of CS and the highest DS values would be reached with high amount of NaOH.
243 Also the interaction term between NaOH and CHPTAC had a positive effect on DS (Fig.1). Based
244 on the results of the cationization experiments, the highest DS (0.40, exp. 8, Table 3) was obtained
245 when the reaction conditions were: 30 °C for the reaction temperature, 8 h for the reaction time, 3
246 for the molar equivalent of CHPTAC to AGU and 3.75 for the molar equivalent of NaOH to AGU.
247 Activation time did not have an effect on the DS.

248 The repeatability of the cationization reactions was tested with the three centre point experiments
249 included in the design (exp. 27, 28 and 29, Table 3). There was some deviation between the DS
250 values of the centre point experiments (RSD% ca. 15%), which can be explained by the
251 heterogeneity of the peel waste.

252 The DS values of the products in this study varied from 0 to 0.40. 8 products of 29 had the DS
253 value 0, which is mainly due to the fact that the low level value for the molar equivalent of NaOH
254 to AGU was 1.25. As mentioned in section 2.8.1 the factor levels were selected based on the
255 literature and some preliminary experiments. Furthermore, NaOH levels were decided to keep as
256 low as possible, since NaOH degrades starch chains and causes the gelatinization of starch. It
257 should be noted, that despite the low amount of NaOH, the cationization reaction happened in two
258 cases (Exp. 1 and 5, Table 3) producing CS with DS of 0.11 and 0.10, respectively.

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Figure 1. The effect of the amount of CHPTAC and NaOH on the degree of substitution of cationized starch, when the reaction temperature (55 °C), reaction time (5.5 h) and the activation time (1.5 h) were at their centre point values.

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274 3.2 Scalability of the cationization experiments

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The scalability of the cationization experiments was studied by repeating two experiments (exp. 1 and 4) in larger scale. These experiments were selected since they had low and high levels of reagents, respectively. Each new experiment was done in duplicate. The scaling of the experiments was 2.5-fold and they were performed in a microwave oven. The reaction time, temperature and

279 activation time were kept constant, 3 h, 80 °C and 0 h, respectively. The scaled-up experiments are
280 presented in Table 4.

281 Both repeated reactions of experiment 1 produced CS with DS 0.16 (Table 4). The DS values
282 were in reasonable agreement with the DS of experiment 1 (DS 0.11, Table 3). However, both
283 repetitions for experiment 4 produced cationized starches with DS values (0.25 and 0.26, Table 4)
284 below the value of the original product (DS 0.33, Table 3). Lower DS values for the repetitions of
285 exp. 4 may be due to poorer mixing of the microwave oven compared to the microwave reactor,
286 combined with high amount of NaOH. NaOH is known to gelatinize starch, which causes the
287 viscosity of the reaction mixture to increase even at low temperature (Yamamoto, Isozumi, &
288 Sugitani, 2005). If mixing is inadequate it may result in lower DS values. Also, the heating
289 mechanisms of the microwave devices used in this study differ from one another. In microwave
290 reactor the irradiation is focused directly to the reaction mixture making the field homogenous. In
291 microwave oven, on the other hand, the irradiation is dispersed throughout the cavity volume
292 making the field less homogeneous. The microwave system rotates the reaction vessel continuously
293 throughout the energy field, which should average the field. (Hayes, 2002) However, the effect of
294 the heating mechanism on the DS values cannot be ruled out.

295

296 Table 4. Reaction conditions^a used in the scaled-up reactions and the DS values of the products.

Exp.	Heating method	CHPTAC ^b	NaOH ^b	DS (%)
1	Microwave	1	1.25	0.16
2	Microwave	1	1.25	0.16
3	Microwave	3	3.75	0.25
4	Microwave	3	3.75	0.26
5	Oil bath	3	3.75	0.48
6	Oil bath	3	3.75	0.44

297 ^a Reaction time, temperature and activation time in each experiment was 3 h, 80 °C and 0 h,
298 respectively

299 ^b Molar equivalent compared to AGU

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301 Finally, the reaction that yielded the highest DS when scaled-up in the microwave oven (exp. 4,
302 Table 3) was repeated as 17-fold with oil bath as the heating method. With oil bath it was easy to
303 monitor the reaction and adjust the mixing if it wasn't proper, which surely enough occurred after
304 ca. 10 min of heating. Indeed, the DS values (0.48 for exp. 5 and 0.44 for exp. 6, Table 4) of the
305 repetition products prepared in an oil bath were much higher than the DS values of the products

