Effect of freezing rate and frozen storage on starch–sucrose–hydrocolloid systems

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Abstract: Model food systems based on starch (100 g kg^{-1}), sucrose (150 g kg^{-1}) and water (750 g kg^{-1}) with and without the addition of a low proportion of hydrocolloid (xanthan gum, guar gum or sodium alginate) were gelatinised, frozen at different rates and stored to analyse textural changes by oscillatory rheometry. Differential scanning calorimetry (DSC) was used to analyse gelatinisation, amylopectin retrogradation and glass transition temperatures. Sucrose had a significant effect on the increase in the gelatinisation temperature as well as on the decrease observed in glass transition values. The onset temperature of the second step of the glass transition, corresponding to the heat capacity change close to ice melting (denoted Tg_{im} in the present work), ranged between −23.0 and −22.2 °C. Rheological viscoelastic tests showed an increase in the dynamic moduli G^* and G' after slow freezing and during storage at −19 °C ($T > Tg_{im}$) in starch–sucrose systems that is related to sponge formation due to amylose retrogradation. DSC studies confirmed that also amylopectin retrogradation occurs during storage; however, samples containing gums did not develop the spongy appearance. Storage at the usual commercial temperatures (close to −18 °C, slightly above Tg_{im}) affects the quality of aqueous starch–sucrose pastes without gums owing to amylose and amylopectin retrogradation. However, when hydrocolloids are included in the formulations, the usual storage conditions allow the maintenance of acceptable textural attributes.

Keywords: sucrose; gelatinised starch suspensions; hydrocolloids; freezing; glass transition; rheology

INTRODUCTION

Formulated food systems based on gelatinised starch may undergo important textural changes related to amylose and amylopectin retrogradation; they can show syneresis (exudate production) during low-temperature conservation and even over the course of frozen storage. These changes may make such products unacceptable to the consumer.[1,2] As starch retrogradation is a recrystallisation process, it is controlled by diffusion and depends on solute mobility in the system. Amylose retrogradation is commonly related to rheological changes in the system;[3,4] amylopectin retrogradation can usually be measured by differential scanning calorimetry.[1–3,5]

By applying the physical chemistry of polymers to foods, many diffusion-controlled deteriorative phenomena have been related to glass transition.[6–8] The latter takes place when a completely amorphous polymer undergoes a transition from a vitreous state to a high-mobility rubbery state where diffusion-controlled changes occur. The effect of water as a plasticiser of food systems is manifested as a depression of the glass transition temperature of amorphous components.[6]

The starch gelatinisation process can be regarded as a fusion of the crystalline starch regions in the presence of enough water and heating. Gelatinisation is kinetically limited owing to the partially crystalline condition of the native starch granule, which establishes a non-equilibrium state.[8,9]

Retrogradation of gelatinised starch can be interpreted as a recrystallisation of amylose and amylopectin occurring between T_g (the glass transition temperature of the gelatinised water–solute matrix) and T_m (the melting temperature, in this case the starch gelatinisation temperature).

The glass transition temperature of the gelatinised matrix depends on the thermal history of the system and its composition. On freezing foodstuffs, water separates as ice and the solute concentration increases in the unfrozen matrix. In starch–water systems, once all freezable water becomes separated as ice during

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freezing, a concentrated matrix of the gelatinised starch and water surrounding the ice crystalline zones is obtained. Maximum ice formation is attained by slow freezing and annealing at a temperature close to the glass transition value; under such conditions an unfrozen matrix which contains maximum solute concentration is obtained. The temperature at which the glass transition takes place in this matrix is denoted T'_g; it depends only on the composition of the system and not on its thermal history.

The formulation of more complex food products often involves the addition of other components such as low-molecular-weight sugars, which play several roles: taste, reduction of water activity, and contribution to texture. The addition of this kind of carbohydrate affects both the starch gelatinisation process and the subsequent retrogradation that may occur during frozen storage; a well-known phenomenon is the shift of the gelatinisation temperature towards higher values, which has been interpreted in several ways by different authors.[6,10–13] The effect of low-molecular-weight sugars on starch retrogradation is controversial; it has been demonstrated in some cases that sugars can inhibit retrogradation,[14,15] and in others that they enhance it.[16] The explanations about their 'plasticiser' or 'antiplasticiser' role in starch-based systems are contradictory, but mostly because the comparisons are made in systems with different water contents.[6,17]

Several authors[7,18–23] reported that many systems containing low-molecular-weight carbohydrates present two successive steps in the DSC thermograms; these two successive steps have been interpreted in different ways. For Slade and Levine,[18] this feature in frozen sugar aqueous solutions represents two successive glass transitions corresponding to different glassy domains: the dilute bulk glass with a lower value of T_g (first step) and the freeze-concentrated glass with a higher value of T_g (second step), denoted T'_g. In more complex aqueous model systems containing proteins–sugars, polysaccharides–sugars and other components, multiple T'_g values have been attributed by Slade and Levine[18] to the coexistence of two distinct aqueous glasses. However, several authors have given other interpretations of this phenomenon. Simatos and Blond[19–21] consider that the two-step increase in heat capacity is representative of a single glass transition with associated relaxation and the second step of the glass transition is superposed to ice melting. For Ablett *et al*[22] the second transition in the DSC thermogram is not a glass transition but is a transition related to the onset of ice dissolution. Shalaev and Franks[23] reported that this second step 'contains a contribution due to heat absorption that accompanies the melting of ice, followed by the dilution of the supersaturated solution phase and associated enthalpy changes'. For all these authors,[21–23] T'_g would be closer to the lower temperature value corresponding to the first heat capacity change.

