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Calorimetric evaluation of the glass transition in hydrated, linear and branched polyanhydroglucose compounds

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By comparing glass transition temperatures, T_g, determined by differential scanning calorimetry (midpoint of heat capacity step at 3°C.mn^{-1}) on powders of varying water contents for different polysaccharides, the influence of molecular weight, degree of branching and (1–4) vs (1–6) glycosidic linkage ratio upon the depression of T_g is illustrated, thus extending results of former studies.

Due to both of the doubts concerning the heat capacity change of water at its glass transition, when dispersed in such media, and limitations of second-order entropy-based derivations, the effect of water plasticization is described by the Couchman's correlation in its degenerated form, which is similar to the Gordon–Taylor formulation.

Strong enthalpy relaxation effects are observed following aging treatments at temperatures below, and even far below T_g. This makes it necessary to erase the history of moisture-conditioned samples and, thus, only the second DSC scan results are presented.

As expected, linear chains appear to favor chain–chain interactions and induce partial crystallinity; branched molecules display lower T_g values, due to chain end effects, as well as flexibility of branching points. The three dihedrals present in α(1–6) linkages seem to depress T_g in a similar fashion to internal plasticization. The case of a linear α(1–4) amylose chain bearing (1–6) grafted fructoses is examined as a first step towards tailored structures, designed to optimize mechanical properties and internal plasticization (as for chemically modified polysaccharides) and inhibit recrystallization. The extension to ciliated structures (sparse brushes) is proposed as a target for biosynthesis optimization. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

The ever increasing interest of food scientists in the concept of glass transition arises from an attempt to improve the description of complex solid systems out of thermodynamic equilibrium (where the use of chemical potentials is not strictly appropriate). Relying on the transfer of polymer science concepts to hydrated biomaterials, the applicability of the kinetic description of amorphous glassy/rubbery systems is being probed using various methods, which unfortunately provide limited dynamical views of what might be called "mobilities" (which are inversely related to friction coefficients or correlation times and are highly dependent on the scale of interest).

It is appropriate to quote the initiators of this refreshing way of interpreting food systems, who pushed forward the idea that water is an ubiquitous plasticizer of biopolymers with less mystifying properties than those of 'bound water' (Van den Berg, 1981; Franks, 1991). The amplification of this line of thinking is found in the efficient overviews of Levine and Slade (1988a, b; Slade and Levine 1991a, b, 1993, 1995) and the references therein, or in proceedings of symposia such as those edited by Blanshard and Lillford (1993) or Fito *et al.* (1994). Various fields such as freeze-drying, cryopreservation, extrusion cooking and baking, have immediately benefitted from such approaches. Recent reviews of existing data were also published in the field of pharmaceutical applications (Hancock & Zografi, 1994) and food science (Roos, 1995). Besides the numerous textbooks referred to by these authors, the study of the universal behaviors of liquids and particularly of polymers (Edwards, 1994), remains a very active field in current physics.

Moreover, it should be mentioned that the polymer science approach to polysaccharides was particularly intense over a quarter of a century ago (Cowie *et al.*, 1969; Whistler & Hilbert, 1944), when chemical derivatives of amylose and cellulose were investigated with the aim of modifying some 'less desirable' intrinsic characteristics of these polymers such as high water affinity and swelling, propensity to recrystallize and high biodegradability. Evidently at that time, environmental consideration and, hence, the need for biodegradable products were less important. What were perceived as weaknesses are becoming "Opportunities and Challenges for New Industrial Uses of Starch" as expressed by the American Association of Cereal Chemists (Doane, 1994; see also Doane *et al.*, 1992).

Among the less dynamic methods of studying glass transition, differential scanning calorimetry (DSC), as well as dilatometry, have a long tradition. One of the advantages of the former is to use a confined sample whose composition may be kept constant during temperature changes. Moreover, the principles are simple and the affordable costs allow their use as an industrial control technique. The heat capacity change detected by this method mainly probes the mobilization of principally translational and rotational degrees of freedom, allowing chain flow in the case of polymers, although specific motions in the structure cannot be precisely identified.

In this study, the behavior of binary low to intermediate moisture polyanhydroglucose compounds has been characterized from the point of view of thermal transitions, essentially from the glassy to rubbery state. The influence of chain length and branching served as a guide to select typical materials, whose sensitivity to plasticization by water was mainly observed at temperatures above freezing in order to avoid the difficulties generated by ice growth kinetics, and freeze concentration in the amorphous residual solutions.

MATERIALS AND METHODS

Materials

Waxy maize starch and potato starch were purchased from Roquette Frères (Lestrem, France). Lintnerized potato starch was prepared by mild acid hydrolysis of potato starch, as described previously (Robin *et al.*, 1974). Amylose was extracted from smooth pea starch (Grinsted) by the *n*-butanol complexation method (Banks & Greenwood, 1975). Leaching of a 2% water/pea starch granule suspension followed the procedure of Miles *et al.* (1985) at 70°C under a nitrogen gas stream for 30min. Subsequent filtration, precipitation by saturation with butanol (8%), followed by cold storage and centrifugation allowed the chains to be obtained in an easily redispersible V-type complex conformation.

Phytoglycogen was extracted from sugary maize su-1 by aqueous dispersion and alcoholic precipitation according to the method of Inouchi *et al.* (1983). Kernels were harvested 45 days after pollination, although the structure is considered to be settled after 15 days.

Pullulan produced at Laval University (Canada) was kindly provided by Dr J.L. Doublier. Dextran T500 was purchased from Pharmacia Chemicals (batch no. 230306). Fructosylated amylose (AMFRU) and amylopectin (APFRU) provided by Professor Richards (University of Montana, USA) are described in the epilogue.

Methods

Beta amylolysis

Beta amylolysis of both amylopectin and phytoglycogen was applied twice, following the procedure of Yuan and Thompson (1994). The β-amylase [α(1-4)maltohydrolase, EC 3.2.1.1] was from Sigma, St Louis, MO, USA. Eventual residual α-amylase activity was not checked because the global properties considered here were not supposed to depend on changes in the high molecular weight of these molecules. The extent of degradation was assumed to be similar to that reported by Kainuma *et al.* (1978), i.e. 58 and 46%, respectively.

Casting

Hot-cast films of potato and waxy maize starches were obtained by drying at 3% (w/v) starch paste on a teflon-coated hot plate at 90°C. Recrystallization was considered to be negligible (no discernible X-ray diffraction pattern) as the rate of drying following casting procedure is much faster than retrogradation kinetics (Kalichevsky *et al.*, 1992). Amylose powders were prepared by rapid casting of 3% (w/v) solutions re-dispersed at 90°C from the butanol complex after bubbling nitrogen until the butanol odor disappeared (Kalichevsky & Ring, 1987). Aliquots were cast on a hot plate heated up to a limit temperature which avoided boiling at the surface (i.e. close to 100°C). The resulting thin films (200mg, 40μm thick) were stored over phosphorus pentoxide overnight. Grinding these dry fluffy films required the use of a cryogrinder (Freezer/Mill, Spex, USA). The shaking tubes which had been used in liquid nitrogen were re-warmed before opening in a dry air stream (dew point below −60°C) to avoid surface re-hydration.

Control of molecular weight

Molecular weight of lintnerized potato starch was determined using size-exclusion chromatography on a superose 12TM column (Pharmacia) after solubilization in 1M KOH as described by Faisant *et al.* (1993).

The molecular weight of amylose was monitored by determining its intrinsic viscosity with a Ubbelholde viscometer. Using the Mark–Houwink–Sakurada intrinsic viscosity relation $[\eta]=K\ M_v{}^a$, the coefficients were $K=6.92\text{ml}\,\text{g}^{-2}\text{mol}^{-1}$ and $a=0.78$ with $[\eta]$ in cm^3g^{-1} measured at 25°C in 0·2M KOH (Banks & Greenwood, 1975). Possibilities of molecular weight alteration during processes, such as casting and dialysis, were also controlled.

Weight-average molecular weights of amylose, phytoglycogen and pullulan were determined using a combination of size-exclusion chromatography and multi-angle laser light scattering (HPSEC-MAS) (Roger & Colonna, 1993). Weight-average molecular weight of waxy maize amylopectin was determined as described by Roger and Colonna (1992) using light scattering.

Water content adjustment

The water content of all samples was adjusted via a vapor phase conditioning over saturated salt solutions (Multon & Bizot, 1991). The method is similar to the Community Bureau of Reference recommendation (Jowitt & Wagstaffe, 1989), except that the temperature was regulated at 25°C±0.1°C by immersion in a water bath and that the sorbostats may be evacuated. This method has been shown to be preferable to other moistening procedures (Gidley *et al.*, 1993).

Pre-drying of samples over phosphorus pentoxide, up to constant weight (±0.05% per 24h), served as the basis for calculating water contents expressed as g of water/g of total material for T_g correlations, and as moles of water per mole of anhydroglucose (g of dry matter/162) for sorption isotherms representation.

