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Effect of calcium chloride on the structure and *in vitro* hydrolysis of heat induced whey protein and wheat starch composite gels

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ABSTRACT

The formation of heat induced whey protein isolate (WPI) and wheat starch (WS) gels in the presence of added calcium chloride (5–192 mM) has been examined. Thermal properties, including the onset temperature of starch gelatinization and protein denaturation, are defined by low amplitude oscillation on shear and modulated temperature differential scanning calorimetry. Upon heating and subsequent cooling, comparison of the storage modulus values bear information on the enhancement of protein aggregation by the electrolyte and the occurrence of phase separation phenomena between the two polymeric constituents in the mixture. Further confirmation of observed trends has been provided by measurements on textural hardness of gels in single cycle compression tests. Porous and aggregated microstructures are identified upon visual examination by environmental scanning electron microscopy. The gels were subjected to *in vitro* enzymatic hydrolysis and the role of calcium in reducing the extent of starch degradation by α-amylase has been established. It is evident from the results that ionic strength in the form of added calcium ions largely influences gelation kinetics of whey protein leading to significant variability in the hydrolytic potential of α-amylase on wheat starch.

1. Introduction

The superior nutritional value, granted GRAS status, and versatile functionality of whey proteins are a part of the appeal in utilizing the additive in many food formulations (Smithers, 2008). Manufacturers most commonly take advantage of its gelation mechanism and emulsification properties to manipulate bulk and interfacial features thus affecting organoleptic properties in end products (Doi, 1993).

Effective incorporation of whey protein is achievable by developing products on a sound technological basis and could directly benefit consumers by providing a healthy alternative to a range of popular convenience foods (Hongsprabhas & Barbut, 1996). This is particularly important as it has been estimated that in excess of a million Australians have obesity and Type 2 diabetes, and about half of those are not aware that they have the condition (Health Insite, 2008). The current society is suffering from 'diseases of choice' where few seem inclined to change their nutritional habits. Food directly influences blood glucose concentrations and response. However, this response is influenced by many factors such as the food form (whole or ground), cooking and processing, particle size and structure.

Heat induced protein gelation primarily occurs *via* partial unfolding of the corpuscular molecule and exposure of non polar and sulfhydryl groups (Lupano & Gonzalez, 1999; Matsudomi, Rector, & Kinsella, 1991; Steventon, Gladden, & Fryer, 1991; Verheul & Roefs, 1998). Aggregation subsequently follows, involving disulphide and non-covalent interactions, i.e. electrostatic and van der waals forces, hydrogen and hydrophobic bonds (Britten & Giroux, 2001; Damodaran, Parkin, & Fennema, 2008; Hoffmann & Van Mil, 1997; Kinsella & Whitehead, 1989; Shimada & Cheftel, 1988; Verheul & Roefs, 1998). The characteristics of the heat set protein gel depends on a multitude of factors including heating rate, holding time and temperature, pH, ionic strength, mineral content and presence of other hydrocolloids forming a rather complex physicochemical environment (Barbut, 1995; Damodaran et al., 2008; Ju & Kilara, 1998; Kuhn, Cavallieri, & Cunha, 2010; Mulvihill, Rector, & Kinsella, 1990; Verheul & Roefs, 1998). Calcium salts are known to be especially effective at enhancing thermal stability and increasing protein aggregation (Kuhn et al., 2010).

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Added calcium influences the degree of network formation through electrostatic shielding of the negatively charged molecules (Baumy & Brule, 1988). Furthermore, the divalent ion binds to the free carboxylic groups of aspartic and glutamic acids to form salt bridges that induce extensive crosslinks. This type of behavior has been of much interest in the structural properties and molecular demixing phenomena of globular protein–polysaccharide mixtures (Kinekawa, Fuyuki, & Kitabatake, 1998). Several developments of this have now emerged, and mixtures of whey protein with hydrolyzed starch or dietary fiber have been engineered in an attempt to create novel product concepts for weight management and pleasant mouthfeel (Yang, Liu, Ashton, Gorczyca, & Kasapis, 2013). Due to thermodynamic incompatibility, heated mixtures of protein with polysaccharides, above ~4% total solids in preparations, result in phase separated networks, and texture can be manipulated based on the polymeric constituent that forms the continuous matrix in the gel (Damodaran et al., 2008).

The present investigation is a fundamental study on the effect of added calcium chloride to heat induced whey protein and wheat starch composite gels. The main focus is on the role of calcium as a potential enhancer of structural behavior in whey protein against the enzymatic degradation of starch in mixtures. The work is based on the hypothesis that understanding the molecular aspects of segregative phase separation between proteins and polysaccharides allows the manipulation of phase behavior that in turn controls textural profile and sugar release in food materials.

2. Materials and methods

2.1. Materials

Native wheat starch was obtained from National Starch and Chemical Co., Bangkok, Thailand, with the powder containing 94.1% starch, 0.3% ash, 0.1% fat and 5.5% moisture on a weight-per-weight basis. Whey protein isolate was supplied by Fonterra Co-operative Group Ltd., Palmerston North, New Zealand. The powder contained 88.71% protein, 0.93% fat, 4.83% moisture and 3.3% ash (w/w). Calcium chloride dehydrate was AnalaR grade and was purchased by Sigma Chemicals, Castle Hill, Australia.

