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Effect of thermal denaturation on the mechanical glass transition temperature of globular protein/co-solute systems

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ABSTRACT

The work prepared high-solid mixtures of whey protein or bovine serum albumin with an amorphous co-solute (glucose syrup) and examined their glass transition behaviour at subzero temperatures. The interest in these condensed matrices was in relation to what extent thermal denaturation and subsequent aggregation of the proteinaceous molecules affects vitrification and, therefore, they were subjected to distinct heating regimes followed by cooling. Small-deformation dynamic oscillation in shear is known to respond to changes in network formation as a function of thermal treatment, albeit published reports thus far focused on low-solid aqueous hydrocolloid samples, and it has been chosen presently to examine the viscoelasticity of their high-solid counterparts. Results were further compared with those from a micromolecular technique, i.e. modulated differential scanning calorimetry. It appears that thermally induced cross-linking is readily recorded in what is known in the literature as the mechanical or network glass transition temperature, whereas the calorimetric T_g is not affected by the extent of polymeric associations in these mixtures. Further, the thermal protocol employed presently results in considerable differences in predictions of the mechanical T_g, which should reflect distinct three dimensional morphologies in these systems of globular protein and co-solute.

1. Introduction

Glass transition can be described as the reversible transformation of an amorphous material from liquid to glassy consistency that exhibits reduced molecular mobility (Badii, MacNaughtan, & Farhat, 2005). Typically, a glassy matrix is characterised by high viscosity of about 10^{12} Pa s, which means that residual flow is *via* an extremely low diffusion rate (Slade & Levine, 1991). Vitrification is a kinetically arrested process depending on the type of solute and external factors including temperature, mechanical stress, hydrostatic pressure or timescale of measurement (Goff, 1994). Characteristic properties of a material undergoing glass transition are free energy, change in volume and stress/enthalpy relaxation that enable measurements with the techniques of mechanical spectroscopy, differential scanning calorimetry, dilatometry, nuclear magnetic resonance and others (Kasapis, 2008).

In food materials, the concept of glass transition has been considered as an index of physicochemical behaviour that reflects the rates of deteriorative molecular processes (Vrentas & Duda, 1978). It has been argued that the architecture of an amorphous matrix in frozen or dehydrated foods influences their stability as well as organoleptic attributes during processing and storage. This has led to intensive studies to unveil the structure–function relationship in high solid biomaterials ranging from small organic molecules (e.g. sugars) to hydrocolloids and their mixtures (Binder, Baschnagel, & Paul, 2003).

Conventionally, the technique of differential scanning calorimetry in micro or modulated mode has been used to follow the process of vitrification and estimate the glass transition temperature (T_g) of a biomaterial (Mazzobre, Aguilera, & Buera, 2003). This is achieved by measuring the difference in energy inputs into a substance and its reference. In the absence of a robust theoretical framework to assign physical significance to a particular point of the sigmoidal change in heat capacity, i.e. identify a single value of T_g, a range of empirical markers has been utilised to demarcate the onset, middle and completion of change in heat flow. It has also been observed that the calorimetric T_g of sugars, for example, a high-dextrose equivalent glucose syrup, depends on the

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concentration of the bulking agent, with the small, but industrially relevant for product development, additions of gelling protein or polysaccharide acting as a mere cross-contamination (Kasapis & Sablani, 2005).

Recently, the aforementioned gelling hydrocolloid/sugar mixtures have been examined for stress relaxation over the glass transition region using small deformation dynamic oscillation in shear (Kasapis, 2008). Recording viscoelastic properties *via* the storage (G') and loss modulus (G'') and utilising the so-called "time–temperature superposition principle" (TTS) allows for synchronisation of the experimental time and temperature at ranges beyond the limits of current technology or availability of laboratory facilities (Ferry, 1980). Further, the approach has been widely used by material scientists to acquire mechanistic understanding of glass transition phenomena through the concept of free volume. The theory argues that irregular packing creates vacant spaces or holes between adjacent polymeric segments, known as free volume, which facilitate molecular motions. On average, free volume within polymer melts counts for up to 30% of the total volume, and it collapses to about 3% of the total volume during cooling at a particular temperature known as the glass transition temperature (Cangialosi, Schut, van Veen, & Picken, 2003).

