Comparative study on analytical tool for cassava starch retrogradation at micro- and macro- molecular structure

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Summary

The retrogradation behavior of cassava starch gel containing 60, 70, and 80% water content was monitored using X-ray diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and compression test. The cassava starch gel was prepared under the heating temperature of 100°C for 30 minutes and then stored at the temperatures of -20, 5, 25, and 45°C. The retrogradation rate at the storage temperature of -20 and 5°C was higher than at 25 and 45°C for all water contents. Monitoring with XRD and FTIR, the initial progress of retrogradation at -20°C was faster than at 5°C for the gel containing 80% water and comparable to that at 5°C for 60 and 70% water content as a result of freeze concentration effect but the retrogradation at -20°C did not proceed with using DSC. The extent of retrogradation at 5°C was the highest after storage for 4 or 8 weeks as monitoring with all techniques. For concentrated gel (60% water), although the initial rate of retrogradation at 25°C monitored using DSC and compression test was slower than at 5°C, the progress of retrogradation delayed later than at 5°C; consequently, the extent of retrogradation at both temperatures was similar after 4 weeks. Using FTIR and compression test, the retrogradation at 25 and 45°C was extremely slow or did not occur in 80% water content system; however, when lowering temperature to 5°C, this progress was noticeable. For ordered structural formation, the B-type polymorph was observed for cassava starch gel stored at -20, 5, and 25°C whereas the A-type polymorph appeared at 45°C with the highest melting temperature and the narrowest range.

Keywords: cassava starch retrogradation, XRD, FTIR, DSC, compression test

Introduction

Starch retrogradation is described as phase transition of starch molecules following gelatinization and considered to be a nonequilibrium polymer crystallization process due to association of starch chains as double helices, and variably order semi-crystalline array of these helices. The rate and extent of retrogradation are dependent on starch structure, starch botanical source, starch concentration, storage temperature and time. Temperature and water dependence of starch retrogradation has been studied in maize starch, waxy maize starch, wheat starch, and rice starch [1-6]. However, these works focused on the

dependence of individual factor. In temperature viewpoint, starch retrogradation occurs at the temperature above glass transition temperature (T_g) at the rate depending on T- T_g appearing the "bell-shaped" behavior, which successfully modeled using the Avrami kinetic equation [1, 3]. At the temperatures below T_g, the retrogradation rate was slower than at storage temperatures above T_g [6]. In aspect of water content, the maximum retrogradation extent was shown at intermediate water content and slightly different for each waxy-type maize starch [2].

Cassava (*Manihot esculenta* Crantz) starch is one of the most important root starch applied in many industries. Upon gelatinization, it exhibited high peak viscosity and low cooled viscosity, imparting a long-texture gel in excess water content [7]. Limited information on the retrogradation behavior of cassava starch has been published. In addition, the interaction of factors affecting starch retrogradation has not been reported which is able to directly deliver to processing and commercial application.

Several methods operating different principles and measuring different properties were reviewed for the study of starch retrogradation [8]. DSC has been extensively used in many studies, representing the retrogradation of small sample size, while mechanical or textural changes is a macroscopic method to monitor a development of supramolecular structure, usually prepared in a larger size. A comparison of studied methods was conducted in a limited number and supplementary information of molecular and textural changes during retrogradation using the same sample, processing and storage history was also limited. Each technique monitoring different structural levels and aspects of retrogradation process provided different sensitivity to the "onset" of the reordering process [5]. Therefore, using different techniques to probe retrogradation is useful to obtain complementary results to more understand the retrogradation phenomenon under various conditions. The objective of this study was to investigate retrogradation behavior of cassava starch affected by the combination of water content and storage temperature using different methods to provide information on different molecular structures.

Materials and Methods

Preparation of retrograded starch

Cassava starch obtained from Sanguan Wongse Industries Co., LTD, (Nakorn Ratchasima, Thailand) was suspended with deionized water for 60, 70 and 80% water content (w/w) in cans (size 300x201) and then equilibrated overnight at the temperature of 30 ± 2°C. The starch suspension was heated in a shaking water bath at 50°C for 15 min, 60°C for 15 min, 70°C for 15 min and 80°C for 15 min. Then it was subjected to thermal treatment in a retort at 100°C for 30 min. Completely gelatinized starch was observed under a polarized light microscope. The cassava gel samples were kept at the storage temperature of -20, 5, 25 and 45°C for 4-8 weeks. The retrogradation of the starch gel samples was monitored using FTIR and compression test. For XRD, the starch gel was lyophilized using a freeze-dryer (Heto PowerDry PL9000, Jouan Nordic A/S, Allerod, Denmark), ground under the temperature of 6-12°C, and then seived. The particle size of powder samples was 75 - 105 μm before measurement.

X-ray diffraction (XRD)

The powdered samples containing 7 - 8% moisture were tightly packed on circular trays. The trays were exposed to monochromatic CuK_α radiation (λ = 1.542 Å) in a Bruker D5005 X-ray powder diffractometer (Bruker GmbH, Germany). It was operated in a reflection mode at 40 kV and 40 mA, with divergence slit of 0.25° and antiscattering slit of 0.25°. The XRD patterns were recorded using diffraction angles (2θ) from 3 to 30° (step size of 0.02° and scanning rate of 0.25°/min). The patterns were collected in two measurements for each sample. The quantitative determination of relative crystallinity, which was referred to degree of retrogradation, was estimated according to *Mizuno* et al. [9] as follows:

$$\text{degree of retrogradation (\%)} = \frac{(X-G)}{(N-G)} \times 100$$

where *X*, *G*, and *N* are the peak area of sample, gelatinized starch and native starch respectively.

Each XRD data set was additionally analyzed by fitting the Avrami kinetic equation:

$$x(t) = x_\infty - (x_\infty - x_o) \exp(-kt^n)$$

where x(t) is measured value at time t, x_o is measured value at t = 0, which was zero, x_∞ is a final value of degree of retrogradation at t = ∞ , k = the rate constant of degree

of retrogradation, and n is the Avrami exponent. The final value (x_∞) and rate constant (k) were calculated by fitting the data set using an iterative, regression analysis method [10].

Fourier Transform Infrared (FTIR) spectroscopy

All FTIR spectra were acquired using a Spectrum GX FTIR spectrometer (PerkinElmer Inc., Boston, MA, USA) equipped with a deuterated triglycine sulphate (DTGS) detector using a horizontal attenuated total reflectance (ATR) accessory with a ZnSe crystal at an angle of incidence of 45° (PerkinElmer Inc., Boston, MA,USA). Starch gel with 2 mm thickness was used to obtain the spectra acquired from 32 scans at the resolution of 4 cm^{-1} against an empty cell as background. The spectrum of water was subtracted from the sample gel spectra in order to eliminate the distorting effect of water in the region 1300 - 800 cm^{-1}. The spectra were baseline corrected in the region 1200 - 800 cm^{-1} and then deconvoluted using an enhancement factor of 2.0 as the retrogradation process was shown to be sensitive in this region [11]. The spectra were collected in six measurements for each sample. The ratio of absorbance intensities (1045:1037 cm^{-1}) was used for monitoring retrogradation.

