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Novel electrospun gelatin-glycerin-ε-Poly-lysine nanofibers for controlling *Listeria monocytogenes* on beef

Lin Lin, Yulei Gu, Haiying Cui*

School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China

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ABSTRACT

In order to improve mechanical properties and extend food preservation performance of nanofibers, electrospun gelatin-glycerin-ε-Poly-lysine nanofiber has been engineered in this study. The optimized ratio of gelatin: glycerin: ε-Poly-lysine was found to be 25: 6: 10 (w/v/v) after the evaluation of electrospinning solutions and nanofiber physical properties. The tensile strength of nanofibers reached 1.63 ± 0.04 MPa with addition of glycerin. The results of FTIR, SEM and AFM confirmed that nanofibers have been fabricated successfully and obtained uniform diameter at 204 nm. TGA and DSC were also analyzed, which revealed the nanofibers have stronger thermal stabilities than pure components. At last, the nanofiber was utilized to preserve beef and exhibited outstanding antibacterial activity against *Listeria monocytogenes* during 10-days storage at 4 °C and 7-days storage at 12 °C, without impact on the surface color and sensory properties. Therefore, electrospun gelatin-glycerin-ε-Poly-lysine nanofiber could be a prospective packaging material in food industry.

1. Introduction

Beef contains high proteins, lipids and appropriate water which acts as “natural media” for microorganisms (Cui, Wu, Li, & Lin, 2017; Cui, Yuan, Li, & Lin, 2017). *Listeria monocytogenes*, gram-positive and non-spore-forming bacteria, widely involved in many food contaminations worldwide (Lin, Gu, Li, Vittayapadung, & Cui, 2018; Lin, Liao, Duraiarasan, & Cui, 2018). Hence, to minimize the potential risk of *L. monocytogenes* contamination, many methods have been applied for beef preservation.

Food packaging is one of the most commonly used methods to hinder bacterial contamination (Lin, Gu et al., 2018; Lin, Liao et al., 2018). Since chemical agents have been confirmed to be toxic and susceptible to permeate into the body (Parisi, Barone, & Caruso, 2015), the preparation of natural packaging material has become an urgent matter. Recently, electrospinning has become an economic, versatile and promising process to produce active nanofibers from synthetic and natural polymers (Ghorani & Tucker, 2015).

Gelatin, one of the most natural protein-based biopolymers, obtained from collagens which existed in the animal bones, connective tissues and skin (Okutan, Terzi, & Altay, 2014). Due to its biodegradability, bioactivity and nontoxicity, gelatin could be recognized as a potential candidate for nanofiber synthesis (Nair & Laurencin, 2007). In food fields, gelatin-based films have been commercially used as wrapping material to maintain food quality and prolong shelf life, especially meat and fishery industries (Nowzari, Shábanpour, & Ojagh, 2013; Ramos, Valdés, Beltrán, & Garrigós, 2016).

However, gelatin nanofibers have some drawbacks such as poor mechanical properties and susceptible to bacteria (Jalaja, Kumar, Dey, Kundu, & James, 2014). These two drawbacks would affect the practical application and result in shortening of shelf life. To solve these problems, the cross-linking treatment is applied to enhance mechanical properties, and the addition of natural preservative agent is utilized to extend food preservation. The cross-linking treatments are mostly based on physical agents (gamma irradiation, ultraviolet) and chemical agents (glutaraldehyde, formaldehyde) (Reddy, Reddy, & Jiang, 2015). However, physical agents and chemical agents have been confirmed to be harmful to human, the researchers are looking for valid and nontoxic cross-linking agent, as well as the natural preservative agent (Balakrishnan & Jayakrishnan, 2005; Jalaja et al., 2014).

Glycerin has excellent biological properties, including non-cytotoxicity and biocompatibility (Pisani, Bottema, Butori, Dani, & Dechesne, 2010). Furthermore, glycerin has been broadly employed as plasticizer during elastic gelatin capsules fabrication process to prevent brittle and keep their elasticity during drying and storage period (Liang et al., 2015; Morsy, Hosny, Reisha, & Elnimr, 2017).

In recent times, ε-Poly-lysine (ε-PL) have been recognized as potential natural antimicrobial agent extracted from *Streptomyces albulus*

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

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and has been accepted as safe food preservative by Food and Drug Administration (FDA, 2004) (GRAS No. 000135; Lin, Gu et al., 2018; Lin, Liao et al., 2018). ε-PL has been applied in many food products, such as egg-based dishes and soft drinks (Hyldgaard et al., 2014) due to its broad spectrum antimicrobial activity against a considerable number of microorganisms, including bacteria, fungi and viruses. Moreover, addition of ε-PL into electrospun nanofibers could not only resist bacterial contamination, but also prolong food preservation period.

The current study aims to explore and optimize the concentration of glycerin and ε-Poly-lysine in electrospun nanofibers for improving mechanical properties and extend food preservation performance. Furthermore, the long-term anti-listeria activity of nanofibers on beef was also investigated, accompanying by surface color and sensory evaluation of beef samples.

2. Materials and methods

2.1. Materials and bacterial culture

Gelatin (type B from porcine skin) was bought from Delong Glue Co., Ltd. (Shanghai, China). Food-grade glycerin (> 99.5%) was purchased from Suzhou Minghua Tangchun Co., Ltd. (Suzhou, China). ε-Poly-lysine (molecular weight 3500–4300, > 98.0% purity) was obtained from Zhengzhou Bainafo Bioengineering Co., Ltd. (Henan, China). Acetic acid (> 99.7% purity) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fresh beef meat (brisket; protein 20.3%, fat 28.8%) was purchased from Metro supermarket.

