

EFFECT OF HYDROCOLLOIDS ON STARCH THERMAL TRANSITIONS, AS MEASURED BY DSC

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Abstract

Differential scanning calorimetry (DSC) was used to analyze the influence of different hydrocolloids (xanthan, guar, and locust bean gums, carboxymethylcellulose and sodium alginate) on the gelatinization of corn starch in systems with starch concentration ranging between 0.1 and 0.7 g starch/g mixture. The reduction of available water produced a shift in gelatinization temperature, especially of the conclusion temperature. The effect was more marked for ionic hydrocolloids.

The influence of hydrocolloids on glass transition temperature (T_g) of gelatinized starch suspensions and on the glass transition temperature of the maximally freeze-concentrated solute/unfrozen water matrix (T'_g) was also studied. T'_g onset values ranged between -4.5 and -5.5°C for corn starch pastes with and without hydrocolloids. Those hydrocolloids that increased the viscosity of the unfrozen matrix inhibited additional ice formation during thawing (devitrification).

Starch concentration and final heating temperature were found to be relevant factors affecting the kinetics of amylopectin retrogradation during frozen storage at -4°C . Xanthan gum failed to prevent amylopectin retrogradation; this observation could be attributed to the fact that gums act outside the starch granule, while amylopectin retrogradation takes place within the granule.

Keywords: annealing, frozen storage, gelatinization, glass transition, hydrocolloids, retrogradation, starch pastes

Introduction

Starch gelatinization and retrogradation have been widely studied, because of the relevance that both phenomena have to food preparation and storage. The roles that amylose and amylopectin play during retrogradation have been thoroughly analyzed by Miles *et al.* [1]. Today, polymer physical chemistry principles are used to help understand starch behavior [2-7]. According to these concepts, water acts as a plasticizer during starch gelatinization, lowering the melting temperature (T_m) of the starch. A glass transition [5] occurs when a completely amorphous polymer undergoes a second-order transition from a metastable amorphous glassy solid state to a rubbery liquid state (with greater mobility) at

a characteristic temperature (T_g). Partially crystalline polymers such as starch show two characteristic transitions, one at T_g for the amorphous component and the other at T_m (which is always higher than T_g for homopolymers), corresponding to a first-order transition from the crystalline solid to the amorphous liquid [8]. Starch retrogradation can be interpreted as a recrystallization occurring at temperatures between T_g and T_m , where nucleation and propagation processes can occur. According to the Williams-Landel-Ferry theory, the driving force for such a recrystallization process is $\Delta T = T - T_g$ [9].

Even before these theories were applied to starch, problems such as retrogradation, syneresis and loss of original texture, inherent to the formulation and preservation of foods based on starch, had already been described [10–16]. To obtain certain textural qualities and avoid undesired phenomena (such as exudate production during storage), different hydrocolloids are often added as stabilizers. These substances have high molecular weights and are highly hydrophilic; they are generally polysaccharides of various origins. In previous work [17–19], the use of xanthan gum in starch-based formulations was shown to reduce syneresis and the development of undesired textures attributed to amylose retrogradation during freezing and frozen storage.

The objectives of the present work were to analyze, by means of DSC: 1) the influence of different hydrocolloids on starch gelatinization and on glass transition temperatures in frozen suspensions of gelatinized starch, and 2) the influence of parameters such as final heating temperature during gelatinization, starch concentration, and presence of xanthan gum on the kinetics of amylopectin retrogradation during frozen storage.

Materials and methods

The following commercial hydrocolloids were used: xanthan gum, guar gum, locust bean gum, carboxymethylcellulose (CMC) and sodium alginate. Commercial corn starch (Refinerías de Maíz, Argentina), containing 0.11 g water, 0.006 g lipids, 0.003 g ash, 0.003 g proteins/g starch, and an amylose/amylopectin ratio of 25/75 (dry basis), was used in all experiments.

DSC

Measurements were performed with a DuPont 910 differential scanning calorimeter, equipped with a series 99 Thermal Analyzer (Wilmington, DE, USA) and a Hewlett Packard XY recorder (San Diego, CA, USA). The DSC cell was calibrated with indium; a coefficient, $E = 0.204$ (mW/mV), was obtained. Temperature corrections were made, as well. Onset, peak and conclusion temperatures (T_o , T_p , T_c) and areas under the curves were determined according to Lund's description [3]. Hermetically sealable, coated aluminum DSC pans

(TA Instruments, New Castle, DE, USA) were used. In the case of gelatinization runs and glass transition determinations, two empty pans (one inside the other) were sealed together and used as the reference. In the case of retrogradation runs, the reference was a pan containing distilled water. Runs were performed at least in triplicate.

To obtain the dry weight of the samples after the runs, the hermetically sealed DSC pans were punctured and dried, to constant weight, in an oven at 120°C.

Gelatinization assays at different hydrocolloid levels

To study the effect of hydrocolloid concentration, the sample pastes were formulated according to the following gum:starch ratios (weight basis): a) 1:10, b) 1:2 and c) 1:1. Total solids content was 0.10, 0.12 and 0.17 (g solids/g mixture), respectively.

Given the solubilization problems associated with hydrocolloids, especially when another hydrophilic component is present in the system (starch in this case), the following procedures were used: in cases a) and b), the hydrocolloid was dispersed in distilled water while the system was under constant agitation with a magnetic stirrer. Once the gum was totally solubilized (homogeneous, with no lumps), finely divided starch was added slowly with continuous agitation. The whole process was performed at room temperature.

In case c), the above procedure did not assure a homogeneous mixture; the hydrocolloid solution trapped so much water that the starch could not be hydrated. Favorable results were achieved by premixing hydrocolloid and starch under dry conditions; then the mixture was slowly sprinkled into water under constant agitation. Once prepared, samples were allowed to equilibrate for 12 h, to permit an adequate interdiffusion of the components and obtain homogeneous systems. Aliquots of about 15 mg were hermetically sealed in pans for DSC analysis of gelatinization. DSC curves were recorded using a heating rate of 10°C min⁻¹, over a temperature range of 20 to 150°C. The recording speed was 1 min cm⁻¹, and the sensitivity was 0.5 mV cm⁻¹.

Gelatinization assays at different starch concentrations and a fixed hydrocolloid concentration

To analyze the influence of hydrocolloids on pastes with different starch concentrations, gum solutions were prepared such that the system's final mass-fraction was 0.01 g gum/g mixture. After gum solubilization, starch was slowly added. Once a homogeneous paste was obtained, pans were filled with 15 mg aliquots for DSC analysis. To obtain homogeneity for pastes with high mass-fractions of starch (>0.50), the gum and starch were dry-mixed prior to solubilization. Control assays were run without hydrocolloid. Each paste was allowed to equilibrate for 12 h before DSC pans were filled.