Sorption isotherms were plotted after fitting the Guggenheim–Anderson–De Boer equation ("GAB"), as previously described (Bizot, 1983). This type of three-parameter model introduced in food science by Van den Berg (1981, 1984) describes localized homogeneous multilayer adsorption mechanisms with two classes of layer: the primary layer, with higher excess adsorption energy than the multilayers themselves, which differ from liquid water. Simple kinetic arguments allow the demonstration of the currently used expression (Strauss *et al.*, 1990):

$$W=[cKa_w\,W_m]/[1-Ka_w].[1-Ka_w+cKa_w]$$

where the water content W is related to water activity a_w via three parameters: (i) W_m = primary adsorbed fraction on the homogeneous family of strong sites; (ii) $c=c'\exp(Hm-Hn)/RT$ (Hm integral heat of sorption of water on primary sites); and (iii) $K=k'\exp(Hl-Hn)/RT$ (Hn integral heat of sorption of water on supplementary layers, Hl heat of condensation).

Simple fitting methods with poor statistical validity can be applied either in a quadratic form ($a_w/W=m1\ a_w{}^*a_w+m2\ a_w+m3$) or a fractional form ($W=m1\ a_w/[(1-m2\ a_w)\ (1-m3\ a_w)]$). Smoothing is usually correct up to $a_w=0.9$.

Differential scanning calorimetry

Differential heat flux calorimeters DSC 111 and 121 from SETARAM (France) were used for all measurements. Temperature and enthalpy calibrations were checked with indium ($T_m=429.8\text{K}$, $\Delta H_m=28.55\text{J/g}$). Pressure-tight stainless steel crucibles (P max = 15MPa) allowed safe manipulations of hydrated products up to decomposition temperature. These crucibles contained about 80mg sample. Due to the thermal lag of the detector and crucibles, the heating rate was kept at 3°C/mn. Most experiments were run once using two successive scans except when outliers were obvious.

The transition temperature was defined as the midpoint of the transition (Wunderlich, 1990); in practice, this is equivalent to the inflection point of most thermograms (Fig. 3). The first scans are systematically complicated by endothermic effects bearing strong analogy with enthalpy relaxation phenomena about 25–50°C above storage temperature (the water conditioning temperature was 25°C over periods exceeding days) producing either pre-peaks or overshoots. Entropy relaxation phenomena have also been observed with polymers (Huzeon *et al.*, 1995) and proteins (Sartor *et al.*, 1994). This was overcome by operating two successive heating scans up to $T_g+30°\text{C}$ after a preliminary measurement when necessary. Most reported values are from the second scan (after cooling at about 30–10°C/mn). Following the heat treatment, the dry matter of the complexes was determined on the open crucibles, by oven drying for 3h at 130°C (accuracy: ±0.3% H_2O dry basis).

A smooth interpolation between the experimental points was obtained by fitting Couchman's model (Couchman, 1987) which describes the dependence of the glass transition upon the composition in polymer blends. For the purposes of the present study we employed the relationship:

$$T_g(Y^w)=\frac{Y^wT^g_w+(1-Y^w)(\Delta C^p_p/\Delta C^w_p)T^p_g}{Y^w+(1-Y^w)(\Delta C^p_p/\Delta C^w_p)}$$

where Y^w is the weight faction of water, ΔC^p_p and ΔC^w_p are the respective specific heat changes of polymer and water at the respective glass transition temperatures: T^p_g and T^w_g. Because of some controversies concerning the glass transition of pure water (Angell, 1988), we selected the classical value $T_g{}^w$ at 134K (Sugisaki *et al.*, 1968), but we let the heat capacity change of water at $T_g{}^w$ be an adjustable constant hidden in the ratio $\Delta C^p_p/\Delta C^w_p$ since the strong character of water may be questionable when dispersed in a polymer matrix. This has been recently discussed by Kalichevsky *et al.* (1993).

X-ray diffraction

Powder X-ray diffraction was used to assess the amorphous nature of some of the samples using a procedure identical to former studies (Godet *et al.*, 1995). This method has a notoriously low sensitivity and spectral resolution is enhanced when the water content is high (around 30%). In order to avoid an eventual retrogradation of highly out-of-equilibrium products, such as hot-cast amyloses, water vapor sorption was replaced by equilibration over methanol/water mixtures 60/40 (w/w). This proved to bring the amyloses below or close to their DSC T_g while allowing good spectral improvement. Higher water fractions induce V-type crystallization (retrogradation), and lower ones increase distortion of the diffractograms particularly at higher diffraction angles. This procedure will be reported elsewhere, accounting for peak displacement, i.e. the amorphous halo maximum is found around 20° instead of 19° in the present case presumably because of V-type helicity.

Data fitting

Kaleidagraph from Synergy Software was used for data fitting and graph generation. The inherent limitations to curve fitting with composite parameters did not allow meaningful statistical analyses.

RESULTS AND DISCUSSION

Chemical and macromolecular characteristics of substrates

Weight-average molecular weights M_w, polymerization degrees and branching characteristics are listed in Table 1. The native potato starch used for casting, contained 23% amylose. In the present study no attention was paid to phosphorus content and the corresponding counter-ion nature, although this point may be important for materials originating from potato starch. The lintnerized potato starch contained, essentially linear, DP 15 short chains as already described (Robin *et al.*, 1974). The molecular weight of pure amylose from pea was 5.9×10^5, with very little intermediate branched material (less than 1%, Kalichevsky & Ring, 1987).

Waxy maize starch is usually considered as nearly pure amylopectin. The values obtained for glycogen and waxy maize amylopectin ($2{\cdot}7\times10^7$ and $3{\cdot}0\times10^8$, respectively) agree with the values given by Tao and Matheson (1993). The weight-average molecular weight of waxy maize amylopectin is usually estimated between 10^7 and 10^8, but is still debated, since some variation in the mean population results from shape effects in solution (Tao and Matheson, 1993), and also from granule heterogeneity as amylopectin from the internal layers of starch granules has been reported to have longer long-B chains than in outer layers (Jane *et al.*, 1994; Jane & Shen, 1993).

Macromolecular characterization of branched amylopectins and glycogen (sometimes described as racemoses) are usually specified by: (i) average chain length (CL); (ii) average external chain length (ECL)—determined by beta-amylolysis of 'A' type chains containing (1–4) linkages as for amylose and terminated by a non reducing glucose unit); (iii) average internal chain length (ICL—'B' type chains bearing (1–6) linkages branching point to 'A' type or other 'B' chains); and (iv) A/B ratio. The figures in Table 1 are from Yun and Matheson (1993) and differ from those of Inouchi *et al.* (1987), yet recent studies demonstrate the highly complex nature of the amylopectin structure (Ong *et al.*, 1994). The branching is larger in phytoglycogen (8.3 vs 5.6%) while A/B ratio (0.7 vs 1.3) and external chain length (6 vs 12) are much smaller by comparison to waxy maize. The primary structure of phytoglycogen extracted from sugary-1 maize (a mutant, deficient in debranching enzymatic activity, which allows pruning of the spontaneously synthetized glycogen like

Table 1. Molecular characteristics of glucans after extraction and preparation and/or modification (CL: chain length; ECL: external chain length; ICL: internal chain length). Literature data are from Yun and Matheson (1993) or Shi and Seib (1992[c])

Polyglucans/averages	M_w	CL in monomeric units	% of 1–6 linkages	A/B	ECL	ICL
Potato starch (cast 90°C)	–	23% linear	–	–	–	–
Pea amylose (leached 70°C)	5.9×10^{5b}	3650	$x < 1\%$	–	–	–
Pea amylose (leached 70°C)	4.1×10^{5a}	2530	–	–	–	–
Pea amylose (after 100°C casting)	3×10^{5a}	1850	–	–	–	–
Lintnerized potato starch	2.3×10^{3}	15	–	–	–	–
Cast waxy maize starch	3×10^{8}	18	5.6%	1.3	12/15[c]	6/8[c]
Su1 maize phytoglycogen	2.7×10^{7b}	12	8.3%	0.7	6	5
AMFRU before grafting	1.9×10^{5a}	1170	–	–	–	–
AMFRU 5.8% (cast 90°C)	1.1×10^{5a}	680	–	–	–	–
Pullulan	1.3×10^{5b}	800	33%	–	–	–
Dextran T 500	4.7×10^{5b}	2900	>95%	–	–	–

[a] Determined by intrinsic viscosity.
[b] Determined by HP SEC MALLS.
[c] Alternate values from Shi and Seib (1992).

structure, Jane *et al.*, 1994) should not be confused with sugary-2 inbred. After maturity, this starch has been reported to contain larger amylose molecules and amylopectin with longer, but less branched long B-chains and smaller intermediate B-chains (Takeda & Preiss, 1993). This substance will be considered in a future study as its low crystallinity and gelatinization enthalpy or retrogradation ability suggest a good aptitude to form homogeneous glasses (Inouchi *et al.*, 1991; Campbell *et al.*, 1994).