When keeping food at a storage temperature below the glass transition value of the freeze-concentrated matrix, diffusion processes are expected to be hindered to prevent certain changes during food conservation. The temperature of the second step, close to ice melting, may be an important temperature to consider with respect to stability, as ice melting induces a dramatic increase in molecular mobility and a decrease in the system viscosity. However, it is a subject of controversy whether this temperature is the true T'_g. In the present work, to avoid confusion, we denote by Tg_{im} the temperature of the second step of the glass transition corresponding to the heat capacity change close to ice melting.

Starch-based formulations are usually stabilised with low proportions of hydrocolloids[24] such as xanthan gum, sodium alginate or guar gum, which help to minimise the negative effects of freezing and frozen storage. In previous works[2,25] we demonstrated the beneficial effect of using small quantities of gums in gelatinised starch aqueous systems with high water content to minimise textural changes. In pastes submitted to slow freezing and abusive frozen storage conditions, hydrocolloids prevent the formation of an elastic, opaque, sponge-like structure and exudate production due to amylose retrogradation.

The objectives of the present work were:

(a) to analyse the effect of sucrose and hydrocolloids (xanthan gum, guar gum and sodium alginate) on the rheological behaviour of gelatinised water–starch systems frozen at different rates and stored at the usual commercial temperatures (close to −18 °C);
(b) to determine thermal transitions in such systems, especially gelatinisation and the glass transition temperature Tg_{im};
(c) to relate amylopectin retrogradation and textural changes due to amylose retrogradation to the location of the thermal transition Tg_{im} with respect to the frozen storage temperature;
(d) to establish whether this thermal transition Tg_{im} is a relevant parameter to maintain the texture quality of starch–sucrose-based foods containing hydrocolloids during frozen storage.

MATERIALS AND METHODS

Commercial corn starch (Molinos Río de La Plata, Argentina) was used in all cases for the starch aqueous system formulations. The hydrocolloids used (xanthan gum, guar gum and sodium alginate) were of commercial type and provided by Saporiti Hnos SACIF (Argentina). Sucrose was provided by Anedra (Argentina).

The selected range of ingredient concentrations was based on common food product formulations such as puddings, custards and other desserts in order to get useful technological applications. The composition of each of the tested samples is summarised in Table 1.

Table 1. Formulation of analysed systems

	Composition (g kg^{-1})			
Nomenclature[a]	Corn starch	Sucrose	Hydrocolloid (type)	Water
CW	100	—	—	900
CW*	200	—	—	800
CW**	250	—	—	750
SW	—	150	—	850
CSW	100	150	—	750
CSW*	200	300	—	500
CSXW	100	150	10 (X)	740
CSXW*	200	300	20 (X)	480
CSGW	100	150	10 (G)	740
CSGW*	200	300	20 (G)	480
CSAW	100	150	10 (A)	740
CSAW*	200	300	20 (A)	480

[a]C, corn starch; W, water; S, sucrose; X, xanthan gum; G, guar gum; A, sodium alginate.

Thermal characterisation of the systems: differential scanning calorimetry assays

The thermal transitions were studied by differential scanning calorimetry (DSC). A Du Pont calorimeter with 910 cell (Du Pont Co, USA) fitted with conventional refrigeration was used; this equipment allows the tempering process to be performed at a controlled temperature. For the gelatinisation tests, starch–water samples containing sucrose and gums were used; CW, CW**, CSW, CSXW, CSGW and CSAW formulations (Table 1) were each analysed in six replicates. CW** samples were formulated with the same water content as CSW samples to analyse the effect of sucrose on the gelatinisation process in systems with the same solid content. The main experimental difficulties were found in the preparation of the starch paste with gums; this stage involved the handling of highly hydrophilic components which competed with each other for the available water. Components were dry-mixed and then slowly added to the predetermined amount of water under constant stirring. Once a homogeneous system was obtained, the DSC hermetic aluminium pans were filled with aliquots of 10–15 mg for the thermal analysis runs. The heating rate for the gelatinisation DSC tests was 10 °C min^{-1}, starting at 20 °C and ending at 120 °C. An empty double pan was used as reference.

To analyse the glass transition of the freeze-concentrated matrix containing the maximum solute concentration, freezing must be carried out slowly to allow maximum possible ice formation, and then the sample has to be annealed. Considering that this thermal transition is characteristic of the matrix with maximum solute concentration regardless of the initial concentration, and that the transition had to be observed with the best possible accuracy, the concentrations were doubled for these tests (Table 1, formulations indicated with one asterisk).

