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The substitution pattern in cationised and oxidised potato starch granules

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Abstract

The distribution pattern of substituents within the granules and the components of two cationised and two oxidised potato starches was studied. The level of crystallinity in wet-cationised (WC) and hypochlorite oxidised (HO) starch granules was similar to that of native starch granules but lower in dry-cationised (DC) and peroxide oxidised (PO) granules. However, the melting temperature of DC granules remained similar to native granules but was decreased in the other samples. With all modified starches, the initial rate of acid hydrolysis (lintnerisation) was increased compared to native granules. The degree of substitution decreased only slightly in WC granules after the lintnerisation, whereas virtually all the substituted glucosyl units in DC starch were hydrolysed already at initial stages. The decrease of substituents in the HO and PO starches was intermediate. The starches were partly resistant to the action of isoamylase and the successive β-amylolysis, suggesting that substituents were found both close to the branches and near the nonreducing ends in the amylopectin component. It is suggested that the DC starch was preferentially cationised at the surface of the granules, whereas WC and oxidised starches were modified throughout the granules.

Keywords: Substitution pattern; Cationised potato starch; Oxidised potato starch; Lintnerisation

1. Introduction

Cationic and oxidised starches are used in paper manufacturing for the improvement of paper quality, surface sizing, and binding of the cellulose fibres. Different methods for the production of these starch derivatives are in use. Cationised starch is commonly prepared by a wet-cationisation method [1], in which an aqueous alkaline granule suspension reacts with a quaternary ammonium reagent (2,3-epoxypropyltrimethylammonium chloride). Preferentially, the D-glucosyl residues of the starch components become mono-substituted at position C-2, though C-3 and C-6 are also substituted and small amounts of disubstituted residues exist [2,3]. The cationisation can, however, also be performed in the absence of a liquid phase, which results in reduced environmental pollution. In this dry-cationisation process [4,5] hydroxide is first sprayed onto the starch, whereafter the

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quaternary amino groups are introduced by spraying the chemicals onto the starch. Common reagents in use to manufacture oxidised starch are sodium hypochlorite and hydrogen peroxide. During the oxidation process the starch components are extensively depolymerised [6–10], but the granules remain intact. The D-glucosyl residues become functionalised with carboxyl and carbonyl groups in different proportions depending on the conditions used [7–9]. Ring-opening without chain cleavage also occurs resulting in di-functionalised moieties at C-2 and C-3 [11].

Comparatively little is, however, known about the location of the substituents on the starch polymers or inside the starch granules. Introduced substituents interfere with the action of various enzymes, which results in different hydrolysis patterns when compared to native starch and has been used for structural investigations of some modified starches [10,12–15]. A conclusion from these studies is that preferentially amylose and the regions around the branches in amylopectin become modified. This largely corresponds to the amorphous parts within the semicrystalline starch granules [16,17]. In a light microscopy study of hydroxypropylated potato starch, Kim et al. [18] concluded that the substituents were mainly distributed in the central region of the granules. However, Vihervaara et al. [19] reported that the substituents in wet-cationised potato starch were evenly distributed in the granules, whereas in dry-cationised starch the outer regions were preferentially modified.

In this investigation we analysed the distribution pattern of substituents in two oxidised and two cationised potato starch granules by different methods. First, the effect on the physical properties of the granules was analysed by X-ray diffraction and differential scanning calorimetry (DSC). Secondly, the amount of substituents was analysed during the solubilisation of the amorphous and less organised parts of the granules in diluted hydrochloric acid (lintnerisation). Finally, the positions of the substituents within the starch components before and after the lintnerisation was studied with enzymatic methods.

2. Experimental

Starches and enzymes.—Native potato starch and the NaClO oxidised (HO) and H_2O_2 oxidised (PO) starches were commercial samples from Raisio Chemicals Oy, Finland. Wet- and dry-cationised (WC and DC, respectively) samples were prepared as described below. Sweet potato β-amylase ((1→4)-α-D-glucan maltohydrolase; EC 3.2.1.2) was from Sigma and the isoamylase of *Pseudomonas amyloderamosa* (glycogen 6-glucanohydrolase; EC 3.2.1.68) was from Hayashibara.

Wet-cationisation.—A slurry of potato starch in water (40% w/v) was kept at 42 °C under constant mixing. The pH was increased to 11.0 with 2.5% NaOH and a commercial etherifying agent (RAISACAT, Raisio Chemicals) containing 2,3-epoxypropyltrimethylammonium chloride was added to the slurry. The pH was maintained at 11.0–11.2 and the reaction was stopped after 20 h by adjusting the pH to 6.5 with 25% H_2SO_4. An aliquot (30 mL) of the slurry was filtered and washed with a 1:1 mixture of EtOH–water (200 mL) and then with EtOH (50 mL). The sample was dried for 1 h at 105 °C and the nitrogen content in the dry sample was analysed using the Kjeldahl method. The degree of substitution (DS) was calculated as follows:

$$\mathrm{DS} = (162.15 \times \mathrm{N\%})/1400 - (\mathrm{N\%} \times M_{\mathrm{subst}})$$

where M_{subst} is the molecular weight of the substituent in the chloride form (151.6). The rest of the slurry was filtered (Schleicher & Shuell, 604 Rundfilter), washed with water, then EtOH, and finally dried in acetone. The dry weight of the samples was measured with a Sartorius Thermo Control balance.

