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Formation and pH-stability of whey protein fibrils in the presence of lecithin

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ABSTRACT

The effect of the soybean lecithin (SL) (0–0.25% w/v) on the kinetics of whey protein (2% w/v) fibrils formation and their stability under different pH values were evaluated. Linear and long fibrils were formed in the presence of SL at concentrations below critical micelle concentration whilst a higher SL content led to fibrils aggregation. The electrophoretic profiles and rate of fibril formation were not affected by the presence of SL. In spite of indicating SL-protein interaction, circular dichroism results showed that SL did not affect significantly the protein secondary structure conformation during heating. The pH-stability of fibrils was evaluated in a pH range from 3 to 7. At pH 3, the fibrils were isolated, but the increase of pH to 5 led to the formation of big aggregates that became more opened at pH 7, mainly in the presence of SL. These results were confirmed by small-angle X-ray scattering profiles. Different from fibrils formed without SL, the secondary structure of fibrils formed in the presence of SL showed no differences increasing pH from 3 to 7. Thus, despite not affecting the fibril growth mechanism and conformation, the presence of SL decreased the protein susceptibility to pH changes broadening the potential application of nanofibrils as a food ingredient. In this context, changes of process variables were carried out in order to enhance whey protein nanofibril formation in the presence of lecithin. Alternatives such as increasing pH, reducing protein-lecithin ratio and the modification of phospholipids composition favored the amyloid-like structure formation.

1. Introduction

Amyloid fibrils are β-sheet-rich and filamentous protein structures with typical high aspect ratio (length/diameter) due to reduced diameters (~10 nm) and lengths of the order of microns (Chiti & Dobson, 2006). Protein nanofibrils self-assembly is considered a simple process which is advantageous when compared to others nanostructures (Sasso et al., 2014). The protein fibril functionality includes increasing viscosity and acting as gelling agent besides stabilizing foams and emulsions (Rossier-Miranda, Schroën, & Boom, 2010), but all these properties are strongly dependent on the aspect ratio of the fibrils (Kroes-Nijboer et al., 2012). The high aspect ratio of fibrillar structures is responsible for their efficient structuring ability since, at low volume fraction or protein content, they are capable of forming a space filling network. The great interest in whey proteins for fibril formation is mainly associated to their low cost and availability (Sasso et al., 2014). Furthermore, replacing β-lactoglobulin (β-lg), which is the major fraction of whey proteins, for β-lg fibrils in food products would be an alternative of decreasing the allergenicity that is caused during its digestion in the gastrointestinal system, providing similar nutritional effect (Bateman, Ye, & Singh, 2011).

Whey protein fibrils are commonly produced at very acidic pH, but the knowledge of pH-stability of fibril systems is essential since most food products show pH values ranging from 4 to 7 (Kroes-Nijboer et al., 2012). Nanofibrils are typically dispersible at certain environmental conditions such as low protein concentrations, low ionic strength and pH far from the protein isoelectric point (pI) (Sagis et al., 2002) that promotes strengthened electrostatic repulsion. In this way, changing these favorable conditions could cause the aggregation of these nanostructures besides modifying the viscosity and turbidity of the nanofibrils dispersion. Improved nanofibrils stability against aggregation at weakened electrostatic

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repulsion was reached after glycation with lactose (Liu & Zhong, 2013) or by electrostatic complexes formation with sodium dodecyl sulfate (SDS), a negatively charged surfactant (Jung, Savin, Pouzot, Schmitt, & Mezzenga, 2008).

Surfactants are the most common additives applied to induce the formation of protein amyloid structures and SDS is the most used for this purpose (Khan, Abdulrehman, Zaidi, Gourinath, & Khan, 2014). Interactions between proteins and surfactants can modify proteins functionality, such as emulsifying capability, which is interesting in food and pharmaceutical industries (Andersen et al., 2009). Lecithin, a zwitterionic surfactant, is an important natural emulsifier (Shukat & Relkin, 2011) which is quite efficient on reducing interfacial tension (Pugnaloni, Dickinson, Ettelaie, Mackie, & Wilde, 2004). Lecithin is composed by a mixture of phospholipids (Michelon, Mantovani, Sinigaglia-Coimbra, de la Torre, & Cunha, 2016), that are important constituents of the cell membrane and can also be naturally found in milk fat globule membrane (Sawyer & Kontopidis, 2000). Ionic surfactants and proteins can bind directly through hydrophobic and electrostatic interactions (Kelley & McClements, 2003), which can modify the stability of a protein-based system under different environmental conditions such as pH or ionic strength (Andersen et al., 2009). Then, the knowledge of nature of surfactant–protein interactions and their influence on whey protein fibril formation is of great importance and essential to define its suitable application in food products.

