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Modulation of physical properties of microfluidized whey protein fibrils with chitosan

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ABSTRACT

There is an increasing market for food and beverage products enriched in proteins and dietary fibers due to their potential health benefits. In this study, the effect of heating (85 °C, 20 min), microfluidization (20,000 psi), and pH (2 to 6.5) on the physicochemical properties of aqueous solutions containing mixtures of whey protein fibrils (7.5%; WPF) and chitosan (0.5%; CN) were examined. Heating of the mixed systems increased their turbidity and apparent viscosity, which was attributed to the formation of protein-rich particles. Interestingly, heating the mixed systems and then applying microfluidization led to phase separation and a lower apparent viscosity, which was attributed to high-pressure disruption of the whey protein fibrils. The pH of the systems significantly influenced their appearance, turbidity, particle size, and apparent viscosity, with high turbidity and viscosity, and large particle size occurring from pH 3.5 to 5.5. These effects were attributed to protein aggregation close to its isoelectric point (pH 5) coupled with electrostatic attraction between anionic groups on the whey protein and cationic groups on the chitosan. The addition of chitosan increased the net isoelectric point from pH 5 for pure whey protein to pH 6 for the mixed system. Overall, this study showed that the appearance and rheological properties of protein-dietary fiber mixtures could be manipulated by heating, microfluidization, and pH adjustment. This information may be useful for designing protein and fiber enriched food and beverage products with desirable physical and sensory properties.

1. Introduction

In recent years, there has been a growth in the demand for functional foods perceived to be better for human health. These may be products that have reduced levels of potentially detrimental food components such as fat, sugar, or salt (Janhøj & Ipsen, 2006; Lim, Swanson, & Clark, 2008) or products fortified with potentially beneficial food components such as proteins, dietary fibers, vitamins, or nutraceuticals (Jones & McClements, 2010; Koo, Chung, Ogren, Mutilangi, & McClements, 2018). As well as having improved nutritional profiles, these products should also have the desired physicochemical properties and sensory attributes, such as appearance, texture, and flavor. To achieve this goal, food scientists are using various structural design approaches to reformulate food products. Edible biopolymers, such as proteins and polysaccharides, are commonly used for this process (Chun et al., 2014; Ghosh & Bandyopadhyay, 2012; Jones, Decker, & McClements, 2009). Mixtures of biopolymers can be assembled into various nano- and micro-structures that provide different functional attributes in foods. The nature of the structures formed is usually controlled by manipulating the molecular interactions between the biopolymers, such as electrostatic, hydrophobic, steric, or hydrogen bonding interactions.

Food proteins and dietary fibers are commonly used in combination to generate specific structures and functional properties (Chun et al., 2014; Jones, Decker, & McClements, 2010b; Li, Wei, Fang, Zhang, & Zhang, 2014; Mohammadian & Madadlou, 2018; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). Proteins are a good source of energy and essential amino acids, whereas dietary fibers may have health benefits such as reduction of constipation, cancer, and heart disease. Examples of protein-polysaccharide combinations studied previously include β-lactoglobulin with pectin (Chanasattru, Jones, Decker, & McClements, 2009; Jones et al., 2009; Jones & McClements, 2008), chitosan (Gilbert, Campanella, & Jones, 2014), carrageenan (Jones, Decker, & McClements, 2010a) and gum arabic (Klein, Aserin, Ishai, & Garti, 2010; Klein, Aserin, Svitov, & Garti, 2010). These mixed biopolymer systems have been shown to have potential applications as fat replacers

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(Janhøj & Ipsen, 2006), for controlling macronutrient digestibility (Chen, Remondetto, & Subirade, 2006), and for encapsulating, protecting and delivering bioactive components to the body (Wichchukit, Oztop, McCarthy, & McCarthy, 2013).

In this study, we were interested in developing model food and beverage systems from a mixture of proteins and dietary fibers. Previously, we fabricated whey protein fibrils by heat denaturing whey protein isolate under controlled conditions (pH 2, 85°C for 20 min) and showed that their functional attributes could be manipulated by controlled microfluidization (Koo et al., 2018). In this study, we examined the impact of mixing chitosan with whey protein fibrils on the physicochemical properties of the systems formed. We postulated that the cationic chitosan molecules would interact with the whey protein fibrils and form structures that would alter the appearance, texture, and stability of the mixtures. The impact of thermal treatment, microfluidization, and pH on the properties of mixed whey protein-chitosan system was examined to determine if their properties could be tuned for particular applications. The results of this study may therefore be valuable for the design of protein and dietary-fiber enriched food and beverage products with specific physical and sensory properties.

2. Material and methods

2.1. Materials

Whey protein isolate (WPI) was provided by PepsiCo (Valhalla, NY, USA). Chitosan (low molecular weight) was obtained from Sigma Aldrich (St. Louis, MO). Hydrochloric acid solutions (6 N and 1 N) and sodium hydroxide solutions (6 N and 1 N) were prepared from 12.1 N hydrochloric acid (HCl) and sodium hydroxide (NaOH) pellets (≥ 97%, Fisher Scientific, Waltham, MA), respectively. All chemicals used were of analytical grade. All solutions were prepared using 18.2 MΩ deionized water prepared using a water purification unit (Milli-Q, EMD Millipore, Billerica, MA).