Dry-cationisation.—The starch (2000 g) was put into a cylindrical container and during vigorous mixing at increased air pressure (1.5 atm) and temperature (60 °C) a 17% (w/v) NaOH soln (180 g) was sprayed onto the starch in small portions. The starch was mixed until it was dry before the cationising chemical was added in small portions. The mixture was allowed to react for 5 h. An aliquot of the granules (30 g) was mixed with EtOH (600 mL) with a blender at 2000 rpm for 30 s and then filtered. This procedure was repeated three times. The starch was then fractionated through two sieves with pore sizes of 500 and 150 μm, respectively, giving a final material with diameter < 150 μm. The fraction was then washed with EtOH and analysed for its nitrogen content. Solid citric acid was added in small portions up to 1% (w/v) to the sample in order to neutralise the remaining NaOH from the cationisation process.

NaClO oxidation.—A starch slurry of 20–30% solids was prepared and the pH was adjusted to 9–10 with NaOH. Active NaClO (20% of dry starch weight) was added into the slurry in small portions and the temperature was kept at 30–40 °C during the oxidation. After 24 h the starch slurry was neutralised to pH 6 with H_2SO_4.

H_2O_2 oxidation.—A starch slurry was prepared as described above. $CuSO_4$ (0.01%) was added as catalyst after which H_2O_2 (3% of dry starch weight) was added in small portions at 30–40 °C. After 24 h neutralisation was carried out as above.

Analysis of carboxyl groups.—The content of carboxyl groups in oxidised starch was analysed titrimetrically as described by Smith [20]. The result was expressed as a percentage of COOH on a dry weight basis.

X-ray diffraction.—Rehydration of the samples (20 mg dry matter) was performed via a vapour phase isopiestic equilibration over a satd salt soln delivering partial water vapour pressures of 0.90 using $BaCl_2$ at 25 °C. Samples were then sealed between two aluminium foils to prevent any significant change in water content during the measurement. Diffraction diagrams were recorded using a transmission technique with a XRG 3000 X-ray generator (Inel Orléans, France) operating at 40 kV and 30 mA. Cu K_{a1} radiation ($\lambda = 0.15405$ nm) was selected using a quartz monochromator. A curved position sensitive detector (Inel CPS120) was used to monitor the diffracted intensities using 3 h exposure periods. The recorded diagrams were normalised at the same total scattering between 3 and 30° (2θ). Crystallisation was determined using the Wakelin's technique [21] with recrystallised amylose and extruded potato starch as B-type and amorphous standards, respectively.

Differential scanning calorimetry (DSC).—An automated heat flux 'differential scanning calorimeter' (SETARAM DSC 121, France) was used for the thermal analysis of 100 mg samples (80% H_2O) sealed in stainless steel high pressure cells. Scans were run between 20 and 180 °C at 3 °C/min. Calibration was checked using indium (429.6 K) and gallium (302.7 K) melting. The reference cell contained dry alumina powder. Enthalpies were expressed in Joules per gram of dry matter.

Lintnerisation.—Starch granules were solubilised with 2.2 M HCl (2.5 g starch/100 mL acid) at 35 °C according to Robin et al. [22]. The starch was stirred regularly and the carbohydrate content in the supernatant was measured daily using the phenol–H_2SO_4 reagent [23]. The lintnerisation was continued to different stages after which the granular residues were collected by centrifugation for 5 min at 1400*g*, neutralised with 0.1 M NaOAc, and washed three times with deionised water. Finally, the residues were suspended in a small volume of deionised water and lyophilised.

Enzymatic analyses.—The starches were analysed by hydrolysis with either isoamylase or β-amylase or by a combination of the two enzymes. A stock soln of the starch (5 mg/mL) was prepared by boiling before dilution with water to a concentration of 3.6 mg/mL. An aliquot (0.9 mL) was treated with 0.1 M NaOAc buffer (0.1 mL, pH 3.5) and freshly diluted ($5\times$) isoamylase (10 μL) and then incubated overnight at room temperature. The sample was boiled for 10 min to inhibit the enzyme activity. In an aliquot (0.3 mL) of the isoamylolysis mixture, the pH was adjusted to 4.8 with 0.005 M NaOAc and the volume was adjusted to 0.59 mL with 0.1 M NaOAc buffer (pH 4.8) before addition of β-amylase (2 μL). The sample was incubated overnight and then boiled. When treated with only β-amylase, the stock soln (0.9 mL) was diluted with 0.1 M NaOAc buffer (0.1 mL, pH 4.8) and the enzyme (2 μL) was then added.

Gel-permeation chromatography.—Samples (0.2 mL) in ~0.5 M KOH were applied onto a column (1×80 cm) of Sepharose CL-6B (Pharmacia) and eluted with 0.5 M KOH at a flow rate of 0.5 mL/min. Fractions (0.5 mL) were analysed with the phenol–H_2SO_4 reagent

Table 1
Characterisation of cationised and oxidised potato starch granules

Sample	N or COOH [a] (weight %)	Crystallinity [b] (± 3%)	Water content (d.b.)	ΔH [c] (J/g)	T_m [c] (°C)
Native starch	0.009 [d]	34	29	18.3	62.3
Cationised [e]					
WC-starch	0.394	34	41	15.6	55.6
DC-starch	0.463	27	34	12.1	62.4
Oxidised [f]					
HO-starch	1.04	34	25	16.9	54.2
PO-starch [g]	0.50	28	50	3.02	56.7
				15.1	69.6

[a] Nitrogen obtained in native starch.
[b] From X-ray diffraction data (Fig. 1).
[c] From differential scanning calorimetry (Fig. 2).
[d] N-content in native starch.
[e] Wet- and dry-cationised starch, respectively.
[f] The HO- and PO-starches were oxidised with NaClO and H_2O_2, respectively.
[g] The sample possessed two endothermic maxima with DSC.