2.2. Methods

2.2.1. Sample preparation

Calcium chloride solutions of 5–192 mM were added to single and mixed dispersions of whey protein and wheat starch. These preparations were as follows (% w/v): 3 WS, 6 WS, 9 WS, 12 WS, 15 WS, 15 WPI, 15 WPI − 3 WS, 15 WPI − 6 WS, 15 WPI − 9 WS, 15 WPI − 12 WS and 15 WPI − 15 WS. Since incubation time can significantly influence protein aggregation and subsequent gel microstructure, powders were dissolved, gently stirred and then immediately used for experimentation (Ju & Kilara, 1998). The above was the preparation of samples used for rheology experiments. For the preparation of samples for texture analysis, microscopy and *in vitro* hydrolysis, gels were prepared by pipetting solutions into 45 °C preheated aluminum molds that were placed into a heated waterbath for 75 min. The temperature for the gelatinization of wheat starch and denaturation of whey protein begins at ~60 °C and ~73 °C respectively (Yang et al., 2013). The temperature at the center of each sample was set to exceed that required for starch and protein to undergo phase transition (~60 °C and ~73 °C respectively) within the first 15 min, after which a constant temperature of 85 ± 1 °C was maintained for the remainder of the heating period.

2.2.2. Rheology

Small deformation dynamic oscillation measurements of elastic modulus (G') and viscous modulus (G'') were performed on shear using a controlled strain AR-G2 rheometer with magnetic-trust bearing technology (TA Instruments, New Castle, DE). Experiments were conducted with a fixed strain of 0.1%, which is well within the linear viscoelastic region of single and mixed preparations of these materials. Parallel-plate measuring geometry was 40 mm in diameter with a gap of 1 mm. Samples were loaded onto the Peltier plate and covered with silicon oil from BDH (50 cS) to minimize evaporation. The entire experimental sequence utilized a scan rate of 2 °C/min, for which the system was first equilibrated at 25 °C for 2 min. The solution was then heated to 85 °C, cooled from 85 to 5 °C, followed by a frequency sweep from 0.1 to 100 rad/s.

2.2.3. Modulated differential scanning calorimetry

Thermal behavior was monitored on a Q2000 MDSC (TA Instruments, New Castle, DE) calibrated with a traceable indium and sapphire standard. An empty pan was used as the reference. Hermetically sealed aluminum pans containing 10 ± 1 mg of the sample were heated from 25 to 95 °C and cooled from 95 to 5 °C at a rate of 5 °C/min. The rate of modulation was 0.53 °C for every 40 s. The instrument interfaced a refrigerated cooling unit to achieve temperatures down to 0 °C and a nitrogen purge cell with a flow rate of 25 mL/min.

2.2.4. Textural analysis

Single cycle compression tests using the TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, England) were performed to determine gel hardness. According to Pons and Fiszman (1996), forces registered from a probe diameter smaller than the sample diameter are often derived from puncture and shear. Thus, a 50 mm diameter cylindrical probe was chosen to compress the samples of equal diameter (50 mm) and 15 mm height. Deformation was set at 80% enabling fracture within the first compression cycle. The compression rate employed throughout the experimentation was 0.1 mm/s at ambient temperature. Data was collected in triplicate for each WPI–WS–$CaCl_2$ combination tested and the results presented are an average ± S.E. of $n = 3$.

2.2.5. Environmental scanning electron microscopy

Micrographs were taken to examine the microstructure of single and binary mixtures of whey protein and wheat starch with varying levels of added $CaCl_2$ using an ESEM system (Fei Quanta 200, Hillsboro, Oregon, USA). Samples were cut into cubes of about 5 × 5 × 2.5 mm dimension whereby the internal surface of the original preparation was exposed to imaging. Observing the microstructure of these high moisture gels requires exposure to a gaseous secondary electron detector (GSED) at an accelerating voltage of 20 kV and pressure of 5.75 torr.

2.2.6. In vitro starch hydrolysis

Enzymolysis was conducted with a closed system utilizing semi permeable dialysis tubes (MW cut off 12–14 kDa). Aliquots were taken within the dialysis tube as well as the dialysate for reading at the end of the 180 min length digestion. Total sugar reduction was calculated as the sum of the two readings measured. The experimental procedure has been adapted from hydrolysis studies by Koh, Kasapis, Lim, and Foo (2009) and Jenkins et al. (1984). In further detail, freshly prepared gels were cut into 2.4 mm diameter × 1 mm height discs. A total of 6 g of these discs were transferred into the dialysis tube along with anhydrous monobasic sodium phosphate buffer (30 mL, 0.05 M) and 1 mL α-amylase (12,000 U). The enzyme utilized in this experiment was an extract from porcine pancreas, purchased from Sigma–Aldrich (Castle Hill, Australia).