Food Control 38 (2014) 88–95

Contents lists available at ScienceDirect

Food Control

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

# Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in ready-to-eat poultry meat

Ali Akbar [a,b], Anil Kumar Anal [a,*]

[a] *Food Engineering and Bioprocess Technology, School of Environment, Recourses and Development, Asian Institute of Technology, P.O. Box 4, Klong Luang, Pathumthani 12120, Thailand*

[b] *Department of Microbiology, Faculty of Life Science, University of Balochistan, Quetta, Pakistan*

ARTICLE INFO

*Article history:*
Received 15 March 2013
Received in revised form
22 September 2013
Accepted 28 September 2013

*Keywords:*
Zinc oxide nanoparticles
*Salmonella*
*Staphylococcus aureus*
Active packaging
Electron microscopy
Ready-to-eat meat

ABSTRACT

Zinc oxide nanoparticles were prepared using hydrothermal synthesis approach. Formation of zinc oxide nanoparticles were confirmed by using UV–Vis spectrophotometer, Fourier transform infrared spectrometer and X-ray diffractometer. The particles size ($\leq$100 nm) and structure of nanoparticles were studied under scanning and transmission electron microscope. The nanoparticles were used against two prominent foodborne pathogens, *Salmonella typhimurium* and *Staphylococcus aureus* and were found highly effective against both of them. The antibacterial activity of the nanoparticles was determined in solid and liquid media using nutrient agar and broth. Zinc oxide nanoparticles loaded active film of calcium alginate was prepared for active packaging against the same foodborne pathogens (*S. typhimurium* and *S. aureus*). Presence and distribution of nanoparticles in active film were confirmed with Fourier transform infrared spectrometer, X-ray diffractometer and scanning electron microscopy. Zinc oxide nanoparticles loaded active films showed antibacterial activity against the target bacteria in Petri dish. The film was also used as an active packaging (a challenge study) in ready-to-eat poultry meat against the same pathogens, and reduced the number of inoculated target bacteria from log seven to zero within 10 days of its incubation at 8 $\pm$ 1 °C.

© 2013 Elsevier Ltd. All rights reserved.

## 1. Introduction

Food safety is the major concern of the consumers and food industries. Foodborne diseases incidence are increasing in developing as well as in the developed countries. Each year in United States of America, there is an estimated 25–81 million foodborne illness cases resulting, approximately 9000 deaths associated to contaminated food consumption (Gomez et al., 2009; Jevsnik et al., 2013). In South East Asia, approximately 22.8 million Salmonellosis cases with a death toll of 37,600 have been estimated each year (Akbar & Anal, 2013a). The use of ready-to-eat (RTE) food is increasing due to its convenience for consumer. The increasing demand for instant and RTE food products is posing challenges for food safety and quality (Jutaporn, Suphitchaya, & Thawien, 2011). The contamination of RTE food with *Salmonella*, *Staphylococcus aureus* and *Escherichia coli* has been repeatedly reported and is considered as a potentially hazardous food worldwide (Akbar & Anal, 2013b; Cho et al., 2011). The increasing antibiotic resistance and re-emerging of infectious diseases is a continuous threat to public health. In order to reduce the number of foodborne infections and prevent the drug resistant bacterial contamination, new strategies are therefore needed to identify and use for its control (Jones, Ray, Ranjit, & Manna, 2008; Li, Sun, Zhang, & Pang, 2013).

The use of active antimicrobial compounds incorporated in packaging material is now getting more attention as the control agents for bacteria in food packaging system. It ensures microbial food safety for consumer, and can be useful for the extension of shelf life of the products (Cho et al., 2011). Contact of active materials with food having the ability to change its composition or the atmosphere around food is an active packaging as per European Union regulations (1935/2004/EC and 450/2009/EC). Such types of packaging inhibit the growth of microorganism present on the surface of food products (Espitia et al., 2012). Recently, Zinc Oxide (ZnO) nanoparticles have been explored as an antimicrobial agent (Bajpai, Chand, & Chaurasia, 2012), and feasible to incorporate in

* Corresponding author. Fax: +66 25246200.
*E-mail addresses:* anil.anal@gmail.com, anilkumar@ait.ac.th (A.K. Anal).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.09.065

the food active packaging system. Zinc oxide is one of the five zinc compound, which is listed as generally regarded as safe (GRAS) by United States food and drug administration (USFDA, 21CFR182.8991). Zinc is a source of micronutrient and plays an important role in body growth and development (Shi, Zhou, & Gunasekaran, 2008).

It has been reported that *S. aureus* is more sensitive to ZnO nanoparticles compared to *E. coli* and *Pseudomonas aeruginosa* (Premanathan, Karthikeyan, Jeyasubramanian, & Manivannan, 2011). The exact mechanism of antimicrobial action of ZnO is still not clear, however some mechanisms of action such as antimicrobial ion release are thought to be one of them (Espitia et al., 2012). Nanotechnology such as use of nanoparticles can provide solutions to the food safety challenges of food industries related to products safety and packaging with economic and environmental friendly ways (Silvestre, Duraccio, & Cimmino, 2011). The present work describe a detailed study of ZnO nanoparticles and its activity against common foodborne pathogens targeted *S. aureus* and *Salmonella typhimurium* and hence tested against the target bacteria in the form of active film. The ZnO nanoparticles were prepared and used against the target pathogens. The prepared nanoparticles were incorporated with alginate based edible film against the test bacteria in RTE poultry meat as a challenge study.