Samples in the DSC pans were gelatinised *in situ* and then frozen at 1.5 °C min^{-1} from 25 to −40 °C, the minimum temperature reached by this equipment. Samples were then heated at 1.5 °C min^{-1} and annealed at −19 °C for 30 min. After this annealing period they were again cooled to −40 °C and reheated to ambient temperature at 1.5 °C min^{-1}. The annealing temperature was selected from preliminary tests where only slow freezing was utilised, and corresponds to a temperature just above the Tg_{im} onset. Cooling and heating procedures were carried out at the same rate (1.5 °C min^{-1}) to avoid relaxation phenomena. Tests were carried out in five replicates, using an empty double pan as reference.

Amylopectin retrogradation by DSC

Recrystallisation of amylopectin is a reversible process that can be analysed by DSC below 100 °C.[3,5]

Amylopectin retrogradation was measured from DSC runs immediately after freezing and after 91 days of storage at −80 and −19 °C. Aliquots (10–15 mg) of the samples used for the rheological assays (see description in the following subsection) were weighed into the DSC pans. Tests were run in duplicate, against a double reference pan, between 20 and 120 °C at a heating rate of 10 °C min^{-1} in order to detect the presence of the amylopectin retrogradation peak.

Amylose retrogradation was not determined by DSC, because the process is not reversible below the maximum attained temperature of 120 °C.

Oscillatory rheological studies and textural analysis

Formulations (Table 1) with hydrocolloids (CSXW, CSGW and CSAW) and without them (CSW) were studied. Two batches (250 g each) were prepared for each formulation by following the mixing procedure described previously. Systems were heated at 90 °C for 15 min in a Haake L thermostatic bath (Haake, Germany) with continuous mechanical stirring. Gelatinisation was controlled through microscopy observations with polarised light (Leitz Ortholux II, Leitz, Germany), which allowed a total loss of birefringence to be verified once the given heating time had elapsed.

Before freezing, each sample was divided into aliquots, placed in high-density polyethylene cylindrical containers 2.5 cm in diameter and 1.5 cm high, and wrapped with Parafilm to prevent dehydration during storage and exudate losses during thawing. Samples were frozen at two different rates: (a) quick freezing with a liquid nitrogen jet in a commercial Mini-Freezer (White Martins Gases Ind, Brazil), and (b) slow freezing in a still-air freezer Gafa S400 at −19 °C (Frimetal SA, Argentina). The quick freezing was recorded by using thermocouples inserted in the thermal centre of control samples placed in different zones of the Mini-Freezer compartment. The temperature sensors were connected to a Keithley Metrobyte DAS-TC/B analogue temperature logger (USA); the mean quick freezing rate measured on the obtained time–temperature curves (in the temperature range between −5 and −20 °C) was 4 °C min^{-1}. The

slow freezing rate was 0.1 °C min^{-1} and was measured using a similar thermocouple system connected to an Omega recorder (USA).

Once the samples were frozen, they were placed in a temperature-controlled cold store chamber −19 °C (±1 °C). During 3 months of storage, two samples from each formulation (different batches) were thawed at different times; thawing was done in a temperature-controlled room at 20 °C. Unfrozen samples as well as quickly frozen samples stored at −80 °C were used as controls.

The rheological behaviour was studied in a Haake RV20 oscillatory rheometer (Haake, Germany) using a plate–plate sensor system with 1 mm gap between plates. Data were analysed using Haake Oscillation 2.0 software. A Haake C thermostatic bath (Haake, Germany) was used to make measurements at 20 °C (±0.1 °C). Two types of rheological tests were conducted: (a) deformation sweeps at a constant frequency to determine the maximum deformation attainable by a sample in the linear viscoelastic range (γ_{lim}), and (b) frequency sweeps (from 0.01 to 10 Hz) at a constant deformation within the linear viscoelastic range. By this procedure the mechanical spectra were obtained, recording the variation in the dynamic moduli G', G'' and G^* as a function of frequency. The G' value is the dynamic elastic or storage modulus, related to the material response as a solid; the G'' value is the viscous dynamic or loss modulus, related to the material response as a fluid. The complex modulus G^* is defined as $(G'^2 + G''^2)^{1/2}$ and represents the global viscoelastic response of the system. Non-linear regression analysis was applied to the experimental data for estimating parameters of G' as a function of frequency.

Objective rheological studies were complemented by visual observations of the samples to test spongy and sandy (grainy) appearances.

Statistical analysis

Statistical tests were carried out by analysis of variance (ANOVA). For simultaneous pairwise comparisons, Tukey's test was chosen. Differences in means were considered significant when $P < 0.05$. All statistical procedures were computed using SYSTAT version 5.0 (SYSTAT Inc, USA).

RESULTS AND DISCUSSION

Starch gelatinisation in the presence of sucrose and hydrocolloids

Gelatinisation temperatures (onset, T_m^o; peak, T_m^p; conclusion, T_m^c) and enthalpies (ΔH) for the different formulations were obtained from DSC thermograms and are listed in Table 2. It has been reported by several authors[8,9,26–28] that the gelatinisation endotherm is preceded by a glass transition, difficult to detect, at a characteristic temperature, occurring in the amorphous regions of the granule that surround the crystalline zones. In our studies no change in heat capacity could be detected in the region that preceded the gelatinisation endotherm.

