International Journal of Biological Macromolecules 89 (2016) 499–506

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Cold-set hydrogels made of whey protein nanofibrils with different divalent cations

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ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form 30 April 2016
Accepted 2 May 2016
Available online 4 May 2016

Keywords:
Cold gelation
Gastric digestion
Chitosan coating

ABSTRACT

Whey protein nanofibrils are gaining interest to fabricate cold-set hydrogels due to their ability to gel at lower concentrations than parent proteins. In the present research, fibrillated protein solution was gelled with three different divalent cation salts including $CaCl_2$, $MnCl_2$ and $ZnCl_2$ and the textural and functional characteristics of the resulting hydrogel samples were studied. Atomic force microscopy indicated that the flexible micron-scaled fibrils with nanometric thickness (up to 8.0 nm) that formed at pH 2.0 underwent breaking in length upon post-formation pH rise to 7.5. Whilst heat-denatured protein solution failed to form self-supporting gel at pH 7.5, fibrillated protein solution gelled by all three types of cations. Fibrillation increased the protein solution consistency coefficient (K) much more than heat denaturation. It was suggested based on Fourier-transform infra-red (FT-IR) spectra that some hydrogen bonds were disrupted by fibrillation. Zn^{2+}-induced gel was firmer, had a higher water holding capacity and a more compact microstructure, as well, required a higher compressive stress to fracture than its counterparts. Nonetheless, the Mn^{2+}- and Ca^{2+}-induced gels disintegrated to a much lesser extent in both pepsin-free and pepsin-present simulated gastric juice than Zn^{2+}-induced sample. Chitosan coating approximately halved the simulated degradability of all gel samples.

1. Introduction

Prolonged heating of whey proteins especially β-lactoglobulin at acidic condition and low ionic strength where the net charge of proteins is high, results in formation of nanofibrillar structures [1]. Veerman et al. [2] developed a new multistep process for Ca^{2+}-induced cold gelation of β-lactoglobulin at very low concentrations. In this method, β-lactoglobulin solution was heated at pH 2.0 to form long linear fibrils. Then, the pH of fibrillar solution was adjusted to 7.0 or 8.0 and fibrils were cross-linked by adding $CaCl_2$. They reported that critical percolation concentration for β-lactoglobulin samples in this process was considerably lower than the conventional gelation methods. The remarkable gelation capability of protein nanofibrils has been ascribed to their high aspect ratio, anisotropic dimensions and ability to form entanglement networks [3].

$CaCl_2$ and NaCl are the most common salts for cold gelation of whey proteins [4]. However, monovalent cations are not convenient for fabrication of gels from β-lactoglobulin nanofibrils since salt bridges formation (such as fibril^{-}...$^{+}$Ca^{+}...$^{-}$fibril) is a prerequisite [2]. Recently, Farjami et al. [5] gelled whey protein nanofibrils solution through slow deacidification by extensive dialysis against water. The obtained ash-free gel had a higher storage modulus and fracture stress than the gels formed via dialysis with $CaCl_2$-enriched waters. All gels had pH values near to the isoelectric point of whey proteins. Alternative cations such as Fe^{2+} [6] and Mg^{2+} [7] have been used to form ionotropically-driven whey protein cold-set gels to acquire nutritional benefits. Zinc (Zn) is an essential dietary element and plays important functions in prenatal and postnatal periods [8]. Manganese (Mn) is another important metal for human health which is essential for metabolism, organs development and antioxidant defense system (metalloenzyme manganese superoxide dismutase) of body [9]. To the best of authors' knowledge, there was no report in literature about cold-set gelation of whey protein nanofibrils by means of $MnCl_2$ and $ZnCl_2$. The aim of the present study was therefore to produce cold-set gels from fibrillated whey protein solution by adding manganese and zinc salts, followed by characterization and comparison of the resulting hydrogel samples with that fabricated via Ca^{2+}-induced cold gelation. Finally, protein gels were coated electrostatically with chitosan to decrease the simulated gastric digestibility of gels.

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http://dx.doi.org/10.1016/j.ijbiomac.2016.05.009
0141-8130/

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) with more than 90% protein content was donated by Arla Food Ingredients (Viby J, Denmark). The enzyme pepsin with protease activity of ≥3000NF units mg^{-1} was obtained from Bio Basic (Bio Basic Inc., Canada). Chitosan with molecular weight of 600–800 kDa was purchased from ACROS (ACROS Organics, Pittsburg, USA). Other used chemicals were analytical grade and supplied from Merck (Darmstadt, Germany).

2.2. Fibrillation of WPI solution

Fibrillation of whey protein isolate was carried out according to the method of Veerman et al. [2]. For this purpose, the pH of fully hydrated whey protein solution (60 mg mL^{-1} containing 0.1 mg mL^{-1} sodium azide as antimicrobial agent) was adjusted to 2.0 by adding HCl 8 M. Afterwards, this solution was heated in a water bath for 5 h at 85 °C under mild condition of stirring. Lastly, for stopping fibril formation, the solution was cooled with ice water and stored at 4 °C for subsequent uses. Since some non-aggregated poly-peptides existed in addition to fibrillar structures in the final solution, we address it as "fibrillated WPI" solution throughout the paper.

