Design and investigation on antimicrobial properties for novel chitosan based active food packaging

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Abstract: Food packaging has attracted a great deal of attention in for sustainable food industry. In the present study, we aimed to develop a chitosan based packaging film and evaluate its ability to inactivate *Salmonella enterica* both in vitro and in actual tests. No inhibition effect on the growth of *Salmonella enterica* was observed within the investigated conditions. The antimicrobial effect of chitosan film is found to be significantly dependent on the ultraviolet A (UVA) light intensity. The surviving cell numbers of *Salmonella enterica* on the film decreased 4 log and 0. 5 log CFU/ml after 3 hours using 20 W daylight fluorescent bulbs. In addition, lettuce was investigated to further evaluate the effect of new packaging system. The results show that the chitosan film could slightly reduce the microbial contamination on the surface of solid food product and thus reduce the risks of microbial growth.
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1. Introduction

Active packaging is a novel packaging method which is incorporated with materials such as antioxidant, antimicrobial and/or nutrients to make an interaction between packaging and food to improve food quality and safety. This method can employ some technology to delay in oxidation and control the respiration rate, moisture migration and microbial growth to prolong the shelf life of food products (Restuccia et al., 2010; Woranuch et al., 2013; Peng et al., 2013). Therefore, active packaging does not only provide a barrier against harmful external factors, but also releases or absorbs substances into food and its environment to maintain, or even improve the condition of food products (Assa, 2015). The substances which have the main role in active packaging can be a component of the packaging system, or directly exist in whole packaging materials. Due to their different operations, they usually consist of various layers or portions (Assa et al., 2015). Active packaging contains some additives depending on their application and packaged food. These additives have some properties such as releasing substances like oxygen, ethanol and carbon dioxide, and antimicrobial activities. The new generation of active packaging is called antimicrobial active packaging. The main function of this packaging is to assist the preservation and to prolong the shelf life of packaged food by suppressing the growth of spoilage microorganisms (Appendini et al., 2002). The active agents for production of antimicrobial packaging include organic acids, fungicides, bacteriocins, enzymes, ceramic materials with silver ions, inorganic gases and planet extract. Nowadays, there is an increasing interest in natural polymers which have inherently various properties for active packaging. Due to their biodegradable characteristics, they can be nominated as an alternative to commodity polymers. In addition, since they have intrinsic antimicrobial and antioxidant properties there is no need for incorporating active agents. Most of these polymers are made from proteins and polysaccharides which can be replaced by some of conventional synthetic packaging materials exerted to preserve food products. Among active packaging sources, chitosan is a biopolymer with special properties such as bio-functional, biodegradability, nontoxic, film forming and antimicrobial activity have made chitosan a promising packaging material. Beside these characteristics, chitosan films are able to postpone the rate of mass transfer, moisture and shelf life (Vaghari et al., 2013). However, the antimicrobial effect of chitosan based polymers needed to be evaluated for their future application in food packaging systems. Therefore, the aim of the present study was to develop chitosan-coated oriented-polypropylene film and evaluate its antimicrobial activity against one of the major food born pathogen, *Salmonella enterica,* to assess its potential use in future active food packaging.

2. Materials and methods

2.1 Preparation of chitosan coated packaging film

Orientedpolypropyrene films with a thickness of

20 µm were prepared and were mixed with isopropyl alcohol and chitosan suspension were gradually coated onto one side of the film using a bar coater (Webster, USA) at 25 °C. The prepared samples were consequently dried in the oven over night.

2.2 Preparation of *Salmonella enterica* cells

Salmonella enterica cells (ATCC 14028) were grown in a 500 ml shake flask containing 300 ml of nutrient broth. Cells were incubated aerobically in a shaker incubator at 37 °C for 24 h. Cells were harvested by centrifugation at 4,000 ×g for 10 min and washed with sterile deionized consequently. The initial population of *Salmonella enterica* cells was approximately ~10^8 CFU/ml.

2.3 In vitro test of film antimicrobial activity

To assess the antimicrobial activity of prepared packaging film, test pieces (a square of 5 cm) of uncoated and chitosan coated film were placed in agar plates under aseptic conditions. One mL of the *Salmonella enterica* solution was pipetted onto each test piece in its Petri dish; the inoculated area was covered with overhead projector film while the Petri dishes were covered with lids. Inoculated samples were placed under light illumination with two 20 W daylight fluorescent bulbs at 20 °C. The ultraviolet (wavelength 315–400 nm) intensities on the surface of the film test piece, as measured by a UVR-400 radiometer was 0.06 mW/cm^2. Samples were taken in duplicates at 60 min intervals for 3 h. Consequently, diluted solutions was plated onto desoxycholate agar for recovery of undamaged cells. The plates were incubated for 24 h at 37 °C to see the growth.

