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Physicochemical properties of cellulose/whey protein fibers as a potential material for active ingredients release

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ABSTRACT

Whey protein isolate – WPI/cellulose fibers were obtained combining formation of cellulose fibers with acid induced gelation of whey protein in the simultaneous process. The mechanical properties of the cellulose fibers were improved by adding whey protein. Regenerated cellulose fibrils exhibited a dense structure with voids of several micrometers pore size. The blend fibers were smoother than cellulose fibers with smaller void spaces and attached protein filaments. On the surface of the regenerated cellulose fibers, formation of hierarchical flowerlike micro/nanostructures was observed. The XRD pattern indicated that the flowerlike microstructure was composed of ammonium copper(II) sulfate hexahydrate. Regenerated cellulose alone and in the mixtures with WPI crystallized partly in the cellulose II monoclinic system. For cellulose/WPI blend reduction in –C–OH groups was observed, which was probably caused by interactions of cellulose with the protein molecules. The obtained blended cellulose/WPI fibers can have tremendous potential use in medicine, tissue engineering and as a material for active ingredients release.

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1. Introduction

Various plant and animal tissues are the sources of biomacromolecules. Proteins, polysaccharides and their compounds are a potential substrate for functional materials (Delben & Stefancich, 1998; Ye & Harte, 2014). Their application reduces environmental waste, production costs, add value to agricultural products and creates biodegradable materials (Zhang, Li, & Yu, 2011). The most common organic polymer is cellulose, representing 1.5×10^{12} tons of the annual biomass production. Cellulose derivatives found application as coatings, laminates, films, sorption media, foods, pharmaceuticals, cosmetics and additives to building materials (Klemm, Heublein, Fink, & Bohn, 2005; Shin et al., 2014; Siro & Plackett, 2010). Cellulose occurs naturally in the form of fibers and the preparation of new fibers with improved mechanical, chemical and biological characteristics has attracted the interest of scientists for many years. In particular, spinning of polymer blends allows to get high-performance composite materials and fibers (Arcidiacono et al., 2002). There is very little research done on new cellulose/protein fibers. Zhang et al. (2011) obtained blend fibers from cellulose and soy protein isolate using the NaOH/thiourea/urea aqueous solution. The addition of protein to the cellulose fiber in the concentration of 10% caused a significant increase in tensile strength and elongation at break. Blending cellulose with soy protein was found to be a useful method to introduce amino acid groups into cellulosic materials. Lee, Bae, Park, and Um (2013) obtained for the first time cellulose nanofibrils blended with silk fibroin. The results indicated that the post drawing ability deteriorated with the increasing cellulose nanofibrils content, probably because of the inhomogeneous dope solution.

Whey proteins are the most common used protein sources for food supplementation. Gelation of these proteins has been very intensively investigated for the last 25 years (Morr & Foegeding, 1990). Apart from traditional heat-induced gelation, the so-called cold gelation process was invented (Barbut & Foegeding, 1993). This process consists of two consecutive steps. In the first step, protein dispersion is pre-heated and soluble aggregates are formed. In the second step gelation is induced by the addition of salts or by lowering the pH (Alting, Hamer, de Kruif, & Visschers, 2000). An acid-induced cold-set gel can be formed by mineral acids, organic acids or by addition of glucono-δ-lactone. Salt-induced whey

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protein gels were lately used a matrices for an active ingredients release (Tomczyńska-Mleko & Mleko, 2014). Aeration process was used to create porous structure with different active ingredients release time. Each year new functional applications for whey proteins are found. Lately whey protein nanoparticles were formed to improve encapsulation of the ingredient which are volatile or susceptible to decomposition (Yi, Lam, Yokoyama, Cheng, & Zhong, 2015). Microspheres created by self-assembly of β-lactoglobulin and egg protein lysozyme were developed as a potential carrier for nutraceuticals (Diarrassouba et al., 2015). Ryan and Foegeding (2015) formed soluble whey protein aggregates used to produce thermally and gravitationally stable beverages. New products for sportsmen and physically active people were developed based on functional properties of whey protein: carbonated beverage with whey protein and creatine and a supplement combining coconut water with whey protein (Tomczyńska-Mleko, 2014a, 2014b).

The longest known solvent for cellulose is the Schweizer's reagent, tetraamminecopper(II) hydroxide, known as “cuoxam”. This reagent was discovered in 1857 and has been used for many years in the cupraammonium process (Schweizer, 1857). Still the alkaline modification is used today, in particular for the preparation of membranes. In polymer analysis both cuoxam and “cuen” (copper(II) hydroxide in aqueous ethylenediamine (en)) methods are used for determination of cellulose polymerization degree (Burchard, Habermann, Klüfers, Seger, & Wilhelm, 1994). In the “cuoxam” method the cellulose fiber is formed in 33% sulfuric acid solution.