2.3. Atomic force microscopy (AFM)

Generation and topography of whey protein nanofibrils were investigated using an atomic force microscope (Nanowizard II instrument, JPK, Germany). Diluting (600 folds) of fibrils solutions prior to microscopy was done by distilled water with same pH (2.0 or 7.5). Then, 20 μL of diluted samples was spread onto a glass slide and subsequently allowed to be air-dried at room temperature. AFM imaging was carried out with a HYDRA-cantilever at a scan rate of 1.2 Hz. Finally, images were processed by JPK Data Processing software version 3.4.15.

2.4. Flow behavior and viscosity measurements

The rheological properties of gel pre-solutions including native WPI, heat-denatured and fibrillated WPI were measured using a rotational viscometer (Model LVDV-II Pro, Brookfield Engineering Inc., USA) at room temperature (24 ± 2 °C) with a cylindrical LV spindle. In each test, about 50 mL of samples was poured into the temperature-controlled measuring vessel and sheared from 1 to 100 s^{-1} within 3 s intervals. Power law model parameters were determined to characterize the flow behavior of solutions by following equation:

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

where η is the apparent viscosity (Pa s), K is the consistency coefficient (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless). Rheocalc software (version 3.2) was used for fitting of obtained data to the rheological model.

2.5. Preparation of divalent cation-induced cold-set hydrogels

After preparing heat-denatured and fibrillated WPI solutions (60 mg mL^{-1}) and adjusting the solutions pH to 7.5 with 1.0 M NaOH, 5 mL of samples were transferred to plastic cylindrical tubes (50 mm height and 15 mm inner diameter). Cold gelation was induced by mixing sample solutions with an appropriate volume of $CaCl_2$, $MnCl_2$ or $ZnCl_2$ stock solutions to a final concentration of 50 mM of cations (Ca^{2+}, Mn^{2+} and Zn^{2+}). Afterwards, samples were stored for 24 h at 4 °C for stabilizing gel network. Gel samples were equilibrated at 25 °C for 2 h prior to characterizations. Heat-denatured WPI solution did not form a self-supporting gel at the protein concentration investigated, hence only the gels made of fibrillated WPI were employed for subsequent experiments.

2.6. Fourier transform infra-red (FT-IR) spectroscopy

FT-IR spectra of lyophilized (Alpha1-2 Lo Plus freeze-drier, Christ Co, Germany) samples including WPI, fibrillated WPI and gels made of fibrillated WPI with different divalent cations were recorded at a wavenumber range of 4000–400 cm^{-1} using a Perkin Elmer spectrometer (FT-IR, Model Spectrum Two, Perkin Elmer Co., MA, USA). The samples were pressed in KBr pellets before spectrum acquisition.

2.7. Textural analysis of hydrogel samples

The firmness of gel samples was assessed by penetration test performed by a universal texture analyzer machine (M350-10CT, Testometric, Lancashire, UK) equipped with a stainless steel cylindrical probe (7.5 mm diameter). Gel samples (15 mm diameter and 30 mm height) were penetrated by the probe to a depth of 15 mm at a constant speed of 10 mm min^{-1}. Firmness is expressed as the maximum force (N) required to penetrate the samples.

Cylindrical hydrogel samples (13 mm diameter and 20 mm height) were subjected to the uniaxial compression to rupture test by compressing the specimens to 75% of their initial height at a constant speed of 10 mm min^{-1}. Compressive stress (σ_c), a measure of the gel strength and shear strain (ε) at fracture were calculated by using the following equations [10]:

$$\sigma_c = \frac{F}{A} \quad (2)$$

$$\varepsilon = -\ln\left(1 - \frac{\Delta H}{H}\right) \quad (3)$$

where F is force at fracture point (N), H is the initial height of uncompressed specimen (m) which fractures after ΔH (m) compression and A is the cross-sectional area of the gel samples (m^2).

2.8. Microstructure

The microstructural features of different gel samples were imaged with a scanning electron microscope (SEM) (Vega, Tescan, USA). For this purpose, small pieces of gels were fixed overnight in a solution of 2.5% glutaraldehyde and then, gradually dehydrated using a series of ethanol aqueous solutions (30, 50, 70, 90 and 100% v/v) for 1 h each time. Dehydrated samples were kept over night in 100% ethanol and coated with a thin layer of gold before imaging.

2.9. Water holding capacity (WHC)

WHC of gel samples prepared by adding different metal chlorides including $CaCl_2$, $MnCl_2$ and $ZnCl_2$ into fibrillated WPI solution was determined according to the method of Remondetto et al. [11], with some modifications. Gel samples fabricated within 15 mL tubes were centrifuged at 1000 for 10 min and WHC was calculated by the following equation:

$$WHC(\%) = \frac{W_g - W_{dw}}{W_g} \times 100 \quad (4)$$

where W_g and W_{dw} are gel weight before centrifugation and drained water weight, respectively.