Onset and peak temperatures as well as enthalpy values in systems with 100 g kg^{-1} starch and 900 g kg^{-1} water (CW) did not differ significantly from those obtained in systems with 250 g kg^{-1} starch and 750 g kg^{-1} water (CW**), since in these high-water starch pastes this difference in water content is not critical.[29] Comparing CW** with CSW samples (Table 1) having the same water content and different solid compositions, a significant shift towards higher gelatinisation temperatures was observed for the system containing sucrose (CSW); however, the enthalpy value was not significantly modified. The increase in gelatinisation temperature due to sucrose can be explained by different means: a reduction in water activity, which could hamper starch gelatinisation;[10] sugar–starch interactions;[11–13] and the smaller plasticising effect of the co-solvent mixture sucrose–water.[3]

The low hydrocolloid concentration used (10 g kg^{-1}) did not significantly affect gelatinisation temperatures when compared with the systems without hydrocolloid; small differences were detected among the systems having alternatively guar, xanthan or alginate. Similar findings were reported in a previous work using starch systems without sucrose.[29] With regard to gelatinisation enthalpy (Table 2), the significantly higher enthalpy value in the system with sodium alginate may be related to the anionic character of this gum and the different interactions with the other components.

Thermal transitions in frozen gelatinised systems

Glass transition close to ice melting (Tg_{im})

Fig 1 shows the typical thawing thermograms of slowly frozen and annealed samples. In these conditions the change in heat capacity observed in the thermograms

Table 2. Effect of addition of sucrose and hydrocolloids on onset, peak and conclusion temperatures T_m (°C) and enthalpies of gelatinisation ΔH (J g^{-1} starch) of aqueous starch pastes

System	T_m^o	T_m^p	T_m^c	ΔH
CW	66.9a	72.0a	78.4a	11.5a
	(0.2)	(0.1)	(0.3)	(0.8)
CW**	66.9a	72.3a	80.2b	11.9ab
	(0.3)	(0.2)	(0.3)	(0.4)
CSW	72.9bc	77.6b	83.6cd	12.1ab
	(0.2)	(0.2)	(0.3)	(0.6)
CSXW	73.3b	78.2bd	84.2c	12.3ab
	(0.3)	(0.2)	(0.4)	(0.4)
CSGW	72.3c	77.0c	82.4d	14.0b
	(0.1)	(0.1)	(0.1)	(0.1)
CSAW	73.3b	78.5d	84.2c	16.1c
	(0.1)	(0.1)	(0.1)	(0.3)

Assays were performed in sextuplicate. Standard error of the mean (SEM) is given in parentheses. Within a column, values with different letters are significantly different ($P < 0.05$).

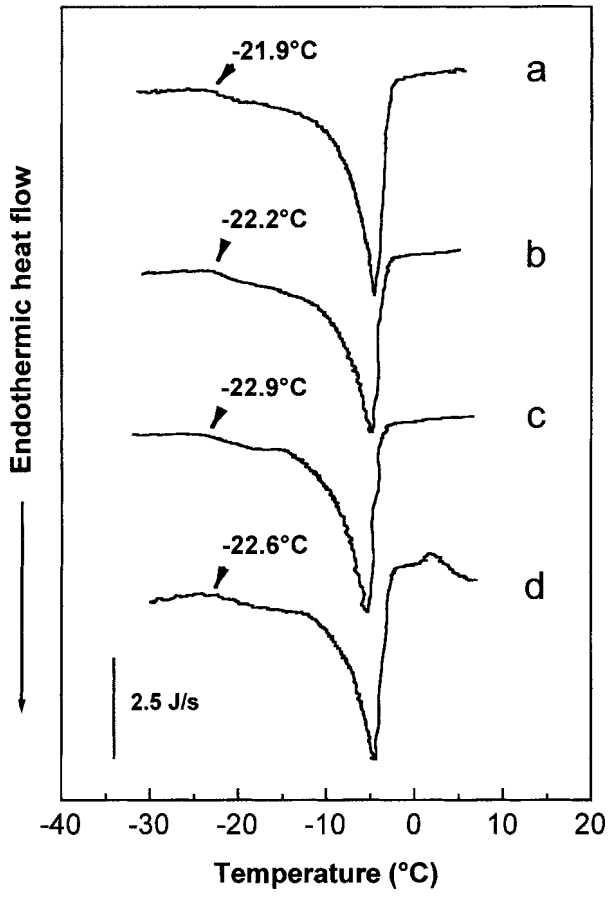

Figure 1. DSC thermograms of various samples frozen at 1.5 °C min^{-1} and annealed for 30 min at $T = -19$ °C: a, CSW* (starch–sucrose–water); b, CSXW* (starch–sucrose–xanthan gum–water); c, CSGW* (starch–sucrose–guar gum–water); d, CSAW* (starch–sucrose–alginate–water). Numbers indicate Tg_{im} onset. See Table 1 for concentrations.

should be attributed to the second step of the glass transition close to ice melting (Tg_{im}). The first step of the glass transition was not visible in the measured temperature range, because the minimum temperature reached by our DSC equipment is −40 °C.

