Structure and Mechanical Properties of Hydroxypropylated Starch Films

David Lafargue, Bruno Pontoire, Alain Buléon, Jean Louis Doublier, and Denis Lourdin*

INRA, UR1268 Unité Biopolymères Interactions et Assemblage, rue de la Géraudière,
F-44000 Nantes, France

Received August 29, 2007; Revised Manuscript Received October 9, 2007

Films of acid-hydrolyzed hydroxypropylated pea starch with average molecular weight M_w ranging from 3.3×10^4 g/mol to 1.6×10^6 g/mol were prepared from 25% (w/w) solution by casting. The structure of the films was investigated by means X-ray diffraction and calorimetry, evidencing a B-type crystalline structure. In similar drying conditions, 25 °C and 40% of relative humidity, the crystallinity varied from 24% for the low molecular weight (A5) to almost none for the highest molecular weight (A160). The influence of the drying temperature was also investigated. A reduction of the crystallinity from 16% to almost none was found when increasing temperature from 25 to 65 °C. The glass transition temperature (T_g) at different water contents was determined. The difference of T_g between the first and the second scan was interpreted by changes in the water distribution between phases into the B-type crystalline structure. Mechanical properties of the films determined by tensile tests and by DMTA in the glassy state showed no effect of the average molecular weight or of crystallinity. In contrast, thermomechanical experiments by DMTA showed that the average molecular weight of the sample influenced the mechanical relaxation and the moduli in the rubbery state.

Introduction

Among all polysaccharides investigated as potential alternatives to materials from oil, such as conventional plastics, starch has attracted a large amount of attention.[1] This is one of the most abundant natural plant polysaccharides and, thanks to its cheapness, renewability, and biodegradability, it can be used as a raw material for the elaboration of biologically degradable materials and, thus, promises an environmental solution to the plastic waste issue.

Starch is composed of amylose, an almost linear macromolecule with a molecular weight ranging from 10^5 to 10^6 g/mol, and amylopectin, a highly branched macromolecule with a higher molecular weight of around 10^7-10^9 g/mol. These two high molecular weight and polydisperse polymers of D-glucose are organized at the native state in semicrystalline granules. Different techniques have been used to produce starch films. Casting, which consists of drying solutions, is an attractive method to prepare free-standing films. From the pioneering work of Wolf et al. (1951) on mechanical and barrier properties of amylose films,[2] many studies have been focused on starch-based films cast from solution or gel state.[3–10] Influence of amylose content on the mechanical properties of starch films has also been investigated.[4,11] It has been found that linear amylose chains confer better mechanical properties compared to branched amylopectin molecules.

It is known that crystallization of polymers strongly depends on molecular weight, branching, concentration, temperature, and the solvent used. Crystallinity of starch films prepared by a casting procedure has been widely studied. A B-type crystalline structure is expected when starch films are cast from aqueous solutions.[12] The effect of the preparation conditions (temperature, relative humidity (RH)) on the structure of a water-cast amylose film has been investigated.[13] All films showed a B-type X-ray diffraction pattern. The effect of temperature, RH, and time of drying have also been investigated on potato starch films.[5,14] It was shown that crystallinity depends on the drying conditions. Films formed at elevated temperature were found to be amorphous, while those obtained after long drying at ambient temperature showed relatively high crystallinity. The crystalline structure has a strong impact on the thermal and mechanical properties of starch films in the rubbery state.[15–17] The increase of their crystallinity has been reported to yield a stiffening of films by increasing elastic modulus, tensile stress, and decreasing the tensile strain. Crystallinity also affects the glass transition temperature (T_g) of amorphous regions of semicrystalline polymers.[18] According to Slade and Levine (1989), the increase of the T_g of starch with increasing the crystallinity has been interpreted by the stiffening effect of crystalline cross-links, which decrease the mobility of chain in amorphous phases.[19,20]

T_g, which can be also affected by the presence of plasticizers,[21] has a major impact on the properties of starch films. Depending on the plasticizer content, the material is in the glassy state or in the rubbery state at ambient temperature, thus determining the material properties and the field of application. Water greatly affects the T_g of starch[22,23] but other small molecules like glycerol,[24] sorbitol[6] are commonly used to plasticize and stabilize starch films. The T_g of starch also depends on the molecular weight.[25] The T_g dependence on the molecular weight has been well described for linear synthetic polymers.[26] T_g usually decreases linearly with increasing the inverse of the molecular weight, which was ascribed to the free volume of polymers.[25] The influence of molecular weight on the mechanical properties of extruded thermoplastic potato starch has been demonstrated in the rubbery state.[27] The lower strain and tearing energy of the low molecular mass materials were attributed to the reduced amylose chain length as well as the degree of branching of the amylopectin molecules, resulting in less effective entangled macromolecules.

---

* Corresponding author. E-mail:lourdin@nantes.inra.fr.

10.1021/bm7009637 CCC: $37.00

Table 1. Average Molecular Weight M_w and Intrinsic Viscosity $[\eta]$ of Hydroxypropylated and Hydrolyzed Pea Starch A5, A15, A30, A60, and A160

sample	avg molecular weight $M_w \times 10^{-4}$ (g/mol)	$[\eta]_{25\ °C\ water}$[a] (ml/g)	Huggins coefficient k_H
A5	4.7	11.9	1.6
A15	15.0	16.1	1.7
A30	33.0	19.8	1.6
A60	59.0	25.1	1.1
A160	160.0	37.8	0.5

[a] $[\eta] = 3.4 \times 10^{-3} \times M_w^{0.33}$.

The use of nonmodified starches for films processing is often limited due to high viscosity of starch pastes and also to the water sensitivity of the resulting films, which reduce their mechanical properties. Moreover, the structure of the film can change upon storage.[10,14] Part of these drawbacks can be overcome owing to physical or chemical modifications.

High viscosity of the starch pastes can be decreased by acid hydrolysis of the starch molecules. In that way, hydrolyzed starch could be dispersed in water at higher concentration than native starch. Modification of starch molecules at the granular state by hydroxypropylation is also particularly interesting. Such chemical modification involving the introduction of hydrophilic groups into starch molecules improves the solubility of starch and the functional properties of starch pastes, such as shelf life, freeze/thaw stability, cold storage stability, cold water swelling, and yields reduced gelatinization temperature, as well as retarded retrogradation. Owing to these properties and their toxicology safety, hydroxypropylated starches have a wide spectrum of applications.[28–30]

Films of hydroxypropylated starch can be prepared easily thanks to a lower solubilization temperature. Advantageous properties for use as packaging materials, especially transparency and flexibility have been evidenced.[31–33] However, the influence of drying conditions and molecular weight on the structure and properties of hydroxypropylated starch films has never been reported.

This work aims at using acid hydrolyzed hydroxypropylated pea starch (HPPS) with different average molecular weights to produce films for casting applications. Influence of molecular weight and casting temperature was thoroughly studied as well as the structural changes occurring during film formation. The resulting structure and mechanical properties were determined using X-ray diffraction (XRD), differential scanning calorimetry (DSC), tensile tests, and dynamic mechanical thermal analysis (DMTA). Pea starch was used because of its relative high amylose content, 34%, which favors good mechanical properties of the films. The overall understanding of the mechanisms involved in film formation as well as the knowledge of the film structure/properties relationships are essential to the development of new plastic materials.

