International Journal of Biological Macromolecules 108 (2018) 444–454

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Preparation and characterization of biocomposite film based on chitosan and kombucha tea as active food packaging

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ARTICLE INFO

Article history:
Received 14 June 2017
Received in revised form
23 November 2017
Accepted 5 December 2017
Available online 6 December 2017

Keywords:
Kombucha tea
Chitosan
Minced beef

ABSTRACT

An active film composed of chitosan and kombucha tea (KT) was successfully prepared using the solvent casting technique. The effect of incorporation of KT at the levels 1%–3% w/w on the physical and functional properties of chitosan film was investigated. The antimicrobial activity of chitosan/KT film against *Escherichia coli* and *Staphylococcus aureus* was evaluated using agar diffusion test, and its antioxidant activity was determined using DPpH assay. The results revealed that incorporation of KT into chitosan films improved the water vapor permeability (from 256.7 to 132.1 g cm^{-2} h^{-1} KPa^{-1} mm) and enhanced the antioxidant activity of the latter up to 59% DPpH scavenging activity. Moreover, the incorporation of KT into the chitosan film increased the protective effect of the film against ultra violet (UV). Fourier transform infrared spectroscopic analysis revealed the chemical interactions between chitosan and the polyphenol groups of KT. In a minced beef model, chitosan/KT film effectively served as an active packaging and extended the shelf life of the minced beef as manifested in the retardation of lipid oxidation and microbial growth from 5.36 to 2.11 log cfu gr^{-1} in 4 days storage. The present work demonstrates that the chitosan/KT film not only maintains the quality of the minced beef but also, retards microbial growth significantly, extending the shelf life of the minced beef meat up to 3 days; thus, chitosan/KT film is a potential material for active food packaging.

1. Introduction

Conventional plastics are derived from petroleum, which entails serious environmental concerns. Biodegradable films and coatings represent an interesting alternative to conventional plastic materials, which is why several biopolymers have been exploited to develop materials for eco-friendly food packaging [1]. In response to the consumer demand and market trends, the area of active packaging is becoming increasingly significant. Active packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product [2]. Active packaging has been applied for fresh and processed foods. One of the common methods to develop active food packaging is to incorporate active compounds, such as antioxidants and antimicrobial agents, into packaging materials.

Chitosan, poly-β-(1/4) N-acetyl-D-glucosamine, yielded from deacetylation of chitin, a biopolymer that is abundant in a variety of crustacean shells, such as crab, crawfish and shrimp shells [3]. Chitosan as one of the most abundant renewable polymers has been studied in various fields due to its unique properties, and one of them is the area of edible coatings and films, which have been emerged as effective and eco-friendly methods to extend the shelf life of food products [4]. Chitosan has been found to be non-toxic, biodegradable, bio functional, biocompatible in addition to antimicrobial characteristics [5–8]. Chitosan has the advantage of being able to incorporate substances such as minerals or vitamins and possesses functional properties as compared with other bio-based food packaging materials [9–11]. Due to chitosan intrinsic antimicrobial properties, it has a great potential for applications as antimicrobial films and coatings [12,13] .The exact antimicrobial action mechanism of chitosan is still unknown, but different mechanisms have been proposed; interaction between positively charged chitosan molecules and negatively charged microbial cell membranes causing the leakage of proteins and other intracellular constituents [14]. Although chitosan is a promising biopolymer, there are limiting factors in food packaging application because of high molecular weight of chitosan which resulted in low sol-

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https://doi.org/10.1016/j.ijbiomac.2017.12.028
0141-8130/

ubility [15]. Chitosan has been also considered as poor water barrier properties due to hydrophilic characteristics. Improvement of antioxidant, antimicrobial, and solubility characteristics of chitosan may lead to further advantages in active food packaging applications [16].

kombucha tea is a refreshing beverage obtained by the fermentation of sugared tea with a symbiosis of *Acetobacters*, including *Acetobacter xylinum*, *Acetobacter xylinoides*, *Bacterium Glu- conicum, Acetobacter aceti, Acetobacter pasteurianus* and various yeasts, such as the *Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Brettanomyces bruxellensis*, *Brettanomyces lambicus, Brettanomyces custersii, Candida* and *Pichia* species [17,18].

The tea fungus broth is composed of two portions; a floating cellulosic pellicle layer and the sour liquid broth. Acetic acid, ethanol, and gluconic acid are the major components of the liquid broth [19], other minor constituents such as lactic acid, glucuronic acid, phenolic components which includes catechin, epicatechin, epicatechin gallate, gallocatechin, epigallocatechin and epigallocatechin gallate [20]. Groups of vitamin B and enzymes are also present [21]; Some of its advantageous effects have already been demonstrated such as: anti-microbial, anti-oxidant, anti-carcinogenic [22], antidiabetic treatment for gastric ulcers [23]. It has also shown to have impact on immune response [24] and liver detoxification [25]. Steinkraus et al [26]. showed that the antimicrobial activity of KT against *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus aurous*, and *Agrobacterium umefaciens* made with a low tea usage level ($4.4\,g\,l^{-1}$) could be attributed to the acetic-acid content.

