Effect of Storage Time on the Retrogradation of Banana Starch Extrudate

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Starch was isolated from banana starch and the retrogradation phenomenon was studied using diverse techniques, including an enzymatic measurement. Wide-angle X-ray scattering (WAXS) showed that the sample stored for 7 h presented small peaks and when the storage time increased the peaks increased in intensity. The type of diffraction pattern found in banana extrudates is typical of the A-type crystal polymorph. The crystallinity index from the diffractograms, showed a plateau after $\sim$20 h of storage. The short-range order measurement with Fourier transform infrared (FTIR) spectroscopy showed that banana starch retrogradation reached a maximum value at approximately 11 h of storage, a value that agrees with the results obtained with differential scanning calorimetry (DSC), because the maximum enthalpy value (approximately 5 J/g) was calculated in the stored sample for 8 h, without changes in the stored samples for more time. Retrograded resistant starch values did not change after 12 h of storage, obtaining the maximum starch retrogradation level. FTIR, DSC, and the enzymatic technique showed the changes at the molecular level in starch during storage; in the case of WAXS, they determine the long-range order that explains the differences found in the starch retrogradation pattern measurement in banana starch.

KEYWORDS: Banana starch; retrogradation; X-ray diffraction; infrared spectroscopy; differential scanning calorimetry

INTRODUCTION

Bananas are grown extensively in both tropical and subtropical regions and is an important food crop. Although the composition of banana fruit has been defined, comparatively little work has been carried out on the starch (*1*, *2*). Chiang et al. (*3*) reported banana starch production on a pilot scale using different temperatures and milling processes. Their results showed that remilling the pomace once could increase the yield of dried starch powder from approximately 46% to 70%. However, the purity of the product dropped from 97% to 94%. The banana is a climacteric fruit and, in México, is consumed when the fruit is ripe. For this reason, many fruits are lost during their commercialization due to deficient postharvest handling. Starch is the principal component of green bananas that is changed during ripening. Lii et al. (*2*) investigated changes in physical and chemical properties of banana starch, as well as banana components, during ripening.

Starch is the major dietary component in all human populations. The consensus on healthy eating habits favors an increment in the proportion of polymeric plant carbohydrates (including starch) in the daily diet. However, in our culture, the main purpose of starch utilization in foods remains aesthetic rather than nutritional. This biopolymer constitutes an excellent raw material to modify food texture and consistency. Not only is the amount of starch important for the texture of a given food product, but starch type is equally critical (*4*).

Starch is deposited in the form of partially crystalline granules, whose morphology, chemical composition, and supermolecular structure are characteristic of each particular plant species. Starch owes much of its functionality to two major high-molecular-weight carbohydrate components, amylose and amylopectin, as well as to the physical organization of these macromolecules into the granular structure (*5*). When starch is cooked in excess water, the granules swell and, at the same time, part of the components solubilize, giving rise to a suspension of swollen particles dispersed in a macromolecular continuous phase (*6*). Starch pastes can be considered as mixtures of swollen and fragmented granules embedded in a continuous phase that contains the macromolecular components leached out from the granule when starch is cooked (*7*). These events are described as starch gelatinization. When starch pastes are stored for minutes or days, retrogradation occurs and is believed to be responsible for the textural and starch digestibility changes that take place during storage of starch-based products

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(*8*). Starch retrogradation is a process when gelatinized starch returns from a solvated, dispersed, or amorphous state to an insoluble, aggregated, or crystalline condition (*9*). This phenomenon is understood as a nonequilibrium, thermo-reversible, recrystallization process, which takes place in three consecutive steps: nucleation, propagation, and maturation. The retrogradation phenomenon varies depending on the starch botanical source, as the starch molecular structure will differ depending on the botanical source and the amylose content. A number of techniques has been used to study starch retrogradation (*10*), among them X-ray diffraction (*8*, *11*), differential scanning calorimetry (*12*, *13*), Fourier Transformed Infrared spectroscopy (*14*−*16*), nuclear magnetic resonance spectroscopy (*8*, *9*) and rheological tests (*17*)

In this work, the retrogradation of banana starch is measured using different analytical techniques and the results will be related to the determination of retrograded resistant starch using a multi-enzymatic procedure. Until today, there have not been studies related with the retrogradation phenomenon in this starch isolated from a nonconventional source.