Moreover, the number of days after pollination may be crucial to the development of the various fractions. Waxy maize amylopectin has rather short average and external chain lengths (21 and 14, respectively as compared to 33 and 20 for potato amylopectin). For 30% solid content gels at 21°C, this has been correlated to its slower retrogradation kinetics which will tend to zero when ECL tends to reach values below 11/12 (Würsch and Gumy, 1994).

Pullulan is considered to be an essentially linear poly(maltotriose) joined end to end by $\alpha(1\text{–}6)$ linkages. The ratio of $\alpha(1\text{–}4)$–$\alpha(1\text{–}6)$ linkage is, thus, about 2/1 (Robyt, 1992). The molecular weight was determined as 1.3×10^5.

Dextran is synthetized by a B512 strain of *Leuconostoc mesenteroides*; it may be considered as formed by (95%) contiguous $\alpha(1\text{–}6)$ linked glucose residues in the main chain, with about 5% $\alpha(1\text{–}3)$ branch linkages bearing either single glucoses or a few long lateral chains (Robyt, 1992). The weight average molecular weight of the T500 commercial sample rechecked by HPSEC-MAALS was 4.7×10^5, and the polydispersity appeared to be better than specified ($M_n/M_w = 1.58$).

Sorption isotherms and water contents

The isotherms of the different compounds are shown in Figs 1 and 2, and are compared to a native potato starch reference. The corresponding GAB coefficients are given in Table 2, including correlation coefficients as calculated by Kaleidagraph, and the root-mean-square of relative deviation between measured and calculated water contents, taking into account all experimental sorption values except some upper ones when mentioned by brackets in Figs 1 and 2.

At low water activities ($0 < a_w < 0.2$), water uptakes of amylose and amylopectin are very similar to those of native potato starch (Fig. 2). Lintnerized and cast potato starches have a lower affinity for water and a behavior close to pullulan and dextran (Fig. 2). This may be attributed to crystallinity for lintnerized starch and possibly to a collapse densification phenomenon for dry cast potato starch compared to the more accessible partially crystalline native granules.

At intermediate water activities ($0.2 < a_w < 0.8$), water uptakes of phytoglycogen, amylose, cast potato starch and amylopectin (in decreasing order) are very similar, but systematically lower than semi-crystalline native potato starch granules. For lintnerized starch and pullulan lower water sorption capacities are observed, if this is correlated to their expected higher mobility, dextran should display an even lower affinity, but this is not the case in our conditions. Similar ranking between amylose and amylopectin (of different origins) was noted by Van den Berg (1981). This may not be easily interpreted, as easier chain/chain interactions (faster retrogradation kinetics) between dispersed linear chains via hydrogen bonded junction zones would suggest that sorption capability should be depressed for dried amylose. On the contrary, if the chain flexibility was the reason for optimum structural collapse, non retrograding more branched phytoglycogen would be expected to have the lowest affinity. In this same range, the isotherms obtained for beta-limit dextrins of amylopectin and phytoglycogen (Fig. 2) are almost identical as expected from their very similar internal topology, while their lower sorption capacity might be related to a more densely branched core. Possibly the interplay between helix/coil inter conversion and the formation of quasi crystalline bundles of helices, alter the balance between intra-intermolecular hydrogen bondings as well as longer distance packing. The sorption affinity as well as the accessibility of these substrates are consequently influenced, by drying histories (equivalent to various quenching kinetics when crossing the glass transition region) and sorption temperature and kinetics. The accuracy required to analyze such minor variations falls quickly within the precision of the conditioning method presently used.

At high water activities ($a_w > 0.8$), the components more prone to form swollen gels (i.e. dextran and pullulan) adsorb very high amounts of water and approach asymptotically the vertical axis, in contrast to the most crystalline sample (lintnerized starch). Our values for dextran are similar to those obtained by Taylor *et al.* (1959) within 1% H_2O (d.b.), but recrystallization of dextrans is to be expected in this domain (Taylor *et al.*, 1959) and effectively occurred when DSC scans were programmed to temperature levels more than about 30°C above T_g. In general, the semi-crystalline or crystalline fractions are expected to display limited sorption: for example, maximum intra-granular water for potato starch amounts to 54% H_2O (d.b.) (BeMiller & Pratt, 1981). In this region of the isotherms, the determination of water uptake is more difficult, especially for a_w ranging from 0.95 to 1 (Taylor *et al.*, 1961), and the values obtained depend upon osmotic, swelling and eventual capillary phenomena; their sensitivity to minor temperature fluctuations would require a higher level of temperature regulation than employed in the present study.

Sorption isotherm data were fitted with the localized homogeneous multimolecular adsorption model GAB, although we are not fully convinced that all adsorption sites are of identical affinity for water. Nevertheless, this model has proved to be robust except for a few exceptions with polysaccharides (Weisser & Liebenspacher,

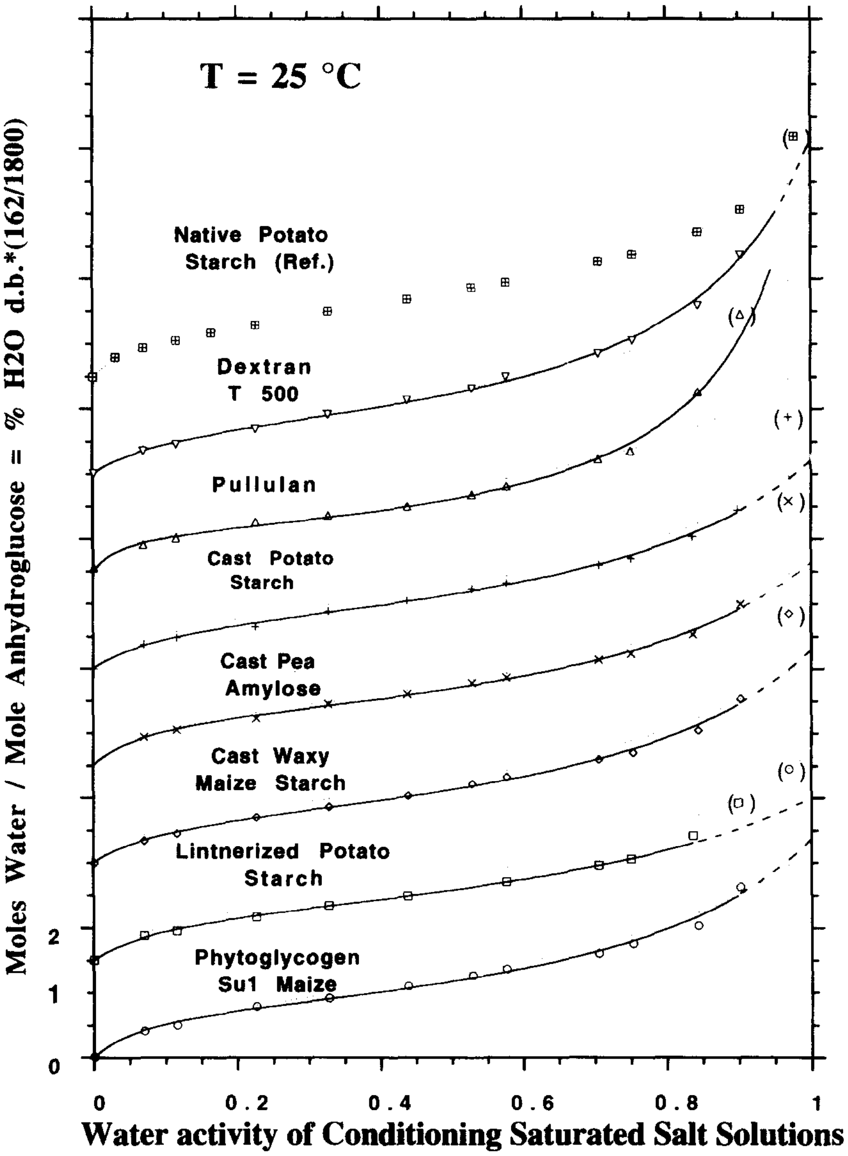

Fig. 1. Comparison of water vapor sorption isotherms (25°C) of polyglycans (successive vertical shifts = 1·5 moles water/mole anhydroglucose).

1989). The general tendency is not totally reflected in W_m values, which should still be considered as the more reliable parameter in this context. Moreover, experimental errors (botanical origin, dry matter determination, pre-treatments and drying conditions) and insufficient statistical validity preclude detailed analysis of parameter values from Table 2 (Toupin *et al.*, 1983; Samaniego-Esguerra *et al.*, 1991). Notably the sorption isotherm of native potato starch granules taken as our internal standard deviates from formerly published values without clear explanation.

