



Amylose involvement in the amylopectin clusters of potato starch granules

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ABSTRACT

The granular organization of common corn and potato starch is characteristically different. The objective of this study was to investigate iodine complex formation with starch lintners from common corn starch (CCS) and potato starch (PS) lintners as a function of water content. Starches were subjected to mild acid hydrolysis (lintnerization). Size exclusion chromatography of the lintners indicated that the linear chains remaining after lintnerization had smaller degree of polymerization in PS lintners than in the corresponding CCS lintners. For both CCS and PS, the absorbance intensity and the wavelength of maximum absorption (λ_{\max}) of molecularly dispersed starches, in diluted iodine solution, decreased with increasing lintnerization extent. When the granular lintners were exposed to iodine vapor, following equilibration to different moisture contents, the reduction in the iodine binding was evident in PS lintners but not in CCS lintners. Furthermore, the iodination partially destroyed the crystallinity of native PS granules but not that of CCS granules. However, B-type crystallinity was still evident in PS lintners. This behavior was attributed to different location of amylose within starch granules, supporting the involvement of amylose in the B-type crystallites of PS, and the independence of amylose from the A-type crystallites of CCS.

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1. Introduction

When observed by optical microscopy, starch granules consist of concentric rings of alternating semi-crystalline and amorphous regions, referred to as growth rings (French, 1984; Jenkins & Donald, 1995). The semi-crystalline growth rings are comprised of alternating amorphous and crystalline 'lamellae'; each lamella is approximately 9–10 nm thick (Jenkins, Cameron, & Donald, 1993). In the crystalline lamellae, the external chains of amylopectin are associated in double helices and are packed together in an array to form clusters (Gallant, Bouchet, & Baldwin, 1997). The amylopectin clusters are considered responsible for the crystalline structure (A- and B-type) of starch. The branch points of the amylopectin molecules are thought to reside in the amorphous lamellae.

Starch granules are hydrolyzed in dilute acids. Acid hydrolysis can be performed with hydrochloric acid, producing lintnerized starches (Lintner, 1886). The amorphous regions (amorphous growth rings and amorphous lamellae) of granular starch are less dense and more susceptible to chemical and enzymatic modification than the crystalline or semi-crystalline regions (Biliaderis, 1998). Diffusion of small water-soluble molecules in the granule also are more likely to occur through the amorphous regions (French, 1984). Therefore, when the starch granule is treated with

acid, the amorphous regions of the granule are preferentially hydrolyzed, leaving intact the more resistant crystalline regions of the granule (Gallant et al., 1997).

The structure of the starch granule depends on the way in which amylose and amylopectin are associated and distributed throughout the starch granule. In contrast to the defined location and role of amylopectin, the location and role of amylose within the granule is poorly defined. An original study (Jane & Shen, 1993), based on cold gelatinization in a calcium chloride solution, proposed that amylose is more concentrated in the periphery of potato starch granule. In contrast, Tatge, Marshall, Martin, Edwards, and Smith (1999), based on the investigation of amylose synthesis in transgenic potato starch granules, suggested that amylose is largely confined to a central region of the granule. Jenkins and Donald (1995) applied small-angle X-ray scattering techniques to investigate the effect of varying amylose content on the internal structure of maize, barley and pea starch species. They hypothesized that amylose is predominately located in the amorphous growth rings, and that the interaction between amylose and amylopectin in these amorphous regions may be the cause of decreased crystallinity. The degree of interaction between amylose and amylopectin may depend on the botanical source of the starch (Oates, 1997), with amylose and amylopectin more closely associated in potato starch than in corn starch (Hoover & Vasanathan, 1994; Saibene, Zobel, Thompson, & Seetharaman, 2008; Zobel, 1988). Gerard, Colonna, Buleon, and Planchot (2002), used mild acid hydrolysis to investigate the location of amylose with respect to the amorphous and/or crystalline regions in maize starch mutants; and reported a greater

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interaction of amylose and amylopectin in high-amylose maize starches than in common corn starch. However, as pointed out by Jane (2006), the present knowledge concerning the location of amylose relative to the amylopectin molecules in common corn starch is contradictory. Based on cross-linking studies, Jane, Xu, Radosavljevic, and Seib (1992) and Kasemsuwan and Jane (1994) suggested the interaction of amylose and amylopectin in common corn starch. On the other hand, Zobel (1988) suggested that amylose exists separate from amylopectin in common corn starch based on criteria of amylose extractability, ease of amylose complex formation, susceptibility of amylose to enzyme attack, and gel setback. Clearly, the location of amylose within starch granules remains one of the unknown facts required to complete our picture of the internal structure of the starch granule.

