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Carbohydrate Polymers 64 (2006) 539–547
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Segmental mobility of polymers in starch granules at low moisture contents

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Received 11 August 2005
Available online 10 January 2006

Abstract

The objective of this study was to determine the moisture content at which the segmental mobility of polymers within a starch granule is restricted. Common corn, waxy corn and high amylose corn starch samples were equilibrated to a final water activity of 0.15, 0.33, 0.75 or 0.97. The samples were then exposed to iodine vapor for 24 h and the color, absorption spectra and X-ray diffraction patterns were measured. Stained and unstained granules were also viewed under a bright field and polarized light microscope. The results demonstrate that successive local transitions occur within a granule with increasing moisture contents. Furthermore, the data shows that at moisture contents of about 13%, iodine is able to penetrate the granule and the resulting complex disrupts the crystalline arrangement within the granules. The differences in extent of mobility of polymers between different starch types can potentially illuminate differences in starch structure and architecture.

Keywords: Iodine–polymer complex; Polymer mobility; Corn starch; Iodine vapor; Low moisture content mobility

1. Introduction

Starch, composed primarily of amylose and amylopectin, is deposited in higher plants in the form of a birefringent, semi-crystalline granule. The crystalline region of the granule is associated with the amylopectin, and the amorphous region is comprised of the branch points in the amylopectin and amylose (Tester, Karkalas, & Qi, 2004). The crystalline and amorphous regions within the granules are organized in an alternating pattern with specific periodicity (Jenkins, Cameron, & Donald, 1993). The particular granule morphology, content of amylose and amylopectin and extent of crystallinity and molecular order within granules from different plant species are primarily genetically controlled (Evans, McNish, & Thompson, 2003; Katz, Furcsik, Tenbarge, Hauber, & Friedman, 1993; Shannon & Garwood, 1984).

The functionality of starch under a hydro-thermal regime is generally accepted to be governed on the basis of polymer science principles including phase and glass transitions (Slade & Levine, 1995). The melting of crystallites is a first order transition reflecting changes in enthalpy, entropy and volume, while glass transition of the amorphous regions is a second-order transition reflecting a discontinuous change in heat capacity (Chung, Lee, & Lim, 2002; Lourdin, Colonna, & Ring, 2003; ten Brinke, Karasz, & Ellis, 1983). At a sufficiently low temperature or amount of plasticizer, the motion of polymer chains in amorphous regions is reduced enough that they are in a glassy state. By heating or adding plasticizers, the mobility of the amorphous polymer is initiated and the polymer becomes viscous, rubbery, and flexible. The change from a glassy to a rubbery state, which reflects an increase in segmental mobility of the polymer backbone, is referred to as glass transition (Slade & Levine, 1995; ten Brinke et al., 1983; Zeleznak & Hoseney, 1987). The functional properties of starches from different species, following various hydro-thermal treatments, also differ based on the length of the polymer, extent of crystallinity, level of amylose and other structural features (Mizuno, Mitsuiki & Motoki, 1998; Roos & Karel, 1991; Slade & Levine, 1995).

The ability of iodine to complex with linear glucan polymers has been used to elucidate the structure of starch polymers and other natural polymers including cellulose. Iodine has also been used to determine starch granule properties following a thermal or enzymatic treatment to understand the impact of a treatment on starch granular matrix (Conde-Petit, Nuessli, Handschin & Escher, 1998; Langton & Hermansson, 1989; Svegmark & Hermansson, 1991; Seguchi, Yasui, Hosomi & Imai, 2000). Rundle & French (1943) on the basis of optical and X-ray diffraction studies showed that the iodine atoms are surrounded by amylose helixes. Moreover, the X-ray studies defined the amylose–iodine complex as

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0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.11.025

a left-handed helical structure with diameter 12.97 Å and a pitch of about 8 Å. The dimensions observed by X-ray diffraction were also confirmed by Gidley & Bulpin (1987). Bates, French and Rundle (1943) reported on the differences between the iodine binding capacity of amylose and amylopectin and showed that the affinity of polysaccharides for iodine varies inversely with the degree of branching of the chain. Only linear branches of amylopectin that are long enough to form helix can develop a complex with iodine in the same manner of amylose (Knutson, 1999); but the short length of these branches originates unstable purple to red color complexes (Rundle & French, 1944). The iodine-binding capacity of pure amylose is reported to be 19–22% of its weight (Banks, Greenwood, & Khan, 1971), whereas for amylopectin it is about 1% (Gerard, Barron, Colonna, & Planchot, 2001). The color of the amylose–iodine complex also changes depending on the degree of polymerization (dp) of the amylose helix range from brown (dp 21–24) to red (dp 25–29), red–violet (dp 30–38), blue–violet (dp 39–46), and finally blue (dp > 47) (John, Schmidt, & Kneifel, 1983).

The glass transition properties of starch granules, starch gels and its derivatives are generally studied by using thermal or thermo-mechanical tools. These estimations, particularly for starch granules, reveal the thermal properties at a macro level of granule organization. Furthermore, these estimations can be complex since they include the combined effects of glass transitions, crystal melting and other order–disorder transitions. Several researchers have also investigated molecular mobility within a glassy matrix by using electron spin resonance or nuclear magnetic resonance spectroscopy (Kou, Dickinson, & Chinachoti, 2000). Shah & Ludescher (1995) have used phosphorescence probes to determine rotational mobility in amorphous sucrose glass system.

It is well known fact that adding a drop of iodine solution to starch granules results in instantaneous development of blue color. The color development implies the mobility of long linear polymers in the granules thus forming a helical complex with iodine ions. We therefore probed the question a little further to determine at what moisture content the mobility of polymer chains is restricted such that they will not complex with iodine. The results reported here are from use of iodine as a tool to elicit another dimension of segmental mobility of polymer chains in starch granules as a function of moisture content.

