

Structural Changes of Injection Molded Starch During Heat Treatment in Water Atmosphere: Simultaneous Wide and Small-Angle X-ray Scattering Study

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ABSTRACT: Native starches with wide varying amylose content were processed by injection molding. The injection-molded materials were conditioned in water for 20 days and sealed in glass capillaries. Simultaneous wide- and small-angle X-ray scattering (WAXS and SAXS, respectively) were recorded during thermal heating using a synchrotron source. Crystallinity, SAXS invariant, Q , and long period, L , were measured as a function of heating temperature. The injection-molding process provokes a destruction of the crystal forms A (cereal starch) and B (tubercle starch) but favors a development of the crystal form V_h . After wet conditioning, WAXS of the injection-molded samples shows again the appearance of the crystal forms A or B, and crystallinity reaches values similar or larger than those of native starch. A constant heating rate (5°C/min) was particularly used for a comparison of potato and corn starch with a similar amylose content. While the crystallinity associated to forms A and B slowly decreases below 55°C and then rapidly decreases until its disappearance at 85–90°C, the invariant shows a maximum around 40°C and rapidly decreases

thereafter. The total nanostructure disappearance occurs at temperatures about 10°C higher for the case of potato starch. In addition, a recovery of the WAXS and SAXS maxima during the subsequent cooling process before reaching room temperature was observed only for potato starch. Analysis of WAXS and SAXS for the rest of the starch materials reveals clear differences in the structural parameters of the samples that cannot be easily explained solely on the basis of the amylose content. Thus, for Cerestar and Roquette, it is noteworthy that there was a continuous decrease of L until its total disappearance as well as the persistence of crystallinity (form B), presumably stabilized by the presence of the V_h structure (12–15%). Real-time crystallization experiments on two *amorphous* injection molded samples, waxy maize (free amylose starch) and potato starch, are also discussed. It is shown that the absence of amylose delays the recrystallization of amylopectine during the experiment. © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 93: 301–309, 2004

Key words: starch; injection molding; WAXS; SAXS

INTRODUCTION

The growing interest in the use of biodegradable polymers is justified because these materials are friendly to the environment. Among the biopolymers, starch is one of the most widely used, not only in the food industry, but also as a material able to substitute the synthetic polymers in some applications. Starch is present in many of the common plants, is cheap, and is easy to obtain and process by conventional methods.^{1,2}

Starch is quite a complex material from a structural point of view, showing several organizational levels. It contains two components: amylose and amylopectin. Amylose, an essentially linear polymer of molecular weight 5×10^5 – 10^6 , is formed by glucose units linked $\alpha(1,4)$. Amylopectin is a branched polymer formed by glucose units linked $\alpha(1,4)$ with 2–4% of branches

linked $\alpha(1,6)$ and has a molecular weight of several millions.^{3,4} The crystalline structure of starch depends on its origin. The so-called A-structure⁵ is typical for starch extracted from cereal plants (wheat, corn, rice, etc.), whereas the B-structure⁶ appears mainly in starch obtained from tubercles (potato, etc.) and also in commercial, rich amylose starches. Some other crystalline structures, i.e., V_h , V_a , or E_h ^{7–9} are present in cereal starches processed under special conditions of temperature and humidity.¹⁰ These structures are typical of the complexes formed between the amylose and the lipids (i.e., fatty acids) present in cereal starch.¹¹

Starch has been widely studied by using different techniques. Wide-angle X-ray scattering (WAXS) has been used to determine the degree of crystallinity of starch obtained from different sources^{12,13} and to calculate the proportion of the A and B polymorphs on pea starch.¹⁴ Donald and coworkers, using a combination of techniques: small-angle X-ray scattering (SAXS), WAXS, small-angle neutron scattering (SANS), and differential scanning calorimetry (DSC), have followed the structural changes that occur during gelatinization for a range of native starches.^{15–18}

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DSC has been very useful to distinguish between the gelatinization and the melting process.¹⁹ Other authors have studied the structural and conformational properties of several starches by means of FTIR²⁰ and NMR.²¹ Recently, dynamic mechanical spectroscopy has been shown to be very fruitful in the elucidation of the gelatinization and gelation processes in corn starch.²² In addition, the interaction of water with starch films,²³ the influence of the processing methods on the structure and surface mechanical properties of potato starch,^{24,25} and its cement clay composites have been investigated.²⁶

In a preceding study, *"in situ"* X-ray scattering results using synchrotron radiation on injection molded starch samples, before and after conditioning in a wet atmosphere, were reported.²⁷ These samples were isothermally treated at selected temperatures under vacuum during the X-ray experiments. Therefore, the water present in the starch was progressively removed. The results obtained showed that both crystalline forms A and B, which were initially destroyed during the injection process, reappeared in the material after its conditioning in a wet atmosphere, probably due to a rearrangement of the chains by the sorption of water molecules.²⁷