Determination of T_g and T'_g

Glass transition temperatures of pastes with 0.50 g starch/g mixture, with and without hydrocolloid, were determined. Pastes were gelatinized by heating in the DSC to 85 or 120°C, to analyze the influence of final heating temperature on T_g . To obtain the T'_g of each system by DSC, samples were subjected to a slow freezing rate and then annealed. Thus, after gelatinization, samples were frozen in the DSC to a final temperature of -27°C, via cooling at 2°C min⁻¹, and after annealing for 30 min at a temperature near the estimated T'_g , they were heated at 2°C min⁻¹ to 20°C. In addition, assays at a higher cooling rate (14°C min⁻¹), and without an isothermal annealing period at -4.5°C, were also performed, to compare the behavior of the different samples.

Determination of kinetic parameters for amylopectin retrogradation

Kinetic studies were performed with pastes containing 0.10 g starch/g mixture, without and with 0.003 g xanthan gum/g mixture, and pastes containing 0.20 g starch/g mixture, without and with 0.01 g xanthan gum/g mixture. Pastes were prepared following the procedure described above. Samples were gelatinized in the DSC at a heating rate of 10°C min⁻¹; two sets of experiments were performed: one set of samples was heated to 85 and the other to 120°C. At the same time, to compare with pastes gelatinized in DSC pans, 400 g batches of the pastes were gelatinized in a thermostated bath, to a final temperature of 85°C. After batch-wise gelatinization, 15 mg aliquots were placed in DSC pans and sealed. Samples in sealed pans were frozen in a chamber at -20°C (freezing rate=5 cm h⁻¹), and after 12 h, they were stored in a -4°C chamber. At different storage times, samples were thawed and run in the calorimeter, over a temperature range of 15 to 80°C, to analyze amylopectin retrogradation, using the same heating and recording conditions as in the gelatinization assays.

Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the influence of concentration and type of hydrocolloid on gelatinization enthalpies and glass transition temperature. The effect of gelatinization conditions was also analyzed. ANOVA was carried out using the SYSTAT program from SYSTAT Inc. (USA), and means comparison analysis was done using Tukey's test [20, 21].

Results and discussion

Gelatinization of starch systems with different hydrocolloid concentrations

Figure 1a shows thermograms for gelatinization of gum-starch samples corresponding to a gum:starch ratio of 1:10 (total solids=0.10 g/g mixture). The

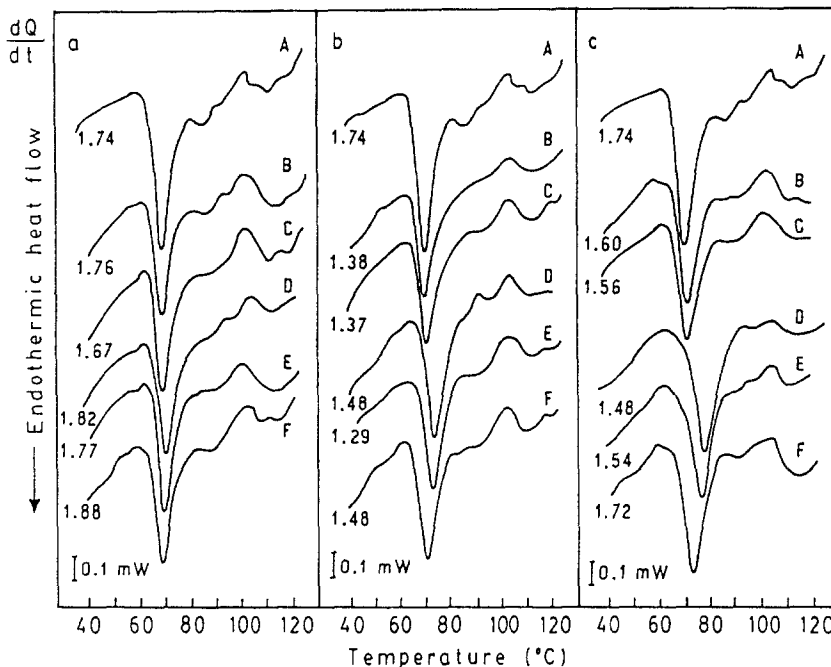


Fig. 1 DSC curves for starch gelatinization. Gum:starch ratio (w/w): a) 1:10, b) 1:2, c) 1:1. Letters on the right of each figure indicate: A) without hydrocolloid, B) with guar gum, C) with locust bean gum, D) with CMC, E) with sodium alginate, and F) with xanthan gum. Numerical values on the left of each figure indicate the weight of dry starch (mg)

shape of the thermograms did not vary among the tested systems. Figures 1b and 1c show thermograms of starch pastes with hydrocolloid:starch ratios of 1:2 (total solids=0.12 g/g mixture) and 1:1 (total solids=0.17 g/g mixture), respectively. The characteristic development of a second transition peak, which appears when starch is gelatinized at low water contents [3, 4, 22], was not observed in these systems. This demonstrates that there was enough water available for both starch and hydrocolloid, regardless of the hydrocolloid used. Similar results were obtained by Ghiasi *et al.* [23] for starch-xanthan gum and starch-protein systems.

The gelatinization enthalpies obtained are shown in Table 1. The analysis of variance showed a significant effect ($P < 0.05$) of gum concentration and gum type. The comparison based on Tukey's test for means did not show significant differences among the enthalpies of pastes with and without different hydrocolloids, when a gum:starch ratio of 1:10 was used. Higher enthalpy values were, in general, observed for pastes with CMC and sodium alginate. A tendency for the gelatinization enthalpies to increase in relation to the gum concentration was observed.

Table 1 Gelatinization enthalpies ($J g^{-1}$) starch

Gum:starch ratio ^(*)	Guar gum	Locust bean gum	CMC	Sodium alginate	Xanthan gum
1:10 ⁽¹⁾	9.4 ^{aA(**)}	10.1 ^{aA}	11.5 ^{aA}	11.7 ^{aA}	11.2 ^{aA}
1:2 ⁽²⁾	10.4 ^{aA}	11.6 ^{aA}	16.3 ^{bB}	16.4 ^{bB}	12.2 ^{abA}
1:1 ⁽³⁾	14.2 ^{bAB}	12.7 ^{aA}	17.5 ^{bB}	15.6 ^{bAB}	14.8 ^{bAB}
without gum			9.6 ^a		

Runs were performed in triplicate. $Se^2=1.249$.