2. Materials and methods

2.1. Materials

Melojel (Common Corn Starch; CCS), Novation 2007 (Waxy Corn Starch; WCS) and Hylon V (High Amylose Corn; HAC) corn starch samples were obtained from National Starch & Chemical Company (Bridgewater, NJ). Drierite (W.A. Hammond Drierite Company Ltd., Xenia), $MgCl_2$ (EMD Chemicals Inc., Gibbstown, NY), NaCl (Fisher Chemicals Inc., Fair Lawn, NJ), and K_2SO_4 (EDM Chemicals Inc., Gibbstown, NY) were purchased. Sodium azide (EM Science, Gibbstown, NY) was added to the K_2SO_4 saturated salt solution (1% w/w) in order to prevent microbial growth. Iodine crystals were purchased from J.T. Baker (Phillipsburg, NJ).

2.2. Sample preparation

Starch samples were equilibrated at room temperature in desiccators (25 cm diameter and 20 cm deep) containing 500 ml of different saturated salt solutions in the base. 2 g of sample was weighed in duplicate and equilibrated to the respective water activity (a_w) for up to 1 month, with final values of 0.15, 0.33, 0.75, and 0.97 by using Drierite, and $MgCl_2$, NaCl, and K_2SO_4 saturated solutions (Greenspan, 1977). Each desiccator held only one set of duplicate samples. Following equilibration, the moisture content of the samples was measured according to the AACC method 44-15A (AACC, 2000). 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 desiccators were covered with aluminum foil during exposure to iodine vapor to eliminate exposure to light.

2.3. Colorimetric analyses

The term 'subtractive mixing' refers to the removal by a sample of part of the incoming light source. The ways in which this light can be removed include absorption and/or scattering. Since the samples analyzed in this set of experiments were powders, we used this definition of complex-subtractive mixing, where there is simultaneous absorption and scattering of light by the sample. The equation that describes the complex-subtractive mixing is the approximated equation derived by Kubelta and Munk:

$$\frac{K}{S} = \frac{(1-R)^2}{2R}$$

where K is an absorption coefficient, S is a scattering coefficient and R is the reflectance of the sample expressed as a fraction between 0 and 1 (Billmeyer & Saltzman, 1981).

A CM 3500-d Spectrophotometer (Konika Minolta Photo Imaging, Mahwah, NJ) was used to evaluate the color and the K/S value of the stained samples. Instrumental color readings are L^*, a^* and b^*. These values are L^* black (−) to white (+), a^* green (−) to red (+), and b^* blue (−) to yellow (+). The K/S value of the samples after exposure to iodine vapor was measured at wavelength range from 400 to 700 nm, at 10 nm intervals, using unstained starch as the target color. Since, the K/S value is defined as the ratio between the absorbance and the scattering coefficients, an increase in K/S reflects an increase in light absorbance and/or decrease in light scattering.

2.4. Light microscopy

A 5 mg portion of starch was dispersed in 0.25 ml of deionized water, and after dispersion one drop was placed on

the microscope slide. Samples were viewed using a light microscope equipped with polarizing filters (BX50, Olympus, Melville, NY) connected to a digital camera (SPOT II, Bioscan, Warrendale, PA). Photographs were taken using the auto-exposure function.

2.5. X-ray powder diffraction

A Kα 1 = 4.54056 Å and Kα 2 = 1.54439 Å radiation was produced by the copper X-ray tube of a Scintag Pad V X-ray powder diffractometer (Scintag, Inc., Cupertino, CA). The starch powders were packed tightly in a quartz zero background plate, of 1.1 square inc dimension (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.

3. Results

3.1. Moisture sorption isotherm

The moisture content of the samples equilibrated above Drierite, and saturated solutions of $MgCl_2$, NaCl, K_2SO_4 are reported in Table 1. CCS had the highest moisture content when equilibrated above Drierite. The moisture content of the samples following equilibration above $MgCl_2$ was not significantly different between the three starch samples. At higher a_w values (0.75 and 0.97), CCS had the lowest moisture content and HAC had the highest moisture content.

3.2. Color development

Significant differences were observed in the color of starch samples following equilibration over Drierite, $MgCl_2$, NaCl, and K_2SO_4 (Fig. 1). Following exposure to iodine vapor, all three starch samples equilibrated over Drierite exhibited a pale yellow color. The L^*, a^* or b^* values were not significantly different between these samples (Fig. 2). However, the L^* and a^* values were higher, and the b^* values were lower than that observed for the respective starch samples that were not exposed to iodine vapor (data not shown) suggesting some deposition of iodine on the granules. The intensity of color development increased with increasing moisture contents. However, the extent of color development was different for the three starches, with significant differences evident even when equilibrated above $MgCl_2$ (~8.4% moisture content).

Table 1
Moisture content of corn starches following equilibration over Drierite (0.15 a_w), $MgCl_2$ (0.33 a_w), NaCl (0.75 a_w), and K_2SO_4 (0.97 a_w)

Samples	Drierite (0.15 a_w)	$MgCl_2$ (0.33 a_w)	NaCl (0.75 a_w)	K_2SO_4 (0.97 a_w)
CCS	2.60 ± 0.24	8.31 ± 0.09	13.32 ± 0.02	18.76 ± 0.15
WCS	1.58 ± 0.04	8.12 ± 0.03	13.85 ± 0.40	23.29 ± 1.68
HAC	1.68 ± 0.19	8.79 ± 0.03	17.48 ± 0.01	25.27 ± 1.92

CCS, common corn starch; WCS, waxy corn starch; HAC, high amylose corn starch.