MATERIALS AND METHODS

Starch Isolation. Unripened bananas (*Musa paradisiaca*) were purchased in the local market of Cuautla, Morelos, México. The starch was isolated using a pilot scale procedure. The fruits were peeled**,** cut into 5−6 cm cubes (100 kg total wt)**,** immediately rinsed in citric acid solution (0.5 g/L), and then macerated at low speed in a Waring blender (10 kg fruit:10 L of solution) for 2 min. The homogenate was consecutively sieved through screens (20 (0.85 mm), 40 (0.38 mm), 100 (0.15 mm), and 200 (0.075 mm) US mesh) until the wash water (distilled) was clean; then it was centrifuged in a semicontinuous centrifuge Veronesi (BSGAR 1500, Verona, Italy) at 10 750 rpm. The sediments from 100 and 200 US mesh were washed, and the sediment was centrifuged a second time. The processes of sieving and centrifuging for isolation and starch purification were carried out three times. The white-starch sediments were dried in a spray dryer (Niro Atomizer, Model P-6.3, Copenhagen, Denmark), with a feed temperature of 130−150 °C, a solid concentration in the feed line of 30−40%, and an outlet temperature of 70−80 °C. The powder was ground to pass a US No. 100 sieve and stored at room temperature (25 °C) in a glass container. Moisture content was determined using 3−4 g of sample in an oven at 105 °C for 14 h.

Extrusion. Nonexpanded ribbons were prepared (30% (±1) moisture content in wet weight basis, wwb) by extrusion through a 1 mm × 30 mm slit die using a Clextral Bc-21 co-rotating intermeshing, twin-screw extruder. The following were the extrusion conditions: screw speed of 300 rpm, solid feed rate of 5 kg/h, temperature profile of 20, 90, 120, and 80 °C. Distilled water was pumped into the second zone at a flow rate of 1.8 L/h. Upon exiting the extruder, the samples were quickly heat-sealed inside aluminum bags and quenched in liquid nitrogen. This was done so that a sample from the same extrusion batch could be used for analysis with different techniques at different times and to allow replications.

Wide-Angle X-Ray Scattering (WAXS). Samples stored at 25 °C for different times were analyzed using a Bruker D5005 wide-angle X-ray diffractometer equipped with a copper source operating at 40 kV and 30 mA producing a Cu K_α radiation with a wavelength of 1.54 Å was used. Data were collected over the 2θ range 4−38° at 0.1° intervals with a scanning rate of 60 s/°. The spectra were holder-subtracted and baseline-corrected over the 4−38° range utilizing the OPUS 3.0 software (Bruker, UK) before calculating the crystallinity indices using the correlation method of Wakelin et al. (*18*).

Fourier Transform Infrared (FTIR) Spectroscopy. The mid-infrared spectra were collected using a Bruker IFS48 infrared spectrometer (Bruker, UK) equipped with a DTGS detector and a heated single reflectance ATR cell with a diamond crystal (Graseby-specac Ltd., UK). A sealed sapphire anvil was used that had a rubber O-ring to minimize moisture loss from the samples during measurements. The

sample was placed onto the ATR Golden Gate cell and sealed with the sapphire anvil from the atmosphere. Additionally, Teflon tape was placed around the edge of the anvil to try to limit any moisture loss from the sample as much as possible. Spectra were collected every 1 h, and duplicate measurements were carried out. For each spectrum, 32 scans were acquired at a resolution of 4 cm^{-1} and co-added. All sample measurements were recorded at a temperature of 25 °C. The ATR cell, excluding the sample compartment that was sealed, was continuously being purged with dry air in order to minimize the water vapor contribution to the spectra. Spectra were acquired using the standard direct mode where the background was recorded with no sample in the ATR cell in order to monitor the changes occurring during starch aging.

Data analysis was carried out using the OPUS 3.0 software (Bruker, UK) To analyze the carbohydrate region of the spectra (1200−800 cm^{-1}), and baseline correction was applied using a single point at 1900 cm^{-1}. The spectra were then deconvoluted (*19*) in the above-mentioned region, where the assumed line shape was Lorentzian, with a deconvolution factor of 750 and a noise reduction factor of 0.2.

Differential Scanning Calorimetry (DSC). The temperature and enthalpy of the melting transition of the extrudate was studied using a differential scanning calorimeter DSC 7 (Perkin-Elmer, UK), previously calibrated with indium and cyclohexane. The samples were weighed (between 2.5 and 3.5 mg sample, dry basis) in an aluminum pan, adding excess water (approximately 3 times more that the amount of sample). The pans were hermetically sealed and allowed to stand overnight on a roller mixer before carrying out the analysis. An empty aluminum pan was used as a reference. The sample was subjected to a heating program over a temperature range from 0 to 95 °C using a heating rate of 10 °C/min. The transition or peak temperature (T_p) and the transition enthalpy (ΔH), were obtained directly using the accompanying Pyris.

