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Effect of a glassy gellan/polydextrose matrix on the activity of α-D-glucosidase

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ABSTRACT

An investigation of the ability of the enzyme α-D-glucosidase to act on the substrate 4-nitrophenyl α-D-glucopyranoside (*p*NPG) while embedded in glassy carbohydrate matrices (deacylated gellan with polydextrose and polydextrose alone) is presented. Physicochemical characterisation of the matrices was achieved using the techniques of modulated differential scanning calorimetry, small deformation dynamic oscillation on shear, Fourier transform infra-red spectroscopy, wide angle X-ray diffraction and scanning electron microscopy. A UV–vis spectrophotometric procedure was adapted for the analysis of the activity of α-D-glucosidase in hydrolysing *p*NPG in the condensed carbohydrate systems. In order to derive a relationship between the structural properties of the matrix and the enzymatic activity, mechanical spectra were recorded using the combined framework of the Williams, Landel and Ferry equation with the time–temperature superposition principle. Theoretical modelling and experimental observations strongly argue for a pronounced effect of the gelling polysaccharide/co-solute mixture on enzymatic activity near the mechanical T_g of the matrix.

1. Introduction

In high solid systems, numerous changes can take place depending on physical state, properties of food materials and the physicochemical environment. During the various processing steps, distribution and storage of food materials, enzymatic reactions may contribute to a significant extent to these transformations, which may be desirable or deleterious. Therefore, it is important to understand these reactions for technological improvement, quality preservation and extension of the shelf life of foods (Drapon, 1985).

As one of the primary components of food, water is involved and affects a number of interactions occurring during the various stages of food production (Roos, 1995). Amorphous food systems exhibit a unique phenomenon which is temperature, time (or frequency) and composition dependent. For example, these are material-specific changes occurring upon heating a "glassy" mechanical solid to a "rubbery" viscous fluid commonly referred to as the "glass transition" (Sperling, 2006). This transition has been discussed as a possible factor affecting the kinetics of enzymatic changes in low-moisture processed foods (Slade & Levine, 1991; Roos & Karel, 1991; Roos, 1998).

A reduction in the translational and rotational motions of component molecules occurs during transition to the glassy state, thus supporting the hypothesis that chemical and biological reactions have reduced rates in glassy systems (Le Meste, 1995; Cardona, Schebor, Buera, Karel, & Chirife, 1997). Extensive literature is available on the effect of temperature on enzymes in amorphous systems including studies by Schebor, Buera, and Chirife, 1996; Mazzobre, Buera, and Chirife, 1997a; Mazzobre, Buera, and Chirife, 1997b; Burin, Buera, Hough, and Chirife, 2002. However, the focus of previous research has been on the thermal resistance or stability of the enzymes as related to the glass transition temperature (T_g) rather than on enzymatic activity. In addition, the interactions between polymer matrices (e.g. proteins or polysaccharides with co-solute at high levels of solids) and the recorded calorimetric or mechanical T_g in relation to enzymatic activity remains to be elucidated.

Microbial exo-polysaccharides have been invaluable ingredients in a variety of applications over a long period. One of them is deacylated gellan, which due to the novel property of forming thermo-reversible gels is gaining importance being utilised in diverse fields encompassing food, pharmaceutical and other industries (Giavasis, Harvey, & McNeil, 2000). It is produced through a fermentation process by a pure culture of *Pseudomonas elodea* yielding an anionic, high molecular weight, polysaccharide gum with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucose residues. The supplier describes it as having good flavour release and gel strength,

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requiring low usage levels (below 0.5% w/w) for most applications (Carlfors, Edsman, Petersson, & Jörnving, 1998; Kelco, 2004; Rozier, Mazuel, Grove, & Plazonnet, 1989). On the other hand, polydextrose is utilised in various products including beverages through to confectionery for both its physiological and technological benefits, e.g. oral health benefit, dietary fibre properties, reducing glycemic impact, prebiotic behaviour, and an ability to act both as humectant and crisping agent in foods (Stowell, 2009).

The present study takes advantage of the distinct structural properties of the aforementioned materials to characterise a high solid mixture (85%) with the aim to develop a model system for entrapping the enzyme-substrate complex of α-D-glucosidase and 4-nitrophenylα-D-glucopyranoside (*p*NPG). This allows examination of enzymatic behaviour in the vicinity of the glass transition temperature as recorded calorimetrically and rheologically for the binary mixture and a single polydextrose matrix.

2. Materials and methods

2.1. Materials

Deacylated gellan powder, Kelcogel trade name, was from Nutrasweet Kelco Company (Santiago, CA). The primary structure of gellan gum is composed of a linear tetrasaccharide repeating unit → [3)-β-D-Glc*p*-(1→4)-βL-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→$_n$. Polydextrose (Sta-Lite III powder) also known as PDX is a D-glucose polymer reaction product with citric acid or phosphoric acid and sorbitol with predominating β (1→6) bond. It was supplied by Tate & Lyle, ANZ, Pvt. Limited (Decatur, IL), as per manufacturer's certificate of analysis; it was of 90% purity with 4% moisture and had passed microbiological testing under the food grade standards. The enzyme used (α-D-glucoside glucohydrolase (EC 3.2.1.20, product number G3651) from *Bacillus stearothermophilus*, Sigma–Aldrich (St. Louis, MO, USA) commonly known as maltase), was as a lyophilised powder containing potassium phosphate buffer salt. Substrate (*p*NPG), potassium phosphate, L-glutathione and calcium chloride were also purchased from Sigma–Aldrich and sodium carbonate was from BDH, Port Fairy, VIC Australia. Milli-Q water from Millipore was used in all experiments.

2.2. Sample preparation

For physico-chemical studies: A clear solution of the polysaccharide was obtained by slowly dispersing weighed amounts of deacylated gellan powder in hot (90 °C) Milli-Q water and stirring constantly on a hot plate for 20 min. Calcium chloride (14 mM) was added to the hot solution to prepare a dispersion containing divalent cations. A weighed amount of polydextrose was separately dispersed in cold Milli-Q water and placed in a preheated water bath at 75 °C until fully dispersed. The temperature of the gellan sample was reduced to 75 °C and the polydextrose solution was carefully added so that no bubbles formed. Small amounts of excess water were removed by evaporation at temperatures close to 75 °C for all the samples. A solution containing only polydextrose was also prepared as described.