Color analyses revealed that the L^* value decreased with increasing moisture content for all three starches (Fig. 2). WCS had a lighter color at all moisture contents when compared to the other two starches. No significant differences were observed for the L^* value between CCS at moisture content higher than 13.3% and HAC higher than 17.5%. The a^* value for WCS increased at a moisture content higher than 8.1% indicating an increase in the intensity observed in the red spectrum (Fig. 2). The a^* value for CCS and HAC peaked at moisture content of 8.3 and 8.8% respectively. The b^* value for WCS peaked at 23.3% moisture suggesting an increase in intensity observed in the blue spectrum when equilibrated above K_2SO_4 (Fig. 2), whereas the b^* values for CCS and HAC decreased around 8% and increased slightly thereafter (Fig. 2).

3.3. Absorption intensity

The intensity of color developed was also reflected in the K/S maxima values of the different starches. Samples equilibrated over Drierite (0.15 a_w) did not show peak K/S intensity at any wavelength measured (Fig. 3(A)). However, following equilibration above $MgCl_2$ solution (0.33 a_w), a significant increase in the K/S value was observed for CCS and HAC with a peak value, while no maxima was observed for WCS. The K/S maxima were at 540 nm for both CCS and HAC and the K/S values exhibited a more than 40 fold increase from about 0.02 to more than 0.8 K/S value (Fig. 3(B)). WCS exhibited K/S maxima when equilibrated above NaCl solution (0.75 a_w) with a broad peak at about 520 nm (Fig. 3(C)). The K/S spectra for CCS and the HAC exhibited a more than 50× increase in K/S value and the wavelength of maximum K/S intensity increased to 570 nm when equilibrated over NaCl. Following equilibration above K_2SO_4 (0.97 a_w), the K/S values increased further for all three starches. WCS exhibited a peak at 520 nm, while the maximum K/S values for CCS and HAC were between 520 and 650 nm (Fig. 3(D)).

A plot of K/S values at 540 and 620 nm against the moisture content of the starches is shown in Fig. 4. At moisture contents corresponding to 0.33 and 0.75 a_w, the absorption values were higher at 540 nm compared to 620 nm for all three starches. Furthermore, CCS and HAC exhibited K/S peak at a moisture content of about 8%, whereas WCS did not exhibit any K/S peak at this moisture content.

3.4. Light microscopy

Following exposure to iodine vapor, no starch samples equilibrated over Drierite (0.15 a_w) or $MgCl_2$ (0.33 a_w) exhibited any staining when observed by using bright field microscopy (BFM) (Fig. 5). CCS and HAC exposed to iodine vapor following equilibration over NaCl (0.75 a_w) and K_2SO_4 (0.97 a_w) solutions stained blue magenta in BFM. However, differences in color were evident between these two samples when equilibrated above NaCl, with HAC exhibiting a darker color than CCS. Heterogeneity in staining intensity was also evident for CCS and HAC equilibrated above NaCl. On the other hand, no differences in color were observed between

Starch	Drierite (0.15 a_w)	$MgCl_2$ (0.33 a_w)	NaCl (0.75 a_w)	K_2SO_4 (0.97 a_w)
Common Corn				
Waxy Corn				
High Amylose Corn				

Fig. 1. Color of the corn starch samples equilibrated over different salt solutions following exposure to iodine. $MgCl_2$, magnesium chloride; NaCl, sodium chloride; K_2SO_4, potassium sulphate.

HAC and CCS following equilibration over K_2SO_4. WCS exhibited a light brown color when equilibrated over K_2SO_4. The presence of blue–magenta granules in WCS equilibrated above NaCl and K_2SO_4 is likely due to contamination with CCS granules.

When observed by using polarized light microscopy (PLM), all the three starch samples equilibrated above Drierite (0.15 a_w) and $MgCl_2$ (0.33 a_w) were bright white with clear maltese crosses visible. CCS equilibrated above NaCl (0.75 a_w) showed pinkish quadrants and black maltese crosses, while granules equilibrated above K_2SO_4 (0.97 a_w) exhibited white quadrants and blue maltese crosses, with only a few granules that stained blue with a darker blue cross. HAC equilibrated above NaCl had pink quadrants and black maltese cross. The intensity of the color of the quadrants was heterogeneous with colors ranging from light pink to dark pink and orange/red color. HAC at 0.97 a_w exhibited darker and very heterogeneous colors: the color of the quadrants varied between pink, blue and orange/red, with black maltese crosses. Furthermore, heterogeneity of color within the same granule was also observed; some granules exhibited pinkish quadrants and a blue area around the dark cross or a blue center. WCS was very bright when observed by using PLM and no color was observed in samples even at the highest moisture content.

Iodine-stained granules containing amylose became less bright with PLM as the iodine concentration increased. The darker granule color and the lighter background of CCS and HAC equilibrated above K_2SO_4 indicated that the intensity of the birefringence had greatly diminished (Evans et al., 2003).

3.5. X-ray powder diffraction

The X-ray diffractograms of CCS, WCS and HAC equilibrated above $MgCl_2$ (0.33 a_w) and K_2SO_4 (0.97 a_w) before and after exposure to iodine vapor are shown in Fig. 6. Unstained starch samples equilibrated above K_2SO_4 showed sharp diffraction peaks. WCS and CCS showed a typical A-type pattern, with strong reflections at 2θ about 15 and 23° and a doublet at 17, 18°. HAC showed a B-type pattern, with the main diffraction peak at about 17° 2θ and a few smaller peaks around 20, 22 and 23°.