(*) by weight, dry basis. Total solids concentration (g solids/g mixture): ⁽¹⁾0.10, ⁽²⁾0.12, ⁽³⁾0.17.

(**) Within each vertical column, values with the same small letter are not statistically different ($P<0.05$). Within each horizontal row, values with different capital letters are statistically different ($P<0.05$).

Table 2 Onset (T_o), peak (T_p) and conclusion (T_c) temperatures and gelatinization ranges (T_c-T_o) for corn starch pastes in the presence of different gums

Gum:starch ratio ^(*)	Gum type	$T_o/$	$T_p/$	$T_c/$	$T_c-T_o/$
$^{\circ}C$					
1:10 ⁽¹⁾	Without gum	65.4 ^{a(**)}	70.8 ^a	76.4 ^a	11.0
	Guar	66.7 ^a	71.0 ^{ab}	77.6 ^a	10.9
	Locust bean	66.5 ^a	71.7 ^{ab}	78.0 ^a	11.5
	CMC	66.5 ^a	72.5 ^b	78.4 ^a	11.9
	Sodium alginate	66.5 ^a	72.5 ^b	78.4 ^a	11.9
	Xanthan	65.9 ^a	71.4 ^a	78.0 ^a	12.1
1:2 ⁽²⁾	Guar	66.6 ^{ab}	72.5 ^b	78.4 ^{ab}	11.8
	Locust bean	67.5 ^{abc}	73.0 ^b	80.1 ^{bc}	12.6
	CMC	69.7 ^c	75.2 ^c	82.5 ^{cd}	12.8
	Sodium alginate	68.4 ^{bc}	75.4 ^c	83.8 ^d	15.4
	Xanthan	67.1 ^{ab}	72.8 ^b	80.9 ^c	13.8
1:1 ⁽³⁾	Guar	66.6 ^{ab}	73.1 ^b	80.3 ^b	13.7
	Locust bean	67.6 ^b	73.6 ^{bc}	80.8 ^{bc}	13.2
	CMC	71.3 ^c	78.9 ^d	87.7 ^d	16.4
	Sodium alginate	70.7 ^{cd}	78.3 ^d	85.7 ^d	15.0
Xanthan	68.4 ^{bd}	75.1 ^c	83.0 ^c	14.6	

Runs were performed in triplicate. $Se^2(T_o)=0.558$, $Se^2(T_p)=0.324$, $Se^2(T_c)=0.676$.

(*) by weight, dry basis. Total solids concentration (g solids/g mixture): ⁽¹⁾0.10, ⁽²⁾0.12, ⁽³⁾0.17.

(**) Within each vertical column, values with the same letters for each gum:starch ratio are not statistically different ($P<0.05$). Comparisons include the control.

Table 2 shows onset temperature (T_o), peak or maximum temperature (T_p) and conclusion temperature (T_c), and also the gelatinization temperature ranges, for the different systems. Gelatinization ranges increased with increasing gum:starch ratio. This peak-widening was due to a shift of T_c to higher temperatures, which may be related to the availability of water for crystalline melting.

T_c can be influenced by the solvent-reducing effect of the polymer/solvent mixture (in this case, starch/water). The greater the availability of water, the lower will be the crystalline melting temperature of the mixture. Large amounts of hydrocolloid could decrease the availability of water to starch, thus explaining an increase in T_c .

ANOVA allowed us to analyze the effect of concentration and type of gum on T_o , T_p and T_c ; results showed that both factors were significant. According to the comparison of means, T_p and especially T_c of pastes with CMC and sodium alginate, at gum:starch ratios of 1:2 and 1:1, were, in most cases, significantly different from those of pastes with other gums. In general, a significant shift in these temperatures was observed, as the hydrocolloid concentration increased.

Concurrent factors, gum concentration and type, could be inferred from the data. The shift of T_o , T_p , T_c for CMC and sodium alginate pastes, particularly at the gum:starch ratios of 1:2 and 1:1, was more marked. Both are anionic hydrocolloids, present as sodium salts. Since they are charged hydrocolloids, while the others are neutral ones, they may interact differently with starch and water [24–26].

Based on these results, only a galactomannan (guar gum) and xanthan gum (as neutral gums) and sodium alginate (as a charged gum) were used in the following assays.

Influence of hydrocolloid addition on the gelatinization of systems with different starch concentrations

Figure 2a shows thermograms for pastes with starch concentrations ranging between mass-fractions of 0.10 and 0.70 (g starch/g mixture). The development of a second gelatinization peak (II), as the amount of available water decreased, was observed. This second transition appears initially as a shoulder of the main peak, at a volume-fraction of water of about 0.60, and then, at lower water concentrations, as an independent peak (II). The complete loss of granule birefringence was achieved at temperatures higher than that of peak II. A shift of this peak to higher temperatures, as moisture content decreased, was observed. At even higher temperatures, the peaks corresponding to order-disorder processes of amylose-lipid complexes (III and IV) were observed [7].

Figure 2b shows an example of the endotherms for starch pastes with xanthan gum. Similar endotherms were obtained for pastes with guar gum and sodium alginate; in each case, the concentration was 0.01 g hydrocolloid/g mixture.

Similar-shaped endotherms were observed for starch pastes with and without hydrocolloid. However, at the same starch concentration, a shift in T_c to higher temperatures was observed, when the gelatinization occurred in the presence of