Therefore, this work aimed to produce whey protein nanofibrils in the presence of soybean lecithin (SL), a surface-active ingredient, evaluating the effect of the surfactant on the kinetics of fibril formation and its stability under pH changes. Afterwards, changes of process variables (pH, protein content and phospholipids composition) were carried out in order to enhance whey protein nanofibril formation in the presence of lecithin. For this purpose, visual appearance, surface charge density, thioflavin T fluorescence, circular dichroism spectroscopy, sodium dodecyl sulfate polyacrylamide gel electrophoresis and small angle X-ray scattering profiles of nanofibrils were evaluated.

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, New Zealand). SL (Lipoid S45) containing >45% w/w phosphatidylcholine (PC), 10–18% w/w phosphatidylethanolamine (PE), <4% w/w lysophosphatidylcholine (LPC) and <3% w/w triglycerides (TG) and purified egg lecithin (EL) (Lipoid E PG) containing 99.6% w/w phosphatidylglycerol (PG), 0.4% w/w phosphatidic acid (PA), <0.1% w/w PC, <0.1% w/w lysophosphatidylglycerol (LPG) and <0.1% w/w TG were purchased from Lipoid GmbH (Germany). Analytical grade reagents were purchased from Sigma Aldrich Co. (St. Louis, USA).

2.2. Determination of critical micelle concentration (CMC)

The SL CMC was determined according to adapted methodology from Wu and Wang (2003). The surface tension of SL aqueous dispersions at pH 2 was measured using a pendant-drop tensiometer Tracker-S (Teclis, Longessaigne, France). These dispersions were prepared at different lecithin concentration in the presence or absence of WPI 2% (w/v). The drop volume was 8 μL and the measurements were performed at 25 °C. Surface tension values were plotted against lecithin concentration. The initial part of the curve showing a reduction of surface tension and the part exhibiting constant values (plateau) were evaluated separately in order to obtain the linear fitting of each part. The intercept where the surface tension started to become constant with increasing lecithin concentration was considered as the CMC. Samples were measured in duplicate at 25 °C.

2.3. Fibrils formation

A WPI stock solution (3% w/w) was prepared by dissolving the protein powder in ultrapure water (Direct-Q3, Millipore, USA) 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, USA). SL stock solution was prepared by dispersing SL in water (1.5% w/v) using magnetic stirring at room temperature until complete dissolution. Stock solutions were mixed using magnetic stirring in order to obtain systems containing a fixed amount of protein (2% w/v) and 0–0.25% (w/v) of SL. The pH was adjusted to 2 and the mixture was heated at 80 °C during 20 h under mild stirring. After the heat treatment, the systems were immediately cooled to room temperature using an ice bath. After that, the pH of fibrillar solutions was adjusted to 3, 5 and 7 using 2 M NaOH. The systems before and after heating were called as Control and Fibril, respectively. In a second moment, the following systems prepared as described above were evaluated: (i) W2 SL0.05 - pH 3: fibrils formed with 2% w/v WPI in the presence of 0.05% w/v SL by heating at pH 3, (ii) W0.5 SL0.05 - pH 2: fibrils formed with 0.5% w/v WPI in the presence of 0.05% w/v SL by heating at pH 2 and (iii) W2 EL0.025 - pH 2: fibrils formed with 2% w/v WPI in the presence of 0.025% w/v EL by heating at pH 2.

2.4. Zeta potential

To determine the surface electric charge density of the fibrils, the dispersions were diluted to a concentration of about 0.05% (w/v) in ultrapure water (Direct-Q3, Millipore, USA) before being placed in the measuring chamber of microelectrophoresis (Zetasizer Nano-ZS, Malvern Instruments Ltd., UK). The Smoluchowski mathematical model was used to convert the electrophoretic mobility measurement into zeta potential values. Samples were measured in triplicate at 25 °C.

2.5. Thioflavin T (ThT) fluorescence

Firstly, ThT fluorescence measurements were carried out to evaluate the nanofibrils formation. Afterwards, ThT fluorescence measurements were carried out in order to evaluate the fibril pH-stability. ThT stock solution (3 mM) was made by dissolving ThT in phosphate buffer (10 mM phosphate, 150 mM NaCl at pH 7). This stock solution was filtered through a 0.22 μm syringe filter and diluted 50-fold in phosphate buffer (10 mM phosphate, 150 mM NaCl at pH 7) before to be used. Fibril dispersions (48 μL) were mixed with 4 mL of this ThT solution during 1 min in order to allow the binding between ThT and the protein. The fluorescence of the samples was measured using a ISS K2 fluorometer (ISS, USA). The excitation wavelength was set on 446 nm (slit width 2 mm) and the emission spectrum was recorded between 470 and 500 nm (slit width 1 mm). The fluorescence intensity peak was determined at 482 nm and the fluorescence intensity of the ThT solution (without reaction) was subtracted as a background. All samples were measured in duplicate.

2.6. Far-UV circular dichroism (CD)

Firstly, far-UV CD was used to investigate the secondary