Effect of co-solute concentration on the diffusion of linoleic acid from whey protein matrices

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A B S T R A C T

Structural relaxation of a delivery vehicle has been identified in the literature as a limiting parameter for the controlled diffusion of a bioactive compound. To further examine the kinetics of controlled release, this study designed a system of homogeneously dispersed linoleic acid in a high-solid whey protein matrix. Amorphous glucose-syrup liquid was added systematically up to about 48% (w/w), with the total solids level in the formulation being kept constant at 80% (w/w), and experimental observations were obtained over a broad timeframe and temperature range. Structural and physicochemical properties of the blend were monitored using dynamic oscillation in-shear, WAXD, FTIR and confocal laser scanning microscopy. A specific chromogenic reaction with UV–vis spectroscopy was employed to follow the release of linoleic acid under perfect sink conditions. Variation in the amount of glucose syrup, as the co-solute, within the high-solid whey protein network affected profoundly the values of the mechanical glass transition temperature. That was reflected in the kinetics of fatty acid diffusion from the glass transition region of our composite materials. Transport phenomena were quantified *via* the concept of effective diffusion coefficient, which increases with higher levels of glucose syrup leading to structural relaxation of the delivery vehicle.

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1. Introduction

In the last century, a plethora of studies documented that quenching of organic liquids may lead to a change in consistency, which is known as a vitreous solid or glass. Hardening takes place over a broad temperature range and without a maximum in the variation of heat capacity (Vreeswijk, Gossink, & Stevels, 1974). Experimental observations led us to understand that glass is a supercooled liquid whose thermal conversion is entirely distinct from the crystalline state, but in the nature of a second-order thermodynamic transition (Ngai, 2000). It is well known from differential scanning calorimetry, for example, that the supercooled thermogram of glycerol is characterised by a step-change in heat capacity with temperature at 190 K (Sou, Nishikawa, Koga, & Tozaki, 2011). In the case of hydrocolloids, which are quite poly-disperse, softening of matrices during the process of devitrification occurs over a large temperature interval centred on a mean value called the glass transition temperature, T_g (Binder, Baschnagel, & Paul, 2003). In the glassy state, which is the temperature range below T_g, materials exhibit high strength, brittleness and ultimately low molecular mobility that slows down dramatically physico-chemical and biological processes (Slade & Franks, 2002).

In view of the nature of this work, it is appropriate to mention concisely the approach used extensively by synthetic polymer scientists, with expertise in thermomechanical measurements, to acquire a mechanistic understanding of glass transition phenomena. This is based on the concept of free volume (v_f) referring to the space between packing irregularities of macromolecules, which is required for their string-like vibrations (Mansilla et al., 2013). Free volume is about thirty percent of the total (free plus occupied) volume in polymer melts, and the theory predicts that it collapses to between two and four percent at the glass transition temperature depending on the rate of cooling and the density of the polymeric glass (Cangialosi, Schut, van Veen, & Picken, 2003). At this point, there is a discontinuity in the linear dependence of total volume with temperature leading to a reduction in the value (obtained from the linear gradient) of the thermal expansion coefficient, a_f (Campbell & Vogt, 2007).

The free volume theory in the form of the Williams, Landel and Ferry (WLF) equation found widespread application not only in

synthetic polymers but also in inorganic materials and low molecular weight organic liquids (Wang et al., 2003). This has prompted calls for utilisation of the equation in hydrocolloids where the process of vitrification might also be governed by free volume, i.e. being independent of chemical features (Dlubek, Fretwell, & Alam, 2000). In the case of whey protein, which is the subject matter of this investigation (and BSA), it was found that high-solid preparations undergo a rubber-to-glass transformation upon cooling following thermal denaturation. Values of T_g were pinpointed in the viscoelastic spectrum using the combined WLF/ free volume approach that were affected considerably by the extent of thermal treatment, i.e. the density of intermolecular associations in the protein network (George, Lundin, & Kasapis, 2014). Introduction of small polyhydroxy compounds, e.g. glucose syrup or lactose, to concentrated whey protein systems reduces the glass transition temperature of the polymer due to the plasticizing effect of co-solute (Dissanayake et al., 2013).