For enzymatic studies: Weighed amounts of gellan powder were slowly dispersed in hot (90 °C) Milli-Q water with constant stirring on a hot plate for 20 min to form a clear solution of the polysaccharide before adding calcium chloride (14 mM). A weighed amount of polydextrose was separately dispersed in potassium buffer (pH 6.8) with stirring for 20 min without heat and then placed in a preheated water bath at 50 °C for 2 h. After a clear solution was obtained, the temperature of the gellan sample was reduced to 50 °C and polydextrose solution carefully added so that no bubbles formed. Following preliminary optimisation and adaptation from the enzyme assay procedure described by Sigma, volumes of substrate (1.2 mL), enzyme (0.1 mL) along with glutathione (0.2 mL) were added to gellan/polydextrose mixtures and small amounts of water was evaporated to bring the final concentration to 85% solids.

2.3. Methods

Small deformation dynamic oscillation: To characterise the development of linear/non-linear viscoelastic parameters as a function of temperature, the technique of small amplitude oscillation on shear was chosen. The data for the elastic (G') and viscous (G'') components of the network can be derived by the technique without destroying the structure of the matrix and for this a controlled strain rheometer (ARG-2) with magnetic thrust bearing technology (TA Instruments, New Castle, DE) was utilised. An environmental test chamber (ETC) and liquid nitrogen were used to study the samples (85% w/w). which were loaded onto the preheated 5 mm measuring geometry of the rheometer at either 75 °C (deacylated gellan and polydextrose) or 25 °C (polydextrose) and covered with silicone oil (50 cS from BDH) to prevent moisture loss.

A temperature ramp was then implemented to sub-zero temperatures of −34 and −42 °C, respectively, for the mixture and co-solute alone at a scan rate of 1 °C/min, frequency of 1 rad/sec and 0.01% strain, which was within the linear viscoelastic region. At the completion of cooling runs, frequency sweeps were recorded in the range of 0.1–100 rad/s with 4 °C temperature intervals to obtain a series of data for theoretical modelling. Overall, the experimental temperature range was able to cover molecular motions covering the glassy state, glass transition region and the flow region. For each experimental preparation, two replicates were analysed with the flow-to-glass transition region being readily reproducible as a function of temperature or time scale of measurement.

Calorimetric measurements: Samples consisting of gellan with polydextrose or polydextrose alone were hermetically sealed in T_{zero} pans and subjected to modulated differential scanning calorimetry (MDSC) measurements (DSC Q2000, TA instruments, New Castle, DE). A refrigerated cooling system attached to the calorimeter enabled achievement of temperatures down to −90 °C. The heat flow signals were calibrated by the use of a traceable indium standard (ΔH_f = 28.3 J/g) and the heat capacity response using a sapphire standard. Samples (8 mg) were heated to 97 °C, cooled to −90 °C and reheated to 95 °C, at a modulation amplitude of 0.53 °C for each period of 40 s. An empty T_{zero} pan was taken as the reference, with nitrogen purge gas at a flow rate of 50 mL/min and systems were scanned at 1 °C/min. Results reported are of individual traces selected as representative of three replicates, which were effectively superposing traces.

Fourier transform infrared spectroscopy (*FTIR*): In order to identify the nature of interactions between the two constituents, FTIR spectra of gellan and polydextrose preparations were obtained using Absorbance mode on a Perkin Elmer Spectrum 100 spectrometer, equipped with MIRacle^{TM} ZnSe single reflection ATR plate (Perkin-Elmer, Norwalk, CT). The wavelength range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1} was investigated and this was corrected against the background spectrum of the solvent at ambient temperature before plotting.

Wide angle X-ray diffraction (*WAXD*): The diffractograms of gellan and polydextrose alone and their mixtures were obtained using a Bruker D_4 Endeavour (Karlsruhe, Germany). Freeze dried samples were placed on the sample magazine and scanned under accelerating voltage and current of 40 kV and 40 mA, respectively, to obtain the raw data in a 2θ range between 5 and 90° in measuring intervals of 0.1°. The Bruker Advanced X-ray Solutions software, DIFFRAC^{plus} Evaluation (Eva), version 10.0 revision 1 was used to analyse the data and then plot the diffractograms.

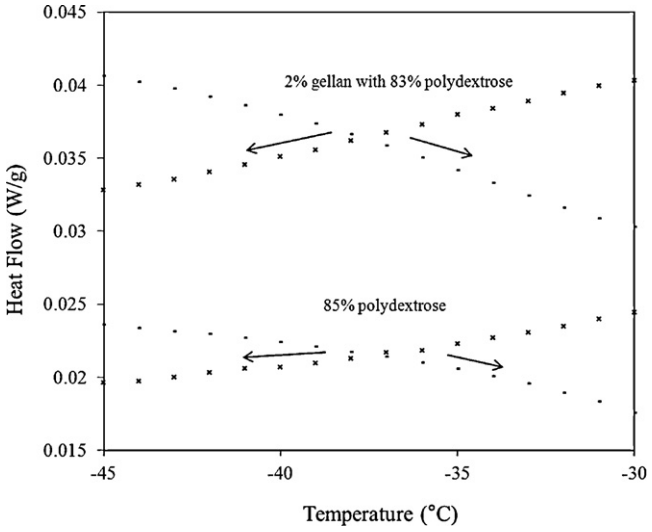

Fig. 1. DSC cooling (—) and heating (×) profiles for 2% gellan with 83% polydextrose, and 85% polydextrose at subzero temperature (scan rate: 1 °C/min).

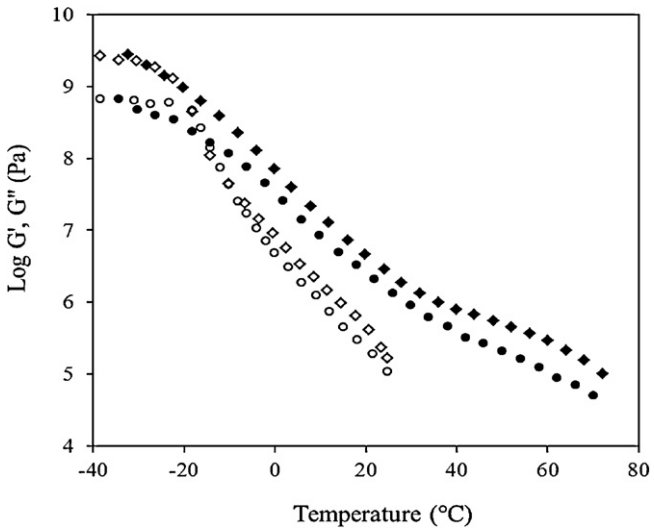

Fig. 2. Cooling profiles of storage and loss modulus for 2% gellan with 83% polydextrose (●, ♦), and 85% polydextrose (○, ◊) scanned at 1 °C/min (frequency: 1 rad/s; strain: 0.01%).