2.2. Formation of whey protein fibrils and chitosan mixtures

Whey protein isolate solution (8% w/w, pH 2) was heated to 85°C for 20 min to denature and form protein fibrils. One portion of the heated solutions was used without further treatment while the other portion was subjected to microfluidization at 20,000 psi for 1 pass (M110-P, Microfluidics, Westwood, MA). The heated whey protein is hereafter termed as whey protein fibrils (WPF) while the heated and microfluidized whey protein fibril system is designated as MF-WPF. The solutions were adjusted to pH 2 to 6.5 for further analysis.

Whey protein fibril and chitosan mixtures were prepared by heat denaturing (85 °C, 20 min) WPI (7.5%, pH 2) and chitosan (CN; 0.5%, pH 2) solution. The heated mixture was cooled with an ice bath until 20 °C, followed by microfluidization at 20,000 psi. The mixture was then adjusted from pH 2 to 6.5 for further analysis.

2.3. System characterization

2.3.1. Visual appearance and turbidity measurement

The visual appearance of the systems was recorded using a digital camera. The turbidity of the systems was measured in 3.5 mL disposable cuvettes (1 cm path length) using a UV/visible spectrophotometer at 600 nm (Cary 100, Agilent Technologies, Santa Clara, CA, USA). Deionized water was used as a blank reference.

2.3.2. Apparent viscosity measurement

The apparent shear viscosity of the samples was measured using a dynamic shear rheometer with a concentric cylinder (cup-and-bob) measurement cell with 26 mm diameter cup and diameter of 25 mm bob (Kinexus Rheometer, Malvern Instruments, Worcestershire, UK) over a range of shear rates (0.1 to 100 s^{-1}) at 25°C. The temperature of the instrument was controlled by the Peltier cylinder cartridge system. The rheological measurements were controlled and analyzed using the instrument software (Kinexus rSpace, Version 1.30, Malvern Instruments, Westborough, MA). The apparent shear viscosity was reported at a fixed shear rate 10 s^{-1} in the Results and discussion section. This shear rate was reported to closely mimic mastication of liquid or semi-solid food and beverage systems and a low shear rate has previously been reported to reduce errors that may result from slippage (Shama & Sherman, 1973).

2.3.3. Microscopy analysis

Protein samples were diluted to 0.05% w/w protein with deionized water (pH 2) prior to carrying out the transmission electron microscopy (TEM) analysis. A drop (5 μL) of diluted protein solution was then deposited on a 400 mesh copper grid with 5 nm of carbon support film (CF400-Cu, Electron Microscopy Sciences, Hatfield, PA). Excess sample was blotted off and subsequently 5 μL of deionized water (pH 2) was used for washing. A drop (5 μL) of 1% uranyl acetate solution was used to negatively stain the sample and excess was blotted off after 2 min. The grids were air dried and placed in a dessicator for storage. TEM micrographs were captured using a transmission electron microscope (JEOL JEM-2000FX, JEOL, Peabody, MA) operating at 200 kV and magnification of 30 k times.

Confocal laser scanning microscopy was also used to examine the microstructure of the systems. All samples containing protein were stained with fluorescein isothiocyanate isomer 1 (FITC, 1 mg FITC in 1 mL dimethyl sulfoxide). 0.2 mL of the fluorescent dye was added to 1 mL of protein sample and mixed well. A drop (0.1 mL) of the stained sample was placed on a microscope glass slide and covered with a glass cover slip. All samples were analyzed with a microscope fitted with a 10× eyepiece and a 60× objective lens (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, USA). The excitation and emission spectrum for the Nile red were 543 nm and 605 nm, respectively and for the FITC were 488 nm and 515 nm, respectively. Microstructural images were taken using an image analysis software (EZ-CS1, Nikon, Melville, NY, USA).

2.3.4. Particle size diameter measurement

The mean particle diameter of the samples was determined using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Samples were diluted with deionized water adjusted to the appropriate pH to prevent particle interaction effects.

2.3.5. Electrophoretic mobility measurement

The electrophoretic mobility (ζ-potential) of the systems was determined using a particle electrophoresis instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Samples were diluted 20-fold with deionized water at the appropriate pH to prevent particle interaction effects.

2.4. Statistical analysis

Replicate systems were prepared for each system, and triplicate measurements were made from each sample ($n = 6$). The mean, standard deviations and statistical differences between means using two-way analysis of variance (ANOVA) were calculated from all replicate readings using Microsoft Excel.

3. Results and discussion

Initially, whey protein fibrils (WPF) were formed by heating whey protein isolate solutions at pH 2 at 85 °C for 20 min (Fig. 1). Subsequent microfluidization of the WPF led to truncated whey protein fibrils. More information on the structure and physical properties of microfluidized WPF formed using this method can be found in our previous paper (Koo

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