Sub-T_g annealing of granular rice starch: effects on enthalpy relaxation and starch–sucrose interactions

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Abstract

Annealing of native rice starch at a temperature somewhat below its effective glass transition temperature (T_g) increased both the gelatinization onset temperature (T_o) and enthalpy (ΔH_G) to their respective quasi-equilibrium values, due probably to free volume and enthalpy relaxation, but had little or no effect on crystallite melting temperature (T_m). "Maximum" ΔH_G was attained well before "maximum" T_o, suggesting that excess volume can continue to be dissipated or redistributed without any substantial change in internal energy. The effect of sucrose on ΔH_G appeared to be dependent on the physical condition of the glassy regions of the starch granules, a significant increase in ΔH_G being observed only when sucrose was added to unannealed granular starch with apparently less stable amorphous regions. Addition of sucrose to annealed starch, which possessed lower energy glassy states, yielded no significant changes in ΔH_G. On the other hand, elevation of both T_o and T_m was obtained on addition of sucrose to either physically aged or unannealed starch.

1. Introduction

The effects of different sugars on starch gelatinization have been extensively studied using differential scanning calorimetry (DSC). While there is no disputing the fact that the presence of sugars elevates the temperature of gelatinization of starch in water, issues relating to the mechanism of the effect and conflicting results pertaining to changes in overall enthalpy of gelatinization (ΔH_G) remain unresolved.

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There appears to be no universally accepted explanation to account for the effect of sugars in delaying starch gelatinization. The ability of sugars to limit the availability of water to starch for gelatinization, through a lowering of relative vapour pressure (RVP) or water activity, and starch–sugar interactions involving formation of "sugar bridges" that stabilize the amorphous regions of the starch granules were the earlier hypotheses proposed by Spies and Hoseney [1]. Slade and Levine [2,3] have adopted a different approach by viewing native starch as a partially crystalline polymer system and the water–sugar mixture as a plasticizing co-solvent that possesses a higher average molecular weight than water alone. The co-plasticizers would thus be less effective than water alone in mobilizing and increasing the free volume of the amorphous fringes in the "fringed micelle" structure of a starch granule, resulting in a smaller depression of the glass transition temperature (T_g). The melting of starch crystallites, which is controlled and preceded by the glass transition [4], would thus occur at higher temperatures in the presence of sugars.

While several workers have observed decreases in ΔH_G and a narrowing of the gelatinization temperature range with increasing sucrose concentration [5–9], the opposite effects have been reported by others [10–12]. On the other hand, Chinachoti et al. [13] observed a reduction in ΔH_G on addition of up to 15% (wet basis) of sucrose to systems at water–corn starch ratios greater than 1:1, but no changes in ΔH_G at lower moisture levels. Unfortunately, the conclusions drawn in some of these studies may be suspect because rigorous statistical analyses were not applied to the data obtained.

Seow and Teo [14] have recently shown that the physical condition of the glassy regions of starch granules is amenable to modification by annealing at temperatures somewhat below the effective T_g associated with gelatinization. Over practical time scales, sub-T_g annealing induces a slow relaxation of the amorphous regions to lower energy glassy states without significantly affecting the melting behaviour of the crystalline regions. One question that arises is: would such time-dependent changes within the glassy regions of granular starch, which occur as a result of sub-T_g annealing, have any effects on starch–sucrose interactions? This study was designed primarily to answer this question and also to resolve the conflicting results in the literature concerning the effects of sucrose on ΔH_G.

2. Experimental

Materials.—Rice starch from a commercial source was used directly. Analytical grade sucrose, purchased from BDH Chemicals Ltd. (Poole, UK), was also used directly without further purification.

The moisture content of the rice starch was determined by drying duplicate 5-g samples to constant weight in an air-oven at 105°C. Drying time was 5 h.

Annealing and DSC measurements.—The thermal properties of two systems were studied, namely: (*1*) a binary 1:2 starch–water (NS) system in which no sucrose was added; and (*2*) a ternary 1:2:1 starch–water–sucrose (AS) system in

which the dry sugar was added to the desired level after sub-T_g annealing of the NS system.

The prepared starch–water mixtures were left to stand for 1 h at room temperature (ca. 25°C) before samples were annealed. At the same time, samples were also taken for DSC zero-time measurements. The latter served as untreated controls.