Experimental Section

Materials. Five samples of hydroxypropylated pea starch with different molecular weight used in this study were prepared by acid hydrolysis and characterized by HPSEC-MALLS by Roquette (Lestrem, France). Their average molecular weight M_w ranges from 5×10^4 g/mol (A5) to 1.6×10^6 g/mol (A160), and the corresponding designations were reported in Table 1. The degree of substitution defined as the average number of hydroxypropylated groups per anhydroglucose unit in the polymer chain was 0.2 ± 0.01.

Methods. *Molecular Weight.* Sample molecular weight characterization was carried out by Roquette with a high-performance size-exclusion chromatograph equipped with a multiangle laser-light scattering detector (Dawn-F, Wyatt Technology, Santa Barbara, CA) and a refractive index detector RI (waters, 410; HPSEC-MALLS-RI). Elution of the starch solution (0.2 M $NaNO_3$ containing 0.02% NaN_3) was carried out at a flow rate of 0.3 mL/min and at a temperature of 75 °C. A total of 100 μL was injected and a refractive index increment of 0.146 mL/g was used for the calculation.

Intrinsic Viscosity. A low shear viscometer (LS 40 from Contraves) was used with a concentric cylinder geometry (inner radius, 6 mm; outer radius, 6.5 mm). A stock solution of 0.010 g/mL was heated at 90 °C in deionized water under stirring to ensure complete solubilization and then diluted at concentrations ranging from 0.010 g/mL to 0.002 g/mL. The dilute solution was filtered through 3 and 0.8 μm cellulose acetate membranes to remove possible granule remnants. All experiments were performed at 25 °C.

Casting. Solutions (25% (w/w)) were prepared by mixing starch powder and deionized water at room temperature and heating at 90 °C over 30 min. To avoid air bubbles, the solutions were stored at this temperature under low stirring and were then stabilized at 50 °C over 30 min. They were poured with a spreader on plastic plates and dried in a cabinet (ERATIS, Bouloc, France) under controlled conditions at 25 °C and 40% RH. Other films were also produced from A15 sample using a starch concentration of 25%. They were dried at various temperature, 35, 45, 55, and 65 °C. All samples were stored for two weeks at 25 °C in a relative humidity of 58% obtained with NaBr saturated salt solution.

Thermomolding. This technique was used to produce films with a thickness of around 700 μm. The cast-films formed at 25 °C and 40% of RH were reduced to powder by a cryogrinder, and the water content was adjusted to 25% (wet basis). After 12 h of equilibration, hydrated samples were heated at 120 °C for 15 min, and pressed into a plate mold ($100 \times 100 \times 1$ mm^3) at 20 MPa. The pressure was maintained during the cooling period to avoid bubbles growth in the film.

Moisture Content. The thermogravimetric method was used to measure the moisture content of the samples (MC, wet basis). Water was extracted from the samples at 130 °C for 90 min.

X-ray Diffraction. XRD were carried out on conditioned powders stored at 58% RH. Approximately 20 mg of sample was sealed between two tape foils to prevent any significant change in water content during the measurement. Diffraction diagrams were recorded on an INEL (Artenay, France) spectrometer working at 40 kV and 30 mA operating in the Debye–Scherrer transmission mode. The X-ray radiation Cu Kα_1 ($\lambda = 1.5405$ Å) was selected with a quartz monochromator. Diffraction diagrams were recorded for 2 h exposure periods, with a curve d position-sensitive detector (INEL CPS 120). Relative crystallinity was determined after normalization of all recorded diagrams at the same integrated scattering between $2\theta = 3°$ and 30°. Spherulitic B-type recrystallized amylose was used as a crystalline standard, after scaled subtraction of our experimental amorphous sample in order to get null intensity in the regions without diffraction peaks. The degree of crystallinity was determined by the method initially developed by Wakelin et al.[34] for cellulose. The crystal size along the *a* axis of the unit cell (lateral crystal size) was approximated from the half-peak width of the (100; $2\theta \sim 5.6°$) reflection using the Scherrer equation:

$$D_{\mathrm{hkl}} = \frac{K\lambda}{\beta \cos\theta} \tag{1}$$

where D_{hkl} is the average length of the diffracting domains normal to the family of planes (hkl), K is a constant usually taken as 0.9 for starch and cellulose,[35] λ is the wavelength, and β is the half-peak width.

Crystallization kinetics during film formation was monitored by recording X-ray diffraction diagrams every 10 min on a Bruker D8 Discover diffractometer. Cu Kα_1 radiation ($\lambda_{\mathrm{Cu\ K\alpha 1}} = 1.5405$ Å), produced in a sealed tube at 40 kV and 40 mA was selected and parallelized using a Gobël mirror parallel optics system and collimated to produce a 500 mm beam diameter. Around 54 mg of solution was introduced at 50 °C in a pierced capsule and was allowed to dry. Weight variations during the drying were recorded thanks to an interfaced balance Mettler Toledo AG285. Drying was performed at 25 °C ± 2 °C and 40% RH ± 1%. The X-ray diffraction data were collected using a two-dimensional GADDS detector. Acquisition time was 600 s, for a total experimental time of 10.5 h.

Differential Scanning Calorimetry (DSC). DSC thermograms were recorded on an automated heat flux T.A. Instruments Q100. The system was calibrated with indium, and about 25 mg of powdered conditioned matter was placed into stainless steel high-pressure cells. Two successive scans were run at 3 °C/min between 25 and 145 °C, separated by a cooling stage.

Mechanical Testing. Large Deformation Tests. Tensile tests were performed on cast-films with an Instron machine, model 1122 with a 100 N tensile load cell. Elongation at break (ϵ_R), tensile strength at break (σ_R), and Young modulus (E) were determined on 5A type dumbbell-shaped specimens according to the ISO-527-2 standard (l_o = 20 mm). The film thickness (90 μm ± 5μm) was determined in five points with a micrometer Mitutoyo (±1 μm). The distance between the supports was set at 50 mm, and the crosshead speed was fixed at 1 mm/min. Each measurement was performed at least five times until sample failure.

Small Deformation Tests. Thermomechanical measurements were performed on thermomolded films on a Dynamic Mechanical Thermal Analyzer (DMTA MK IV, Rheometric Scientific, U.S.A.). The dual cantilever bending mode was used at a frequency of 1 Hz, a strain of 0.1%, and a heating rate 3 °C/min from 25 to 125 °C. Strips (22 × 10 × 0.7 mm^3) were coated with a silicone-based hydrophobic grease to limit dehydration during experiment. The temperature of the glass-rubbery relaxation was determined at the beginning of the falling of the E' modulus corresponding to the molecular mobility associated with the calorimetric glass transition of the material.