Thermogram interpretation

General shape

Typical DSC scans of amylose and amylopectin, including heat capacity changes (ΔC_p) measured at the transition temperature (second scan), are shown in Fig. 3. As already mentioned, the transition temperature $T_g^{1/2}$ was defined as the midpoint of the transition (equivalent in practice to the inflection point of most thermograms), and we systematically rely on the second scan to eliminate enthalpy relaxation pre-peak or overshoot, as well as to improve sample homogeneity and standardize the cooling history. This choice is somewhat arbitrary and some errors may be introduced when recrystallization or other structural changes take place. For example, drier dextran samples displayed exothermal drifts before T_g in the first scan obtained after conditioning and industrial treatments; further scans were reproducible. Other temperatures are defined in Fig. 3 and will be used in a following section.

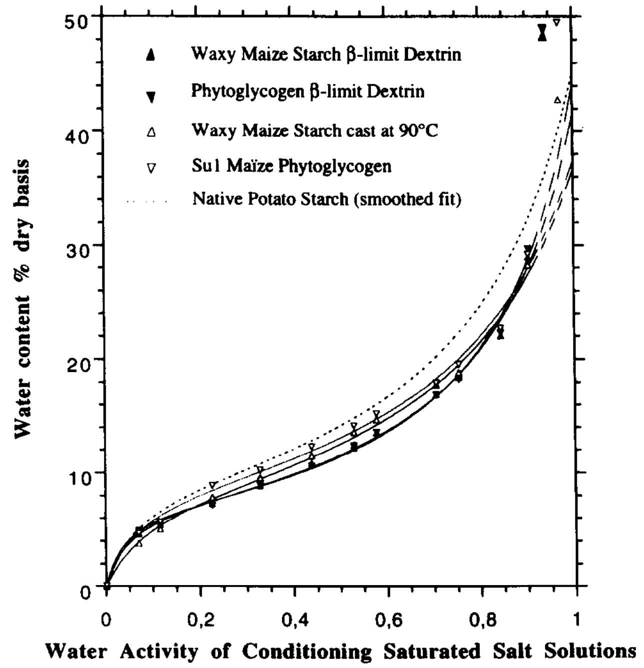

Fig. 2. Water vapor sorption isotherms (25°C) of β-limit dextrins for branched glucans.

Table 2. Values of the fitting parameters for the homogeneous, localized, multi-molecular adsorption model of Guggenheim/Anderson/deBoer using the water contents of the polyglucans conditioned in sorption at 25°C from the dry state for glass transition determinations. The %RMS is expressed for relative water contents error (measured-fitted)/measured

Polyglucans/G.A.B. parameters:	W_m[a]	C	K	R	%RMS
Native potato starch granules	0.789	11.85	0.85	0.9989	3.09
Potato starch (cast 90°C)	0.796	13.57	0.758	0.9991	3.2
Pea amylose (cast 100°C)	0.817	18.03	0.747	0.9987	2.75
Waxy maize starch (cast 90°C)	0.747	21.15	0.767	0.9979	5.01
Lintnerized potato starch	0.736	16.48	0.749	0.9992	2.74
Su-1 maize phytoglycogen	0.716	8.77	0.714	0.9995	3.5
Pullulan	0.628	28.03	0.915	0.9983	6.6
Dextran T500	0.789	11.8	0.850	0.9988	3.1
Phytoglycogen β-limit dextrin	0.623	30.39	0.84	0.9979	3.1
Amylopectin β-limit dextrin	0.645	24.34	0.829	0.998	2.7
AMFRU 5.8%	0.826	11.41	0.691	0.998	3.7
APFRU 4.6%	0.755	14.19	0.729	0.999	3.4

[a] moles H_2O/moles anhydroglucose.

Heat capacity change at T_g

In the range 10–15% H_2O (w.b.), we may rank our products in the following order with decreasing heat capacity change (usually upon the second DSC scan, in J/g): dextran (0.51) < pullulan (0.35) < β-limit dextrin of phytoglycogen (0.32)≤β-limit dextrin of amylopectin (0.30) < amylopectin (0.27) < phytoglycogen (0.20) ≤amylose (0.20). More precise heat capacity measurements on amylopectin (when related to the known heat capacity of 100 micron alpha alumina particles) gave data about 30% smaller than that of Noel and Ring (1992) who used a more precise calorimeter for this purpose. Studying a series of malto-oligomers, the heat capacity increment at T_g has been estimated by extrapolation as $0{\cdot}47\,\mathrm{J\,g^{-1}K^{-1}}$ for high molecular weight compounds, considering this value to be independent of temperature (Orford *et al.*, 1989). As noted by Kalichevsky *et al.* (1993), most ΔC_p obtained from ordinary thermograms yields very scattered data. Limitations due to thermal contacts, irreproducibility inside and outside sample pans, free space allowing vapor migrations and absence of inert interstitial liquid to improve thermal conductivity (Runt & Harrison, 1980), render absolute heat capacity determinations difficult, particularly for wet powders.

Transition behavior of substrates

In general, we have not been able to obtain the complete collapse of amylose particles inside the DSC pan despite efforts to maximize the amorphous character of amylose films by rapid casting of diluted dispersions at high temperature (≤100°C). For amylopectin, either sintering of particles or complete collapse, resulting in a cylindrical solid, was obtained after heating above T_g. Consequently, we believe that amylose particles are always partially crystalline particularly at their outer-layers where chain reorganization is presumably favored. In fact, the glass transition of amorphous polymers at surfaces is depressed because of increased chain end concentration drained by surface tension effects, and this leads locally to a higher conformational entropy (Mayes, 1994). Therefore, our T_g values could be overestimated even if no crystallinity was detectable by X-ray powder diffraction measurement before the first DSC scan (Fig. 4a).

Heat capacity change increment (ΔC_p) *between first and second scans*

In the second DSC scan, we have generally observed a reduction in heat capacity increment for amylose or amylopectin (Fig. 3) which, furthermore, remained constant for some of the other substrates. This could be interpreted as recrystallization taking place during the transition (we expected a behavior similar to the retrogradation studies of Joupilla & Roos, 1995) with no nucleation delay and immediate, but limited propagation, as X-ray diffraction patterns (of mixed allomorphs) appear to be weak (Fig. 4b). Upon further heat treatment, the entanglement of chains and their crosslinking by the crystallites could preclude the further chain slippage necessary to improve crystallinity. This point requires further investigation, but the data for amylose may not be

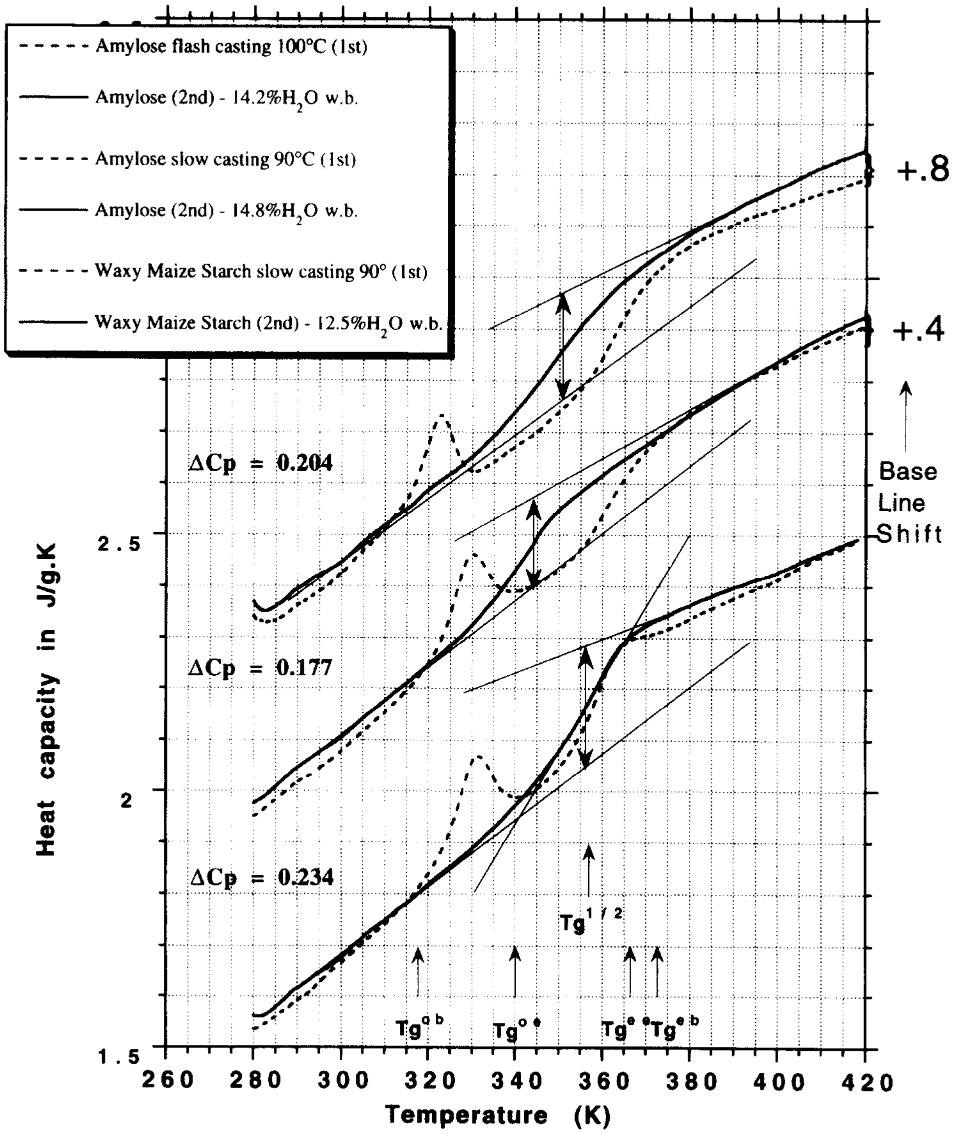

Fig. 3. Heat capacity of hydrated amylose and amylopectin (two successive DSC scans at 3°/mn) and definition of transition parameters.

