

Retrogradation of Potato Starch as Studied by Fourier Transform Infrared Spectroscopy

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Retrogradation kinetics for a potato starch-water system (10% w/w gel) was monitored by Fourier Transform Infrared spectroscopy and compared with waxy maize starch. The spectra showed the C-C and C-O stretching region ($1300\text{--}800\text{ cm}^{-1}$) to be sensitive to the retrogradation process. A multi-stage process was observed during the retrogradation of potato starch and characterized as the formation of short- and long-range order. The first stage was characterized as the formation of helices and the fast formation of crystalline amylose regions. The second stage was described as the induction time for amylopectin helix aggregation. Stage three was described as the helix-helix aggregation and the crystallization of amylopectin. The overall first order calculated rate constant of potato starch was $(9.6 \pm 1.4) 10^{-3} \text{ h}^{-1}$. The calculated rate constant were in agreement with the known difference in retrogradation kinetics of waxy maize and potato starch. The effects were explained by the differences in retrogradation rate of amylopectin and amylose. Potato starch consists of amylose as well as amylopectin. Whereas amylose crystallization occurs within a few hours, amylopectin crystallization is slow and takes a few weeks.

Durch Fourier-Transform-Infrarotspektroskopie untersuchte Retrogradation von Kartoffelstärke. Die Retrogradationskinetik eines Kartoffelstärke-Wassersystems (10% w/w gel) wurde durch Fourier-Transform-Infrarotspektroskopie ermittelt und mit wachsiger Maisstärke verglichen. Die Spektren zeigten, daß die C-C und C-O-Streckungsbereiche ($1300\text{--}800\text{ cm}^{-1}$) empfindlich gegenüber dem Retrogradationsprozeß sind. Während der Retrogradation von Kartoffelstärke wurde ein Mehrstufen-Prozeß beobachtet und als Bildung von Kurz- und Langbereichsordnung charakterisiert. Die erste Stufe wurde als die Bildung von Helices und die feste Bildung kristalliner Amylosebereiche charakterisiert. Die zweite Stufe wurde als Induktionszeit für die Amylopectin-Helix-Aggregation beschrieben. Die dritte Stufe wurde als die Helix-Helix-Aggregation und die Kristallisation von Amylopectin beschrieben. Die als Gesamt-Erstordnung berechnete Geschwindigkeitskonstante für Kartoffelstärke betrug $(9.6 \pm 1.4) 10^{-3} \text{ h}^{-1}$. Die berechnete Geschwindigkeitskonstante stimmte überein mit der bekannten Differenz zwischen der Retrogradationskinetik von wachsiger Mais- und Kartoffelstärke. Die Einflüsse werden erklärt durch die unterschiedlichen Retrogradationsgeschwindigkeiten von amylopectin und Amylose. Kartoffelstärke besteht sowohl aus Amylose als auch aus Amylopectin. Während die Amylosekristallisation innerhalb von wenigen Stunden abläuft, ist die Amylopectinkristallisation langsam und benötigt einige Wochen.

1 Introduction

The application of thermoplastic starch (TPS) in biodegradable plastics is subject of growing attention. A disadvantage of these materials is the loss of mechanical properties during time due to ageing and retrogradation which is caused by changes in water content, changes in short-range structure and crystallization. It is of importance to study the effect of the difference of native starch types used in thermoplastic starch materials on ageing of TPS. The retrogradation of starch containing materials have commonly been studied by X-ray diffractometry (XRD), differential scanning calorimetry, rheological methods, and light scattering [1-10]. However, these methods give no direct information on the structural changes at the molecular level. Even the XRD is limited to the long-term appearance of the final, crystalline form of retrograded materials. More recently, non-invasive methods such as Raman [11-15], NMR [16-20] and Fourier Transform Infrared (FT-IR) spectroscopy [21-26, 30] have been used. FT-IR spectroscopy coupled with sampling methods such as attenuated total reflection (ATR) is used as a powerful method to study polysaccharides [27-29]. Conformational changes due to retrogradation during storage can be monitored by analysis of the observed band-narrowing process and of the observed intensity changes of conformational sensitive bands in the $1300\text{--}800\text{ cm}^{-1}$ region. The kinetics of this process were measured by quantification of the extent of the band narrowing and of the time-dependent intensity changes of specific bands.