Compression test

The retrograded starch gel prepared in cans was evaluated for textural properties as uniaxial compression force using an Instron universal testing machine (Model 5560, Instron Corp., Canton, MA, USA) with a load cell of 5 kN. The retrograded gel was compressed at a speed of 1 mm/sec for a distance of 10 mm with a cylinder probe (20 mm diameter). Three measurements were conducted.

Differential Scanning Calorimetry (DSC)

Cassava starch (8 - 16 mg) was weighed in a DSC pan (50 µl aluminum pan, PerkinElmer Inc., Connecticut, USA) and deionized water was added to obtain the water content of 60, 70 and 80% (w/w). The pan was sealed and equilibrated for 12 hours at room temperature (27 ± 2°C). In order to obtain starch gelatinization, the heat treatment was conducted using a Pyris Diamond DSC (Perkin Elmer Inc., Connecticut, USA). All pans were heated from 25 to 95°C at a rate of 20°C/min and then cooled to 25°C. A rescan from 25 to 95°C at a rate of 20°C/min was performed to ensure complete gelatinization. Then, the samples were removed from the DSC and stored at different temperatures. After the

starch gels were aged for certain periods of time, the sample pans were heated from 5 to 95°C at a rate of 10°C/min to determine thermal transition of retrogradation using an empty pan as a reference. Temperature and heat flow calibration was conducted using indium. The percentage of retrogradation was determined as follows:

$$\text{degree of retrogradation (\%)} = \left(\frac{\text{Enthalpy of retrograded starch}}{\text{Enthalpy of native starch}}\right) \times 100$$

The enthalpy associated with the gelatinization of native starch was performed in the same procedure as retrogradation enthalpy.

Statistical Analysis

For FITIR and compression test, the data used for analysis were the ratio of the measured value of retrograded samples at time t to that of gelatinized starch (t = 0) in order to normalize the measured value of gelatinized starch containing different water contents. The duplicated experiments were conducted using Full Factorial Experiment in Completely Randomized Design. The Analysis of Variance and Duncan's multiple range test were performed by using SPSS software (SPSS for windows release 10.0.1, standard version, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Retrogradation monitored using XRD

Retrogradation behavior of retrograded cassava starch followed by change in relative crystallinity was shown in Fig. 1. The degree of retrogradation of retrograded starch samples increased and reached plateau as storage for 8 weeks. The degree of retrogradation was fitted to the Avrami equation in order to obtain the retrogradation kinetic values: rate constant, k, and a final value of degree of retrogradation as illustrated in Tab. 1. Storage of cassava starch gel containing 60, 70, and 80% water content at the low temperature of 5 and -20°C accelerated the reorganization of starch to become an ordered structure, showing greater degree of retrogradation than that of 25 and 45°C ($p<0.01$). The higher k of retrogradation for storage at the low temperatures was also consistent with this result, which was similar to other reports [4, 12]. At the low storage temperatures, the k of retrogradation of gel with 60% water was higher than that of 70 and 80% water. As noticed in Fig. 1, the initial rate of retrogradation at -20°C was higher than that at 5°C in the first

period of storage (2 - 6 days) for the gel containing 80% water. In addition, the storage time required to reach the plateau of retrogradation at 80% water was the shortest while it required longer time for gel containing 70 and 60% water respectively. It indicated that during beginning of storage, starch crystallization occurred very fast at -20°C, especially at 80% water content. *Hsu* and *Heldman* [6] observed a low retrogradation rate of rice starch at -15°C using DSC technique and concluded that T_g and diffusion limitation were factors controlling the rate of retrogradation. However, T_g of starch gel could change during low-temperature storage as a result of concentration changes [13]. Basically, the storage of cassava gel at -20°C should allow starch crystallization proceed on the least extent. However, it showed a great retrogradation extent similar to 5°C during the first period, probably due to these explanations: (i) The overall rate of crystallization of partially crystalline polymers exhibited a maximum between T_g and melting temperature (T_m) as resemble to a bell curve [14]. Therefore, the lower the storage temperature the higher the rate of crystallization as presented in this study due to the extent of supercooling (T_m-T), where the T_m range of retrograded cassava starch at -20 °C was 43 - 70°C. (ii) During the first period of storage, a part of the water remained unfrozen and plasticized the freeze-concentrated phase, which, in this case, did not yet become a glass. As starch mobility was considerably reduced due to an additional factor of high concentration, starch association was extremely induced. Upon storage, the transformation of small ice was expected, which facilitated the formation of higher concentrated regions, thereby favoring retrogradation [15]. For the starch gel containing 80% water, more amount of water became ice and less unfrozen water content existed in the matrix of unfrozen phase. No starch crystallization appeared in the latter period faster or approached plateau earlier because of lower water diffusion. The plateau value of degree of retrogradation at -20°C for all water contents was about 16-17% which was similar to the predicted final values in Tab. 1, suggesting that the retrogradation of cassava starch at -20°C did not proceed in the latter period of storage.

At 5°C storage temperature, the average degree of retrogradation of the gel with 70 and 80% water content was higher than that of 60% water for 8 week storage ($p<0.05$). In contrast, the k and final value at 60% water content was greater. They were not in agreement because of the length of storage time. Unlimited storage time was required to

obtain the final value of degree of retrogradation of 30% for the gel containing 60% water, suggesting that starch crystallization could slowly continue after 8 week storage. At 5°C, the higher water content (70 and 80%) would increase the mobility of amylopectin chains through water plasticization, thereby favoring starch retrogradation. The role of water also reduced T_g of amorphous regions and T_m of crystalline regions though promoting molecular motion. Under low T-T_g condition, nucleation was fast and crystal growth was kinetically restricted and slow, resulting that crystallization began rapidly and thereafter was delayed by the formation of rigid amorphous regions next to the early crystallites [16]. On the contrary, at 45°C storage the gel containing 80% water showed lower retrogradation extent than that of 70 and 60% water ($p<0.05$), indicating that the higher water content could delay the retrogradation behavior. *Lewen* et al. [17] did not observed an increased enthalpy of retrogradation of dilute starch gel (<20% starch). These led to the fact that high water constituent was an additional factor to delay recrystallization of cassava starch gel stored at high temperature.

