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Edible coating based on whey protein isolate nanofibrils for antioxidation and inhibition of product browning

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ABSTRACT

Whey protein isolates (WPI) have been studied for film formation because of their favorable functional properties and industrial surplus; however, little is known regarding whey protein nanofibrils (WPNFs) as raw material for edible films. In the present study, WPNF-based edible coatings plasticized with glycerol (Gly) and trehalose (Tre) were studied. WPI (5%) can self-assemble into nanofibrils at 80 °C for 10 h under 220 rpm constant magnetic stirring. Compared with that of WPI, the hydrophobic and antioxidant activities of WPNFs were significantly increased. The microstructures and functions of WPI/Gly, WPNF/Gly, and WPNF/Gly/Tre films were characterized by scanning electron microscopy and atomic force microscopy, and by analyzing their contact angles, film thickness, transparency, moisture content, and solubility in water. Results showed that the formation of WPNFs increased surface smoothness, homogeneity, continuity, hydrophobicity, and transparency, and decreased the moisture content and water solubility of the films. To evaluate the performance of the edible coating, coated fresh cut apples were analyzed during storage. We found that the coatings containing WPNF (5%, w/v), Gly (4%, w/v) and Tre (3%, w/v) afforded the best protective action toward retarding the total phenolic content, browning, and product weight loss. Overall, the low cost, high biocompatibility, and consumer acceptance in taste tests support the ready application of these optimized WPNF films for commercial purposes.

1. Introduction

The fresh-cut market trend has increased the demands to the food industry for seeking new strategies to increase the storability and shelf life and to enhance the microbial safety of fresh produce (Martins, 2015). The technologies of edible films (EFs) and coatings (ECs) have been considered as potential approaches for meeting this demand. An EF constitutes a preformed thin layer, made from edible components, which, once formed, can be placed on or between food components, whereas ECs comprise a thin layer of edible material formed as a coating on a food product (Fakhouri, Martelli, Caon, Velasco, & Mei, 2015; Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2016); generally these, as part of the food, can be eaten along with the products (Kuorwel, Cran, Orbell, Buddhadasa, & Bigger, 2015; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). Owing to the beneficial characteristics of edibility and biodegradability, EFs and ECs can be used to complement or replace traditional materials in order to reduce traditional polymeric packaging, the majority of which are of petrochemical origin (Fakhouri et al., 2015). Therefore, EFs and ECs are currently receiving much attention.

ECs can keep fruits and vegetable fresh by improving the retention of color, acid, sugar, and flavor components to extend shelf life and retain nutritional quality (Fakhouri et al., 2015; Kerch, 2015). Biopolymers such as polysaccharides, proteins, and lipids can be used for the formation of EFs and ECs (Jahed, Khaledabad, Almasi, & Hasanzadeh, 2017; Martelli et al., 2017; Niamlang, Tongrain, Ekabutr, Chuysinuan, & Supaphol, 2017; Schmid, Sängerlaub, Wege, & Stäbler, 2015). Among these, protein-based materials appear to be most attractive because they also provide nutritional value (Galus & Kadzińska, 2016). In particular, whey protein isolates (WPI) can be made into flexible and transparent films with excellent barrier function for gas, aroma compounds, and oil as compared to the films made with polysaccharides and lipids. However, these films have low mechanical properties and high water vapor permeability owing to the high degree of hydrophilic amino acids in their structure (Umaraw & Verma, 2017).

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To produce dense and strong films with good mechanical resistance that constitute an excellent barrier, physical, chemical, or enzymatic protein denaturation is required. Notably, WPI can self-assemble into semiflexible amyloid-like protein nanofibrils (WPNFs) when heated at low ionic strength and low pH (Loveday, Su, Rao, Anema, & Singh, 2011, 2012). Upon the formation of WPNFs, the properties such as gelation, emulsification, foam stability, antioxidant activity, and digestibility become significantly improved, yielding a class of high-performance biomaterials (Adamcik et al., 2010; Kroes-Nijboer, Venema, & Van, 2012; Loveday, Su, Rao, Anema, & Singh, 2012; Mohammadian & Madadlou, 2016). Furthermore, fibrils can form entanglement networks that increase bulk viscosity with relatively little protein, which makes them potentially economical ingredients for modifying texture in food and biomedical products. However, although WPNFs exhibit excellent gelation, little information is available regarding the use of WPNFs as raw material for EFs. In this sense, the application of WPNFs may provide new approaches for EF and EC generation.

In addition, to reduce film brittleness and increase flexibility, extensibility, toughness, and tear resistance, hydrophilic plasticizers are generally added to the film-forming protein dispersion (Ramos et al., 2013). Plasticizers lead to decrease hydrogen bonding between polymers and increase intermolecular spacing, thus improving flexibility, extensibility, toughness, and tear resistance of the film (Osés, Fernández-Pan, Mendoza, & Maté, 2009; Schmid, 2013; Talens & Krochta, 2010). Glycerol (Gly) is the most widely used plasticizer for whey protein films, providing control of moisture transfer, respiration rate, and oxidation processes, and extending shelf life (Han, Hwang, Min, & Krochta, 2008; Osés et al., 2009; Silva, Garcia, Amado, & Mauro, 2015). Hence, Gly was chosen as the plasticizer in the present study.

