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Gelatinization and freeze-concentration effects on recrystallization in corn and potato starch gels

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Abstract—Freeze-concentration of starch gels was controlled by temperature and gelatinization with glucose and lactose. The aim of the study was to evaluate the effects of freezing temperature and gel composition on starch recrystallization behaviour of corn and potato starch gels (water content 70%, w/w) in water or glucose or lactose (10%, w/w) solutions. Starch gels were obtained by heating in differential scanning calorimetry (DSC). Samples of starch gels were frozen at −10 °C, −20 °C and −30 °C for 24 h and, after thawing, stored at +2 °C for 0, 1, 2, 4 and 8 days. The extent of starch recrystallization was taken from the enthalpy of melting of the recrystallized starch by DSC. Freezing temperatures, glucose, lactose and the origin of the starch affected the recrystallization behaviour greatly. The recrystallization of amorphous starch during storage was enhanced by freeze-concentration of gels at temperatures above T'_m. Molecular mobility was enhanced by unfrozen water and consequently molecular rearrangements for nucleation could take place. Further storage at a higher temperature enhanced the growth and the maturation of crystals. In particular, glucose decreased the T'_m of the gels and consequently lower freezing temperatures were needed to reduce enhanced recrystallization during storage. Freeze-concentration temperatures also showed a significant effect on the size and the perfection of crystals formed in starch recrystallization.

Keywords: Retrogradation; Freezing; Starch; Potato; Corn; Gelatinization

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1. Introduction

Starch is the major polysaccharide in plants and exists in starch granules. Starch component polysaccharides, amylose + amylopectin, are partially crystalline and partially amorphous systems.[1,2] The extent of crystallinity in starch is around 15–35%.[3] The crystalline regions in granules of common starches appear in branched glucose units of the amylopectin molecules within the granules. Amylose, the second and linear polysaccharide of starch, is largely amorphous and randomly distributed between the amylopectin clusters.[1,4,5] The packing of amylose and amylopectin within the granules has been reported to vary among starches from different species. X-ray diffraction studies have revealed the presence of ordered and partially crystalline structures within starch granules.[1,6] Cereal starches (rice, wheat and corn) exhibit the typical A-type X-ray pattern, whereas, tuber starches show B-form and legume starches, the mixed state pattern known as type C.[7]

Starch processing often includes starch gelatinization, that is, irreversible swelling or even disruption of the starch granules, depending upon the severity of the treatment applied. Collapse of crystalline order associated with gelatinization within the starch granules is one of the most important irreversible changes. Others include granule swelling, pasting, loss of optical birefringence, uncoiling and dissociation of the double helices,

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and starch solubility.[8–10] Gelatinized starches undergo changes also in cooling and storage, which are generally termed as ‘retrogradation’. These changes affect quality, acceptability and shelf life of starch-containing food and other materials.[11]

Starch retrogradation is a temperature- and time-dependent phenomenon, which involves reassociation of molecules in an ordered structure. This, under favourable conditions, results in a crystalline order leading to partial crystallization of amylose and amylopectin. This can be detected from a change in the appearance of a starch gel or paste, from changes in X-ray diffraction properties and from a melting endotherm in DSC scans, which shows an increasing size with increasing storage time. Starch crystallization is a non-equilibrium process, the rate of which is determined by diffusion (i.e., temperature and concentration) and by polymer structure (linear or branched).

It is important to distinguish between the short-term development of a gel structure via amylose crystallization and long-term reordering of amylopectin, which is a much slower process involving recrystallization of the outer branches of this polymer.[12–14] For common starches containing both amylose and amylopectin, a composite gel network forms, consisting of swollen amylopectin filling an interpenetrating amylose gel matrix.[13] During long-term storage, amylopectin recrystallizes, thus increasing the rigidity of the swollen granules which, in turn, reinforces the continuous amylose phase. The slow crystallization of amylopectin was referred to as a nucleation-limited growth process, which occurred above glass transition in a mobile, viscoelastic, fringed-micelle network.[3] The temperature dependence of the kinetics of crystallization (either isothermal or non-isothermal) is well known. The quasi-bell-shape function of the logarithm of crystallization rate is known to go to zero towards the lower limit of glass transition and melting at higher temperatures.[3,15]

In recent years, evidence has become increasingly compelling that molecular mobility, and thus all diffusion-controlled events, may become severely restricted around the glass transition of an amorphous material. Below glass transition, in the glassy state, translational molecular movement is not possible and crystallization cannot take place. The crystallization rate approaches a maximum at a temperature between glass transition and equilibrium melting temperature, T_m, of starch crystallites.[2,16] According to Morris,[11] the net rate of crystallization (nucleation and growth) has a maximum value at a temperature $T \approx 1/2(T_g + T_m)$. Accelerated crystallization can also be achieved by allowing nucleation to occur at a low temperature followed by storage close to melting temperature, which increases the rate of crystal growth.[2]

Laine and Roos[17] studied the effect of water content and T–T_g on the extent of crystallization of gelatinized corn starch at fairly low water contents and high storage temperatures. Crystallization was found to occur above T_g, but the extent of crystallization, as determined from the limiting enthalpy value after 10 days with DSC, increased as the T–T_g increased from 20 °C to 75 °C. It was interesting to note that the melting temperature of recrystallized starch gels was also observed to depend on storage temperature. These differences were attributed to the perfection of the crystals formed,[2] which may also explain the differences in melting enthalpies observed by Laine and Roos.[17] It was concluded that starch retrogradation is a typical polymer crystallization process that depends on the amount of plasticizer and the temperature in relation to T_g and T_m.