[23]. The column was calibrated [24] with dextrins of known average degree of polymerisation (DP). The DP of the samples was calculated as $\Sigma c_i/\Sigma(c_i/\mathrm{DP}_i)$, in which c_i is the carbohydrate concentration and DP_i is the DP of fraction i.

High-performance anion-exchange chromatography (HPAEC).—Ion-exchange chromatography was performed on a Dionex HPLC system (series 4500i, Dionex, USA) with a BioLC Gradient Pump and with pulsed amperometric detection (PAD). The samples (in concentrations of 1.1, 1.7, or 3.3 mg/mL) were filtered and injected (25 μL) onto a CarboPac PA-100 anion-exchange column (250 × 4 mm) in combination with a CarboPac PA-100 guard column and eluted at a flow rate of 1.0 mL/min. The gradient was a slight modification of that described by Koch et al. [25] and included eluent A (150 mM NaOH) and eluent B (150 mM NaOH containing 500 mM NaOAc). The column was equilibrated and the sample was applied at 75% A and 25% B. The sample was then eluted with a linear gradient of eluent B in four steps as follows: from 0–1 min a gradient of eluent B from 25–34%; 1–6 min from 34–45%; 6–55.4 min from 45–67%; and 55.4–80.4 min from 67–90%.

3. Results

The nitrogen content of the wet-cationised (WC) potato starch granules (Table 1) represented a degree of substitution of 0.048 that are used in commercial applications. The degree of substitution of the dry-cationised (DC) sample was 0.056. The sodium hypochlorite (HO) and the hydrogen peroxide oxidised (PO) potato starch samples were identical to those characterised previously [10,15] and their content of carboxyl groups was 1.04% and 0.50% (d.b.), respectively.

The recorded X-ray diffraction diagrams and corresponding crystallinities are given in Fig. 1 and Table 1, respectively. All samples

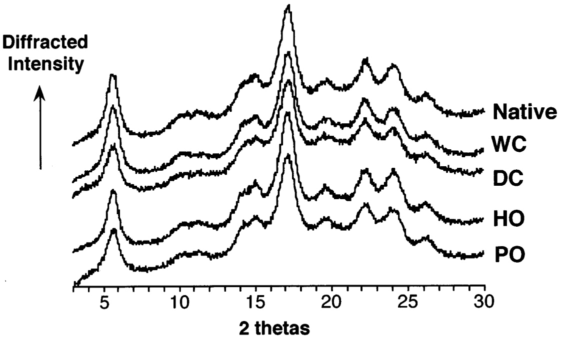

Fig. 1. X-ray diffractograms of native, wet-cationised (WC), dry-cationised (DC), NaClO oxidised (HO), and H_2O_2 oxidised (PO) potato starch.

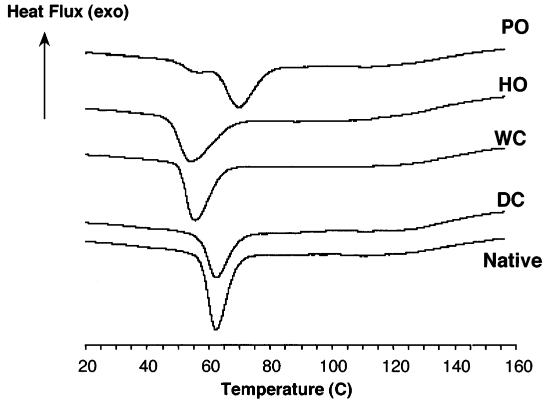

Fig. 2. DSC thermograms of H_2O_2 oxidised (PO), NaClO oxidised (HO), wet-cationised (WC), dry-cationised (DC), and native potato starch.

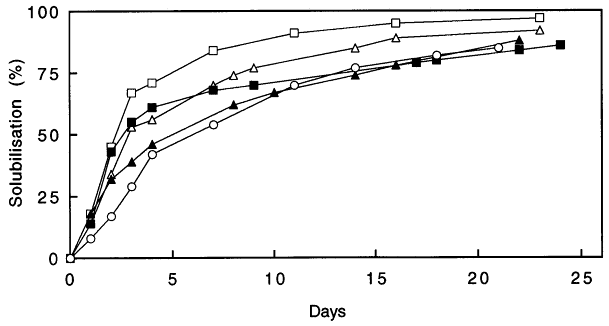

Fig. 3. Lintnerisation of native potato starch granules (○) in comparison with wet-cationised (■), dry-cationised (▲), NaClO oxidised (□), and H_2O_2 oxidised (△) starches.

possessed the pure B-type pattern that is characteristic for potato starch granules. The relative crystallinity was not decreased by the chemical modification in the HO and WC samples, while it decreased slightly for PO and DC starches (Table 1). The different samples were measured at high water contents, because the B-type crystallinity is highly sensitive to water content [26]. Some of the chemical modifications used induced very high water sorption capacities, as for PO and WC starch. Nevertheless, our measurements can be taken in account since the resulting water content of modified starches was always higher than 30%, the hydration level above which there is no more increase of crystallinity with water content, except for HO-starch, which was as crystalline as the native starch.