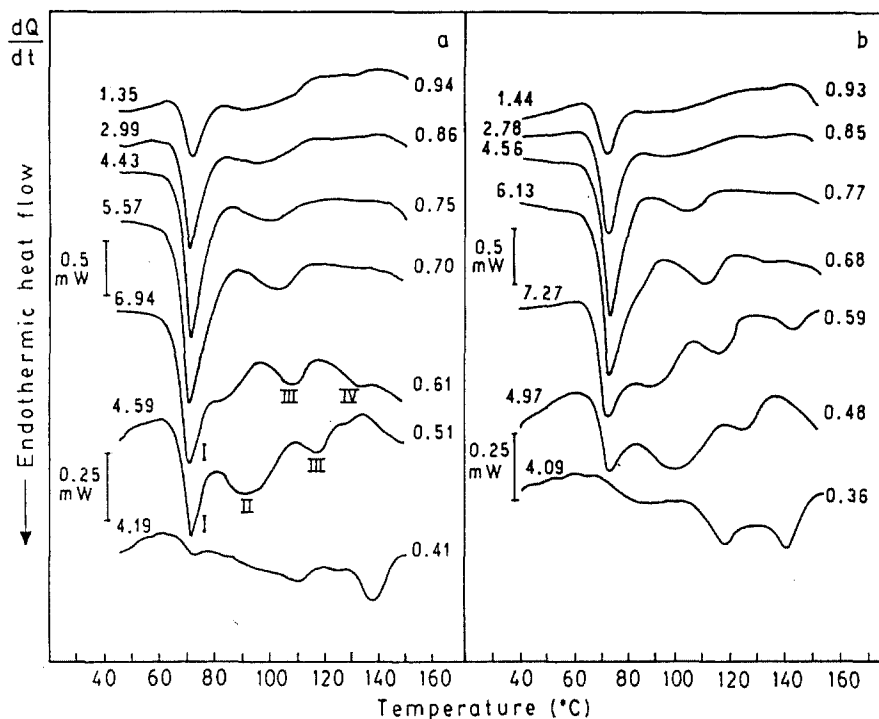


Fig. 2 DSC curves for starch gelatinization in systems with different volume fractions of water: a) without hydrocolloid, b) with xanthan gum (mass-fraction=0.01 g gum/g mixture). Numerical values on the right side of each figure indicate the volume fraction of water; those on the left side indicate the weight of dry starch (mg). The upper scale in mW corresponds to the first five thermograms and the lower scale to the last two curves

gum. The presence of hydrocolloid seemed to affect the availability of water for starch during gelatinization, similarly to a reduction in water content. With regard to gelatinization enthalpies, except for pastes with a mass-fraction of starch of 0.65–0.70 (g starch/g mixture), which had lower gelatinization enthalpies, the other pastes showed enthalpies that did not differ significantly, either among themselves or with the controls. Figure 3 demonstrates that this concentration range corresponded, in almost every case, to the maximum gelatinization enthalpy. The trend observed in this plot of ΔH as a function of water content is similar to that obtained by other authors [22, 27].

With regard to the nature of the two endotherms, different models have been proposed. One emphasized the lack of cooperativity of crystalline melting in low water-content systems [28], while another suggests that the development of two endotherms corresponds to two different types of melting, related to different degrees of stability of the crystals in the starch granule [29].

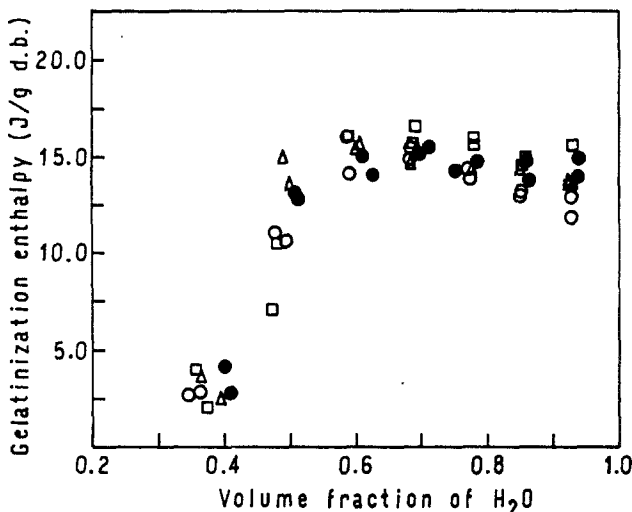


Fig. 3 Gelatinization enthalpy as a function of the volume fraction of water in starch pastes (●) without hydrocolloid, (○) with xanthan gum, (Δ) with guar gum, (□) with sodium alginate. In each case, the hydrocolloid mass-fraction was 0.01 g gum/g mixture

Biliaderis *et al.* [4] have proposed that such multiple-melting profiles reflect melting and reorganization processes occurring simultaneously during dynamic heating in the DSC. Biliaderis *et al.* [4] and Slade and Levine [5], among other authors, have postulated the non-equilibrium nature of this melting process. As with other partially crystalline polymers, starch structures are in metastable states and are therefore amenable to perfection (annealing) upon plasticization by water and thermal treatments [4]. These processes, related to melting of starch crystals, are highly influenced by the water content and become less favored with increasing moisture content. Slade and coworkers [30, 31] stressed the existence of a second-order transition (glass transition) that precedes the melting of starch crystallites. These workers also emphasized the critical role of water in plasticizing the glassy regions of starch and thereby lowering their effective T_g . For some starch samples, DSC gives direct evidence for a glass transition that is characterized by an incremental change in heat capacity, ΔC_p . However, for most granular starches, this transition is not always evident, presumably because of the heterogeneous nature of the amorphous phase [4].

Under thermodynamic equilibrium conditions, the effect of a solvent to depress the melting temperature (T_m) of a polymer can be expressed using the Flory-Huggins equation [2]. This equation relates the T_m of the polymer to the volume-fraction of plasticizer (water in this case), according to:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{RV_u}{\Delta H_u V_1} (v_1 - X_1 v_1^2) \quad (1)$$

where R is the gas constant, V_u/V_1 is the ratio of the molar volume of the repeating unit to the molar volume of the solvent, T_m^0 is the polymer's equilibrium melting point in the absence of dilution, T_m is the melting point of the polymer-solvent mixture, X_1 is the Flory-Huggins polymer-solvent interaction parameter, v_1 is the volume-fraction of water, and ΔH_u is the melting enthalpy of the repeating unit. When $X_1=0$ (no polymer-solvent interaction), the equation yields a linear relationship between the volume-fraction of water in the samples (v_1) and $1/T_m$.

The Flory-Huggins equation has been found to describe reasonably well DSC melting data for starch, particularly at water volume-fractions lower than 0.7 [3, 4]. However, its theoretical validity has recently been questioned [4, 5, 8] on the basis that true equilibrium conditions are not met during starch gelatinization. Several processes occur simultaneously during heating: crystal melting, annealing and crystallization [4, 5, 7]. Nevertheless, from a practical viewpoint, the Flory analysis is useful for the comparison of the thermal stability of starch materials, and to allow an estimation of the apparent T_m under various moisture-content and heating-rate conditions [32].

To compare the effects of the different gums on gelatinization, our data treatment was performed according to the Flory-Huggins equation, considering it as a phenomenological equation without a thermodynamic basis.