Gelatinized starch systems are important components of a variety of frozen materials, such as starch gels desserts. Starch can also be gelatinized prior to lyophilisation of pharmaceutical systems. Frozen materials need to be characterized in terms of glass transition of the maximally freeze-concentrated state, T'_g.[18,19] Maximum freezing leaves an unfrozen matrix with solute concentration of C'_g in the unfrozen phase. This unfrozen phase is plasticized by unfrozen water and it has a glass transition onset temperature at T'_g.[18] Maximum freeze-concentration requires annealing, or isothermal holding, within a narrow temperature range between the glass transition and ice initial melting temperature where time-dependent ice formation can occur.[18,20,21] The initial melting temperature of ice within the maximally freeze-concentrated system is referred to as T'_m. This temperature corresponds to a temperature at which ice formation ceases as a result of kinetic limitations and it is observed as onset temperature of ice melting in heating of maximally freeze-concentrated systems.[18] T'_g and T'_m are initial concentration independent parameters. However, both T'_g and T'_m, depend on the molecular weight of solutes.

Roos and Karel[22] reported that the T'_g and T'_m coincided for high molecular weight carbohydrates, suggesting that the glass transition and the onset of ice melting in gelatinized starch may occur at the same temperature. There are numerous studies that emphasize a temperature below T'_g as an optimum storage temperature to avoid staling or undesirable changes in starch gels during frozen storage.[23] Nucleation could be promoted during freezing at temperatures above T'_g and when the frozen gel was thawed crystal growth rate could be enhanced. In fact, freezing/thawing is well known factors accelerating crystallization. This treatment is used in several starch-based products, to achieve desired textural characteristics. There is a general consensus that starch crystallization contributes significantly to staling or undesirable firming of bread and other starch-based products.[24–28] It may also be a factor affecting the quality of frozen dough and bread.[29]

In this work, the influence of a freezing pre-treatment of starch gels on their crystallization kinetics after thawing (+2 °C) was studied. The aim of the study was to evaluate the effect of freezing temperature, as a parameter controlling the extent of freeze-concentration, on starch recrystallization and the influence of starch gel composition using starch gels with glucose and lactose. These sugars have substantially different T'_g and T'_m values and freezing properties.[19]

2. Results and discussion

2.1. Gelatinization and recrystallization characteristics of starch systems

Table 1 presents the gelatinization enthalpy of the starches used in this work. Potato starch showed a swelling enthalpy, obtained from the starch–water mixture, of 18.90 ± 0.15 J/g of starch. This was notably higher than that of corn starch, 13.34 ± 0.12 J/g of starch. Granule shape and percentage of large and small granules have been reported to affect the gelatinization enthalpy values of starches.[30,31]

The initial gelatinization temperature of potato starch was 55.1 ± 0.4 °C, significantly lower than that of corn starch, 58.8 ± 0.3 °C. The differences in transition temperatures between the different starches may be attributed to the differences in the degree of crystallinity which provides structural stability and makes the granule more resistant towards gelatinization.[32,33] Singh and Singh[34] and Kaur et al.[35] also reported lower transition temperatures and higher gelatinization enthalpies for large and irregular or cuboidal potato starch granules. The higher transition temperatures for corn starch may result from the more rigid granular structure and the presence of lipids.[33] Amylose content in starch has also been related to the onset temperature of gelatinization. More heat is needed to initiate melting in the absence of amylose-rich amorphous regions and consequently the gelatinization temperature is higher.[33,36]

The endothermic transition of starches after gelatinization and storage at 2 °C appeared at notably lower transition temperatures than initial gelatinization. Some authors have reported that transition temperatures for retrogradated starches are 10–26 °C lower than those for gelatinization of starch granules.[31,37,38] The decrease in the melting temperatures suggested that smaller and/or less perfect crystalline regions were formed.[2,39,40] In the present study, differences between onset gelatinization and melting of recrystallized starch temperatures of 26 °C for potato starch and 29–30 °C for corn starch were obtained. It was remarkable that similar onset melting temperatures were obtained for recrystallized starch in corn and potato starches, around 30 °C, although the peak temperature of potato was notably higher than that of corn starch. The difference between the peak and the onset temperatures, which indicated broadness of the transition, was around 17 °C for corn starch and 26 °C for potato starch. This could be related to a higher imperfection and lower size of crystals that formed during potato starch recrystallization.[39]

The addition of glucose and lactose showed a significant effect on starch gelatinization. As shown in Table 1, these components increased both the onset and peak gelatinization temperatures and the heat of gelatinization. This has been considered to be due to (1) the ability of sugars to reduce water availability;[41] (2) starch–sugar interactions that stabilize the amorphous regions;[42–44] and (3) the increase in free volume and the antiplasticization effect of sugar in comparison with pure water.[45] The effect of the disaccharide lactose on swelling temperature was higher than that of glucose. However, the difference between the effect of glucose and lactose on gelatinization temperature was significant only when peak temperatures were analyzed. This was probably a consequence of the lower standard deviation obtained for peak temperature. A similar effect of sugars was observed in the onset temperatures of the melting of recrystallized starch. However, this was rather a

Table 1. Gelatinization enthalpy and onset and peak temperatures of gelatinization and melting of recrystallized starch from corn and potato starches doughs prepared only with water or with glucose or lactose solutions[a,b]

	Gelatinization			Retrogradation	
	T_{ONSET} (°C)	T_{PEAK} (°C)	$\Delta H_{\mathrm{NORMAL}}$ (J/g of starch)	T_{ONSET} (°C)	T_{PEAK} (°C)
Corn					
Water	58.80 ± 0.29^{c}	67.75 ± 0.07^{d}	13.34 ± 0.12^{a}	29.73 ± 0.75^{a}	47.07 ± 0.77^{a}
20% Glucose	60.86 ± 0.27^{d}	69.85 ± 0.07^{e}	14.16 ± 0.11^{b}	$31.18 \pm 0.72^{a,b}$	$48.40 \pm 0.71^{a,b}$
20% Lactose	61.50 ± 0.28^{e}	70.59 ± 0.07^{f}	14.00 ± 0.11^{b}	31.48 ± 0.76^{b}	48.77 ± 0.75^{c}
Potato					
Water	55.09 ± 0.37^{a}	61.52 ± 0.10^{a}	18.90 ± 0.15^{c}	29.57 ± 0.98^{a}	55.30 ± 0.97^{d}
20% Glucose	56.52 ± 0.40^{b}	63.39 ± 0.10^{b}	19.64 ± 0.16^{d}	$30.42 \pm 1.07^{a,b}$	56.40 ± 1.06^{d}
20% Lactose	57.35 ± 0.40^{b}	63.98 ± 0.10^{c}	19.18 ± 0.16^{c}	$31.10 \pm 1.10^{a,b}$	57.05 ± 1.06^{d}

[a] The water content was always 2.5 g/g of starch and the sugar content was 20% (w/w dry basis). Data are the mean ± confidence interval.