Annealing was carried out by holding ca 2-g samples (accurately weighed) in air-tight screw-capped tubes in a water bath maintained at 50 ± 0.1°C. Tubes were withdrawn at intervals of time and cooled immediately to room temperature. In the case of the NS system, samples were immediately taken for DSC measurements. For the AS system, however, the required weight of sucrose was added to the contents of the appropriate tube and thoroughly mixed with a spatula in order to ensure dissolution of sucrose before samples were taken for DSC measurements.

Samples of ca. 10 mg (accurately weighed to ± 0.01 mg) were filled into hermetic aluminium pans which were then hermetically sealed. DSC measurements were carried out using a Du Pont 2910 differential scanning calorimeter, and transition temperatures (onset, T_o; peak, T_p [$= T_m$]; conclusion, T_c; glass transition, T_g) and overall gelatinization enthalpy (ΔH_G, expressed as J/g dry starch) were determined as described by Seow and Teo [14]. Statistical analysis on the data was carried out as appropriate.

A heating rate of 10°C min^{-1} was used throughout the study and an empty aluminium pan with two lids was used as the reference. Heat-flow and temperature calibrations were performed using pure indium. DSC measurements were carried out at least in duplicate at each interval of time.

3. Results

The effects of duration of annealing at 50°C on the shapes of DSC thermograms of the NS and AS systems are shown in Figs. 1A and B, respectively. The thermal transitions associated with starch gelatinization are labelled as the G transition and the M_1 endotherm, the former being associated with the glassy-to-rubbery state transformation and the latter being ascribed to melting of starch crystallites [4,14]. In both NS and AS systems, the G transition became increasingly more distinct and narrower with time of annealing as T_o was shifted to higher temperatures, an effect similarly observed by others [8,14–19]. Since the M_1 endotherm was more or less unaffected, the overall effect was a narrowing of the gelatinization range ($T_c - T_o$) caused primarily by changes in the lower temperature (G) endotherm. The thermal transitions associated with gelatinization of the AS system occurred over a higher temperature range than those of the NS system.

The unannealed ternary starch–water–sucrose sample appeared to produce DSC thermal profiles in which the apparent endotherm associated with glass transition was slightly more prominent and occurred over a much higher temperature range than in the control binary starch–water mixture. It therefore appears
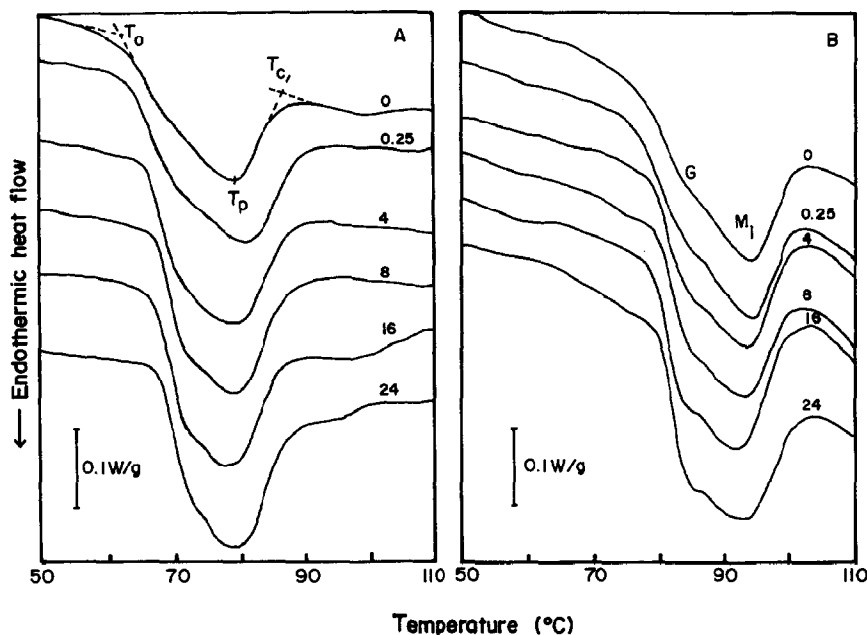
Fig. 1. DSC thermograms of the NS system (A) and AS system (B), labelled with time of annealing in hours. All thermal curves are normalised to 1 g of dry starch.

that both sub-T_g annealing and the presence of sucrose have parallel effects on the amorphous regions of the starch granules, albeit to different extents. However, while the M_1 endotherm associated with crystallite melting was shifted to a higher temperature range in the presence of sucrose, its position was practically unaffected by sub-T_g annealing. This notable difference suggests that the mechanisms by which these two factors affect the thermal transitions associated with starch gelatinization are probably not the same.