Scanning electron microscopy (*SEM*): Micrographs of gellan and polydextrose matrices were obtained using FEI Quanta 200 ESEM (Hillsboro, OR, USA) in order to study the network morphology and phase topology of the samples under investigation. A high-vacuum mode at an accelerating voltage of 30 kV, pressure of 0.54 mbar, spot size 5 and working distance between 9.2 and 11.2 mm was utilised to image freeze-dried and gold-plated preparations.

Estimation of enzyme activity using UV–vis spectroscopy: Polysaccharide and co-solute matrices containing the enzyme-substrate complex were immediately cooled to the required temperature in an ARB fridge-freezer (temperature range of −18 to 10 °C). Temperature was authenticated by the use of a thermocouple. Samples were individually packed using aliquots of 0.1 g in small containers. These were then equilibrated for an hour before inactivating enzymes by addition of 8 ml of 100 mM sodium carbonate solution and vortexing vigourously for 1 min. Absorbance was recorded at 400 nm on a Lambda 35 UV-vis spectrophotometer (Perkin-Elmer, Singapore). Measurements of enzyme activity at each experimental temperature were carried out in triplicate and average values are reported in terms of nanokatals per gram of the matrix.

3. Results and discussion

3.1. Estimation of the glass transition temperature using MDSC

The molecular mechanisms involved in different relaxations including glass transition are commonly investigated by the use of MDSC as it is a versatile and sensitive technique in providing the total heat flow along with the reversible (kinetic) and non-reversible (heat capacity) components (Lopez, Champion, Blond, & Le Meste, 2005; Rabel, Jona, & Maurin, 1999). In the present study, the technique allowed us to observe the phenomenon of glass transition, when the condensed systems of gellan with polydextrose, and polydextrose alone (total solid concentration in both cases of 85%, w/w) were subjected to heating and cooling cycles at the controlled scan rate of 1 °C/min (Fig. 1).

During cooling, the heat capacity and volume of the gel decreases, as a result of reduced thermal motion and molecular mobility, so that the calorimetric T_g can be derived as the central point of the onset and endset of the heat capacity change. This was observed at approximately -38 °C for both polydextrose and its mixture with gellan. The similar values of T_g for both samples confirm the suggestion of Goff, Caldwell, and Stanley (1993) and Roos (1993) that the thermal spectrum of proteins or polysaccharides in the presence of a high sugar environment is dominated by the vitrification of the latter. It appears, therefore, that in mixtures of low polysaccharide and high sugar, the former merely acts as "cross contamination" and the calorimetric T_g can be predicted by the total level of solids in these systems.

3.2. Characterisation of viscoelastic behaviour in single and mixed systems of polydextrose and deacylated gellan

Calorimetry in the preceding paragraph elucidated micromolecular aspects of vitrification phenomena. The purpose in this section is to investigate the macromolecular effects of addition of 2% deacylated gellan (14 mM $CaCl_2$) to a concentrated (83%, w/w) polydextrose preparation. This approach has been adapted from the "synthetic polymer approach", which commonly follows the viscoelastic properties of three dimensional structures by performing small deformation dynamic oscillation or stress relaxation experiments. Fig. 2 depicts the variation of storage (G') and loss (G'') modulus of thermally reversible mechanical profiles for condensed single polydextrose samples and polydextrose/gellan mixtures at a total solids level of 85% (w/w).

There is a strong thermal effect on the behaviour of both systems over the temperature range of 75 to −40 °C resulting in three distinct regions, i.e. the rubbery plateau for the binary mixture, followed by the glass transition and glassy state for both systems. The storage modulus develops five orders of magnitude from 10^5 Pa in the rubbery plateau at the beginning of the cooling run to about $10^{9.5}$ Pa at the end of the cooling run for the mixture. Mechanical traces are displaced to higher temperatures, an outcome which argues that the process of vitrification is rapid, as compared to single polydextrose preparations. The latter exhibit a crossover of the two modulus traces at about −20 °C, being indicative of the onset of the glassy state, which is considered as an empirical index of the mechanical glass transition temperature.

It appears that the values of mechanical T_g are distinct from those recorded in Fig. 1 from calorimetry observations. The latter are consistent with recorded estimates for small molecule polyhydroxyl compounds in condensed matrices (Biliaderis, Lazaridou, & Arvanitoyannis, 1999; Kalichevsjy, Jaroskiewicz, Ablett, Blanshard, & Lillford, 1992; Slade & Levine, 1991). Differences in the predictions between the two techniques could be due to several factors