Following iodination, all starches exhibited similar X-ray patterns as the original unstained samples. Moreover, no differences were observed in the diffractograms of stained and unstained samples equilibrated over $MgCl_2$. However, the scattering intensity for almost every diffraction peak of CCS and HAC equilibrated above K_2SO_4 decreased following exposure to iodine vapor, except that at $2\theta = \sim 20°$. This peak is attributed to the formation of the V-type complex (Cheetham & Tao, 1998). Therefore, by visual comparison of this trend with the pattern shown by the unstained samples, we conclude that the degree of crystallinity in CCS and HAC equilibration above K_2SO_4 decreased following exposure to iodine vapor, presumably with the formation of a V-type complex. WCS, however, retained its native X-ray diffraction pattern following iodination, with no apparent change in the degree of crystallinity even at the highest moisture content; i.e. a typical V-type iodine complex diffraction peak was not observed.

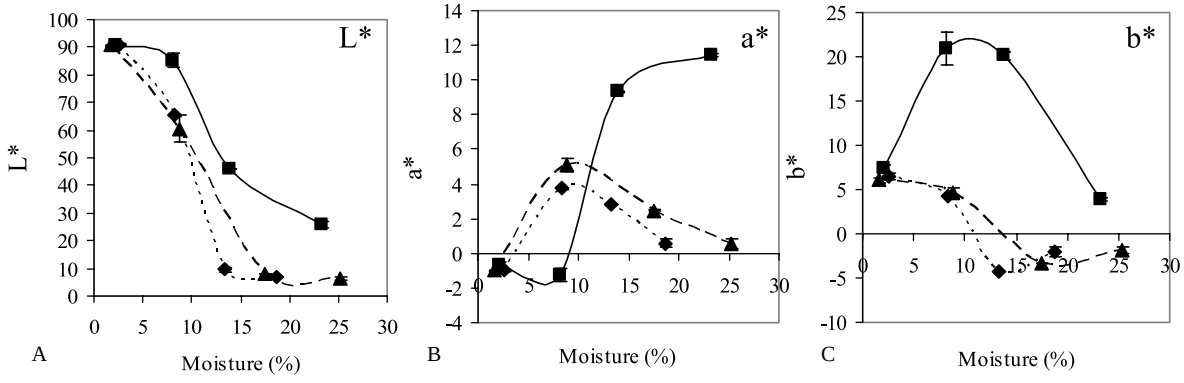

Fig. 2. L^* (A), a^* (B), and b^* (C) values of waxy (—■—), common (… ♦ …), and high amylose (- - -▲- - -) corn starch samples as a function of moisture content following exposure to iodine vapor.

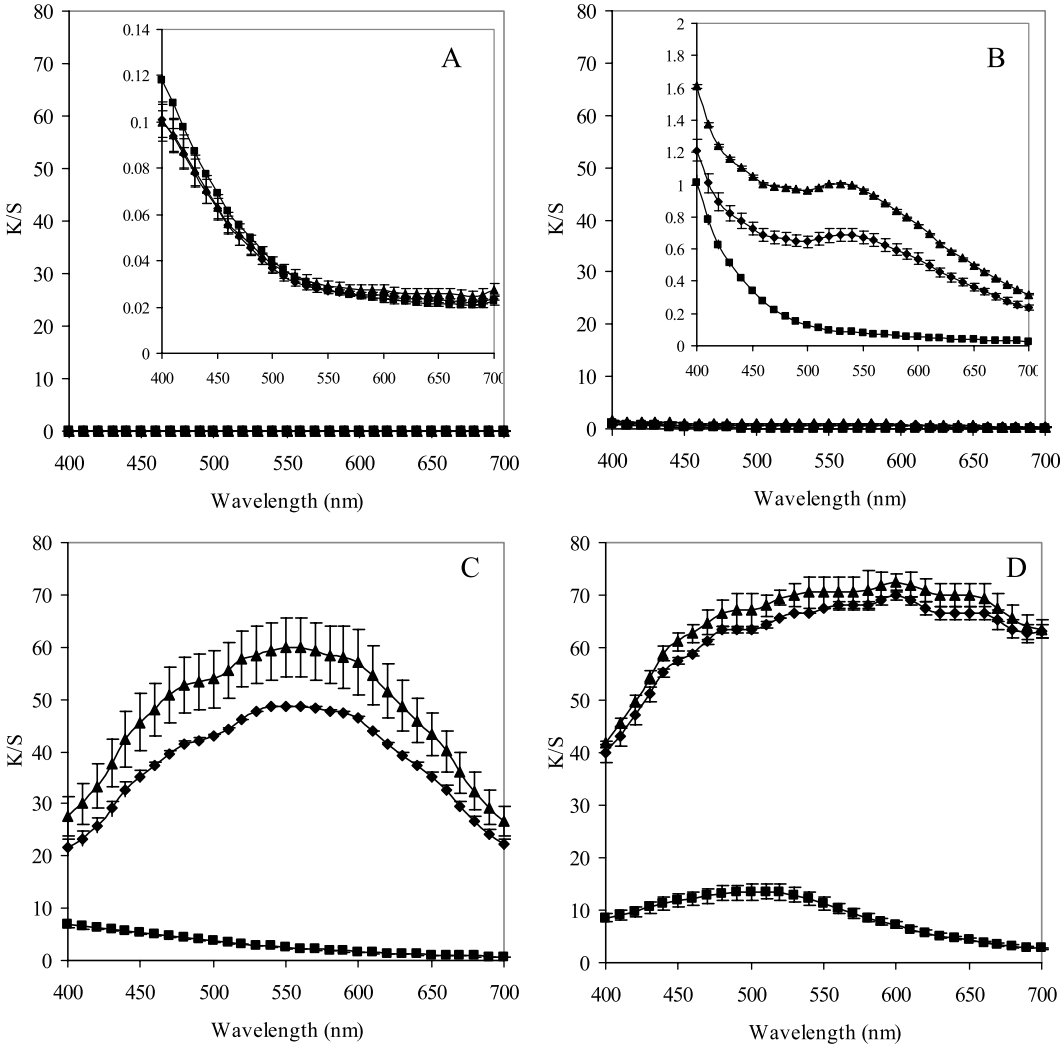

Fig. 3. *K/S* value of waxy (■), common (♦), and high amylose (▲) corn starch samples exposed to iodine vapor following equilibration over Drierite (A), $MgCl_2$ (B), NaCl (C), and K_2SO_4 (D) saturated solutions. The insets in Figs. (A) and (B) are enlarged.

