Carbohydrate Polymers 198 (2018) 225–232

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Antibacterial activity of PEO nanofibers incorporating polysaccharide from dandelion and its derivative

Lin Lin[a], Yulin Zhu[a], Changzhu Li[b], Lei Liu[c], Duraiarasan Surendhiran[a], Haiying Cui[a,*]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Department of Bioresource, Hunan Academy of Forestry, Changsha 410007, China
[c] Institute for Advanced Materials, Jiangsu University, Zhenjiang 212013, China

ARTICLE INFO

Keywords:
Dandelion
Polysaccharide
Carboxymethylation
Antibacterial activity
Nanofiber

ABSTRACT

A water-soluble antibacterial polysaccharide from dandelions (PD) was chemically modified to obtain its carboxymethylated derivative (CPD). The degree of substitution of CPD was 0.455. Fourier transform infrared (FTIR) spectra analysis, zeta potential, particle size and rheological test verified the carboxymethylation of PD, accompanying with the change of physicochemical properties. Moreover, *Listeria monocytogenes* treated with 10 mg/mL PD and CPD achieved 1.96 and 3.29 log CFU/mL reduction in population, respectively. Subsequently, PD and CPD were incorporated into polyethylene oxide (PEO) nanofiber matrix to fabricate antimicrobial nanofibers. The prepared nanofibers were characterized by scanning electron microscope, atomic force microscope and FTIR. Finally, both PD/PEO and CPD/PEO nanofibers exhibited favourable antibacterial effect on *L. monocytogenes*, with an improved antibacterial activity of CPD/PEO nanofibers than PD/PEO nanofibers. In conclusion, this study demonstrated PD and CPD could be applied to the fabrication of antibacterial food packaging.

1. Introduction

Pathogen contamination of instant foods without appropriate packaging methods is the main reason for food deterioration and spoilage which results in the loss of nutrition and flavor. *Listeria monocytogenes* is one of the most common food-borne pathogenic bacteria. Once the *Listeria monocytogenes* contaminated foods are ingested by people, especially those with low immunity, severe food-borne illness such as meningitis, septicemia, and diarrhea etc., are likely to be induced (Cui, Wu, Li, & Lin, 2017a). However, due to the capability of surviving and adapting to adverse environments such as low moisture, high salinity, presence of chemical substance and even refrigeration condition, it is hard to completely eliminate the growth of *L. monocytogenes* during food processing and packaging. As a consequence, 94% hospitalization and fatality rate caused by bromatoxism are associated with *L. monocytogenes* contamination (Lin, Zhang, Zhao, & Cui, 2016). In this regard, there is an urgent need to develop an effective food packaging method to restrain the growth of *L. monocytogenes* and increase the shelf life of packaged foods.

In recent years, electrospinning technique has gained increased attentions as a promising technique in food packaging industries due to its ability to produce functional nanofibers and nanofibrous matrix (Monteiro et al., 2015). Electrospun nanofibers have many unique properties such as high surface area, nanoporous structure, excellent mechanical properties and easy encapsulation of functional components due to their nanoscale diameter (Wen et al., 2016). With the frequent occurrence of food safety incidents caused by pathogen contamination, the electrospun nanofibers with antibacterial activity are imminently required in food packaging industries. The antibacterial electrospun nanofibers can be processed by incorporating a certain antimicrobial substance into a polymer nanofibrous matrix through electrospinning process (Aytac, Yildiz, Kayaci-Senirmak, Tekinay, & Uyar, 2017). Numerous polymers such as polyethylene oxide (PEO), polylactic acid and polyglycolic acid have been successfully applied in the fabrication of nanofibers matrix through electrospinning process (Cui, Wu, Li, & Lin, 2017b). However, many chemical antiseptics are soluble in organic solvents. If they were used to spin antimicrobial nanofibers in food packaging, thus public health may be threatened. Therefore, water-soluble antimicrobial agents with natural, nontoxic and biodegradable properties are preferred to produce antibacterial electrospun nanofibers for the concerns of food safety and human health.