Changes in T_o of the two systems with time of annealing at 50°C are shown in Fig. 2. Both the binary and ternary systems exhibited similar trends in that T_o increased relatively sharply over the first 10 hours of annealing before levelling off.

It is interesting to note that changes in overall enthalpy of gelatinization (ΔH_G) did not appear to parallel changes in T_o. Table 1 shows that, in the case of the NS system, there appeared to be an immediate increase in ΔH_G after 15 min of annealing. Analysis of variance (followed by Duncan's multiple range test), performed on the 0 h, 15 min, and 24 h data based on quintuplicate measurements, showed this increase to be significant at the 5% level of probability. The value of ΔH_G did not vary much more upon further annealing of the NS system. In contrast, no significant change ($P < 0.05$) in ΔH_G was evident after annealing of the AS system for 15 min. Extending the annealing time to 24 h did, however, produce a significant increase in ΔH_G when compared with zero-time data.

Statistical analysis was also applied to the zero-time, 15 min, and 24 h data in order to study the effects of sucrose on the overall enthalpy associated with
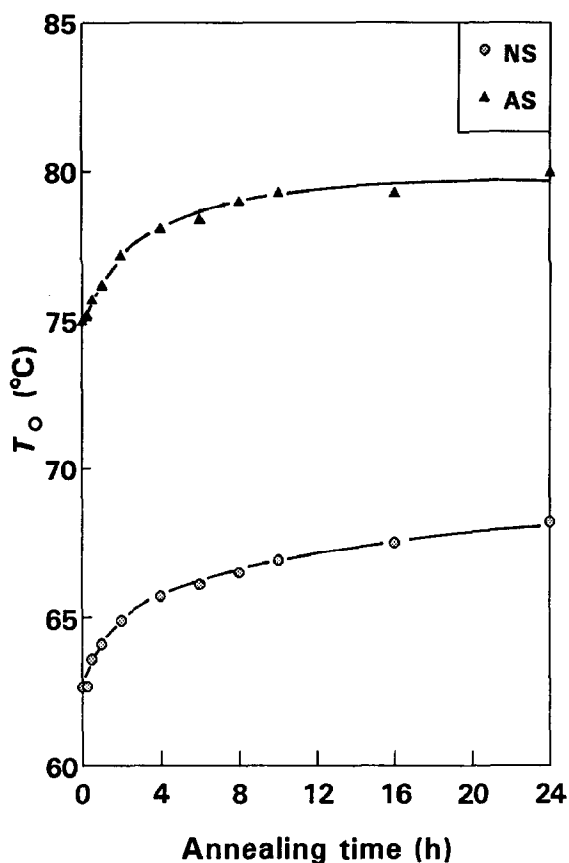
Fig. 2. Changes in onset temperature of gelatinization (T_o) of NS and AS systems with time of annealing at 50°C.

Table 1
Comparative effects of time of annealing at 50°C on changes in overall enthalpy of gelatinization (ΔH_G) in NS and AS systems

Annealing time (h)	ΔH_G (J/g) [a]	
	NS	AS
0	12.2 [a]	13.7 [a]
0.25	15.3 [b]	14.7 [a,b]
0.5	14.2	14.6
2	14.2	14.2
4	15.7	14.1
8	15.7	13.6
16	14.4	14.8
24	15.2 [b]	15.5 [b]

[a,b] Mean of five determinations at zero time, 0.25 h, and 24 h. The other data are means of duplicate determinations. Means within a column with the same letter are not significantly different at $P < 0.05$.

Table 2
Effects of sucrose on the overall enthalpy associated with gelatinization of granular rice starch after sub-T_g annealing at 50°C for various periods of time

System	ΔH_G (J/g)		
	0 h	0.25 h	24 h
NS	12.2±0.5 [a]	15.3±0.7 [a]	15.2±0.1 [a]
AS	13.7±0.8 [b]	14.7±0.6 [a]	15.5±0.5 [a]

[a,b] Mean of 5 determinations±standard deviation. Means in a column with the same letter are not significantly different at $P < 0.05$.

gelatinization. The results are given in Table 2. Addition of sucrose to untreated starch was found to significantly increase ($P < 0.05$) ΔH_G as shown by a comparison of the zero-time data. Beyond 15 min of annealing, however, the ΔH_G value for the NS system was not significantly different from that of the AS system.