4. Discussion

4.1. Color development

The depression of glass transition temperature of a polymer with increasing plasticizer content is well known (Slade & Levine, 1995). These values, however, reflect the transitions of polymers at a supramolecular level, i.e. at the granular level. In this study, the development of color of samples equilibrated at different moisture contents indicates successive transitions or multiple local transitions within starch granules. The intensity of the color developed in the stained samples is reflective of the size of the helix and the number of iodine ions complexed by the polymers. Mobility of longer polymer chains results in the formation of longer helixes, thus resulting darker colors (Banks & Greenwood, 1975).

Differences in chain mobility are observed even when the three starch samples are equilibrated above the same salt solution. The most visible differences were observed between samples equilibrated above $MgCl_2$ (0.33 a_w), with corresponding moisture content of 8.1% for WCS, 8.3% for CCS and 8.8%

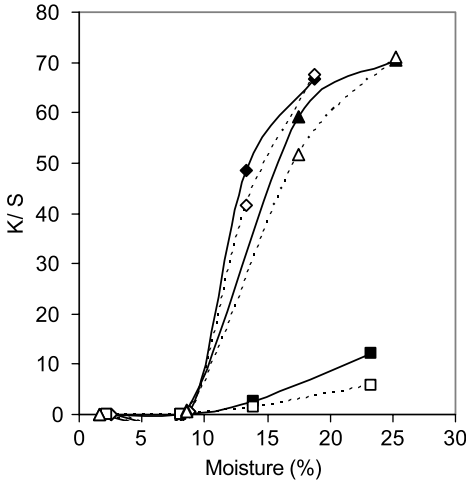

Fig. 4. *K/S* values at 540 nm (filled markers) and 620 nm (empty markers) as a function of moisture content for waxy corn starch (■), common corn starch (♦), and high amylose corn starch (▲) exposed to iodine vapor following equilibration.

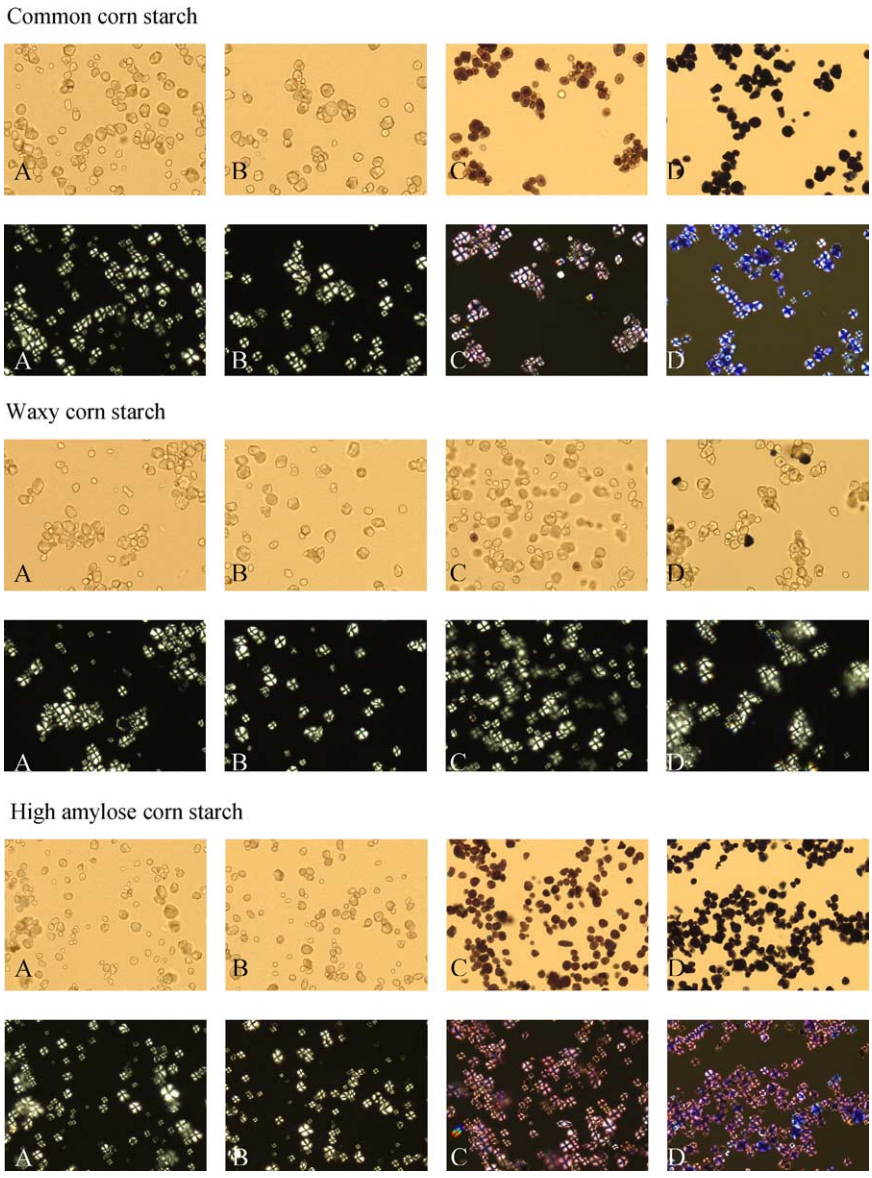

Fig. 5. Light microscopy of Common corn starch, Waxy corn starch, and High amylose corn starch samples stained with iodine vapor. For each sample, the same field is shown for bright field (top frame) and between crossed polarizers (bottom frame). A = Drierite (0.15 a_w); B = $MgCl_2$ (0.33 a_w); C = NaCl (0.75 a_w); D = K_2SO_4 (0.97 a_w).