Results

Intrinsic Viscosity. Intrinsic viscosity [η] of samples in dilute aqueous solutions is summarized in Table 1. It was obtained using both the Huggins and the Kraemer equations:

$$\frac{\eta_{sp}}{C} = [\eta] + k_H[\eta]^2C + \cdots \quad (2)$$

$$\frac{\ln\ \eta_r}{C} = [\eta] - k_K[\eta]^2C + \cdots \quad (3)$$

The intrinsic viscosity [η], representing the hydrodynamic volume of coil polymers, varies from 37.8 mL/g for A160 to 11.9 mL/g for A5 (Table 1). These values are relatively low compared to those usually obtained for starch polymers. In similar solvent conditions, much higher values have been reported, 150 mL/g[36] and 130 mL/g[37–39] for amylose and amylopectin, respectively. This reflects the molecular weight decrease of starch components induced by hydrolysis. The dimensionless Huggins coefficient k_H reported in Table 1 accounts for hydrodynamic pair interactions between the macromolecules and describes the solvent quality. It ranged from 0.5 for A160 to values higher than 1 for the other samples. Values of ~0.5 are found in Θ conditions and approach ~0.3 in good solvents. Usually, $k_H > \sim 0.8$, as observed for A60 (k_H = 1.1) or A30, A15, and A5 ($k_H \sim 1.5$) indicates that the polymer chains are prone to aggregation. This suggests that the low molecular weight samples are more able to aggregate.

From data presented in Table 1, the coefficient of the Mark–Houwink equation, $[\eta] = KM^{\alpha}$, was also determined

$$[\eta] = 3.4 \times 10^{-3} \times \bar{M}_w^{0.33} \quad (4)$$

This calculated α value is consistent with the results of Granath and co-workers (1969) who reported α = 0.35 for hydroxyethyl starch from maize.[40] This exponent α gives some information about the polymer structure. It can vary from 0 for hard sphere, 0.8 for coil-like behavior in good solvent, to a value close to 2 for rod-like polymers. A value lower than 0.5 is characteristic of branched polymers of large molecular weight. This evidence the major contribution of the branched amylopectin to the hydrodynamic behavior, which is consistent with the amount of amylopectin in pea starch (around 66%).

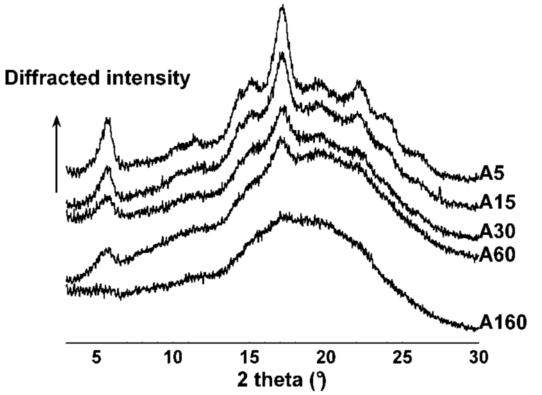

Figure 1. X-ray diffraction diagrams of films obtained by casting at 25 °C and 40% RH. Water content of films: 13.5 ± 0.2%.

Structural Properties. It is important to note that the lowest M_w A5 samples did not form true films by casting, the sample being totally cracked at the end of the drying process. Therefore, tensile experiments could not be performed on this sample. However, X-ray analysis and calorimetric measurements were performed on the powdered sample, enabling structural characterization of A5 samples. XRD diagrams of the different samples produced by casting at 25 °C and 40% RH are shown in Figure 1. The average water content of the films was about 13.5% (±0.2). Except for the A160 film, all spectra display a B-type crystalline structure with main characteristic diffraction peaks at $2\theta = 5.6$, 15, 17, and 22°. It is rather surprising that hydroxypropylated chains crystallized in a similar way as reported for cast films obtained from nonmodified starch.[5,8] Such a crystallization phenomenon has never been described for hydroxypropylated starch films. Both peak sharpness and peak intensity increase with decreasing M_w. The relative crystallinity and lateral crystal size are given in Table 2. The crystallinity decreases from 24% for A5 to 16% for A15, 12% for A30, and 6% for A60, whereas A160 films were almost amorphous. Simultaneously, the lateral crystal size varies from 9 nm for A5 to 5 nm for A60.

Figure 2 shows the diffraction diagrams recorded during the drying of the A15 solution at 25 °C and 40% RH. No diffraction peak was observed on the initial starch suspension. After one hour drying, the main characteristic peaks of the B-type structure (5.6° and 17°) appeared. The spectral resolution enhanced during drying due to both crystallization and reduction of X-ray absorption with decreasing water content. Water is known to play a major role on starch crystallinity and is an integral part of B-type crystals. B-type starch crystallinity has been shown to increase with the moisture content and to reach a maximum at 33% of water content.[41] A maximum diffraction intensity was reached at a drying time of 9 h corresponding to an average water content of 16%. Then, no further changes were observed except a decrease of the intensity of the peak at 5.6°, probably due to the loss of water. The final water content reached in these drying conditions was 11%. A similar experiment was carried out on A5 solutions for which crystallinity appeared sooner and also stopped sooner than A15 (8 h instead of 9 h), evidencing the higher ability of shorter chains to reorganize.

Table 2. XRD and DSC Data of A160, A60, A30, A15, and of A5 Films Formed at 25 °C and 40% of RH

		XRD data		DSC data			
drying conditions	sample	degree of crystallinity (±3%)	lateral crystal size[a] (nm)	T_g from the first scan (T_{g_1}) (°C)	T_g from the second scan (T_{g_2}) (°C)	melting temp T_m (°C)	melting enthalpy ΔH_m (J/g)
25 °C, 40% RH	A160			79	77		
	A60	6	5.0	77	73	114	0.5
	A30	12	6.1	79	70	112	1.0
	A15	16	8.0	75	65	109	1.8
	A5	24	9.0	75	62	107	2.5

[a] From the Sherrer equation.

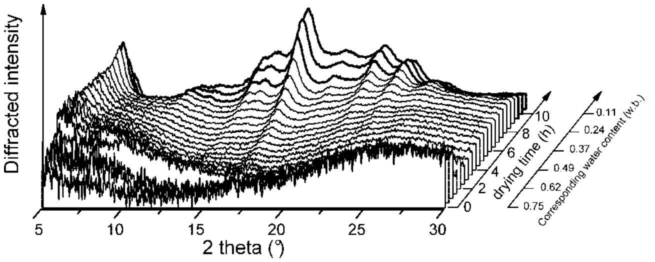

Figure 2. Crystallization kinetics of A15 films during film formation at 25 °C and 40% RH.

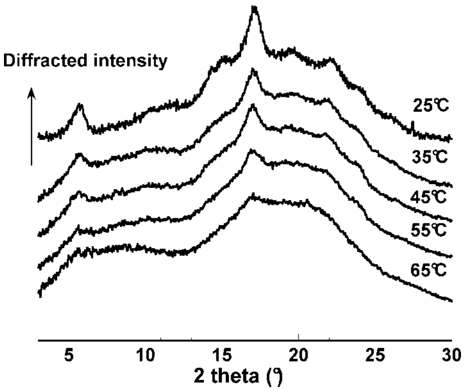

Figure 3. X-ray diffraction diagrams of A15 films obtained by casting at 25, 35, 45, 55, and 65 °C.