considered as representative of a truly amorphous state, which may be more closely approximated via a freeze drying technique. Sintering of starch particles without fusion of granules has also been noted for starch, as long as the temperature does not reach the completion of the highest endotherm around 170°C where crystalline nuclei seem to have disappeared (Willenbücher *et al.*, 1993). This could be connected to the bifurcation between gelation and precipitation of dilute amylose solution around a critical temperature of 160°C (Doublier *et al.*, 1992). To our knowledge no crystallinity was ever reported even at ranges below X-ray detectability employing techniques such as CPMAS NMR, but again the decrease in ΔC_p should imply some re-organization.

Extension of the transition

Generally on most DSC traces (Fig. 3) the extent of the transition is about twice as large, when evaluated from the onset of the base line departure T_g^{ob} to the base line completion temperature T_g^{eb}, than when taken from extrapolated temperature values (intersection between the tangents of base lines and inflection point of the transition: T_g^{oe}, T_g^{ee}). The first scan transition is usually sharper, particularly for amylose which recrystallizes during this slow heating/cooling cycle. Without giving an explicit formulation to describe the cooperativity of the transitions, we obviously observed (Table 3) that amylose displays an extended transition suggesting inhomogeneity and low cooperativity, while pullulan or dextran behave much more like synthetic polymers (for an industrial poly-methyl-metacrylate: $\Delta T_g = 14$°C in our equipment). For products unable to crystallize, such as phytoglycogen and beta-limit dextrins or even substituted amylose (?), the transition develops over a wider temperature range than for products rich in short linear chains or branches which may couple the motions

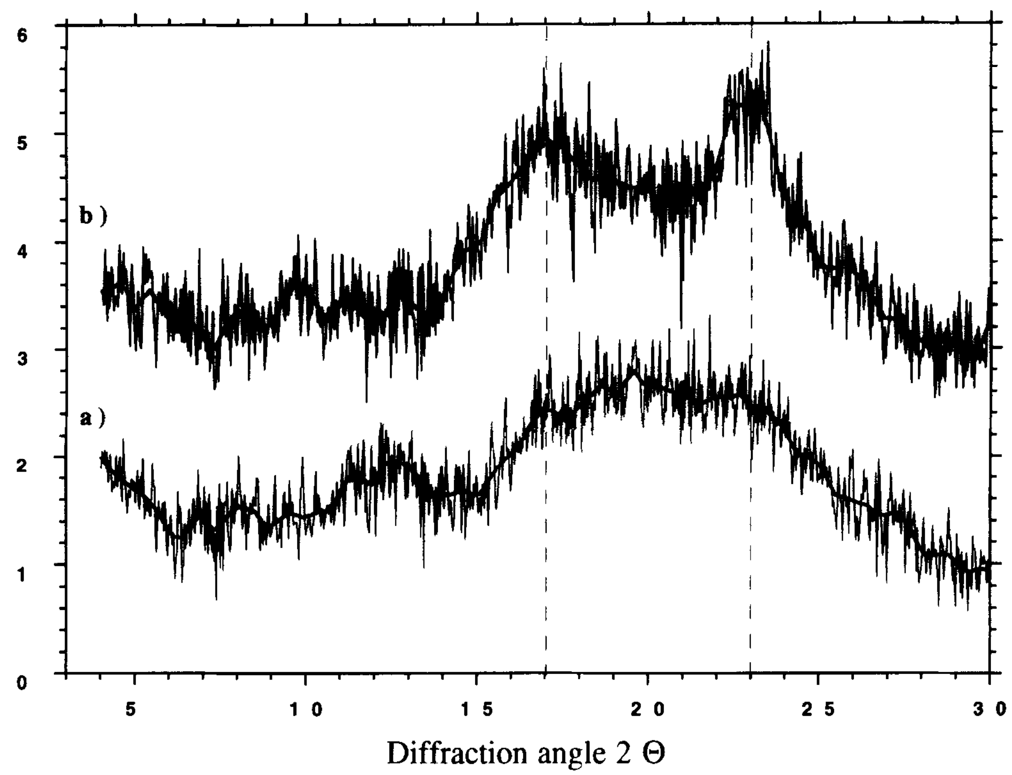

Fig. 4. Smoothed X-ray diffractograms of amylose powders conditioned by exposure to the vapor of a methanol/water (60/40) mixture: (a) amylose cast at 100°C ground in liquid nitrogen; (b) same amylose recrystallized after DSC Scan at 13% H_2O w.b. up to 150°C.

Table 3. Calculated 'dry polymer' glass transition temperature and fit estimated heat capacity change for the pure polymer using Couchman's correlation; comparison with the approximated experimental specific heat increment at 10% H_2O w.b. The width of the transition is evaluated between extrapolated values for onset and completion temperatures

Estimated parameters (% w=content in % H_2O w.b.) Water-plasticized polyglucans (y)	Tg^{yo} (°K) @ 0% w	$\frac{\Delta C_p^y}{\Delta C_p^w}$	ΔC_p^y calculated (a)	$\Delta C_p\, y+w$ @10% w (±20%)	T_g exp. @10% w (±3°)	R Reg. Coeff. Couchman	ΔT_g Extr. @ 10% w $T_g^{\circ}-T_g^{e}$ (±3°)
Pea amylose (cast 100°C)	605	0.145	0.265	0.20	400	0.998	41
Potato starch (cast 90°C)	589	0.145	0.265	0.2	389	0.999	19
Waxy maize starch (cast 90°C)	558	0.161	0.295	0.22	384	0.999	18
su-1 maize phytoglycogen	533	0.174	0.318	0.25	376	0.998	26
Lintnerized potato starch	410	0.238	0.436	0.6	320	0.992	19.5
Dextran T500	462	0.246	0.450	0.51	359	0.998	11
Pullulan	488	0.180	0.329	0.30	352	0.998	14
Amylopectin β-limit dextrin	542	0.155	0.284	0.26	372	0.999	33
Phytoglycogen β-limit dextrin	539	0.150	0.267	0.32	366	0.999	34
AMFRU 5.8%	502	0.183	0.335	0.17	364(380)	0.991	31
APFRU 4.6%	516	0.175	0.320	0.18	370	0.997	20

([a]) Heat capacity change at T_g for water in carbohydrates taken from Hatley and Mant (1993): ($\Delta C_p^w = 1.83$ J/g.°).

between locally associated bundles. Probably some distinction between long range and short range associations should be introduced.

General trends

Figures 5 and 6 show the relationship of T_g with water content for the different studied compounds. The general shape of the curves is similar, showing an approximately parallel decline of T_g with water content. The fitting parameters for Couchman's model are summarized in Table 3, where an easier comparison of curves level may be obtained from the T_g values at 10% H_2O w.b. Amylose (long linear chains) presents the highest T_g with an estimated value for the pure polymer T_g^{yo} at 605K in the dry state (Fig. 5). The curve of potato starch (77% amylopectin/23% amylose) lies between those of amylose and amylopectin, while the values of the readily soluble phytoglycogen are about 5–10°C below amylopectin. Resulting from external chains elimination, the β-limit dextrins of waxy maize amylopectin and su-1 maize phytoglycogen show a respective depression from their parent compound values. In the 1–4 linkage rich series, lintnerized potato starch (Fig. 5) takes the lowest position, but for 1–6 rich polymers, the relationship for dextran is not parallel to the other compounds and pullulan yields the lowest values over the practical range of hydration (Fig. 6). A similar T_g relationship with hydration was found for fructosylated amylose and amylopectin, in the limited range investigated (0.13–0.25g/g H_2O w.b.). The amounts of water necessary to achieve a T_g close to room temperature (at a 100s time scale) were found to be between 0.14g/g for lintnerized starch and 0.21 g/g for amylose and potato starch.