for HAC (Fig. 1). The light orange color developed in WCS implies the mobility of linear polymers with a degree of polymerization (dp) greater than 6 glucose units, which is the minimal number of glucose units needed to form a complex (Rundle & French, 1943). The *K*/*S* scan of WCS equilibrated above $MgCl_2$ did not show an absorption maximum (Fig. 3(B)), but the *K*/*S* values were about 1 log greater than that observed for starch granules equilibrated over Drierite (0.15 a_w). In contrast, the HAC exhibited distinct blue color at 0.33 a_w and with similar moisture content as WCS at 0.33 a_w (Table 1). The absorption maximum of 540 nm for HAC at 0.33 a_w reflects the mobility of a linear polymer chain of about 36 dp (Banks et al., 1971) at moisture content of 8.7%. CCS at 0.33 a_w had a color between HAC and WCS, although the wavelength of absorption maximum was the same as that observed for HAC. The slightly lower L^* values observed for all three starches when equilibrated over Drierite, compared to starch samples not exposed to iodine vapor, suggests that iodine is deposited on the granules. However, this iodine does not form a complex as is evident by the negligible signal observed when *K*/*S* was measured. It is also likely that no color was developed because of the limited moisture content (Murdoch, 1992).

The subsequent increase in intensity of color developed and the increasing absorption maximum reflect increasing chain mobility with increasing moisture contents for all starch types. At 0.97 a_w and corresponding moisture content of about 25.3, 23.3 and 18.8% for HAC, WCS and CCS, respectively, the samples exhibited the typical color expected for these starches. We further extrapolated, using data reported by Banks et al. (1971), the potential length of the iodine–polymer complex based on the absorption maximum recorded at each moisture

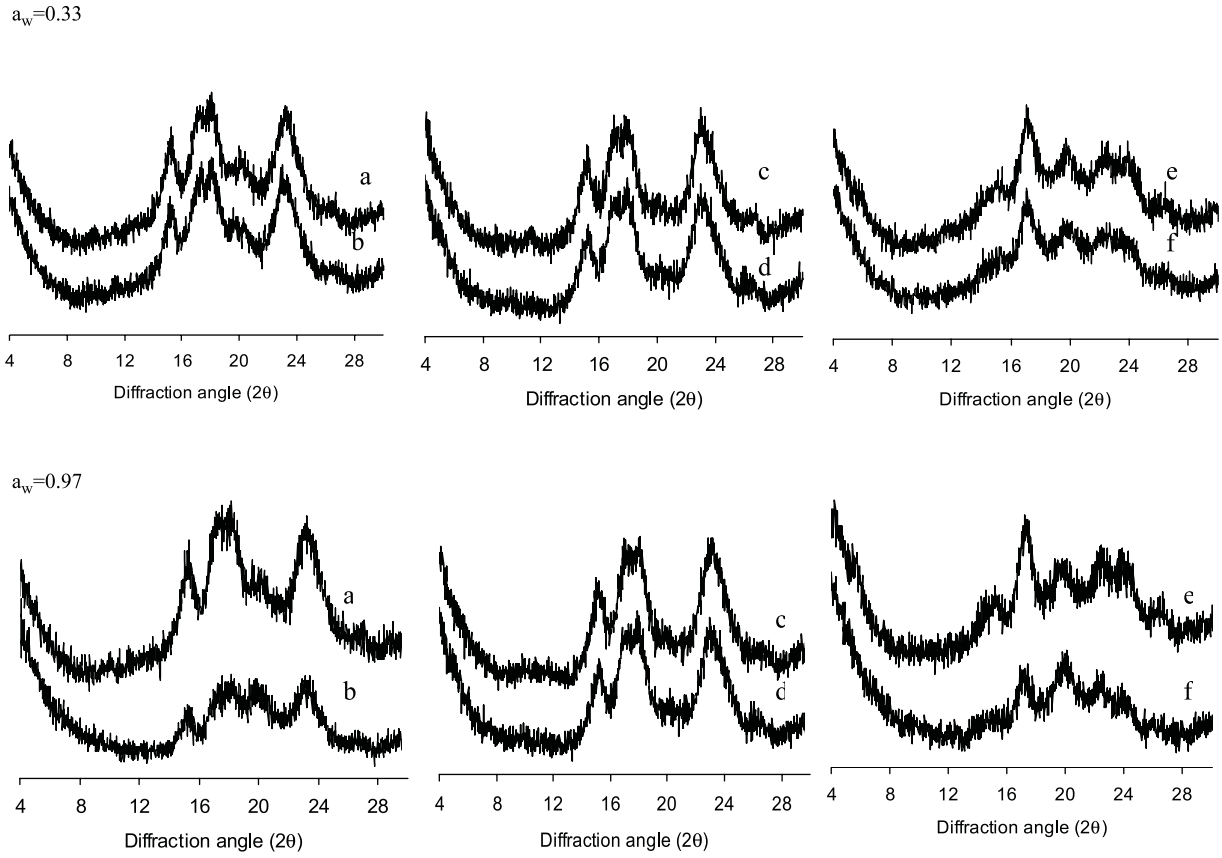

Fig. 6. The effect of iodination on the X-ray powder diffraction spectra of corn starches. (a) common corn starch; (b) common corn starch following exposure to iodine vapor; (c) waxy corn starch; (d) waxy corn starch following exposure to iodine vapor; (e) high amylose corn starch; (f) high amylose corn starch following exposure to iodine vapor.