The starch-water system (10% w/w gel) is used as a model for the complex thermoplastic starch materials. Amylose and amylopectin are the main constituents of starch. Amylose is a linear molecule whereas amylopectin is a highly branched polymer. The amylose-to-amylopectin ratio strongly determines the ageing and the starch structure in TPS and thus the mechanical properties. Research is focused on native potato starch, a mixture of amylose and amylopectin. The results are compared with the data found for a waxy maize starch-water system (10% w/w gel) [30]. Waxy maize starch consists of pure amylopectin whereas potato starch is a mixture of amylose and amylopectin.

2 Experimental

2.1 Preparation of Gels

The 10% (w/w) starch gel was prepared by gelatinization of potato starch (var. Farina, 79% amylopectin on the basis of dry material and 17% water on the basis of total mass, AVEBE, Veendam, The Netherlands), dispersed in deionized water. The gelatinization was performed in a Contraves Rheomat 115 Viscometer, modified for preparation of standard gels, at standard conditions (Temperature profile: logarithmic incline from 40° to 95°C during 30 min). The hot solution was poured into a preheated (65°C) ATR-cell and sealed to prevent water loss. The samples were stored at 5°C after the first 10 h of experiments. Thimerosal, 0.01% (w/w), (Mercury- ρ -car-

boxyphenyl)-thio]-ethyl sodium salt) (Sigma Chem Co.) was added as a preservative.

2.2 X-ray diffractometry

XRD patterns were measured by using a Philips powder diffractometer (Model PW 3710). The X-ray generator was operated at the $\text{CuK}\alpha$ wavelength of 1.542\AA . Measurements of diffracted intensities were made over the angular range of 2.5 to $20^\circ \theta$ at ambient temperature.

2.3 FT-IR spectroscopy

All FT-IR spectra were obtained on a Digilab FTS-60A spectrometer (BIO-RAD) equipped with a deuterated triglycine sulfate detector (DTGS) operated at a mirror velocity of $0.3\text{ cm}\cdot\text{s}^{-1}$. The number of interferograms being co-added for each Fourier transformation was 64 for the initial measurements and 256 for the measurements from 5h on at 4 cm^{-1} resolution. An ATR cell (BIO-RAD) with a ZnSe crystal was used. Spectra were run at irregular intervals up to 400h. The ATR cell was allowed to equilibrate at room temperature before each measurement. A spectrum of the empty cell was used as the background. The spectrum of water was subtracted from all starch gel spectra in order to eliminate the distorting effect of water in the region $1300\text{--}800\text{ cm}^{-1}$.

Resolution enhancement was applied to all resulting spectra by using the deconvolution technique described by Cameron et al. [31] and modified for use on the Digilab FTS-60A. The assumed line shape was *Lorentzian* with a half-width of 15 cm^{-1} . The resolution enhancement factor (K) was optimized at 1.5. Absorbance ratio measurements were made on the resolution enhanced spectra.

2.4 Data treatment

The FT-IR data were analyzed by fitting an *Avrami* first-order equation [31], to each data set:

$$\phi = (A_L - A_t) / (A_L - A_0) = \theta^{-k t^n} \quad (1)$$

where ϕ is the fraction of the total change in measured property still to occur; A_0 , A_t and A_L are experimental values of the absorbance ratio at time zero, t and infinity, respectively, k is the rate constant and n is the *Avrami* exponent. The *Avrami* exponent was assumed to be $n=1$. The fits were obtained by means of an iterative, non-linear least squares procedure.

3 Results and Discussion

The viscogram of the potato starch gel is shown in Figure 1. For potato starch the onset-gelatinization temperature is 62°C and the peak-gelatinization temperature is 66°C . The peak viscosity is $8.0\text{ Pa}\cdot\text{s}$. The onset-gelatinization temperature for the waxy maize starch gel is 67°C and the peak gelatinization temperature is 74°C . The peak viscosity is $2.7\text{ Pa}\cdot\text{s}$ [30].

The FT-IR spectrum of potato starch before gelatinization is shown in Figure 2. The spectra at selected time points after gelatinization are shown for the potato starch gel in Figure 3. All spectra show eight major distinct bands in the region of $1300\text{--}800\text{ cm}^{-1}$ at similar frequencies. In Table 1 the band frequencies are summarized at time zero and at the last time point. The spectrum of native potato starch show three separated bands in the region of 1100 to 900 cm^{-1} located at 1047 cm^{-1} , 1018 cm^{-1} and 994 cm^{-1} . These absorbances are related to the amount of crystalline starch and to the water content (unpubl.