Previous studies showed that antioxidant activity of chitosan could be improved through incorporating of natural substances, such as plant extracts [27], green tea extract [28], rosemary essential oil [29], Rosemary and oxygen scavenger [30], grape seed extract [31], and the addition of vitamin E and α- tocopherol [32,33], thymus moodier or thymus piper Ella essential oils [34] in to chitosan-based film. KT could be used as a promising source of antimicrobial and antioxidant ingredients to incorporate chitosan film. Therefore, The main objective of this study was to i) produce chitosan film containing KT, ii) assess the antibacterial and antioxidant activities of chitosan films containing KT, iii) investigate the effect of KT on physical properties of chitosan film and iv)application of chitosan film containing KT as potential active packaging to extend shelf life of minced beef.

2. Material and methods

2.1. Materials

Black tea (*Camellia sinensis L.*) and tea fungus samples were purchased from local markets in Iran. Chitosan (with molecular weight of 310000–375000 Da and 75–85% deacetylation degree) was purchased from Sigma Aldrich (USA). DPpH (2,2-diphenyl-1 picryhydrazyl), TBA (thiobarbituric acid), acetic acid, and methanol were also obtained from Sigma Aldrich (USA). Peptone water, Plate Count Agar, Baird Parker agar, and egg yolk tellurite were obtained from HiMedia Laboratories (Bombay, India). All other chemicals were analytical grade and from Merck (Darmstadt, Germany).

2.2. Preparation

2.2.1. Preparation of KT

Four gram of black tea, were added to 1l boiling water and allowed to infuse for about 5 min after which the infusions were filtered through sterile sieve. Sucrose (100 gr) was dissolved in hot tea and then was left to cool to room temperature. A certain amount of tea (200 ml) was poured into 500 ml glass jars that had been previously sterilized at 121 °C for 20 min. The cool tea was inoculated aseptically with 3% (w/v) of freshly grown tea fungus that had been cultured in the same medium for 35days and 10% (v/v) of previously fermented liquid tea broth. The jar was carefully covered with a clean cloth and fastened properly. The fermentation was carried out in a dark incubator at 24 ± 3 °C for about 18 days [35].

2.2.2. Preparation of films

Chitosan film was prepared by dissolving chitosan (high molecular weight, 310000–375000 Da, 75–85% deacetylated, Sigma-Aldrich, Steinheim, Germany) in an acetic acid aqueous solution (1% v/v, Sigma-Aldrich,Steinheim, Germany) while stirring on a magnetic stirrer/hot plate, following the indications of Ojagh et al. [16] with some modifications. The chitosan solution was stirred at room temperature until it was completely dissolved for 24 h. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper to remove any undisclosed particles. Then, KT at the concentrations of 1%, 2%, and 3% (w/w) was added to chitosan aqueous solution. The solution stirred for 60 min at 300 rpm and 30 °C and then the appropriate amount of solution was distributed into petri dishes for casting and dried at 50 °C and 30% relative humidity for 24 h. The peeled films were kept in a chamber at room temperature and 75% relative humidity for 48 h prior to experimental use.

2.3. Characterization

2.3.1. Chemical analysis of KT

Chemical analysis of KT compounds was determined by the method described by Malbaša et al. procedure [36]. Dry matter content was measured after drying at 150 °C for 24 h. Ash content was measured after mineralization at 550 °C. The pH of the samples was measured with an electronic pH meter (Orion 290A, ThermoFisher Scientific, USA). Acidity of KT was determined by titration with a standard solution of sodium hydroxide and phenolphthalein as indicator [37].

2.3.2. DPpH radical scavenging assay

DPpH assay performed according to Yen & Chen [38] procedure. A certain amount of KT (0.025 ml) was diluted to 4 ml of methanol and then 0.6 ml of DPpH solution (a-diphenyl-b-picrylhydrazyl, 1 mM in methanol) was added. The mixture was then incubated at room temperature for 30 min and then the absorbance at 517 nm was determined [38].

The antioxidant activity of the chitosan/KT films was evaluated by assaying the scavenging of free radical of DPpH following the method of Blois [39], Siripatrawan & Harte [28],Wang et al. [40] with some modification. 3 ml of film extract solution were mixed with 1 ml of 1 mM methanol solution of DPpH . After shaken in an oscillator for 1 min, the mixture was incubated in dark at room temperature for 30 min. The UV absorbance of the DPpH assay solution at 517 nm was measured.

The scavenging capacity of KT and films was calculated by the following equation:

$$\text{DPPH scavengi ng activity } (\%) = \frac{A_{DPPH} - \text{As}}{A_{DPPH}} \times 100 \quad (1)$$

Where A_{DPpH} is the absorbance value at 517 nm of the methanol solution of DPpH and As is the absorbance value at 517 nm of the DPpH assay solution. Tests were performed three times for each specimen and the average values were taken.

2.3.3. Determination of the amount of total phenolic

The amount of total phenolic compounds was determined by the method described by Jayabalan et al [41]. 0.1 ml of KT was transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by addition of distilled water. Afterward, 1 ml