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Electrospun thyme essential oil/gelatin nanofibers for active packaging against *Campylobacter jejuni* in chicken

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ABSTRACT

Campylobacter jejuni (*C. jejuni*) contamination on poultry surface posed great threats to meat industry and human health. In order to control the propagation of *C. jejuni*, the gelatin nanofibers containing thyme essential oil/β-cyclodextrin ε-polylysine nanoparticles (TCPNs) were engineered. Firstly, TCPNs were successfully fabricated via ionic gelation. The prepared TCPNs showed excellent antimicrobial activity against *C. jejuni*, which caused membranolysis and protein leakage of *C. jejuni*. Subsequently, TCPNs were incorporated into gelatin nanofibrous matrix via electrospinning to produce antimicrobial nanofibers. Uniformly disordered fibrous structure with good continuity and fine diameter distribution was observed. Finally, the aerobic bacterial count, thiobarbituric acid, total volatile basic nitrogen, pH, color and texture of meat samples packaged with the nanofibers were detected. The results implied the TCPNs embedded gelatin nanofibers had a promising prospect in meat preservation without impact on sensory evaluation.

1. Introduction

Poultry and their further products are prevalent foods in our daily life due to their abundant nutrition and satisfactory flavor. However, the prevalence of poultry also provides the ideal seminary for the infections of food-borne pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella* and *Campylobacter jejuni* (*C. jejuni*) etc. *C. jejuni* is a zoonotic pathogen that exists as a symbiotic microorganism in the alimentary canal of the poultry (Agunos, Waddell, Leger, & Taboada, 2014). These *C. jejuni*, which are harbored by the alimentary canal of poultry, may leak and burst through external channel during the slaughter process of poultry, resulting in the carcass contamination of meats. The *C. jejuni* in contaminated meat is reckoned as the primary causative agent of human campylobacteriosis, accounting for 8.4% of the diarrheal diseases expressed as 7.5 million DALY (Disability Adjusted Life Years) (Umaraw, Prajapati, Verma, Pathak, & Singh, 2017). Unfortunately, *C. jejuni* on these contaminated meats may not be inactivated completely during processing due to its high antimicrobial resistance rates against some frequently-used antibiotic including cephalosporins, quinolones and gentamicin etc (Silva, Targino, Mendonça, Sant'Ana, & Hungaro, 2017). As a consequence, 214779 and 135000 *Campylobacter* cases were respectively reported in the European Union and USA in 2014, posing a great threat to human health and huge loss in money (Skarp, Hanninen, & Rautelin, 2016). Therefore, a safe and natural antibacterial substance that can not induce resistance of bacteria should be utilized to replace chemical antibiotic in meat industries.

Thyme essential oil (TEO), a plant secondary metabolite with natural, safe and non-toxic properties, is refined from *thymus vulgaris*. TEO has been proved with excellent antibacterial effect against a broad-spectrum of bacteria including *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Salmonella* (Cui, Ma, Li, & Lin, 2016). Nevertheless, undesirable deficiencies such as volatility, hydrophobicity and special flavor are associated with the TEO when exposed to air condition in its free form, which limits its application in food preservation. Such deficiencies can be overcome by encapsulating TEO into β-cyclodextrin (β-CD) using a co-precipitation method (Samperio et al., 2010).

However, the electroneutral nature of β-CD restrains the potential of TEO/β-CD inclusion complex as antibacterial agent against *C. jejuni*, because it can not absorb onto negatively charged bacterial cell wall. This problem can be solved by the adsorption of cationic biopolymers onto the surface of TEO/β-CD inclusion complex via ionic gelation (Pant & Negi, 2018). Herein, ε-polylysine (ε-PLY), a cationic biological metabolite with excellent antibacterial effect, as well as biodegradability, biocompatibility and biosecurity is selected to prepare TEO/β-CD ε-polylysine nanoparticles (TCPNs) (Lu, Zou, Xu, & Li, 2018). Furthermore, the existence of $-NH_2$ along the ε-PLY chains assists the binding of nanoparticles onto negatively charged bacterial cell wall,

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which accelerates the apoptosis of bacterial cells (Muhammad et al., 2017).

Since *C. jejuni* mainly contaminates the surface of meats and meat products, there is an imperative need to employ antimicrobial packaging to suppress the reproduction of *C. jejuni* on meat surface. Making use of electrospinning technique to produce antibacterial nanofibers gains increased attentions in antimicrobial packaging, due to the porosity, high surface to volume ratio and design flexibility of the resulting nanofibers (Aytac, Yildiz, Kayaci-Senirmak, Tekinay, & Uyar, 2017). Antibacterial nanofibers can be obtained by incorporating TCPNs into polymer matrix using electrospinning technique. Up to now, various synthetic polymers such as polyethylene oxide (PEO), polylactic acid (PLA) and polyvinyl alcohol (PVA) have been applied as fiber-forming substances (Manikandan, Mani, Jaganathan, Rajasekar, & Jagannath, 2017). In comparison with these synthetic polymers, gelatin, as a natural biomolecular polymer extracted from connective tissue in animals, is preferred due to its higher biosecurity.

Based on above-mentioned reasons, thyme essential oil was selected as the antibacterial agent to prepare TCPNs in this study. Subsequently, TCPNs were incorporated into gelatin nanofiber matrix to fabricate TCPNs embedded gelatin nanofiber (TEGN). The morphology and antibacterial effect of TEGN against *C. jejuni* were investigated as well.

2. Materials and methods

2.1. Materials

Thyme essential oil was purchased from J.E International (Caussols, France). β-cyclodextrin was provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). ε-polylysine was bought from Silver-Elephant Bio-engineering Co., Ltd (Zhejiang, China). Gelatin was obtained from Henan Boyang Gelatin Co., Ltd (Henan, China). Columbia blood agar medium (CBA) was purchased from Haibo Biotechnology Co., Ltd (Qingdao, China). *Campylobacter jejuni* ATCC 33291 was obtained from China Center for Industrial Culture Collection (Beijing, China).