The DSC thermograms are shown in Fig. 2 and the corresponding data in Table 1. Melting temperatures of native and DC starches were rather similar around 62 °C, while those of WC and HO starch were lower (54–55 °C). The melting behaviour of the PO-sample was more complex with a main endotherm at 69 °C (enthalpy 15 J/g) and a smaller one at 56 °C (enthalpy 3 J/g). The chemical modification induced a decrease of the melting enthalpy compared to native starch, especially for the DC-sample.

When treated with diluted hydrochloric acid, the native potato starch granules solubilised by rates that have been described as typical for several types of starches [22,27–30]. The initial, fast rate lasted for 4 days up to a degree of solubilisation of ~40%, after which a slower rate of solubilisation continued (Fig. 3). When ~70% of the granules had been solubilised, the lintnerisation became very slow and after 21 days 85% of the starch had been solubilised. The initial stage of lintnerisation was faster with the cationised samples. With the WC-starch, the slower stage started at approximately the same solubilisation level (~40%) as with the native granules, and the final degree of solubilisation was very similar to the native sample. For the DC-starch the rate decreased already after 2 days and the granules were then solubilised by a pattern similar to the native granules.

The oxidised starches were also lintnerised at a faster rate than native granules, and the HO-starch was more susceptible to the acid than the PO-starch (Fig. 3). Different from the cationised samples, however, the initial stage of lintnerisation continued up to higher levels of solubilisation. With the PO-starch the second stage started when 53% of the granules had been solubilised, whereas with the HO-starch this stage was obtained at 67%. The lintnerisation then continued at apparently similar rate as with the native granules and 92 and 97% of the PO and HO starches, respectively, was solubilised after 23 days.

The remaining nitrogen content was analysed in the granular residues of the two cationised starches after a low and a high level of lintnerisation (LL and HL, respectively, when ~25 and ~80% of the granules had been solubilised). In the WC-starch the relative nitrogen content had decreased to 91% at the LL-level and at the HL-level the value remained constant (Table 2). The changes within the DC-starch granules were very different. Already at the LL-level the residual nitrogen content was only 2% of the original and later only trace amounts remained. The content of the carboxyl groups in the oxidised starches also decreased during lintnerisation. When 80% of the starch had been solubilised, the residual granules of both samples contained 64% of the original level.

The composition of the starch granules was analysed enzymatically by debranching with isoamylase and by successive β-amylolysis. The unit chain distribution of the debranched native potato starch is shown in Fig. 4(a). Chains with a DP > 300 represented the amylose component [10], whereas shorter chains were mostly of amylopectin origin. A small amount of chains resistant to the attack of β-amylase was found among the shorter chains in this sample (Fig. 4(b)) and were probably phosphorylated [31,32]. The molecular distribution of the isoamylolysates of the cationised samples possessed increased amounts of material in the DP-range 100–1000, showing that the debranching was not complete (Fig. 4(a)). A small increase of the material with DP > 1000 was also observed.

Table 2
Characterisation of modified potato starch granules before lintnerisation and in residual undissolved granules

Sample [a]	Solubilised carbohydrates (%)	N or COOH (weight %)	Relative content of substituents (%) [b]
Native starch	0	0.009 [c]	
LL	30	n.a. [d]	
HL	74	n.a.	
WC-starch	0	0.394	100
LL	28	0.357	91
HL	81	0.356	90
DC-starch	0	0.463	100
LL	23	0.011	2
HL	72	0.002	0
HO-starch	0	1.04	100
LL	29	0.73	70
HL	81	0.66	64
PO-starch	0	0.50	100
LL	24	0.43	86
HL	80	0.32	64

[a] The samples were solubilised to a low and high lintnerisation level (LL and HL, respectively).
[b] Content in granule residues in comparison to original level before lintnerisation.
[c] N-content in native starch.
[d] n.a., not analysed.

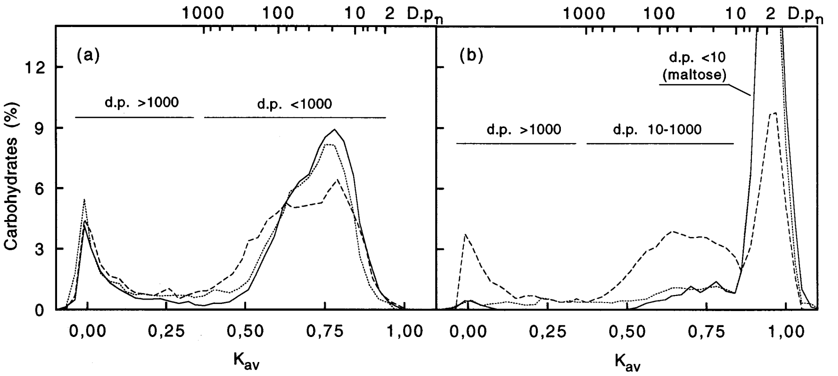

Fig. 4. Molecular weight distribution on Sepharose CL-6B of (a) isoamylolysates of native (—), wet-cationised (---), and dry-cationised (···) starches, and (b) the hydrolysates after successive β-amylolysis.