The water volume-fraction of samples (v_1) was considered as the ratio of the total volume of water to the sum of the volumes of the different components. T_c in the thermograms was taken as the melting temperature, T_m ; in the case of the presence of a second peak, T_c of peak II was used. The experimental data and the straight line obtained for the system of starch without hydrocolloid are shown in Fig. 4. In the case of pastes with hydrocolloids, the values do not lie on the straight line representing the data for pastes without hydrocolloid; this is due to the shift in T_c to higher temperatures in the presence of gum. The effect of gums can be attributed mainly to the fact that they limit (due to their hydrophilic character) water availability during gelatinization. Therefore, this effect was greater as the volume-fraction of water decreased. No differences were observed when the volume-fraction of water was higher than 0.7.

Glass transition

After starch gelatinization, an amylose matrix filled with granules of different degrees of fragmentation (depending on the thermal history) is obtained [33]. During freezing of gelatinized starch suspensions, only part of the total water freezes, and the rest remains unfrozen. The amount of ice depends on the thermal history. T'_g represents the solute-specific, subzero T_g of the maximally freeze-concentrated, amorphous solute/unfrozen water matrix surrounding the ice crystals in a frozen solution [8]. C'_g is the solute concentration of the T'_g glass [8].

With regard to heating conditions, no significant differences in T'_g were observed among the pastes heated to 85 or 120°C. Figure 5 shows typical curves obtained during thawing of gelatinized starch pastes (heated to 120°C during ge-

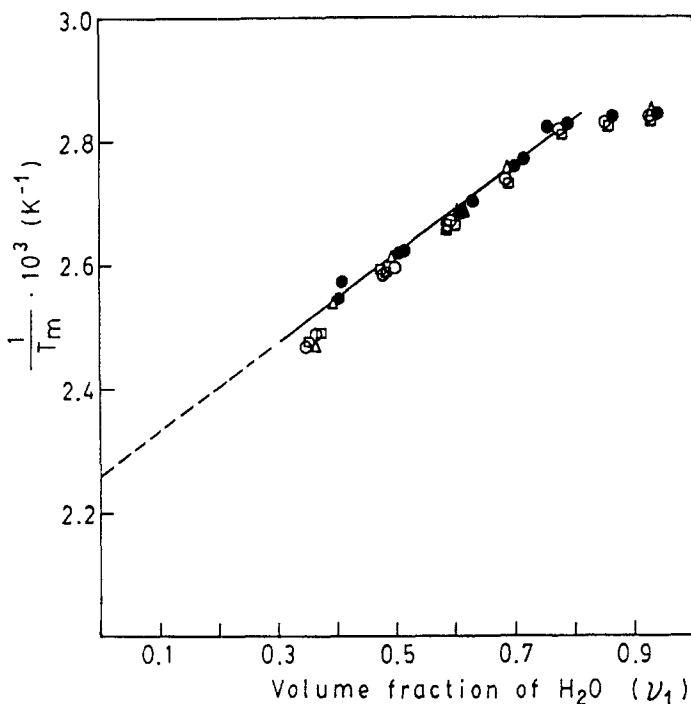


Fig. 4 Reciprocal of the melting temperature (T_m^{-1}) as a function of the volume-fraction of water for starch pastes: (●) without hydrocolloid, (○) with xanthan gum, (Δ) with guar gum, (□) with sodium alginate. In each case, the hydrocolloid mass-fraction was 0.01 g gum/g mixture. The straight line corresponds to the Flory-Huggins equation, applied to the data for starch without hydrocolloid

latinization) with and without hydrocolloid. Before the ice-melting peak, the characteristic change in C_p due to the glass transition was observed. In the case of starch pastes without gum (Fig. 5a), and pastes with sodium alginate (Fig. 5d) both without annealing, an exothermic peak can be observed between the ice-melting endotherm and the glass transition temperature. This peak was attributed to additional ice crystallization (devitrification) [37]. This ice crystallization cannot take place as long as the water-starch-hydrocolloid matrix is in the glassy state ($T < T_g$). When the temperature exceeds T_g , pastes move into the rubbery state of increasing molecular mobility, thus allowing additional ice formation to occur [34, 37].

The absence of an exothermic devitrification peak in the systems with xanthan or guar gum without annealing (Figs 5b and 5c) may be related to the higher viscosity of the pastes with these gums, which delays the formation of additional ice, compared to the paste with sodium alginate.

Maximum ice crystallization was obtained by annealing samples between the T_g^{mv} and T_g^{ov} (approximately -4.5°C) at a slow cooling rate. No exothermic peak

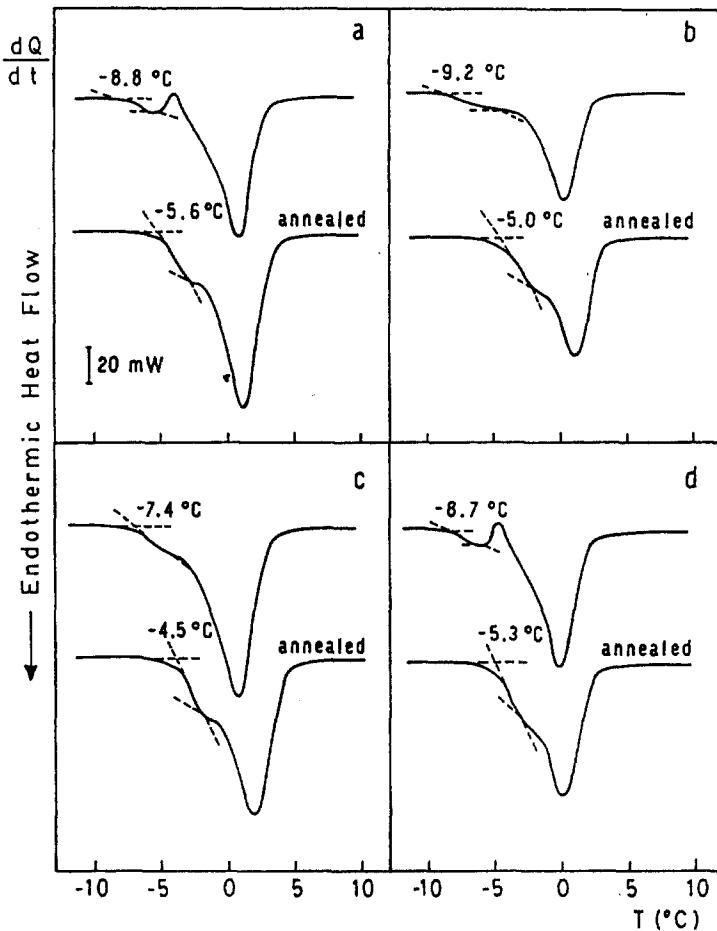


Fig. 5 DSC curves obtained during thawing of starch pastes (mass-fraction = 0.50 g starch/g mixture): a) without hydrocolloid, b) with xanthan gum, c) with guar gum, and d) with sodium alginate. Mass-fraction of hydrocolloid = 0.01 g gum/g mixture. In each case, the upper curve corresponds to a sample without annealing, and the lower curve to a sample annealed for 30 min at -4.5°C . The temperature shown corresponds to the onset value

for additional ice formation was observed in any case. Similar results were obtained by Roos and Karel [35]. This additional ice formation during thawing of systems stored at $T < T_g^o$ is one of the main factors in food deterioration [37].