The ability of starch to complex with iodine is commonly used for the characterization of starch molecules from various botanical sources (Knutson, 1999). The amylose–iodine complex consists of a single helical inclusion complex, which can crystallize in a V-type crystalline pattern (Rundle & French, 1943). The traditional spectrophotometric approach consists of measuring absorbance as a function of wavelength for dispersed starches in a diluted iodine solution. The color and wavelength of maximum absorbance (λ_{\max}) of the complex vary accordingly to the degree of polymerization (DP) of the polymer chain. As the length of the glucan chain increases, the λ_{\max} also increases (Banks & Greenwood, 1975). For granular starches, light scattering as well as absorption occurs. In two previous publications (Saibene & Seetharaman, 2006; Saibene et al., 2008) we investigated iodine complex formation with starch molecules in native granular starches as a function of water content by using the Kubelka and Munk theory (Kubelka & Munk, 1931). According to this theory, a powder sample such as granular starch is considered as a continuous, turbid medium. When the incident light strikes on the surface of a sufficiently thick layer of starch granules, a fraction of light that is scattered is described by a scattering coefficient (S), while the fraction of light that is absorbed is described by an absorption coefficient (K). The ratio K/S is determined by measuring the reflectance (R) of the sample (Kubelka & Munk, 1931; Lindberg & Laude, 1974).

The objective of this study was to investigate iodine complex formation with starch molecules in common corn starch (CCS) and potato starch (PS) linters as a function of water content. The goal of this study was to potentially highlight structural differences in the granular arrangement of CCS and PS.

2. Materials and methods

2.1. Materials

Commercial starch from common maize endosperm (Melojel®) was a gift from National Starch and Chemical Company (Bridge-water, NJ). Commercial starch from potato (Kosher® FGPS) was a gift from Avebe (Veendam, The Netherlands). These starches are referred to as CCS and PS, respectively.

Drierite® (W.A. Hammond Drierite Company Ltd., Xenia, OH), MgCl_2 (EMD Chemicals Inc., Gibbstown, NY), NaCl (Fisher Chemicals Inc., Fair Lawn, NJ), K_2SO_4 (EDM Chemicals Inc., Gibbstown, NY), and sodium azide (EM Science, Gibbstown, NY) were purchased. Iodine crystals were purchased from J.T. Baker (Phillipsburg, NJ).

2.2. Starch lintnerization

Starch granules (CCS and PS) were treated with 2.2 M HCl (5 g starch/100 mL) at 29 °C. The slurry was stirred periodically everyday.

Small aliquots (2 mL), taken at intervals, were centrifuged at $1000 \times g$ for 5 min, and the supernatants analyzed for carbohydrates by using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Following treatment for 2 h, 2 days, 6 days, and 21 days a sample of the slurry was centrifuged (15 min at $1000 \times g$), and the granular residues (lintners) were washed with water ($2 \times$), neutralized with 0.1 N NaOH, washed with water ($3 \times$), and finally washed once with acetone. The lintners were then dried at room temperature, ground in a mortar, and sieved (openings 125 μm).

2.3. Sepharose CL-2B chromatography

Native starches and lintners (1 g db) were dispersed in 20 mL of 90% (v/v) DMSO at room temperature with constant stirring overnight. Following dispersion, 4 vol of ethanol were added and the mixture was centrifuged at $1000 \times g$ for 15 min at room temperature. The supernatants were discarded and the pellets were washed once with ethanol and once with acetone. The precipitates were then dried at room temperature.

Dispersed starch samples (15 mg) were dispersed in 2 mL of 0.1 M NaOH for 1–2 days. The dispersed starches were then diluted with 5 mL of water and loaded into a Sepharose CL-2B (Supelco Chromatography Products, Bellefonte, PA) SEC column (74 cm \times 2.5 cm, Pharmacia Fine Chemicals, Sweden) using gravity flow. The nominal fractionation range for dextrans is 100,000–20,000,000 MW.

For each sample, 550 mL of eluent was collected as 5 mL fractions, at a flow rate of 20–30 mL/h. The void volume and salt volume of the column were determined using a mixture of 1 mg of waxy corn starch and 1 mg of glucose. Every third SEC fraction and every SEC fraction within 10 fractions of the peak were examined for total carbohydrate and iodine binding λ_{\max} , according to Klucinec and Thompson (1998).

2.4. Iodine binding of dispersed starches

The iodine binding of the dispersed starches was determined by using the method described by Klucinec and Thompson (1998). All samples were scanned from 400 to 800 nm by using a HeLios Alpha UV–visible Spectrophotometer (Thermo Spectronic, Rochester, USA). The blue value of the starches was defined as the absorbance at 635 nm. The λ_{\max} was the peak absorbance value over the range of wavelengths examined.

2.5. K/S spectra of granular samples exposed to iodine vapor

2 g of starch or lintner samples were equilibrated to the respective water activity (a_w), with final values lower than 0.15, and to 0.33, 0.75, and 0.97 a_w using Drierite®, or saturated solutions of MgCl_2 , NaCl, and K_2SO_4 (Greenspan, 1977), as described by Saibene and Seetharaman (2006). Following equilibration, the moisture content of the samples was measured according to the AACC method 44-15A (AACC, 2000). To determine the iodine binding, a thin layer of the equilibrated starch sample (0.2 g) was spread in a standard plastic weighing dish, placed in the corresponding a_w desiccator, and exposed to iodine vapor generated from 2 g of iodine crystals for 24 h at room temperature. The K/S values over a wavelength range from 400 to 700 nm of the samples after exposure to iodine vapor were measured as described in Saibene and Seetharaman (2006) with a CM 3500-d Spectrophotometer (Konika Minolta, Mahwah, NJ).