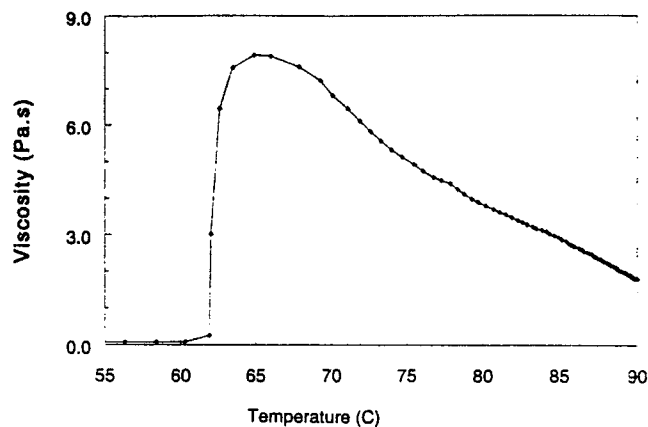


Figure 1. Viscogram of the potato starch gel.

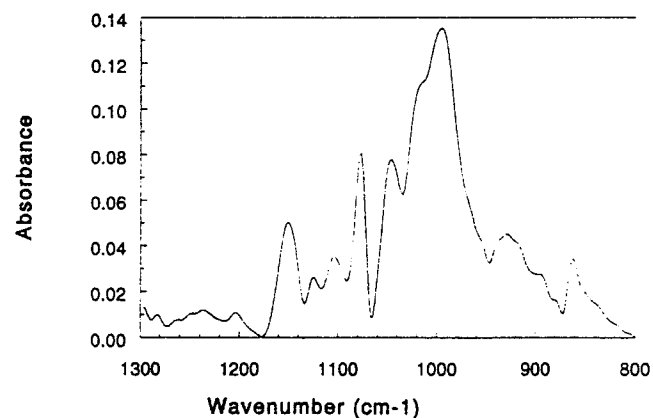


Figure 2. Infrared spectra of a potato starch with 18% water before gelatinization.

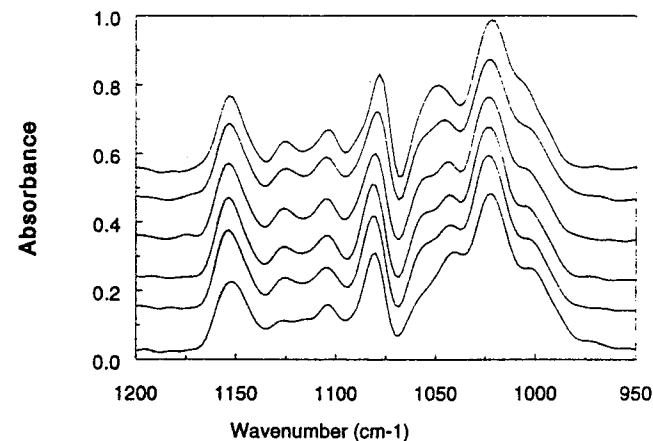


Figure 3. Infrared spectra for the potato starch gel (10%w/w) at selected time points during retrogradation; from bottom to top after: 0, 2, 20, 50, 165 and 360h.

Table 1. The FT-IR Bandfrequencies (in the region $1300\text{--}800\text{ cm}^{-1}$) of the Potato Starch Gel at Various Time Points During Retrogradation at 5°C .

Time (h)	Band (cm^{-1})							
	1	2	3	4	5	6	7	8
0	1152	1126	1104	1081	1053	1040	1022	1003
					sh*	sh		sh
360	1153	1126	1105	1079	1053	1046	1022	1002
					sh			sh

*sh = shoulder

results). Gelatinization of starch results in the convergence of these separated bands to one intense broad band in this region with a maximum intensity at 1022cm^{-1} and a noticeable shoulder on the high-frequency side. During retrogradation for several weeks, this broad line becomes resolved into three bands around 1053 , 1022 and 1000cm^{-1} (with a minimum of the intervening valley at 1035cm^{-1}). The most pronounced changes in the spectra occur at 1000 (peak), 1035 (valley) and 1053 (peak) cm^{-1} .