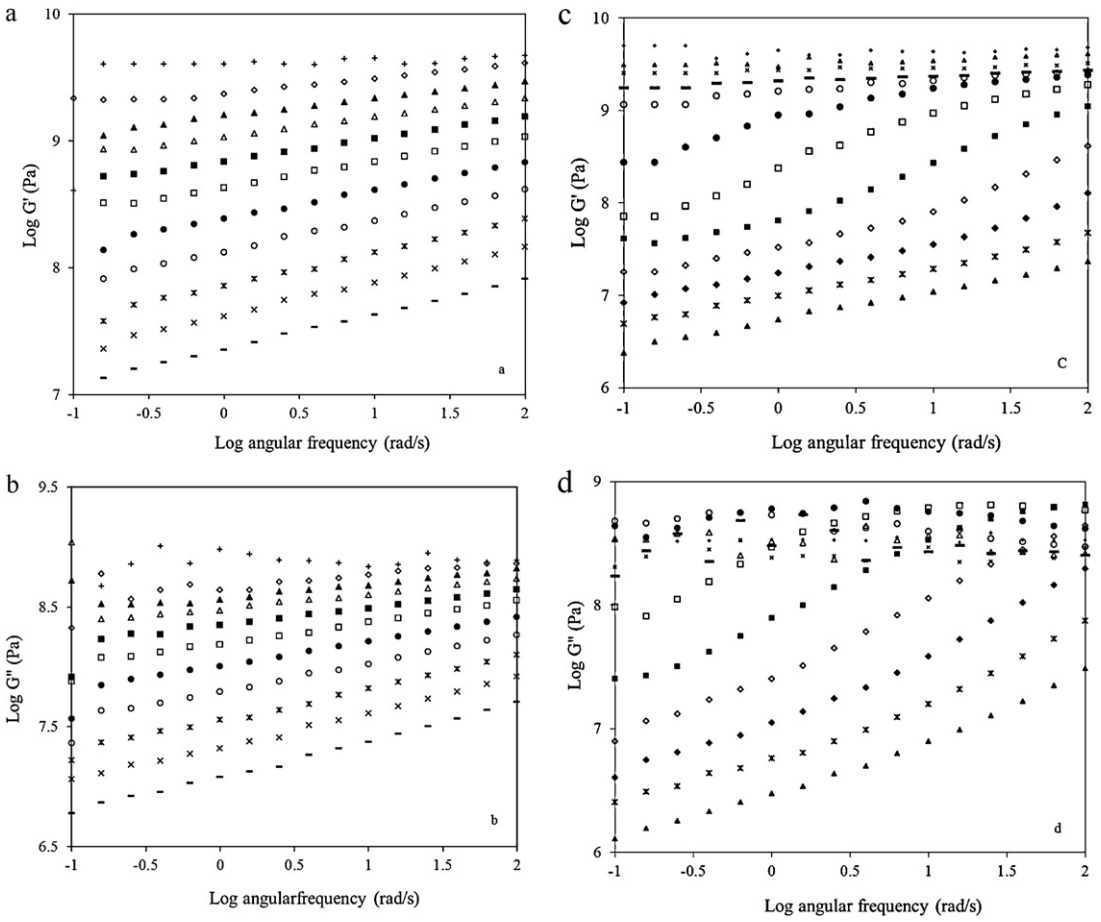

Fig. 3. Frequency variation of G' (a) and G'' (b) for 2% gellan with 83% polydextrose, the lowest curve is taken at 6 °C (−), other curves successively upwards 2 °C (×), −2 °C (*), −6 °C (○), −10 °C (●), −14 °C (□), −18 °C (■), −22 °C (Δ), −26 °C (▲), −30 °C (◊), −34 °C (+), and frequency variation of G' (c) and G'' (d) for 85% polydextrose, the lowest curve is taken at 4 °C (▲), other curves successively upwards 0 °C (*), −4 °C (♦), −8 °C (◊), −12 °C (■), −16 °C (□), −20 °C (●), −24 °C (○), −28 °C (−), −32 °C (×), −36 °C (Δ), −40 °C (+).

including those induced by the analysis of experimental data (e.g. definition of T_g in the DSC curve), choice of parameters in mechanical tests and, significantly, coupling of distinct structural units with particular relaxation times in the two modes of experimentation.

3.3. Quantitative exploration of the structural properties of gellan at high levels of polydextrose

To provide a means of identification of the molecular dynamics involved in secondary transitions, we evolved mechanistic explanations via the time-temperature superposition principle (TTS). Glass formation is such transformation taking place from a solid- to a liquid-like consistency, where there is a change in 'state' but not in "phase". In the present work, TTS was utilised in Fig. 3(a–d) by recording a series of mechanical spectra within the accessible frequency range of 0.1–100 rad/s for both gellan with polydextrose and polydextrose alone. These were taken at a constant temperature interval of 4 °C upon heating the binary gel from −34 to 6 °C and from −40 to 4 °C for polydextrose. As observed from the data at low temperatures, mechanical spectra remain relatively flat, but transform into a steep drop in modulus with increasing frequency at high temperatures (e.g. −36 and 0 °C, respectively in Fig. 3d).

Next, an arbitrary reference temperature was selected within the glass transition region (T_0 = −10 and −16 °C, respectively for binary and single preparations) and remaining mechanical spectra were shifted horizontally along the log frequency axis. This provides a relationship between viscoelasticity and reference temperature of the heating or cooling run as long as the frequency of the former is multiplied by a shift factor, a_T. For TTS to be applicable, it is critical that the mechanical spectra of G' and G'' generate the same factors a_T and superimpose thoroughly, otherwise modelling should be rejected.

3.4. Theoretical modelling to interpret the mechanical data

Mechanical spectra recorded for both materials could be readily superposed in Fig. 4a. Superposition yields composite (or master) curves of viscoelasticity spanning sixteen decades of frequency from 10^{-4} to 10^{11} rad/s in Fig. 4a. This is the analogue of the temperature profile discussed in Fig. 2 by reproducing the transition zone and glassy state. Generated shift factors were first modelled with the Arrhenius rate law that describes the temperature dependence of molecular parameters in various chemical and physical reactions (Hrma, 2008). The reaction rate is proportional to $\exp(E_a/RT)$, where E_a is the activation energy of the molecular process and is expressed for a set of two different temperatures, as follows:

$$\log\ a_T = \frac{E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \quad (1)$$

This type of analysis generates a straight line, with the gradient reflecting the activation energy of relaxation processes. As illustrated in Fig. 4b, good linear fits are obtained for the low temperature ranges, which according to Fig. 2 cover the glassy state.