2.6. X-ray powder diffraction

A $\text{K}\alpha_1 = 4.54056 \text{ \AA}$ and $\text{K}\alpha_2 = 1.54439 \text{ \AA}$ radiation was produced by the copper X-ray tube of a Scintag Pad V X-ray powder diffrac-

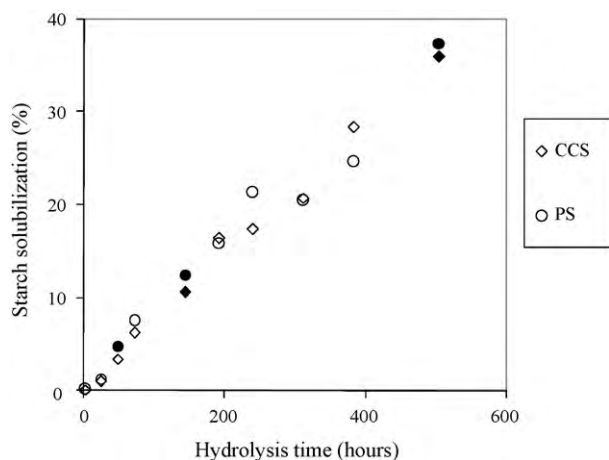


Fig. 1. Progress of linterization of common corn and potato starch at 29°C. Filled symbols mark the samples that were used for further analysis.

tometer (Scintag, Inc., Cupertino, CA). The starch or lintner powders were packed tightly in a quartz zero background plate, 1.1" square (Gem Dugout, State College, PA). The samples were exposed to the X-ray beam with the X-ray generator running at 35 kV and 30 mA. The scan was run at an interval of 0.02 and at a rate of 2°/min (Cheetham & Tao, 1998).

3. Results

The extent of solubilization of starch from CCS and PS in diluted hydrochloric acid as a function of time is shown in Fig. 1. The temperature of the lintnerization process was 29°C. Bertoft (2004) reported that the lintnerization temperature must be below 35°C in order to prevent annealing. The extent of solubilization of the samples that were taken for further analysis is reported in Table 1. CCS and PS showed similar extent of susceptibility to mild acid hydrolysis.

Table 1
Solubilization extent (%) of common corn (CCS) and potato starch (PS) following 2 h, and 2, 6, and 21 days of hydrolysis.

Samples	Hydrolysis time			
	2 h	2 days	6 days	21 days
CCS	0.10 ± 0.01	3.4 ± 0.2	10.6 ± 0.2	36.0 ± 0.1
PS	0.14 ± 0.02	4.8 ± 0.3	12.5 ± 0.3	37.4 ± 0.2

Values are mean ± standard deviation based on four replicates of the lintnerization procedure.

Table 2

Iodine binding properties of native common corn (CCS) and potato starch (PS) respective lintners following 2 h, and 2, 6, and 21 days of hydrolysis.

Starch sample	Blue value ^a	λ_{\max} ^b
CCS		
Native	0.40 ± 0.01	622 ± 0.7
2 h	0.36 ± 0.01	620 ± 1.4
2 days	0.29 ± 0.03	589 ± 2.8
6 days	0.20 ± 0.01	576 ± 0.7
21 days	0.14 ± 0.01	561 ± 2.8
PS		
Native	0.31 ± 0.01	609 ± 1.4
2 h	0.32 ± 0.01	599 ± 0.1
2 days	0.22 ± 0.01	575 ± 0.1
6 days	0.13 ± 0.01	541 ± 2.1
21 days	0.06 ± 0.01	510 ± 2.1

Values are mean ± standard deviation based on two replicates of the dispersion procedure.

^a Measured at 635 nm.

^b Wavelength of maximum absorption.

The Sepharose CL-2B chromatograms of the native starches and the respective lintners are shown in Fig. 2. The void volume peak of the 2 h lintners was smaller than the corresponding peak observed for the native starches, but no difference was observed for the iodine binding wavelength maximum (λ_{\max}) of the void volume peaks. No void volume peak was observed for any sample exposed to lintnerization for 2, 6, and 21 days (Fig. 2). For these samples, the glucose standard peak, identifiable in the native starches and in the 2 h lintners chromatograms at approximately fraction 87, could not be distinguished from the peak shown by the samples. For CCS, the highest λ_{\max} measured following 2, 6, or 21 days of lintnerization was about 600 nm. For PS, the λ_{\max} decreased with increasing hydrolysis time; and after 21 days of lintnerization the λ_{\max} remained between 515 and 490 nm for the entire chromatogram (Fig. 2).

The iodine absorption spectra of dispersed starches and the respective lintners in solution are shown in Fig. 3. The iodine binding properties are shown in Table 2. For both CCS and PS, the blue value and the λ_{\max} decreased as the acid hydrolysis progressed.

The moisture content of granular native starches and lintners equilibrated above Drierite® pellets (<0.15 a_w), or saturated solutions of MgCl₂ (0.33 a_w), NaCl (0.75 a_w), K₂SO₄ (0.97 a_w) are shown in Fig. 4. Native PS and PS lintners exhibited significantly higher moisture contents compared to CCS starches and lintners when equilibrated at 0.75 and 0.97 a_w .