The extent of retrogradation after storage for 4 weeks referred to the later period of crystallization where retrogradation process slightly increased was another concerned point. For the concentrated starch gel (60% water), although storage at 25°C showed a higher rate of retrogradation than at 45°C, the degree of retrogradation in the latter period of storage time became approach and their final values of degree of retrogradation were equivalent. Regarding the phenomenon of cassava starch recrystallization at these storage temperatures, the ordered part of gel structure, which formed earlier in a greater extent, would limit structural arrangement or crystallization process in a later period. In contrast, the slower crystallization at 45°C gradually continued and, later, it did not be limited. Therefore, the degree of retrogradation at both temperatures turned to be equivalent finally. The event at 45°C in later period of storage time could be considered to be annealing process [18]. After the nuclei were already formed, annealing at higher temperature but below T_o induced physical arrangement of the crystallite. This reformation led to growth of more perfectly ordered structure, perhaps imparting a more extent of crystallinity. This phenomenon showed a less degree of influence in the gel containing 70% water and did not exhibit in a soft gel of 80% water (Fig. 1b and 1c). For a dilution system, the interaction

between water and thermal plasticization was a driving force to inhibit the continuity of starch association. However, if lowering temperature to 25°C, which decreased molecular mobility of starch chains, the crystallization was able to develop and, then, a final value of degree of retrogradation at 25°C was higher than that of -20°C for the gel containing 80% water. The same principle of explanation that was previous discussed could be applied as well as the recrystallization of gel at -20°C was inhibited as aforementioned.

Retrogradation monitored using FTIR

Absorbance ratio in the region sensitive to the amount of ordered or crystalline starch was successfully used to follow the kinetics of starch retrogradation in a short-range order of structural change [19]. Fig. 2 showed that the progressive retrogradation of all cassava starch gels was noticed within 4 week storage. The FTIR result indicated a similar effect of water content and storage temperature to that of XRD. During initial storage (0-4 days), a higher absorbance ratio at -20°C was found in 70 and 80% water content when compared to 5°C while this retrogradation behavior was only observed in 80% water content for XRD. At -20°C, absorbance ratio increased substantially and underwent plateau within 2 days for 80% water content and 3 days for 70% water content, but it was found to be 8 and 14 days in XRD for 80 and 70% water content respectively. This result concurs with the work of *Bello-Perez* et al. [20] in that retrogradation behavior monitoring using FTIR reached a maximum value earlier than that using XRD, due to monitoring structural changes from different molecular distance scales. Retrogradation was a multi-stage process: (i) the helix formation and fast formation of crystalline amylose regions, (ii) the indication time for amylopectin helix aggregation, and (iii) the helix aggregation and crystallization of amylopectin [19]. As discussed in XRD, aging the gel at -20°C led to a phase change of water into ice formation. The higher concentration of starch in the system was a consequence. The onset temperature of glass transition temperature (T'_g) within maximally freeze-concentrated solution of gelatinized starch was reported to be -11°C for gelatinized corn starch and the concentration of solid in the freeze-concentrated unfrozen material was estimated to be 73% (w/w) [16]. Thus, gel stored at -20°C which was initially more concentrated than that at 5°C showed a higher initial rate of retrogradation for both

XRD and FTIR. However, the gel containing 60% water stored at 5°C showed a more extent of retrogradation than that at -20°C for the entire period of storage time for FTIR result, where it was different from XRD. This could be due to the reason that FTIR was more sensitive to structural damage of gel from ice crystal formation.

At the storage temperature of 5°C, the absorbance ratio of gel with 70% water was greater than that of 80% water ($p<0.05$), which was not similar to the retrogradation behavior monitored using XRD. At 25 and 45°C, the retrogradation extent of 80% water treatment was significantly lower than that of 60 and 70% water, supporting the combined effect of water and thermal plasticization to delay starch retrogradation. For high water system (80%), the starch gel stored at 5°C continuously induced structural reorganization and it did not show a tendency to become leveling-off. The same retrogradation behavior was also observed in the gel containing 70% water at 25°C. In the case of concentrated gel (60% water), the extent of retrogradation at 25°C increased and then equal to that at -20°C on day 5. These events suggested that FTIR was a sensitive method which could detect a minor structural change and showed different retrogradation behavior from XRD in particular cases.

Retrogradation monitored using Compression test

Fig. 3 showed a change in gel firmness suggesting a progressive retrogradation in terms of macromolecular level. In general, temperature water content and storage time showed a significant effect on gel network, similar to XRD and FTIR. For the gel containing 80% water, the initial extent of retrogradation at -20°C was slightly above that at 5°C (Fig. 3c), while it was obviously higher for XRD and FTIR. For 60% water content, the extent of retrogradation at -20°C after leveling-off was extensively lower than that at 5°C as compared to XRD and FTIR. These indicated that the ice crystal formation in starch gel damaged a gel network, resulting in a reduction of gel strength. Therefore, a mechanical test is not appropriate for studying retrogradation at subfreezing temperature. Furthermore, as monitoring macroscopic structural level the cassava starch gel at 45°C did not show retrogradation for all water contents, which was different from XRD and FTIR. Thus, compression test is not a proper method to follow cassava starch retrogradation at

relatively high temperature storage due to its less sensitivity on minor changes at macromolecular structure.

For concentrated gel, the gel at 25°C showed an unusual retrogradation behavior different from XRD and FTIR (Fig. 3a). Although an increase in gel firmness at 25°C was slower than at -20 and 5°C, the retrogradation at 25°C maintained regularly until the extent of retrogradation matched that of -20 and 5°C and it was higher later. The retrogradation behavior of 25°C and -20°C was similar to FTIR but the matching date of FTIR was earlier, suggesting textural changes occurred in the latter stage of retrogradation. In addition, an increase in water content to 80% delayed retrogradation progress at 25°C. This behavior was more pronounced with using compression test, as compared to XRD and FTIR techniques.

Retrogradation monitored using DSC

Fig. 4 illustrated that the retrogradation behavior of concentrated gel monitored using DSC at different temperatures showed a more similar tendency to that found in compression test. Using DSC, the degree of retrogradation at 25°C was higher than that at -20°C for entire period of time and that of -20°C was relatively constant after the first day of storage with a low value (~5-6%). The phenomena of retrogradation at -20°C using DSC method was considerably different from XRD, FTIR and compression test due to the fact that a sample size of DSC method was considerably smaller. A shorter time to obtain a frozen temperature (-20°C) was expected. Rapid cooling also caused a faster rate of retrogradation during storage [6]. Therefore, the retrogradation at -20°C in DSC pans would undergo equilibrium earlier than other techniques.