Table 3
Characterisation of components in debranched native and modified potato starches

Parameter	Native	Cationised		Oxidised [a]	
		WC	DC	HO	PO
After isoamylolysis					
Components of DP > 1000					
Weight %	16	21	21	3	3
Components of DP < 1000					
Weight %	84	79	79	97	97
DP	26	36	34	23	23
Successive β-amylolysis					
β-Amylolysis limit (%) [b]	90	38	82	64	75
Components of DP > 1000 [c]					
Weight %	1	16	4		
Components of DP 10–1000 [c]					
Weight %	9	46	14	36	25
DP	29	39	38	32	29

[a] Data from Ref. [10].

[b] The amount of maltose produced.

[c] Dextrins of high or low molecular weight that were resistant to β-amylolysis.

Thus, the samples contained dextrins that were resistant to the action of the isoamylase. When subjected to the successive β-amylolysis, an increased amount of dextrins that also remained resistant to the β-amylase attack was observed (Fig. 4(b)). These dextrins were divided into a group of high molecular weight (DP > 1000) and a group of lower molecular weight from DP 10 ~ 1000. Smaller oligosaccharides were not resolved from the large peak of maltose.

In the debranched native starch the material with DP < 1000 represented 84% of the total carbohydrate content (Table 3). Because the potato starch contained ~ 21% amylose [10], this included the unit chains of the amylopectin fraction together with a part of the shorter chains from the amylose. The proportion of the low-molecular-weight fraction decreased to 79% in the cationised starches, though the proportion of material within the DP range 100–1000 was higher in the WC-sample than in the DC-starch (Fig. 4(a)). The average DP of the low-molecular-weight material increased from 26 in the native starch to ~ 35 in the cationised samples, reflecting the increase of resistance to isoamylase.

The β-amylase produced 90% of maltose (the β-limit value) from the debranched native sample (Table 3). The enzyme was clearly blocked in its action after cationisation. From the WC-sample only 38% of maltose was produced. The resistant dextrins with DP 10–1000, that in the native starch possessed 9% of the weight amount, increased to 46% in the WC-sample. The average DP of this material increased from 29 to 39. A large part of the apparent amylose fraction (DP > 1000) was also resistant to β-amylolysis. The production of maltose from the isoamylolysate of the DC-sample was much higher than after wet-cationisation and was, in fact, more close to that obtained with the native sample (Table 3). The fraction of resistant dextrins with DP 10–1000 represented only 14% of the carbohydrates, but the average DP was similar to the WC-sample. Only traces of β-amylase resistant material at the void volume was detected.

The molecular composition of the oxidised starches was characterised previously [10] and some of the results are summarised in Table 3. They possessed most of the carbohydrates as dextrins with DP < 1000 after debranching. The average DP of this material was lower than in the cationised starch.

The molecular weight distribution of isoamylolysates and successive β-amylolysis was also analysed after the lintnerisation (Fig. 5). In accordance with other reports [22,27,30] the molecular weight was reduced drastically already after a low level of lintnerisation. At later stages the material was mostly corresponding to the short linear chains of amylopectin that participate in the crystalline lamellae within the starch granules [22,33] (Fig. 5(a,c,e)). The data are collected in Table 4, in which also the average DP of the starch residues before the debranching is included. All samples possessed resistant dextrins after β-amylase attack (Fig. 5(b,d,f)) and the β-limit values of the modified samples increased at all stages of lintnerisation (Table 4). Therefore, the amount of resistant dextrins decreased. The average DP of these dextrins was similar to that obtained with the isoamylase alone.

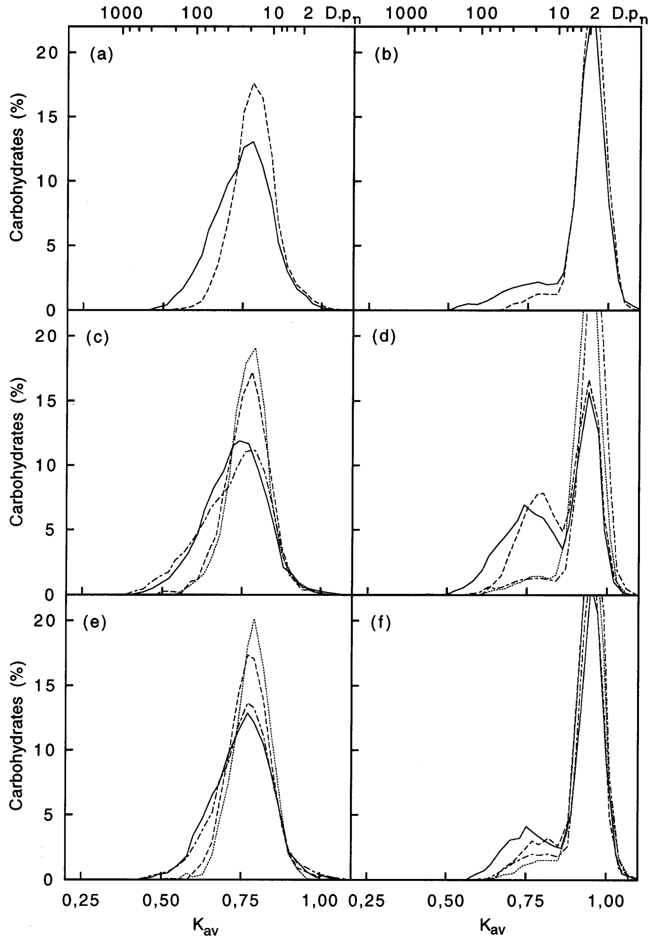

Fig. 5. Molecular weight distribution on Sepharose CL-6B of lintnerised starches. (a) Isoamylolysates of native starch granule residues at a low (LL; —) and high (HL; ---) lintnerisation level and (b) after successive β-amylolysis. (c) The isoamylolysates of residues from wet-cationised starch at the LL (—) and HL (---) levels and the dry-cationised starch at LL (-··-) and HL (···) levels and (d) after successive β-amylolysis. (e) The isoamylolysates of residues from NaClO oxidised starch at the LL (—) and HL (---) levels and H_2O_2 oxidised starch at LL (-··-) and HL (···) levels and (f) after successive β-amylolysis.