XRD diagrams of A15 cast-films obtained at different drying temperatures from 25 to 65 °C are shown in Figure 3. The average water content of the films varied from 13.7% for films formed at 25 °C to 11.5% for films formed at 65 °C (Table 4). Except the films formed at 65 °C, which are almost amorphous, all A15 films exhibit a B-type crystalline structure (Figure 3). The relative crystallinity varies from 16% for films formed at 25 °C to 4% for those obtained at 55 °C, as shown in Table 3. Lateral crystal size is equal to 8 nm for all films whatever the relative crystallinity.

Calorimetric Properties. The DSC thermograms recorded for A5, A15, A30, A60, and A160 films prepared in standard conditions (25 °C, 40% HR) are shown in Figure 4. An endotherm around 50 °C is observed for all samples during the first heating scan (dotted lines). This endotherm is usually attributed to the enthalpy relaxation resulting from the physical aging of the polymer during storage.[42] This has been previously observed in the case of starch.[43,44] Another endotherm appears at around 110 °C except for A160. In agreement with diffraction measurements, it was attributed to the melting of the crystalline phase. The important width of this endotherm could indicate a wide distribution of crystal sizes. The absence of melting endotherm for A160 is related to the amorphous state observed by XRD. The melting temperature decreases from 114 °C for A60 (6% of crystallinity) to 107 °C for A5 (24% of crystallinity; Table 2). The related melting enthalpy, ΔH_m, also reported in Table 2, increases from 0.5 J/g for A60 to 2.5 J/g for A5. T_g determined on the second scan increases from 62.0 °C for A5 to 76.7 °C for A160 as reported in Table 3. These T_g values are noticeably lower than in the first scan, the difference increases with increasing film crystallinity.

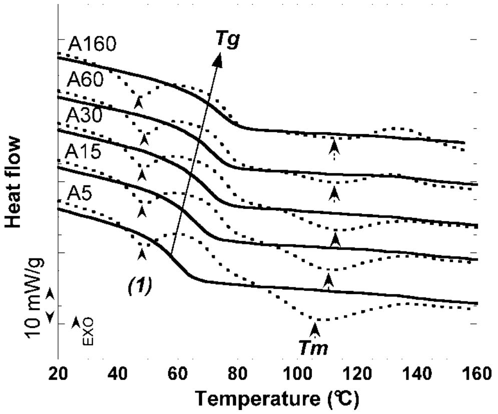

Figure 4. DSC thermograms from cast-films stored at 25 °C and 58% of RH. First scan: dotted lines. (1) Indicated by arrows: enthalpy relaxation. Melting temperature T_m is indicated by dotted arrows. Second scan: continuous lines; shift of glass transition temperature T_g indicated by arrow. Water content in films: 13.5 ± 0.2%.

T_g of the A15 films prepared in standard conditions (25 °C, 40% RH) was also determined at different moisture contents (MC) from 8% to 23% after conditioning the films at RH varying from 7% to 90% (Figure 5). T_g decreases with increasing water content from 132 °C for 8% to 13 °C for 23% MC. The T_g variations as a function of moisture content was fitted using the Gordon–Taylor equation[45] on the basis of the equation usually employed to predict water plasticization of carbohydrate materials

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \qquad (5)$$

where w_1 and w_2 are the respective weight fractions of the A15 sample and water, T_{g_1} is the T_g of the anhydrous polymer, T_{g_2} represents the T_g of amorphous water taken as −139 °C,[46] and k is a constant related to the strength of polymer-diluent interactions. T_g of A15 sample in the dry state was calculated at 271 °C and k was 6.1. Comparable T_g values have been reported on amylopectin films[3,10] and on hydroxypropylated films from pea starch.[33]

DSC data of the A15 films cast at different drying temperatures (from 25 to 65 °C) are reported in Table 3. The melting temperature increases from 109 °C for films prepared at 25 °C (16% crystallinity) to 125 °C for those prepared at 55 °C (4% of crystallinity). The melting enthalpy associated to this endotherm decreases from 1.8 J/g for films prepared at 25 °C to 0.16 J/g for those prepared at 55 °C (Table 3) due to the low

Table 3. XRD and DSC Data of A15 Films Formed at Drying Temperature from 25 to 65 °C

		XRD data		DSC data			
drying conditions (°C)	sample	degree of crystallinity (±3%)	lateral crystal size (nm)	T_g from the first scan T_{g1}(°C)	T_g from the second scan T_{g2}(°C)	melting temp T_m (°C)	melting enthalpy ΔH_m (J/g)
25	A15 (25)	16	8	74	65	109	1.8
35	A15 (35)	10		80	71	117	1.6
45	A15 (45)	8		80	74	121	0.75
55	A15 (55)	4		86	81	125	0.16
65	A15 (65)			87	83		

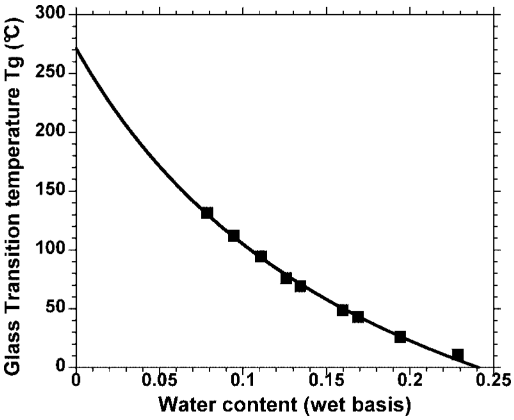

Figure 5. Glass transition temperature T_g as a function of water content for A15 films (■) fitted with the Gordon and Taylor model (continuous line; see text).

Table 4. Experimental Water Content and Calculated Water Content Form T_g Values During the First Scan[a]

casting temp	degree of crystallinity (%)	exptl water content (%)	calcd water content from T_{g1} (%)
35	10	13.6	12.4
45	8	12.7	12.4
55	4	12.3	11.7
65	0	11.5	11.7

[a] Using the Gordon and Taylor equation (see text) for A15 films as a function of the casting temperature.

crystallinity of films formed at higher temperature. T_g values from the second heating scan are also reported in this table. T_g increased from 65 to 83 °C for drying temperature from 25 to 65 °C (Table 3). Here again, significantly different T_g values were obtained between the first and the second scan, the deviation being larger for higher crystallinities.

It is, thus, clearly demonstrated from XRD and DSC analysis that structural and thermal properties of the cast films from HPPS vary to a relatively large extent depending on the molecular weight and the drying temperature.

Mechanical Behavior. Typical stress–strain curves of the A160, A60, A30, and A15 cast-films obtained at 25 °C and 40% RH are shown in Figure 6. All films displayed a rather brittle mechanical behavior even if a slight plastic deformation is observed. Overall, the mechanical properties of the films reported in Table 5 are similar. The tensile properties of A15 films produced at different temperatures whose crystallinity varied from 16% to almost none are also reported in this table. The Young modulus is around 0.86 GPa, the tensile stress at break (σ_R) around 34 MPa, and the strain at break (ϵ_R) was 9%. This indicates that both crystallinity and M_w do not have a significant influence on the mechanical properties of the films in the glassy state.