[b] Values in the same column with some letter in common means they are not significantly ($p < 0.05$) different (Tukey HSD test).

tendency than a significant effect. The standard deviation of these values was very high as a result of the broadening of these peaks and the difficulty of interpretation of onset temperatures with high accuracy. The presence of sugars also significantly increased ($p < 0.05$) starch gelatinization enthalpy. Glucose had the most pronounced effect with a 6% increase compared to the original corn swelling enthalpy and a 4% for potato starch. Spies and Hoseney[43] suggested that sugar could form crosslinks (sugar bridges) between starch chains in the amorphous regions of starch granules, which restricted starch swelling and thereby stabilized these regions. As chain flexibility is restricted by the crosslinks, more energy is required to pull the starch crystallites apart.

Glucose and lactose showed a significant effect on initial freezing temperature of starch gels (see Fig. 1). As shown in Table 2, the onset melting temperature of the maximally freeze-concentrated (T'_m) starch gels prepared with water were at −14 °C, both for corn and potato starches. However, when the gel was prepared with lactose the solution's T'_m decreased by 5 °C to −19 °C. Glucose decreased T'_m by around 10 °C to −25 °C. These additions of sugar corresponded to a sugar content of 20% (w/w dry basis) in the starch gel. The stronger effect of glucose compared to lactose was related to their freezing temperature depression in water.

2.2. Starch crystallization during storage of starch gels

The extent of starch crystallization was estimated from the enthalpy of the melting of recrystallized starch as a function of storage time at 2 °C (Fig. 2). The Avrami relationship[46,47] was fitted to experimental data leading to the continuous lines shown in Figure 2. This relationship is often used to model starch crystallization kinetics.[40,48] The results shown in Figure 2 indicated that the melting enthalpy increased dramatically during the first 1 or 2 days of storage, depending on the type of

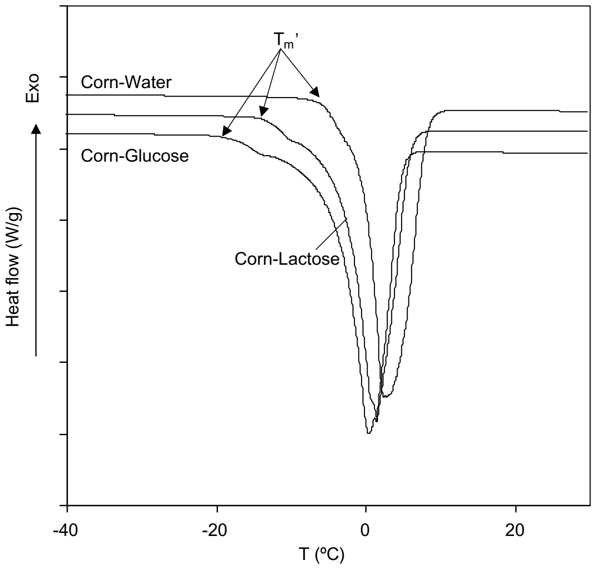

Figure 1. Ice melting endotherms obtained after annealing to determine the onset of ice melting temperatures, T'_m of corn starch gels with and without lactose or glucose.

Table 2. Values of the initial melting temperature of the maximally freeze-concentrated solution (T'_m) of gels prepared with corn or potato starches and water or 10% sugar solutions[a]

	T'_m (°C)		
	Water	Glucose	Lactose
Corn			
Average	−14.4	−24.7	−18.8
S.D.	0.1	0.1	0.2
Potato			
Average	−14.2	−24.8	−19.5
S.D.	0.2	0.1	0.2

[a] The water content was always 2.5 g/g of starch and the sugar content was 20% (w/w dry basis).

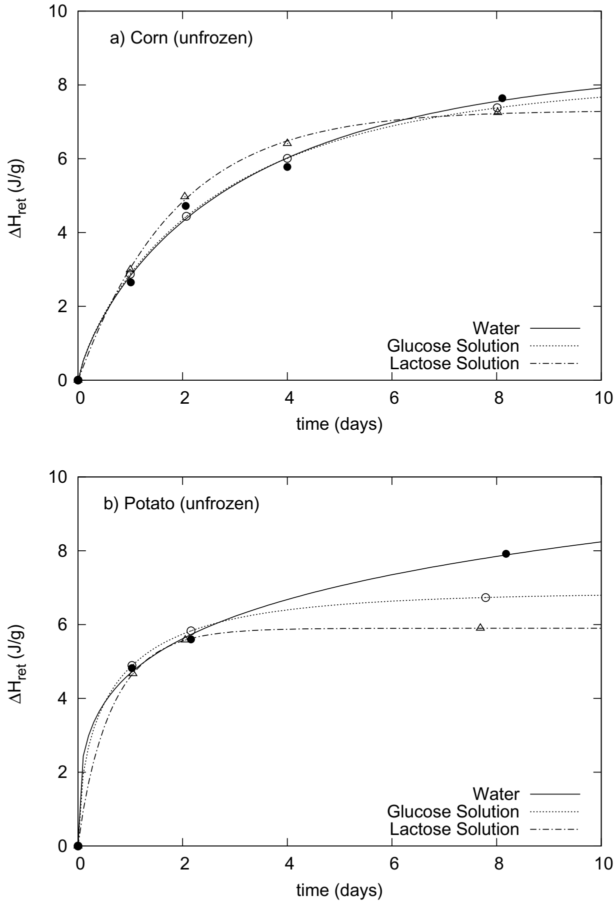

Figure 2. Melting enthalpy (J/g of starch) of recrystallized starch versus storage time at 2 °C of starch gels prepared with or without 20% glucose or 20% lactose; (a) corn starch, (b) potato starch. The continuous lines correspond to exponential equations that resulted from fitting the experimental values to the Avrami equation.[46,47]