T_g correlation

Couchman's type of models appear to allow reasonable extrapolation to the dry state, which cannot be physically reached because of thermal decomposition starting around 180°C. However, the quality of the model fitting does not justify the hypotheses necessary to its derivation, such as the second-order transition nature of the glass transition, the continuity of entropy at T_g or the linear variation of ΔC_p with $1/T$. Numerous attempts to extend this type of formulation and account for the specific interactions (such as hydrogen bonds) have led to second or higher power equations (Couchman, 1991; Kwei *et al.*, 1987) which may be re-cast as virial-like expansions (Schneider & Di Marzio, 1992). The resulting improvements in fitting ability require more adjustable parameters and limit predictability. Moreover, due to some controversies concerning the glass transition of pure water (Angell, 1988) we selected the classical value value T_g^w at 134K (Sugisaki *et al.*, 1968), but allowed the heat capacity change of water at T_g^w to be an adjustable constant hidden in the $\Delta C_p^p/\Delta C_p^w$ since the strong character of water may be questionable when dispersed in a polymer matrix.

The present formulation is identical to the traditional Gordon–Taylor expression (Gordon & Taylor, 1952) based on free volume arguments where the heat capacity ratio $\Delta C_p^p/\Delta C_p^w$ should be replaced by a ratio involving materials densities and changes in thermal expansivities $\rho^w.\Delta\alpha_p^w/\rho^p.\Delta\alpha_p^p$. Its robustness was judged satisfactory for pharmaceutical solids (Hancock & Zografi, 1994) or food systems (Kalichevsky *et al.*, 1993).

This equation was, nevertheless, used to calculate the heat capacity changes ΔC_p^y at T_g in the dry state taking arbitrarily the value of Hatley and Mant (1993) for ΔC_p^w (Table 3). These values were found to vary similarly to measured DSC data at 10% H_2O w.b., although extrapolated values were under-estimated for lintnerized potato starch, dextran and beta-limit dextrin of phytoglycogen and over-estimated for fructosylated substrates.

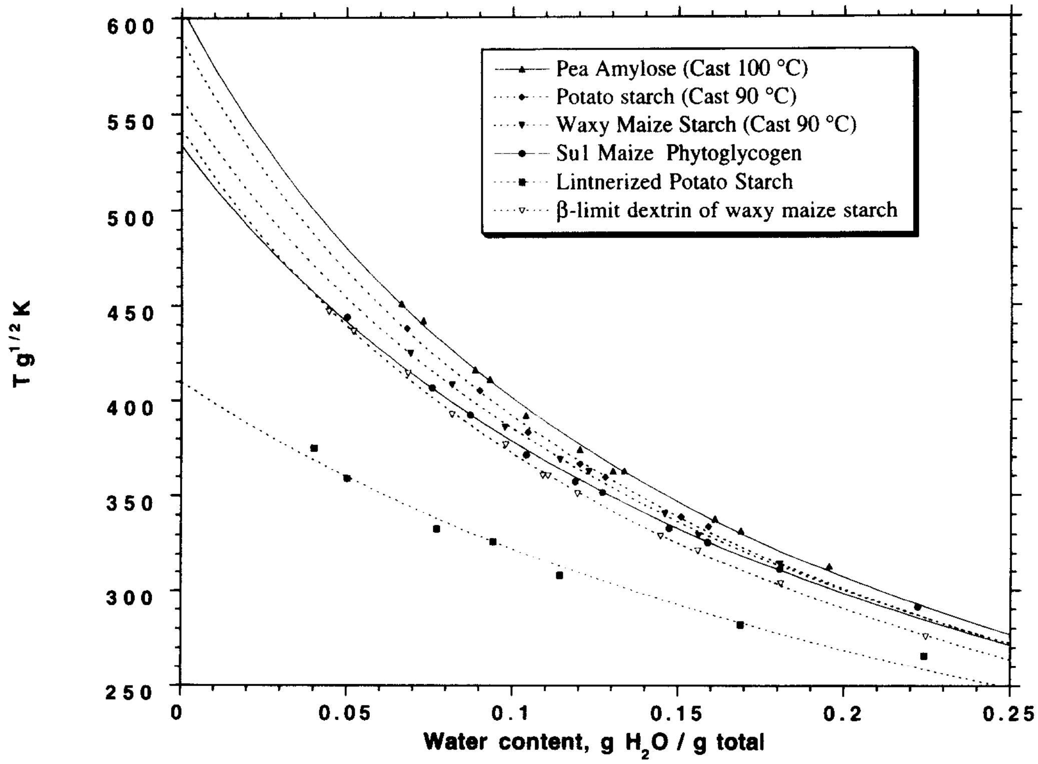

Fig. 5. Calorimetric glass transition (at 3°C/mn) of starch sub-fractions vs water content.

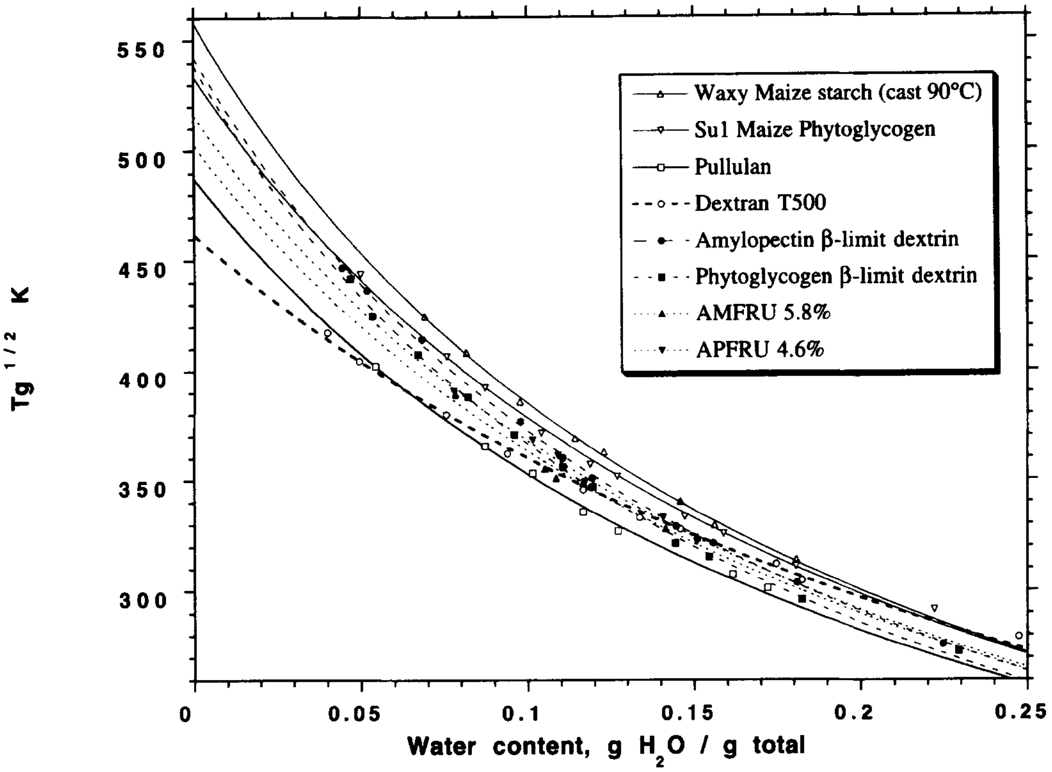

Fig. 6. Effect of (1–6) linkages on the calorimetric glass transition (at 3°C/mn) of some (1–4) and (1–6) linked polysaccharides vs water content.

The present measurements on our products as well as literature data indicate that this model has greater fitting difficulties when strong curvature is needed either for drier products, or at higher water contents where plasticizing by water levels off. This is apparent for our lintnerized starch and appears strikingly, for instance, with elastin (Lillie & Gosline, 1993) when their composite data of DSC and mechanical spectroscopy (shifted to account for characteristic frequency scaling), are submitted to fitting. The wide range probed in this study shows the need for a decreasing curvature at water contents approaching C_g' (Levine and Slade, 1988a), so that correct fitting is obtained only by letting the water glass transition temperature operate as a free parameter which finally converges to values only slightly below T_g' (Levine & Slade, 1988a). As with many polymer science studies, the investigation of a too narrow window leads to modeling data with limited theories resulting in only a local 'tangent' to the real phenomena. For lower hydration, water may exert an equivocal role via: (i) flexible intercalation (through hydrogen bond bridges); and (ii) antiplasticization (obstruction of free volume) as suggested by the specific volume maximum around 6% H_2O (d.b.) for starches (Sala & Tomka, 1993; Haine *et al.*, 1985), and electron spin resonance studies in polar polymers (Roozen *et al.*, 1991). However, such a tendency was not detected in the present study which mainly concerned higher hydration levels.