For both starches, the native granular samples and lintners equilibrated above Drierite® did not exhibit a K/S peak at any wavelength measured (data not shown). Following equilibration at 0.33 a_w , all the samples exhibited a significant increase in the K/S inten-

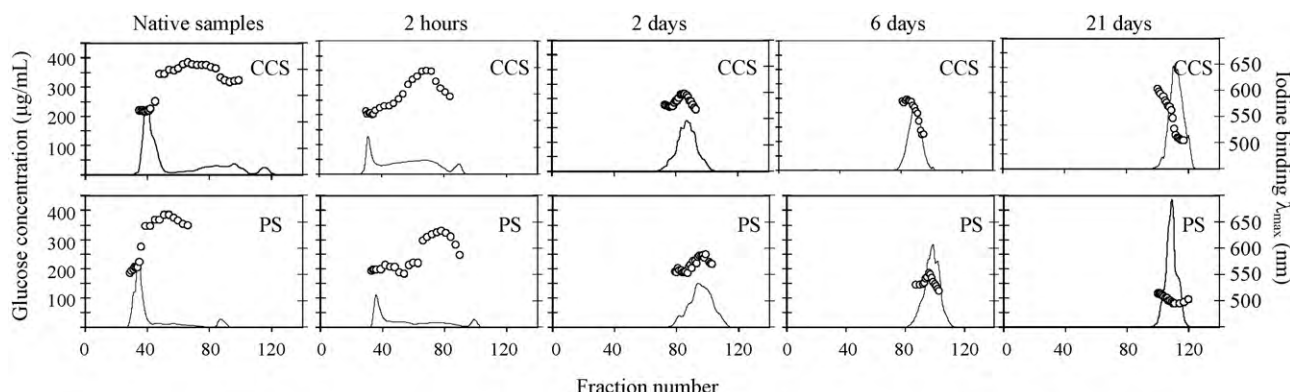


Fig. 2. Size exclusion chromatograms of native starches and lintners following 2 h, and 2, 6, 21 days hydrolysis. Common corn starch (CCS); potato starch (PS).

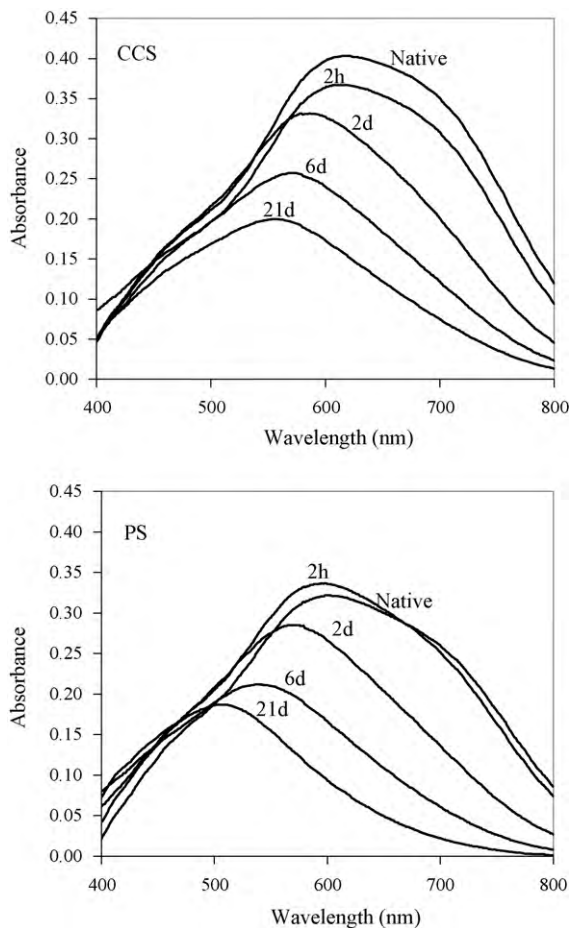


Fig. 3. Iodine binding by dispersed native starch granules and lintners. Native, no lintnerization; 2 h, 2 h hydrolysis; 2 d, 2 days hydrolysis; 6 d, 6 days hydrolysis; 21 d, 21 days hydrolysis.

sity; however, the values recorded were still exceptionally low (Fig. 5A). The K/S maximum of the lintnerized CCS samples equilibrated at a_w of 0.75 decreased with increasing hydrolysis (Fig. 5B). The K/S intensity for the PS lintners sharply decreased with increasing wavelength (Fig. 5B), and no peak of K/S intensity was evident.

When the samples were equilibrated at 0.97 a_w , CCS lintners showed somewhat higher K/S intensities than the native sample

(Fig. 5C). The K/S spectra for the CCS lintners had similar K/S intensities (between 50 and 80 K/S) at all wavelengths, with exception of the 2-day CCS lintners, which showed greater variation (the fluctuations were not statistically significant ($p < 0.05$)). Compared to CCS, PS lintners showed peculiar K/S spectra (Fig. 5C). The 2 h and 2-day PS lintners showed no differences in the K/S intensity when compared to the native PS sample. No clear K/S peak was detected for these samples. On the other hand, the K/S spectra of the 6 and 21-day PS lintners exhibited a decrease in the K/S intensity at wavelengths higher than 560 and 530 nm, respectively, from about 60–15 K/S .

