Antibacterial Properties of Tannic Acid Incorporated Rice Starch-gelatin Film

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Abstract. Many food products either handled chill or frozen are frequently packed in plastic-based materials, although these packaging materials are of environmental concerns due to their non-biodegradable characteristics. However, for a short handling period at chill temperatures, for products such as beef, chicken or fish sausages, safety of consuming the products is the utmost important issue. Hence, these products are formulated with preservatives such as nitrates to prolong their shelf-life. This brings in the concept of employing active packaging material as an additional measure to ensure minimal surface contamination to the packaged foods for better shelf-life. Therefore, the objective of this paper is to determine the antibacterial activity of tannic acid when incorporated in rice starch-gelatin biodegradable/edible film at 0 to 0.6% concentrations. The antibacterial activities of the incorporated film were carried out against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* for the duration of sixteen days under ambient temperatures (~28 °C). Upon storage, the increasing concentration of tannic acid incorporation resulted in a significant (p<0.05) increase in inhibition effect as evidence by the zone of inhibitions obtained. The zones of inhibition were 4.5-7 mm for *Escherichia coli*, 0-7 mm for *Salmonella typhimurium*, 0-6 mm in *Listeria monocytogenes* 0.5-4.5 mm for *Bacillus cereus*. The result also showed that *E. coli* O157:H7 was most sensitive to the presence of tannic acid than *L. monocytogenes*, *S. typhimurium*, and *B. cereus*. Film incorporated with 0.45 and 0.6% tannic acid were the films with the highest antibacterial activities. Higher incorporation with tannic acid is limited by the darkening of the film and stiffer texture. Tannic acid can be a potential antibacterial ingredient in active biodegradable/edible packaging formulation.

Keywords: biodegradable film, tannic acid, rice starch-gelatin film, antibacterial activity

1. Introduction

Biodegradable/edible films function as a barrier to moisture, gas, aroma, and lipid besides protecting a food product after the primary package is opened [1]. The degradability of these films is so rapid as such they will help reduce the environmental pollution issue unlike their counterpart the non-degradable plastic films. Starch-based films have minimum barrier to moisture [2] low permeability to oxygen [2]-[4]. Therefore, they form weak brittle films and hence limit their application as a food packaging material with good functional properties. However, physical and/or chemical modifications could improve the overall performance of the film [3]. Proteins such as soy proteins, whey protein and gluten have been used in various modifications of the starch-based films to influence their barrier properties. Gelatin could also be used in modifying the barrier properties of starch-based films. Starch-based films and coatings have been reported to be used for produce packaging such as strawberries, mushrooms and other vegetables [5].

Tannic acid, a gallic ester of D-glucose is known for its antioxidant capacity because of their multiple phenolic groups that is able to interact with biological macromolecules [6]. It exists in a range of fruits and plants and is listed as a ‘generally recognized as safe’ (GRAS) food additive [7] and [8]. Several researchers have reported that tannic acid has antimicrobial activity against foodborne pathogens such as *Escherichia coli* and *Listeria monocytogenes* [8]-[11]. The application of biodegradable film with tannic acid incorporation for prevention of wound infection was recently reported [12]. There is no previous report for

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the usage of tannic acid in rice starch-fish gelatin edible films to impart additional functional properties to the film for food application. Therefore, the objective of this paper is to determine the effect of tannic acid incorporation in the rice starch-gelatin film on the microbial inhibition properties of the film against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* which could be reflective of their potential to be used as active packaging for the reduction of surface contamination in actual food application system. The effectiveness of the tannic acid incorporation in the film was studied at ambient temperature of approximately 28 °C.

2. Material and Methods

2.1. Film formation and Observation

Aqueous solutions of 5 % (w/w) rice starch and the addition of fish gelatin (1 %) including 40 % sorbitol (w/w, starch and gelatin blend basis) were prepared with the addition of tannic acid at different concentrations (0.15, 0.30, 0.45, and 0.60 %). The film mixtures were then adjusted to about pH 8 to 9 by Na2CO3. The mixture was thoroughly mixed before casting into plastic petri dishes (15 cm×15 cm×1.5 cm) and was allowed to dry in a ventilated oven at 65 °C around 24 h. Dried films were peeled off and kept in desiccators (RH 56 % at 28 °C) until use.

2.2. Storage Study of Film

The prepared films were cut into 6 mm diameter discs and stored in the respective desiccators for the total duration of sixteen days. They were removed at pre-determined intervals and placed on streaked agar of the different tested microbes prior to standard incubation time as described in the antimicrobial test. The initial day was designated as day one (D1).