GENERAL DISCUSSION

Molecular weight and chain length effects—(the longer, the higher the T_g?)

The glass transition temperature of linear polymers increases with increasing molecular weight. In this work, extrapolated $T_g^{y^\circ}$ varies from 410K for lintnerized potato starch ($M_w \cong 2.3\ 10^3$) to 605K for pea amylose ($M_w \cong 5.9\ 10^5$). In the same way, amylopectin which has longer external chains than phytoglycogen presents a higher $T_g^{y^\circ}$ (558K vs 533K), but in this case, the effect of the branching ratio participates in the same direction. Extrapolation of glass transition values from oligomers to homopolymers is generally obtained according to Fox and Flory (1950): $T_g = T_g^\infty - K_g M^{-1}$. Following this procedure on oligomers, Orford *et al.* (1989) deduced the T_g of amylose and amylopectin as 500±10K. However, these values differ substantially from our fitting extrapolation ($T_g^{y^\circ}$ amylose = 605K) an also from the figures of Cowie *et al.* (1969) on a series of internally plasticized amylose esters extrapolated to unsubstituted values (T_g amylose = 590K). In the 1970s, Cowie (1975) and Cowie and Henshall (1976) following Boyer's studies (Boyer, 1992) further pointed out that three regimes of molecular weight dependence of T_g were generally observed when plotting T_g vs log x (where x is the number of chain segments). These three domains correspond to: (i) independent oligomeric behavior; (ii) polymeric behavior ($1/M_w$ dependence) up to the critical chain entanglement threshold (typically around DPn = 175 for substituted amyloses); and (iii) infinite high polymer plateau.

In the case of glycogen, greater motional flexibility which results in longer NMR spin–spin relaxational times (narrower linewidth) has been reported for chain end protons such as H4 (Zang *et al.*, 1991). The influence of chain ends on the mobility of interior glucose rings has also been investigated in solution by Brant *et al.* (1990) for malto- and isomalto-oligomers by determining the mean spin-lattice relaxational time of endocyclic carbons of the reducing chain end as a function of chain length; an asymptotic value was found to be about seven or eight monomers at low concentrations, while the co-operative unit for segmental motion was evaluated to about twice that number. However, within the time window accessible by ^{13}C NMR (125.7MHz), the variations in spin-lattice relaxation times of interior residues in oligomeric chains are apparently dominated by mechanisms depending more on viscous damping as related to concentration effects than on conformational flexibility (Kadkhodaei *et al.*, 1991). For lintnerized starches, further characterization of residual branching content will be provided in future work as these could provide extra mobility.

For the commercial dextrans which we used, a further comparison with higher molecular weight products ("T2000" M_w # 2×10^6 with a bimodal distribution) yielded T_g values systematically 5°C above those of "T500". Being well above the entanglement regime, we suspect that some primary structural changes took place, such as a 1–3 linkages preferential debranching during hydrolytic preparation. For lower molecular weights the T_g depression appeared to be minor.

(1–6 links) and branching effects—(the more branched, the lower the T_g?)

The effect of branching as a T_g depressor is known and is often described in terms of internal plasticization. The T_g depression observed, respectively, for amylopectin vs amylose (−10/−20°C) and phytoglycogen vs amylopectin (−5/−10°C) can be interpreted in terms of internal plasticization by α(1–6) branching (Slade & Levine, 1991a). The presence of α(1–6) links induces higher flexibility by a co-monomer effect and explains the intermediate behavior of the high molecular weight linear pullulan. Moreover, each branching generates one dangling non-reducing chain end per external chain, bringing an overall higher mobility to neighboring residues; this effect is involved in the rationalization of the T_g vs $1/M_w$ correlation in the framework of a free volume description (Eisenberg, 1984).

The α(1–6) branching point in amylopectin has been shown to allow a very efficient packing, with only minor conformational strains, thanks to the three rotational dihedrals (Buléon & Tran, 1990). However, the initial flexibility of this linkage may not introduce as much mobility (internal plasticization) as expected, once its conformation has been fixed by the combined interactions between glucose residues of each of the propagating amylose chains arranged in the double helical conformation. Only for short independent branches (2–4?) may we expect a significant improvement in mobility.

Non-systematic improvement of chain mobility vs side chain length could, however, be expected, since α(1–6) grafted maltose units (or a β-limit dextrin stump?) could fold back towards the main chain as a double helix precursor (Buléon & Tran, 1990) adopting compact conformation such as for panose (Imberty & Perez, 1988). The compactness of panose is also inferred from the density of its anhydrous crystals (d = 1.62) as compared to other sugars and alditols usually ranging from 1.47 to 1.64 (except for exceptionally symmetric packings (Angyal & Craig, 1994)). The density of glycogen and β-limit dextrins should, therefore, be tested. In the present case, we do observe an improvement of internal plasticization for the beta-limit dextrins whose T_g values are about 10°C lower than their parent molecules for approximately a doubling of their (1–6) content, if one considers the hydrolysis ratio.

Flexibility—(the more flexible, the lower the T_g?)

Presently, the correlation between primary structure characteristics and glass to plasticized melt transition is still being refined for organic polymers via simulation, correlation or theoretical methods (Binder, 1992). Common sense prompts that (1–6) linkages offering three rotational degrees of freedom instead of two for (1–4) linkages, should introduce more flexibility in the linear chains and additional dangling chain end effects for dendrimers.

Classically, one may follow Flory who considers a structureless amorphous state where random coils in the molten state behave as if they were in a theta solvent, ignoring any short range ordering (Boyer, 1992). Many attempts to correlate glass transition temperatures to cohesive energy density and chain stiffness factors have been developed (Lu & Jiang, 1991). For our three linear high polymers, if we postulate similar cohesive energy as related to hydrogen bonding capabilities (same number of hydroxyl groups per monomer and similar sorption properties), then the chain stiffness (Elias, 1984) taken as the value of Kuhn's statistical segment length b_K ($b_K = 2\times$persistent length) should be proportional to T_g. The hydrodynamic evaluations of Pavlov *et al.* (1994) would then suggest that T_g amylose > T_g pullulan > T_g dextran following the values of b_K amylose = 2.3nm > b_K pullulan = 1.8nm > b_K dextran = 1.3nm, relying on SAXS values for pullulan in water (Muroga *et al.*, 1987). However, as they used an additivity rule for flexibilities, their argument turns out to be circular for our concern. Using light scattering, orders of magnitude were obtained for amylose b_K amylose = 5.6nm (in water; Ring *et al.*, 1985) or 6.4nm (in KCl 0.1M; Roger & Colonna, 1992), while neutron scattering experiments on long amylose chains give b_K amylose of about 5nm in D_2O at 40°C and 8.4nm in 0·1M KOD at 25°C (P. Roger, private communication, 1995). With better intercomparability molecular modeling, Brant and Burton (1981) estimated values of persistence length (as mean end to end vector), i.e. amylose = 2*2.8nm > b_K pullulan = 2*1.3nm > b_K dextran = 2*0.6–1·3nm. These values are likely to be updated thanks to improvements in conformational energy map calculations (Brady & Schmidt, 1993; Dowd *et al.*, 1994). In any case, extrapolating solution data or short time scale simulations to the glass transition region will remain very questionable.

Crystallinity effect (the more crystalline, the higher the T_g)

Chain–chain association via crystalline zones may be considered as equivalent to crosslinking and is, thus, presumed to increase T_g and reduce the heat capacity increment by reducing 'mobility' (Chang, 1992). This mobility decrease occurs in the interfacial or interlamellar rigid amorphous fraction bordering crystallites (Sauer & Hsiao, 1995), and the relative amplitude of the heat capacity change at T_g has been proposed as a crystallinity index.

Nevertheless, the influence of crystallinity on T_g is probably not straightforward. Whilst parallel increase of both properties has been observed more often than not (Ellis *et al.*, 1984; Jin *et al.*, 1984, Ten Brinke *et al.*, 1983), the opposite dependence has also been reported (Wunderlich, 1990). These effects are probably related to the respective size and distribution of crystalline and amorphous domains when the matrix is mobilized at the glass transition. Few large crystalline domains (compared to chain entanglement distances) would be expected to remain independent of the amorphous fraction, or even introduce more free volume in the matrix, if their density is higher than the bulk due to packing effects and, consequently, the T_g may be unchanged or reduced. On the contrary, a high density of small crystalline junction zones could similarly increase T_g due to crosslinking.