starch, and then increased more slowly with further increases in storage time. Starch crystallization occurred more quickly in potato than in corn systems. After one day of storage, the melting enthalpy in potato starch gel was 4.8 J/g of starch, which represented about 70% of the maximum enthalpy obtained after eight days of storage. Corn starch required two days of storage to develop a similar crystallization extent.

Some authors have reported a higher initial tendency of potato starch gels towards crystallization than corn, rice and wheat starches.[49] In spite of this, approximately the same maximum enthalpies were attained for corn and potato starch gels after eight days of storage, 7.9 versus 7.6 J/g of starch. Melting enthalpies after levelling-off were presumed to indicate the extent of crystallization.[39] This means a lower recovery of crystallinity in 8 days in potato starch than in corn starch, 42% versus 57%.

Jouppila and Roos[39] and Jouppila, Kansikas and Roos[40] have shown that crystallization in starch occurred to a levelling-off extent that was dependent on water content and storage temperature. As water content and storage temperatures were maintained constant in the present study, the effect of the origin of starch in its crystallization could also be concluded. The amylose content has been reported to be one of the influential factors for starch crystallization[50–53] and traditionally linked to a greater crystallization tendency in starches.[54] Amylopectin also plays an important role in starch crystallization during refrigerated storage.[55] Higher perfection and the size of crystals formed during recrystallization could also explain the higher melting enthalpies found in starch gels.[3]

Crystallization kinetics of starch gels at 2 °C was not notably affected by sugars. Especially at short storage times, no significant differences between the presence and the absence of sugar in melting enthalpies of recrystallized starch were obtained. However, at 8 days of storage, a small effect of sugars on reducing starch crystallization could be observed, especially in potato starch. The effect of sugars as suppressors of the crystallization of starch molecules has been emphasized by many authors[56–58] although the effectiveness of different sugars often varies between reports, even there are studies that state that sugars, such as fructose, increased the recrystallization of wheat starch[59] or corn starch systems.[60] Generally, these differences in reported effects of sugars can be ascribed to the differences in starch variety, sugar concentration, aging temperature and time,[57] as was observed in our work in relation to starch source and storage time.

2.3. Starch crystallization in prefrozen starch gels

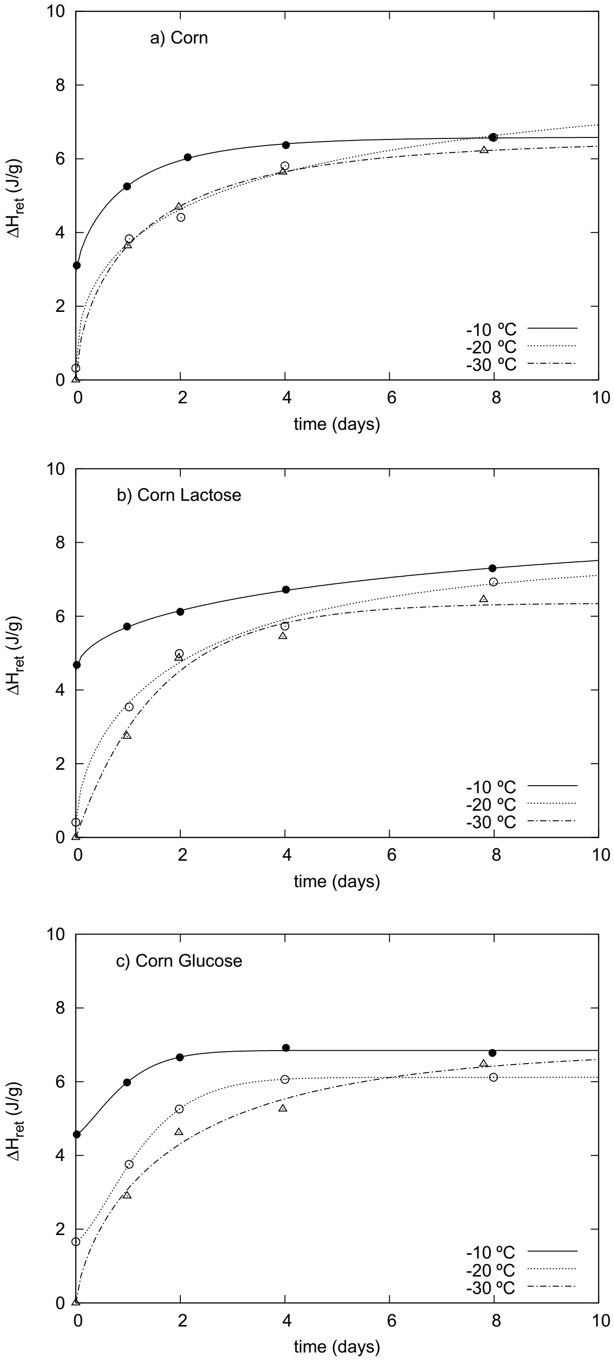

Figure 3. Melting enthalpy (J/g of starch) of recrystallized starch versus storage time at 2 °C of prefrozen/thawed corn starch gels at freezing temperatures of −10 °C, −20 °C and −30 °C prepared with (a) water; (b) lactose; (c) glucose. The continuous lines correspond to exponential equations that resulted from fitting the experimental values to the Avrami equation.[46,47]