Dandelion, as a valuable herbal medicine natived to the Northern hemisphere, has been recorded in many Chinese traditional pharmacopoeias for its diuretic, anti-rheumatic and anti-inflammatory properties (Schutz & Carle, 2006). Dandelions contain many active ingredients, of which polysaccharides extremely possess of high

* Corresponding author.
E-mail address: cuihaiying@ujs.edu.cn (H. Cui).

https://doi.org/10.1016/j.carbpol.2018.06.092
Received 3 February 2018; Received in revised form 12 June 2018; Accepted 20 June 2018
Available online 21 June 2018
0144-8617/

commercial values. Polysaccharides from dandelions (PD), as a kind of water-soluble bioactive components, have been proved with predominant anti-oxidant, anti-diabetic, anti-coagulation, human immunity-enhancing properties (Cai, Wan, Yi, & Luan, 2017) and antibacterial effects (Wang, 2014). Recent studies reported that PD could obviously enhance androgenic hormone, playing the effect of hepatic protective role, insulin stimulant, cure indigestion, anticancer as well as antioxidant (Li et al., 2017). Moreover, the active hydroxyl groups on the PD backbone provide excellent active sites for chemical modifications, thereby helping in introducing some changes in PD properties.

Chemical modifications of polysaccharides to produce polysaccharide derivatives have catched increasing concerns from both research and practical point of view. Many previous researches have revealed that specific polysaccharide derivative such as sulphated, carboxymethylated and acetylated polysaccharides exhibited better functional performances than unmodified polysaccharides. For instance, Li et al. (2017) found that cholesterol-lowering activity of the polysaccharide extracted from *Morchella angusticepes* Peck was enhanced after carboxymethylation. Tan, Li, Dong, Chen, and Guo, (2017) reported that improved antifungal properties were observed when novel cationic chitosan derivatives were loaded with quaternary ammonium and phosphonium salts. Another recent study conducted by Zhang, Wu, Kang, Yu, and Liu, (2017) demonstrated that sulphated derivative of *Aconitum coreanum* polysaccharide possessed better inhibitory effect against human breast cancer.

Herein, this is the first study to ever report the antibacterial effect of carboxymethylated PD (CPD). Besides, the antibacterial nanofibers were fabricated by incorporating PD and CPD as antimicrobial substances into the PEO nanofibrous matrix, followed by a series of characterization analysises. As a proof of concept, the antibacterial activity of nanofibers against *Listeria monocytogenes in vitro* was evaluated as well.

2. Materials and methods

2.1. Materials

Polysaccharide from dandelions (PD) was purchased from Ciyuan Biotech Co., Ltd (Shanxi, China). Chloroacetic acid was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Polyethylene oxide (PEO) with molecular weight of 900,000 was purchased from Zhengzhou Jinlong Chemical Co., Ltd (Zhengzhou, China). *L. monocytogenes* was provided by China Center for Industrial Culture Collection (Beijing, China). This strain was inoculated in the peptone-yeast-glucose (PYG) broth and cultured at 37 °C for 48 h in a orbital shaker to obtain log-phase bacteria. All other reagents were purchased from commercial suppliers and were analytical grade.

2.2. Carboxymethylation of PD

Carboxymethylation of PD was carried out according to the method recorded by Leite et al. (2017) with slight modifications. In brief, 2 g of PD was dissolved in 20 mL NaOH solution (30%, w: w) and stirred until complete dissolution, then the mixture was placed in a shaker for 2 h at 40 °C. Subsequently, 1.5 g of chloroacetic acid dissolved in 20 mL ethanol (75%) was added into above mixture and allowed them to react at 40 °C for 3 h in a shaker. After the reaction, the resulted mixture was neutralized to pH 7 using 90% acetic acid solution. Finally, the mixture was dialyzed against tap water for 48 h and distilled water for 24 h, concentrated, precipitated by ethanol overnight and freeze-dried to make carboxymethylated PD (CPD).