2.3. Antimicrobial Test

The disc-diffusion assay was carried out according to [13] and [14]. Films’ inhibition against *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 19115), *Salmonella typhimorium* (ATCC 14028), and *Bacillus cereus* (ATCC 33019) were conducted. Each microbial culture was incubated at 37 °C in tryptic soy agar (TSA) (Becton Dickinson, Maryland, USA) for 18 h with shaking at 120 rpm. Cell numbers were adjusted to 5 logs CFU/ml with peptone water (Becton Dickinson, Maryland, USA) before their use. Each culture was homogenously streaked on to Mueller-Hinton agar (Becton Dickinson, Maryland, USA) and a disc of 6 mm in diameter of rice starch-gelatin films of various tannic acid concentrations were then placed on the agar and the plates were incubated at 37 °C for 18 h. The clear zone around the film disc on the media was measured. Film without tannic acid incorporation was run separately as the control. The test was carried out three times for each microbe evaluated. The inhibition zone reported was the clear zone diameter after the deduction of the diameter of the film discs.

2.4. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using Minitab release 16. The one-way ANOVA was used to analyze the effect of rice starch-fish gelatin edible films incorporated with tannic acid. The Tukey’s test with 95 % confidence interval was used for mean comparison when a significant variation was found by the ANOVA test.

3. Results and Discussion

3.1. Film Observation

The appearance of plasticized-rice starch-fish gelatin films was slightly opaque and smooth, but rice starch-fish gelatin films incorporated with tannic acid were brownish and stiffer than the untreated film. This is due to the cross-linking property of tannic acid itself (Fig. 1).
Fig. 1: Rice starch-fish gelatin film without tannic acid (a) and incorporated with tannic acid (b)

3.2. Antimicrobial Activity

Control film without tannic acid exhibited no inhibitory influence on the growth of tested microbes. Film incorporated with tannic acid at concentration from 0.15 to 0.60 % per disc demonstrated antimicrobial activities against *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *B. cereus*. Fig. 2-Fig. 5 show the inhibition zone plates against the tested microbes.

![Fig. 2: Agar diffusion inhibition zone of *Escherichia coli*](image1)

![Fig. 3: Agar diffusion inhibition zone of *Listeria monocytogenes*](image2)
With increasing tannic acid concentration the zone of inhibition increased significantly (p<0.05). Previous researches had demonstrated that tannic acid have antimicrobial activity on an extensive range of microorganisms as well as foodborne pathogens such as *E. coli* 0157:H7 and *L. monocytogenes* based on log reduction of their growth; however, the studies reported were not done with edible films [8], [11] and [15]. The zone of inhibition were 4.5-7 mm for *Escherichia coli* (Fig. 6), 0-6 mm in *Listeria monocytogenes* (Fig. 7), 0-7 mm for *Salmonella typhimurium* (Fig. 8) and 0.5-4.5 mm for *Bacillus cereus* (Fig. 9). The result also showed that *E. coli* O157:H7 was more sensitive to the tannic acid present than *L. monocytogenes*, *S. typhimurium*, and *B. cereus*. This variation might reflect differences in cell surface structures between Gram-negative and Gram-positive bacteria. The outer cell membrane of bacteria containing lipopolysaccharide may delay some water-soluble antimicrobial molecules’ ability to influence cytoplasmic membranes. The cause of stabilization of lipopolysaccharide layer is divalent cations such as Mg2+ and Ca2+ ions. There is a strong chelating property of these divalent cations with tannic acid [8]. Tannic acid penetration removed these cations which would be the lipopolysaccharide cell surface layer of Gram-negative bacteria’s destabilization but would not have any effect on Gram-positive bacteria. On the membrane, the antimicrobial effect’ mode of tannic acid might be correlated to its inactivation ability of enzymes, microbial adhesions, mineral uptake, and cell envelope transport proteins [8], [9], and [12]. In contrast, gram-positive bacteria has preventive barrier against water-soluble tannic acid due to their thick peptidoglycan layer.

There was no significant (p> 0.05) inhibition against *S. typhimurium* and *B. cereus* in lower concentrations of tannic acid, 0.15 and 0.30 % (Fig. 8 and Fig. 9) respectively. There was significant inhibition against all studied microbes in higher concentration of tannic acid (0.45 and 0.6 %); however, there was no significant difference between the inhibitory effects of the two concentrations of tannic acid against the studied microbes.

On the overall, by day sixteen, moderate to strong inhibition could not be obtained for *L. monocytogenes*, *S. typhimurium* and *B. cereus* where the tannic acid concentration were less than 0.3, 0.4 and 0.4 percent incorporation, respectively.
Fig. 6: Zone of inhibition for *E. coli*

Fig. 7: Zone of inhibition for *L. monocytogenes*

Fig. 8: Zone of inhibition for *S. typhimurium*

Fig. 9: Zone of inhibition of *B. cereus*

4. Conclusions
The addition of tannic acid to the base film resulted in a stiffer and darker film biodegradable/edible rice starch-fish gelatin film with increasing concentration of tannic acid incorporation. Tannic acid incorporation in the rice starch-gelatin film can render antibacterial property to the film for food application. Hence, the film studied can be used as an active packaging material. The starch-gelatin film incorporated with tannic acid at 0.45 and 0.60 % showed better inhibition against *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, and *B. cereus* activities. *E. coli* is most sensitive among the four studied microbes.

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6. References