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The role of structural relaxation in governing the mobility of linoleic acid in condensed whey protein matrices

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ABSTRACT

The classical limiting case of simple diffusion as described by Fick's second law was examined in the transport of a small molecule, linoleic acid, through a condensed polymer matrix, whey protein. Experimental protocol was based on small-deformation dynamic oscillation in-shear, wide angle X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, FTIR micro-spectroscopy imaging, ANS fluorescence spectroscopy, and the sulfo-phospho-vanillin assay. This mass transfer problem for the omega-6 fatty acid was examined in relation to whey protein forming a glassy system with a glass transition temperature, T_g, of −16 °C. Diffusion followed a more complicated pattern than Fick's equation that could be described at temperatures above T_g with the so-called "anomalous transport". The diffusion coefficient of linoleic acid was estimated within the glass transition region and glassy state of the whey protein network delineated with changing environmental temperature. The free-volume theory of transport was then considered to provide a useful vehicle for rationalising molecular motion and, in doing so, we established a generalised relationship between diffusion coefficient of bioactive compound and fractional free volume of polymeric matrix.

1. Introduction

The concept of diffusion that emerged from physical sciences, with a paradigmatic example being the heat induced Brownian motion, is of increasing interest in functional food and nutraceutical manufacturing for the delivery of biofunctionality (Korsmayer & Peppas, 1981). Orally ingested embodiments for health benefit or medicinal use are hardly stable or equilibrium systems yielding "surprising" changes in structural morphology and consistency during storage and consumption. Clearly, advanced-formulation engineering requires that the time dependence of the statistical distribution of bioactive compounds in the three dimensional lattice is accurately followed by a differential diffusion equation (Slade & Levine, 1991). Solutions to this problem should primarily deal with the rate of molecular transport, which is governed by the diffusivity in the surrounding environment and the concentration gradient between adjacent demixed phases.

Small-molecule diffusion is driven by a chemical potential difference in the interfacial area of flat surfaces or decrease in Gibbs free energy, as shown by the classical depiction of a molar free energy diagram where diffusant transport occurs along its concentration gradient (Chantawansri, Yeh, & Hsieh, 2015). It is widely recognised that Adolf Fick in 1855 was the first to elaborate mathematical descriptions through his first law that applies to steady state systems, i.e. where the concentration of diffusant molecules remains constant. A more relevant case in model biological materials and food preparations, however, is that of Fick's second law following concentration changes with time (Rahman, Al-Marhubi, & Al-Mahrouqi, 2007). This allows the description of diffusion kinetics as applied, for example, in a number of practical cases including homogenisation of lipids whose segmental mobility in a glassy bread matrix is temperature dependent (Roudaut, Van Dusschoten, Van As, Hemminga, & Le Maste, 1998).

Spin-offs of understanding the meaning and application of Fick's second include the estimation of activation energy (4–8 kcal/mol) for the diffusion of lipids through cell membranes, and their diffusion coefficient (10^{-8} – 10^{-11} cm^2/s) in the bulk or along several defects of the gel phase in processed foods (Derzko & Jacobson, 1980). More recently, however, it became apparent that small-molecule diffusion through a glassy polymer often cannot be rationalised on the basis of a concentration-dependent diffusion coefficient. This "anomalous effect" is readily observed once

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spheres, cylinders or slabs of a polymeric material are placed in contact with a solvent, with the ensuing sink-diffusion model exhibiting an upward curvature in the relationship of diffusant weight increase ($\Delta\omega$) *versus* $t^{1/2}$ (Singh & Chauhan, 2009). In contrast, classical Fickian kinetics argues for an initial proportionality between $\Delta\omega$ and $t^{1/2}$.

Today, anomalous transport is known to describe non-Fickian kinetics and commonly involves cross-linked polymers in the glass transition region. This limiting condition is designated as "Case II", as opposed to "Case I" for Fickian kinetics, and is characterised by a much higher bioactive compound mobility than the segmental relaxation rate, with the polymer relaxation becoming the rate determining step (Crank, 1975). Therefore, analysis of important mass transfer problems for the functional foods industry requires knowledge of the polymer network characteristics in relation to the temperature dependence of diffusion coefficient in polymer-solvent systems (Dissanayake et al., 2012).

Synthetic polymer research has considered the free-volume theory of transport as a useful expedient for describing the physical picture of polymer-network effects on molecular motion. Polymer relaxation creates expanded holes, which are filled by molecules or "jumping units" of a bioactive compound, and this fluctuation in the local free volume allows diffusion with the formation and disappearance of holes (Tramon, 2014). The present investigation examines the aforementioned conceptual approaches on a high-solid matrix of whey protein that is capable of creating structures to hold bioactive microconstituents (Hudson, Daubert, & Foegeding, 2000). Experimental data obtained for the diffusion of linoleic acid in the polymeric system will be used to carry out a critical discussion of the applicability and predictive capabilities of the combined free volume/molecular diffusion theory in this type of materials.