## 2. Materials and methods

### 2.1. Zinc oxide nanoparticles preparation and characterization

The ZnO nanoparticles were prepared by hydrothermal synthesis approach following Bajpai et al. (2012) with slight modification. Aqueous solutions (100 mL) of sodium hydroxide (Merck, Germany) with molar concentration of 0.2 M and zinc chloride (Merck, Germany) solution of 0.1 M were prepared. Sodium hydroxide (0.2 M) solution was added drop wise to aqueous zinc chloride (0.1 M, 100 mL) solution under constant stirring (100 rpm). The mixture solution was heated at 60 °C for 2 h in water bath. Following the heating, the reaction mixture was left standing overnight (12 h) at room temperature and filtered through Whatman filter number one. The precipitate obtained was kept in hot air oven at 60 °C for 48 h to ensure the complete formation of ZnO nanoparticles. The powdered nanoparticles were used for further experiments. The formation of ZnO nanoparticles were confirmed by UV–Vis spectrophotometer (Unicam) in the range of wavelength 300–550 nm, Fourier transform infrared (FTIR) spectroscopy in the range of 500–4000 $cm^{-1}$, using FTIR spectrometer (Nicolet, Avatar 360). X-ray diffraction (XRD) patterns were observed in the range of $2\theta$ values from $20^{\circ}$–$80^{\circ}$ with PANalytical X'pert PRO, X-ray diffractometer. Morphology and size of the nanoparticles were observed under scanning and transmission electron microscope SEM (JEOL JSM-6301F, Japan)/TEM (FEI, TECNAI T20, Japan).

### 2.2. Preparation and enumeration of target bacteria

The target bacteria *S. aureus* (TISTR 029) and *S. typhimurium* (TSTIR 292) were grown in nutrient broth at 37 °C for 24–48 h aerobically. The number of the target bacteria were always adjusted using McFerland standards and optical density value. Fresh cultures (18 h) of target bacteria in nutrient broth were washed three times with sterile normal saline and adjusted to $10^6$–$10^7$ CFU/mL using McFerland standards and spectrophotometer for each experiment. Standard plate count (SPC) method was used for target bacteria enumeration in all test and control samples by preparing tenfold (1:10) initial dilution in sterile saline water and then further diluted serially. The 1 mL amount from each decimal dilution was seeded over specific agar media for each target bacteria and then incubated at 37 °C for 24–48 h (Akbar & Anal, 2013b; Fernández-Pan, Carrión-Granda, & Maté, 2014).

### 2.3. Antibacterial activity of zinc oxide nanoparticles

Zinc oxide nanoparticles (stock solution 10 mg/mL in de-ionized water) were used for further studies of antibacterial activity. Antibacterial activity of the pre-synthesized ZnO nanoparticles was determined against the target foodborne pathogens *S. aureus* and *S. typhimurium* as described below.

Bacterial growth inhibition of the target bacteria were analysed by inoculating and spreading 100 μl of target bacteria ($10^6$–$10^7$ CFU/mL) on the surface of nutrient agar (Merck, Germany) supplemented with different concentrations ranging from 0.01, 0.1, 0.5, 1, 2, 3 and 4 mg/mL of ZnO nanoparticles. The target bacterial growth were observed after 16–24 h incubation at 37 °C. In a similar experiment, nutrient broth (Merck, Germany) supplemented with the same concentrations of the ZnO nanoparticles were inoculated with the 100 μl of target bacteria ($10^6$–$10^7$ CFU/mL) and incubated at 37 °C for 24–48 h. Growth of the target bacteria in nutrient broth were observed visually by measuring turbidity with the help of spectrophotometer and SPC method. Nutrient agar and broth without ZnO nanoparticles were used as control.

### 2.4. Morphological changes and nanoparticles interaction with cells by transmission electron microscopy

The effect of ZnO nanoparticles on cell surface morphology and its interaction with the target bacterial cells were studied under TEM at center of nano-imaging (CNI), Mahidol University Thailand. Fresh culture 100 μl ($10^6$–$10^7$ CFU/mL) of target bacteria were inoculated to 4.9 mL nutrient broth supplemented with 0.5 mg/mL ZnO nanoparticles and incubated at 37 °C for 8–10 h and were termed as test, whereas ZnO nanoparticles free nutrient broth were used as a control to observe the normal cell morphology of the target bacteria. The nutrient broth (test and control) containing the target bacterial cells were centrifuged at 5000 rpm for 10 min and were used to prepare samples for electron microscopy using the following procedure.

The cells were primarily washed twice and fixed with 2.5% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer pH (7.2–7.4) at 4 °C for overnight, and subsequently fixed with 1.0% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C for 1 h. The samples were dehydrated with 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol at 4 °C, two times for 15 min each. The samples were embedded, trimmed, sectioned, stained, and observed with TEM at acceleration voltage 120 kV.

### 2.5. Preparation of zinc oxide nanoparticles loaded active film

A defined amount (0.7 g) of sodium alginate (Viv Interchem Co. Ltd. Thailand) was mixed by stirring for 6 h with 30 mL de-ionized water. The calcium chloride (Ajax Finechem, Australia) solution (1 mL, 5% w/v) was dropped into 30 mL of alginate solution, followed by mixing 1 mL of glycerol (Union Chemical Co. Ltd. Thailand). The defined quantities (1, 2, 3 and 4 mg/mL) of ZnO nanoparticles were mixed finally according to the initial sodium alginate and de-ionized water mixture volume. The final mixture solution was kept mixing for 5–10 min and then poured on the clean glass Petri dishes (15 cm diameter) and spread evenly with the help of glass spreader. The plates were kept in hot air oven at 45 °C for 12 h, followed by linking again with 1 mL of 5% calcium chloride by spreading over the film for 1 min and then washed three times subsequently with distilled water, and re-dried in oven

Download English Version:

https://daneshyari.com/en/article/6392039

Download Persian Version:

https://daneshyari.com/article/6392039

Daneshyari.com