As observed in Table 3, values found for the onset temperature (Tg°_{im}) varied between −23.0 and −22.2 °C for tempered samples containing sucrose with and without gums. Non-significant differences were observed among these systems. A peak of additional ice formation before ice melting was not observed, because the freezing process led to maximum ice formation. Previous reports showed that in starch–water gelatinised systems without sucrose, T'_g onset is located between −4.5 and −5.5 °C; small quantities of gums did not significantly shift the T'_g value.[29]

The decrease in glass transition temperatures caused by sucrose addition in frozen systems, whether annealed or not, was described by several authors as a plasticising effect of low-molecular-weight sugars.[30–33] It has been stated[18,34] that foods containing high amounts of low-molecular-weight sugars show considerable freezing temperature depression and contain higher amounts of unfrozen water at lower temperatures than foods based on polymeric compounds with the same solid content. Nishinari *et al*,[35] studying non-annealed systems with agarose, concluded that sugars and polyols decreased the amount of frozen water in agarose gels, thus increasing plasticisation and molecular movement of polymer chains, which was evidenced by a shift in T_g to lower temperatures.

Considering that the obtained Tg_{im} values in our tested systems are below −20 °C, the commonly used frozen storage conditions (approximately −18 °C) could allow deteriorative phenomena to develop because of the higher molecular mobility at $T > Tg_{im}$. For this reason, amylopectin and amylose retrogradation were studied in the present work.

Amylopectin retrogradation

Retrogradation of amylopectin was not detected by DSC in any of the just frozen samples containing starch, sucrose and gums, irrespective of the freezing rate used (quickly or slowly frozen). At the end of frozen storage (91 days at −19 °C), all the slowly frozen samples evidenced amylopectin recrystallisation. Gum addition did not prevent amylopectin retrogradation and had no significant effect on either temperature range or enthalpy values. This result can be related to the fact that, during starch gelatinisation, amylopectin remains inside the granule, and hydrocolloids do not affect its retrogradation.

DSC endotherms showed retrogradation peaks with mean temperature ranges varying between an onset temperature of 43.7 °C (SEM = 0.5 °C) and a final temperature of 60.8 °C (SEM = 0.6 °C). The enthalpy mean value was 3.1 J g^{-1} (SEM = 0.3 J g^{-1}), being similar for the different formulations irrespective of the type of gum used.

Control samples stored for 91 days at −80 °C did not show amylopectin retrogradation. The obtained results confirm that amylopectin retrogradation can occur even at a temperature near −20 °C when starch

Table 3. Onset, medium and conclusion glass transition temperatures (°C) of concentrated aqueous starch–sucrose–hydrocolloid systems submitted to slow freezing and annealing

System	Tg^{o}_{im}	Tg^{m}_{im}	Tg^{c}_{im}
CW*	−5.2[a]	−4.1[a]	−3.1[a]
	(0.5)	(0.4)	(0.4)
CSW*	−22.9a	−20.8a	−18.7a
	(0.3)	(0.2)	(0.2)
CSXW*	−22.2a	−19.2a	−16.1b
	(0.3)	(0.4)	(0.9)
CSGW*	−23.0a	−20.3a	−17.5ab
	(0.7)	(0.7)	(0.6)
CSAW*	−22.3a	−19.6a	−17.0ab
	(0.4)	(0.4)	(0.5)

Assays were performed in quintuplicate. SEM given in parentheses. Within a column, values with different letters are significantly different ($P < 0.05$).

[a] 500 g kg^{-1} starch, 500 g kg^{-1} water—values reported in Ref 20.

systems contain sucrose and Tg_{im} is below this storage temperature. This phenomenon was not observed in our previous works using samples formulated only with starch and water, because the glass transition in such systems is located close to −5 °C.[2,25] Additionally, in starch systems without sucrose the presence of a hydrocolloid (xanthan gum) did not prevent amylopectin retrogradation during frozen storage at a temperature close to the glass transition, similarly as the hydrocolloids in the present work.

Effect of freezing rate and frozen storage on the rheological behaviour of the systems

Textural changes causing deterioration in frozen starch pastes, eg sponge formation, can be related to amylose retrogradation; it occurs at $T > T'_g$ owing to the mobility of the molecular chains. In previous works[2,25] we reported that, in starch–water gelatinised systems, storage at −20 °C did not affect the texture; this was attributed to the fact that the storage temperature was below T'_g (∼−5 °C).

When a starch gel is submitted to freeze–thaw treatments, water is separated because of the tendency of starch molecules to reassociate to form insoluble aggregates. Textural changes such as sponge formation and a weepy or grainy appearance[1] are easily appreciated by visual analysis of the tested samples.

The rheological behaviour of the tested samples is observed in Fig 2; the complex dynamic modulus (G^*) was plotted as a function of frequency at a constant deformation ($\gamma = 4\%$) within the linear viscoelastic range. The following samples were analysed: unfrozen, control samples that were quickly frozen and stored at −80 °C, slowly or quickly frozen samples (without storage) and slowly or quickly frozen samples that were stored at −19 °C (results shown correspond to one of the batches prepared in each case; those of the other batch had a similar tendency); G' and G'' were also determined for the same samples.

Fig 2a corresponds to a sample containing only starch, sucrose and water (CSW), while Figs 2b–2d show the effect of gum addition (samples CSXW, CSGW and CSAW of Table 1).