At 45°C, no retrogradation was observed in the first period. It began on day 14 and continued to increase. This retrogradation behavior was considerably different from XRD, FTIR and compression test, indicating that the retrogradation followed by DSC at high storage temperature combined with small content of sample lagged behind XRD and FTIR. For water content aspect, 60% water cassava gel demonstrated the highest extent of retrogradation, followed by 70 and 80% water system respectively ($p<0.05$) (Fig. 4b), where it was similar to the compression test result.

At 5°C storage temperature, the degree of retrogradation following by DSC method was extensively higher than that using XRD, implying that DSC was more sensitive to monitor degree of retrogradation. DSC was believed to measure enthalpy of dissociation of double helices while XRD was used to monitor the crystallinity of ordered structure which existed in a small amount for both native and retrograded starch [5].

Thermal property and polymorphism

Beside retrogradation behavior, storage of starch gel under various water contents and temperatures would manipulate different structural formation. From DSC measurement, storage at higher temperature resulted in upper onset (T_o) (Fig. 5a), peak (T_p) and conclusion (T_c) of melting temperature (data not shown) of retrograded starch gel in an order of 45>25>-20>5°C ($p<0.01$). This observation was consistent with other reports and suggested that the crystallites formed at higher storage temperature were bigger and/or more perfect [1, 4]. In addition, T_o, T_p and T_c of retrograded starch stored at 45°C were higher than those of native cassava starch, which were 66.3, 72.3 and 79.8°C respectively. However, T_c-T_o of all retrograded samples (Fig. 5b) was larger than that of native starch, indicating that the super-helical structure present in native starch was not resumed in retrograded starch and the size of the crystalline domains in retrograded starch was smaller [21]. As noticed in Fig. 7, the diffractograms of all retrograded starch samples were broad and flat and their relative crystallinities were low, as compared to native starch. However, when compared to other temperatures, T_c-T_o of 45°C was the lowest, implying that it contained more homogeneous crystallites as a result of annealing process as aforementioned [22]. In the case of -20°C, the crystallites formed at this temperature were more perfect and homogeneous than those of 5°C due to a result of higher concentrated starch matrix facilitating molecular arrangement. Starch recrystallization was considered to be governed by consecutive three step mechanism of nucleation, propagation and maturation [14]. Storage at 5°C more favored nucleation; consequently, the highest extent of retrogradation existed but the least perfect crystallites were formed.

In the aspect of water content, T_o and T_p of gel containing 60% water was lower than those of 70 and 80% water ($p<0.05$) (data not shown). The T_c-T_o at 60% water content was also larger (Fig. 6). Thus, in a higher water system, water acting as plasticizer

facilitated structural reorganization to be more ordered, in that the crystallites were more perfect.

The comparative XRD patterns of native and retrograded starch were presented in Fig. 7. Native cassava starch showed an A-type pattern. At different storage temperatures, diffractogram of retrograded starch stored at -20, 5 and 25°C contained peaks at 2θ of 17 and 23°, which were a typical pattern of retrograded starch (B-type), while a small peak at 2θ of 17 and 18° was noticed for the gel stored at 45°C, which resembled the A-type. It suggested the influence of storage temperature on the formation of polymorphism. The polymorphism of recrystallized starch was essentially dependent on water content and storage temperature. The A-polymorph was favored in condition of low water content and higher storage temperature, as opposed to the B-polymorph, which was favored at higher water content and lower storage temperature [5]. At the same storage temperature, water content and time did not affect the diffractogram pattern in the present result.

Conclusions

Cassava starch underwent different retrogradation behaviors under different storage conditions. In excess water system, the lower storage temperature was, the higher the rate and extent of retrogradation for long term storage, except subfreezing temperature storage. Storage at subfreezing temperature had a consequence of higher concentrated gel that facilitated the initial progress of retrogradation but this influence was less pronounced in small sample size such as DSC method. Although the rate of retrogradation during the first period of storage was high for the lower water content, the maximum extent of retrogradation could be obtained in a higher water content system with low temperature storage depending on storage time and monitoring techniques. This can be extended to an application for obtaining high amount of retrograded starch for enzyme resistant starch production.

The combined effect of water content and storage temperature on retrogradation behavior appeared some differences with various monitoring techniques, suggesting that each technique which probed reordering process with different ranges of structure could have sensitivity and limitation on different systems. However, it was obvious that FTIR was sensitive to changes in short-range molecular order and precede XRD which detected long-

range order structure of molecular packing into crystalline lattices. At macromolecular structure, the development of textural firmness, which was monitored by uniaxial compression test, occurred in a latter stage of retrogradation. For relatively high water content and high temperature storage, FTIR and compression test were not appropriated methods to follow the retrogradation process due to their less sensitivity.

Storage temperature had an influence on polymorphism of recrystallized cassava starch. The high temperature storage of 45C led to the formation of the A-type polymorph with more perfect and homogeneous crystalline structure.

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Tab. 1 The rate constant and final values of degree of retrogradation monitored using XRD and calculated by fitting the Avrami kinetic equation to the data.

Water content [%, w/w]	Storage temperature [°C]	Rate constant, k [day^{-1}]	Final value [%]
60	-20	0.620	16.91
	5	0.343	30.07
	25	0.211	12.86
	45	0.060	12.98
70	-20	0.135	16.48
	5	0.141	21.88
	25	0.082	13.84
	45	0.045	12.96
80	-20	0.157	16.8
	5	0.113	24.3
	25	0.041	18.07
	45	0.044	9.41

List of Legend

Fig. 1. Relative crystallinity of cassava starch gel containing (a) 60%, (b) 70%, and (c) 80% water content stored at -20°C (○), 5°C (▽), 25°C (□), and 45°C (◇) as a function of time. The Avrami equation was fitted to the data as shown by different lines.

Fig. 2. FTIR Absorbance ratio of cassava starch gel containing (a) 60%, (b) 70%, and (c) 80% water content stored at -20°C (○), 5°C (▽), 25°C (□), and 45°C (◇) as a function of time.

Fig. 3. Compression force ratio of cassava starch gel containing (a) 60%, (b) 70%, and (c) 80% water content stored at -20°C (○), 5°C (▽), 25°C (□), and 45°C (◇) as a function of time.

Fig. 4. Degree of retrogradation of cassava starch gel (a) containing 60% water content stored at -20°C (○), 5°C (▽), 25°C (□), and 45°C (◇) and (b) containing 60% (○), 70% (▽), and 80% (□) water content at 5°C.

Fig. 5. Melting characteristic of retrograded cassava starch containing 60% water content stored at -20°C (○), 5°C (▽), 25°C (□), and 45°C (◇) as a function of time.