2. Materials and methods

2.1. Materials

2.1.1. Whey protein isolate

It was a microfiltration-isolated powder from Fonterra Co-operative Group Ltd (Palmerston North, New Zealand), and contained 90.4% protein (N x 6.38), 4.7% moisture with the minor addition of carbohydrate (0.9%), fat (1.0%) and minerals (3.0%). The material has been tested for microbiological contamination, which was <10 cfu/g for yeast and mould, and the aerobic plate count was <10,000 cfu/g. Physical tests showed 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. Linoleic acid (cis-9,cis-12-octadecadienoic acid)

The fatty acid was obtained from Sigma Aldrich Co (Sydney, Australia). It is a high purity material (≥98.5% by GC) with an average molecular weight of 280.45 g/mol and density of 0.902 g/ml at 25 °C.

2.1.3. Reagents

8-Anilino-1-naphthalenesulfonic acid ammonium salt (ANS), with more than 97.0% purity and HPLC grade, was purchased from Sigma Aldrich Co (Sydney, Australia). Analytical reagents for the phosphate buffer, i.e. potassium dihydrogen phosphate (≥99.5% purity) and disodium hydrogen phosphate anhydrous (99.0% purity) were purchased from BDH Chemicals Ltd, Poole, UK.

2.2. Methods

High-solid sample preparation: Whey protein dispersion was prepared by adding 30% powder in Millipore water at room temperature with constant stirring on a magnetic plate. The preparation was continuously stirred for 2 h until perfectly dispersed and then stored overnight at 4 °C for complete hydration and removal of air bubbles. Sample was removed from the refrigerator and stirred for another 15 min at ambient temperature before adding 1% linoleic acid. Mixing was extended for another 30 min for thorough dispersion using a conventional magnetic stirrer. The mixture was further homogenised for 3 min at 3000 rpm with a laboratory homogeniser (Ultra-Turrax T25, IKA-Labortechnik, Staufen, Germany). Preparation was then concentrated in a rotary evaporator at 40 °C to achieve a level of 80% (w/w) solids. Concentrated matrix of 80% whey protein excluding linoleic acid was also prepared as the standardised system.

2.3. Experimental analysis

2.3.1. Rheological measurements

Viscoelastic properties of condensed samples of 79% whey protein isolate with 1% linoleic acid (and 80% whey protein isolate) were analysed using small-deformation dynamic oscillation in shear with the Advanced Rheometer Generation 2 (AR-G2 from TA Instruments, New Castle, DE) equipped with magnetic trust bearing technology. Samples were loaded onto the Peltier plate of the instrument at 25 °C with a set gap of 1000 μm and a 10 mm parallel-plate measuring geometry. Sample edges were covered with silicone oil (BDH, 50 cS) to minimize moisture loss. To monitor changes in storage (G') and loss (G'') modulus with temperature, a constant scan rate of 1 °C/min, oscillatory frequency of 1 rad/s and strain of 0.01%, which was tested to be within the linear viscoelastic region (LVR), were applied throughout the experimental routine (maintaining a normal force of 0.08 N throughout).

This controlled strain rheometer was connected to a 60 L liquid nitrogen tank to provide purging nitrogen gas, which, in this work, cooled the samples down to −38 °C. Frequency-sweep data were collected from the lowest experimental temperature to 6 °C within a range of angular frequencies of 0.1–100 rad/s at constant temperature interval of four degrees centigrade. Storage and loss modulus were plotted against reduced angular frequency data to obtain the master (or composite) curve of viscoelasticity. The principle of time-temperature superposition (TTS) was then applied to estimate the so-called mechanical glass transition temperature (T_g) using appropriate modeling.

2.3.2. Fourier transform infrared spectroscopy (FTIR)

The technique was employed to identify potential alteration in chemical fingerprints as a result of adding fatty acid to the high-solid system. Samples of 79% whey protein with 1% linoleic acid were thus prepared along with single preparations of 80% whey protein and linoleic acid, which served as the standard. Work was performed using a Perkin Elmer Spectrum 100 with MIRacle™ZnSe single reflection ATRplate (Perkin Elmer, Norwalk, CT). All materials were scanned within the range of 600–4000 cm^{-1} with a resolution of 4 cm^{-1} averaged over thirty two scans, and each experimental sample was analysed in triplicate.

2.3.3. Wide angle X-ray diffraction (WAXD)

Bulk structure of 79% whey protein with 1% incorporated linoleic acid in comparison with whey protein powder and 80% high-solid protein preparation was examined using a Bruker D4 Endeavour (Karlsruhe, Germany). Freeze-dried samples were loaded onto the X-ray measuring compartment, scanned at the 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 with DIFFRAC^{plus} Evaluation (Eva), version 10.0, revision 1 and each experimental sample was

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