Table 3 shows mean values of onset, midpoint and conclusion glass transition temperatures (T_g^o , T_g^m and T_g^c) for samples with and without annealing. Annealed pastes showed a shift in T_g^o , T_g^m and T_g^c to higher values, yielding the onset, midpoint and conclusion glass transition temperatures of the maximum

Table 3 Glass transition temperatures (onset, midpoint and conclusion) for gelatinized corn starch pastes containing 0.50 g starch/g mixture and 0.01 g gum/g mixture, with and without annealing

	System	$T_g/^\circ\text{C}^{(*)}$	$T_g'/^\circ\text{C}^{(***)}$
$T_{\text{onset}}/$	without gum	-8.1 ^{ab(***)}	-5.2 ^a
T_g^o	with xanthan	-8.1 ^{ab}	-5.2 ^a
	with guar	-7.5 ^a	-4.5 ^a
	with alginate	-9.0 ^b	-5.5 ^a
$T_{\text{Midpoint}}/$	without gum	-6.4 ^a	-4.1 ^a
T_g^m	with xanthan	-6.7 ^{ab}	-4.0 ^a
	with guar	-5.9 ^a	-3.6 ^a
	with alginate	-7.7 ^b	-4.5 ^a
$T_{\text{Conclusion}}/$	without gum	-4.7 ^a	-3.1 ^a
T_g^c	with xanthan	-5.1 ^a	-2.9 ^a
	with guar	-4.6 ^a	-2.5 ^a
	with alginate	-6.4 ^b	-3.3 ^a

Runs were performed at least in triplicate. $\text{Se}^2(T_g^o)=0.232$, $\text{Se}^2(T_g^m)=0.183$, $\text{Se}^2(T_g^c)=0.184$.

(*) T_g =glass transition temperature (without annealing).

(**) T_g '=glass transition temperature of the maximally freeze-concentrated matrix (with annealing).

(***) Comparisons were made among T_g or T_g ' values for different systems, analyzing independently the onset, midpoint and conclusion temperatures. Within each four grouped figures, values followed by the same letter are not significantly different ($P < 0.05$).

matrix concentration (T_g^{or} , $T_g^{m'}$ and $T_g^{c'}$, respectively). The variance analysis showed a significant effect of annealing and the type of gum used.

The means comparison test ($P < 0.05$) for pastes without annealing did not give significant differences for T_g^o between each paste with gum and that without gum. With regard to T_g^m and T_g^c , only the paste with alginate differed significantly from the control paste.

The presence of gums did not change the T_g' values (onset, midpoint or conclusion). A $T_g' = -10(\pm 5)^\circ\text{C}$ was reported to be characteristic of high molecular-weight biopolymers at or above moisture contents near 0.30 g/g ($W_g' = 0.25$ –0.30 g water/g mixture) [36]. Below T_g' , diffusion-limited processes will not occur, on a practical time scale, in ice-containing frozen food systems [37]. As can be observed, T_g' did not vary significantly among systems with different gums, which yielded values between -4.5 and -5.5°C for T_g^{or} and between -2.5 and -3.3°C for $T_g^{c'}$. These values were similar to those reported by Slade and Levine for starch-water pastes [5, 37]. Huang *et al.* [38] reported T_g' values between -5 and -10°C for different rice starch varieties. A $T_g' = -12^\circ\text{C}$ for white bread was obtained by Le Meste *et al.* [39] using TMA.

Influence of different parameters on starch retrogradation at subzero temperature

Starch retrogradation is a recrystallization process involving both glucose polymers, linear amylose and branched amylopectin. Amylose retrogradation is a short-term recrystallization [1], irreversible below 140–160°C [4]. Amylopectin retrogradation is a long-term recrystallization, reversible below 100°C [1, 4].

In the present work, only amylopectin retrogradation was quantified by DSC. In previous work [17, 18], the effect of freezer-storage temperature on starch retrogradation was analyzed in detail. Storage temperatures above the glass transition allow amylopectin and amylose retrogradation to occur, because there is enough molecular mobility for polymer associations to occur [19, 33, 37].

Concentration effects

Several authors have pointed out the importance of starch concentration in retrogradation [40]. Figure 6 shows retrogradation enthalpy values obtained after storage at -4°C for 45 days, for gelatinized pastes with different corn starch contents. This storage time was selected, according to previous work, to allow maximum retrogradation [19]. The characteristic bell-shaped plot indicates that there is a range of moisture values that produces maximum retrograded fractions.

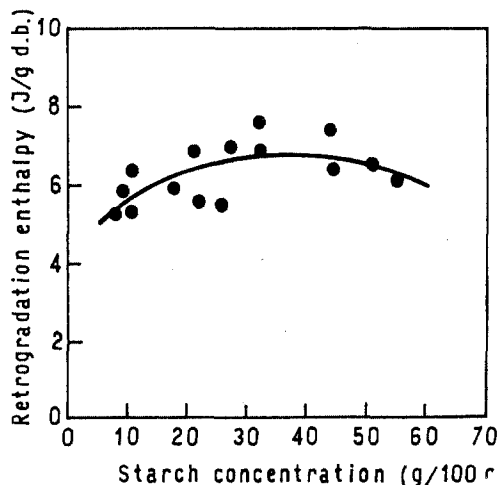


Fig. 6 Amylopectin retrogradation enthalpies for starch pastes without hydrocolloid, stored for 45 days at -4°C , as a function of starch concentration

Kinetics of amylopectin retrogradation: effect of thermal history, concentration and hydrocolloid addition

Figures 7a and 7b show micrographs of gelatinized starch pastes (mass-fraction=0.10 g starch/g mixture) heated to a final temperature of 85°C , either

batch-wise (Fig. 7a) or in DSC pans (Fig. 7b). Non-granular disintegration was observed in both cases, although samples gelatinized batch-wise showed slightly more swollen granules with uniform and rounded borders than did those heated in DSC pans. These findings suggests that the morphology of DSC-gelatinized granules is equivalent to that of batch-gelatinized ones. Pastes with xanthan gum (starch mass-fraction=0.10 g starch/g mixture, xanthan gum mass-fraction=0.003 g gum/g mixture) gave similar results (Fig. 7c). Figure 7d shows that similar granular integrity was maintained in higher-concentration pastes (starch mass-fraction=0.20 g starch/g mixture, xanthan gum mass-fraction=0.01 g gum/g mixture).