In the studied substrates, amylose and lintnerized potato starch are the most prone to recrystallize during cooling, but probably yield very different structural features, with a 'fringed micellar' like arrangement for high DP amylose, and poorly crosslinked small crystals for lintnerized potato starch. For substituted amyloses, partial crystallization resulted in a T_g increase (Cowie & Henshall, 1976). Amylopectin is known to recrystallize more slowly than amylose (Goodfellow & Wilson, 1990), due to its high molecular weight and high level of branching. More specifically, the approach of Würsch and Gumy (1994) has revealed that recrystallization of amylopectin is strongly inhibited by shorter stubs (DP 2–5) generated by partial β-amylolysis or as contained in some waxy varieties (DP 6–9) (Shi & Seib, 1992), but its promotion begins for smooth segments of DP 11 or longer. This is in line with observations showing that linear segments induce or accelerate recrystallization even in amylose/amylopectin mixtures (Jane & Chen, 1992) or maltodextrin mixtures as observed by Schierbaum *et al.* (1992), when such media experience sol/gel transitions.

For starch, the behavior during retrogradation strongly depends on the conditions of pasting. In our case, pasting was carried out at 150°C and the disruption of the granule is expected to be complete, yielding a mixture of amylose and amylopectin dispersed in water. These two polymers are known to be incompatible in these conditions and reorganize separately (Miles *et al.*, 1985; Kalichevsky & Ring, 1987). Some doubts remain, however, concerning the elimination of residual nuclei which require heating above 160°C (Doublier *et al.*, 1992).

In the case of pullulan and dextran, their crystallinity was verified by powder X-ray diffraction on the original product, yielding a featureless diffractogram of amorphous pattern with an unique broad reflection centered at 0.51nm. However, room temperature recrystallization is to be expected at higher hydration levels (Taylor *et al.*, 1959) occurring in smooth regions of the polymer chains (Guizard *et al.*, 1984). During DSC scans, exothermic recrystallization was detected above 100°C at 11.5% H_2O (w.b.) resulting in a shift of the glass transition to lower temperature (!) and a decrease in the heat capacity increment upon rescanning. Guizard *et al.* (1984) have also described an anhydrous, high temperature crystalline polymorph, while their report of rheological studies by Russian teams provides an evaluation of the glass and melting transition temperatures of anhydrous forms at 140 and 245°C, respectively. Because of such recrystallizations, the heating scans were adjusted whenever possible, so that the maximum temperature of DSC scans remained about 20–30°C above the completion of the glass transition domain in order to minimize distortions. Further studies should be pursued in order to monitor recrystallization effects more precisely.

Despite the difficulties of determining T_g on thermograms (i.e. the transition amplitude decrease with the reduction of amorphous domains), low moisture amylose has shown lower T_g values upon second DSC scans, while samples with water content above 12% have higher T_g values. The eventual decrease of the molecular weight and a possible plasticization by some compounds produced upon degradation have not been checked. Therefore, the trend observed in this specific case requires further investigation with due account to local ordering such as provided by small angle X-ray scattering for electronic density fluctuations and CPMAS NMR for the identification of the packing type of double helices with respect to the asymmetric repeating unit (Gidley, 1992). More generally, the proportion of helical conformation as related to the chemical shifts in the solid state (Durran *et al.*, 1995) should be investigated.

Blending

Although de-mixing appears in solutions of amylose and amylopectin (Kalichevsky & Ring, 1987), their blends apparently behave as compatible with a single calorimetric glass transition spanning a similar temperature range (12–25°C for amylopectin, 25–50°C for amylose and 15–30°C for potato starch film). However, this could be confirmed by modulated differential scanning calorimetry which provides increased resolution (Song *et al.*, 1995). For potato starch film (sorption), our values fall between amylose and amylopectin, reasonably following the proportionality rule of DiMarzio (1990) i.e.: $T_g = B1\ T_g1 + B2\ T_g2$, where B*i* are the fractions of flexible bonds brought by each polymer. Incidentally, our data parallel that of Zeleznak and Hoseney (1987), but are 30°C higher than their pregelatinized wheat starch. Our definition of $T_g1/2$ (second scan) being identical, the discrepancies must concern the nature and origin of starch, the drying procedure (degradation) and the scanning rate 10°C/mn instead of 3 in the present paper. The same potato starch was also extruded with about 25% H_2O (w.b.) and the yielded first scan T_g values were similar to within 4°C of the cast product, when both samples were conditioned in desorption (data not shown). Unfortunately, significant recrystallization took place, increasing T_g values by some 20–40°C compared to samples in the sorption mode, and further studies would be needed.

PROPOSAL FOR PRIMARY STRUCTURE OPTIMIZATION

From experience gained by studies relating structure to starch plastics properties (Van Soest, 1996), we may consider that a reduced propensity to crystallize and internal plasticization by branching are expected to improve processibility of essentially linear amylose chains. The challenge now becomes to bio-synthesize linear limbs with regularly positioned lateral stumps, say every 10 residues. Copolymers of smooth linear amylodextrin portions (prone to the formation of small crystallites or junctions zones), interspaced by glucosylated panose of optimized length (between 2 and 10 monomeric units), should lead to the design of a slightly grafted comb which we may call 'ciliated' amylose.

For the synthesis of such an optimal substrate, three routes may be considered.

(1) A chemical pathway to comb-like D-glucans, bearing variable branching (Seymour *et al.*, 1979) of single (1–6)-linked D-glucopyranosyl groups, has been explored (Pfannemuller *et al.*, 1976). Surprisingly, however, only β-D linkages were obtained, despite Helferich's conditions favorable to α-D-glucosylation (Helferich & Zirner, 1962).

(2) Combination of enzymes with branching (Boyer *et al.*, 1982), elongation or transfer activities may also yield some optimal primary structures with suitable branching ratio and smooth chain lengths. Thus, further studies concerning internal self-plasticization and end chains effects should include truncated dendrimers such as limit dextrins from β-amylase (EC 3.2.1.2 leaving maltosyl or maltotriosyl stubs) or cyclomaltodextrinase (EC 3.2.1.54 leaving glucose stumps, Bender (1994)) on normal and su-2 amylopectins as well as phytoglycogen. They should also deal with comb-like linear chains with grafted oligo-glucosyl stumps separated by smooth sequences, allowing tailoring of chain–chain associations before choosing biosynthetic pathways to obtain optimal structures. Chemo-enzymatic grafting is also a possible route for branched cyclodextrins grafted by reverse activity of debranching enzyme (EC 3.2.1.68), such as isoamylase or pullulanase. This is only one of the routes to explore, since in the case of non-food uses, such as biodegradable films production, most patents have been concerned with chemical modifications, blending, hydrophobic complexing and plasticizer optimization.

(3) Finally, controlling starch biosynthesis via genetic modifications may be of use. Enzymes involved in su-2su-2su-2 maize mutants have been shown to modify amylopectin, to favor longer internal B chains (Takeda & Preiss, 1993) and similarities have been found for starch biosynthesis (Maddelein *et al.*, 1994) on unicellular green alga mutants (*Chlamidomonas reinhardtii*). These st-3 carrying mutants (i.e. lacking one of the two soluble starch synthase enzyme SSII, EC2.4.1.21) synthesize amylopectin enriched in longer internal B chains and in short external stubs ($CL < 9$) (Fontaine *et al.*, 1993).

EPILOGUE

Although we have not yet attempted to synthesize glycosylated glucans, donated samples of fructosylated amylose AMFRU (5.8%) and amylopectin APFRU (4.6%), prepared by Professor Richards, have been studied. These samples can be prepared by reacting fructosyl cation to hydroxyl nucleophiles during acid thermolysis of sucrose, either in DMSO solution

(Richards & Vandenburg, 1995) or from a water-restricted molten mixture (Manley-Harris & Richards, 1993, 1995). Grafted amylose or amylopectin are soluble, and no retrogradation was observed (as expected), due to the anticrystallizing effect of bulky side groups along the main chain. Both products have a sorption capacity very similar to amylopectin (within 1.2% H_2O). DSC traces are spread over large temperature ranges so that the glass transition takes place over a 35–50°C range for APFRU and a 40–60°C range for AMFRU. In the water content ranges studied, both products also have identical T_g values (±5°C) and behave similarly to β-limit dextrins. Thus, fructosylation decreases T_g by 15–8°C below the original amylopectin in the 8–15% water content region, but more significantly, by 35–40°C for amylose over the same range with only a two-fold molecular weight reduction, as inferred from intrinsic viscosity determinations (Table 1).

Conclusion

We have tried to explore the structure/property relationship between polyanhydroglucose branching pattern and water plasticization using a calorimetric measurement of the glass transition. Considering this criteria as connected to global mobility of polymeric chains, we show examples of T_g depression by more mobile segments such as chain ends and (1–6) branching points or linkages. From a polymer science point of view, all trends seem orthodox. At our present stage of understanding, tailored ciliated amylose structures to optimize local internal plasticization and steric hindrance partially preventing chain–chain association are more interesting as an academic exercise than an economical opportunity for industrial applications. However, when not limiting the use of modified starch and polysaccharides to low cost applications, Professor Richards may not admit so abruptly that "I am through with AMFRU".

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