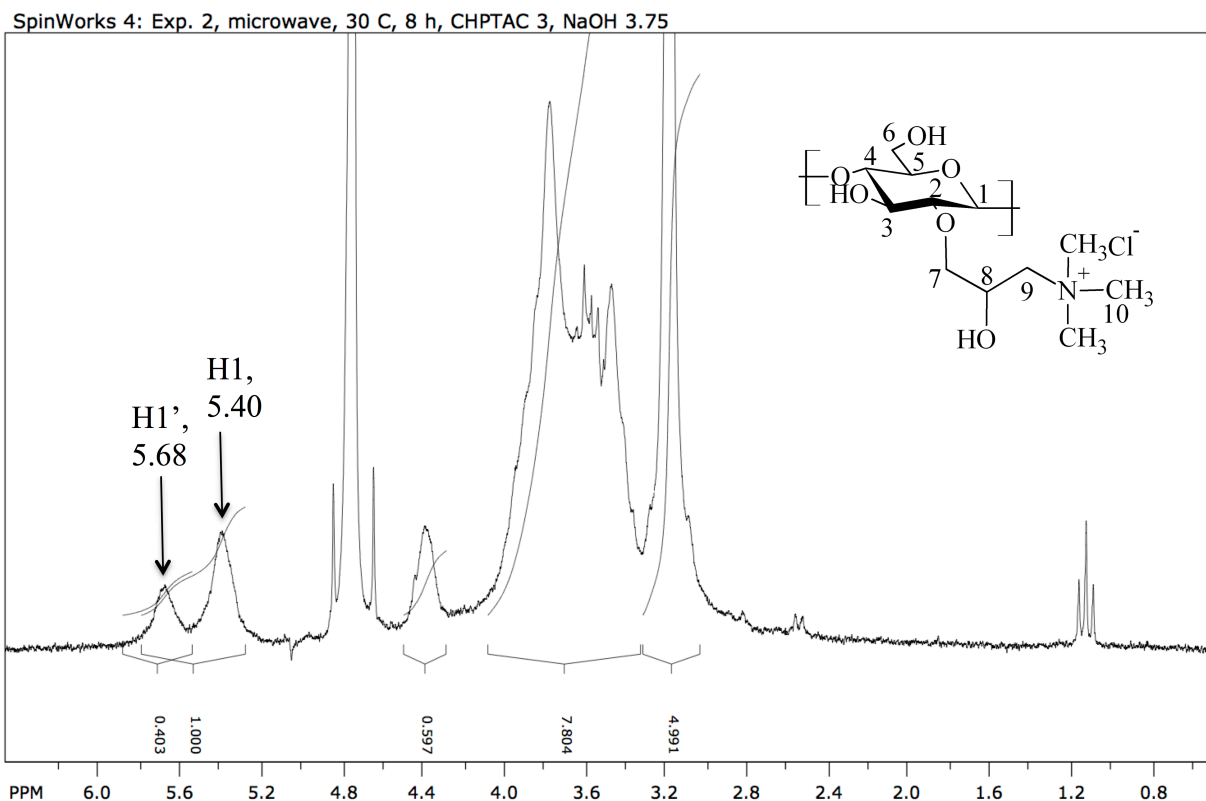
306 prepared in the microwave oven (exp. 3 and 4, Table 4). The DS values of exp. 5 and 6 were also
307 considerable higher than the DS value of the repeated experiment 4 indicating that the mixing in the
308 microwave reactor may not have been sufficient.

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310 3.3 Characterization of the cationized products with ^1H NMR, FTIR and FESEM

311 The structure of the prepared cationized starches was studied by ^1H NMR. The degree of
312 substitution of the cationized products is commonly determined by elemental analysis or the
313 Kjeldahl method (Krentz et al., 2006; Bendoraitiene et al., 2006). In this study, it was suspected that
314 the cationized products might contain some trace amounts of proteins or other nitrogen containing
315 components originating from the PW starting material, which could distort the DS values.

316 Therefore, DS of the reaction products was determined by using the ^1H NMR spectra. A typical ^1H
317 NMR spectrum of cationized product is presented in Figure 2 and the ^1H NMR spectra of native
318 potato starch, PW starting material and cationized products with DS 0.10 and 0.25 as
319 Supplementary material (Fig. S3, S4, S5 and S6, respectively). The OH group at C-2 is the most
320 reactive group of AGU due to the adjacent anomeric carbon C-1. Therefore, the substitution of
321 starch started at that position (Heinze et al., 2004; Lappalainen et al., 2015). The H-1 signal of the
322 unmodified AGU was at ca. 5.40 ppm (Figs. 2 and S3-S6). The cationization at C-2 changed the
323 chemical environment of H-1 and shifted the signal of H-1 at ca. 5.68 ppm (marked as H-1', Figs. 2,
324 S5 and S6). The spectra of all cationized products included the signals of H-1 (at 5.40 ppm) and H-
325 1' (at 5.68 ppm), indicating that starch had cationized in part of the glucose units. As the combined
326 integrals of H-1 and H-1' was set to 1, then DS corresponded to the integral of H-1'. (Lappalainen
327 et al., 2015) The increase in the integral of H-1' corresponds to the increase in the DS values, which
328 can clearly be seen in ^1H NMR spectra. The determined DS values of the cationized products are
329 presented in Table 3.