Deviation from Arrhenius predictions is noted at higher temperatures, where rationalisation of molecular events was attempted

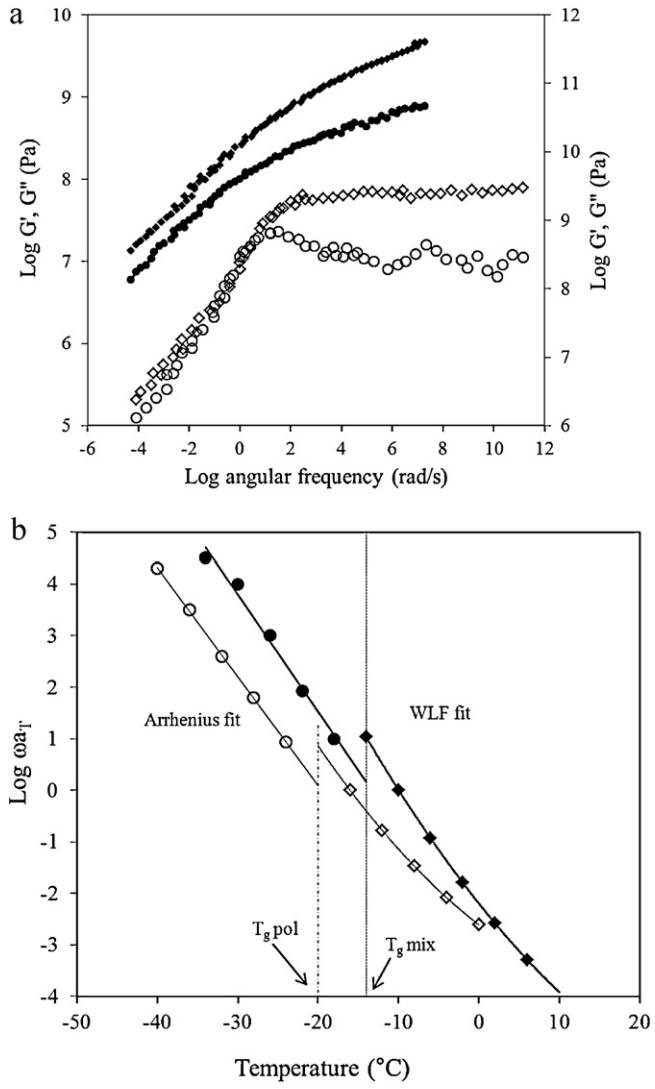

Fig. 4. (a) Master curve of gellan/polydextrose, at the reference temperature of −10 °C, for the reduced storage and loss modulus (●, ♦) as a function of reduced frequency of oscillation along with the master curve of 85% polydextrose (○, ◊) at the reference temperature of −16 °C, and (b) temperature variation of the factor a_T within the glass transition region (●) and the glassy state (■) for gellan/polydextrose and the single polydextrose preparation (○, □), with the solid lines reflecting the WLF and modified Arrhenius fits of the shift factors throughout the vitrification regime and dashed lines pinpointing the T_g predictions (all data from the frequency sweeps of the preparations in Fig. 3).

using the concept of molecular free volume. This approach assumes that the space available to a group of molecules is the total volume actually taken by the molecules (sum of volume due to the molecular oscillations and van der Waals radii) plus large scale vibrations (Meinders & van Vliet, 2009). The mathematical expression of free volume theory is given by Williams, Landel and Ferry (WLF equation) in the following (Ferry, 1980):

$$\log\ a_T = -\frac{C_1^0 (T - T_0)}{C_2^0 + T - T_0} \tag{2}$$

where, C_1^0 and C_2^0 are the WLF constants at T_0 and relate to the free volume theory as follows:

$$C_1^0 = \frac{B}{2.303 f_0} \quad \text{and} \quad C_2^0 = \frac{f_0}{\alpha_f} \tag{3}$$

where, f_0 is the fractional free volume (the ratio of free to total volume per gram of material), α_f is the thermal expansion coefficient and B is usually taken as one for simplicity.

Utilisation of the combined WLF/free volume framework yields good fits for the temperature dependence of shift factors at the upper range of temperatures, which from Fig. 2 correspond to the glass transition region of our samples. This threshold of behaviour where large configurational adjustments contributing to changes in free volume are superseded by a barrier to rotation with a constant activation energy can be considered as the mechanical T_g (−20 and −14 °C for polydextrose and its mixture with gellan, respectively).

Eq. (2) and (3) permit calculation of the fractional free volume and thermal expansion coefficient at T_g being 0.021 and 3.88×10^{-4} deg^{-1} for the mixture, and 0.039 and 7.22×10^{-4} deg^{-1} for polydextrose. Values are within the ranges reported earlier for amorphous synthetic polymers and diluted systems (Tsui, Paraskos, Torun, Swager, & Thomas, 2006). Experimental observations in Fig. 2 are consistent with theoretical modelling in Fig. 4 arguing that mechanical profiles in the rubber-to-glass transition are strongly influenced by the network forming polysaccharide. The magnitude of the gellan contribution to rheological properties is thus represented by the concept of mechanical (or "network") T_g being distinct from the corresponding T_g values for polydextrose obtained rheologically and by calorimetry.

3.5. Phase morphology and interactions in gellan/polydextrose mixtures

Scanning electron microscopy (SEM) was used to obtain images in Fig. 5a and b. Gellan in aqueous media forms uniformly spread aggregates of a helical configuration that can be readily visualised as thick aggregates protruding from the featureless background. These structures are not readily identifiable upon addition of 83% polydextrose, an outcome which is accompanied by the evolution of a distinct amorphous structure. Similar phase morphology has been reported in micrographs of gellan with sugars obtained using transmission electron microscopy (Kasapis, 2006; Kasapis, Abeysekera, Atkin, Deszczynski, & Mitchell, 2002). Tangible evidence from microscopy supports the case of amorphicity in gellan/polydextrose samples put forward via thermomechanical analysis and theoretical modelling in the preceding sections.

To look for possible interactions between the two constituents of our mixture, Fourier transform infrared spectroscopy (FTIR) was employed. Infrared spectra obtained are depicted in Fig. 6a. Results are consistent with those reported by Mickova, Copikova, and Synytsya (2007), and Sudhamani, Prasad, and Sankar (2003) for polydextrose and gellan, reporting a variety of molecular events that correspond to specific chemical linkages within each material: O—H stretching (3500 cm^{-1}), C—H stretching (2900 cm^{-1}), C—O stretching of aldehyde (1627 cm^{-1}) and stretching vibration of the COC glycosidic linkage (1180–930 cm^{-1}) for polydextrose. A significant absorption peak at wave numbers of approximately 3463 cm^{-1} for gellan is attributed to a major OH group signal. In conclusion, these profiles do not provide evidence of chemical interactions between the two components of the mixture under the present experimental conditions.