The native and the WC-starch were also analysed qualitatively by HPAEC after lintnerisation to the high level (Figs. 6 and 7, respectively). The distribution of dextrins in the lintnerised native starch possessed a major group of short chains around DP 14 and another group of dextrins with DP 23 ∼ 40 (Fig. 6(a)). When subjected to isoamylolysis the latter group was only slightly debranched into the short chains (Fig. 6(b)) confirming the distribution obtained by gel-permeation chromatography (Fig. 5(a)). After successive β-amylolysis the resistant dextrins were resolved as two groups with DP ranges estimated to be 4 ∼ 8 and 9 ∼ 20 (Fig. 6(c)). The lintnerised WC-sample also possessed a major group around DP ∼ 14 and a second, minor group of larger dextrins (Fig. 7(a)). The profile was, however, considerably more complex than that of the native starch, suggesting a range of neutral and cationised molecular species and their actual DP could only be roughly estimated. The profile was slightly changed after the isoamylase treatment (Fig. 7(b)). The composition of resistant dextrins obtained by successive β-amylolysis was also complex (Fig. 7(c)) but spanned an apparently similar DP range as in the native starch. The two lintnerised starches were also treated by β-amylase without a prior isoamylolysis. The hydrolysis mixture of the native starch possessed a major group of β-limit dextrins at DP 5–7 in addition to smaller amounts of dextrins with DP up to ∼ 20 (Fig. 6(d)). Trace amounts of larger limit dextrins seemed, however, also to be present. The β-amylolysis mixture of the cationised sample was complex and possessed considerably more limit dextrins with DP 8 ∼ 20 (Fig. 7(d)).

4. Discussion

Starch granules are build up of alternating semicrystalline and amorphous rings, of which the former constitute regular layers of thin crystalline and amorphous lamellae [17,34,35]. When starch granules are treated in hydrochloric acid, it is the amorphous rings that are preferentially degraded [36]. 40% of the native

Table 4
Characterisation of the components in native and modified potato starches at low lintnerisation (LL) and high lintnerisation (HL) levels

Parameter	Native		Cationised [a]				Oxidised [b]			
			WC		DC		HO		PO	
	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL
Before isoamylolysis										
DP	40	18	46	22	91	19	37	23	43	21
After isoamylolysis										
Weight %	100	100	100	100	100	100	100	100	100	100
DP	20	16	27	18	21	19	21	18	21	18
Successive β-amylolysis										
β-Amylolysis limit (%) [c]	81	94	52	61	92	93	73	83	87	90
Components of DP > 10 [d]										
Weight %	17	6	47	39	9	9	24	17	12	9
DP	27	18	26	20	21	20	23	19	21	21

[a] Wet- and dry-cationised samples.
[b] NaClO and H_2O_2 oxidised samples.
[c] The amount of maltose produced.
[d] Dextrins resistant to β-amylolysis.

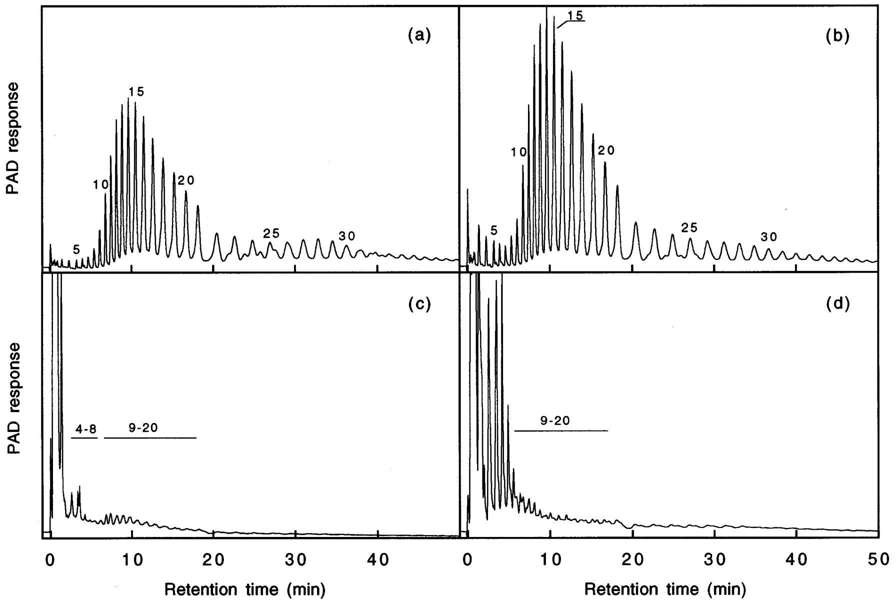

Fig. 6. HPAEC-PAD chromatograms of native potato starch residues at (a) a high lintnerisation level and (b) the sample after isoamylolysis, (c) after isoamylolysis + β-amylolysis, and (d) after β-amylolysis. The concentration of the samples applied to the column was (a,b) 1.1, (c) 1.7, and (d) 3.3 mg/mL.

potato starch was hydrolysed by a fast, initial rate (Fig. 3), which therefore mainly should represent the amount of material included in the amorphous rings [22,27]. However, all of the starch material had depolymerised extensively already at the low level of lintnerisation (LL-level) when only 30% of the granules had solubilised (Table 4), which showed that the acid also penetrated the amorphous lamellae within the semicrystalline rings already at early stages. When ~ 70% of the granules had been solubilised, the lintnerisation rate became very slow (Fig. 3), which corresponded roughly to the crystallinity determined experimentally by X-ray diffraction. The DSC data obtained for the native product was characteristic for a normal potato starch with a melting temperature of 62 °C and a rather high melting enthalpy, also consistent with the degree of crystallinity.