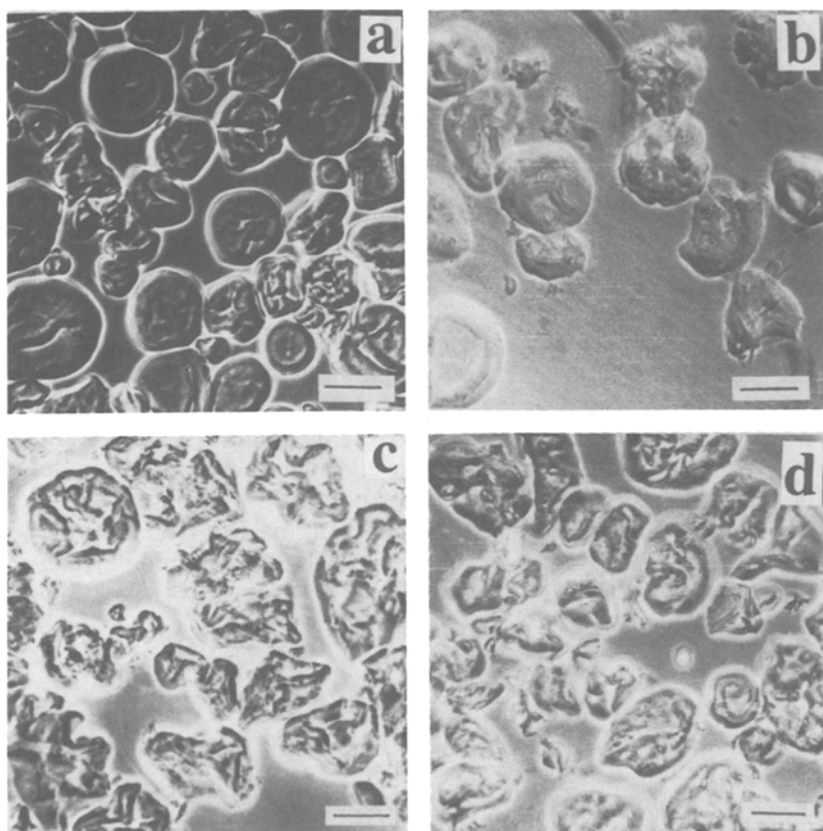


Fig. 7 Micrographs of starch pastes heated to 85°C: a) gelatinized batch-wise, b), c) and d) gelatinized in DSC pans; a) and b) mass-fraction of starch=0.10 g starch/g mixture, c) mass-fraction of starch=0.10 g starch/g mixture and mass-fraction of xanthan gum=0.003 g gum/g mixture, d) mass-fraction of starch=0.20 g starch/g mixture and mass-fraction of xanthan gum=0.01 g gum/g mixture. Scale bar=20 μ m

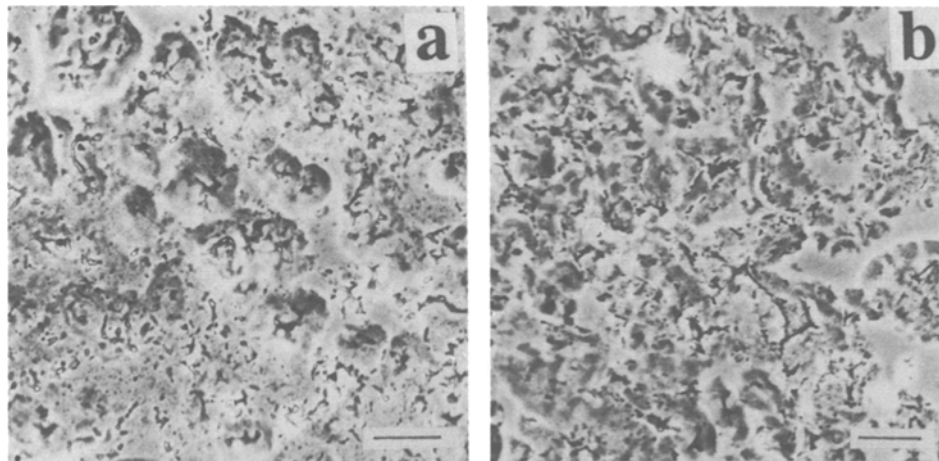


Fig. 8 Micrographs of starch pastes (mass-fraction=0.20 g starch/g mixture) heated in DSC pans to 120°C: a) without xanthan gum, b) with xanthan gum (mass-fraction=0.01 g gum/g mixture). Scale bar=20 μm

Figures 8a and 8b show starch granules in a paste containing 0.20 g starch/g mixture, gelatinized in DSC pans heated to 120°C. A greater disintegration of the granules was observed; no marked differences between pastes with and without xanthan gum were observed.

Xanthan gum, at the concentration levels used, had no significant influence on starch-granule breakdown. Heating conditions had a significant effect on granule integrity. High final temperatures, such as 120°C, resulted in a marked degree of granule breakdown. The two final temperatures, 85 and 120°C, were selected to produce different situations, with potentially different numbers of amylopectin nuclei able to recrystallize (retrogradation) during storage.

Retrogradation enthalpies were obtained from the DSC curves for samples stored at -4°C for different times. Enthalpy values were modelled with the Avrami equation [41–43], as follows:

$$\Delta H = \Delta H_{\infty} e^{-kt} \quad (2)$$

where ΔH is the retrogradation enthalpy (J g^{-1}), ΔH_{∞} is the maximum retrogradation enthalpy (J g^{-1}), k is the kinetic constant of retrogradation (days^{-1}), and t is the storage time (days).

Figure 9 shows the data and curves obtained using the Avrami model. No significant differences between amylopectin retrogradation enthalpies, for pastes heated to 85°C batch-wise or in DSC pans (all stored at -4°C), were observed.

Table 4 shows ΔH_{∞} and k constants for the different pastes. The retrogradation kinetic constants (k) for pastes with 0.10 and 0.20 g starch/g mixture were significantly different. However, the maximum enthalpies did not differ signifi-

lar mobility of the non-frozen matrix at -4°C ; pastes with lower starch concentrations may exhibit higher mobility, leading to a higher retrogradation rate. The presence of xanthan gum did not significantly affect the results.