Provided that the presence of water is fundamental for the development of crystallinity in starch, in the present work we have performed new experiments on injection-molded starch samples of different origin. These samples were *"wet conditioned"* during 20 days and then subjected to thermal treatments in presence of water atmosphere, by using sealed glass capillaries. The aim of the present study is twofold: 1) to follow the crystallization process of two kinds of starch, potato and waxy maize, in a wet atmosphere at different increasing temperatures and 2) to study the structural variations originated in injection-molded samples of different starches, after wet conditioning during 20 days, by heating them at constant heating rate or in a stepwise manner. The influence of the crystalline structure V_h on the destruction of the crystalline A and B forms upon heating is also discussed.

EXPERIMENTAL

Materials

The starch materials investigated are collected in Table I. Samples referred to as Cerestar and Roquette are two particular types of corn starch. The relative amylose content (linear polysaccharide) refers to the amylopectine (branched, high-molecular polysaccharide) as the main component of the starch.

Injection-molded starch samples with a 4×4 mm cross section were obtained by means of elongational flow injection molding. The samples were prepared from longitudinal 1-mm-thick cuts from the center of

TABLE I
Starch Materials Investigated and Amylose Content %

Starch type	Amylose content (%)
Waxy maize	0
Potato	26
Corn	29
Cerestar	68
Roquette	70
Pea	76

the bar. A set of these samples was previously wet-conditioned for 20 days. The cuts of the samples were introduced in glass capillaries (2 mm diameter) partially filled with water. A steel rod (1 cm length) allowed the sample to be kept above the water level during experimentation. Glass capillaries were easily sealed over a match flame before each experiment.

Techniques

Simultaneous WAXS and SAXS experiments were performed using the synchrotron radiation source at the A2 beam line of HASYLAB (Hamburg, Germany). A wavelength of 0.15 nm was used. The accumulation time per frame was 30 s for both WAXS and SAXS detectors. The latter was located, on the X-ray path, 180 cm away from the sample. Each glass capillary was mounted into the sample holder and maintained under rotary pump vacuum during the experiment. The samples were first held at room temperature for 2 min and then were heated according to two heating procedures: 1) subjected at constant heating rate (5°C/min) from room temperature up to a temperature of about 110°C followed by cooling down (10°C/min) to room temperature; and 2) heated up in a stepwise manner at increasing temperature in 5-min steps up to a nominal temperature of 150°C. The time required to change the temperature between two steps was about 1 min.

The sample temperature calibration was carried out by recording the WAXS patterns of standard organic compounds of known melting points (Azobenzol, m.p. 68°C, and Benzanilide, m.p. 163°C) in glass capillaries.

X-ray scattering curves were analyzed by means of a curve fit program. Particularly, WAXS traces were analyzed taking into account the polymorphism of starch. Thus, a partial crystallinity attributed to either A or B crystal forms was calculated for all consecutive frames, neglecting the contribution from the crystal form V_h .

Experimental SAXS patterns were corrected by the Lorentz factor. The area of the SAXS maximum and its angular position were measured to calculate the cor-