contents for all three starches (Table 2). Since, as mentioned before, the *K*/*S* value is defined as the ratio between the absorbance and the scattering coefficients, an increase in *K*/*S* reflects an increase of light absorbed and/or decrease in light scattered. The *K*/*S* values obtained in this study can be therefore related to absorbance values measured in previous works (Banks et al., 1971). CCS and HAC exhibited absorption maxima of 540, 570 and 600 nm, at 0.33, 0.75 and 0.97 a_w, respectively and this corresponds to polymer chain mobility with a dp around 36, 51 and 105, respectively. WCS on the other hand exhibited a maximum of only 520 nm, corresponding to 30 dp even at 0.97 a_w. At the highest a_w treatment, the *K*/*S* for CCS and HAC continue to remain high at 700 nm and therefore, suggest mobility of chains greater than dp 1500. It is likely that the granules have transitioned into their rubbery state at this a_w and corresponding moisture content, thereby facilitating the mobility of even long chains (ten Brinke et al., 1983; Zelenzak & Hoseney, 1987). The very low *K*/*S* value at 700 nm for WCS at 0.97 a_w, suggests that WCS has fewer long chains of about a dp of 1500 or greater.

4.2. Bright field and polarized light microscopy

The differences in color and staining intensity of the different starch types, at higher moisture contents, when viewed by using BFM, suggests that iodine is able to penetrate the granule and that polymer segments within the granule are able to complex with iodine. The darker bluish color observed

Table 2
Length of the iodine–polymer complex based on the absorption maximum recorded at each moisture content for all three starches

Sample	Common corn starch				Waxy corn starch				High amylose corn starch			
Moisture (%)	2.60	8.31	13.32	18.76	1.58	8.12	13.85	23.29	1.68	8.79	17.48	25.27
λ_{max} (nm)	–[a]	540	570	600	–	–	520	520	–	540	570	600
DP[b]	–	36.4	50.7	105	–	–	28.9	28.9	–	36.4	50.7	105

λ_{max}, Wavelength of maximum *K*/*S* value; DP, degree of polymerization.
[a] No peaks observed.
[b] Values extrapolated from Banks et al. (1971).

at higher moisture contents suggest the formation of complex between the lipid-free amylose and iodine. However, granules equilibrated over $MgCl_2$ (0.33 a_w) did not exhibit any color when viewed under a microscope, while differences in color development were evident when viewed directly (Fig. 1). It is likely that at 0.33 a_w, the iodine–polymer interaction is only on the surface of the granules while at higher a_w or moisture content, iodine penetrates into the granules.

The nature of the polymer–iodine complex within the granules is further illustrated when viewed under polarized light. When viewed by using PLM, only regions of the granule that contain molecules with a net radial orientation contribute to the blue color of the light quadrants of the maltese cross (Evans et al., 2003). With the increasing iodine concentration, the increase in absorbance from complexes of either amylose or amylopectin would lead to darker granules with a diminished ability to observe colors under the polarizer. Furthermore, the heterogeneity in color within a granule indicates heterogeneity of structure within a granule. A dark color of the granule can potentially be due to strong absorbance due to oriented absorbing molecules, weak birefringence due to lack of orientation or some combination of the two.

CCS exhibited a single color throughout the granule when viewed under PLM suggesting a uniform radial orientation of then helical amylose. HAC granules on the other hand, had a heterogeneous color distribution, suggesting a lack of radial arrangement of the amylose within the granules. In addition HAC granules exhibited pink color when viewed under PLM as has been reported earlier (Evans et al., 2003). The pink color in HAC starch could be due to inefficient binding of iodine by amylose even if it were radially oriented. The combination of the dark color in BFM and the pink color in PLM is consistent with a lack of orientation of the amylose–iodine complexes. The complexes with amylopectin, being radially oriented, are responsible for the light and color observed by PLM.

4.3. X-ray powder diffraction

Data from the X-ray diffraction patterns at higher moisture contents further confirms the observation that iodine is able to penetrate the granule and form a complex that disrupts the crystalline arrangement within the granule. Cheetham & Tao (1998) also reported that the formation of the amylose–iodine complex disrupts the crystallinity of the granules. Furthermore, the increase of the relative intensity at 20° 2θ in CCS and HAC exposed to iodine vapor following equilibration over K_2SO_4 (0.97 a_w) is attributable to the formation of the V-type complex (Cheetham & Tao, 1998). However, the absence of the loss of crystallinity in WCS even at higher moisture content suggests that the disruption of crystallinity in amylose containing starches is due to the complex formation between iodine and lipid-free amylose.

5. Conclusions

The data presented in this study highlight several interesting observations within the context of glass transitions in starch granules. The observations allow for the following specific conclusions:

1. Sequential mobility of increasingly longer linear glucan polymers occurs within a granule with increasing moisture content. The lowest moisture content at which mobility was observed was at 8.1% wherein potentially a 36 dp polymer chain was able to complex with iodine.
2. The chain length of the mobile segments was different for different starch types and the transition occurred at different moisture contents.
3. At lower moisture contents it is likely that the iodine–polymer complex occurs only at the surface, while at higher moisture contents, the complex formation is able to disrupt the crystalline structure within the granule.
4. The structural and architectural differences between different starch types and mutants can be further probed by using iodine as a tool at low moisture contents.

Studies are underway to further elaborate possible structural differences in the starch granules from different botanical sources.