Wide angle X-ray diffraction (WAXD) was also employed and diffractograms obtained are presented in Fig. 6b. According to Payne, McCormick, and Francis (1999), a broad peak recorded at 22° with shouldering until 50° for the polydextrose and gellan/polydextrose samples (as observed currently) is the fingerprint of amorphous materials, with their characteristic dense morphology resulting from the processing conditions of freeze drying used in the preparation of these samples. Therefore, the experimental observations of rheology, calorimetry and the working framework of glass transition theory employed in this investigation are

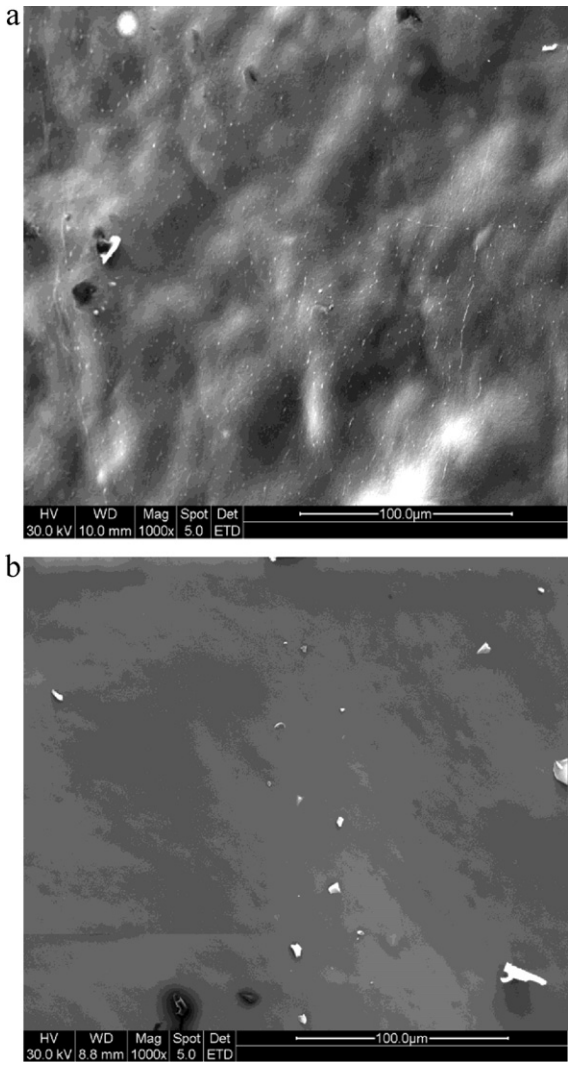

Fig. 5. Micrographs for (a) 2% gellan and (b) 2% gellan with 83% polydextrose (1000× magnification).

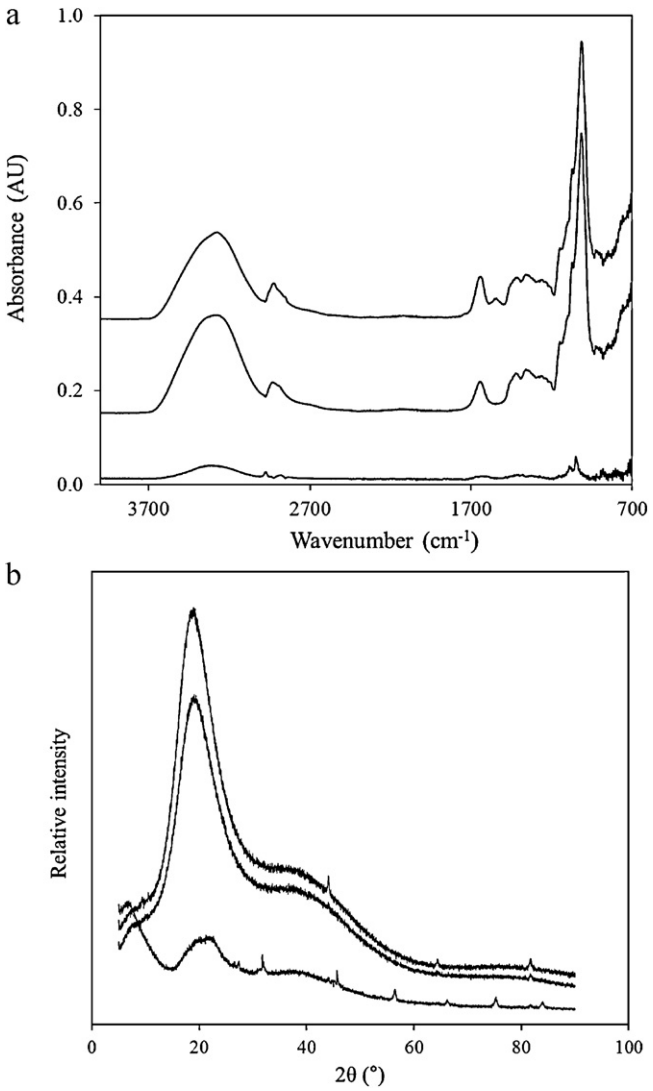

Fig. 6. (a) FTIR spectra and (b) X-ray diffractograms, the lowest curve is for 2% gellan and others successively upwards are for 2% gellan with 83% polydextrose, and 85% polydextrose alone.

further supported by the absence of sharp peaks in the diffractogram confirming that the constituents and mixture of this investigation being amorphous.

3.6. The activity of enzyme embedded within the glassy carbohydrate matrices

In order to facilitate the utilisation of the techno- and biofunctional benefits of gellan and polydextrose, as reviewed, a novel series of experiments was designed to evaluate the possibility of using these as entrapping agents for enzymes while seeking to broaden our understanding of the behaviour of an enzyme within the matrix in the vicinity of the glass transition temperature. Following the characterisation of the gellan/polydextrose matrix, the enzyme α-D-glucosidase was chosen and its activity upon the substrate *p*NPG using a UV-vis spectrophotometer was monitored in order to allow measurements within the matrix held at various temperatures.

For this, a series of preliminary experiments was used to modify and adapt a "spectrophotometric stop rate determination method" described by Sigma Chemical Co. (2012) to suit our project aims and conditions. The critical conditions of temperature (≤50 °C), time of preparation (approximately 45 min) and pH (6.8) were rigorously maintained to assist in the assay

Table 1
Absorbance recorded for the polydextrose matrix and in combination with gellan.

