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Assessing the potential of whey protein fibril as emulsifier

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ABSTRACT

The effect of pH and mechanical processes on non-heated whey protein isolate (WPI) and WPI fibril was evaluated from structural, physical and emulsifying properties. Fibril aggregation was observed increasing pH from 2 to 7 whilst mechanical processes with high energy density resulted in fibril shortening and smaller aggregates. Despite affecting fibril morphology, mechanical processes did not affect significantly the protein secondary structure conformation and interfacial tension at aqueous protein dispersion-oil interface. However, increasing pH resulted in loss of ordered secondary structure, decreased interfacial tension and surface hydrophobicity of fibril. Fibril dispersions showed shear-thinning behavior but thixotropy was only observed at pH 7. Fibril-stabilized emulsions at pH 7 were more stable due to the higher viscosity and faster migration of fibrils to the interface when compared to fibrils at low pH, resulting in steric hindrance stabilization. Therefore, changes on WPI fibrils resulted in varied structural and emulsifying properties.

1. Introduction

Amyloid fibrils are β-sheet-rich and filamentous protein structures with reduced diameters (~10 nm) and lengths of the order of microns, which leads to a typical high aspect ratio (length/diameter) (Kroes-Nijboer et al., 2012). Fibrillar protein aggregates are promising anisotropic nanostructures for food, pharmaceutical and cosmetic applications due to their gelling ability (Akkermans et al., 2008b; Jiang et al., 2016) and interfacial properties (Jordens et al., 2014; Rühs et al., 2012, 2013; Wan et al., 2016). In general, fibrils for use in food products have been produced from globular proteins derived from different sources such as pea (Munialo et al., 2014), egg (Lara et al., 2012; Pearce et al., 2007), soy (Akkermans et al., 2007; Wan et al., 2016), kidney bean (Tang et al., 2010) and milk (Akkermans et al., 2008a, 2008b; Kroes-Nijboer et al., 2012; Mantovani et al., 2016). β-lactoglobulin (β-lg) is the unique protein fraction that can form fibrils among whey proteins by a simple process consisting of heating at high temperatures for several hours under strongly acidic conditions and low ionic strength (Bolder et al., 2006). However, the high cost of purified ingredients as β-lg limits their application in food products. Whey protein isolate (WPI) is a food ingredient rich in β-lg, which is widely used as emulsifying/stabilizing agent (Guzey and McClements, 2006). In this study, WPI was used because of its high relevance for practical applications in food industry.

The length distribution of protein fibrils is important for application purposes (Peng et al., 2016; Serfert et al., 2014). Therefore, changes on protein fibril morphology and, consequently, on fibrils properties can be desirable. Previous studies have reported the effect of applying shear mechanical forces (Akkermans et al., 2007, 2008a, 2006) or agitation (Klunk et al., 1999) on the formation of protein fibrils. The production of amyloid fibrils by ultrasonication-induced spontaneous fibrillation of β_2-microglobulin (Chatani et al., 2009; Ohhashi et al., 2005), a tripeptide (Maity et al., 2011), bovine serum albumine (BSA), myoglobin and lysozyme (Stathopulos et al., 2004) expands the range of food, biotechnological and medical applications. The effect of ultrasonication on fibril formation using collagen extracted from the skin of grass carp has also been assessed (Jiang et al., 2016). However, modifying the length distribution of protein fibrils during production is a challenge, which could be overcome exposing mature fibrils to an external shear field (Kroes-Nijboer et al., 2010).

The fracture of preformed fibrils produced from β-lg or WPI into

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shorter fibrils was observed after these nanostructures have been subjected to elongational flow (Kroes-Nijboer et al., 2010), rotor–stator-dispersion (Peng et al., 2016), high pressure homogenization (Jung and Mezzenga, 2010; Serfert et al., 2014) and microfluidization (Oboroceanu et al., 2011). The effect of ultrasonication on preformed fibrils of β_2-microglobulin (Chatani et al., 2009) and insulin (Milto et al., 2013) has also been reported, but the effect of sonication on WPI fibrils has not been fully investigated. Moreover, pH change arises as an alternative to the application of mechanical forces aiming WPI fibril shortening (Akkermans et al., 2008b; Mantovani et al., 2016). The fibril size reduction may prevent the occurrence of bridging flocculation in fibril-stabilized emulsions, leading to a better covering efficiency around of small oil droplets (Serfert et al., 2014). Previous studies have reported the use of fibrils in emulsions stabilized by native whey proteins to induce a depletion-flocculation process, improving emulsion stability (Blijdenstein et al., 2004; Peng et al., 2016). However, the potential of fibrils produced from WPI as a single emulsifier for emulsion stabilization has not been fully investigated, as well as the combined influence of pH and ultrasound on structural and emulsifying properties of preformed WPI amyloid fibrils.

Thus, the present study aimed to investigate the effect of modifying WPI fibrils by pH changes and mechanical processes on their morphology and emulsifying properties. Investigations included the characterization of fibrillar systems in terms of morphology, secondary structure conformation, surface hydrophobicity, interfacial tension, rheology and emulsifying activity, compared to non-heated WPI.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) (protein content of 90.6 ± 0.5% w/w) was obtained from New Zealand Milk Products (ALACEN 895, Wellington, New Zealand). Other reagents of analytical grade were purchased from Sigma Aldrich Co. (St. Louis, USA). Soybean oil (Soya, Bunge Alimentos S.A., Gaspar, Brazil) was purchased in the local market.