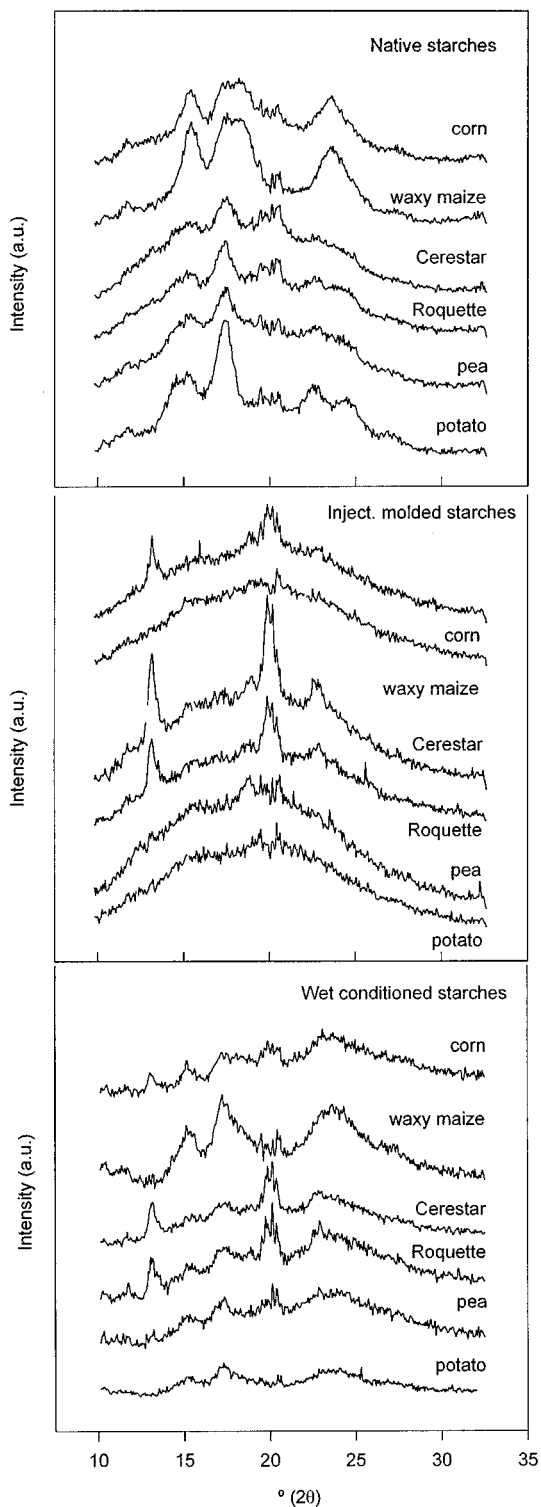


Figure 1 WAXS diffractograms, recorded at room temperature using wavelength $\lambda = 0.15$ nm for different starches in native powder form (top), after mold injection process (middle), and after wet conditioning for 20 days the mold injection samples (bottom).

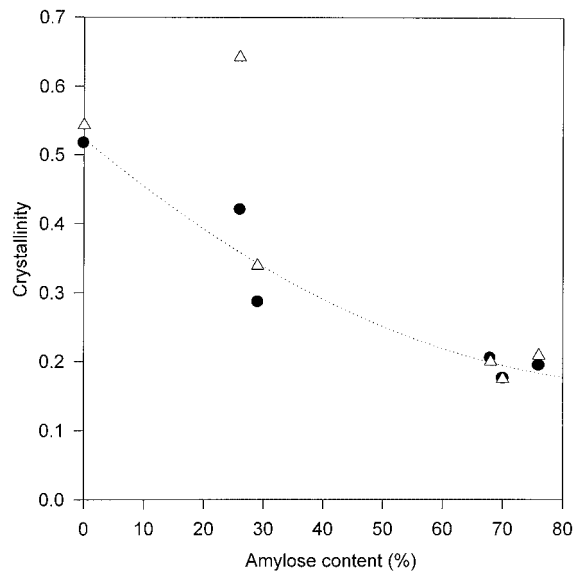


Figure 2 Variation of the crystallinity associated to A or B structures with the amylose content of different starches. Circles, native starch; triangles, mold injection samples after wet conditioned for 20 days.

responding values of the invariant, Q , and the long period, L , respectively.

RESULTS AND DISCUSSION

Structure of native starch before and after injection molding

Figure 1 illustrates the WAXS patterns, recorded at room temperature, for native starch samples, as supplied in powder form (top) and for the corresponding injection-molded samples (middle). Native starch exhibits two different types of diffraction patterns that are related to the crystal form A (two upper curves), predominant in cereal plants (corn), and the crystal form B (four lower curves), more abundant in tubercles (potato) and in high-amylose starches.^{28,29} The injection-molding process provokes important structural changes as revealed by the X-ray diffraction patterns (Fig. 1, middle). The reflections related to the crystal forms A and B disappear completely after processing. Nevertheless, due to the injection-molding process, new reflections appear for some of the samples investigated. These new reflections can be associated to the V_h crystal form. It is known that the latter crystal form develops in the case of starch containing lipid compounds that complex the amylose component.¹¹

Figure 2 shows the crystallinity measured for the native starch samples (Fig. 1, top) as a function of amylose content (circles). One sees that the crystallinity is higher for the starch sample free of amylose (waxy maize) and seems to decrease for starch with

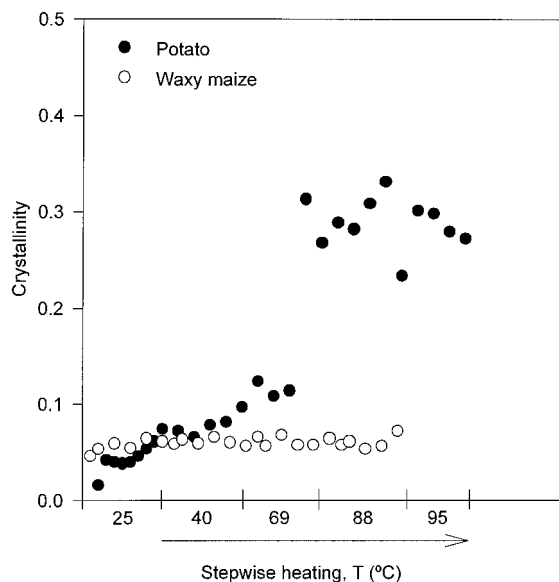


Figure 3 Development of crystallinity in injection-molded potato and waxy maize starch during heating in sealed glass capillaries containing some amount of water.

increasing content of amylose. Other researchers have found similar results in a series of corn starch samples with different amylose contents.^{28,29} As amylose is a linear polymer in contrast to the branched, high-molecular amylopectine, the results of Figure 2 are somewhat unexpected. Therefore, the role of amylopectine during the starch crystallization seems to be relevant.