X-ray diffractograms of the starch samples and of the 21-day lintners equilibrated above K_2SO_4 (0.97 a_w) are shown in Fig. 6. The crystalline pattern of all starches before iodine exposure remained unchanged on lintnerization: CCS lintners exhibited the typical A-type X-ray spectrum; while PS lintners exhibited a B-type X-ray spectrum.

Before exposure to iodine vapor, the 21-day CCS and PS lintners showed sharper diffraction peaks than the native samples. The doublet peak at 17° and 18° 2θ and the doublet peak at 22° and 24° 2θ became more obvious in the lintnerized CCS and PS sample, respectively.

The exposure to iodine vapor of both starches equilibrated at 0.97 a_w caused a reduction in the diffraction intensities. A reduction of the scattering intensity for the main diffraction peaks (15° , 23° , 17° and 18° 2θ) of CCS was observed following iodination of the samples, with exception of the weak peak at about 20° 2θ . For CCS it appears that the pattern (Fig. 6A) could be a combination of A + V patterns, while for PS the pattern was only B, consistent with earlier reports (Saibene et al., 2008; Zobel, 1988). B-type crystallinity is evident in all PS patterns, with exception of the native PS sample exposed to iodine (Fig. 6B). In this case, the B structure as indicated by peaks at 23° and 24° 2θ , is partially destroyed.

4. Discussion

4.1. Effect of starch lintnerization on the molecular size distribution

For all the CCS and PS lintners, the elution peak shifted towards higher fraction numbers with increasing lintnerization time, indicating a reduction of the molecular size (Fig. 2). Among all the samples, the void volume peak, constituted by amylopectin molecules, was present only for the native starches and the 2 h lintners.

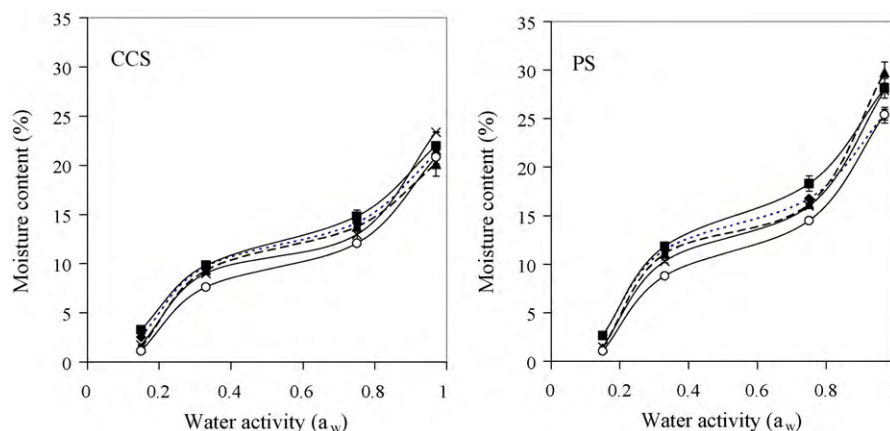


Fig. 4. Moisture content of common corn (CCS) and potato (PS) native granular starches and lintners, following equilibration above Drierite ($<0.15 a_w$), and $MgCl_2$ (0.33 a_w), NaCl (0.75 a_w), and K_2SO_4 (0.97 a_w) saturated salt solutions. Native granular samples ($\cdots \blacklozenge \cdots$), 2 h lintners ($\text{—} \blacksquare \text{—}$), 2 days lintners ($\text{---} \blacktriangle \text{---}$), 6 days lintners ($\text{--}\times\text{--}$), 21 days lintners ($\text{—} \bigcirc \text{—}$).