Retrograded Resistant Starch. The retrograded resistant starch (RRS or RS3) content was measured as the starch remnants in enzymatically obtained dietary fiber residues, according to the so-called "Lund method" as modified by Saura-Calixto et al. (*20*). In brief, the sample (100 mg) was suspended in 10 mL of phosphate buffer (0.08 M, pH 6.9) and incubated with 10 μL of α-amylase (Termamyl Novo A/S, Copenhagen) in a boiling water bath for 35 min. Incubation with 100 μL of protease at 60 °C for 35 min and thereafter with 60 μL of amyloglucosidase A-3176 (Sigma Chemical CO., St. Louis, MO) at 60 °C for 30 min. The mixture was centrifuged at 3000 g for 15 min, the residue was washed with alcohol and acetone, centrifuged, and the residue resuspended in 3 mL of distillated water and 3 mL of KOH 4 M; the pH was adjusted to 4.75 and incubated with 60 μL of amyloglucosidase (Boehringer, Mannheim, Germany) at 60 °C for 30 min. The mixture was centrifuged at 3000 g for 15 min, and the supernatant was used for glucose oxidase peroxidase assay (SERA-PAK Plus, Bayer de México, S. A. de C. V., Edo. de México).

Statistics. Results were expressed by mean values ± standard error of the three separate determinations. The comparison of means was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the SPSS statistical program (v. 2.03, SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Wide-Angle X-Ray Scattering (WAXS). WAXS measurements performed after storage of the samples for 0 and 3 h were diffuse (**Figure 1**). This pattern is typical for amorphous systems, indicating that the crystalline structure was disorganized during the extrusion process due to complete starch gelatinization. The sample stored for 7 h show broad peaks at $2\theta = 17°$ and 23°. When the storage time is increased to 14 h and more, the peaks mentioned above increased in intensity and a peak at $2\theta = 15°$ appears as well (**Figure 1**, see arrows). This type of diffraction pattern is typical of the A-type crystal polymorph. However, for the banana starch, where the native crystal polymorph was found to be a mixture of the A- and B-types, on retrogradation at 25 °C, the polymorph formed was the A-type. In a study using gelatinized potato starch, when the
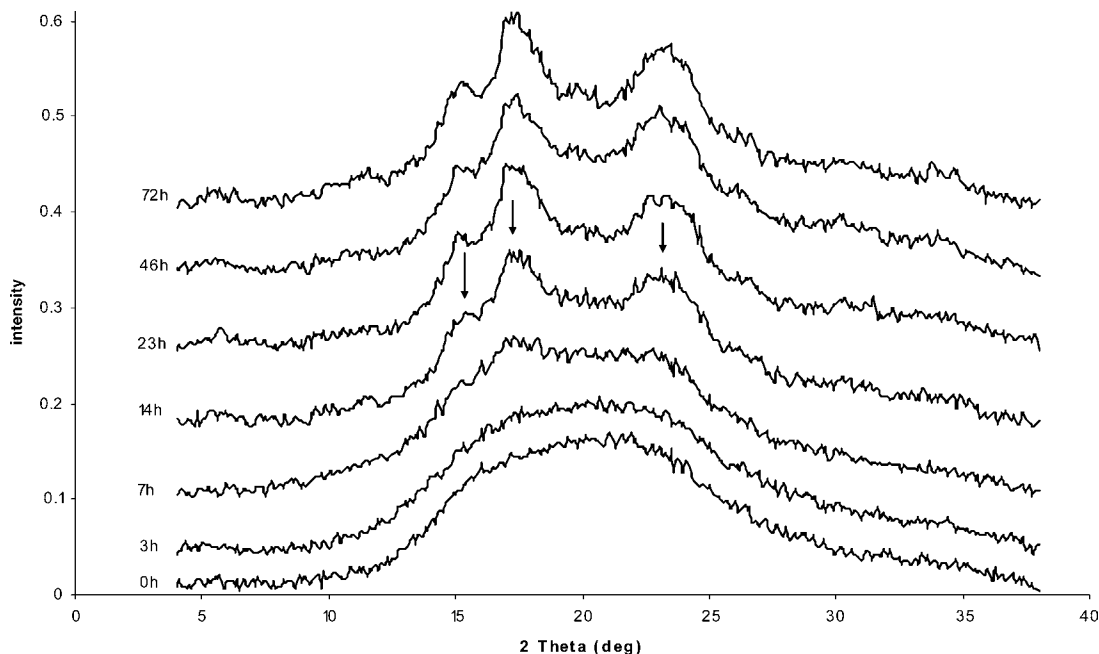
Figure 1. X-ray diffractograms of retrograded starch extrudates stored at 25 °C for different times.
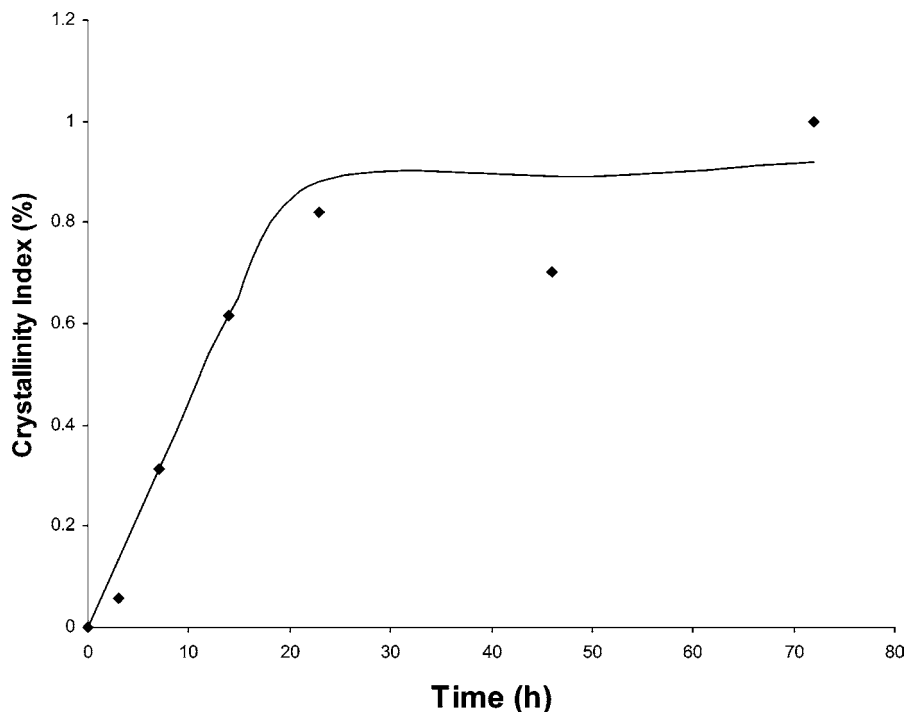