Crystallization of amorphous injection-molded starch in water atmosphere

From Figure 1 (middle), one sees that the injection-molded samples of potato and waxy maize are *amorphous*. Both samples were selected to study the structure development induced by water vapor in real time at increasing temperatures. The samples were placed in sealed glass capillaries partially filled with water as described above. The time elapsed between the preparation of each sample in the capillary and the beginning of the X-ray experiment was less than 10 min. Figure 3 illustrates the variation of the crystallinity during the heating of the potato and waxy maize samples at 10-min steps. While for waxy maize (free of amylose content) no crystallinity variation is detected, in the case of potato starch some crystallinity changes are observed. In the latter case, a jump of crystallinity at 69°C from 0.12 up to 0.3 and, then, a leveling of crystallinity at higher temperatures was observed.

Concerning the SAXS results, waxy maize does not present any scattering maximum in contrast to potato starch, which exhibits a SAXS maximum even at room temperature. Figure 4 illustrates the structural changes occurring in potato starch expressed by Q and

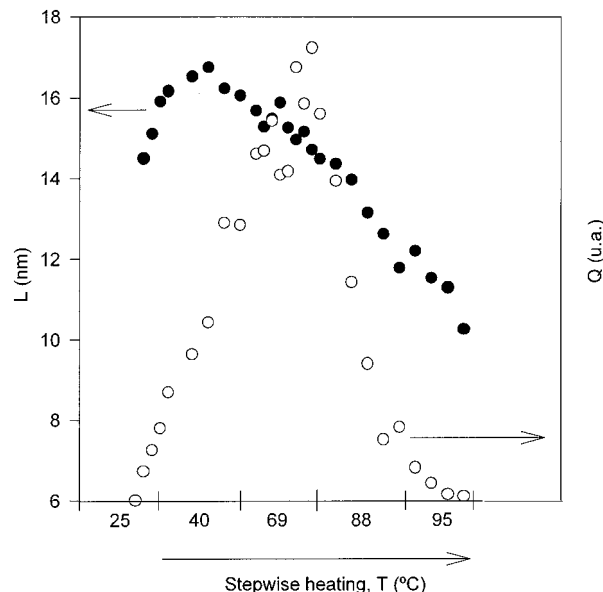


Figure 4 Variation of the long period, L , and SAXS invariant, Q , for potato starch during crystallization (as in Fig. 3).

L variations. The invariant Q continuously increases till 69°C, starts to decrease rapidly at 88°C, and finally vanishes at 95°C. On the other hand, L shows a maximum value (16.5 nm) at 40°C during the first heating and then it decreases until a value near 10 nm is reached.

In light of the above results, it is additionally convenient to describe the structure variation through analysis of the coherent crystal size. Figure 5 illus-

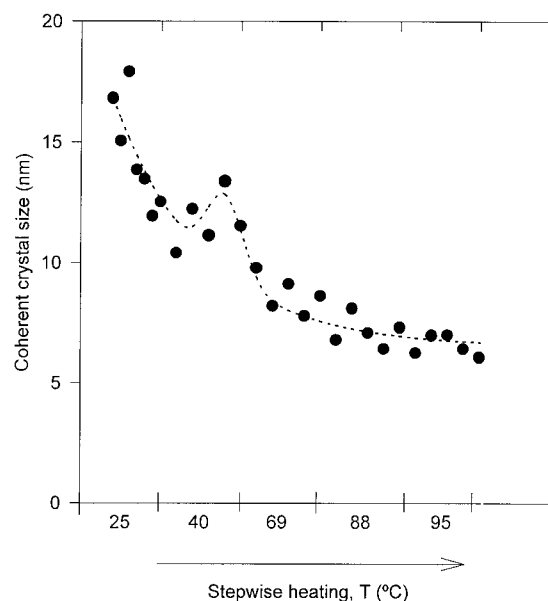


Figure 5 Variation of the crystal size of the reflection at 16.9° of the B structure observed for potato starch during crystallization (as in Fig. 3).

trates the crystal size derived from the integral width of the peak at 16.9° (2θ), corresponding to the crystallographic distance of 0.534 nm between the (300) planes,⁶ as a function of heating temperature. A dotted line is drawn to guide the view of scattered data. According to these results, the first crystals appearing at room temperature are the largest ones. With increasing annealing temperature the average crystal size decreases continuously until a value of about 6 nm is reached. At 40°C the crystal size seems to pass through a relative maximum value. This maximum in crystal size occurs when L reaches a maximum (Fig. 4) and the crystallinity is still low (Fig. 3).