85% Polydextrose						
Time (min)	Temperature (°C)					
	−8	−10	−12	−14	−16	−18
60	0.476	0.441	0.453	0.461	0.442	0.429
60	0.415	0.438	0.440	0.451	0.440	0.421
60	0.462	0.456	0.441	0.424	0.441	0.419
Average	0.451	0.445	0.444	0.445	0.441	0.423
SD	0.031	0.009	0.007	0.019	0.001	0.005
2% Gellan with 83% polydextrose						
Time (min)	Temperature (°C)					
	−8	−10	−12	−14	−16	−18
60	0.639	0.637	0.438	0.373	0.369	0.344
60	0.658	0.589	0.439	0.352	0.333	0.304
60	0.652	0.575	0.440	0.325	0.344	0.314
Average	0.650	0.600	0.439	0.350	0.349	0.321
SD	0.009	0.032	0.001	0.024	0.018	0.020

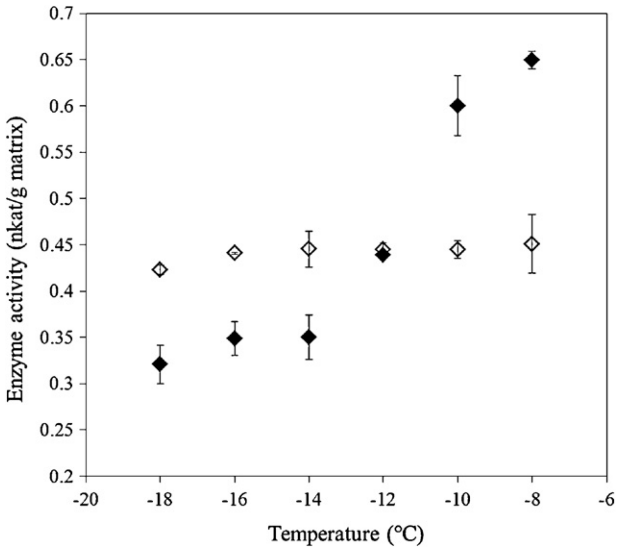

Fig. 7. The influence of temperature on the activity of α-D-glucosidase in a matrix of 2% gellan with 83% polydextrose (♦) in comparison to 85% polydextrose (◊). Enzyme activity is expressed in terms of nanokatal per gram of the matrix.

of α-D-glucosidase. A clear trend was observed for the release of the hydrolysis product recorded after 60 min of equilibration, which was sufficient to control the conditions of low moisture and low temperatures to which the matrix and enzyme were subjected (Table 1). The enzymatic activity expressed in terms of nanokatals/gram of the matrices was calculated from the average values after 60 min of observation from data in Table 1 using Eq. (4) which is based upon that described by Sigma:

$$\text{Activity (nanokatals/g)} = \frac{(\Delta A_{400})(8)(10^6)}{(18.3)(10^3)(3600)(0.1)} \quad (4)$$

where, ΔA_{400} is the change in absorbance during incubation at the particular temperature, 8 is the volume of $NaCO_3$ used (mL), 10^6 converts milli to nanomoles, 18.3 is the millimolar extinction coefficient of *p*-nitrophenol at 400 nm, 10^3 expresses volumes in units of litre, 3600 is the time of observation (s), and 0.1 is the amount of the matrix taken for analysis (g).

Results presented in Fig. 7 depict the effect of the gellan polysaccharide on the ability of α-D-glucosidase to act on its substrate (*p*NPG) in the vicinity of the T_g value established in earlier phases of this study (−14 °C). Little effect of temperature on activity was observed below T_g, contrasting with the trend at increasing temperatures above this index. In the case of the matrix consisting of only polydextrose, the enzyme activity curve remains relatively flat throughout the range of temperatures trialled. It is noted that the T_g of the polydextrose system (−20 °C) is well below the experimentally accessible temperatures for the enzymatic assay investigated here. This indicates that the gellan polysaccharide by accelerating the vitrification process of the matrix considerably affects enzymatic phenomena in these systems.

Recent reports on the effect of gelling polysaccharides on bioactive components and their mobility have described the diffusion of caffeine in high solid matrices of glucose syrup and glucose syrup/κ-carrageenan (Jiang & Kasapis, 2011; Kasapis & Shrinivas, 2010). The current findings confirm the previous observations emphasising the role of network glass transition temperature in diffusional processes of bioactive compounds and proteins. Whilst it has long been known that enzymes have limited activity within low moisture systems, this is a systematic study of the effects of temperature on the activity of an enzyme in the vicinity of the glass transition temperature that has been estimated using theoretical modelling. Therefore the mechanical glass transition temperature has the potential to be applied in the control not only of the mobility of bioactive compounds but also of enzymatic activities that might influence product attributes. There are direct implications for quality control from this work in the processing of biomaterials in addition to traditional considerations based on T_g values established using calorimetric techniques.

4. Conclusions

Physicochemical characterisation in this study demonstrates that a dilution of the gellan helix, visible in the aqueous state, occurs when high amounts of polydextrose are added to preparations. There are no chemical interactions between the two constituents in the mixture, which is amorphous in nature and converts upon cooling from the rubbery to the glassy consistency. Further examination of the binary system argues that the calorimetric glass transition temperature depends on the high concentration of the co-solute, on the other hand, the presence of gellan influences the mechanical T_g. This particular polysaccharide/co-solute arrangement has a pronounced effect on substrate mobility and the hydrolytic effect of the enzyme, α-D-glucosidase. Results indicate that the mechanical T_g should be considered and utilised as an effective tool in the quality control and development of novel formulations with desirable structural properties and biofunctionality.

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References

Biliaderis, C. G., Lazaridou, A., & Arvanitoyannis, I. (1999). Glass transition and physical properties of polyol-plasticiszed pullulan-starch blends at low moisture. *Carbohydrate Polymers*, *40*, 29–47.

Burin, L., Buera, M. P., Hough, G., & Chirife, J. (2002). Thermal resistance of β-galactosidase in dehydrated dairy model systems as affected by physical and chemical changes. *Food Chemistry*, *76*, 423–430.

Cardona, S., Schebor, C., Buera, M. P., Karel, M., & Chirife, J. (1997). The thermal stability of invertase in reduced-moisture amorphous matrices in relation to glassy state of trehalose. *Journal of Food Science*, *62*, 105–112.

Carlfors, J., Edsman, K., Petersson, R., & Jörnving, K. (1998). Rheological evaluation of Gelrite® in situ gels for opthalmic use. *European Journal of Pharmaceutical Sciences*, *6*, 113–119.

