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Preparation and characterization of protocatechuic acid grafted chitosan films with antioxidant activity

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ABSTRACT

In this study, novel chitosan (CS) based active films were developed by grafting natural phenolic antioxidant of protocatechuic acid (PA) onto chitosan backbones. The physicochemical, mechanical and antioxidant properties of three PA grafted CS (PA-g-CS) films with different grafting ratios were characterized. The transparent PA-g-CS films exhibited yellowish color with thickness ranging from 44.1 to 48.6 μm. By contrast with CS film, PA-g-CS films with medium and high grafting ratios showed decreased moisture content, water vapor permeability; however, increased water solubility and tensile strength. The barrier to UV light of CS film could be improved by grafting with PA. An increase in the total color difference was observed for PA-g-CS films, indicating PA-g-CS films were more colored. Among four kinds of films tested, PA-g-CS film with medium grafting ratio exhibited the highest tensile strength (45.7 MPa) and elongation at break (5.05%). According to thermogravimetric analysis, the maximum rates of weight loss for PA-g-CS films appeared at 291.8–294.0 °C. Antioxidant activity assays showed that PA-g-CS films had both dose-dependent and time-dependent scavenging activity on DPPH radical. Our results suggest that PA-g-CS films are expected to serve as novel antioxidant food packaging materials.

1. Introduction

Nowadays, there is an increasing interest to develop bio-based active films in order to improve food preservation and to reduce the use of chemical preservatives (Aider, 2010). The basic materials used to prepare bio-based active films are polysaccharides, proteins and lipids compounds derived from plant and animal resources (Dutta, Tripathi, Mehrotra, & Dutta, 2009). In general, polysaccharide films made from starch, alginate, cellulose ethers, chitosan (CS), carrageenan and pectin can provide efficient barriers against oils and lipids, and exhibit good gas barrier properties (van den Broek, Knoop, Kappen, & Boeriu, 2015; Vieira, da Silva, dos Santos, & Beppu, 2011). Among various polysaccharides, CS is the deacetylated product of chitin (the second most abundant polysaccharide in nature) obtained by alkaline treatment. Owing to its non-toxic, biodegradable, biocompatible, antimicrobial and good film forming property, CS has been widely used for the production of edible films in food packaging (Alishahi & Aïder, 2012; Elsabee & Abdou, 2013; Kerch, 2015).

The cationic character of CS offers an opportunity to establish electrostatic interactions with other compounds. CS has two types of reactive groups in its structure, the free amine groups and the hydroxyl groups (at C_3 and C_6 carbons). Due to these structural functional groups, CS can be chemically modified to broaden its applications (Shukla, Mishra, Arotiba, & Mamba, 2013). In recent years, several efforts have been made to graft natural phenolic antioxidants including gallic acid (GA), ferulic acid (FA), caffeic acid (CA), and catechin onto CS backbones (Cirillo et al., 2016; Oliver, Vittorio, Cirillo, & Boyer, 2016). Phenolic compounds are known to possess potent antioxidant properties mainly because they can act as free-radical scavengers (Dai & Mumper, 2010). Therefore, the introduction of phenolic groups into the CS structure allows obtaining new matrices with satisfied antioxidant properties and extending CS based films' functional properties. Till now, three kinds of graft copolymerization methods including the carbodiimide chemical cross-linking, free radical mediated grafting and enzyme catalyzed reactions have been frequently adopted to synthesize phenolic grafted CS (Aljawish, Chevalot, Jasniewski, Scher, & Muniglia, 2015; Curcio et al., 2009; Liu, Lu, Kan, & Jin, 2013; Liu, Wen, Lu, Kan, & Jin, 2014; Pasanphan & Chirachanchai, 2008; Xie, Hu, Wang, & Zeng, 2014).

Recent studies have demonstrated that phenolic grafted CS can

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be applied as antioxidant, antimicrobial, adsorptive and encapsulation agents, as well as food packing materials with wide applications in food, pharmaceutical and environmental industries (Hu et al., 2016; Liu et al., 2014, 2015, 2016; Schreiber, Bozell, Hayes, & Zivanovic, 2013). However, only little attention has been paid to the preparation and characterization of phenolic grafted CS film (Aljawish et al., 2016; Nunes et al., 2013; Schreiber et al., 2013; Wu et al., 2016). Generally, the film's property is closely related to the type of grafting method used, film making procedure and the type of phenolic compounds grafted. Nonetheless, the effect of grafting ratio on the physicochemical, mechanical and bioactive properties of phenolic grafted CS film is still unclear.

Protocatechuic acid (PA) is a natural phenolic antioxidant existing in fruits, edible plants and vegetables (Reis et al., 2010). In our previous work, PA was grafted onto CS by a carbodiimide mediated chemical cross-linking reaction. The structural characterization of PA grafted CS (PA-g-CS) was investigated by several instrumental methods (Liu et al., 2016). Results showed that PA was grafted onto CS through amide and ester linkages. As compared to CS, PA-g-CS exhibited altered physical property (e.g. surface morphology, crystallinity and thermal stability) and enhanced antioxidant activity. In this study, PA-g-CS films with different grafting ratios were prepared. The physicochemical (physical appearance, thickness, moisture content, water solubility, water vapor permeability, light transmittance and color), mechanical (tensile strength and elongation at break) and antioxidant properties of PA-g-CS films were characterized. Effect of grafting ratio on the PA-g-CS film's properties was also evaluated. Our results provide novel information on the characteristics of phenolic grafted CS film.