2.3. Determination of degree of substitution

The degree of substitution (DS) of CPD was determined by the method of De Paula, Heatley and Budd, (2015) with slight modifications. In brief, 10 mg of CPD was desiccated at 100 °C for 1 h then cooled to room temperature. Subsequently, the desiccative CPD was added into 3 mL of ethanol solution(70%, v: v) and stood for 5 min. After that, the mixture was diluted with 10 mL distilled water and 50 mL of 0.5 mol/L NaOH solution, accompanying by vibration until the mixture was absolutely dissolved. Then the residual NaOH was titrated against 0.1 mol/L HCl using phenolphthalein as an indicator.

2.4. Characterizations of PD and CPD

2.4.1. Fourier transform infrared spectrum (FTIR)

Characteristic features of PD and CPD were detected by an infrared spectrometer (IR Prestige-21, Shimadzu Corporation., Tokyo, Japan). One milligram of PD or CPD was blended with 100 mg KBr and pressed into a disc. FTIR spectra of PD and CPD were obtained in the range of 400-4000 cm^{-1} under the resolution of 4 cm^{-1} from an average of 16 scans using the transmission mode.

2.4.2. Particle size, zeta potential and polydispersity index of PD and CPD

Particle size, zeta potential and polydispersity index (PDI) of PD and CPD solutions were measured using a dynamic light scattering zetasizer (Nano ZS90, Malvern Instruments, Malvern, UK). The measurements were performed in triplicate.

2.4.3. Analysis of rheological properties

The rheological behaviors of PD and CPD were monitored by a stress controlled rheometer (Bohlin Instruments, Inc., Cranbury, NJ, USA) with a parallel-plate geometry (40 mm diameter; 2 mm gap) using the strain controlled mode. Samples were loaded on the plate and the exposed edge was sealed with liquid paraffin to minimize evaporation. Samples were heated from 25 °C to 85 °C at the rate of 5 °C/min. The temperature scanning test was performed in an oscillatory mode at a fixed frequency of 1 Hz with a maximum strain of 2%. The storage modulus (G') and loss modulus (G'') were measured as a function of temperature over triplicate runs to investigate dynamic rheological properties of different samples.

2.4.4. Time-killing analysis of PD and CPD against L. monocytogenes

Time-killing analysis was carried out by the method of our previous study (Cui, Yuan, Li, & Lin, 2017) to compare the antibacterial ability of PD and CPD against *L. monocytogenes*. 10 mg/mL of each PD and CPD were added into phosphate buffer solution (PBS, 0.03 M, pH 7.2) containing 10^5−10^6 CFU/mL of test bacterial suspension in two tested tubes. The bacterial suspension without PD or CPD treatment was considered as a control. All samples were incubated at 37 °C and the residual bacteria were counted by the plate count method every day for 3 days. All the experiments were performed in triplicate.

2.4.5. Transmission electron microscopy (TEM)

The morphological changes of *L. monocytogenes* treated with PD or CPD was detected by a transmission electron microscopy (TEM) (Model-JEM-2100, JEOL, Tokyo, Japan). The *L. monocytogenes* samples were separately dyed with 3% (v/v) phosphotungstic acid and dried, a microscopic observation was then carried out to obtain the micrographs (Cui, Zhang, Zhou, Zhao, & Lin, 2015).

2.5. Preparation of electrospinning nanofibers

In order to incorporate PD or CPD into nanofiber matrix, PEO was selected as the nanofiber-forming substance to prepare PD/PEO and CPD/PEO nanofibers. In brief, 3% (w: v) total amounts of PD/PEO (1: 1) and CPD/PEO (1: 1) mixtures were dissolved in a 20 mL deionized water to prepare spinning solutions (Cui, Wu, Wu et al., 2017). Subsequently, spinning solutions were transferred into a syringe with a needle tip of 0.50 mm inner diameter. The needle was placed vertically at 15 cm above a collecting plate coated with an aluminum foil and