Drapon, R. (1985). Enzyme activity as a function of water activity. In D. Simatos (Ed.), *Properties of water in foods in relation to quality and stability* (pp. 171–189). Lancaster: Nighoff, M. Publishers.

Ferry, J. D. (1980). *Viscoelastic properties of polymers*. New York: John Wiley and Sons., pp. 264–320.

Giavasis, I., Harvey, L. M., & McNeil, B. (2000). Gellan gum. *Critical Reviews in Biotechnology*, *20*, 177–211.

Goff, H. D., Caldwell, K. B., & Stanley, D. W. J. (1993). The influence of polysaccharides on the glass transition in frozen sucrose solutions and ice cream. *Journal of Dairy Science*, *76*, 1268.

Hrma, P. (2008). Arrhenius model for high-temperature glass-viscosity with a constant pre-exponential factor. *Journal of Non-Crystalline Solids*, *354*, 1962–1968.

Jiang, B., & Kasapis, S. (2011). Kinetics of a bioactive compound (caffeine) mobility at the vicinity of the mechanical glass transition temperature induced by gelling polysaccharide. *Journal of Agricultural and Food Chemistry*, *59*, 11825–11832.

Kalichevsjy, M. T., Jaroskiewicz, E. M., Ablett, S., Blanshard, J. M. V., & Lillford, P. J. (1992). The glass transition of amylopectin measured by DSC, DMTA and NMR. *Carbohydrate Polymers*, *18*, 77–88.

Kasapis, S. (2006). The morphology of the gellan network in a high-sugar environment. *Food Hydrocolloids*, *20*, 132–136.

Kasapis, S., & Shrinivas, P. (2010). Combined use of thermomechanics and UV spectroscopy to rationalize the kinetics of bioactive compound (Caffeine) mobility in a high solids matrix. *Journal of Agricultural and Food Chemistry*, *58*, 3825–3832.

Kasapis, S., Abeysekera, R., Atkin, N., Deszczynski, M., & Mitchell, J. R. (2002). Tangible evidence of the transformation from enthalpic to entropic gellan networks at high levels of co-solute. *Carbohydrate Polymers*, *50*, 259–262.

Kelco. (2004). Biopolymers. In *Gellan gum gel (G^3) a new self-lubricated, easy to swallow, oral dosage form*. (rev 10/99).

Le Meste, M. (1995). Mobility of small molecules in low and intermediate moisture foods. In G. Barbosa-Cànovas (Ed.), *Food preservation by moisture control; fundamental and applications*. USA: Technomic, pp. 210–223.

Lopez, E. C., Champion, D., Blond, G., & Le Meste, M. (2005). Influence of dextran, pullulan and gum Arabic on the physical properties of frozen sucrose solutions. *Carbohydrate Polymers*, *59*, 83–91.

Mazzobre, M. F., Buera, M. P., & Chirife, J. (1997a). Protective role of trehalose on stability of lactase in relation to its glass and crystal forming properties and effect of delaying crystallization. *Lebensmittel-Wissenschaft & Technologie*, *30*, 324–329.

Mazzobre, M. F., Buera, M. P., & Chirife, J. (1997b). Glass transition and thermal stability of lactase in low-moisture amorphous polymeric matrices. *Biotechnology Progress*, *13*, 195–199.

Meinders, M. B. J., & van Vliet, T. (2009). Modeling water sorption dynamics of cellular solid food systems using free volume theory. *Food Hydrocolloids*, *23*, 2234–2242.

Mickova, K., Copikova, J., & Synytsya, A. (2007). Determination of polydextrose as a fat replacer in butter. *Czech Journal of Food Science*, *25*, 25–31.

Payne, J. A., McCormick, A. V., & Francis, L. F. (1999). A study of stress development in aqueous gelatin coatings. *Journal of Applied Polymer Science*, *73*, 553–561.

Rabel, S. R., Jona, J. A., & Maurin, M. B. (1999). Applications of modulated differential scanning calorimetry in performulations studies. *Journal of Pharmaceutical and Biomedical Analysis*, *21*, 339–345.

Roos, Y. H. (1993). Melting and glass transition of low molecular weight carbohydrates. *Carbohydrate Research*, *238*, 39–48.

Roos, Y. H. (1995). *Phase transitions in foods*. San Diego: Academic Press., p. 360.

Roos, Y. H. (1998). Phase transition and structure of solid food matrices. *Current Opinion on Colloid and Interface Science*, *3*, 651–656.

Roos, Y. H., & Karel, M. (1991). Phase transitions of mixtures of amorphous polysaccharides and sugars. *Biotechnology*, 49–53.

Rozier, A., Mazuel, C., Grove, J., & Plazonnet, B. (1989). Gelrite®: A novel, ion activated, in situ gelling polymer for ophthalmic vehicles. *Effect on bioavailability of timolol. International Journal of Pharmaceutics*, *57*, 163–168.

Schebor, C., Buera, M. P., & Chirife, J. (1996). Glassy state in relation to thermal inactivation of the nezyme Invertase in amorphous dried matrices of trehalose, maltodextrin and PVP. *Journal of Food Engineering*, *30*, 269–282.

Sigma Chemical Co. (2012). Enzymatic assay of α-D-glucosidase (EC 3.2.1.20), Available from: http://www.sigmaaldrich.com/catalog/search?interface=All&term=SSNITR01&lang=en&region=AU&focus=documents&N=0+220003049+219853060+219853286&mode=match%20partialmax Accessed 21.04.12.

Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, *30*, 115–360.

Sperling, L. H. (2006). *Introduction to physical polymer science* (4th ed.). New York: Wiley, J. and Sons. (chapter 8)

Stowell, J. D. (2009). Polydextrose. In S. S. Cho, & P. Samuel (Eds.), *Fibre ingredients* (pp. 173–201). London: CRC Press.

Sudhamani, S. R., Prasad, M. S., & Sankar, U. K. (2003). DSC and FTIR studies on gellan and polyvinyl alcohol (PVA) blend films. *Food Hydrocolloids*, *17*, 245–250.

Tsui, N. T., Paraskos, A. J., Torun, L., Swager, T. M., & Thomas, E. L. (2006). Minimization of internal molecular free volume: A mechanism for the simultaneous enhancement of polymer stiffness, strength, and ductility. *Macromolecules*, *39*, 3350–3358.