The initial stage of the lintnerisation of the WC-starch granules was faster, but continued up to the same level (40%) as for native granules (Fig. 3). This suggested that the cationic substituents made the most amorphous parts of the granules more susceptible to the hydrochloric acid. After this, however, the rate decreased fast and the final level of solubilisation became similar as for the native granules. Therefore, the more organised areas inside the granules seemed unaffected by the substitutions in terms of their acid lability. This agreed with the estimated crystallinity level, which was the same as in the native granules (Table 1). The relative content of cationic groups remained, however, at a high level in the residual granules after the lintnerisation (Table 2), showing that the substituents were evenly distributed and found in all parts of the granules. The melting temperature of WC was around 7 °C less than that of the corresponding native potato starch, which could be related to depolymerisation, increasing solubility, or modified process of gelatinisation. The enthalpy of the WC-granules was slightly lower than for native granules, which showed that the cationic substitutions affected some physical properties of the organised areas in the granules without changing the overall crystallinity.

The DC-sample was very different from the WC-starch, despite their similar degree of substitution. The sample lost practically all cationic glucosyl residues already during the initial lintnerisation stage (Table 2), suggesting that in the dry process the reagent only can penetrate the granules to a limited degree. However, the relative crystallinity and the melting enthalpy decreased after dry-cationisation (Table 1). The dry process, therefore, affected the physical organisation of the starch components in the granules, even if the cationic substituents were found only locally. Nevertheless, the melting temperature was less affected, which could mean that depolymerisation, if any, was limited to the external parts of the granules resulting in local degradation of crystalline structures.

The oxidised starch samples were extensively depolymerised during their preparation. The average degree of polymerisation of the HO- and PO-starches was only 177 and 130, respectively [10]. Despite this fact, the relative crystallinity in the granules obtained by X-ray

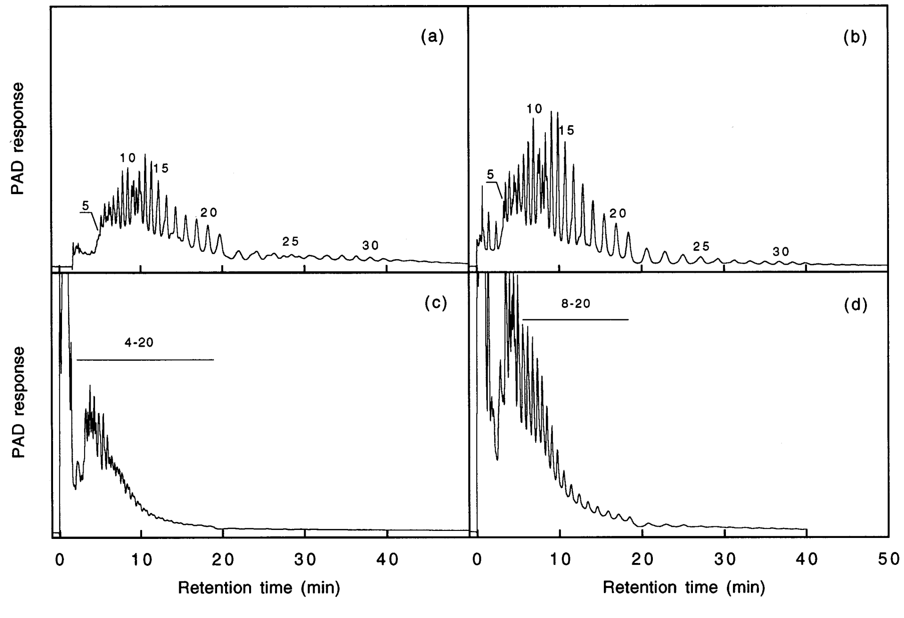

Fig. 7. HPAEC-PAD chromatograms of residues of wet-cationised potato starch at (a) a high lintnerisation level and (b) the sample after isoamylolysis, (c) after isoamylolysis + β-amylolysis, and (d) after β-amylolysis. The concentration of the samples applied to the column was (a,b) 1.1, (c) 1.7, and (d) 3.3 mg/mL.

diffraction remained at almost the same level (Fig. 1 and Table 1) with only a 6% decrease for PO when compared to native starch. The crystallinity and the melting enthalpy determined for HO were very close to those of native starch, which could show that the most organised parts had not been damaged. However, the melting endotherms of PO and HO were completely different (Table 1 and Fig. 2). The lower melting temperature observed for HO and the small PO endotherm were consistent with the depolymerisation, but the main endotherm observed at higher temperature for PO is more difficult to explain. Such a high temperature could be due to either a more difficult swelling or hydration process with the chemical groups grafted on the polymers, or a slight cross-linking induced by the oxidation reaction. Besides carboxyl groups, the oxidation also leads to an introduction of carbonyl groups into the starch [8]. In fact, the PO-starch contained nearly a double amount of these groups (0.41 weight %) compared to the HO-starch [15]. This could possibly explain the unusual behaviour in the DSC.