Figures 3 and 4 show the effect of a prefreezing/thawing treatment of starch gels on their crystallization kinetics during refrigerated storage. The freezing temperatures applied during 24 h to starch gels, −10 °C, −20 °C or −30 °C, greatly affected the crystallization behaviour once the gels were thawed and stored at +2 °C. The effect of freezing temperature on the extent of starch recrystallization was evident over the whole storage

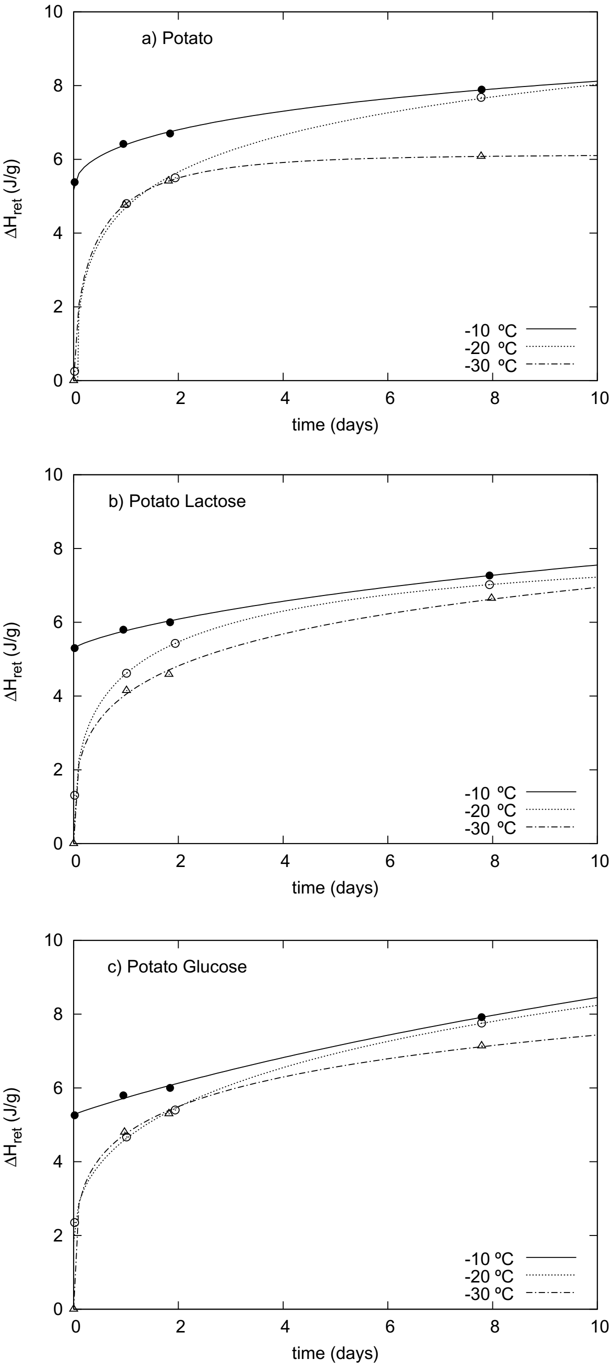

Figure 4. Melting enthalpy (J/g of starch) of recrystallized starch versus storage time at 2 °C of prefrozen/thawed potato starch gels at freezing temperatures of −10 °C, −20 °C and −30 °C prepared with (a) water; (b) lactose; (c) glucose. The continuous lines correspond to exponential equations that resulted from fitting the experimental values to the Avrami equation.[46,47]

period. The biggest differences were observed during the first days of storage.

The presence of sugars in starch also showed a marked effect on crystallization kinetics of the gels. The initial state of starch gels (zero points in Figs. 3, 4) were taken from thermograms obtained for gels immediately after 24 h of freezing prior to storage at 2 °C. Prefrozen starch gels showed some recrystallization due to freeze-concentration in the prefreezing step. Figure 3a shows enthalpies for corn starch gels without sugar. It can be observed that freezing at −30 °C did not enhance crystallization. This means that during the 24 h freezing at −30 °C nucleation and recrystallization were reduced. The same was observed at −20 °C, as the initial enthalpy, 0.32 J/g of starch, was minimal. The crystallization curves obtained after freezing at −30 °C or −20 °C were very similar and both showed exactly the same shapes as those obtained for gels without prefreezing.

Very different crystallization behaviour was obtained when −10 °C frozen corn starch gels were stored at 2 °C. The most remarkable fact was the high initial enthalpy appearing as a result of freezing without storage at 2 °C. This was 3.2 J/g of starch, corresponding to those obtained after one day for gels frozen at −20 °C or −30 °C or without prefreezing. This enthalpy increased further during storage. This increase was fast during the first two days and then levelled off. Gels prefrozen at −10 °C showed in one day a melting enthalpy of 5.3 J/g of starch, similar to gels stored four days subsequent to freezing at −20 °C or −30 °C or unfrozen. In two days, the gels had doubled the extent of crystallization. Independent of the initial extent of crystallization, prefrozen and control gels showed similar melting behaviour at the end of storage. It can be concluded that freeze-concentration at −10 °C had an important effect on the crystallization kinetics but not on the final extent of recrystallization. That was probably more dependent on the storage temperature.[16,39,40] The effect of freezing temperature on starch crystallization during the storage was related to the freezing temperature and the T'_g and T'_m of starch gels. When water–starch gels with a T'_m of −14 °C were frozen at −10 °C, they were not maximally freeze-concentrated. In this condition, molecular mobility of amorphous compounds, as gelatinized starch, was enhanced by unfrozen water and consequently molecular rearrangements for nucleation and growing of crystals could occur.[3]

Figures 3b and c show the enthalpy corresponding to the melting of crystals formed during the storage of prefrozen sugar–corn starch gels. The three curves in Figure 3b, corresponding to lactose–starch gels, were totally similar to those of control gels. At zero storage time only gels frozen at −10 °C showed notable enthalpy values of 4.7 J/g of starch. This initial crystallization led to similar differences to that noticed for gels prefrozen at −20 °C and −30 °C for the water–corn starch gels. The initial melting enthalpy of gels prefrozen at −20 °C, 0.41 J/g of starch, was near to the detection limit. Differences in results, as shown in Figure 3c, were obtained for glucose–corn starch gels. In this case, not only gels frozen at −10 °C but also those frozen at −20 °C showed initial enthalpies of 4.6 and 1.7 J/g of starch, respectively.