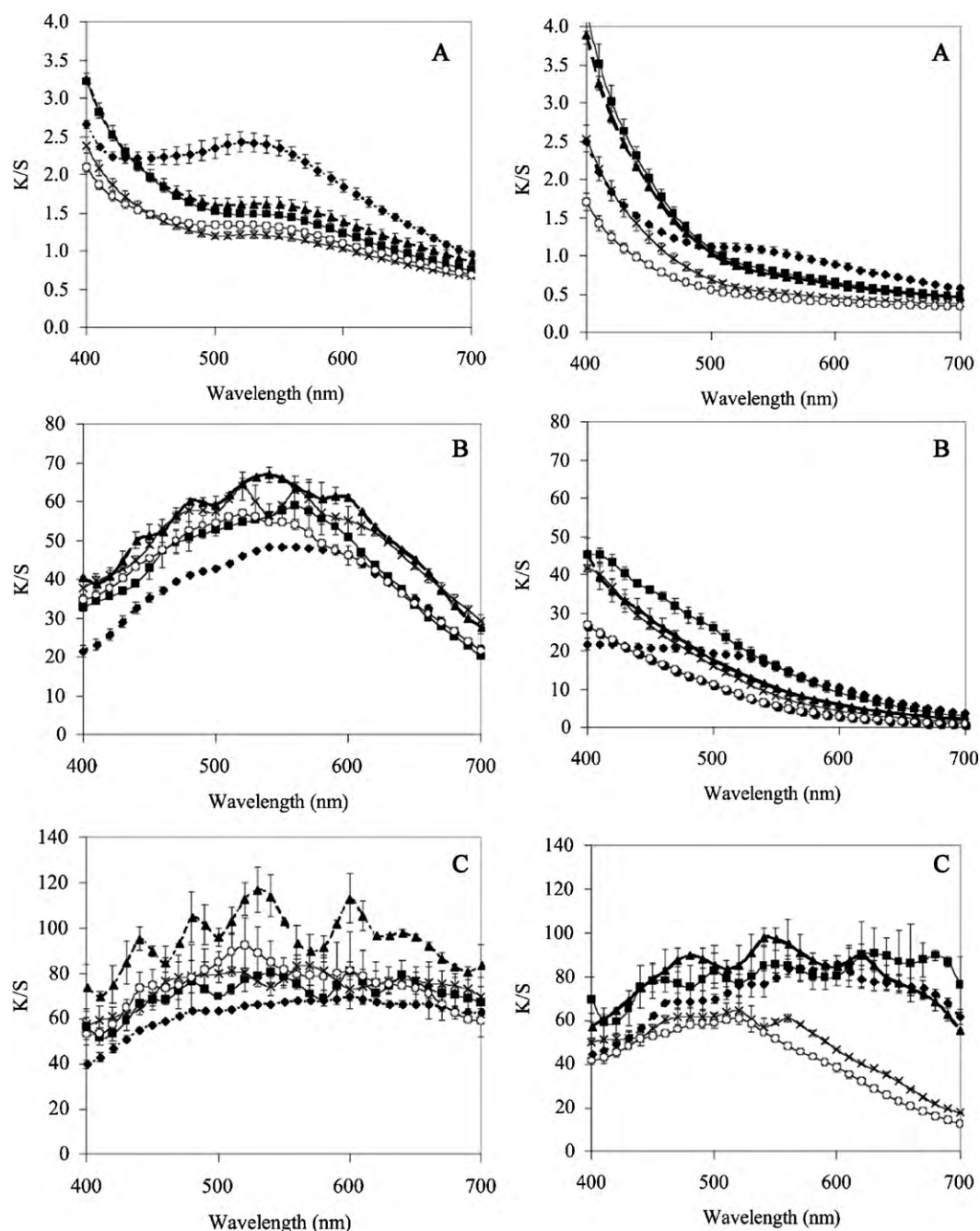


Fig. 5. K/S spectra of common corn (graphs on the left) and potato (graphs on the right) native granular starches and lintners exposed to iodine vapor following equilibration above (A) MgCl_2 (0.33 a_w), (B) NaCl (0.75 a_w), (C) K_2SO_4 (0.97 a_w). Native granular samples ($\cdots \circ \cdots$), 2 h lintners ($\text{—}\blacksquare\text{—}$), 2 days lintners ($\text{—}\blacktriangle\text{—}$), 6 days lintners ($\text{—}\times\text{—}$), 21 days lintners ($\text{—}\circ\text{—}$).

Acid attack is non-specific, and it randomly degrades glycosidic bonds of the amylose and amylopectin molecules, primarily within the amorphous regions (Kainuma & French, 1971). As a result, molecules with a broad range of elution volume are generated. The void volume peak of the 2 h lintners represents the portion of the branched molecules generated by the hydrolysis of amylopectin molecules, which had a volume too big to be separated by the chromatographic gel used in the experiment ($\text{MW} > 2 \times 10^7$).

The reduction of the void volume peak of 2 h lintners was accompanied by a decrease in the λ_{max} of the fractions eluting after the void volume peak. Amylose hydrolysis would generate shorter linear chains with a broad range of DP, due to the non-specificity of the acid attack. The shorter the DP of the glucan polymer generated by

amylose hydrolysis, the lower the λ_{max} (Banks & Greenwood, 1975). However, the reduction of the λ_{max} could be also attributed to the elution of the smaller branched molecules ($\text{MW} < 2 \times 10^7$) resulting from amylopectin lintnerization, concomitant to the linear chains generated by amylose hydrolysis.

The absence of the void volume peak for samples lintnerized for 2 days and more (Fig. 2) indicates substantial amylopectin hydrolysis for both CCS and PS lintners. Aside from the similarity in the SEC elution profiles of the CCS and PS samples lintnerized for 2 days and more, the 6 and 21-day lintners obtained from CCS and PS appeared to have some structural differences based on the λ_{max} values (Fig. 2). For CCS 6 and 21-day lintners, the first fractions eluted had λ_{max} at about 600 nm. By size exclusion chromatography, for