4. Discussion

The present study was conducted using a rice starch that exhibits rather unusual DSC gelatinization thermal profiles, in that the lower temperature or G transition appears as a small shoulder at the leading edge of the dominant higher temperature or M_1 endothermic peak over a relatively wide range of moisture contents (50–80 wt %) [14]. In contrast, most other starches show a prominent G endotherm, with the M_1 transition appearing as a trailing shoulder, at intermediate water contents, and a single peak at high water contents. It has been suggested by Biliaderis et al. [20] that this lower temperature shoulder of such an atypical rice starch owes its existence to a glass transition that occurs prior to crystallite melting. Based on the results of previous annealing experiments, Seow and Teo [14] have suggested that the G endotherm is, in all probability, the manifestation of a glass transition associated with enthalpy relaxation, and the onset temperature of gelatinization (T_o) may thus be taken as the operative or effective onset glass transition temperature, as suggested earlier by Slade and Levine [21]. It is worthwhile noting that this glass transition occurs over a relatively narrow range of temperature, rather than at a single sharp temperature, due probably to structural variations among starch granules and the inherent inhomogeneity of the glassy regions within a granule [4]. The size and sharpness of the G "endotherm", relative to that of the M_1 endotherm, will likely be governed by many factors including genetic makeup, conditions affecting starch biosynthesis, molecular organization in the starch granules, the extent of plasticization [3], the method of starch extraction, the presence of other constituents, and the thermal history of the material [3]. Discussion of the results of the present study will be based on the above interpretation of the origin of the thermal transitions associated with starch gelatinization.

The increasing prominence of the lower temperature shoulder and the shift of T_o to higher temperatures in the DSC thermograms of the NS and AS systems with increasing time of annealing, as shown in Figs. 1 and 2, may be ascribed to free volume and enthalpy relaxation (i.e., the decrease in excess volume and enthalpy) on sub-T_g annealing. As a result, there is a narrowing of the glass transition temperature range and an increase in the enthalpy recovered during subsequent heating over the gelatinization range. The apparent endothermic peak, also called the heat-flow or T_g overshoot [22], obtained on heating the aged material through the glass transition range thus becomes even larger and sharper, reflecting enhanced homogeneity and/or metastability in the glassy regions of the starch granules. Such non-equilibrium behaviour of the glassy state is commonly observed in amorphous or partially crystalline synthetic polymers [22–28]. Therefore, structural and property changes can and do occur when such materials are annealed at temperatures not too far below their glass transition temperatures. Biopolymer [4,29,30] and low molecular weight glassy systems [31–33] also exhibit this phenomenon. Recently, Shogren [34] has shown that embrittlement of gelatinized starch can occur as a result of free volume relaxation during sub-T_g aging.

Both T_o and ΔH_G in the NS and AS systems appeared to increase to apparent "equilibrium" values on hydrothermal aging at 50°C. Since the glassy state is a metastable state, no true equilibrium has actually been achieved [3]. The physical aging process will presumably continue, albeit at an extremely low rate. An interesting feature of this non-equilibrium behaviour of the glassy state in granular starch is that, while it took more than 10 h for T_o to increase to its quasi-equilibrium value on hydrothermal aging at 50°C, "maximum" enthalpy relaxation appeared to occur after only 15 min of annealing. This could mean that excess volume, originally quenched into the system, can continue to be dissipated or redistributed (and, by implication, conformational rearrangement and packing of polymer molecules can go on) without any substantial change in internal energy. Thus, an increase in T_o on sub-T_g annealing of granular starches at any particular temperature need not necessarily be accompanied by an increase in overall gelatinization enthalpy, as this would depend on the nature and magnitude of the excess thermodynamic properties in the amorphous regions of the starch granules under study which, in turn, would be affected by the thermal history of the material, such as the conditions prevailing during flour milling or starch isolation. This is probably the reason why, on isothermal annealing of a wide variety of granular starches below their respective onset gelatinization temperatures, increases in ΔH_G have been reported in some cases while little or no change has been observed in others [16,17]. Decreases in ΔH_G would only be evident at annealing temperatures above T_g for two main reasons: (1) the apparent enthalpy associated with the glassy-to-rubbery state transformation would no longer contribute to ΔH_G, and (2) melting of less thermostable (deformed or imperfect) crystallites may occur during above-T_g annealing [14,21].