Experimentation partnered with theoretical modeling of structural properties has been used as a useful avenue of developing technofunctionality for further explorations in biomaterials of added value. These include the design of a delivery vehicle for biofunctionality, which is a subject of steadily increasing scientific and technical importance in food and nutraceutical industries. Future research and development in these materials will deal with the optimisation of formulations for novel dosage forms of bioactivity. Within this broad area of high future potential, the present communication aims to study the kinetics of diffusional mobility of an essential fatty acid, linoleic acid, in relation to the content of plasticizing glucose syrup in a high-solid matrix of whey protein.

2. Materials and methods

2.1. Materials

2.1.1. Whey protein isolate
The powder was obtained from Fonterra Co-operative Group Ltd (Palmerston North, New Zealand). It contained 90.4% protein (N x 6.38) and 4.7% moisture with the minor addition of carbohydrate, fat and minerals at 0.9, 1.0 and 3.0% (w/w), respectively. As a standard whey protein, it is mainly a mixture of β-lactoglobulin (~65%), α-lactalbumin (~25%), bovine serum albumin (~8%) and immunoglobulins. It passed microbial tests, with the estimated amounts of yeast and mould being less than 10 cfu/g and 10,000 cfu/g, respectively. Physical tests showed a bulk density of 0.34 g/ml for the powder and pH 6.9 of 5% (w/w) solution at 20 °C.

2.1.2. Glucose syrup
It was purchased in a viscous liquid form at ambient temperature from Edlyn Foods Pty Ltd (Melbourne, Australia). The total level of solids is 81% (w/w) and concentrations in this investigation refer to dry solids. About forty-five percent glucose residues is present as reducing end groups contributing to an overall dextrose equivalent, DE, of 42 for the sample. A trace of sodium is detected at about 0.005% (w/w).

2.1.3. Linoleic acid (cis-9,cis-12-octadecadienoic acid)
It was purchased from Sigma Aldrich Co (Sydney, Australia). This fatty acid is a high purity product of ≥98.5%, as confirmed by GC analysis with an average molecular weight of 280.45 g/mol and density of 0.902 g/ml at 25 °C.

2.1.4. Other reagents
Sulfo-phospho-vanillin (SPV) chemicals, i.e. a mixture of vanillin (99.0%), ethyl acetate (≥99.0%), phosphoric acid (85.0%), and sulphuric acid (≥98.0%) met AR standards for use in the chromogenic assay of this work, and were obtained from Sigma Aldrich Co (Sydney, Australia). The same supplier also provided Nile red and acetone (≥99.5%) for lipid staining.

2.2. Sample preparation

High solid mixtures of whey protein/glucose syrup/linoleic acid were formulated using a systematic variation in the ratio of protein to co-solute: 100:0, 80:20, 70:30, 60:40, 40:60 and 0:100 (w/w) to make up 79% (w/w) total solids plus 1.0% (w/w) linoleic acid. In doing so, Milli-Q water was added to glucose syrup at ambient temperature and stirred thoroughly on a magnetic plate until a clear and homogeneous solution was obtained. An appropriate amount of whey protein was included to make up a dispersion of 30% (w/w) solids. That was stirred for 2 h and stored overnight at 4 °C to facilitate hydration and removal of air bubbles. Samples were collected and stirred for 15 min at ambient temperature before adding 1.0% linoleic acid. Tertiary preparations were mixed for another 30 min to enhance homogeneity and subjected to a homogenisation step for 3 min at 3000 rpm with a laboratory homogeniser (Ultra-Turrax T25, IKA-Labortechnik, Staufen, Germany). Finally, they were transferred to the evaporation flask of a rotary evaporator and concentrated up at 40 °C to achieve a level of 80% (w/w) solids. Concentrated matrices of 80% solids without linoleic acid were also prepared as the reference material.