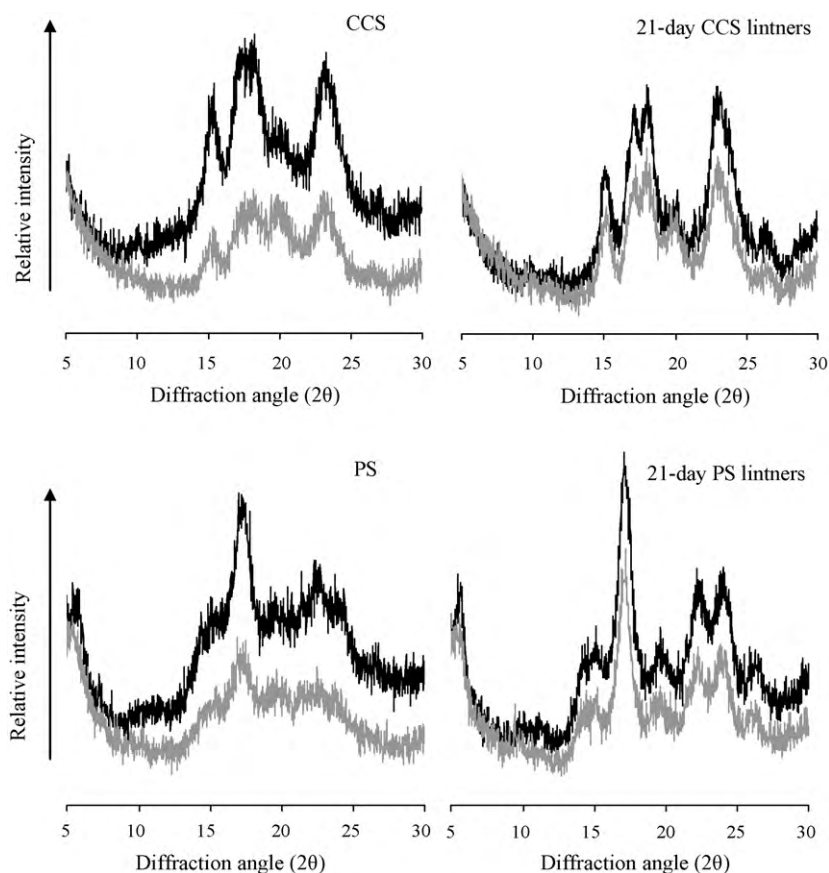


Fig. 6. Effect of iodination on the X-ray powder diffraction spectra of common corn starch (CCS) and potato starch (PS) and 21-day lintners equilibrated above K_2SO_4 (0.97 a_w). The black diffractogram in each graph represents the unstained starch samples; the grey diffractogram represents the starch sample following exposure to iodine vapor.

native starch granules, the proportion of molecules eluting at the void volume peak is generally considered to be amylopectin. The remaining proportion of molecules, with λ_{max} value about 600 nm, is generally considered amylose (Klucinec & Thompson, 1998). For the CCS 6 and 21-day lintners, the first fractions eluted appeared to behave similarly to amylose, as indicated by the similarity in the λ_{max} value for the proportion of molecules eluting after the void volume peak for native CCS (Fig. 2). According to the values extrapolated from Banks and Greenwood (1975), linear chains with DP more than 100 would show λ_{max} values similar to amylose. The presence of such long linear chains in the CCS lintners, even following 21 days of hydrolysis, indicates that amylose molecules in CCS are at least partially protected from the acid attack.

For the PS 6 and 21-day lintners, the λ_{max} measured were lower than 550 and 520 nm, respectively. The lower λ_{max} observed for the 6 and 21-day PS lintners suggested that the chains capable of forming a complex with iodine that remained after lintnerization were shorter than the amylose in native PS (Fig. 2). Thus, although CCS and PS showed similar susceptibility to lintnerization (Fig. 1), it appears that the amylose in PS is more susceptible to acid hydrolysis than the amylose in CCS.

4.2. Effect of starch lintnerization on the iodine binding

The traditional spectrophotometric approach describes the iodine binding ability of starch polymer chains in a dispersed flexible state, when they can freely interact and complex with iodine. For both CCS and PS, the absorbance intensity and the λ_{max} decreased with increasing lintnerization (Fig. 3). This behavior reflects the reduction in molecular size shown by size chromatography (Fig. 2).

The reduction of the molecular size did not seem to affect the wavelength of K/S intensity of the granular CCS samples equilibrated at 0.97 a_w (Fig. 5). Differences between the solution absorption spectra from transmission spectroscopy and the K/S spectra from reflectance spectroscopy possibly reflect the physical constraints of the starch polymers in the granular arrangement, as previously explained (Saibene et al., 2008). For the CCS samples equilibrated at 0.97 a_w (moisture content about 21%), the amorphous regions have transitioned into their rubbery states (Zeleznek & Hoseney, 1987), thereby facilitating the mobility of even the longest polymer chains remaining in the lintners. The fact that native CCS and the respective lintners exhibit similar K/S intensities may indicate that similar chain mobility was maintained by the polymers within the granular arrangement, even following lintnerization.

PS samples showed similar trends in the absorbance (Fig. 3) and K/S spectra for samples equilibrated at 0.97 a_w (Fig. 5C). In fact, for both spectrophotometric approaches, the 6 and 21-day PS lintners showed the lowest intensities, and lowest wavelengths of absorption and K/S maxima. The 6 and 21-day PS lintners showed much lower intensities at wavelengths higher than 600 nm than the native PS sample and the 2 h PS lintners, which indicates that the linear chains remaining after extended lintnerization had shorter iodine-complexible chain segments than in native PS.

4.3. Effect of starch lintnerization and iodine binding on the X-ray diffractograms

The decreased intensity in the A and B X-ray diffraction patterns of samples exposed to iodine is likely due to the absorption of scattered radiation by the heavier iodine atoms (Saibene et al., 2008).