2.2. Fibril formation

A WPI stock solution (2% w/v) was prepared by dissolving the protein powder in Milli-Q water using magnetic stirring at room temperature for 2 h, ensuring complete dissolution of the protein. The pH of protein solution was adjusted to pH 2 with 3 M HCl. Afterwards, the stock solution was filtered through 0.45 μm low-protein adsorbing filters (Millex-HV®, Millipore, Billerica, USA) and heat-treated at 80 °C for 20 h under mild stirring. After that, the systems were immediately cooled to room temperature using an ice bath. After fibrillization, these systems consisted of fibrils and unconverted peptides (Akkermans et al., 2008b; Kroes-Nijboer et al., 2012). Nevertheless, we address this solution as 'fibril system' in the remaining part of the paper. Then, the pH of part of the fibrillar dispersion was adjusted to 7 using 2 M NaOH. Mechanical treatments of fibril dispersions were performed according to a previous work (de Figueiredo Furtado et al., 2017). WPI fibril dispersions (2% w/v) at pH 2 and pH 7 were then subjected to shearing in a rotor-stator homogenizer (SilentCrusher M, Heidolph, Schwabach, Germany) for 3 min at 5,000 rpm. Afterwards, an ultrasonic processor (QR 750 W, Ultronique, Campinas, Brazil) with a 13 mm diameter probe of titanium was used to produce ultrasound-treated aqueous fibril dispersions. The power, frequency and sonication time were fixed at 300 W, 20 kHz and 3 min, respectively. The temperature of the samples was measured before and after sonication and did not exceed 30 °C. The pH of the protein solutions was measured before and after sonication using a pH meter (Metrohm 827, Metrohm, Herisau, Switzerland). Non-processed fibril dispersions (without mechanical treatment) and subjected only to the first step in a rotor-stator were also evaluated. Therefore, fibrils were classified into three categories according to their exposure to mechanical treatment: non-processed (NP), rotor-stator dispersion (RS) and rotor-stator dispersion followed by ultrasound-treatment (UT). Aqueous dispersions of non-heated WPI were prepared as described above but without the heat treatment step (fibrillation). The dispersions of non-heated WPI were called as 'control systems', since they were subjected to the same mechanical process conditions of fibrils.

2.3. Emulsion preparation

The aqueous phase of oil-in-water (O/W) emulsions consisted of 2% (w/v) WPI dispersion, before (Control) or after fibrillation process (Fibril), without mechanical treatment or subjected to (i) shearing in a rotor-stator homogenizer and (ii) shearing in a rotor-stator homogenizer followed by sonication as previously described in section 2.2. O/W emulsions were prepared at 25 °C by homogenizing the soybean oil with the aqueous phase using a rotor-stator homogenizer (SilentCrusher M, Heidolph, Schwabach, Germany) for 3 min at 14,000 rpm according to a previous work (Mantovani et al., 2017). The oil content was fixed at 20% (v/v). The emulsions were evaluated by creaming stability, optical microscopy and mean droplet size.

2.4. Whey protein isolate fibril evaluation

2.4.1. Transmission electron microscopy (TEM)

Electron micrographs of WPI fibril dispersions were taken using a Libra 120 transmission electron microscope (Carl Zeiss AG, Oberkochen, Germany) operating at 120 kV equipped with an in-column OMEGA energy filter and an Olympus CCD camera 14 bits with 1376 × 1032 resolution. A droplet of the diluted sample was put onto a carbon support film on a copper grid. The excess was removed after 5 min with a filter paper. Afterwards, a droplet of uranyl acetate (2% w/v) was put onto the grid. After 1.5 min the excess was removed again with a filter paper.

2.4.2. Zeta potential

WPI dispersions, before (Control) and after fibrillation process (Fibril), were diluted in Milli-Q water (0.05% w/v) before being placed in the measuring chamber of microelectrophoresis (Zetasizer Nano-ZS, Malvern Instruments Ltd., Malvern Hills, UK) to determine their surface electric charge density. The electrophoretic mobility measurement was converted into zeta potential values using the Smoluchowski mathematical model. Samples were measured in triplicate at 25 °C.

2.4.3. Far-UV circular dichroism (CD)

Far-UV CD was used to investigate the secondary structure of WPI before (Control) and after fibrillation process (Fibril). Far-UV CD spectra of 0.1 mg/mL protein dispersions were obtained and recorded at 25 °C in the spectral range from 190 to 260 nm with a Jasco J-810 spectropolarimeter (Jasco Corp., Tokyo, Japan), using a quartz cuvette with an optical path of 0.1 cm. The spectral resolution was 0.5 nm, and the scan speed was 100 nm/min, with a response time of 0.125 s at a bandwidth of 1 nm. Twenty scans were accumulated and averaged, and the spectra were corrected using a protein-free sample. The mean residue ellipticity $[\theta]$ (deg.cm^2/dmol) was calculated as shown in Eq. (1).