Fig. 6. Melting characteristic of retrograded cassava starch containing 60% (○), 70% (▽), and 80% (□) water content stored at 5°C as a function of time.

Fig. 7. X-ray diffraction pattern of native and retrograded cassava starch (a) 60% water content stored at 45°C, (b) 80% water content stored at 25°C, (c) 80% water content stored at -20°C, and (d) 80% water content stored at 5°C.

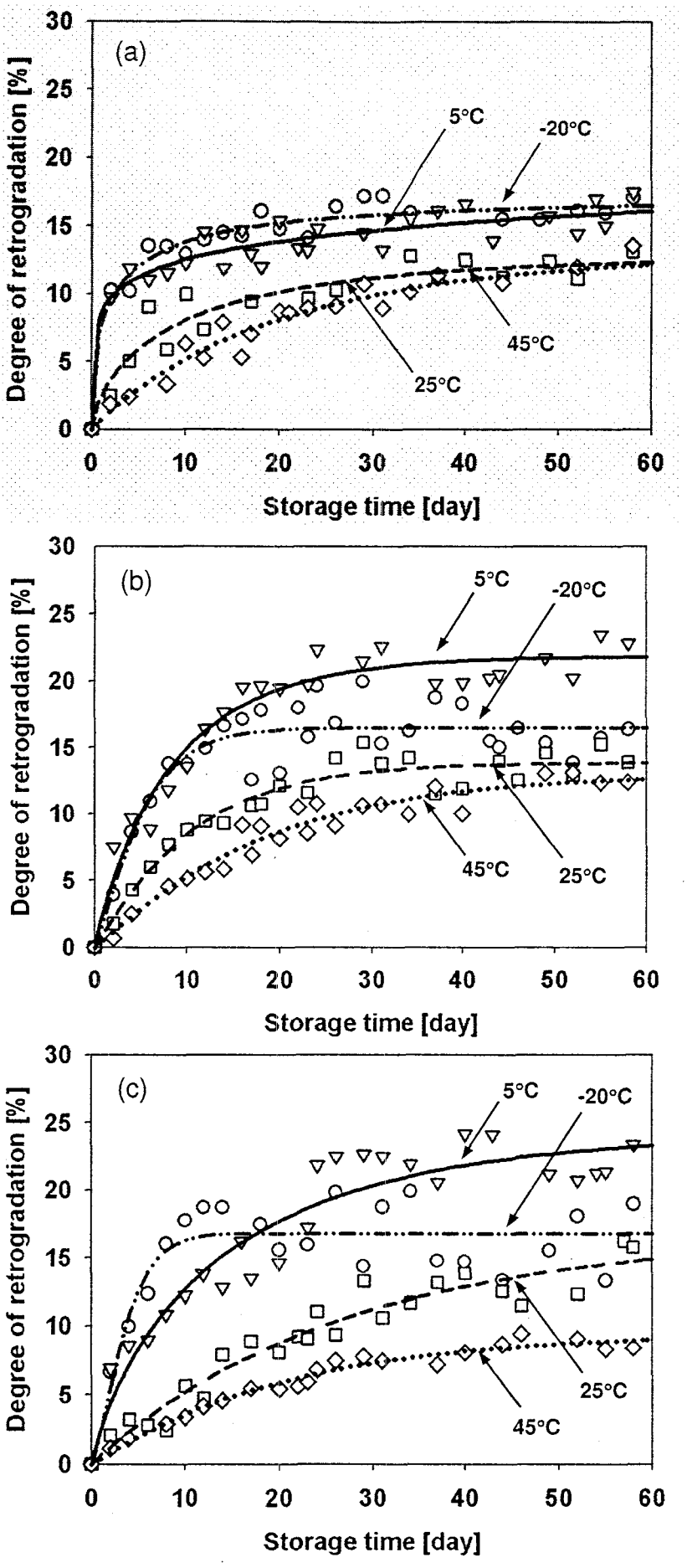
(a)
5°C
-20°C
45°C
25°C
Degree of retrogradation [%]
Storage time [day]
(b)
5°C
-20°C
45°C
25°C
Degree of retrogradation [%]
Storage time [day]
(c)
5°C
-20°C
25°C
45°C
Degree of retrogradation [%]
Storage time [day]

Fig. 1.

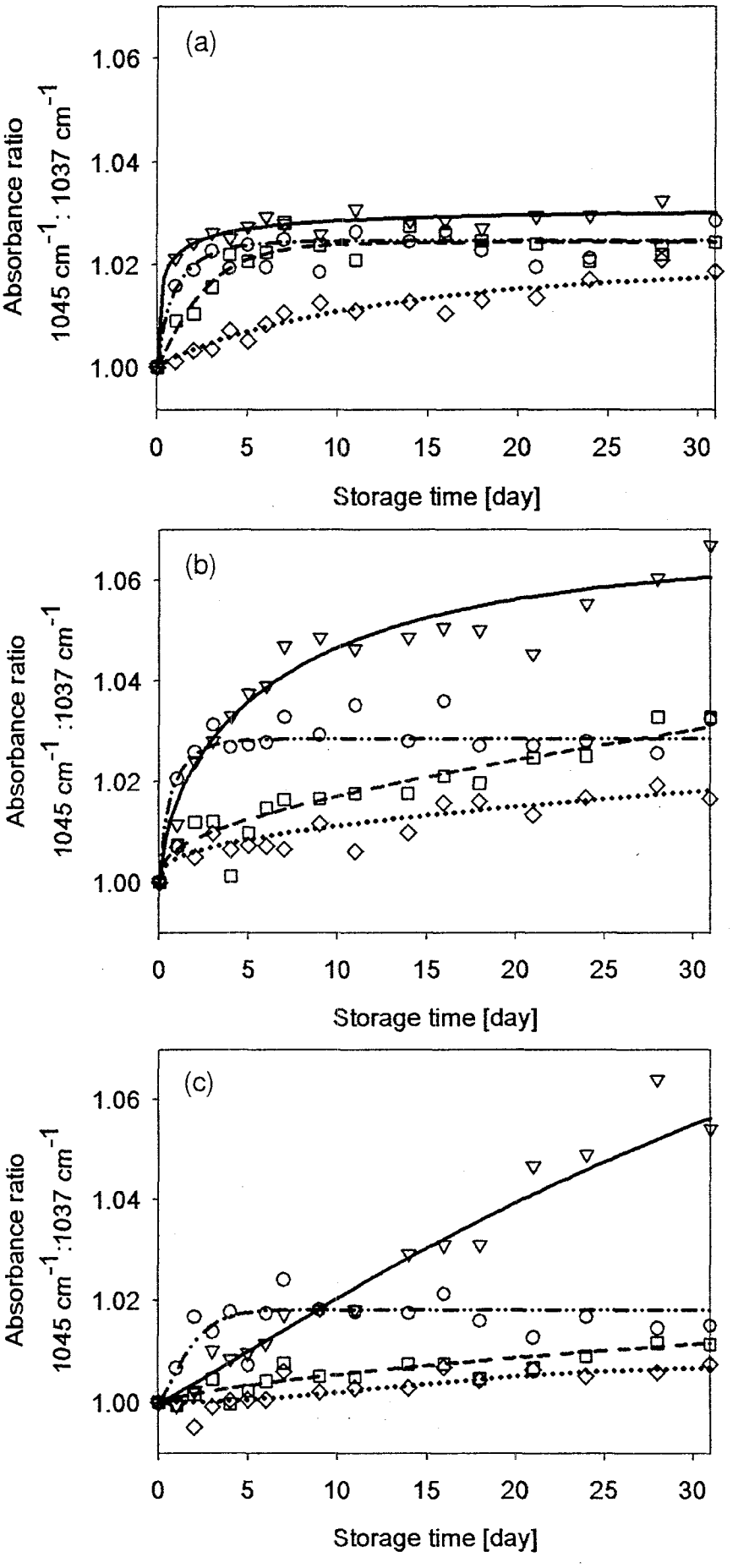
(a)
Absorbance ratio
1045 cm-1 : 1037 cm-1
1.06
1.04
1.02
1.00
0
5
10
15
20
25
30
Storage time [day]
(b)
Absorbance ratio
1045 cm-1 :1037 cm-1
1.06
1.04
1.02
1.00
0
5
10
15
20
25
30
Storage time [day]
(c)
Absorbance ratio
1045 cm-1 :1037 cm-1
1.06
1.04
1.02
1.00
0
5
10
15
20
25
30
Storage time [day]