Figure 2. Change in the crystallinity index for banana extrudates stored for different times.

starch sample was stored at 22 °C for 14 days (35% moisture (wwb)), showed the same X-ray diffraction pattern (B-type) as the native sample. In that study, it was mentioned that high storage temperatures produce a change in the diffraction pattern of the retrograded samples to the A-type (*11*). These results shown that the polymorph formed depends on the storage temperature and starch type. The changes in long-range ordering occurring over time were calculated using the crystallinity index **(Figure 2**) from the diffractograms, where a plateau was reached after ∼20 h of storage. Banana starch shows a fast retrogradation kinetic that could be related to the amylopectin chain length, as shown in waxy maize starches, where they reported that amylopectin with longer chains has a higher retrogradation rate than those with a shorter chain, as measured by DSC (*13*). The

correlation method, developed by Wakelin et al. (*17*) showed that when the storage time increased, the crystallinity in the sample increased too. The crystalline standard was the sample stored for 72 h (line at 45°), and the amorphous standard was that at 0 h (horizontal line). Differences in the banana starch extrudates can be observed with time. It is important to mention that the starch botanical source plays an important role in the retrogradation phenomenon, but molecular studies are necessary for explaining this behavior and can determine which starch type should be used for a specific application.

Fourier Transform Infrared (FTIR) Spectroscopy. The deconvoluted spectra in the range 800−1200 cm^{-1}, acquired shortly after extrusion (0.5 h) and after 24 h of storage for the banana starch extrudate are shown in **Figure 3**. This region is
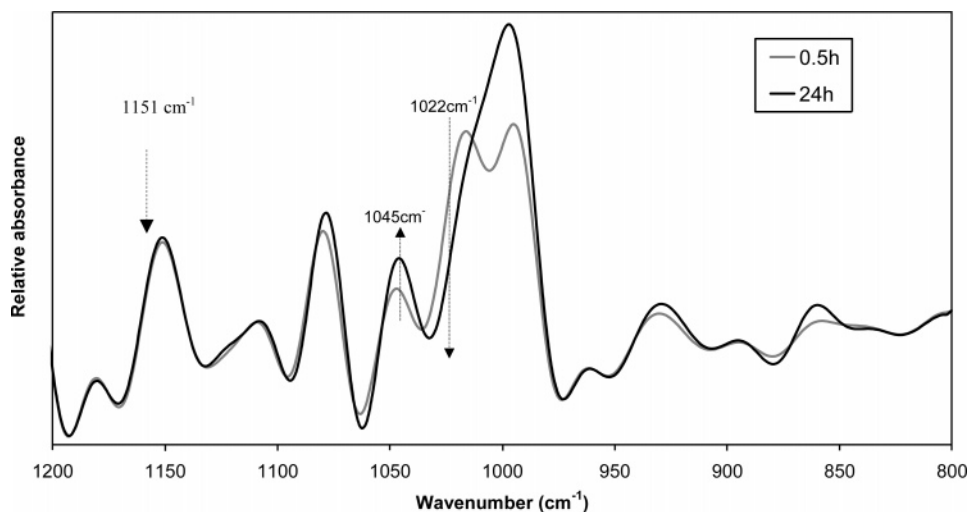
Figure 3. Mid-IR deconvoluted spectra of banana starch extrudates.
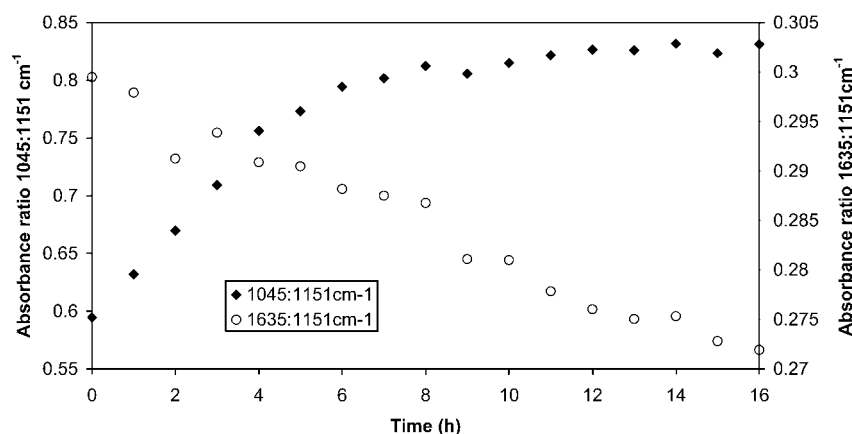

Figure 4. Changes in absorbance ratio for banana extrudates stored at 25 °C for different times.
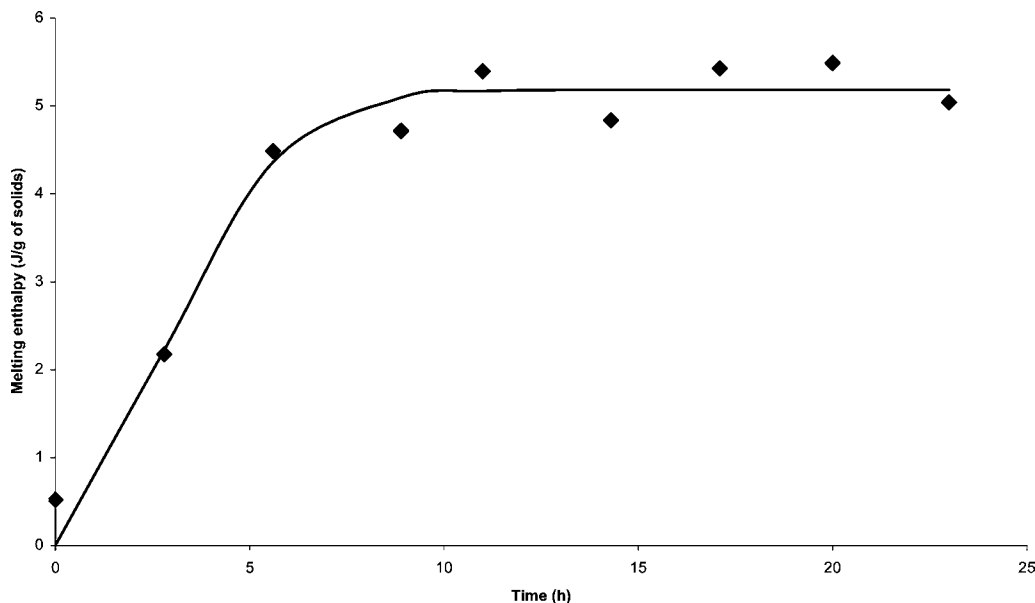