Thermal stability of the structure developed in wet-conditioned injection-molded starch

Injection-molded samples of different starch (Table I) were wet conditioned for 20 days and then sealed in glass capillaries prior to their investigation by simultaneous WAXS and SAXS. Figure 1 (bottom) shows the WAXS diffractograms for the wet-conditioned starch samples recorded at room temperature. Besides the reflections from V_h , present in some injection-molded samples before wet conditioning (Fig. 1, middle), other reflections related to the crystal forms A (two upper curves) and B (four lower curves) reappear. By comparison of the three plots of Figure 1 it can be concluded that the typical crystalline nature of native starch (crystal forms A and B) is destroyed through the injection-molding process, though crystallinity is restored upon wet conditioning of the samples. The crystallinity levels reached after wet conditioning, without the contribution of V_h peaks, are shown in Figure 2 (triangles). It is shown that starch samples with higher amylose content recover almost exactly their original crystallinity level. Most remarkable is the higher crystallinity value restored in the case of potato starch.

Following our previous investigation on the thermal stability of the starch crystal forms,²⁷ we present here a more reliable study by using samples in sealed glass capillaries. In addition to potato and corn starch, this study is extended to other starch materials (Table I). In the case of potato and corn starch samples we have used a constant heating rate of $5^\circ\text{C}/\text{min}$ followed by a cooling rate of $10^\circ\text{C}/\text{min}$. A stepwise heating mode was used for the rest of the samples.

Figure 6 presents the crystallinity variation measured for the crystal forms A (corn) and B (potato) upon heating followed by a cooling process. During heating a similar behavior is observed for both starch types: first, a slow crystallinity decrease till 55°C , followed by a rapid decrease until the total disappearance of crystallinity at $\sim 85^\circ\text{C}$ is observed. The disappearance of crystallinity occurs at temperatures 5–10 degrees lower in the case of corn starch. However,

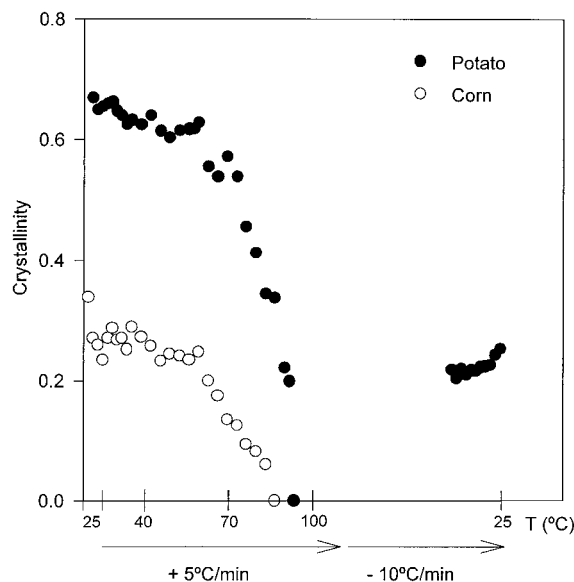


Figure 6 Variation of the crystallinity of wet-conditioned samples of corn and potato starch during heating at $5^\circ\text{C}/\text{min}$ and consecutive cooling at $10^\circ\text{C}/\text{min}$.

during cooling of the samples to room temperature, the behavior of both samples is different. Indeed, while potato starch recovers some crystallinity, corn starch does not.

As far as SAXS is concerned, Figures 7(a) and (b) show the variation of the invariant Q and the long period L during the heating and the cooling cycles, respectively. For a better comparison of the data we have presented the Q values normalized to unity. In both cases, Q first slightly increases and passes through a maximum during heating at about 40°C . At higher temperatures, Q decreases more rapidly for corn starch than for potato starch. For corn starch Q becomes zero at a temperature about 15°C lower than potato starch. As pointed out for the crystallinity derived from WAXS, during cooling (Fig. 6), the SAXS maxima only reappear in the case of potato starch. The corresponding L values derived from SAXS maxima are presented in Figure 7(b). The initial L value for corn starch is larger than for potato starch. For both samples, L increases first slowly and thereafter more rapidly until the final disappearance. For corn starch, L becomes much larger than for potato starch. After cooling the samples, potato starch shows larger L values as derived from the incipient SAXS maxima observed. In Table II, the structure recovered after X-ray experiments and 2 days storage, at room temperature, of glass capillary samples is compared with that for the wet conditioned samples for 20 days. The total crystallinity includes the contribution of the V_h structure as well. Thus, the difference between columns 2 and 3 offers information about the amount of V_h structure developed during the injection-molding process.

