LWT - Food Science and Technology 61 (2015) 638–643

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Combined sterilizing effects of nano-ZnO and ultraviolet on convenient vegetable dishes

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ARTICLE INFO

Article history:
Received 3 May 2012
Received in revised form
16 December 2014
Accepted 19 December 2014
Available online 30 December 2014

Keywords:
Convenient vegetable dishes
Ultraviolet radiation
Nano-ZnO
Non-thermal sterilization
Response surface methodology

ABSTRACT

To develop a new microbial control method for ready-to-eat convenient dishes, a combined treatment of nano-ZnO and UV treatment was tested. Here, the bacteriostasis of nano-ZnO treatment was studied and compared with potassium sorbate and sodium benzoate treatments. The results showed that microbial growth rate was substantially reduced under nano-ZnO treatment for convenient dishes. Moreover, the combined effect of UV irradiation and nano-ZnO, as a non-thermal treatment, was highly researched to improve loss of texture, color and nutrition constituents of green vegetable. According to the Response Surface Methodology (RSM), nano-ZnO was applied at 0.04 g/Kg, and UV intensity and time were 196.98 V and 47.79 min respectively. The applied treatment was found successful as it showed an effective control of microorganisms along with a slight change in physical and chemical characteristics compared to traditional thermal sterilization.

1. Introduction

The convenient dishes are known as a popular type of food, appreciated because of their pleasant taste, nutritional value and suitable price. They are a kind of ready-to-eat vegetable products that can be obtained from the fresh vegetables through selecting, washing, peeling, cutting, blanching, processing, packaging and sterilizing. During their storage period, it is not easy to preserve the natural attributes of vegetables, since frequent processing cause mechanical injuries of tissues, leading to water loss and color changes (browning or discoloration). Furthermore, the formation of exudates rich in minerals, sugars, vitamins and other nutrients may support the growth of autochthonous microbiota. Oliveira, Souza, Bergamini, and Martinis (2011) reported that psychotropic aerobic bacteria were found at 96.7% in the ready-to-eat minimally processed vegetables while coliforms were detected at 81.5%. The growth of microorganism such as aerobic bacteria, coliforms, mold and yeast are the main spoilage microorganisms in convenient dishes. Traditional thermal sterilization methods such as autoclaving and pasteurizing are able to inhibit the growth of microorganism effectively, but it leads to the loss of nutrient component, texture and color of the products. Therefore, a non-thermal sterilization technology needs to be developed.

Nano-science and nanotechnology marked this century. Their applications in the area of agriculture and food are relatively scarce compared to their use in the field of drug delivery and pharmaceuticals (Sozer & Kokini, 2009). The high performance of nanoparticles is due to their high surface area/volume ratio, thus increases the antimicrobial activity of metal nanoparticles (Damm, Neumann, & Münstedt, 2006). Among various nano-metals, zinc oxide (ZnO) nanoparticles have received considerable interests of both academics and industrialists owing to their attractive physicochemical properties. Due to physical and chemical stabilities, lower cost, ease of availability and non-toxicity, ZnO has become the focus point as a photocatalyst with UV irradiation. The antimicrobial activity of ZnO under UV irradiation is considered to be a result of the generation of hydrogen peroxide (H_2O_2) from its surface (Li et al., 2011). Moreover, the FDA has been currently listed ZnO as a generally recognized as safe (GRAS) material (Jin, Sun, Su, Zhang, & Sue, 2009). It was reported that ZnO, as a food nutrient enhancer, could be added into powdered drinks (6 mg–18 mg per 100 g) and in Western-

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http://dx.doi.org/10.1016/j.lwt.2014.12.042
0023-6438/

style cakes and biscuit (5 mg–8 mg per 100 g) in the 19th announcement of China's Ministry of Health in 2009. Therefore, the addition of nano-ZnO was controlled at less than 5 mg/100 g in this study. In previous research on ZnO coating, Emamifar, Kadivar, Shahedi, and Zad (2010) used the antimicrobial nanocomposite packages containing Ag and ZnO as an alternative non-thermal technology in fresh orange juice in order to extend the shelf life. However, there is few research conducted on nano-ZnO applied to food directly. The main objective of this study was to evaluate the capabilities of non-thermal treatment using both ultraviolet radiation and nano-ZnO on convenient vegetable dishes.

2. Materials and methods

2.1. Materials

Green soy bean with good maturity (5 kg) was purchased from a local market in Wuxi, China. Sodium carbonate, zinc nitrate, absolute ethyl alcohol, agar and peptone were supplied by the Shanghai Chemistry Reagent Company, Shanghai, China. Beef extract used for microbiological culture was collected from Shanghai Changyang Biochemical Pharmaceutical Factory, Shanghai, China. Sodium carbonate, zinc nitrate, absolute ethyl alcohol are analytical reagent and agar, peptone, beef extract are biological reagent.