Figure 5. DSC melting enthalpy measured during storage at 25 °C.

comprised of a series of bands, mostly the result of C−O and C−C stretching vibrations, and is reported to be a very sensitive to the physical state of carbohydrates (e.g. *21*). The bands at 1045 and 1022 cm^{-1} are sensitive to the amounts of ordered and amorphous regions, respectively (*15*). The band at 1151 cm^{-1} was used as an internal correction standard (*14*). The ratio

of the bands at 1045 and 1151 cm^{-1} was used to follow the change in short-range order during the banana starch retrogradation. The absorbance ratio of the banana starch extrudate stored for different time periods is shown in **Figure 4**, where it is possible to observe that the ratio increases with increasing storage times, reaching a plateau after ∼11 h. The samples were

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Table 1. Retrograded Resistant Starch (RRS) of Banana Starch Extrudates Stored for Different Times[a]

storage time (h)	RRS (%)
0	1.24 ± 0.06
4	1.97 ± 0.14
8	2.59 ± 0.06
12	5.04 ± 0.06
16	4.87 ± 0.06
20	5.1 ± 0.06
24	5.25 ± 0.06

[a] Data are means ± SD; $n = 3$. Means in columns not sharing the same setter are significantly different ($p < 0.05$).

run in situ inside the ATR cell. This was attempted to minimize the moisture loss from the samples; however, it could not be entirely prevented. To monitor the moisture loss occurring during the experiment, the ratio of the bands at 1635 and 1151 cm^{-1} from the deconvoluted spectra were plotted against time, as shown in **Figure 4**. The band at 1635 cm^{-1} is related to O−H *def* and is thus directly related to the moisture content of the sample. A linear correlation has been found to exist between the moisture content of a retrograded starch sample and the band ratio 1635:1151 cm^{-1} (*22*). This ratio decreases from ~0.3 to 0.27, which equates to a moisture loss of ~4−5% (total wet basis). It was believed that a loss in moisture from the sample at this temperature would decrease the retrogradation kinetics, as was also observed to be the case for waxy maize starch (*8*).

Differential Scanning Calorimetry (DSC). The DSC results of the melting of the retrograded samples in excess water showed an increase in the melting enthalpy on storage, believed to be related to the order formed in the sample during retrogradation. The changes in melting enthalpies obtained upon storage (**Figure 5**) showed that a plateau is reached after ~8 h, a value that agrees quite well with that obtained for FTIR, which also monitors short-range ordering. In the case of X-ray results, the crystallinity was only constant after 20 h of storage, as this technique is only sensitive to changes in the long-range order of the sample. The overlapping of amylose chains in order to obtain an ordered structure and realignment of amylopectin chains upon recrystallization are carried out within ~24 h, with the order being less perfect in the retrograded sample (plateau $\Delta H = 5$ J/g of solids in excess water) than in the native starch ($\Delta H = 23$ J/g of solids in excess water).

Retrograded Resistant Starch (RRS). The banana starch extrudates presented increased RRS values with the storage time (**Table 1**). This technique was used for measuring the retrograded starch formed in the sample during storage. The RRS values after 12 h were constant, indicating that starch retrogradation in the sample reaches the maximum value and no more starch chain association is carried out upon storage. These results agree with those found with FTIR (~11 h) and DSC (~8 h). These techniques can be complementary for starch retrogradation measurements, and it is possible to know the behavior of starch in food systems during storage.

Conclusions. It was found that the banana starch extrudate, when stored at 25 °C and at 30% moisture content (wwb), retrograded from its native mixture of the A- and B-polymorphs, to the A-polymorph. The techniques used, WAXS (~20 h), FTIR (~11 h), DSC (~8 h), and the enzymatic method (~12 h), indicated that the retrogradation was completed within the first ~20 h of storage. The maximum retrogradation enthalpy obtained with the DSC was ~5 J/g of solids, which was much lower than that obtained for the native starch, indicating a lower and less perfect degree of order in the retrograded sample. The

RRS value after 12 h of storage was approximately 5%. This research aims to further the knowledge of the retrogradation properties of this relatively novel starch.

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