2.3. Experimental analysis

2.3.1. Rheological measurements
These were performed on a controlled strain rheometer (AR-G2 from TA Instruments, New Castle, DE) equipped with a magnetic thrust bearing technology. The instrument was connected to 60 L liquid nitrogen tank to provide purging nitrogen gas for efficient subzero cooling. All samples were loaded onto the parallel plate geometry (10 mm diameter) of a Peltier device at 25 °C. Measuring gap of the two stainless-steel plates was 1000 μm and silicone oil (BDH, 50 cS) was added at the edges to minimize sample moisture loss. Constant cooling rate of 1 °C/min, strain of 0.01% (within the linear viscoelastic region of the material; LVR), normal force at 0.08 N and oscillatory frequency of 1 rad/s during a temperature ramp from 25 to −55 °C were carried out to record the storage (G') and loss (G'') modulus values in-shear. Mechanical spectra were also obtained at regular temperature intervals of 6 °C within the angular frequency range of 0.1–100 rad/s. Experimental work includes triplicate runs that yield effectively overlapping mechanical spectra.

2.3.2. Fourier transform infrared spectroscopy (FTIR)
Spectra were recorded using Perkin Elmer Spectrum 100 FTIR Spectrometer (Norwalk, CT). Condensed samples of 79% whey protein/glucose syrup with 1% linoleic acid at the compositions described earlier, and single whey protein or glucose syrup at 80% solids (reference systems) were examined. They were placed on the surface of MIRacle[TM]ZnSe single reflection ATR plate, scanned from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} and averaged over thirty two scans. Each measurement was performed in triplicate.

2.3.3. Wide angle X-ray diffraction (WAXD)
Freeze-dried tertiary mixtures and single globular protein or co-solute systems at a total solid level of 80% were examined for X-ray diffraction with Bruker D4 Endeavour (Karlsruhe, Germany). Diffraction patterns of bulk samples were subjected to accelerating voltage of 40 kV and current of 40 mA using a position sensitive detector (PSD) within the 2θ range of 5–90° in measuring intervals of 0.1°. Data were converted using DIFFRAC[plus] Evaluation (Eva),
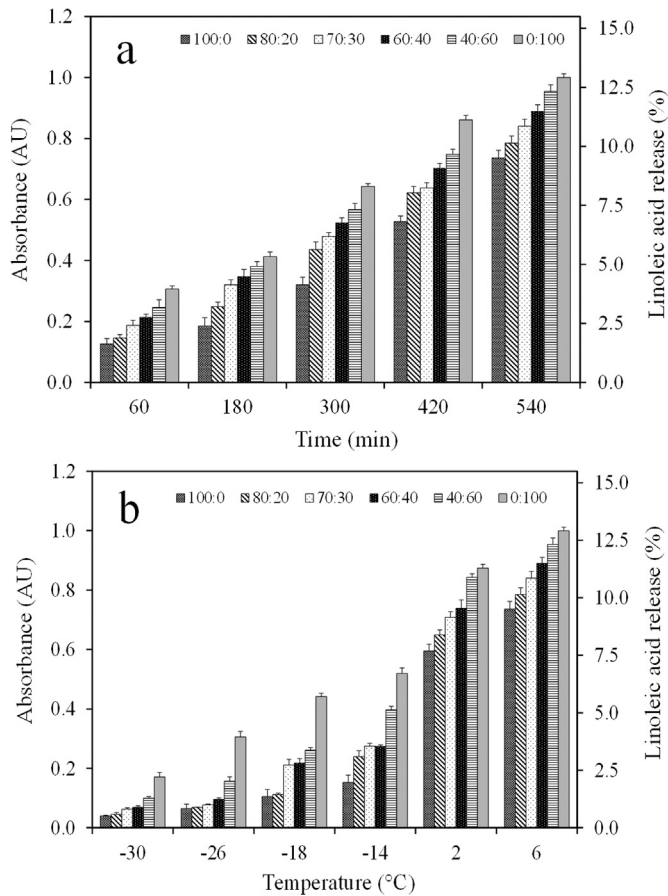
Fig. 7. Absorbance and percentage release of linoleic acid to ethyl acetate as a function of (a) time for mixtures comprising whey protein/glucose syrup at 6 °C as indicated and (b) temperature for the same mixtures after 9 h diffusion.
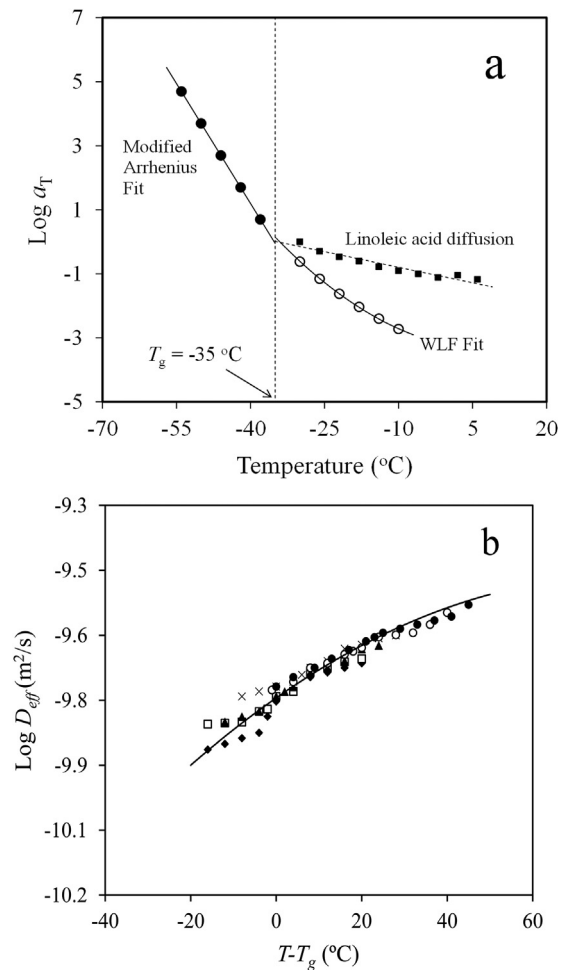