Lately Ahmadi, Madadlou, and Sabouri (2015) fabricated whey protein cold-set gels loaded with cellulose particles. Cellulose crystals weakened the texture of whey protein gels. There is no report in the literature on mixed cellulose/whey protein fibrils. The aim of this research was to combine for the first time formation of cellulose fibers with acid induced gelation of whey protein in the simultaneous process. Mixed cellulose/whey protein co-polymers have been formed by dissolving cellulose in tetraamminecopper(II) hydroxide and subsequent fibers forming in 33% sulfuric acid solution. Rheological properties of obtained cellulose/whey protein co-polymers have been investigated. The structure of cellulose/WPI fibers has been evaluated using scanning electron microscopy, transmission electron microscopy, X-ray diffraction and IR-Raman spectroscopy.

2. Materials and methods

2.1. Materials

Whey Protein Isolate (WPI) (88.0% protein) was purchased from Arla Foods Ingredients (Viby, Denmark). WPI composition was determined following methodology for total nitrogen (Kjeldahl), fat (Soxhlet), lactose, water and ash (gravimetrically) using the AOAC methods (AOAC, 1990). Mineral analysis was performed by an atomic absorption spectrometry using a Varian Spectra 280 FS (Varian, Inc., Palo Alto, USA). WPI composition is presented in Table 1. Concentration of Cu^{2+} ions in the mixed cellulose/WPI fibers was evaluated by an atomic absorption spectrometry using a Varian Spectra 280 FS (Varian, Inc., Palo Alto, USA). Cellulose (MN-Cellulose powder 300, average particle size − 10 μm) was obtained from Macherey, Nagel & Co, Düren (Germany).

2.2. Preparation of protein solution and Schweizer's reagent

Whey protein isolate dispersion (8.5% protein w/w) was made by hydrating in distilled water and mixing using a magnetic stirrer. Dispersions were heated in water bath for 30 min at 80 °C. After heating the dispersions were cooled down in tap water. 100 cm^3 of ice-cooled 25% aqueous ammonia solution was added to 5 g of $Cu(OH)_2$ (Aldrich, technical grade). The solution was stirred for about 1 h and then used for cellulose dissolution. 1.0 g of cellulose was added to 30 g of Schweizer's reagent and mixed for 30 min. After this time 15 g of distilled water was added or 15 g of preheated whey protein isolate dispersion was added. The blend contained 2.17% of cellulose and 2.77% of WPI. After dissolution, the flask was opened to insert a vacuum desiccator to vent the solution for about 15–20 min using a vacuum pump.

Table 1
Chemical composition of whey protein isolate.

Ingredient	Concentration
Protein	88.0%
Water	5.5%
Ash	4.2%
Fat (Soxhlet)	0.1%
Lactose	0.1%
Na	0.54%
K	1.34%
Ca	0.05%
Mg	0.03%
P	0.24%
Cl	0.05%
Cu	2.2 ppm
Fe	17 ppm
Pb	0.29 ppm
Cd	0.05 ppm
As	0.02 ppm

2.3. Fibers formation

Deaerated cellulose, cellulose/WPI or preheated WPI solution was put into a syringe with a needle and injected slowly to a solution of 33% sulfuric acid. During the injection the dispersion precipitated into a fiber which was wound onto a baguette. The fiber was rinsed with distilled water, 5% solution of ammonia and again with distilled water. For rheological measurements discs (35 mm diameter and about 2–3 mm thick) were obtained and measured after an access of water was removed using filter paper. The fibers were dried for 15 h at 40 °C.

2.4. Dynamic oscillatory measurements

Rheological properties were measured using RS300 (Thermo-Haake, Karlsruhe, Germany) rheometer with a serrated parallel steel plate geometry (35 mm diameter, 2 mm gap size) to limit the potentiality of sliding effects. Precipitated discs were analyzed by frequency sweeps in the 0.1–10 Hz range in the linear viscoelastic region (at 0.01 strain evaluated previously by strain sweeps).

2.5. Scanning electron microscopy (SEM)

SEM images were obtained using a scanning electron microscope VEGA II LMU (Tescan, Canberra, USA). Samples of the fibers were fixed by immersion in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer. The samples were dehydrated in serial dilutions of ethanol and acetone and dried at the critical point in liquid carbon dioxide. Preparations were coated with gold using a vacuum evaporator EMITECH K550x (Emitech, Ashford, United Kingdom).

2.6. Transmission electron microscopy (TEM)

TEM images were obtained using FEI Tecnai Spirit G2 microscope (FEI, The Netherlands) at an acceleration voltage of 120 kV.

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