L. monocytogenes ATCC 19115 was purchased from China General Microbiological Culture Collection Center (Beijing, China). This strain was stored in liquid paraffin wax at 4 °C and cultured with shaking at 25 °C for 48 h.

2.2. Preparation of spinning solutions

Firstly, the gelatin (25%, w/v) was dissolved in acetic acid solution (30%, v/v) with magnetic stirrer (85-1, Shanghai Zhiwei Co., Ltd, Shanghai, China) for 30 min. Different volumes of glycerin (0%, 2%, 4%, 6%, 8%, and 10%, v/v) were added into gelatin solution dropwise. Subsequently, ε-PL (1.0 mg/mL) stock solution was prepared. The ε-PL solution with a concentration of 10% (v/v) was appended into gelatin-glycerin solutions. Finally, the gelatin-glycerin-ε-PL solutions were stirred for 1 h to acquire homogeneous solutions. A pure gelatin solution was prepared as a control sample. Different ratio of gelatin: glycerin: ε-PL solutions were prepared (gelatin: glycerin: ε-PL; 25: 0: 0, 25: 0: 10, 25: 2: 10, 25: 4: 10, 25: 6: 10, 25: 8: 10, 25: 10: 10, w/v/v) and their properties were determined and then proceeded to electrospinning process.

2.3. Properties of spinning solutions

2.3.1. Electrical conductivity

The electrical conductivity was measured by a portable multi-parameter analyzer (DZS-718, Shanghai Precision Science Instrument Co., Ltd, Shanghai, China) at room temperature.

2.3.2. Viscosity

The viscosity of electrospinning solutions was measured by a rotational rheometer (DHR-1, Waters Technologies Co., Ltd, Shanghai, China) with plate-plate sensor (diameter 40 mm, gap 1 mm) (Aydogdu, Sumnu, & Sahin, 2018). The sample was placed on the plate and the edges were carefully trimmed by a spatula. The shear rate range varied between 0.1 s^{-1} to 200 s^{-1}.

2.4. Preparation of electrospun nanofibers

The electrospun nanofibers were prepared by the electrospinning apparatus (SNAN-01, Electrospinning Setup, MECC Co., Ltd., Fukuoka, Japan). In the beginning, the homogeneous solutions (gelatin / glycerin / ε-PL) was transferred into a glass syringe with a needle tip of 0.50 mm inner diameter. A voltage of 25.0 kV was put into use between the syringe and the collector. The flow rate of spinning solution was modulated to 0.2 mL/h. Nanofibers were collected on the aluminized foil (20 cm × 20 cm) which was placed at 15 cm vertical distance to the needle tip (Cui, Wu et al., 2017; Cui, Yuan, Li et al., 2017). In order to facilitate comparison, gelatin nanofiber was also prepared.

2.5. Evaluation of nanofiber physical properties

2.5.1. Nanofiber thickness

An electronic digital micrometer (Guangdong, China) with the accuracy of 0.001 mm was applied to measure nanofiber thickness (Pankaj et al., 2014). The measurements were done in at least ten random positions of nanofibers.

2.5.2. Moisture content

For determination of moisture content, nanofibers were weighed before and after drying in an air oven at 110 °C until a constant weight was achieved. The moisture content (%) was calculated according to the following equation (1):

$$Moisture\ content = \frac{M_0 - M_1}{M_0} \times 100\% \quad (1)$$

Where M_0 and M_1 represent for initial and dried samples (mg) (Laurila & Lauhanen, 2010).

2.5.3. Water solubility

Nanofibers were cut into 20 mm × 20 mm before drying at 110 °C to achieve constant weight and measured as the initial weight. Afterwards, nanofibers were put into distilled water at room temperature for 15 min, respectively. Undissolved nanofiber pieces were taken out and dried at 110 °C until the constant weight to determine the final weight. The water solubility of nanofiber was calculated using the equation (2):

$$Water\ solubility = \frac{W_0 - W_1}{W_0} \times 100\% \quad (2)$$

Where W_0 and W_1 stand for the weight of initial and final samples (mg) (Samira, Thuan-Chew Tan, & Azhar, 2014).

2.5.4. Contact angle

The hydrophilicity of nanofiber surface was studied by contact angle measurements. A contact angle goniometer was utilized to measure the static contact angle (CA100 A, Shanghai Innuo Precision Instruments Co., Ltd. Shanghai, China) (Honarvar et al., 2017).

2.5.5. Water vapor permeability (WVP)

The WVP of nanofibers were surveyed according to Cui, Yuan, and Lin, (2017). Nanofiber samples were sealed on the top of permeation cups containing distilled water. The internal relative humidity (RH) of permeation cups was 80%. Then test cups were put into a desiccator cabinet. The weight of each cup was recorded daily for 8 days. The WVP was figured out through equation (3):

$$WVP = \frac{\Delta m \bullet X}{A \bullet \Delta t \bullet \Delta P} \quad (3)$$

Where $\Delta m/\Delta t$ is weight of moisture loss per unit of time (g/h), X is thickness of nanofiber (mm), A is nanofiber area exposed to moisture transfer (2.0096×10^{-4} m^2), ΔP is the differential water vapor partial pressure across the nanofiber (1.753 kPa).

2.5.6. Mechanical properties

Mechanical properties of nanofiber samples included tensile strength (TS, MPa) and elongation at break (EAB, %). The properties

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