The thermomechanical properties of the thermomolded films have been measured by DMTA from 25 to 125 °C. The overall results are reported in Figure 7 for A5, A15, and A160. The molding temperature (120 °C) was chosen to obtain amorphous starch films, which was further checked by X-ray diffraction (not shown). At 25 °C the flexural storage modulus E' was similar at about 1.7×10^9 Pa for all samples. A dramatic drop in E' simultaneously with a peak in tan δ were experienced at around 65−85 °C corresponding to the main mechanical relaxation associated to the glass transition. In agreement with calorimetric experiments, the temperature of the E' modulus drop and tan δ decreased with decreasing M_w. Although the mechanical behavior in the glassy state at 25 °C was similar for all samples, the rubbery modulus was lowered when decreasing the molecular weight.

Discussion

Crystallinity. The crystallization of hydroxypropylated starch during casting, as evidenced by XRD and DSC, was not expected owing to the presence of hydroxypropyl groups, susceptible to prevent the starch macromolecules from associating. However, calculated Huggins coefficient K_H from intrinsic viscosity in diluted solutions indicated the aggregation tendency of the low molecular weight samples. The B-type crystallinity, observed by XRD, is usually observed during film formation from nonmodified starch solutions at temperatures close to room temperature.[5,8]

Molecular weight has a strong impact on the crystalline structure of the films. Here, A160 film ($M_w$around 1.6×10^6g/mol) were amorphous, while the A5 films (M_w around 4.7 g/mol) had a crystallinity of 24%. Such a behavior is well-known for synthetic polymers[47,48] and has also been evidenced for enzymatically synthesized amylose.[49] It requires more time to the longer chains to reorganize, due to entanglement and viscosity effects, whereas the shorter chains are very prone to reorganize. This result clearly demonstrates how important the role of the molecular weight is on the structure of hydroxypropylated starch films formed by casting.

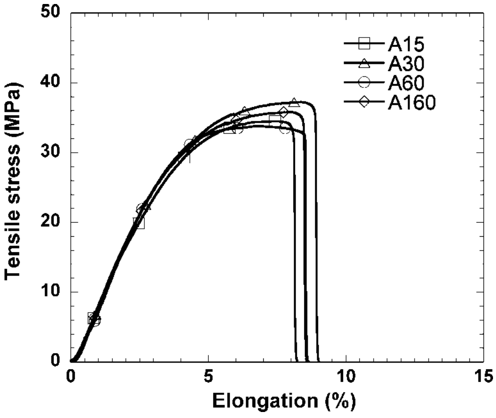

Figure 6. Tensile-strain curves of the films cast at 25 °C and 40% of relative humidity. Water content: 13.5% ± 0.2%.

Table 5. Young Modulus (E), Tensile Strength at Break (σ_R), and Percentage of Elongation at Break (ϵ_R) of A5, A15, A30, A60, and A160[a]

sample	degree of crystallinity (±3%)	Young modulus (E) (GPa)	tensile strength at break (σ_R) (MPa)	elongation at break (ϵ_R) (%)
A160		0.83 ± 0.08	35.4 ± 3.2	8.5 ± 1.9
A60	6	0.86 ± 0.07	30.4 ± 2.2	8.22 ± 1.8
A30	12	0.90 ± 0.05	38.4 ± 4.5	8.62 ± 1.8
A15	16	0.88 ± 0.02	34.9 ± 2.1	8.3 ± 1.2
A15 (35)	10	0.90 ± 0.10	35.3 ± 5.4	6.1 ± 2.0
A15 (45)	8	0.86 ± 0.12	30.6 ± 5.6	7.0 ± 1.6
A15 (55)	4	0.92 ± 0.09	33.0 ± 4.7	6.5 ± 1.7
A15 (65)		0.95 ± 0.11	35.4 ± 4.9	7.1 ± 2.1

[a] Calculated from tensile measurements at 25 °C. Water content in samples 13.5% ± 0.2. Average molecular weight M_w and crystallinity of films are reminded.

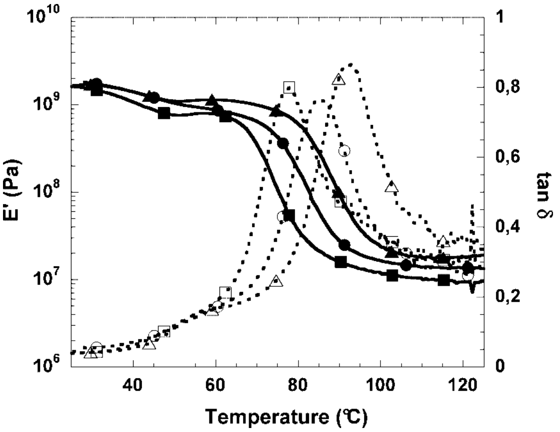

Figure 7. Dynamic thermomechanical properties of thermomolded films: A160 (triangles), A15 (circles), A5 (squares); storage flexural modulus (E', continuous line); and tan δ (dotted line) as a function of temperature at 1 Hz, 0.1% strain.

Casting temperature also plays a significant role on the crystalline structure of the film. Crystallinity of A15 films varied from 16% to almost none when the drying temperature ranged from 25 to 65 °C. Rindlav et al. (1997, 2002) investigated the role of the film formation conditions (temperature, RH, and time) on the crystallinity and morphology of potato starch films. They demonstrated that films formed at 50 °C were almost amorphous. This was explained by the crystallization phenomenon located between the glass transition temperature, T_g, and the melting temperature, T_m, at a rate depending on $T-T_g$.[10,20,50] They also highlighted that films formed at high RH had a higher B-type crystallinity than the films formed at low RH. Increasing RH slows down the drying; therefore, starch molecules have more time to reorganize. We assume that the crystalline structure of hydroxypropylated films is the result of the competition between the rate of dehydration and the rate of crystallization, itself depending on the molecular weight. At high temperature, drying rate is too important and starch chains have no water and no time enough to reorganize, resulting in amorphous films.

Thermal Transitions: T_g and T_m. Calorimetric analysis of hydroxypropylated films evidenced the role of molecular weight M_w and crystallinity on the T_g.

A decrease of M_w resulted in a T_g decrease. This is consistent with the T_g−molecular weight relationship, well-described for synthetic polymers[26] and also reported for starchy materials.[25] In the present work, the highest molecular weight sample (A160: 1.6 × 10^6 g/mol) had a T_g of 77 °C. Our data are slightly higher than those reported by Bizot et al. (1997)[3] on amylopectin films of comparable molecular weight. These values are also in agreement with other studies on starch and amylopectin films.[10,22] As expected, the melting enthalpy ΔH_m, associated to the crystalline phase, increased with the crystallinity. The melting temperature T_m decreased with decreasing molecular weight, whereas the crystallinity increased. This is attributed to a smaller chain length involved in the crystalline domains. Because no diffraction peak characteristic of the (00*l*) planes is present in the B-type diagram, it is not possible to have information on the crystal size along the chain axis from XRD, contrary to the lateral crystal size, which can be drawn from the (100) peak at $2\theta = 5.6°$. Nevertheless, such crystallization behavior is general for all polymers with a slower rate of entanglement in a longer crystal for high molecular weights and higher ability of short chains to reorganize quicker into larger crystals. The increase in melting temperature observed for A15 films with increasing drying temperature could correspond to nucleation/propagation effects, propagation being favored at higher temperature.