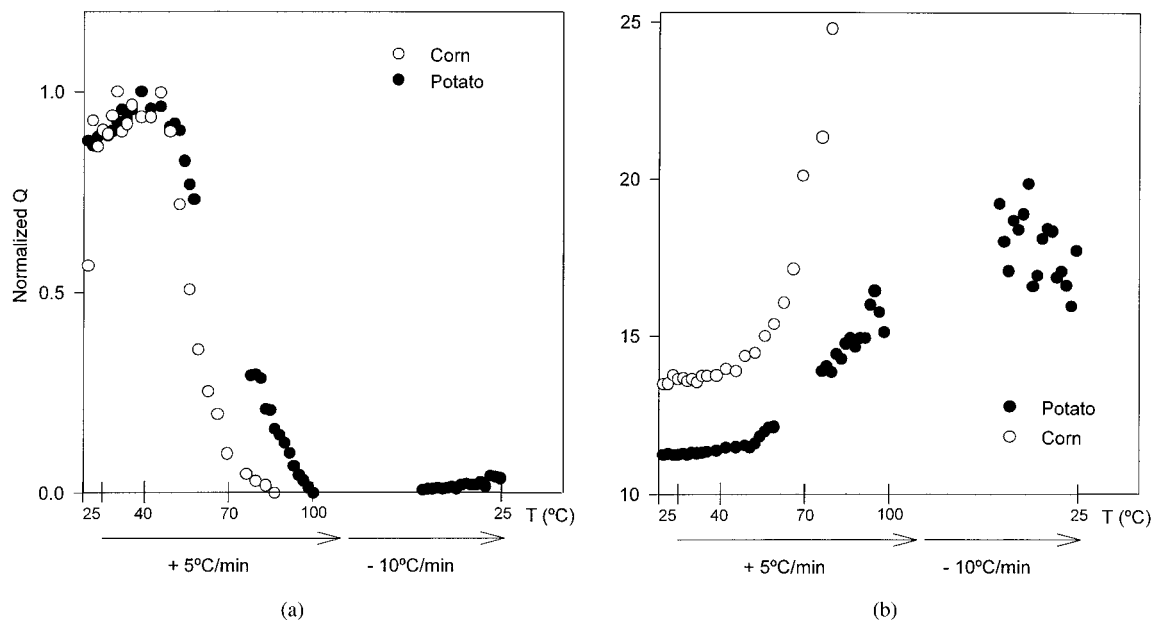


Figure 7 (a) Variation of the normalized Q invariant for potato and corn starch as described in Figure 6. (b) Variation of L for potato and corn starch as described in Figure 6.

Waxy maize and the three-amylose rich starches (Table I) were thermally treated using a similar stepwise heating method. Concerning the WAXS results, Figure 8 shows the variation of the recovered crystal form (A, B) with thermal treatment. Data for Roquette, which are similar to those observed for Cerestar, were omitted in this figure for clarity. Starch samples with a high content of amylose exhibit very low crystallinity values (20%) in contrast to that of starch-free amylose. However, for all samples the crystallinity is progressively destroyed with increasing temperature. While for waxy maize and pea starch the crystallinity disappears totally during heating at 114°C, for Cerestar and Roquette some crystallinity still remains even for the higher temperature used (about 150°C). The corresponding SAXS data regarding Q and L are presented

TABLE II
Total Crystallinity, α_T , A or B Crystallinity, and Long Spacings, L , for the Samples Investigated

Starch type	Molded injection samples after conditioning for 20 days			Same samples after heat treatment and 2 days storage at R. T. in sealed capillaries		
	α_T	α_A or α_B	L (nm)	α_T	α_A or α_B	L (nm)
Waxy maize	54.3	54.3	11.1	14.3	14.3	8.6
Potato	64.2	64.2	11.2	60.7	60.7	12.7
Corn	36.5	33.9	13.5	28.9	24.1	12.5
Cerestar	35.4	20.0	12.7	18.0	8.9	—
Roquette	29.8	17.5	13.3	17.9	10.5	—
Pea	24.4	20.9	13.0	—	—	—

in Figures 9(a) and (b), respectively. Again the Q values are normalized to 1 for all of the samples [Fig. 9(a)]. Q values decrease rapidly during the first thermal treatment, although with a clearly different rate for the samples investigated. It is worth mentioning the persistence of the SAXS maximum for pea starch in relation to the other samples [Fig. 9(a)]. On the other hand, the variation of L [Fig. 9(b)] is quite different for these starch samples. In Cerestar and Roquette, L de-