2.2. Preparation of nano-ZnO and determination of its characterization

Nano-ZnO was synthesized by a microwave-assisted aqueous solution method (Al-Gaashani, Radiman, Tabet, & Daud, 2011; Ye, Zuo, Du, Xie, & Li, 2010). Briefly, 50 mL (1 mol/L) of sodium carbonate (Na_2CO_3) and 50 mL (1 mol/L) of zinc nitrate ($Zn(NO_3)_2$) aqueous solutions were dissolved in deionized water under magnetic stirring (CJJ78-1, Jintan Dadi Automation Instrument Co., Jintan, China) at room temperature. Then drops of Na_2CO_3 aqueous solution was gradually added to the $Zn(NO_3)_2$ aqueous solution under constant magnetic stirring and constant temperature of 75 °C in a water-bath for 2 h until a white precipitate was formed. The soluble inorganic salts were removed after filtering and the precipitate were washed away using deionized water. After washing with absolute ethyl alcohol for three times, stewing for 24 h in order to displace the water followed-on. Then the precipitates were transferred into a porcelain crucible, covered and loaded into the turntable of the microwave oven (Qingdao Haier Co., Ltd., Qingdao, China) to irradiate the precipitates. To get a white nano-ZnO powder, microwave oven was set at 150 W for 8 min first and at 750 W for 30 min.

Nano-ZnO suspension was prepared by dissolution in absolute ethyl alcohol continuously stirred by a magnetic stirrer for 12 h followed by ultrasonic treatment (KQ-250B, Kunshan Ultrasonic Instrument Co., Ltd., Shanghai, China) for 20 min at room temperature. Absorbance of the nano-ZnO solution was measured with a UV–vis spectrophotometer (UV-2600, Shanghai Tianmei Biochemical Instrument Co., Ltd., Shanghai, China) at a rate of 50 nm min^{-1}. The particle size distribution was also evaluated by a Zeta-sizer nano (ZS, Malvern Co., Worcestershire, UK) while the nano-ZnO suspensions were stayed at 37 °C for 1d, 7d and 30d respectively with the aim to evaluate the dispersion stability of ZnO nanoparticle suspensions.

2.3. Preparation of convenient dishes samples

The green soy beans were hulled and blanched at 100 °C for 2 min then washed by cold water (about 0 °C) to inactive enzymes well such as peroxidase and polyphenol oxidase, cooked in soy oil at 120 °C for 5 min, then cooled. Subsequently the samples were poured into the broth with the solid–liquid ratio of 1: 4. The broth containing nano-ZnO (various concentrations according to the different experimental conditions mentioned later) and flavorings, involving salt (30 g/kg), sugar (40 g/kg, from a local market in Wuxi, China), were sterilized with a high pressure steam sterilizer (LD2X–50FBS, Shen An Instrumentarija, Shanghai, China) at 121 °C for 15 min in advance. The solid–liquid ratio was 7:3 and each 50 g of mixture were vacuum packed. All samples were stored in a constant temperature room (SPX, Nanjing Experimental Instrument Co., Ltd., Nanjing, China) at 37 °C for 16 days and analyzed at an interval of 4 days.

2.4. Antimicrobial activity of nano-ZnO

In order to investigate the antimicrobial characteristics of nano-ZnO, four dosages of nano-ZnO (0 g/kg, 0.01 g/kg, 0.03 g/kg, 0.05 g/kg) were applied to the convenient dish samples respectively. Sodium benzoate (1.0 g/kg) and potassium sorbate (0.5 g/kg) were also added as a contrast sample, as the maximum dosages limit provided by GB2760-2011 Chinese Standard. The total bacterial number (TBN), total coliforms, yeast and molds were tested to evaluate the microbial control of nano-ZnO to preserve convenient dishes.

2.5. Effect of UV light on photocatalytic reactions of nano-ZnO

A comparative study was carried out with nano-ZnO at 0.04 g/kg to evaluate the effect of UV light on photocatalytic reactions of nano-ZnO. Three samples were respectively placed in darkness, sunlight lamp and UV lamp (SW-CJ-2D, Suzhou Jinghua Equipment Co., Ltd., Suzhou, China) for 60 min and both sunlight lamp and UV lamp distance were 30 cm. The total bacteria number (TBN) was tested to evaluate this research.

2.6. Autoclaving and pasteurization

In order to compare the combined effects of UV and nano-ZnO on the physical and chemical characteristics with traditional thermal sterilization, autoclaving and pasteurization were used. Samples were treated with a high pressure steam sterilizer (LD2X–50FBS, Shen An Instrumentarija, Shanghai, China) at 121 °C for 15 min and a water bath (Medical Instruments Wuchang, Shanghai, China) at 90 °C for 30 min.

2.7. Microbial analysis

About 10 g of samples was aseptically removed from each package, placed in a stomacher bag (S400, Shanghai Scientific Instrument Co., Ltd., Shanghai, China), diluted with NaCl (8.7 g kg^{-1}) solution and homogenized. Serial dilutions in sterile saline solution were plated onto appropriate media. The media and conditions were the following: Plate Count Agar (PCA) in cubated at 37 °C for 48 h for the total bacteria number (TBN). Sabouraud media for yeast and molds incubated at 25 °C for 120 h. Violet Red Bile Agar (ARBA) incubated at 37 °C for 24 h for total coliforms (Costa, Conte, Buonocore, & Del Nobile, 2011). All the tests were performed twice on two different batches and results were expressed as colony-forming units (CFU) per gram.