Fig. 2.

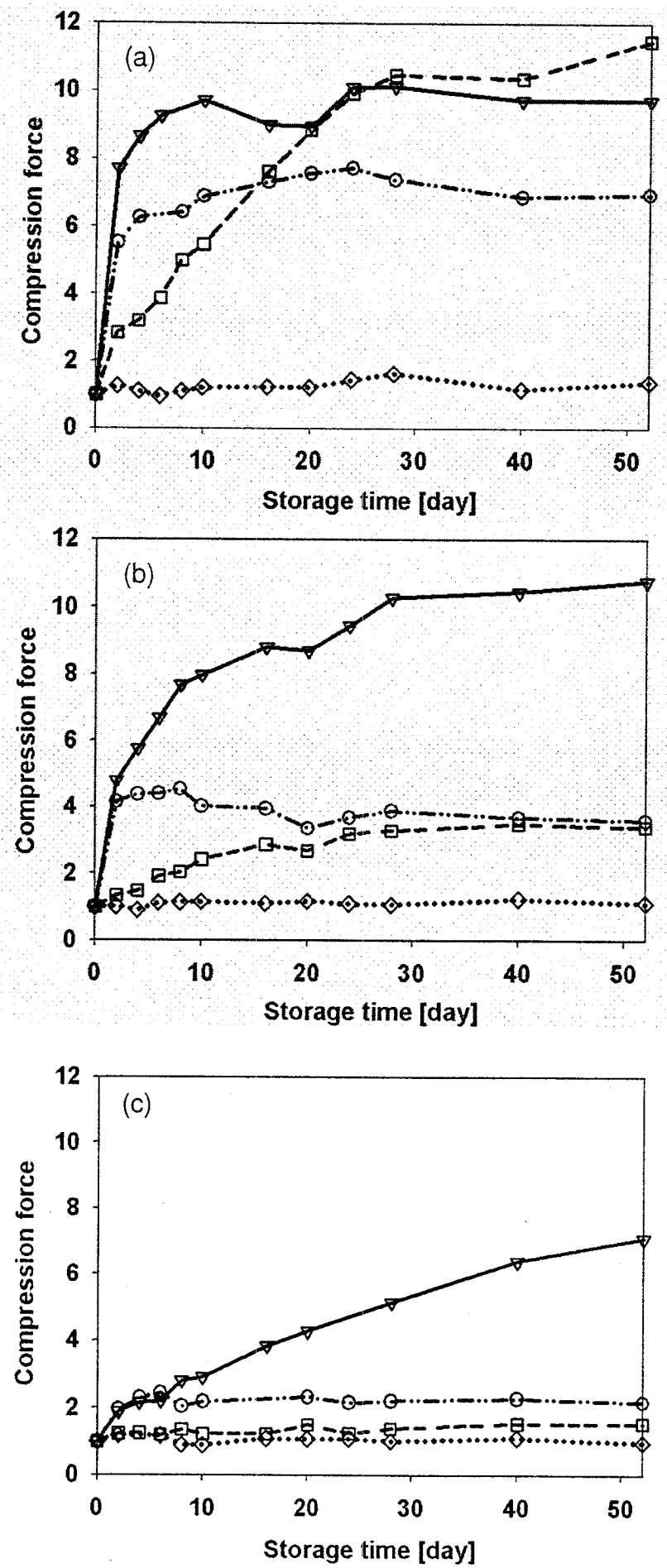
(a)
Compression force
Storage time [day]
(b)
Compression force
Storage time [day]
(c)
Compression force
Storage time [day]

Fig. 3.