The influence of crystallinity on T_g was observed for all films on the first DSC scan. We observed for A15 films conditioned at 58% that T_g decreased with increasing the crystallinity. These results are not consistent with previous studies on potato[51] and amylopectin,[23] suggesting an increase in T_g with increasing the crystallinity. This effect has been usually explained by the restriction of the mobility in the amorphous phase in contact with crystals. However, for the A15 films dried at different temperatures, we observed that both hydration and crystallinity of the film varied. We found that water content increased with increasing the crystallinity of the films (Table 4). Moreover, the difference between T_g from the first scan T_{g1} and from the second scan T_{g2} (after melting of the crystalline structure and subsequent quenching) increased with increasing crystallinity.

A similar behavior has been reported for unmodified starch films. Bizot et al. (1997)[3] suggested a potential plasticization by compounds resulting from some degradation. Rindlav et al. (1997)[5] explained the T_g differences by a release of water from the crystalline domains or by a difference in the local order in the amorphous phase.

It has been demonstrated that water molecules are present inside the B-type crystalline structure.[52,53] In that case, the differences between T_{g1} and T_{g2} could be linked to a different water content, water being a good plasticizer of starch. To explain the differences between T_g data from the first scan and the second scan, the water content present in the B-type crystalline domains of hydroxypropylated samples of the present study was calculated for A15 films. This was performed owing to calculated T_g data from the Gordon and Taylor model between 0% and 30% of water content and using the following equation

$$W_T = \chi_c \times W_c + (1 - \chi_c) \times W_A \tag{6}$$

where W_T is the total water content; χ_c is the crystallinity; W_c is the water content in the crystalline phase; and W_A is the water content in the amorphous phase.

The calculated water contents corresponding to T_{g1} are reported in Table 4. As expected, the water content associated to T_{g1} was lower than from T_{g2}. From the difference between calculated water contents from T_{g1} and T_{g2}, we can estimate the amount of water present in the crystalline domains using eq 6. The W_c value was close to 20%. Imberty and Perez[50] demonstrated that the channel between six double helices in the B-type crystalline structure contains about 36 water molecules per three−glucose unit corresponding to a water content of 27%. This would mean that in our case the crystalline domains are not totally filled by water molecules.

These results show that both crystallinity and distribution of water between the amorphous and the crystalline phases play an important role in the physical properties of hydroxypropylated starch films. Differences between T_g from the first and the second scan can be explained by the presence of water inside the crystalline domains. During the second scan, water was released from the crystalline phase during the melting is available to plasticize the amorphous phase and thus to decrease T_g. This explains the increase in the differences between T_{g1} and T_{g2} with increasing crystallinity. The role of water on crystalline and amorphous regions depending on its localization seems to be the more important parameter. Even if water is present in the crystals, the increase in crystallinity leads to a decrease of T_g on the first scan. This might indicate that a part of water present in crystals also participates to the plasticization of the amorphous phase during the heating scan.

Structural Reorganization During Casting: Origin of the Crystallinity. Crystallization of hydroxypropylated starch during casting experiments has never been described before. It was expected that the grafted propylene oxide groups would make the association of chains in double helices and their packing into a regular array more difficult. It has been demonstrated that hydroxypropyl groups are primarily introduced into the starch chains in the amorphous regions composed mainly of amylose.[54] It was confirmed that amylose is modified to a greater extent than amylopectin in hydroxypropylated maize and potato starches and that the modification of amylopectin occurs close to the branch points.[55,56] Consequently, due to the heterogeneity of grafting, crystallization could involve amylopectin chains or nonsubstituted amylose chains. This might confirm the possibility of amylopectin chains to crystallize. Another explanation is that hydroxypropyl groups could also be present in the crystalline domains but lay within the central cavity present between the double helices in the B-type hexagonal packing. Apparently, this cavity was not completely filled up by water in our conditions. It has been demonstrated for potato starch that some phosphate groups might be present close to this central cavity.[57] Molecular modeling calculations have allowed the approach to organization of some covalently bound phosphate groups into amylopectin crystalline domains.[58] The resulting model describes that, when phosphate groups were present on C-6 hydroxyl groups of the glucose unit, they are aligned in the double helical grooves. In this position they do not affect the double helix formation nor the interhelical packing. In the present study, it is difficult to support this hypothesis for hydroxypropyl groups, owing to the lack of information about the potential presence of propylene oxide groups in the crystalline domains.

The melting temperatures are similar to the values reported in crystalline amylopectin of waxy maize starch[23] and potato starch films[8] at similar water content. Because the melting temperature for recrystallized amylose films at the same water content was higher,[43] we could argue that the melting endotherm corresponded to the melting of recrystallized amylopectin. However, it is usually observed that amylopectin films do not crystallize by casting, whereas amylose films are very prone to reorganize into a crystalline structure.[8] The occurrence of cocrystallization between amylose and amylopectin has been postulated in starch systems.[59] Some authors demonstrated the role of amylose on the formation of B-type crystallinity in thermomolded potato starch.[17] They mentioned that amylose could induce crystallization of amylopectin. The cocrystallization phenomenon could result from the entanglement in double helices of amylose chains, amylopectin chains, or amylose and amylopectin chains. The broadness of melting endotherms might be ascribed to this heterogeneity inside cocrystals.

Impact of Molecular Weight and Crystallinity on the Mechanical Properties of Films. From DSC and XRD analysis, it was shown that the films produced by casting were semicrystalline and that the crystallinity level was controlled by the molecular weight and the film formation temperature. Tensile tests performed on cast-films in the glassy state at room temperature evidenced a similar behavior for all films, whatever the crystallinity and molecular weight. An increase in the relative crystallinity of the polymer usually results in increased Young modulus and tensile stress and a decrease of elongation. However, previous studies dealing with plasticized and thermomolded starch films have shown a strong influence of molecular weight and crystallinity on the mechanical properties of starch materials in the rubbery state, but no significant influence was evidenced in the glassy state.[16,27] DMTA analysis performed on hydroxypropylated thermomolded films permitted to confirm the influence of the molecular weight on the glassy and rubbery state. In the glassy state, the mechanical properties of all samples were similar. At this temperature, the molecular mobility is reduced and the molecular weight does not influence the mechanical properties. However, the temperature of the drop of E' and the peak of tan δ, corresponding to the mechanical relaxation associated to the glass transition, was lowered while decreasing the molecular weight. In the rubbery zone, the E' modulus decreased with decreasing the molecular weight. These results are consistent with previous studies of van Soest et al. (1996), confirming that the molecular weight of starch only affect the mechanical properties in the rubbery state. Chains of high molecular weight are entangled and can be stretched more than shorter chains. However, the explanation of this phenomenon was difficult to interpret in the present case due to the presence of both amylose and amylopectin. Although the linear amylose molecules can easily be entangled, the branched structure of amylopectin suggests that only the out chains can participate in the formation of entangled structure with amylose and also with the other amylopectin chains. Acid hydrolysis is known to affect mainly the linear amylose chains, but the chain length and the degree of branching of amylopectin may be changed. The reduction of the chain length leads to entanglements in smaller domains, explaining the decrease of rubbery plateau with the molecular weight.