2. Materials and methods

2.1. Reagents and chemicals

CS (deacetylation degree of 71% and molecular weight of 2.5×10^5 Da) and Folin-Ciocalteu reagent were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide (NHS), 2-(*N*-morpholino)ethanesulfonic acid (MES), PA and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Synthesis of PA-g-CS

Three grafted copolymers (named PA-g-CS I, PA-g-CS II and PA-g-CS III with low, medium and high grafting ratios, respectively) were synthesized according to the method of Liu et al. (2016). Briefly, CS was grafted onto PA by an EDC/NHS coupling method in the MES buffer solution (pH 5.5). The grafting reaction was carried out in an ice bath for 1 h and then at room temperature for 12 h. The reaction molar ratios of CS:PA:EDC:NHS were 1:1:1:1, 1:3:3:3 and 1:5:5:5 for PA-g-CS I, PA-g-CS II and PA-g-CS III, respectively (Table 1). The reaction products were dialyzed against distilled water for 72 h using dialysis bags with molecular weight cut-off (MWCO) of 8000–12,000 Da, followed by centrifugation at 10,000 rpm for 30 min and lyophilized to afford final grafted copolymers. The obtained grafted copolymers were checked to be free of unreacted PA and any other compounds by high performance liquid chromatography (HPLC) according to our previous report (Liu et al., 2013). The grafting ratios of PA-g-CS I, PA-g-CS II and PA-g-CS III were determined as 61.64, 190.11 and 279.69 mg/g by the Folin–Ciocalteu method (Liu, Jia, Kan, & Jin, 2013).

2.3. Preparation of PA-g-CS films

PA-g-CS films were prepared according to the method of Wang et al. (2011) with some modifications. Firstly, the film forming solution was prepared by dissolving 2 g of PA-g-CS sample in 100 ml of 2% (v/v) acetic acid solution and stirred at room temperature overnight. The sample solution was centrifuged at 10,000 rpm for 20 min to remove any insoluble particles, and then mixed with 2 ml of glycerol. After 1 h of stirring, the film forming solution was treated ultrasonically with ultrasonic power of 200 W at frequency of 40 KHz for about 30 min to remove air bubbles. Then, a total volume of 80 ml film forming solution was cast onto a self-designed Plexiglas plate (24 × 24 cm) and then dried at 30 °C with 50% relative humidity for 48 h in a humidity chamber. Finally, the dried film was peeled from the plate and stored at 25 °C in desiccators containing saturated solution of $Ca(NO_3)_2$ (50 ± 2% relative humidity) for at least 48 h prior to tests. CS film used as control was prepared in the same way.

2.4. Characterization of PA-g-CS films

2.4.1. Film thickness

The film thickness was determined with a Mitutoyo No. 293-766 digital micrometer (Mitutoyo Absolute, Tester Sangyo Co. Ltd., Japan). The thickness was measured in ten randomly selected locations on each film and then an average value was calculated.

2.4.2. Moisture content

Moisture content of film sample (approximate 50 mg) was determined by measuring weight loss of film upon drying at 105 °C until constant weight. Three replicates were performed for each sample. Moisture content (%) was calculated by the following equation:

$$\text{Moisture content}(\%) = \frac{(M_w - M_d)}{M_w} \times 100 \quad (1)$$

where M_w was the weight of film conditioned in 50% relative humidity to moisture equilibrium, and M_d was the weight of film after being dried at 105 °C.

2.4.3. Water solubility

For water solubility test, film was firstly dried at 105 °C to constant weight. The dried film was then cut into square piece (5 cm × 5 cm), accurately weighed (M_i) and immersed in 100 ml of distilled water at 25 °C for 24 h under constant agitation (Jouki, Yazdi, Mortazavi, & Koocheki, 2013). Afterwards, the remaining

Table 1
The reaction conditions and grafting ratios of PA-g-CS I, PA-g-CS II and PA-g-CS III.

Designated PA-g-CS	Reaction conditions	Grafting ratios (mg/g)*
PA-g-CS I	CS: 0.15 M, PA: 0.15 M, EDC: 0.15 M, NHS: 0.15 M	61.64 ± 3.96^c
PA-g-CS II	CS: 0.15 M, PA: 0.45 M, EDC: 0.45 M, NHS: 0.45 M	190.11 ± 10.14^b
PA-g-CS III	CS: 0.15 M, PA: 0.75 M, EDC: 0.75 M, NHS: 0.75 M	279.69 ± 18.52^a

*Values are given as mean ± standard deviation (SD). Different letters in the same column indicate significantly different ($p < 0.05$).