As previously reported (Saibene et al., 2008), the iodine exposure of native CCS and native PS enhanced the intensity at $20^\circ 2\theta$ only for CCS. Similarly, following iodine exposure of the 21-day lintners, the weak peak at $20^\circ 2\theta$ became relatively more prominent for the CCS lintners but not for the PS lintners (Fig. 6). The diffraction peak at $20^\circ 2\theta$ indicates V-type crystallinity. This conformation is not compatible with associated double helices (Biliaderis, 1998). Only the iodine-complexible chain in the amorphous regions of the granule would have sufficient mobility to complex with iodine and associate in V-type crystallinity without resulting in a weakening of the double helical A structure observed in the X-ray diffractogram. Thus, the appearance of the V-type crystallinity in the CCS lintners exposed to iodine vapor suggests that the iodine-complexible chains remaining after lintnerization were located primarily within the amorphous regions of the granule.

For the PS samples, the iodine exposure resulted in a weakening of the B-type crystallinity in the region of the double peaks at 23° and $24^\circ 2\theta$ for native PS but not for the 21-day PS lintners (Fig. 6). We previously hypothesized (Saibene et al., 2008) that the partial loss of the B crystalline structure, mainly due to amylopectin, is somehow due to the complexation of iodine with amylose. The mechanical stress resulting from the complexation of iodine with the amorphous portions of the amylose chains partially involved in the crystalline structure of PS would lead to the partial disruption of the double helical B structure observed in the X-ray diffractogram. Because the sharp double peak at 23° and $24^\circ 2\theta$ in the 21-day PS lintners (Fig. 6) was maintained following iodine exposure, it appears that the iodine-complexible chains in the PS lintners would be independent of the crystalline B structure.

4.4. Involvement of amylose in crystallites

The amorphous regions of starch granules are less dense and therefore more susceptible to chemical modification than the more highly organized crystalline regions (Biliaderis, 1998). Thus, the starch-iodine complex formation is more likely to occur in the amorphous regions of the granule. Because starch lintnerization randomly degrades glycosidic bonds of the amylose and amylopectin molecules primarily within the amorphous regions (Kainuma & French, 1971), the iodine-complexible chains would be expected to get smaller with increasing lintnerization extent. Although the X-ray diffraction evidence was consistent with the idea that amylose is mainly located in the amorphous regions of CCS granule, based on the molecular size distribution and iodine binding analysis evidences, it was suggested that amylose in CCS may be partially protected from the acid attack. As suggested by Kainuma and French (1971), all glycosidic linkages of a double helix are buried in the interior of the helix and they could not be contacted by hydrated protons. Thus, some amylose chains in the amorphous regions of the CCS granules may be protected from acid hydrolysis under the conditions used in this study, if present in a helical conformation. These remaining amylose chains may still maintain the ability to complex with iodine. At present, insufficient evidence is available to exclude whether the formation of linear helices within the amorphous regions of CCS granules may result from ordering of the newly released chains formed during the lintnerization.

Since the crystalline A structure in native or lintnerized corn starch was not affected neither by the acid treatment nor by the iodine adsorption (Fig. 6), it appears that the amylose chains remaining in the CCS lintners are not incorporated in the crystalline A structure. We suggest that the amylose in corn is likely to be located in the amorphous regions of the granule, mainly independent of the amylopectin crystallites, consistent with an earlier report (Zobel, 1988). Because only an estimated half-fraction of the amylopectin molecules is organized enough to be involved in crystallites (Gerard et al., 2002), the interaction of amylose and

amylopectin in common corn starch, as argued by Jane et al. (1992) and Kasemsuwan and Jane (1994), may be limited to the amorphous regions of the CCS granule.

For PS, findings shown in the present study are consistent with a partial involvement of amylose in the amylopectin crystallites, as supported by Zobel (1988). The amylose chains that co-crystallize with the external chains of amylopectin would act as a cross-linker between the crystalline lamellae. The complexation of the amorphous portions of the amylose chains with iodine may pull the amylopectin chains out of register in the crystalline packing, and thus partially disrupting the double helical B structure in the process, as observed in the X-ray diffractogram of native PS (Fig. 6). For the amylose molecules traversing through consecutive lamellae, the portions of the molecules located within the amorphous lamellae would be exposed to hydrolysis during the lintnerization treatment. Thus, with increasing lintnerization extent, the segments of amylose able to bind iodine will get smaller, as indicated by the molecular size distribution and iodine binding analysis. The mobility of these linear segments newly formed during the lintnerization treatment would be less restricted by the cross-linking effect between crystalline lamellae, and could bind iodine without stressing the crystalline packing, maintaining the integrity of the B-type crystallinity.

5. Conclusion

The molecular size distribution, iodine binding and X-ray diffraction analysis of the native starches and the respective lintners elucidate differences in the location of amylose within the amorphous or crystalline regions of PS and CCS. Data are consistent with a partial involvement of amylose in the B-type crystallites of PS. For CCS, the amylose would be located within the amorphous regions of the granule, independent of the amylopectin crystallites, free to complex with iodine without disruption of the A-type crystallinity.

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