Conclusions

This study demonstrated that hydroxypropylated and hydrolyzed samples of pea starch crystallized in B-type crystal structure during the casting process. In similar casting conditions, 25 °C and 40% of RH, the degree of crystallinity increased from almost none to 24% with decreasing molecular weight, the shorter chains being more prone to reorganize compared to the higher chains. The casting temperature also enabled the control of the crystallinity of films from 16% at 25 °C to almost none at 65 °C. DSC analysis showed that water redistribution between amorphous and crystalline phase enable to explain quite accurately the variation of T_g of starch films between first and second scans. However, such modification of the structure did not influence the mechanical properties of the films in the glassy state, but affected the rubbery behavior. This study contributes to further information about starch film formation process. The control of the molecular weight and of the casting temperature on modified samples enable to obtain desired structural properties. Theses results may be useful to control the physical properties of modified starch for applications in the pharmaceutical industry as aqueous coating. Even if mechanical properties of the films were comparable in this conditions of storage (RH, 58%), we can argue that mechanical behavior could be noticeably modify at different temperatures and relative humidity.

Acknowledgment. Roquette is gratefully acknowledged for supplying the samples and determining their molecular weight by HPSEC-MALLS. The authors thank Joelle Davy and Colin Roberts for their skilled assistance on calorimetric measurements and mechanical measurements, respectively. This work was financially supported by the National Network on Materials and Processes (RNMP) from the French Ministry for research and the Department of Trade and Industry.

References and Notes

(1) Shogren, R. L.; Fanta, G. F.; Doane, W. M. Development of starch-based plastics—A re-examination of selected polymer systems in historical perspective. *Starch/Staerke* **1993**, *45* (8), 276–280.

(2) Wolff, I. A.; Davis, H. A.; Cluskey, J. E.; Gundrum, L. J.; Rist, C. E. Preparation of films from Amylose. *Ind. Eng. Chem.* **1951**, *43* (4), 915–919.

(3) Bizot, H.; Le Bail, P.; Leroux, B.; Davy, J.; Roger, P.; Buléon, A. Calorimetric evaluation of the glass transition in hydrated, linear, and branched polyanhydroglucose compounds. *Carbohydr. Polym.* **1997**, *32* (1), 33–50.

(4) Lourdin, D.; Della Valle, G.; Colonna, P. Influence of amylose content on starch films and foams. *Carbohydr. Polym.* **1995**, *27* (4), 261–270.

(5) Rindlav, A.; Hulleman, S. H. D.; Gatenholma, P. Formation of starch films with varying crystallinity. *Carbohydr. Polym.* **1997**, *34* (1–2), 25–30.

(6) Gaudin, S.; Lourdin, D.; Le Botlan, D.; Ilari, J. L.; Colonna, P. Plasticisation and mobility in starch-sorbitol films. *J. Cereal Sci.* **1999**, *29* (3), 273–284.

(7) Myllarinen, P.; Partanen, R.; Seppala, J.; Forssell, P. Effect of glycerol on behaviour of amylose and amylopectin films. *Carbohydr. Polym.* **2002**, *50* (4), 355–361.

(8) Rindlav-Westling, A.; Stading, M.; Gatenholm, P. Crystallinity and morphology in films of starch, amylose, and amylopectin blends. *Biomacromolecules* **2002**, *3*, 84–91.

(9) Rindlav-Westling, A.; Stading, M.; Hermansson, A.-M.; Gatenholm, P. Structure, mechanical, and barrier properties of amylose and amylopectin films. *Carbohydr. Polym.* **1998**, *36* (2–3), 217–224.

(10) Jouppila, K.; Roos, Y. H. The physical state of amorphous corn starch and its impact on crystallization. *Carbohydr. Polym.* **1997**, *32* (2), 95–104.

(11) Bader, H. G.; Goritz, D. Investigations on high amylose corn starch films 3. Stress−strain behavior. *Starch/Staerke* **1994**, *46*, 435–439.

(12) Katz, J. Abhandlungen zur physikalischen Chemie der Starke und der Brotbereitung. *Z. Physik. Chem.* **1930**, *150*, 37–59.

(13) Unbehend, J. E.; Sarko, A. Light scattering and X-ray characterization of amylose films. *J. Polym. Sci.* **1974**, *12*, 545–554.

(14) Myllarinen, P.; Buleon, A.; Lahtinen, R.; Forssell, P. The crystallinity of amylose and amylopectin films. *Carbohydr. Polym.* **2002**, *48* (1), 41–48.

(15) van Soest, J. J. G.; Vliegenthart, J. F. G. Crystallinity in starch plastics: Consequences for material properties. *Trends Biotechnol.* **1997**, *15* (6), 208–213.

(16) van Soest, J. J. G.; Hulleman, S. H. D.; de Wit, D.; Vliegenthart, J. F. G. Changes in the mechanical properties of thermoplastic potato starch in relation with changes in B-type crystallinity. *Carbohydr. Polym.* **1996**, *29* (3), 225–232.

(17) Hulleman, S. H. D.; Kalisvaart, M. G.; Janssen, F. H. P.; Feil, H.; Vliegenthart, J. F. G. Origins of B-type crystallinity in glycerol-plasticised, compression-moulded potato starches. *Carbohydr. Polym.* **1999**, *39* (4), 351–360.

(18) Jin, X.; Ellis, T. S.; Karasz, F. E. The effect of cristallinity and crosslinking on the depression of the glass transition temperature in nylon 6 by water. *J. Polym. Sci., Part B: Polym. Phys.* **1984**, *22* (10), 1701–1717.

(19) Slade, L.; Levine, H. In *A Food Polymer Science Approach to Selected Aspects of Starch Gelatinization and Retrogradation. In Frontiers in Carbohydrate Research-1: Food Applications*, Millane, R. P., BeMiller, J. N., Chandrasekaran, R., Eds.; Elsevier Applied Science: London, 1989; pp 215–270.

(20) Roos, Y. H., *Phase Transitions in Foods*. Academic Press: San Diego, New York, Boston, London, Sydney, Tokyo, Toronto, 1995.

(21) Slade, L.; Levine, H. Water and the glass transition—Dependence of the glass transition on composition and chemical structure: Special implications for flour functionality in cookie baking. *J. Food Eng.* **1995**, *24* (4), 431–509.

(22) Zeleznak, K. J.; Hoseney, R. C. The glass transition in starch. *Cereal Chem.* **1987**, *64* (2), 1211–124.

(23) Kalichevsky, M. T.; Jaroszkiewicz, E. M.; Ablett, S.; Blanshard, J. M. V.; Lillford, P. J. The glass transition of amylopectin measured by DSC, DMTA, and NMR. *Carbohydr. Polym.* **1992**, *18*, 77–88.

(24) Lourdin, D.; Coignard, L.; Bizot, H.; Colonna, P. Influence of equilibrium relative humidity and plasticizer concentration on the water content and glass transition of starch materials. *Polymer* **1997**, *38* (21), 5401–5406.