In the case of unfrozen samples with and without gums the dynamic elastic modulus G' was analysed as a function of frequency to find out whether the samples are gels or solutions or else highly viscous dispersions.[36] For comparative purposes an empirical equation of the type $G' = a\ f^b$ was applied to the frequency (f) sweep tests as suggested by Steffe.[37] The a and b parameters varied according to the rheological characteristics of the system. Steffe[37] reported typical values for a and b of 0.00028 Pa s^b and 1.66 respectively for a dilute solution. For a gel the values were 5626 Pa s^b and 0.0371 respectively, and intermediate values correspond to concentrated solutions (a = 16.26 Pa s^b and b = 0.840).

The rheological behaviour of unfrozen samples (CSW, CSXW, CSGW and CSAW) and the a and b parameters of the G' vs f equation are shown in Table 4. The different rheological behaviours observed in the various unfrozen formulations were caused by the

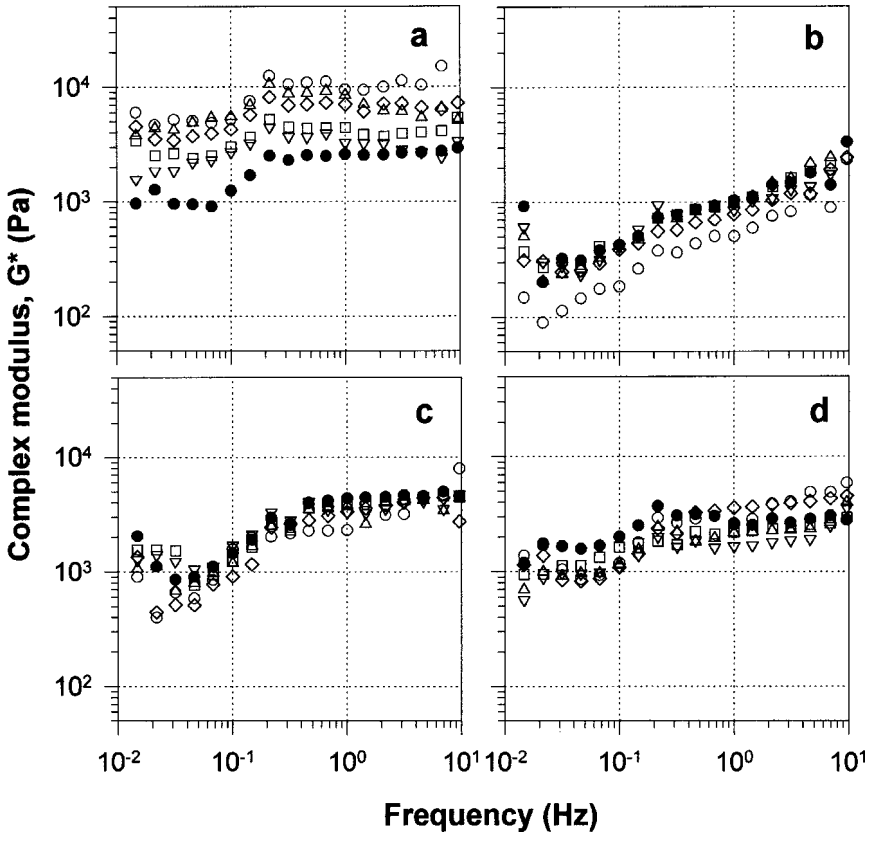

Figure 2. Frequency sweeps (at constant deformation $\gamma = 4\%$) at several times and storage temperatures for various samples: a, CSW (starch–sucrose–water); b, CSXW (starch–sucrose–xanthan gum–water); c, CSGW (starch–sucrose–guar gum–water); d, CSAW (starch–sucrose–alginate–water). See Table 1 for concentrations. Symbols: ●, unfrozen, □, quickly frozen without storage; △, quickly frozen and stored for 91 days at −19 °C; ▽, quickly frozen and stored for 91 days at −80 °C; ◇, slowly frozen without storage; ○, slowly frozen and stored for 91 days at −19 °C.

Table 4. Mechanical spectral characteristics for aqueous starch–sucrose–systems without and with hydrocolloids, and parameters of equation $G' = a\, f^b$

System	*Crossover frequency*	a *(Pa s^b)*	b
CSW	$G' > G''$ in whole tested range	1959 ± 285[a]	0.235 ± 0.080
CSXW	1 Hz	695 ± 138	0.420 ± 0.128
CSGW	0.1–0.2 Hz	3016 ± 568	0.293 ± 0.103
CSAW	$G' > G''$ in whole tested range	2240 ± 343	0.175 ± 0.083

[a] Confidence limits of the parameter (95%).

specific textural characteristics imparted by each gum and, probably, by higher or lower swelling of the starch granules during gelatinisation. The starch–sucrose–water (CSW) sample and alginate-added system (CSAW) showed a gel-like behaviour (weak gel) where the storage modulus G' (elastic response) remained constant and always above G'' (viscous response); in this case, G' almost coincided with G^*. Xanthan or guar gum addition modified this behaviour; these systems showed a crossover frequency below which G'' is higher than G', whereas above that value the behaviour is reversed ($G'' < G'$). The complex modulus G^* coincides with G'' below the crossover frequency and with G' above it. The results obtained indicate that the behaviour of the system with xanthan gum (CSXW) approaches that of a semi-concentrated entangled solution of macromolecules. The behaviour of the starch system containing guar gum (CSGW) is closer to that of a gel, as observed from the *a* and *b* parameters.