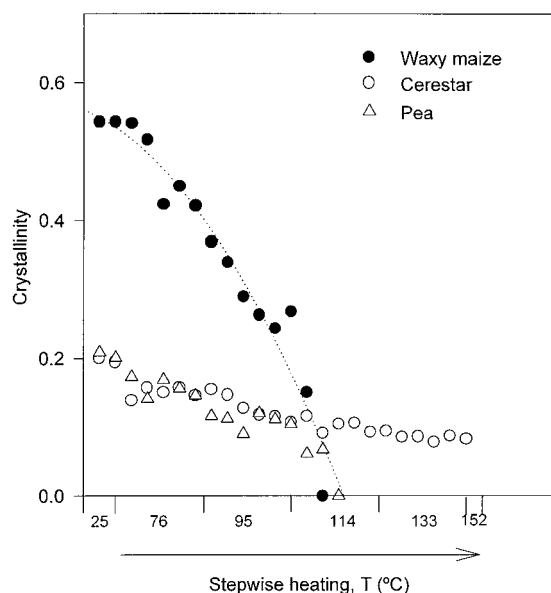


Figure 8 Variation of the crystallinity of wet-conditioned starch samples upon treatment at different temperatures for 5 min.

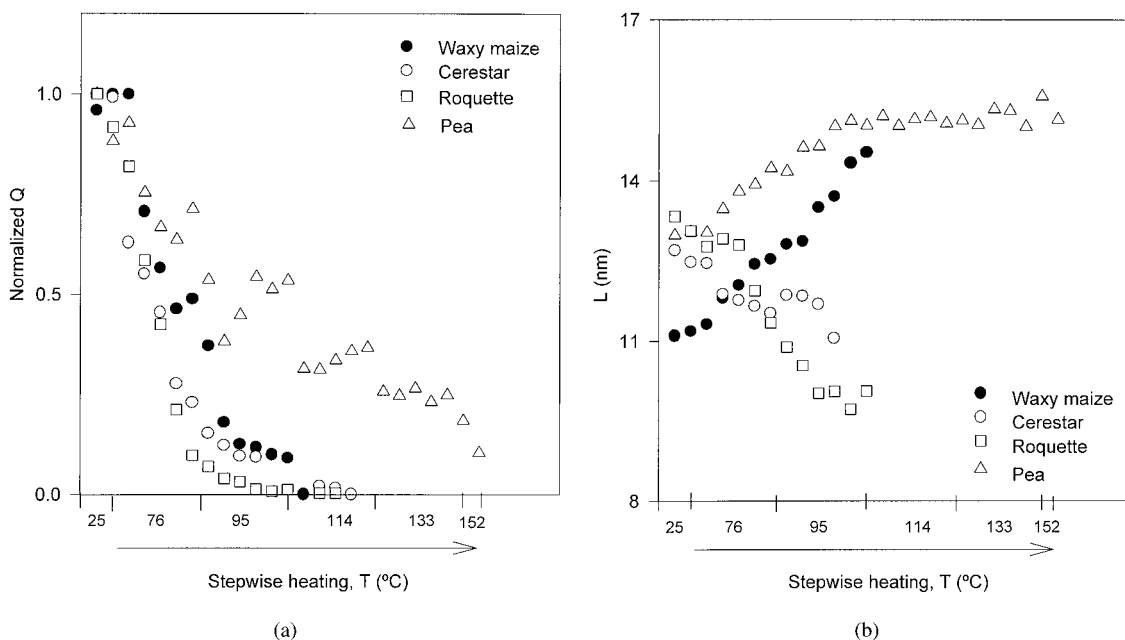


Figure 9 (a) Variation of the normalized Q values of samples described in Figure 8. (b) Variation of the long period of samples described in Figure 8.

creases continuously with increasing thermal treatment in contrast to the L increase observed for all other starch materials [Figs. 7(b) and 9(b)]. The maximum L value observed before the disappearance of the SAXS maximum is around 15 nm. This value is smaller than that detected for corn and potato starch [Fig. 7(b)]. In pea starch, L increases, reaching a maximum value at temperatures near 100°C, and remains constant for higher temperatures, whereas other starch samples show a vanishing tendency of the scattering at small angles. The structural variation reported in Figures 8 and 9(a) and 9(b) reveals obvious differences between samples rich in amylose content: pea starch (persistence of the SAXS maximum after destruction of the crystal form B) and Cerestar and Roquette (persistence of the crystal form B after vanishing the SAXS maximum).