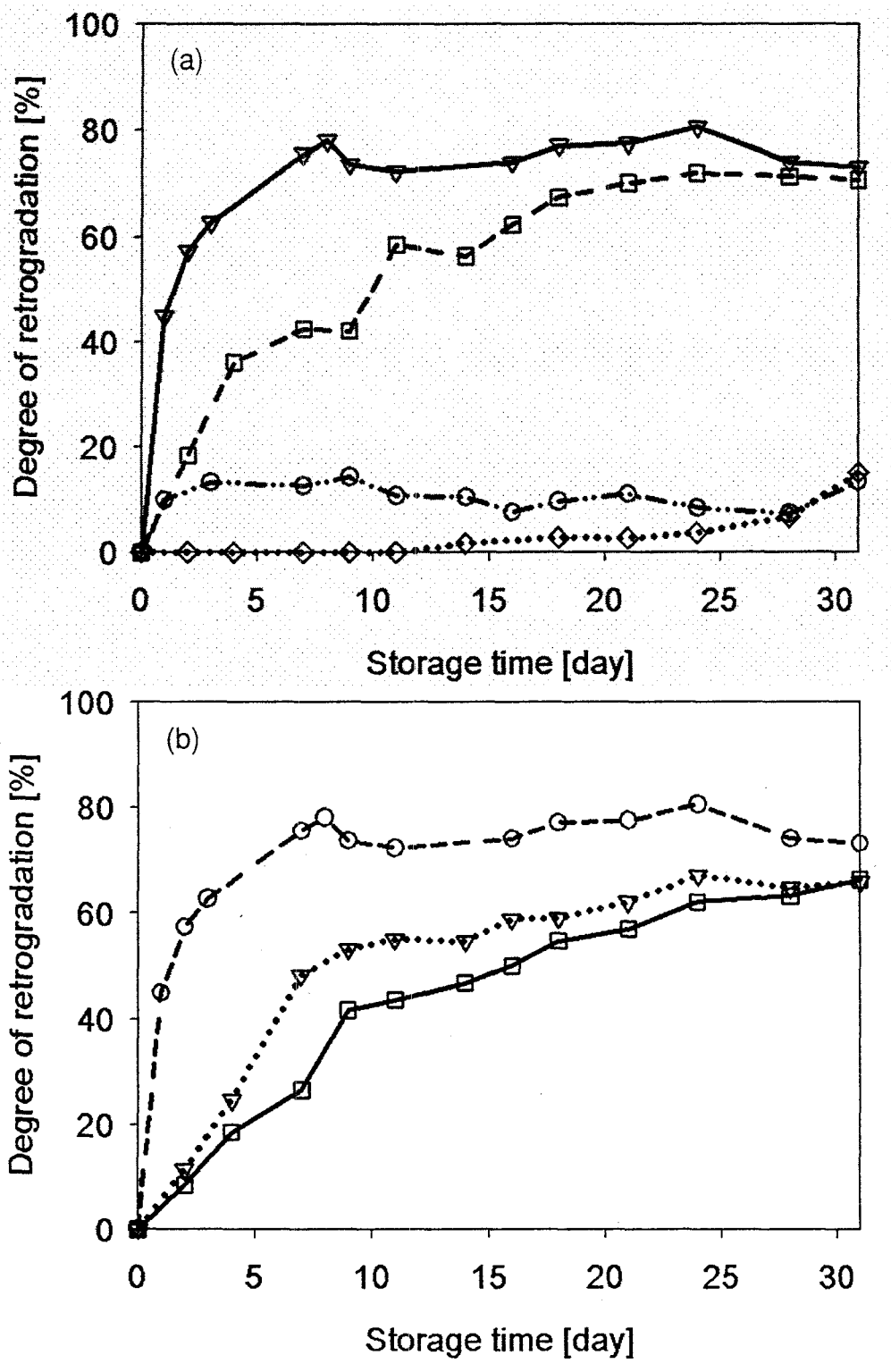
(a)
Degree of retrogradation [%]
100
80
60
40
20
0
0
5
10
15
20
25
30
Storage time [day]
(b)
Degree of retrogradation [%]
100
80
60
40
20
0
0
5
10
15
20
25
30
Storage time [day]

Fig. 4.

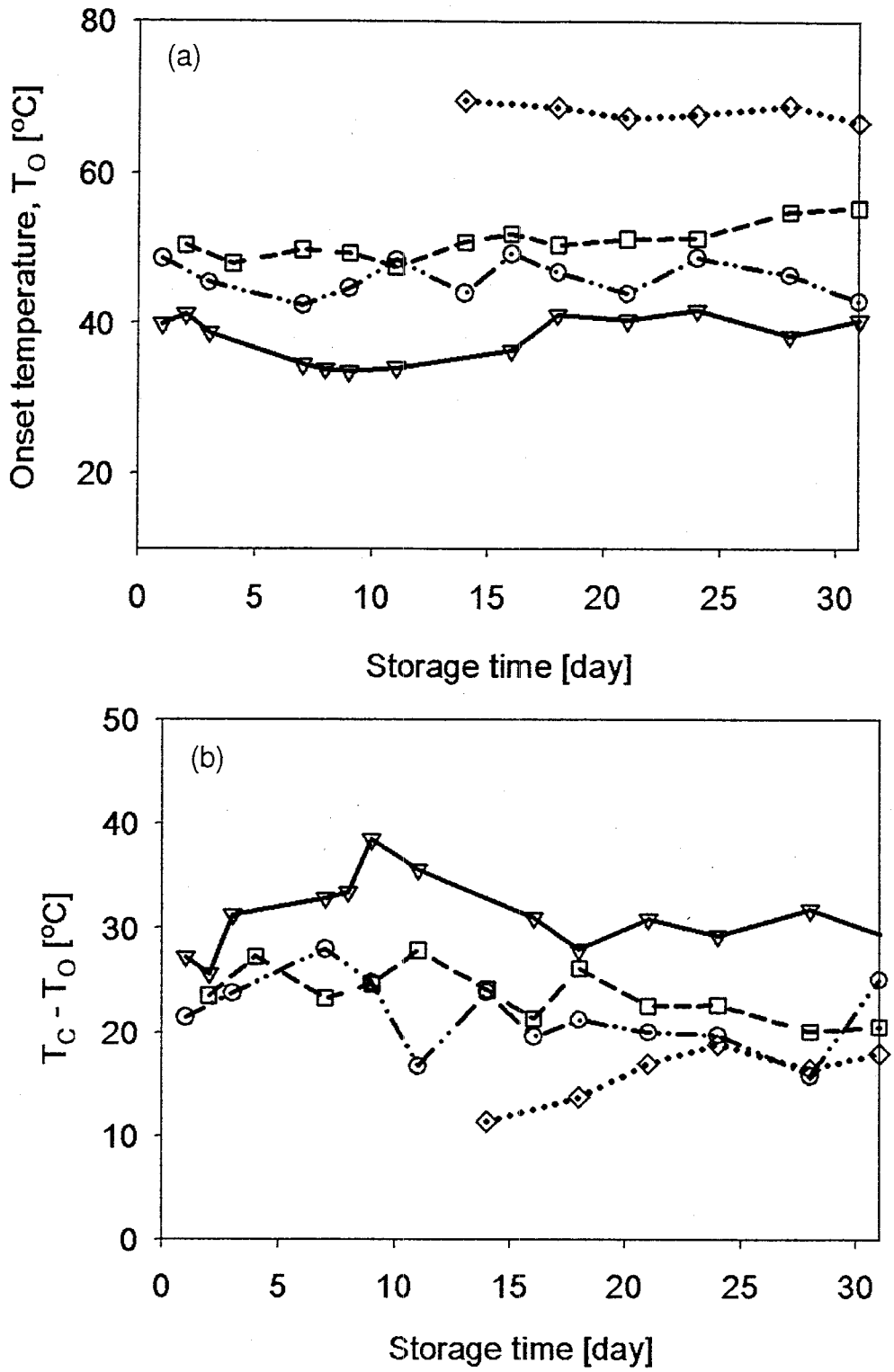
(a)
Onset temperature, T_O [°C]
80
60
40
20
0
5
10
15
20
25
30
Storage time [day]
(b)
$T_C - T_O$ [°C]
50
40
30
20
10
0
0
5
10
15
20
25
30
Storage time [day]

Fig. 5.