330
331 Figure 2. A typical ^1H NMR spectrum of the cationized potato peel waste (DS 0.40).

332

333 The attachment of the cationic group to the waste starch backbone was confirmed also with FTIR.
334 The spectra for native potato starch, dried potato peel waste and cationized product (DS 0.40) is
335 shown as Supplementary material (Fig. S7). In the spectrum of native potato starch and potato peel
336 waste, the characteristic absorptions for C–O bond stretching vibrations appear at 1148, 1076 and
337 994 cm^{-1} or 1010 cm^{-1} , respectively. The characteristic C–H stretching vibration appear at 2932 cm^{-1}
338 and 2921 cm^{-1} , respectively and the band resulting from the stretching vibration of the hydroxyl
339 groups (O–H) at 3297 cm^{-1} . (Wang et al., 2009; Pal et al, 2005; Fang, Fowler, Sayers & Williams,
340 2004) The spectrum of the cationized product is similar to that of potato peel waste and native
341 potato starch. The spectrum has the characteristic starch backbone bands and also additional bands,
342 arising from the C–N stretching vibrations of the quaternary ammonium groups, at 1476 and 1416
343 cm^{-1} . These additional bands can be considered as proof of the attachment of the cationic moiety to
344 the waste starch backbone. (Pal et al., 2005; Wang et al., 2009)

345 The morphology of dried PW and cationized products with DS of 0.10, 0.24 and 0.40 (exp. 5, 21
346 and 8, respectively, Table 3.) were analysed with FESEM (Supplementary material, Fig. S8). The
347 PW sample consisted mainly of large, oval potato starch granules with well-defined integrity.

348 However in the samples of the cationized products the starch granules were completely
349 disintegrated. Apparently the cationization reaction destroyed the granular structure completely
350 even though the reaction temperatures in reactions were not high (30, 55 and 30 °C, respectively).

351

352 3.4 Sulfate removal from aqueous solution with ultrafiltration and cationized starch enhanced
353 ultrafiltration

354 The sulfate removal experiments with ultrafiltration method were begun by studying the influence
355 of the regenerated cellulose ultrafiltration membrane on the SO_4^{2-} retention. The experiments were
356 done without CS. The operating conditions of these comparison experiments were identical to the
357 experiments with CS. The results, presented in Table 5, showed that the membrane caused some
358 retention of SO_4^{2-} ions. However the retention was only 28% at the best (exp. 2, Table 5).

359

360 Table 5. Reaction conditions^a used in sulfate removal experiments with ultrafiltration method and sulfate removal
361 percentages.

Exp.	SO_4^{2-} -S (mg/l)	pH	Removal (%)
1	17	4	15
2	17	8	28
3	42	6	15
4	67	4	14
5	67	8	18

362 ^a Each solution was mixed 15 min at room temperature after pH adjustment

363

364 The cationized starch enhanced ultrafiltration experiments were performed with the CS prepared
365 by the scaled-up oil bath reaction (DS 0.44, Exp. 6, Table 4). The prepared CS was initially in a salt
366 form with Cl^- anion as a counterion for the quaternary ammonium group. Hence it was thought that
367 the removal of SO_4^{2-} occurred mainly through an anion exchange between the Cl^- counterion and
368 the SO_4^{2-} ion. The same removal mechanism has been observed for example with Cr(VI) removal
369 (Sánchez & Rivas 2011). The results for the SO_4^{2-} removal experiments as well as the conditions for
370 each experiment are presented in Table 6. The removal-% was determined with ICP-OES by
371 comparing the SO_4^{2-} -S concentration of the initial SO_4^{2-} solution to the SO_4^{2-} -S concentration of the
372 solution that was treated with cationized starch enhanced ultrafiltration. The model was fitted to the
373 data and then improved by excluding the coefficients, which did not have an effect on it. According
374 to the analysed data, the molar equivalent of CS to SO_4^{2-} and the SO_4^{2-} ion concentration had a
375 statistically significant effect on the SO_4^{2-} removal (Supplementary material, Tables S5 and S6).