Final remarks

The crystallization results obtained, particularly for potato starch (Figs. 3–5), represent a detailed picture of the structural changes occurring on the *amorphous* processed material. During the first minutes at room temperature, the starch in water atmosphere “relaxes” due to the incorporation of water molecules, which facilitate a chain rearrangement, giving as a result the appearance of a certain crystalline order (Fig. 3). Later on, still at room temperature, order in the nanoscale is also developed ($L = 14.5$ nm) (Fig. 4). During the first thermal treatment (40°C), although the crystallinity level is very low (<10%),

the Q invariant reaches a 50% of its total variation (Fig. 4) whereas the long period shows a maximum. According to Figure 5 the crystalline order initially appears in relatively large aggregates of “parallel segments” of helical chains that are more susceptible to crystallize. At higher temperatures, smaller aggregates and less parallel chain segments can crystallize, thus reducing the average crystal size. The apparent maximum observed in crystal size during heating at 40°C could be related to a thickening of the first crystals formed.

During the treatment at 70°C a rapid increase of crystallinity is accompanied by the highest value of the Q invariant as expected. On the other hand, L continuously decreases. This could be interpreted in terms of the crystallization of thinner crystals outside or inside the lamellae stacks.

Regarding the thermal stability of the structures developed in injection-molded materials, the comparison of results obtained for potato and corn starch, for similar amylose content, reveals important differences that should, in principle, be attributed to different structures developed in the injection-molded samples (Fig. 1, middle). Thus, the presence of structural order in corn starch, due to the crystal form V_{hv} , which remains nearly constant upon thermal treatment,²⁷ seems to be responsible for the different “destruction kinetics” observed [Figs. 7(a) and (b)] for corn and potato starch. One possibility is that the smaller amount of crystal form A (30%) in corn starch, in contrast to a larger amount of form B (65%) in potato starch (Fig. 6), could be associated to less perfection of

form A, which is growing together with form V_h . In addition, the thermal treatment applied (5°C/min) reveals a clear temperature transition (around 55°C) in the destruction kinetics of both crystal forms A and B. This finding could be related to a partial relaxation of the amylose network.³⁰

There is an apparent contradiction between the variation of L during crystallization of potato (Fig. 4: L decreases with increasing T) and during the thermal heating [Fig. 7(b): L increases with T] of wet conditioned samples (potato and corn starch). This could be due to the two opposite processes involved in both experiments. Thus, during the crystallization process, smaller and less perfect crystallites are being incorporated to the initially defined structure based on the larger, more perfect crystallites, thus reducing the average L values. On the contrary, the wet conditioned sample initially shows a crystallized structure. Upon heating, the latter loses first the smaller and less perfect crystals and consequently should show larger L values during heating [Fig. 7(b)].

It is noteworthy the particular behavior shown by starch samples rich in amylose content (Table I). The three samples are very low crystalline materials. This suggests that amylose is not capable of building up a crystalline structure in contrast to amylopectine (see Figs. 2 and 8). Furthermore, L during the thermal treatment [Fig. 9(b)] decreases for Cerestar and Roquette. It is worth mentioning that both materials considerably develop the V_h structure upon the injection-molding process (Fig. 1). As this structure is not affected by the thermal treatment,²⁷ one may think that after wet conditioning the V_h structure is relaxed and "filled" with crystallites of the grown B structure. The preferential destruction of the B crystals with thermal treatment could be the reason of the L decrease observed for Cerestar and Roquette [see Fig. 9(b)]. In addition, the persistence of the B form in these starch materials (Fig. 8) could be related to some coexistence or interaction between, both, B and V_h structures, the latter providing a higher thermal stability for the B crystals. Accordingly, the stacks of more spaced B crystals, contributing to the initial larger long period, are gradually destroyed with increasing temperature while, at higher temperatures in the 76–95°C range, the B stacks contributing to the smaller long period are better preserved through the V_h structure. Finally, at temperatures higher than 95°C, the stacking of crystals, as revealed by SAXS, disappears, although crystals giving rise to the WAXS pattern persist (Fig. 8).

CONCLUSIONS

1. The crystallinity of native starch decreases with increasing content of amylose. Both crystal

forms A and B are destroyed through the injection-molding process, but they can be restored upon wet conditioning of samples. For starches with similar amylose content, the recovery of crystallinity is less favored for the starch with the V_h form developed.

2. For wet conditioned materials, the thermal stability of both crystal forms A and B seems to be dependent on the crystallinity level reached, which, on the other hand, is dependent on the V_h structure. The A and B structures collapse around 90–100°C. After cooling the samples, a partial recovery of the corresponding crystal forms A or B is observed, provided long storage times are used (Table II). The quicker recovery observed for the structure of potato starch, in relation to other starches, can be related to the absence of the crystal form V_h .
3. For amylose-rich samples, in which the V_h structure is more developed (Cerestar and Roquette), the crystal form B is only partially destroyed at the highest temperatures reached.
4. The low crystallinity values observed for the rich amylose starches support the main role of amylopectine in the nanostructure developed for starch materials.
5. The comparison of the results obtained for waxy maize and potato starch indicates that amylose is not relevant for a final crystallinity development, though it speeds up the crystallization process (retrogradation) in amorphous starch.

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