Spectral changes can be divided into two observations: band narrowing and changes in absorbance intensities of specific bands. Band narrowing is caused by ordering of the polymers and a reduction of the number of conformations. This results in a smaller distribution of bond energies which is measured as more pronounced and defined peaks. Changes of intensities bands are due to changes in specific starch conformations such as long-range ordering and crystallinity. The time-dependent spectral changes in the potato starch gel are shown in Figure 4 on a logarithmic time scale for the absorbance ratio

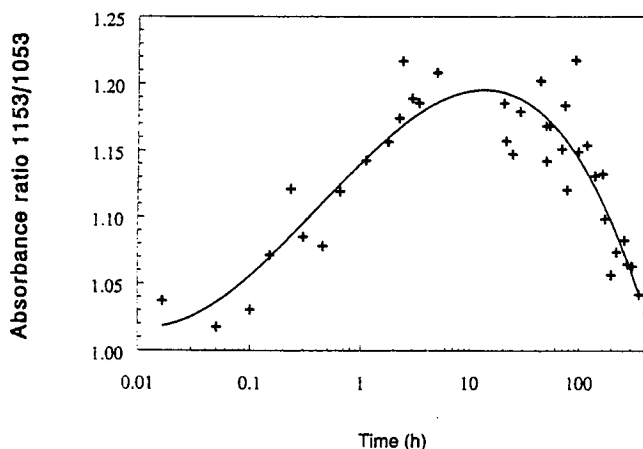


Figure 4. Spectral changes over time for the potato starch gel (10%w/w) for the absorbance ratio 1153/1053.

1153/1053. The absorbance ratio of band intensities between selected peaks were measured at different time points. The use of ratios allows the compensation of path-length differences that might arise from incomplete coverage of the ATR crystal between different samples. There different steps are clearly visible: from 0 to 1h the ratio increases, from 1 to 10h there is a plateau region, and from 10h on the ratio declines. It has to be concluded that a multi-stage process occurs during retrogradation. Stage 1 has been described [15, 24, 32] as a purely conformational ordering, possibly involving the formation of double helices of amylose or in the amylopectin branches within a single polymer molecule. Amylose aggregation and crystallization occurs also within this period [23]. This results in a band narrowing in the spectrum and in a decline of the band intensity at 1053cm^{-1} and thus an increase of the ratio 1153/1053 in which 1153cm^{-1} is taken as an internal correction standard. Stage 2 is understood as the induction time for onset of amylopectin helix aggregation and crystal growth. Stage 3 is described as the primary amylopectin aggregation and crystallization step. The band at 1053cm^{-1} , which is linearly related to the amount of long-range ordering and crystallinity [26], will increase as a result. This crystallization has been confirmed by the appearance of diffraction in XRD measurements for the dried gels during the 360h of retrogradation (see Figure 5). The crystal structure is B-type. This fourth step, during which the absorbance at 1000cm^{-1} (this band is very sensitive to water content [26]) increases, involves phase-separation of water (syneresis). This syneresis of water

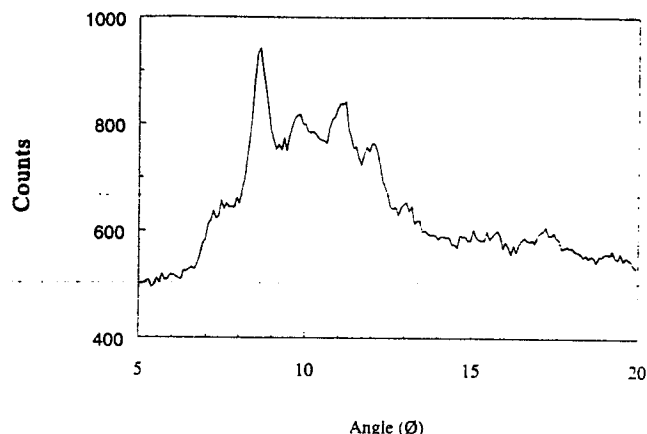


Figure 5. X-ray diffractogram of potato starch after 3 weeks of retrogradation.

occurred only for the waxy maize starch gel during the time span of IR-measurements (360h) [30].

Retrogradation kinetics

The rate of structural changes (kinetics) have been determined by analysis of the ratios 1053/1035. This ratio combines the effects of band narrowing and changes in crystallinity during retrogradation. In Figure 6 the ratio 1053/1035 is plotted against time over the period 0–400h for the potato starch gel. The overall-retrogradation kinetics of the first three stages, i.e. the total process of forming of helices, aggregation and crystallization, can be accurately described by a first-order Avrami fit for the ratio 1053/1035 (see Figure 6). An apparent smooth change over time in the spectroscopic parameters is observed. The overall-reaction rate constants k (Avrami-fit) for the absorbance ratio 1053/1035 are given in Table 2. A_1 and A_0 are de-