(25) Orford, P. D.; Parker, R.; G., R. S.; Smith, A. C. Effect of water as a diluent on the glass transition behaviour of malto-oligosaccharides, amylose and amylopectin. *Int. J. Biol. Macromol.* **1989**, *11*, 91–96.

(26) Fox, T. G.; Flory, P. Second-order transition temperatures and related properties of polystyrene. 1. Influence of molecular weight. *J. Appl. Phys.* **1950**, *21*, 581–591.

(27) van Soest, J. J. G.; Benes, K.; de Wit, D.; Vliegenthart, J. F. G. The influence of starch molecular mass on the properties of extruded thermoplastic starch. *Polymer* **1996**, *37* (16), 3543–3552.

(28) Wurzburg, O. B. Modified Starches. In *Food polysaccharides and their applications*, Stephen, A. M., Ed.; Marcel Dekker, Inc.: New York, 1995; pp 67–97.

(29) Lefevre, P.; Francois, A.; Facon, P.; Quettier, C.; Parissaux, X. Film-forming starchy composition. WO2005/060944 A1, 2005.

(30) Singh, J.; Kaur, L.; McCarthy, O. J. Factors influencing the physico-chemical, morphological, thermal, and rheological properties of some chemically modified starches for food applications—A review. *Food Hydrocolloids* **2007**, *21* (1), 1–22.

(31) Roth, W. B.; Mehltretter, C. L. Some properties of hydroxypropylated amylomaize starch films. *Food Technology* **1967**, *21*, 72–74.

(32) Vorwerg, W.; Dijksterhuis, J.; Borghuis, J.; Radosta, S.; Kroger, A. Film properties of hydroxypropyl starch. *Starch/Staerke* **2004**, *56* (7), 297–306.

(33) Lafargue, D.; Lourdin, D.; Doublier, J.-L. Film-forming properties of a modified starch/κ-carrageenan mixture in relation to its rheological behaviour. *Carbohydr. Polym.* **2007**, *70* (1), 101–111.

(34) Wakelin, J. H.; Virgin, H. S.; Crystal, E. Development and comparison of two X-ray methods for determining the crystallinity of cotton cellulose. *J. Appl. Phys.* **1959**, *30*, 1654–1662.

(35) Klug, H.; Alexander, L. *X-ray procedures*; Wiley-Interscience.: New York, 1954; pp 491–538.

(36) Burchard, W. Das viskositatsverhalten von amylose in verschiedenen losungsmitteln. *Makromol. Chem.* **1963**, *64*, 110–125.

(37) Bello-Perez, L. A.; Paredes-Lopez, O.; Roger, P.; Colonna, P. Amylopectin—Properties and fine structure. *Food Chem.* **1996**, *56* (2), 171–176.

(38) Wang, L. Z.; White, P. J. Structure and properties of amylose, amylopectin, and intermediate materials of oat starches. *Cereal Chem.* **1994**, *71*, 263–8.

(39) Hizukuri, S.; Takeda, Y.; Maruta, N.; Juliano, B. Molecular structures of rice starch. *Carbohydr. Res.* **1989**, *189*, 227–35.

(40) Granath, K. A.; Stromberg, R.; De Belder, A. N. Studies on hydroxyethyl starch. *Starch/Staerke* **1969**, *10*, 251–256.

(41) Buléon, A.; Bizot, H.; Delage, M.; Multon, J. Evolution of crystallinity and specificity gravity of potato starch versus water ad- and desorption. *Starch/Staerke* **1982**, *32*, 361–366.

(42) Hutchinson, J. M. Physical aging of polymers. *Prog. Polym. Sci.* **1995**, *20* (4), 703–760.

(43) Shogren, R. L. Effect of moisture content on the melting and subsequent physical aging of cornstarch. *Carbohydr. Polym.* **1992**, *19* (2), 83–90.

(44) Borde, B.; Bizot, H.; Vigier, G.; Buleon, A. Calorimetric analysis of the structural relaxation in partially hydrated amorphous polysaccharides. II. Phenomenological study of physical ageing. *Carbohydr. Polym.* **2002**, *48* (2), 111–123.

(45) Gordon, M.; Taylor, J. S. Ideal copolymers and the second-order transitions of synthetic rubbers I. Noncrystalline copolymers. *J. Appl. Chem.* **1952**, *2*, 493–500.

(46) Sugisaki, M.; Suga, H.; Seki, S. Calorimetric study of the glassy state. IV—Heat capacity of glassy water and cubic ice. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2591–2599.

(47) Flory, P. *Principles of polymer chemistry*; Cornell University Press: Ithaca, New York, 1953.

(48) Ergoz, E.; Fatou, J. G.; Mandelkern, L. Molecular weight dependence of the crystallization kinetics of linear polyethylene. I. Experimental results. *Macromolecules* **1972**, *5* (2), 147–157.

(49) Potocki-Veronese, G.; Putaux, J.-L.; Dupeyre, D.; Albenne, C.; Remaud-Siméon, M.; Monsan, P.; Buléon, A. Amylose synthesized in vitro by amylosucrase: Morphology, structure, and properties. *Biomacromolecules* **2005**, *6*, 1000–1011.

(50) Slade, L.; Levine, H. Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, *30* (1), L-360.

(51) Mizuno, A.; Mitsuiki, M.; Motoki, M. Effect of crystallinity on the glass transition temperature of starch. *J. Agric. Food Chem.* **1998**, *46* (1), 98–103.

(52) Imberty, A.; Perez, S. A revisit to the three-dimensional structure of B-amylose. *Biopolymers* **1988**, *27*, 1205–1221.

(53) Buleon, A.; Colonna, P.; Planchot, V.; Ball, S. Starch granules: Structure and biosynthesis. *Int. J. Biol. Macromol.* **1998**, *23* (2), 85–112.

(54) Blanshard, J. M. V. *Starch granule structure and function: a physicochemical approach. In Starch: Properties and Potential*; Galliard, T., Ed.; Wiley: Chichester, 1987; pp. 16–54.

(55) Kavitha, R.; BeMiller, J. N. Characterization of hydroxypropylated potato starch. *Carbohydr. Polym.* **1998**, *37* (2), 115–121.

(56) Shi, X.; BeMiller, J. N. Effect of sulfate and citrate salts on derivatization of amylose and amylopectin during hydroxypropylation of corn starch. *Carbohydr. Polym.* **2000**, *43* (4), 333–336.

(57) Blennow, A.; Nielsen, T. H.; Baunsgaard, L.; Mikkelsen, R.; Engelsen, S. B. Starch phosphorylation: A new front line in starch research. *Trends Plant Sci.* **2002**, *7* (10), 445–450.

(58) O'Sullivan, A. C.; Perez, S. The relationship between internal chain length of amylopectin and crystallinity in starch. *Biopolymers* **1999**, *50* (4), 381–390.

(59) Mestres, C.; Colonna, P.; Buleon, A. Gelation and crystallisation of maize starch after pasting, drum drying, or extrusion cooking. *J. Cereal Sci.* **1988**, *7*, 123–134.

BM7009637