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Fundamental studies on the structural functionality of whey protein isolate in the presence of small polyhydroxyl compounds as co-solute

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ABSTRACT

The present work deals with the changing network morphology of whey protein isolate (15%, w/w) in the presence of glucose syrup (co-solute) with concentrations ranging from 0% to 65% (w/w) in 10 mM $CaCl_2$ solution, thus producing formulations with a total level of solids of up to 80% (w/w). Denaturation behaviour and aggregation of whey protein systems were investigated using small deformation dynamic oscillation on shear, micro and modulated differential scanning calorimetry, and confocal laser scanning microscopy. A progression in the mechanical strength of protein aggregates was observed resulting from enhanced protein–protein interactions in the presence of glucose syrup. Addition of the co-solute resulted in better thermal stability of protein molecules by shifting the process of denaturation to higher temperature, as observed by calorimetry. Observations are supported by micrographs showing coherent networks with reduced size of whey protein aggregates in the presence of high levels of glucose syrup, as opposed to thick and random clusters for systems of whey protein by itself. Glass transition phenomenon was observed for condensed protein/co-solute systems, which were treated with theoretical concepts adapted from synthetic polymer research to pinpoint the mechanical glass transition temperature.

1. Introduction

Whey protein is a by-product of cheese manufacture being primarily a mixture of β-lactoglobulin (50%), α-lactalbumin (20%), bovine serum albumin (5%) and immunoglobulins (13%) (de la Fuente, Singh, & Hemar, 2002; Mercade-Prieto & Gunasekaran, 2009). These globular proteins are widely used in food, pharmaceutical and health care products because of their unique functionalities of foaming capacity, emulsion stabilising, solution thickening and gel formation. The functional characteristics are altered by the influence of solvent quality, mechanical forces, processing temperature, etc. (McClements, 2002).

Within food products, whey proteins are often dispersed in an aqueous phase along with other low molecular weight co-solutes such as sugars, minerals and alcohols (McClements, 2002). These co-solutes can alter the molecular and functional characteristics of the complex system by binding to protein surface groups, but the precise nature of interaction depends on type and concentration of co-solute (Kumosinski, 1990; Timasheff, 1998). Previous studies in low solid systems have shown that sugar induces thermal stability to globular protein by extending their denaturation temperature (Jou & Harper, 1996; Lee & Timasheff, 1981). The proposed mechanism for the stability of globular proteins in the presence of sugar is attributed to sugar molecules being preferentially excluded from the protein surface. This is a process that reduces the thermodynamic affinity of the protein molecules for the composite solvent of water with various co-solutes (Arakawa & Timasheff, 1982; Timasheff, 1993).

To provide functionality in the form of a desirable gelling property, globular protein must be capable of aggregating to produce a three dimensional network that can act as a suspending medium of various ingredients in end products (Mulvihill & Kinsella, 1987; Zeigler & Foegeding, 1990). Native whey protein does not form gels when dispersed in water at ambient temperature, as the intermolecular repulsive forces dominate attractive interactions. Upon heating, the globular protein unfolds and exposes the hydrophobic core, leading to increased protein surface hydrophobicity, which is sufficient to promote aggregation and subsequent gel formation. Presence of sugar alters the above physicochemical process by increasing the unfolding temperature of protein molecules and changing the magnitude of the attractive and repulsive forces within the system (Record, Zhang, & Anderson, 1998). This influences the physicochemical properties of the gel including appearance, texture, stability and water holding capacity (Chanasattru, Decker, & McClements, 2008).

Condensed polysaccharide/sugar systems form rubbery gels at ambient temperature, which upon cooling to sub-zero temperatures undergo vitrification to produce materials with a brittle glassy consistency (Almrhag, George, Bannikova, Katopo, & Kasa-

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pis, 2012). Their relaxation processes have been monitored with the theoretical framework of free volume theory that predicts a mechanical glass transition temperature with physical significance (Kasapis, Al-Alawi, Guizani, Khan, & Mitchell, 2000). The approach has been adapted from well established research in amorphous synthetic polymers, which allows determination of viscoelastic properties of materials as a function of an extended range of temperatures or time scale of observation (Dannhauser, Child, & Ferry, 1958).

Our current work focuses on the gelation and vitrification behaviour of whey protein in the presence of glucose syrup as co-solute, within a concentration range that eventually produces condensed systems, allowing treatment of viscoelastic properties on the basis of a glass transition theory. As far as we are aware, there is no corresponding work in the literature on globular protein/sugar systems at high levels of solids, and we aim in this study to compare structural properties with those reported earlier for the polysaccharide/sugar counterparts.

2. Materials and methods

2.1. Materials

2.1.1. Whey protein isolate

The material used was a product from MG Nutritionals, Murray Goulburn Co-operative Co Ltd., Vic, Australia. According to the supplier, the composition of whey protein was reported to be 91.3% protein, 0.7% fat, 3.5% moisture, 3.8% ash and 0.44% lactose. The bulk density of the powder was reported to be 0.45 g/ml and produced a microbial standard plate count of 9,900 cfu/g. Solution of 10% (w/w) of whey protein in distilled water gave a pH value of 6.3.