Fig. 8. (a) Spectroscopic shift factors of fatty acid diffusion in comparison to the mechanical shift factors of the whey protein/glucose syrup matrix at 40:60 (w/w) as a function of temperature and (b) Diffusion coefficients of linoleic acid in whey protein/glucose syrup matrices at 100:0 (◆), 80:20 (☐), 70:30 (▲), 60:40 (X), 40:60 (◯) and 0:100 (●) as a function of T-T_g.

released at initial, t and infinite time, respectively; D_{eff} is the effective or apparent diffusion coefficient of the bioactive material that travels only in the L direction (Choi et al., 2005; Peppas & Peppas, 1994; Tutuncu & Labuza, 1996; Wang, Wu, & Lin, 2008).

Fig. 8b illustrates the values of diffusion coefficient as a function of temperature difference from the T_g estimates, i.e. T-T_g, for all samples of this investigation from equation (3). Gratifyingly, a smooth polynomial progression is obtained, especially for temperatures within the glass transition region, i.e. $T > T_g$, leading to higher molecular mobility. This is confirmed in Table 1 where D_{eff} increases from 1.63 to 2.50 x 10^{-10} m^2/s with increasing addition of glucose syrup at −16 °C, which is the highest recorded glass transition temperature and corresponds to the condensed whey protein matrix.

Our results add to the work by Champion, Hervet, Blond, Le Meste, and Simatos (1997) who reported higher fluorescein diffusion in sucrose-water solutions with increasing temperature and moisture content. Contreras-Lopez, Champion, Hervet, Blond, and Le Meste (2000) argued that at least 10% polysaccharide (dextran, gum arabic or pullulan) is needed to have a significant effect on retarding the diffusion of sucrose in aqueous systems depending on the type of polymer. Diffusion of oxygen and various aroma compounds was also examined in relation to the physical ageing and glass transition temperature of natural polymers and edible films (Miller & Krochta, 1997; Tiemblo et al., 2001).

4. Conclusions

The diffusion of linoleic acid molecules from the whey protein/glucose syrup matrix was examined during vitrification of the condensed preparations. Results unveil the effect of rising molecular mobility in composite structures by increasing the level of small-molecule co-solute. It was documented that addition of distinct amounts of glucose syrup plasticised effectively the whey protein network, an outcome which was reflected in reduced values of the glass transition temperature. Generalised Fickian Law was utilised to generate effective diffusion coefficients, which increased smoothly with higher temperatures within the glass transition region of experimental preparations. It appears that the combined framework of glass transition and diffusion theory is robust enough to predict the lipid-to-matrix mobility for a broad temperature range above T_g. Thus, the effect of different levels of plasticiser on the diffusional kinetics of linoleic acid through condensed whey-protein networks was quantified.

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