No significant differences in the retrogradation kinetic parameters (k and ΔH_{∞}) were found for pastes with different starch and xanthan gum concentrations, heated to 120°C and stored at -4°C . In this case, the effect of starch concentration was not significant. Thus, the values were combined for regression

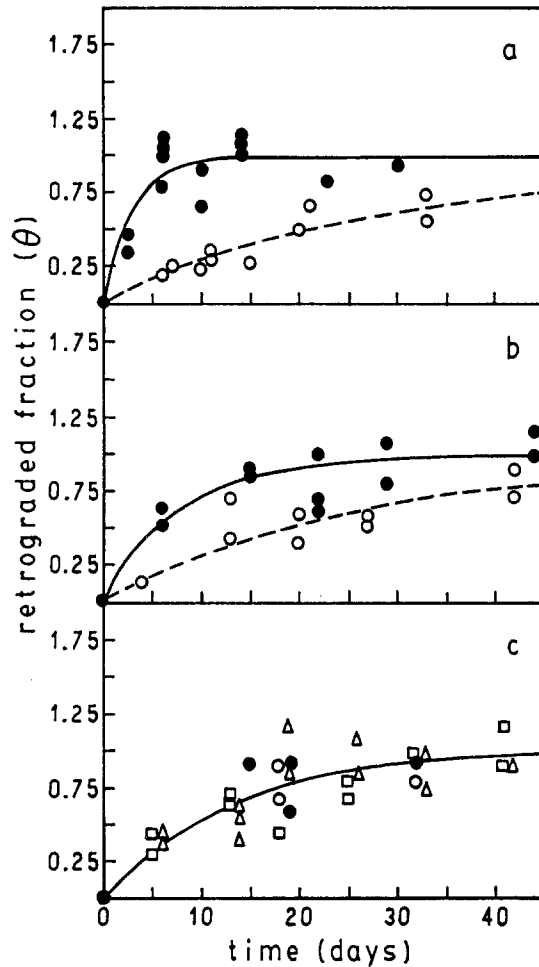


Fig. 9 Retrograded fraction of starch ($\theta = \Delta H_t / \Delta H_{\infty}$) as a function of storage time in: a) starch pastes heated to 85°C ; (\bullet) 0.10 g starch/g mixture, (\circ) 0.20 g starch/g mixture; b) starch pastes heated to 85°C ; (\bullet) 0.10 g starch/g mixture and 0.003 g xanthan gum/g mixture, (\circ) 0.20 g starch/g mixture and 0.01 g xanthan gum/g mixture; c) starch pastes heated to 120°C ; (\bullet) 0.10 g starch/g mixture, (\circ) 0.20 g starch/g mixture, (Δ) 0.10 g starch/g mixture and 0.003 g xanthan gum/g mixture, (\square) 0.20 g starch/g mixture and 0.01 g xanthan gum/g mixture

Table 4 Amylopectin retrogradation kinetic parameters (ΔH_∞ and k) of Avrami equation for corn starch pastes with xanthan gum stored at -4°C

Starch mass-fraction, g starch/g mixture	Without xanthan gum		With xanthan gum ^(*)	
	$\Delta H_\infty/\text{J g}^{-1}$	k/d	$\Delta H_\infty/\text{J g}^{-1}$	k/d
0.10	3.751	0.330	4.134	0.125
	(± 0.556)**	(± 0.187)	(± 1.071)	(± 0.053)
0.20	3.801	0.034	4.854	0.038
	(± 2.927)	(± 0.040)	(± 1.093)	(± 0.020)

(*) gum mass-fraction: 0.003 g gum/g mixture in pastes with 0.10 g starch/g mixture and 0.01 g gum/g mixture in pastes with 0.20 g starch/g mixture.

(**) values within parentheses indicate confidence limits (95%).

analysis, yielding $\Delta H_\infty = 4.439 \pm 0.644$ (J g^{-1}) and $k = 0.078 \pm 0.031$ (d^{-1}) at the 95% confidence level.

The final heating temperature has a marked effect on granular integrity; at 120°C , a high degree of granular disintegration is observed. Even though there would be fewer amylopectin nuclei able to crystallize, molecules would be more exposed; this could reduce the differences between pastes with different starch concentrations.

The presence of gum did not prevent amylopectin retrogradation in corn starch, regardless of the final heating temperature used (85 or 120°C). This could be attributed to the fact that the gum acts outside the starch granule, and the possibility to interact with the amylopectin would be very small.

Conclusions

Hydrocolloid addition showed a significant effect on corn starch gelatinization. A tendency for the gelatinization enthalpies to increase with gum concentration (1:10, 1:2, 1:1 g gum/g starch) was observed. In systems with a starch mass-fraction of 0.10, availability of water was not sufficiently reduced by the presence of gums, and the development of a second transition peak in the DSC curves (characteristic of starch gelatinization at low water content) was not observed. However, the presence of hydrocolloids produced a shift in gelatinization temperature, especially of the conclusion temperature (T_c); this effect was more obvious with charged gums (CMC, sodium alginate). Gelatinization temperature ranges increased with increasing gum:starch ratio.

When starch mass-fraction was increased to 0.70, the development of the second gelatinization peak was observed for pastes with and without hydrocolloid. The shift in T_c to higher temperatures, for the samples with hydrocolloids, was evidenced by the Flory-Huggins analysis, which was utilized as a phenomenological equation.

Glass transition onset temperatures ranged between -7.5 and -9.0°C , depending on the type of hydrocolloid. Those hydrocolloids that produced high viscosities, such as xanthan and guar gum, inhibited additional ice formation during thawing, which is one of the causes of food damage during frozen storage. When samples were annealed at temperatures close to T_g' , maximum ice crystallization was obtained, and no exothermic peak for additional ice formation was observed in any case. The glass transition onset temperatures for annealed samples (T_g'') did not vary significantly among systems with different gums, yielding values between -4.5 and -5.5°C .

With regard to the kinetics of amylopectin retrogradation, starch concentration and final heating temperature were found to be relevant factors. No significant differences in the retrogradation kinetic parameters of the Avrami equation (k and ΔH_{∞}) were found for pastes with different starch and xanthan gum concentrations (0.10 and 0.20 g starch/g mixture and 0.003 and 0.01 g gum/g mixture), heated to 120°C . The presence of gum that acts outside the starch granule did not prevent amylopectin retrogradation, regardless of the final heating temperature used (85 or 120°C).

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