The effect of freezing rate and frozen storage can also be analysed in Fig 2. The complex dynamic modulus G^* of the just frozen samples containing starch and sucrose without gums (CSW) increased with respect to the unfrozen sample having the same composition (Fig 2a). This increase is significantly higher for slow freezing than for quick freezing and is related to structural changes, particularly sponge formation due mainly to amylose retrogradation at low freezing rates.

In systems containing gum (CSXW, CSGW and CSAW), no spongy structure was observed in just slowly frozen samples; quickly frozen samples hardly differed from the unfrozen ones. Systems with starch, sucrose and xanthan or guar gum that were frozen at low rates showed values of G^* slightly lower than those of the unfrozen samples in the whole range of tested frequencies (Figs 2b and 2c). These results could be attributed to structural changes related to large-ice-crystal formation. In alginate-added systems, G^* increased with respect to the unfrozen samples only at frequencies higher than 1 Hz.

For the different formulations, Table 5 summarises the effect of freezing rate on the different rheological parameters obtained from deformation sweeps at 1 Hz. The linear viscoelastic range varied with sample formulation and was non-significantly affected by quick freezing. However, under slow freezing, the ranges of linear viscoelasticity tended to decrease, this effect only being significant for systems containing guar gum. Similar findings have been reported by Navarro *et al*,[38] who found a decrease in the linear viscoelastic range in starch–water pastes after both quick and slow freezing. A lower linear viscoelastic range denotes that the structural resistance to the applied deformation is reduced.

Table 5 also shows G^* values at 1 Hz frequency and the results from visual observations previously described.

The effect of frozen storage at −19 and −80 °C can be analysed from the G^* data of Fig 2. For each tested system, samples were thawed at different storage times to study their behaviour during deformation and frequency sweeps. The dynamic complex modulus G^* increased in the quickly frozen starch–sucrose–water (CSW) samples after 91 days of storage at −19 °C; this increase can be attributed to amylose retrogradation during storage. However, this behaviour was not observed in quickly frozen CSW samples stored at −80 °C, whose G^* values practically coincided with those of the just quickly frozen sample. In the slowly frozen CSW systems, negligible changes were observed after 91 days of storage at −19 °C, because sponge was mainly formed during freezing.

The effect of frozen storage can also be observed in

Table 5. Effect of freezing rate on linear viscoelastic range and dynamic complex modulus G^* (measured in deformation sweeps at 1 Hz) and macroscopic characteristics of aqueous starch–sucrose–hydrocolloid systems after thawing

	Unfrozen samples			*Quickly frozen samples*			*Slowly frozen samples*		
System	γ_{lim} *(%)*	G* *(Pa)*	*Texture*	γ_{lim} *(%)*	G* *(Pa)*	*Texture*	γ_{lim} *(%)*	G* *(Pa)*	*Texture*
CSW	9.2a (0.9, 2)	2529a (43, 6)	NS	9.3a (0.3, 2)	4373b (179, 9)	NS	7.8a (0.5, 2)	9034c (167, 9)	S
CSXW	7.5a (0.5, 2)	895a (36, 10)	NS	7.6a (0.1, 2)	758b (25, 10)	NS	6.5a (0.1, 2)	631c (20, 9)	NS
CSGW	9.3a (0.3, 2)	4006a (93, 3)	NS	9.0a (0.7, 2)	3568b (62, 4)	NS	4.9b (0.5, 2)	3423b (63, 6)	NS
CSAW	11.0a (0.4, 2)	2071a (30, 5)	NS	11.1a (0.5, 2)	1830b (19, 11)	NS	9.6a (0.6, 2)	3231c (59, 4)	NS

SEM, *n* (number of measurements) given in parentheses. Within a row, corresponding values with different letters are significantly different ($P < 0.05$).
S, sponge formation; NS, no sponge formation.