2.4 Effect of coated film on *Salmonella enterica* inoculated onto lettuce

Fresh lettuce cooled overnight and were cut into pieces. Samples were dipped in 100 ppm sodium hypochlorite solution for 4 min. consequently, lettuce pieces were then inoculated with *Salmonella enterica* at a concentration of ~108 CFU/ml for 4 min at 25 °C, and the excess solution was shaken off. Experiments were conducted in duplicated and plates were incubated for 24 h at 37 °C. The number of viable cells was presented as CFU/g cut lettuce.

3. Result and Discussion

3.1 Pilot-scale experiment

The experiment on the antimicrobial activity of the chitosan based film were conducted. As can be concluded from Figure 1, the surviving number of *Salmonella enterica* cells on the surface of uncoated and chitosan treated samples resulted in no decrease after 3 h of experimental period. This suggests that the antimicrobial testing method was not successful in inactivation of *Salmonella enterica.* The effect of UVA light intensity on the inactivation of several bacteria has been previously reported in the literature (Fujita and Suzuki, 1978; Tuveson et al., 1988; Yoshimura et al., 1996; Bintsis et al., 2000). UVA light can significantly damages cells through the oxidative stress caused by oxygen radicals within the cells (Bock et al., 1998). The results of the present study show that the inactivation rate of bacteria with chitosan coated film plus UVA light is higher than when UVA is just used. This behaviour reveals the effective synergetic effect of chitosan-coated packaging film with UVA light.

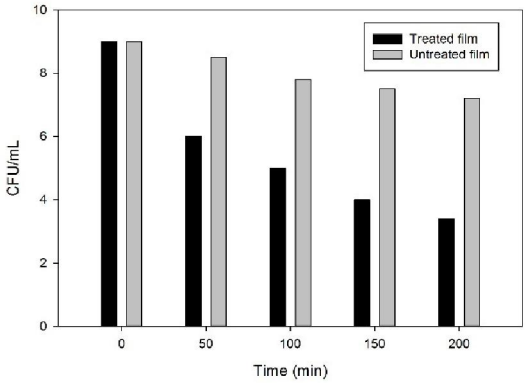

Figure 1. Inactivation of cells under different conditions.

The cell count studies of *Salmonella enterica* in the in vitro test using chitosan-coated film and uncoated film were similar as shown in Figure 2. The data showed that the antimicrobial effect of chitosan-coated film is clearly independent of the particle size of chitosan. This result may be due to partial agglomeration of particles on the surface of the plastic film which could not enhance the *Salmonella enterica* inactivation effect. Generally, it is accepted that the high surface area of chitosan is important in increasing the gas-phase photocatalytic activity of volatile organic compounds (Anpo et al., 1987; Maira et al., 2000).

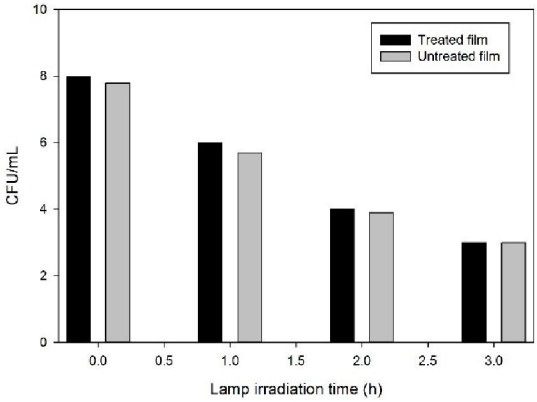

Figure 2. Inactivation studies under light illumination.

In this study, the activity of chitosan-coated film on gas-phase photocatalytic oxidation of ethylene was prefunded; however, no such trend was observed in the photo-catalytic disinfection of *Salmonella enterica*. The inactivation rate of *Salmonella enterica* with chitosan-coated film was dependent on the UVA light intensity derived as can be concluded from Table 1. When the UVA light intensity was decreased from 1 to 0.05 mW/cm^2 the antimicrobial efficacy of coated film was decreased. This can be justified by the OH radical formation on the surface of the coated film (Ghaz-Jahanian et al., 2013). In the actual test, the cell number of *Salmonella enterica* obtained from the lettuce packed in a chitosan-coated film decreased from 6 to 5 log CFU/g after 24 h of storage. In addition, the number of *Salmonella enterica* from cut lettuce packed in uncoated film after 48 h was slightly higher than the initial concentration. The result suggests that the chitosan-coated film cannot effectively reduce the microbial contamination on the surface area of solid food products in food packaging.

Table 1. Surviving cells under illumination for 3h.

Sample	Light Intensity (mW/cm^2)	Initial cell number	Final cell number
Treated	<0.05	8±0.1	7.4±0.3
Untreated	<0.05	8±0.1	7.6±0.2

In conclusion, based on the both in vitro and actual tests, chitosan-coated film showed negligible antimicrobial effect on *Salmonella enterica* inactivation. The chitosan-coated active film exhibited a slight bactericidal effect when exposed to UVA light. The extent of inactivation of the film was completely relevant to the UVA intensity. The photo-catalytic. The number of *Salmonella enterica* cells on cut lettuce packed in the chitosan coated film plus UVA light was lower than that subjected to UVA light alone. This study shows the need for further improvements in chitosan based food packaging antimicrobial properties

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