In many synthetic polymers, stress or enthalpy relaxation during sub-T_g annealing has been reported to exhibit a linear dependence on the logarithmic annealing time [25,27]. In the present study, this was found to be also true where the
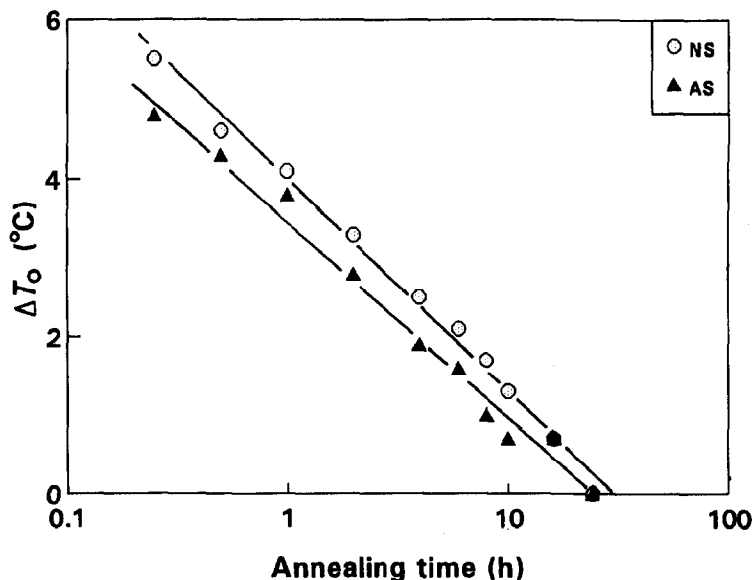
Fig. 3. The approach of T_o to its pseudo-equilibrium value on sub-T_g annealing.

approach of T_o to its apparent "equilibrium" value, in both NS and AS systems, is concerned. This is shown in Fig. 3 which is a plot of ($\Delta T_o = T_{oe} - T_{ot}$) versus $\log_{10}$ annealing time, where T_{oe} is the onset glass transition temperature of the "equilibrium" glassy state (taken arbitrarily at 24 h) and T_{ot} is the effective onset glass transition temperature after an annealing time, t.

Table 1 shows that addition of sucrose (at the level studied) to granular rice starch, physically aged or unannealed, increased T_o by a more or less constant amount ranging from 11.8–12.8°C. Although both sub-T_g annealing and the presence of sucrose had the same effect of elevating T_o, the mechanisms involved appear to be different. Free volume relaxation in the glass, as obtained through sub-T_g annealing over practical time scales, would elevate T_o without significantly affecting starch crystallite melting temperature (T_m) as shown in the present study and previously by Seow and Teo [14], whereas an increase in T_m was clearly evident when sucrose was added to either annealed or untreated starch. This, coupled with the fact that the same extent of T_o increase was obtained even in samples where the major part of the excess volume had already been dissipated by sub-T_g annealing, suggests that the primary mechanism responsible for elevation of T_o in the presence of sucrose is the "antiplasticizing" effect of the water–sucrose co-solvent as compared to water alone as proposed by Slade and Levine [2,3,21]. Addition of sucrose decreases the free volume of the starch–plasticizer mixture, thereby leading to decreased segmental motion of starch chains in the amorphous regions of the starch granules and, in turn, an elevation of T_g and consequently also of T_m [21].

While the addition of sucrose to physically aged granular rice starch, in which the glassy state enthalpy had attained a quasi-equilibrium value, did not significantly ($P < 0.05$) affect ΔH_G, its presence in the original (untreated) material produced a significant increase in ΔH_G (Table 2). Such an increase could arise through stabilization of the glassy regions of the starch granules as a result of starch–sugar interactions, as suggested by several workers [1,12]. The contribution of such interactions, which have been observed using such techniques as electron spin resonance [35] and nuclear magnetic resonance [36,37], to the elevation of T_o, although apparently restricted to materials with relatively large excess volume and internal energy, cannot be totally discounted.

The reason for the lack of change in ΔH_G when sucrose was added to aged granular starch is as yet unclear. It is possible that the high degree of packing and low segmental mobility of starch chains in the stabilized amorphous regions of the starch granules after sub-T_g annealing would not permit any significant degree of starch–sucrose interactions. Further studies are obviously warranted. What is apparent, however, is that the effect of sucrose addition on the overall enthalpy of starch gelatinization would depend largely on the physical condition of the amorphous regions of the starch granules which can be altered, intentionally or adventitiously, by exposure to heat below the effective T_g. In effect, a three-component starch–water–sucrose system may actually have a lower ΔH_G than the corresponding two-component starch–water system if the amorphous regions of the starch granules in the latter are in a thermodynamically more stable state. On this basis, it would not be surprising, therefore, to find that contradictory observations concerning the effect of sucrose on ΔH_G have been reported in the literature, since the thermal histories of the granular starches studied can be presumed to be different.

Acknowledgment

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