376 pH, the squared terms of the factors and the interaction terms between the factors did not have a
377 statistically significant effect on the SO_4^{2-} removal so they were excluded from the model.

378

379 Table 6. Reaction conditions^a used in full 2^3 factorial design sulfate removal experiments with cationized starch
380 enhanced ultrafiltration method, the run order of the experiments and sulfate removal percentages.

Exp.	Run order	SO_4^{2-} -S (mg/l)	CS ^b	pH	Removal (%)
1	9	17	1	4	36
2	1	67	1	4	31
3	3	17	10	4	74
4	5	67	10	4	67
5	7	17	1	8	49
6	6	67	1	8	32
7	4	17	10	8	74
8	10	67	10	8	67
9	2	42	5.5	6	58
10	11	42	5.5	6	58
11	8	42	5.5	6	54

381 ^a Each solution was mixed 15 min at room temperature after pH adjustment

382 ^b Molar equivalent of cationized starch compared to SO_4^{2-}

383

384 The highest SO_4^{2-} removal (74%) was reached with 15 minutes contact time when the SO_4^{2-}
385 concentration was low and cationized starch concentration high (exp. 3 and 7, Table 6). However,
386 high removal percentage (67%) was reached also with high SO_4^{2-} concentration as long as the CS
387 concentration was high as well (exp. 4 and 8, Table 6). Anyhow, the retention of SO_4^{2-} ions was
388 considerably higher with CS than without it.

389 Potato peel waste contains also small amounts of other polysaccharides besides starch, e.g.
390 cellulose and hemicellulose (Arapoglou et al., 2010). It is possible that also these polysaccharides
391 were cationized during the starch cationization experiments, even though they were not observed in
392 ¹H NMR or FTIR spectra. Therefore, other cationized polysaccharides may have contributed to the
393 SO_4^{2-} removal along with CS. However, from the utilization point of view it is insignificant whether
394 SO_4^{2-} removal is due to CS or partly due to other polysaccharides as well.

395 The results of the SO_4^{2-} removal experiments conducted in this study are in accordance with the
396 SO_4^{2-} experiments reported in literature. Cao et al. (2011) achieved 79% sulfate removal with
397 modified rice straw with 2 h contact time, when the initial SO_4^{2-} -S concentration was 33 mg/l.

398 Runtti et al. (2016) used Fe-modified carbon residue as an adsorbent for SO_4^{2-} and achieved 89%
399 removal in 24 h with initial SO_4^{2-} -S concentration of 17 mg/l. When commercial activated carbon
400 was used as an adsorbent (initial SO_4^{2-} -S concentration 67 mg/l), 11% removal was achieved in 24 h
401 (Runtti et al., 2016). Iakovleva et al. (2015) utilized kaolinite and calcite containing pulp and paper
402 waste and achieved 99% sulfate removal, when the initial SO_4^{2-} concentration was 20 mg/l.
403 However, the contact time was long, 480 h (Iakovleva, Mäkilä, Salonen, Sitarz & Sillanpää, 2015).

404

405 Conclusions

406

407 In this study, we have shown that the starch-rich food industry waste stream, potato peel waste, can
408 be modified by cationization to produce CS. Furthermore, PW can be used in the synthesis as
409 received from the peeling factory without any pre-treatment. The cationization reactions were
410 performed by applying experimental design in order to study the effect of the reaction conditions on
411 the DS of CS. According to the results the DS was mainly affected by the increasing amount of
412 NaOH and the cationization reagent, CHPTAC. NaOH and CHPTAC also had an interaction effect
413 on the DS. The reaction time and temperature, as well as the activation time did not have a
414 statistically significant effect on the DS. The highest DS (0.40) was obtained when the reaction
415 conditions were: 30 °C for the reaction temperature, 8 h for the reaction time, 3 for the molar
416 equivalent of CHPTAC to AGU and 3.75 for the molar equivalent of NaOH to AGU. Activation of
417 peel waste was not needed for the highest DS value.

418 The prepared CS combined with ultrafiltration technique was used in preliminary SO_4^{2-} removal
419 experiments. The experiments were performed utilizing experimental design in order to study the
420 effect of reaction conditions on SO_4^{2-} removal. According to the analysed data SO_4^{2-} removal,
421 which was 74% at highest, was mainly affected by the amount CS and the initial SO_4^{2-} ion
422 concentration. The results hence suggest, that the CS prepared from PW could be used e.g. as a
423 biodegradable and economic adsorbent for water purification or in the removal of nutrients from
424 aqueous solutions. Presently, the nutrient removal experiments are on-going.

425

426 Declaration of interest

427

428 Conflict of interest: none.

429

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435

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