2.2. Preparation of TEO/β-CD ε-polylysine nanoparticles (TCPNs)

2.2.1. Preparation of TEO/β-CD inclusion complex

The TEO/β-CD inclusion complex was obtained using a co-precipitation method established by Celebioglu and Uyar (2010) with slight modifications. In brief, 8 g of β-CD was added into 80 mL of deionized water and maintained at 60 °C for 2 h under stirring. Subsequently, 1.2 mL of TEO/ethanol (1:1, v:v) mixed solution was added onto above β-CD solution in dropwise to make the final ratio of TEO: β-CD (w:w) at 1:8, and maintained at 50 °C for 2 h under continuous agitation. After that, the resulting mixture was sonicated at 100 W, 20 °C for 30 min. Finally, after cooling the mixture to room temperature, the refrigerant solution was placed in a refrigerator at 4 °C overnight. The precipitated TEO/β-CD was acquired by vacuum-filtration using 0.45 μm microfiltration membranes, and the precipitate was washed with deionized water and ethanol for several times, heated in a vacuum oven at 40 °C until constant weight. The dry powder collected was named as TEO/β-CD inclusion complex.

2.2.2. Preparation of TEO/β-CD ε-polylysine nanoparticles

The processing method of TEO/β-CD ε-polylysine nanoparticles was carried out by an ionic gelation method (Pant & Negi, 2018). In general, TEO/β-CD inclusion complex (1 g) and sodium tripolyphosphate (25 mg) were added into a 50 mL deionized water and stirred for 60 min. The electronegative phosphate group in sodium tripolyphosphate can be used as a bridge ion between ε-polylysine and β-CD inclusion complex. Afterwards, ε-polylysine (2 mg/mL in deionized water) was added into the above mixed solution dropwise (1 mL/min) and was continuously stirred for another 60 min. The resulting mixture was regarded as TEO/β-CD ε-polylysine nanoparticles (TCPNs) suspension.

2.3. Fourier transform infrared spectroscopy (FTIR)

The functional groups and chemical structure of TEO, β-CD, ε-polylysine, TCPNs, gelatin nanofibers and TCPNs embedded gelatin nanofibers (TEGNs) were analysed by an infrared spectrometer (Nicolet is50, Thermo Electron Corporation., Massachusetts, USA). FTIR spectrogram was obtained in the range from 650 to 3500 cm^{-1} under the resolution of 4 cm^{-1} from an average of 16 scans.

2.4. Antibacterial activity

The antibacterial effect of TCPNs against *C. jejuni* in chicken soup was evaluated using a previous method (Cui, Zhao, & Lin, 2015). Commercially available chicken was added into 2 times volume of distilled water and blended into slurry. Then the slurry was filtered through 4 layers gauze and the filtrate was sterilized by an autoclave at 121 °C for 30 min. Subsequently, the prepared TCPNs (1%, v:v) were added into above chicken soup containing 10^{4-5} CFU/mL *C. jejuni*. The samples without treatments were regarded as control, and samples treated with equivalent TEO (0.4 mg/mL), TEO/β-CD inclusion complex and ε-polylysine (0.02 mg/mL) were tested for comparisons. All samples were cultured in an anaerobic jar with a micro-aerobic bag at 40 °C. The residual population of *C. jejuni* was monitored every day for 5 days (to ensure the maximum activity of *C. jejuni*) using Columbia blood agar medium by the plate count method.

The observations of inhibition zone of TCPNs against *C. jejuni* were also performed to assess the antibacterial effect. In general, 100 μL of bacterial suspension (10^{5-6} CFU/mL) of *C. jejuni* was inoculated and spread on the Columbia blood agar medium. The filter paper was cut into circular (0.8 cm in diameter) and preliminarily sterilized under UV radiation for 2 h, then placed in the middle of the plates. Drawing 2 drops of TCPNs (1%, v:v) suspension onto one of the circular filter paper, the other one without any treatment was regarded as the control. The plates were cultured in an anaerobic jar with a micro-aerobic bag at 40 °C for 48 h. The antibacterial effect of TCPNs was measured by the zone of inhibition against *C. jejuni*.

2.5. Antimicrobial mechanism

2.5.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to detect the soluble protein content and protein leakage from *C. jejuni* (Wang, Chang, Yang, & Cui, 2015). Log-phase bacteria suspension of *C. jejuni* was treated with 1% (v:v) TCPNs, samples without treatment were seen as control. All samples were centrifuged at 5000 rpm, 4 °C for 10 min, the precipitated *C. jejuni* were rinsed and re-suspended in 10 mL of sterile water, sonicated at 150 W for 5 min to break the cells, boiled for another 8 min, finally again centrifuged at 5000 rpm, 4 °C for 10 min to collect the soluble protein of *C. jejuni*. 100 μL of the collected sample was mixed with 25 μL of the loading buffer (250 mmol/L Tris-HCl (pH 6.8), SDS (10%, w/v), bromophenol blue (0.5%, w/v), glycerine (50%, v/v) and β-mercaptoethanol (5%, v/v)), then the mixture was boiled for 8 min, cooled immediately and centrifuged at 8000 rpm, 4 °C for 10 min. The generated supernate was collected and analysed by the SDS-PAGE.

2.5.2. Transmission electron microscopy (TEM)

TEM was utilized to observe the morphology and cell integrity of *C. jejuni* treated with TCPNs. Untreated sample was tested as a control. All samples were separately dyed with 3% (v/v) phosphotungstic acid and dried, a microscopic observation was then carried out to obtain the micrographs.