Earlier, Jiang, Kasapis, and Kontogiorgos (2011) demonstrated that the molecular weight of solute is one of the governing factors affecting the mechanical glass transition temperature. In doing so, they employed four distinct molecular fractions of gelatin ($M_w = 318$, 284, 229 and 197 kDa) in mixture with glucose syrup as co-solute. Fractions with higher molecular weight exhibited earlier vitrification, as opposed to the low molecular weight counterparts, at a total solid level of 80% in preparations (T_g variation of about sixteen degrees centigrade seen in tan δ maxima). The effect of increasing concentration of single hydrocolloid systems on the glass transition temperature has also been examined as is the rheological pattern of vitrification in binary hydrocolloid mixtures (Altay & Gunasekaran, 2012; Whitehouse, Ashby, Abeysekera, & Robards, 1996). As far as we are aware, this is the first study that explores the relationship between mechanical T_g and extent of cross-linking in the globular protein network as a result of distinct regimes of thermal denaturation.

2. Materials and methods

2.1. Materials

2.1.1. Whey protein isolate (WPI)

The powder used in this investigation was purchased from MG Nutritionals, Murray Goulburn Co-operative Co. Ltd., Vic, Australia. Ingredient composition according to the supplier was 91.3% protein, 0.7% fat, 3.5% moisture, 3.8% ash and 0.44% lactose.

2.1.2. Bovine serum albumin (BSA)

That was a product from Sigma–Aldrich Pty. Ltd., NSW, Australia. It came in the form of a lyophilised powder of albumin from bovine serum with 98% purity and molecular weight of approximately 66 kDa, as observed by electrophoresis.

2.1.3. Glucose syrup

The high viscosity solution was purchased from Cerestar (Manchester, UK) with a dextrose equivalent (DE) of 42. As per information from the supplier, the level of solids was 82% (w/w) and the solution remained amorphous upon cooling to subzero temperatures due to its polydisperse nature. That was confirmed from the relationship between degree of polymerisation and surface area (%) of the glucose syrup spectrum using gel permeation chromatography (Tsoga, Kasapis, & Richardson, 1999). Percentages in preparations of this investigation refer to dry solids (w/w).

2.2. Methods

2.2.1. Sample preparation

High solid systems of whey protein isolate/glucose syrup and bovine serum albumin/glucose syrup were formulated using 15% (w/w) protein with 65% (w/w) co-solute. A total solids content of eighty percent in formulations was used to achieve extensive vitrification at the low cooling rate (1 °C/min) of this study, and it was based on earlier observations that lower levels of solids (<70%) in hydrocolloid/co-solute mixtures yield partially amorphous matrices due to ice formation (Kasapis & Al-Marhoobi, 2005).

Glucose syrup was mixed with 10 mM $CaCl_2$ solution using a magnetic stirrer until a clear solution was obtained. To that, required amounts of WPI or BSA were added in batches at ambient temperature. Protein/co-solute solutions were further stirred for a couple of hours to facilitate dispersion and refrigerated overnight to ensure proper hydration. All samples were analysed using rheology or calorimetry following overnight storage, as detailed below:

2.2.2. Rheology

Three distinct experiments were performed in the rheological part of the work:

i) Protein/co-solute mixtures were heated to 85 °C at 1 °C/min followed by an isothermal period at that temperature for 30 min and a cooling run to subzero temperatures at the same scan rate. This working protocol ensured extensive denaturation and subsequent aggregation of the globular protein molecules.
ii) To obtain a partially denatured protein network, materials were heated to 85 °C, without implementing the isothermal run, and cooled to subzero temperatures at 1 °C/min.
iii) Native samples devoid of any kind of heat treatment were cooled from ambient to subzero temperatures at 1 °C/min.

During these experimental routines, measurements were taken using small deformation dynamic oscillation in shear with a controlled strain rheometer AR-G2 (TA Instruments, New Castle, DE). A parallel plate geometry of 10 mm diameter was engaged, and the edges of samples were covered with silicone oil (50 cS) to minimise moisture loss. A constant frequency of 1 rad/s and strain of 0.01% (i.e. within the linear viscoelastic region) were applied throughout the heating followed-by-cooling routine. Finally, samples were heated once more from subzero temperatures, and frequency sweeps were performed within the experimentally accessible range of 0.1–100 rad/s at a temperature interval of four degree centigrade.

In the discussion of the manuscript, the following abbreviations are used: extensively denatured, partially denatured and native whey protein/co-solute systems are: W1, W2 and W3, respectively. Similarly, extensively denatured, partially denatured and native bovine serum albumin/co-solute systems are: B1, B2 and B3, respectively. All thermal routines (isochronal tests) were complemented by frequency sweeps (isothermal tests) and carried out in duplicate to yield effectively overlapping traces. For each experimental preparation (e.g. W1) there were cooling and heating runs and an addition of eight frequency sweeps bringing the total number of replicates for all samples to one hundred and twenty. This good volume of experimental results allowed valid implementation of the time–temperature superposition principle to derive master curves of viscoelasticity for prediction of glass transition temperatures. Modelling in the glass transition region was