As for the cationic starches, the initial lintnerisation rate was higher after oxidation of the starch granules (Fig. 3). Different from the cationic starches, however, the fast rate continued to higher levels. For the HO-starch, which contained more carboxyl groups than the PO-starch, the fast rate continued up to a higher solubilisation level (67%) that corresponded to the level of crystallinity and to the level at which the native granules became very resistant to the acid. From this level, however, the HO-starch continued to solubilise into almost completeness. The same pattern was displayed by PO, but the final level of solubilisation was somewhat lower, corresponding with the lower content of carboxyl groups in this starch. Both samples lost a part of their carboxyl groups (and probably also carbonyl groups, though these were not analysed because of too small amounts of samples) during the lintnerisation. In proportion, the samples lost more substituted glucosyl residues than the wet-cationised starch, which suggested that the anionic groups, in combination with the depolymerisation of the starch components, made the crystallities available to the acid.

The resistance to isoamylase attack possessed by the cationised starches (Table 3 and Fig. 4) suggested that the substituents had been introduced to D-glucosyl residues at or close to the branches in the amylopectin component. The oxidised starches were apparently extensively debranched as shown earlier [10]. The successive treatment with β-amylase resulted in lower β-limit values of all starch derivatives and the DP of the resistant fragments was higher than the average DP of the material obtained by the preceding isoamylolysis. This suggested that the substituents in these resistant dextrins were close to the non-reducing end. A substituent distribution at both the non-reducing ends and the branches could be explained by the penetration of the reagents into the amorphous lamellae within the semi-crystalline rings. This should also explain why the crystallinity was almost untouched, since mainly the two edges of the crystalline lamellae are modified. As the amorphous lamellae are preferentially modified, they could be mobilised earlier during heating, which explains the lower melting temperature observed in DSC. The proportion of resistant material in the DC-starch was much smaller than in the WC-sample, which showed that only a part of the starch had been derivatised, in agreement with the extensive loss of cationic groups during the lintnerisation. However, because the DS was similar to that of the WC-sample, the cationised components of DC-starch had a considerably higher local concentration of the substituents.

All modified starches were better debranched after lintnerisation and the mixtures contained less of β-amylase resistant dextrins (Fig. 5 and Table 4), which was in agreement with the reduced levels of substituents. The DP of the β-limit dextrins remained the same as before the debranching, which showed that dextrins substituted at the non-reducing end also remained at the HL-level.

The molecular-weight-distribution profiles obtained by HPAEC-PAD of the native potato starch at the HL-level was similar to that reported for potato Nägeli amylodextrins obtained by treatment of granules with sulphuric acid [33]. A part of the dextrins with DP 23 ~ 40 remained after isoamylolysis (Fig.

6(a,b)), which also seems to have been the case with the Nägeli amylodextrins [33]. When treated with β-amylase, this group of dextrins disappeared (Fig. 6(c)), suggesting that they were linear in nature, at least at their non-reducing end side. When the lintnerised starch was hydrolysed with the β-amylase without prior debranching, much of branched dextrins with DP 4-8 were obtained (Fig. 6(d)). This kind of dextrins have also been described by others in both lintnerised starches [22,27,30] and Nägeli amylodextrins [33,37].

The very complex profiles obtained by HPAEC-PAD of the lintnerised WC-starch could not be resolved in detail, but it suggested that the cationic groups predominated in the DP range $5 \sim 12$ (Fig. 7(a)), in which mostly linear dextrins should be found [22,30,33]. The pattern of individual peaks remained therefore almost identical in this area after the debranching (Fig. 7(b)), and the β-amylolysis of both the debranched and undebranched samples resulted in a predominance of material at DP $4 \sim 8$ (Fig. 7(c,d)). This showed that a large part of the cationic groups were found at the reducing side of the crystalline lamellae. However, in accordance with the gel-permeation chromatogram (Fig. 5(d)), β-limit dextrins were also obtained in the DP range $8 \sim 20$, especially of such dextrins that corresponded to half the thickness of the crystalline lamellae (DP $8 \sim 13$). Apparently, a small part of the comparatively large and charged cationic groups were found as substituents even in the middle part of the crystalline lamellae. The crystals in potato starch are of the B-type, in which six double-helices are arranged around a central cavity that is filled with water [38]. This cavity could allow the cationising reagents to penetrate deep into the crystalline lamellae and also provide a space for the cationic groups. In comparison, it is interesting to notice that a similarly treated wet-cationised waxy-maize starch sample lost a major part of its substituents after lintnerisation [39], indicating that less groups had penetrated into the crystalline lamellae. This starch is of the A-type, in which the central cavity is filled with a double-helix instead of water [38].

In conclusion, the dry-cationisation process results in local derivatisation of the potato starch granules, probably preferentially at the granular surface, whereas wet-cationisation results in a derivatisation of the starch components throughout the whole granules. The oxidation process results in a similar derivatisation of all starch components, but causes also an extensive depolymerisation, which results in a higher acid lability of the granules. Substitutions appear at, or close to, the non-reducing end of the carbohydrate chains of the starch components and in the vicinity of branches in the amylopectin, thereby blocking the action of β-amylase and isoamylase.

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