These results were in agreement with the previous ones and were also related to the T'_m measured for lactose and glucose–corn starch gels used in this work (18.8 °C and 24.7 °C, respectively). When gels were prefrozen at −20 °C, only those with 20% lactose could be near the glassy state. However, gels with 20% glucose were only partially frozen and therefore, molecular mobility and recrystallization could take place rapidly. Gels prefrozen at −10 °C with glucose or lactose attained, after 8 days of storage, a higher melting enthalpy than that of gels frozen at −20 °C and −30 °C. This can be explained by the increase in nucleation and crystal growth rates during the freezing at −10 °C. The extent of crystallization at storage temperatures under low T–T_g was proposed to be low because, while nucleation was fast, crystal growth was kinetically restricted and slow, because molecular mobility was slow. Under high T–T_g conditions, crystal growth was fast, but nucleation occurred slowly.[39,45] A temperature of −10 °C is well above glass transition temperature, T'_g, and T'_m. This relatively low level of freeze-concentration and high amount of unfrozen water can substantially enhance nucleation and crystals growth.[23] The prefreezing induced crystallization further enhanced the growth and maturation of crystals during further storage at 2 °C.

Figures 4a–c show the crystallization during the storage of prefrozen potato starch gels. The study with potato starch gels confirmed similar results already observed for corn starch with regard to the effect of sugars and freezing temperature. Only minor differences were observed: Potato starch gels with 20% lactose prefrozen at −20 °C showed some initial crystallization prior to storage at 2 °C. With corn starch gels, this was only observed when glucose was present. The initial melting enthalpy of lactose–potato starch gels was of 1.3 J/g of starch after freezing at −20 °C and 5.3 J/g of starch when the freezing temperature was −10 °C. Likewise, the initial melting enthalpy of glucose–potato starch gels was of 2.4 and 5.3 J/g of starch after freezing temperatures of −20 °C and −10 °C, respectively.

It can also be confirmed from the curves of Figure 4 that melting enthalpies were always higher for freezing at −10 °C and lower for freezing at −30 °C. In agreement with the nucleation and crystal growth theories prefreezing at −10 °C allowed the formation of a high number of nuclei that probably had started to grow at the end of the freezing period. Further storage at 2 °C enhanced the propagation of nuclei leading to a fast growth of crystallites in the early storage time. Unfrozen gels or gels prefrozen at temperatures under T'_g started the storage period without nuclei formed.[45]

The prefreezing temperature showed a significant ($p < 0.05$) effect on the broadness of the melting peaks obtained after storage at 2 °C, both in corn and potato starch gels. The values of differences between the peak and the onset temperatures ($T_p - T_o$) that indicated the broadness of the transition, showed the following order: $-10\ °C < -20\ °C < -30\ °C$. The broadness of melting peaks of unfrozen samples was always higher. The mean value of ($T_p - T_o$) for −10 °C prefrozen corn starch gels was 15.8 ± 0.9 °C, and for unfrozen gels of 19.0 ± 1.0 °C. In potato starch gels, these values were 25.6 ± 1.3 °C and 28.3 ± 0.9 °C, respectively. This indicated that samples prefrozen at −10 °C could have a higher degree of perfection of crystallites.[39,61]

In summary, we have proved that the crystallization of amorphous starch during refrigeration storage is enhanced by prefreezing treatment at temperatures above T'_m. Molecular mobility is enhanced by unfrozen water and consequently molecular rearrangements for nucleation and growing of starch crystals can occur. The prefreezing induced crystallization further enhanced the growth and the maturation of crystals during further storage at 2 °C. This explained the faster crystallization kinetics found both in corn and potato starch gels prefrozen at −10 °C and the higher crystallization extent achieved after eight days of storage. A freezing pretreatment of starch gels at temperatures below T'_g, as −30 °C, have no effect on corn and potato starch gels crystallization. The presence of 20% glucose or lactose in starch gels markedly changes the effect of prefreezing on starch crystallization kinetics, especially in short-term storage. This can be predicted from the effect of sugars on Tm′ and T'_g that in gelatinized starch will coincide with the same value. The knowledge of T'_m and T'_g gives a useful control tool for the desirable freezing conditions of products based on starch gels.

3. Experimental

3.1. Materials

Corn starch (moisture content of 12.1%, amylose content 25–27%) and potato starch (moisture 17.3% and amylose content of 21–23%) were obtained from Cargill (Mechelen, Belgium). Glucose and lactose monohydrate grade reagent were obtained from Sigma Chemical Co. (Saint Louis, MO, USA).

3.2. Sample preparation

Starch dispersions (corn or potato starch) with 6–9 mg of dried starch and water, or 10% (w/w) glucose or lactose solutions, were directly prepared in aluminium DSC (Differential scanning calorimetry) pans (40 μL). The water contents were adjusted with a microsyringe to give a constant H_2O–starch ratio of 2.5 ± 0.1 g H_2O/g starch solids and a sugar:starch ratio, when sugar was present, of 0.26 ± 0.02 g glucose or lactose/g starch solids which corresponded to 20.6 g sugar/100 g dry solids. Samples were weighted using a Mettler AG245 (Mettler Toledo, Schwerzenbch, Switzerland) electronic balance (0.01 mg accuracy). The gelatinization of starches was achieved by heating suspensions in the DSC furnace.