Numerous studies have been published regarding modification of the whey protein film structure by addition of active ingredients such as tocopherol, ascorbic acid, and trehalose (Tre) for improving the functional properties of edible films (Albanese, Cinquanta, & Di Matteo, 2007; Giosafatto et al., 2014). Tre, as a natural generally recognized as safe (GRAS) molecule, has been used mainly to preserve the aroma and color of dried fruits, and can decrease the biological activity in vegetables owing to its properties of water replacement, glass transformation, and chemical stability (Albanese et al., 2007; Komes, Lovrić,Ganić, Kljusurić, & Banović, 2005; O'Donnell & Kearsley, 2012). Accordingly, Tre is suitable to be used as a food additive to facilitate protein dispersion, thus forming a coating on the fruit surface.

Hence, the objective of this study was to develop WPI into WPNFs for ECs incorporated with Gly and Tre to enhance the quality of fresh-cut apple slices and to evaluate the effect of the films with respect to antioxidant, barrier, and surface properties. The film microstructures were also observed, for which the main chemical and physical parameters during storage at 4 °C were monitored.

2. Materials and methods

2.1. Materials

WPI (>91.5%) was purchased from Hilmar Industries (Hilmar, CA, USA). Treha, 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Aladdin Reagent Co. (Shanghai, China). 2-[4-(dimethylamino) phenyl]-3,6-dimethylbenzothiazolium chloride (ThT) and Congo Red were purchased from Sigma (St. Louis, MO, USA). 1-anilinonaphthalene-8-sulfonate (ANS) was purchased from Fluka (Buchs, Switzerland). Apples from the “Fu shi” cultivar were purchased from a local market (Harbin, China) in January 2017.

2.2. Preparation and characterization of WPNFs

2.2.1. Preparation of WPNFs

WPNFs were prepared according to a modified method described in our previous study (Liu, Feng, Feng, & Li, 2016). WPI was dissolved in deionized water at 5% (w/v). The pH of the solution was adjusted to 2.0 with 3 M hydrochloric acid and the mixture was stirred at room temperature for 30 min, and then centrifuged at 9000×*g* for 15 min at 4 °C, (Z236HK Hermle, Wehingen, Germany). The supernatant was vacuum-filtered with fiber membrane (0.45-μm, Aladdin, Shanghai, China) to remove undissolved proteins. WPNFs were obtained by incubating the filtrate at 80 °C for 10 h under 220 rpm constant magnetic stirring.

2.2.2. Negative stain transmission electron microscopy (TEM)

WPNF samples were ultrafiltrated using a method reported previously to reduce the background in TEM images (Bolder, Vasbinder, Sagis, & van der Linden, 2007). WPNF solution (100 μL) was added to 4 mL HCl at pH 2.0 in a centrifuge filter with nominal cutoff of 100 kDa and previously washed with HCl solution (pH 2.0). The filter was centrifuged at 3000×*g* for 15 min and the retentate topped with 4 mL HCl solution (pH 2.0). Filtration was carried out three times in total, and the final retentate was transferred to a 1.5-mL plastic tube with 1 mL of HCl at pH 2.0. The final dilution of WPNFs was approximately 10-fold.

WPNFs were then transferred to a special copper mesh on a carbon film, allowed to stand for 20 min, and touched against filter paper to soak away excess sample. A droplet of 2% uranyl acetate was then added to the dried copper and held for 8 min, any excess being removed as before. Electron micrographs were observed using TEM (H-7650 transmission electron microscope, Hitachi, Tokyo, Japan).

2.2.3. Thioflavin T (ThT) fluorescence assay

ThT fluorescence analysis was performed according to Biancalana, Makabe, Koide, and Koide (2009). A stock solution of 3.0 mM ThT in phosphate-NaCl buffer (10 mM phosphate and 150 mm NaCl, pH 7.0) was filtered through a 0.22-μm syringe filter. Working solution was prepared by diluting the ThT stock solution 50-fold in phosphate-NaCl buffer. In the assay, 48 μL of the test sample was mixed with 4 mL ThT working solution, vortexed for 1 min, and analyzed using a PE LS-55 Fluorescence Spectrometer (Perkin Elmer, Boston, MA, USA). Each scan was repeated 5× at a 412-nm excitation wavelength, 10 nm emission slit width, 10 nm excitation slit width, 200 nm/min scanning speed, and 470–600 nm scan range.

2.2.4. Determination of surface hydrophobicity

ANS (8-anilino-1-naphthalenesulfonic acid)-based measurement of surface hydrophobicity is the most appropriate way to assess proteins and determine the overall three-dimensional structure in solution (Cardamone & Puri, 1992). ANS spectroscopy was performed according to the method described in our previous study (Liu, Liu, Feng, & Li, 2014). The WPNF solution was gradually diluted to 0.005–0.1% with 0.1 M phosphate buffer (pH 7.0). ANS working solution (8 mM ANS and 0.01 M phosphate buffer at pH 7.0) was mixed with the simple at a concentration of 17:1 and the mixture was shaken for 3 min. The fluorescence spectra were obtained using a LS-55 fluorescence spectrometer. The excitation wavelength was 370 nm, the emission spectrum was 400–600 nm, with 10 nm emission slit width, 10 nm excitation slit width, and 500 nm/min scanning speed. The protein concentration was determined using Folin phenol reagent according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Surface hydrophobicity can be calculated from the initial slope of the