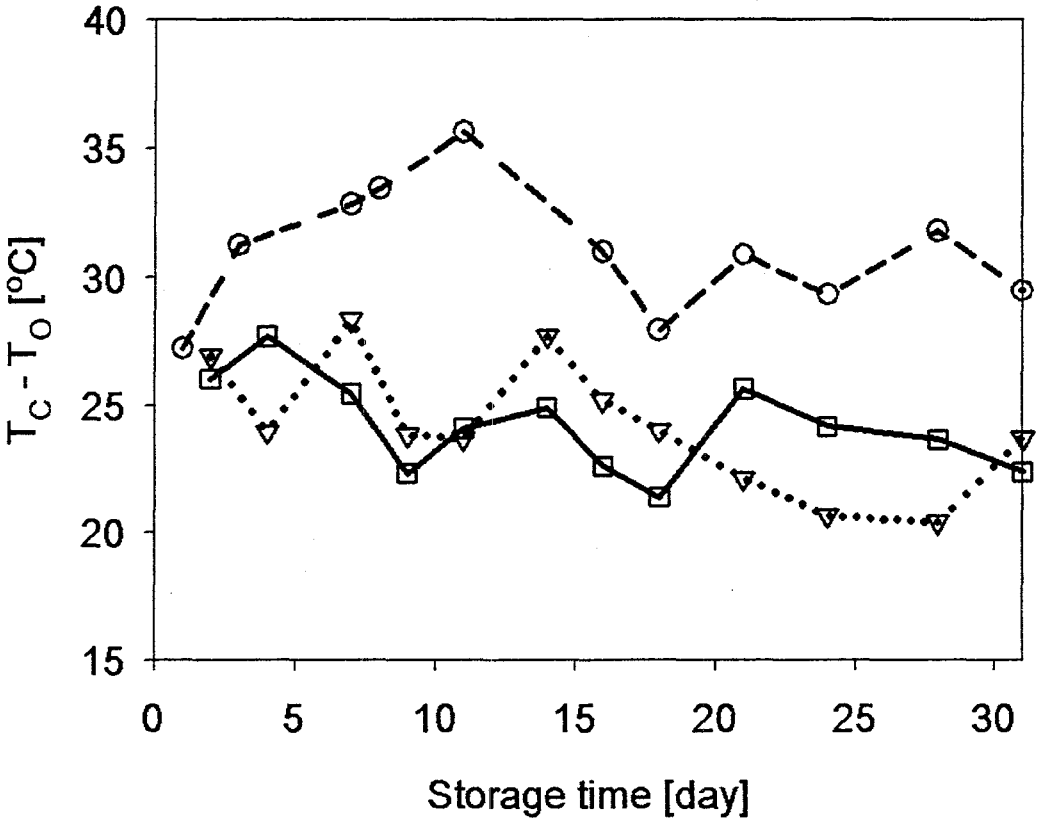

Fig. 6.

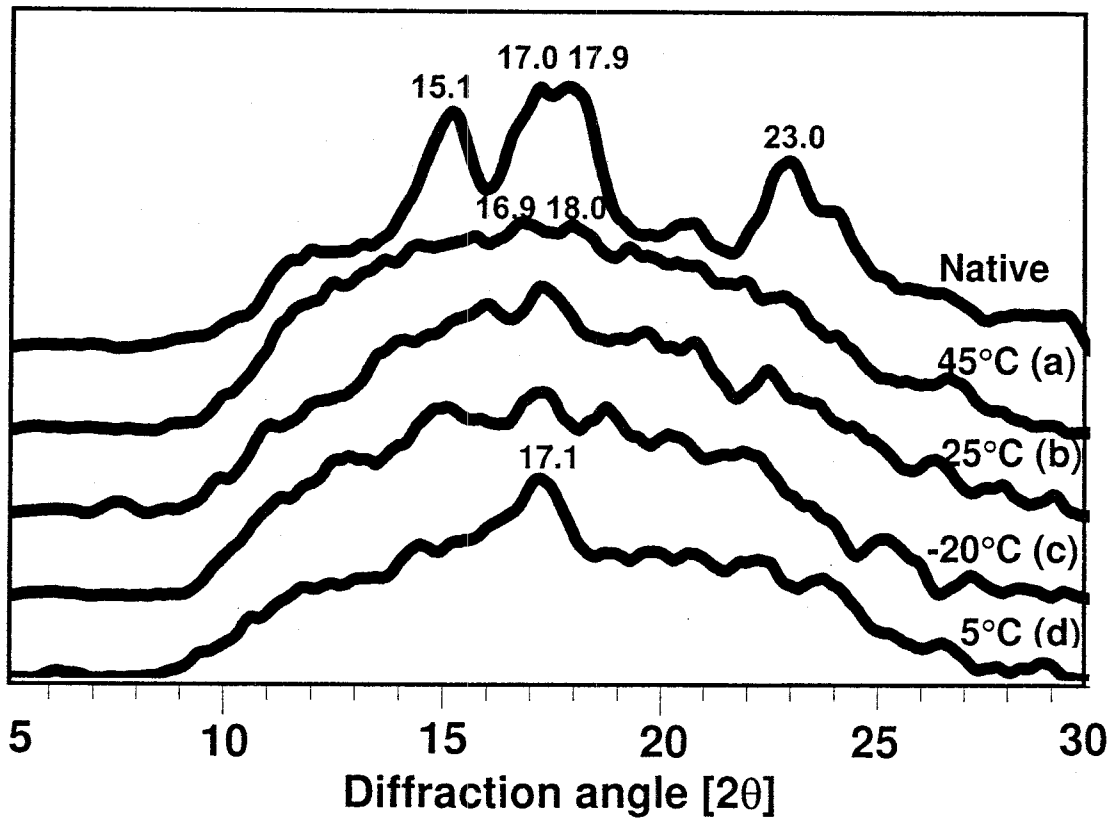
17.0 17.9
15.1
23.0
16.9 18.0
Native
45°C (a)
25°C (b)
17.1
-20°C (c)
5°C (d)
5
10
15
20
25
30
Diffraction angle [2θ]

Fig. 7.