References

AACC (2000). *Approved methods of American association of cereal chemists* (10th ed.). St Paul, MN: The Association.

Banks, W., & Greenwood, C. T. (1975). The reaction of starch and its components with iodine. In W. Banks, & C. T. Greenwood (Eds.), *Starch and its components* (pp. 67–112). Edinburgh: Edinburgh University Press.

Banks, W., Greenwood, C. T., & Khan, K. M. (1971). The interaction of linear, amylose oligomers with iodine. *Carbohydrate Research*, *39*, 25–33.

Bates, F. L., French, D., & Rundle, R. E. (1943). Amylose and amylopectin content of starches determined by their iodine complex formation. *Journal of the American Chemical Society*, *65*, 142–148.

Billmeyer, F. W., & Saltzman, M. (1981). The coloring of materials in industry. In F. W. Billmeyer, & M. Saltzman (Eds.), *Principles of color technology* (pp. 133–172). New York: Wiley.

Cheetham, N. W. H., & Tao, L. (1998). Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohydrate Polymers*, *36*, 277–284.

Chung, H. J., Lee, E. J., & Lim, S. T. (2002). Comparison in glass transition and enthalpy relaxation between native and gelatinized rice starches. *Carbohydrate Polymers*, *48*, 287–298.

Conde-Petit, B., Nuessli, J., Handschin, S., & Escher, F. (1998). Comparative characterization of aqueous starch dispersions by light microscopy, rheometry and iodine binding behaviour. *Starch/Stärke*, *50*, 184–192.

Evans, A., McNish, N., & Thompson, D. B. (2003). Polarization colors of light iodine-stained maize starches for amylose-extender and related genotypes in the W64A inbred line. *Starch/Stärke*, *55*, 250–257.

Gerard, C., Barron, C., Colonna, P., & Planchot, V. (2001). Amylose determination in genetically modified starches. *Carbohydrate Polymers*, *44*(1), 19–27.

Gidley, M. J., & Bulpin, P. V. (1987). Crystallisation of malto-oligosaccharides as model of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydrate Research*, *161*, 291–300.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards Section A—Physics and Chemistry*, *81A*(1), 89–96.

Jenkins, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke*, *45*, 417–420.

John, M., Schmidt, J., & Kneifel, H. (1983). Iodine–maltosaccharide complexes: Relation between chain-length and colour. *Carbohydrate Research*, *199*, 254–257.

Katz, F. R., Furcsik, S. L., Tenbarge, F. L., Hauber, R. J., & Friedman, R. B. (1993). Behavior of starches derived from varieties of maize containing different genetic mutations: Effects of starch genotype on granular morphology. *Carbohydrate Polymers*, *21*, 133–136.

Knutson, C. A. (1999). Evaluation of variations in amylose–iodine absorbance spectra. *Carbohydrate Polymers*, *42*, 65–72.

Kou, Y., Dickinson, L. C., & Chinachoti, P. (2000). Mobility characterization of waxy corn starch using wide-line ^{1}H nuclear magnetic resonance. *Journal of Agricultural and Food Chemistry*, *48*, 5489–5495.

Langton, M., & Hermasson, A. M. (1989). Microstructural changes in wheat starch dispersions during heating and cooling. *Food Microstructure*, *8*, 29–39.

Lourdin, D., Colonna, P., & Ring, S. G. (2003). Volumetric behaviour of maltose–water, maltose–glycerol and starch–sorbitol–water systems mixtures in relation to structural relaxation. *Carbohydrate Research*, *338*, 2883–2887.

Mizuno, A., Mitsuiki, M., & Motoki, M. (1998). Effect of crystallinity on the glass transition temperature of starch. *Journal of Agricultural and Food Chemistry*, *46*, 98–103.

Murdoch, K. A. (1992). The amylose–iodine complex. *Carbohydrate Research*, *233*, 161–174.

Roos, Y., & Karel, M. (1991). Water and molecular weight effects on glass transitions in amorphous carbohydrates and carbohydrates solutions. *Journal of Food Science*, *56*, 1676–1681.

Rundle, R. E., & French, D. (1943). The configuration of starch in the starch–iodine complex. III. X-ray diffraction studies of the starch–iodine complex. *Journal of the American Chemical Society*, *65*, 1707–1710.

Rundle, R. E., & French, D. (1944). On the nature of the starch–iodine complex. *Journal of the American Chemical Society*, *66*, 2116–2120.

Seguchi, M., Yasui, T., Hosomi, K., & Imai, T. (2000). Study of internal study of waxy wheat starch granules by KI/I2 solution. *Cereal Chemistry*, *77*, 339–342.

Shah, N. K., & Ludescher, R. D. (1995). Phosphorescence probes of the glassy state in amorphous sucrose. *Biotechnology Progress*, *11*, 540–544.

Shannon, J. C., & Garwood, D. L. (1984). Genetics and physiology of starch development. In R. L. Whistler, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch: Chemistry and technology*. New York: Academic Press.

Slade, L., & Levine, H. (1995). Water and the glass transition—dependence of the glass transition on composition and chemical structure: Special implications for flour functionality in cookie baking. *Journal of Food Engineering*, *24*, 431–509.

Svegmark, K., & Hermansson, A. M. (1991). Distribution of amylose and amylopectin in potato starch pastes: Effects of heating and sharing. *Food Structure*, *10*, 117–129.

ten Brinke, G., Karasz, F. E., & Ellis, T. S. (1983). Depression of glass transition temperatures of polymer networks by diluents. *Macromolecules*, *16*, 244–249.

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science*, *39*, 151–165.

Zeleznak, K. J., & Hoseney, R. C. (1987). The glass transition in starch. *Cereal Chemistry*, *64*, 121–124.