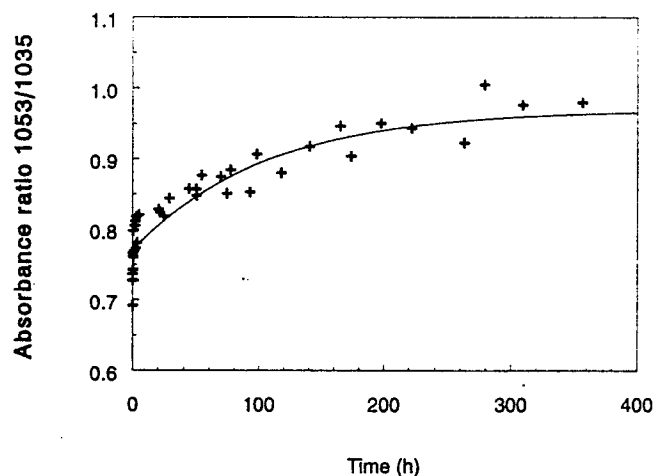


Figure 6. Spectral changes of potato starch gels for the absorbance ratio 1035/1035; The data are fitted with a first-order Avrami equation.

Table 2. The Kinetics Parameters of Waxy Maize and Potato Starch Gels^{a)}.

Starch	A_L	A_0	$k \times 10^3$ (h^{-1})
Waxy maize ^{b)}	1.18 ± 0.01	0.69 ± 0.01	4.1 ± 0.1
Potato	0.98 ± 0.02	0.77 ± 0.02	9.6 ± 1.4

^{a)} The parameters were calculated with an Avrami-fit. The data were fitted with variable k , A_L and A_0 . ^{b)} Taken from literature [30].

rived for the gels with an Avrami-fit with free k , A_1 and A_0 . the overall (first-order Avrami) reaction rate constant k clearly shows a increase in the retrogradation rate of potato starch in terms of short-range structural changes compared to waxy maize starch. The differences in initial rate, which is higher for potato starch, can be explained by the differences in retrogradation rate of amylose and amylopectin. The differences are too large to be accounted for by variations in lipid and fatty acids contents between waxy maize and potato starch. Also the observed higher viscosity in the potato starch gel can not explain the increased rate because this would have lead to a lower retrogradation rate because of a decreased starch chain mobility. Amylose retrogradation is a fast process which is completed within a few hours as observed with FT-IR spectroscopy [24]. XRD measurements show also earlier formation of crystallites in potato starch as compared to waxy maize starch (see Figure 7). Rapidly retrograded amylose, forming an ordered matrix on a molecular level, can act as seed nuclei for amylopectin molecules, accelerating the aggregation and crystallization of amylopectin.

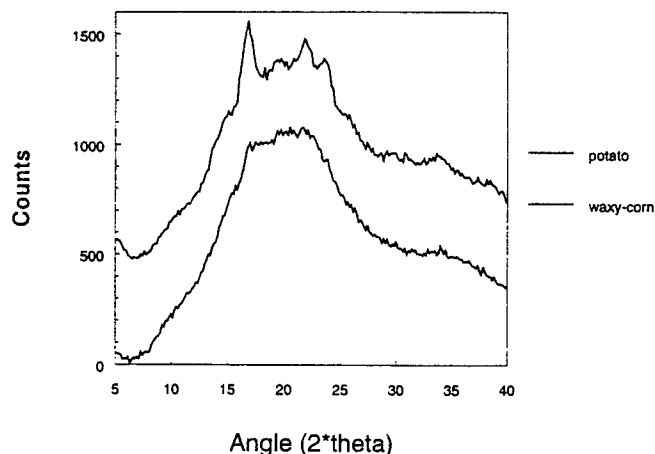


Figure 7. X-ray diffractogram of potato (top) and waxy maize (bottom) starch after 1 week of retrogradation.

4 Conclusions

FT-IR spectroscopy can effectively distinguish differences on a structural level in starch materials. Good quality spectra are obtained for the 10% w/w starch/water systems. Major differences in the infrared spectra are obtained on gelatinization and subsequent retrogradation of waxy maize and potato starch/water systems. The spectra showed that bands in the region $800\text{--}1300\text{cm}^{-1}$ are sensitive to starch polymer conformation. these spectral differences are consistent with gelatinization, and thus granule and crystallite melting, followed by a multi-stage process of retrogradation. The data obtained from the time dependence of the C-C and C-O stretching vibrational modes are explained by a multi-stage process of formation helices, aggregation and crystallization. The calculated rate constants for the latter process clearly indicate differences in retrogradation rate of waxy maize and potato starch due to differences in retrogradation rate of amylose and amylopectin.

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