3.3. Storage conditions

Three different freezing temperatures, -30 ± 1 °C, -20 ± 1 °C and -10 ± 2 °C, were used to gelatinize starches with or without glucose or lactose added. After 24 ± 1 h, starch gels were defrosted for 5 min at room temperature (≈22 °C) and stored at 2 ± 1 °C, for 0, 1, 2, 4 and 8 days. Potato starch gels were stored for 0, 1, 2, and 8 days. Experiments with unfrozen gelatinized starch were also carried out. The freezing temperatures were chosen after determining the T'_m of starch gels. Hence, ice melting in the systems followed the order: $-30\ °\mathrm{C} < T'_{\mathrm{m\ starch+glucose}} < -20\ °\mathrm{C} < T'_{\mathrm{m\ starch+lactose}} < T'_{\mathrm{m\ starch}} < -10\ °\mathrm{C}$. T'_m was used as it determines the temperature above which dilution of the gels occurs as a result of ice melting.[22] It was assumed that T'_g was close to T'_m in storage.[22] Triplicate analyses were carried out for samples at all storage conditions.

3.4. Differential scanning calorimetry (DSC)

A Mettler Toledo-27331 (Schwerzenbch, Switzerland) equipped with a liquid nitrogen cooling system and nitrogen purge gas was used in the experimental work. The gelatinization of starch was achieved in heating from 15 °C to 105 °C and measured using DSC pans with starch–water at a heating rate of 5 °C/min. An empty pan was used as a reference. The DSC was calibrated for temperature using *n*-hexane (melting point, −95.0 °C), mercury (melting point, −38.8 °C), H_2O (melting point, 0.0 °C), gallium (melting point, 29.8 °C) and indium (melting point, 156.6 °C) and for heat flow using *n*-hexane (ΔH_m, 151.8 J/g), mercury (ΔH_m, 11.4 J/g), H_2O (ΔH_m, 334.5 J/g) gallium (ΔH_m, 80 J/g) and indium (ΔH_m, 28.45 J/g).

The ice melting temperature of the maximally freeze-concentrated system was obtained from specific tests. It was taken after annealing of samples for 15 min at a temperature slightly below T'_m.[22] Pans were firstly scanned over the gelatinization temperature region, then cooled at a rate of 20 °C/min to −80 °C, heated again until the annealing temperature and cooled to −100 °C. The final scan was at 5 °C/min to 25 °C. The T'_m values are the average of three determinations. The annealing temperatures were chosen for each system at 2 °C below the onset ice melting temperature, T'_m, in preliminary tests. Starch recrystallization was evaluated from DSC endotherms and enthalpies obtained by DSC during the scanning from 0 °C to 105 °C at a heating rate of 5 °C/min. The onset and peak temperatures and the melting enthalpy of the systems were determined using STAR^e thermal analysis software, version 6.0 (Mettler Toledo, Schwerzenbch, Switzerland). The endotherms were taken as signals of the melting of recrystallized regions of starch components with size corresponding to the heat of melting and crystallinity.

3.5. Statistical analysis

To assess significant differences among samples, a multiple comparison analysis of samples using the program Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, MD, USA) was done. The HSD Tukey test was used to describe means with 95% confidence.

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References

1. Blanshard, J. M. V. Starch Granule Structure and Function: A Physicochemical Approach. In *Starch: Properties and Potential*; Galliard, T., Ed.; John Wiley & Sons: Chichester, 1987; pp 16–54.
2. Slade, L.; Levine, H. A Food Polymer Science Approach to Selected Aspects of Starch Gelatinization and Retrogradation. In *Frontiers in Carbohydrate Research*; Millane, R. P., BeMiller, J. N., Chandrasckaran, R., Eds.; Elsevier Applied Science: London, 1989; pp 25–270.
3. Roos, Y. H. *Phase Transitions in Foods*; Academic Press: San Diego, CA, 1995.
4. Zobel, H. F. *Starch* **1988**, *40*, 1–7.
5. Zobel, H. F. *Starch* **1988**, *40*, 44–50.
6. Galliard, T.; Bowler, P. In *Starch Properties and Potential*; Galliard, T., Ed.; John Wiley & Sons: Chichester, 1987; pp 55–78.
7. Sarko, A.; Wu, H.-C. H. *Starch* **1978**, *30*, 73–78.
8. Atwell, W. A.; Hood, L.; Lineback, D.; Varriano-Martson, E.; Zobel, H. *Cereal Food World* **1988**, *33*, 306–311.
9. Hoover, R. *Carbohydr. Polym.* **2001**, *45*, 253–267.
10. Stevens, D. J.; Elton, G. A. H. *Starch* **1981**, *23*, 8–11.
11. Biliaderis, C. G. *Can. J. Physiol. Pharmacol.* **1991**, *69*, 60–78.
12. Morris, V. J. *Trends Food Sci. Technol.* **1990**, *1*, 2–6.
13. Miles, M. J.; Morris, V. J.; Oxford, P. D.; Ring, S. G. *Carbohydr. Res.* **1985**, *135*, 271–278.