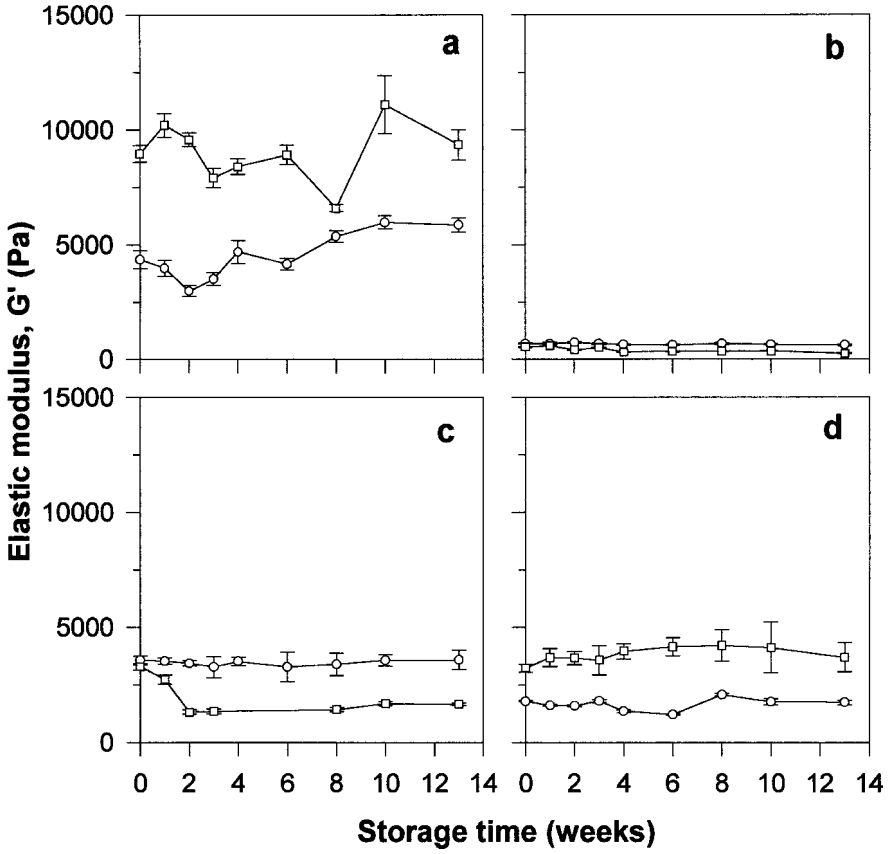

Figure 3. Elastic modulus G' as a function of frozen storage time at −19 °C for various samples: a, CSW (starch–sucrose–water); b, CSXW (starch–sucrose–xanthan gum–water); c, CSGW (starch–sucrose–guar gum–water); d, CSAW (starch–sucrose–alginate–water). See Table 1 for concentrations. Symbols: ○, quickly frozen samples; □, slowly frozen samples. Error bars are calculated for a confidence level of 95%.

Fig 3, where G' values (elastic response) at 1 Hz are plotted as a function of storage time. Fig 3a corresponds to the CSW system; the initial values of G' (just frozen samples) show the effect of freezing rate. Slow freezing produced higher G' values that can be associated with the spongy structure. Quick frozen samples showed lower G' values that increased during storage owing to sponge development; the addition of gums modified this behaviour. As was shown in Fig 2, the effect of freezing rate is less marked when xanthan and guar gums are added to the systems than for alginate pastes. In alginate systems, slow freezing gives firmer gel structures than quick freezing, similarly to samples without gums. In the case of pastes with xanthan or guar gum, the slowly frozen samples showed lower G' values, demonstrating that the sponge structure was not formed; however, these samples showed the development of a sandy appearance during frozen storage. Frozen storage practically did not change the initial G' values in systems with added gums.

In our study, systems without gums undergo a significant increase in gel firmness and thus in G' value mainly as a consequence of freezing. Hydrocolloid addition minimises these textural changes during both freezing and storage, even under slow freezing. Amylose chains are linear and longer than amylopectin ones. During starch gelatinisation, amylose is released outside the granule and forms an external matrix; thus it is more exposed to interaction with other macromolecules such as gums. Hydrocolloids could act in different ways. Hydrocolloid–amylose interactions probably compete against amylose–amylose interactions, thus preventing amylose retrogradation.[25] Additionally, hydrocolloids can make systems in the rubbery state more viscous, decreasing molecular mobility and preventing retrogradation-related sponge formation.[29]

CONCLUSIONS

A model starch system containing 100 g kg^{-1} starch, 150 g kg^{-1} sucrose and 750 g kg^{-1} water showed a higher gelatinisation onset temperature (72.9 °C) in comparison to systems without sucrose but with (i) the same solid content (250 g kg^{-1} starch, 750 g kg^{-1} water) and (ii) the same starch content (100 g kg^{-1} starch, 900 g kg^{-1} water). Hydrocolloid addition (10 g kg^{-1}) in the range of tested concentrations did not have any significant effect on the gelatinisation temperature.

The temperature of the second step of the glass transition, corresponding to the heat capacity change close to ice melting (Tg_{im}), was mainly affected by sucrose and was shifted towards lower values (onset between −23 and −22 °C depending on the system). Thus commercial storage temperatures (∼−18 °C) lie above Tg_{im} in starch–sucrose systems, allowing molecular mobility; this fact led to amylose and amylopectin retrogradation and, as a consequence, to textural changes. Small quantities of gums (in the ranges used in common formulations) did not change

the Tg_{im} of the samples, but they had an important role in minimising structural damage. This was verified by rheological viscoelastic tests where an increase in the dynamic moduli G^* and G' after slow freezing and during storage at $-19\,°C$ ($T > Tg_{im}$) was observed in starch–sucrose systems, related to the sponge-like structure formation due to amylose retrogradation. Hydrocolloid addition did not prevent amylopectin retrogradation, but it inhibited the development of the spongy matrix that is related to amylose retrogradation. As amylose is released outside the granule during gelatinisation and forms an external matrix, it is more exposed to interaction with hydrocolloids. Amylopectin remains inside the starch granule during gelatinisation, and hydrocolloids do not affect its retrogradation.

From the results of the present work it can be concluded that storage at the usual commercial temperatures (close to $-18\,°C$, slightly above Tg_{im}) affects the quality of aqueous starch–sucrose pastes without gums owing to amylose and amylopectin retrogradation. When hydrocolloids are included in the formulations, the usual storage conditions allow the maintenance of acceptable textural attributes.

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