2.1.2. Glucose syrup

Glucose syrup used for this investigation was a product of Cerestar (Manchester, UK) with a dextrose equivalent (DE) of 42. The total level of solids was 82% and percentages in formulations of this investigation refer to dry solids. According to the data provided by the supplier, with the aid of gel permeation chromatography, the polydisperse nature of the glucose syrup is established ranging from glucose molecules to high molecular weight fractions.

2.2. Methods

2.2.1. Sample preparation

Whey protein/glucose syrup samples were formulated on the basis of a fixed concentration of whey protein (15%, w/w) with varying concentration of glucose syrup from 0 to 65% (w/w). The required amount of glucose syrup was mixed with 10 mM $CaCl_2$ solution using magnetic stirrer. After obtaining a clear sugar solution, whey protein was added to the system as batches at ambient temperature. Samples were stirred for a further two hours to ensure proper dissolution. Following thorough mixing, they were stored overnight at 4 °C to ensure proper hydration. All analyses were performed at the natural pH of whey protein (~6.3).

2.2.2. Rheological characterisation

Measurements of systems from low to intermediate level of total solids (15–65%, w/w) were executed on MCR 301 rheometer (Anton Paar, Virginia, USA) using a cup and bob measuring geometry with dimensions of 28.66 and 26.66 mm in diameter, respectively. Under small amplitude oscillatory mode, viscoelastic properties of the samples were analysed at varying conditions of temperature and frequency. They were loaded at 25 °C and a solvent trap was placed to prevent moisture loss. Samples were heated to 85 °C at 1 °C/min, kept at that temperature for 30 min, and then cooled to 5 °C at the same scan rate using a constant frequency of 1 rad/s and strain amplitude of 1%.

To investigate the mechanical relaxation of high solid systems (80% total solids, w/w), a control stress rheometer, AR-G2 (TA Instruments, New Castle, DE) attached with an environmental test chamber that controls temperature was employed. Viscoelastic measurements of the sample were made using 5 mm parallel plate geometry, with edges being covered with silicone oil from BDH (50 cS) to prevent moisture loss. Condensed samples were heated to 85 °C at 1 °C/min, followed by an isothermal run for 30 min at that temperature. System was then cooled to −15 °C at the same scan rate using a frequency of 1 rad/s and strain of 0.01%. Upon subsequent heating from subzero temperatures, frequency sweeps were performed within the range of 0.1–100 rad/s, at an interval of four degrees centigrade, and results were modelled theoretically for estimation of the mechanical glass transition temperature.

2.2.3. Modulated differential scanning calorimetry

Thermal measurements of samples were performed with Q 2000 (TA instruments, New Castle, DE). The instrument used a refrigerated cooling system to achieve temperatures down to −90 °C and a nitrogen DSC cell at 50 ml/min to purge condensation. Samples were loaded in hermetic aluminium pans. Calibration of the heat flow signals, using a traceable indium standard (ΔH_f = 28.3 J/g), and the heat capacity response, with a sapphire standard, enabled accurate measurements. Samples were weighed (~10 mg) and analysed at a modulation amplitude of 0.53 °C at every period of 40 s. They were equilibrated at 25 °C and then heated to 95 °C at 1 °C/min. Condensed sample with 80% (w/w) total solids was cooled to −90 °C at the same scan rate, following the aforementioned thermal treatment, to observe the enthalpic relaxation of the system.

2.2.4. Confocal laser scanning microscopy

This technique assisted with producing tangible evidence of the changing morphology of protein systems in the presence of glucose syrup. Samples were stained with rhodamine B dye and placed on a concave glass slide to obtain fluorescent emission from the protein molecules. A glass coverslip was glued along the four edges covering the sample surface to prevent moisture loss and the temperature was raised to 85 °C at scan rate of 1 °C/min using a peltier platform. Specimens were scanned with N PLAN L 20.0 × 0.4 DRY objective lens from 553 to 619 nm using argon lasers.

3. Results and discussion

3.1. Observations on the changing network characteristics of whey protein with increasing additions of glucose syrup

In the present investigation, a fixed amount of whey protein isolate was utilised in mixture with an extended range of glucose syrup as the co-solute, giving us the opportunity to investigate the denaturation and aggregation behaviour of this material in an aqueous and high solid environment. Small deformation mechanical measurements were carried out first to observe the unfolding and subsequent profile of network formation in these systems ranging from low to intermediate levels of solids.

As shown in Fig. 1, during the initial heating regime a dramatic increase in the values of storage modulus (G') can be observed as the experimental temperature approaches 70 °C. This intensity of thermal treatment leads to the exposure of nonpolar amino acids that were originally located in the interior of the globular molecule dispersed in low viscosity solutions (Mulvihill & Donovan, 1987). Exposed nonpolar groups create strong and intermolecular interactions of hydrophobic nature leading to aggregation of whey protein