14. Ring, S. R.; Colonna, P.; I'Anson, K. J.; Kalichevsky, M. T.; Miles, M. J.; Morris, V. J.; Oxford, P. D. *Carbohydr. Res.* **1987**, *162*, 277–293.
15. Cornford, S. J.; Axford, D. W. E.; Elton, G. A. H. *Cereal Chem.* **1964**, *41*, 216–229.
16. Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, New York, 1953.
17. Laine, M. J. K.; Roos, Y. Water Plasticization and Recrystallization of Starch in Relation to Glass Transition. In *Proceedings of the Poster Session, International Symposium on the Properties of Water, Practicum II*, Argaiz, A., López-Malo, A., Palou, E., Corte, P., Eds.; Universidad de las Américas: Puebla, 1994; pp 109–112.
18. Roos, Y. H.; Karel, M. *Int. J. Food Sci. Technol.* **1991**, *26*, 553–566.
19. Roos, Y. H. *Carbohydr. Res.* **1993**, *238*, 39–48.
20. Roos, Y. H.; Karel, M. *Cryo-Lett.* **1991**, *12*, 367–376.
21. Tananuwog, K.; Reid, D. S. *J. Agric. Food Chem.* **2004**, *52*, 4308–4317.
22. Roos, Y. H.; Karel, M. *J. Food Sci.* **1991**, *56*, 1676–1681.
23. Slade, L.; Levine, H. *CRC Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 115–360.
24. D'Appolonia, B. L.; MOrad, M. M. *Cereal Chem.* **1981**, *58*, 186–190.
25. Knightly, W. H. *Baker's Dig.* **1977**, *51*, 52–56.
26. Kulp, K.; Ponte, J. G. *Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 1–48.
27. Maga, J. A. *Crit. Rev. Food Technol.* **1975**, *5*, 443–486.
28. Seow, C. C.; Thevamalar, K. Problems Associated with Traditional Malaysian Starch-based Intermediate Moisture Foods. In *Food Preservation by Moisture Control*; Seoww, C. C., Ed.; Elsevier Applied Science: London, 1988; pp 233–252.
29. Ribotta, P. D.; León, A. E.; Añón, M. C. *Food Res. Int.* **2003**, *36*, 357–363.
30. Stevens, D. J.; Elton, G. A. H. *Starch* **1971**, *23*, 8–11.
31. Yuan, R. C.; Thomson, D. B.; Boyer, C. D. *Cereal Chem.* **1993**, *70*, 81–89.
32. Barichello, V.; Yada, R. Y.; Coffin, R. H.; Stanley, D. W. *J. Food Sci.* **1990**, *54*, 1054–1059.
33. Singh, N.; Singh, J.; Kaur, L.; Singh Sodhi, N.; Singh Gill, B. *Food Chem.* **2003**, *81*, 219–231.
34. Singh, J.; Singh, N. *Food Chem.* **2001**, *75*, 67–77.
35. Kaur, L.; Singh, N.; Sodhi, N. S.; Gujral, H. S. *Food Chem.* **2002**, *79*, 177–181.
36. Fredriksson, H.; Silverio, J.; Andersson, R.; Eliasson, A. C.; Aman, P. *Carbohydr. Polym.* **1998**, *35*, 119–134.
37. Baker, L. A.; Rayas-Duarte, P. *Cereal Chem.* **1998**, *75*, 301–303.
38. White, P. J.; Abbas, I. R.; Johnson, L. J. *Starch* **1989**, *41*, 176–180.
39. Jouppila, K.; Roos, Y. H. *Carbohydr. Polym.* **1997**, *32*, 95–104.
40. Jouppila, K.; Kansikas, J.; Roos, Y. H. *Carbohydr. Polym.* **1998**, *36*, 143–149.
41. Derby, R. I.; Miller, B. S.; Miller, B. F.; Trimbo, H. B. *Cereal Chem.* **1975**, *52*, 702–713.
42. Lelievre, J. *Polymer* **1976**, *17*, 854–858.
43. Spies, R. D.; Hoseney, R. C. *Cereal Chem.* **1982**, *59*, 128–131.
44. Hansen, L. M.; Setser, C. S.; Paukstelis, J. V. *Cereal Chem.* **1989**, *66*, 411–415.
45. Levine, H.; Slade, L. Influences of the Glassy and Rubbery States on the Thermal, Mechanical and Structural Properties of Doughs and Baked Products. In *Dough Rheology and Baked Product Texture*; Faridi, H., Faubion, J. M., Eds.; AVI: New York, 1990; pp 157–330.
46. Avrami, M. *J. Chem. Phys.* **1940**, *8*, 212–224.
47. Sperling, L. H. *Introduction to Physical Polymer Science*; Wiley: New York, 1986.
48. Hsu, C.-L.; Heldman, D. R. *J. Food Process Eng.* **2005**, *28*, 506–525.
49. Singh, J.; Singh, N.; Saxena, S. K. *J. Food Eng.* **2002**, *52*, 9–16.
50. Baik, M. Y.; Kim, K. J.; Cheon, K. C.; Ha, Y. C.; Kim, W. S. *J. Agric. Food Chem.* **1997**, *45*, 4243–4248.
51. Gudmundsson, M.; Eliasson, A. C. *Carbohydr. Polym.* **1990**, *13*, 295–315.
52. Fan, J.; Marks, B. P. *Cereal Chem.* **1998**, *75*, 153–155.
53. Kaur, L.; Singh, N.; Shodi, N. S. *Food Chem.* **2002**, *79*, 183–192.
54. Whistler, R. L.; BeMiller, J. N. Starch. In *Carbohydrate Chemistry for Food Scientists*; Whistler, R. L., BeMiller, J. N., Eds.; Eagan Press: Eagan Press St. Paul, MN, 1996; pp 117–151.
55. Yamin, F. F.; Lee, M.; Pollak, L. M.; White, P. J. *Cereal Chem.* **1999**, *76*, 175–181.
56. Biliaderis, C. G.; Prokopowich, D. J. *Carbohydr. Polym.* **1994**, *23*, 193–202.
57. Bello-Perez, L. A.; Paredes-Lopez, O. *Starch* **1995**, *47*, 83–86.
58. Kohyama, K.; Nishinari, K. *J. Agric. Food Chem.* **1991**, *39*, 1406–1410.
59. Cairns, P.; Miles, M. J.; Morris, V. J. *Carbohydr. Polym.* **1991**, *16*, 355–365.
60. Wang, Y.-J.; Jane, J. *Cereal Chem.* **1994**, *71*, 527–531.
61. Chang, S.-M.; Liu, L.-C. *J. Food Sci.* **1991**, *56*, 564–566.