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Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract

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ABSTRACT

The aim of this work was to study the effects of drying methods and conditions (i.e., ambient drying, hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying within the temperature range of 70–90 °C at an absolute pressure of 10 kPa) as well as the concentration of galangal extract on the antimicrobial activity of edible chitosan films against *Staphylococcus aureus*. Galangal extract was added to the film forming solution as a natural antimicrobial agent in the concentration range of 0.3–0.9 g/100 g. Fourier transform infrared (FTIR) spectra and swelling of the films were also evaluated to investigate interaction between chitosan and the galangal extract. The antimicrobial activity of the films was evaluated by the disc diffusion and viable cell count method, while the morphology of bacteria treated with the antimicrobial films was observed via transmission electron microscopy (TEM). The antimicrobial activity, swelling and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract. The electron microscopic observations revealed that cell wall and cell membrane of *S. aureus* treated by the antimicrobial films were significantly damaged.

1. Introduction

Microbial deteriorations are responsible for enormous losses of foods. Various chemical and physical means have been developed to help alleviate these undesirable phenomena. Nowadays, there is a considerable interest in the possibility of using edible films to delay or to prevent growth of microorganisms since these films can serve as carriers for a wide range of food additives, including antimicrobial agents, which can extend the shelf-life of foods. Among many materials that can be used to form edible films, chitosan (β-(1,4)-2-amino-2-deoxy-D-glucose) is one of the most promising as it has a good ability to form film and due to its biodegradability, biocompatibility and non-toxicity (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004).

Among many possible natural antimicrobial agents that can be incorporated into edible films, galangal (*Alpinia galanga* Linn.) or "khaa" in Thai is one of the most promising. Galangal is a traditional spice used extensively for flavoring and medicinal purposes. Galangal extract has also proved to be an effective natural antimicrobial agent against some food poisoning bacteria, e.g., *Staphylococcus aureus* (Mayachiew & Devahastin, 2008a). The main compounds of galangal extract are the terpenes, which have potential antimicrobial activity (Burt, 2004; Cowan, 1999; Holley & Patel, 2005; Mohammed & Al-Bayati, 2009).

Recently, many types of antimicrobial edible films have been developed and used to inhibit growth of microorganisms, resulting in an ability to prolong the shelf-life of foods. For example, Seydim and Sarikus (2006) investigated the antimicrobial properties of whey protein isolate (WPI) films containing oregano, rosemary and garlic essential oils against *Escherichia coli* O157:H7, *S. aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Lactobacillus plantarum*. The results suggested that spice extracts exhibited antimicrobial activity in WPI based edible films. Maizura, Fazilah, Norziah, and Karim (2007) assessed antimicrobial films prepared from partially hydrolyzed sago starch and alginate and incorporated with lemongrass oil. The films containing lemongrass oil were effective in inhibiting the growth of *E. coli* based on the zone of inhibition assay. Sivarooban, Hettiarachchy, and Johnson (2008) found that soy protein edible films incorporated with grape seed extract, nisin and EDTA were effective against *L. monocytogenes*, *E. coli* and *Salmonella typhimurium*.

Drying is one of the most challenging steps in the production of edible films. It is well known that different drying methods and conditions affect the properties and functionalities of edible films differently (Mayachiew & Devahastin, 2008b; Srinivasa, Ramesh,

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Kumar, & Tharanathan, 2004). Recently, Mayachiew and Devahastin (2008b) investigated the effects of drying methods and conditions, namely, ambient drying (~30 °C), hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying (LPSSD) within the temperature range of 70–90 °C at an absolute pressure of 10 kPa, on the physical properties of selected chitosan film. It was found that LPSSD at 70 °C led to films with higher tensile strength and percent elongation than the films dried by other drying methods and at other drying conditions. Ambient dried and LPSSD films had higher crystallinity than the films dried by vacuum drying. However, no information is so far available on the effects of these (or any other) different drying methods and conditions on the antimicrobial activity and other physico-chemical properties of chitosan films incorporated with galangal (or any other) extract.

The aim of this study was therefore to assess the effects of drying methods (i.e., ambient drying, hot air drying, vacuum drying and LPSSD) and conditions (drying temperature of 70, 80 and 90 °C) on the antimicrobial activity of chitosan films incorporated with galangal extract by both the disc diffusion and viable cell count methods. Fourier transform infrared (FTIR) spectroscopy was also performed to investigate functional group interaction between chitosan and the added active agent. Degree of swelling of the films was measured to help explain the release behavior of the films. The mechanism of the extract and of the antimicrobial films to inhibit bacterial cell growth was observed using transmission electron microscopy (TEM).

2. Materials and methods

2.1. Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.2%) was obtained from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Glycerol was purchased from Carlo Erba (Val de Reuil, Italy) while acetic acid was obtained from Merck (Darmstadt, Germany). For antimicrobial tests, Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB), Mueller Hinton agar (MHA) and buffer peptone water were purchased from Difco (Detroit, USA). Galangal rhizomes were purchased from a local market.

2.2. Preparation of chitosan solution

1.5% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under constant stirring at 300 rpm using a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at room temperature for 24 h. 25% glycerol (w/w chitosan) was then added into the chitosan solution; stirring was continued at room temperature for 1 h. After mixing the solution was centrifuged for 15 min at 12,400 rpm by a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities and bubbles in the solution.

2.3. Preparation of galangal extract

Galangal powder (10 g dry basis), dried by a tray dryer at 40 °C with particle size between 125–425 μm, was extracted with 100 mL of 95% (v/v) ethanol (Oonmetta-aree, Suzuki, Gasaluck, & Eumkeb, 2006). The extract was filtered through a filter paper (Ø110 mm, Cat. No. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated by a rotary evaporator (Resona Technics, model Labo Rota 300, Gossau, Switzerland) at 40 °C for 10 min and kept at 4 °C in a dark bottle until its use (Mayachiew & Devahastin, 2008a).

2.4. Preparation of antimicrobial chitosan films

Galangal extract was added to the chitosan solution at concentrations of 0.3, 0.6 and 0.9 g/100 g. These concentrations were selected based on a minimum inhibitory concentration (MIC) of the extract against *S. aureus* (Mayachiew & Devahastin, 2008a). The final concentrations of galangal extract in the films were 126, 252 and 378 mg/g film, respectively. The mixture was homogenized by a bench top homogenizer (Ika® Works (Asia), model T 25 basic, Selangor, Malaysia) at 9500 rpm for 2 min. The film solution (21 g) was poured on an acrylic plate with dimensions of 13 × 10 cm to cast an antimicrobial film. Drying of the film was performed by four methods, which are ambient air drying (~30 °C), hot air drying at 40 °C, vacuum drying and LPSSD at 70, 80 and 90 °C at 10 kPa, following the methods of Mayachiew and Devahastin (2008b). After drying the films were conditioned for at least 48 h in a desiccator at a relative humidity (RH) of 53% containing saturated salt solution of magnesium nitrate (Ajax Finechem, Seven Hills, Australia).

2.5. Antimicrobial activity evaluation

2.5.1. Microorganism and cultural methods

S. aureus (ATCC 25923) was obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The stock culture was maintained by regular subculture on TSA slants at 4 °C and transferred monthly. A loopful of bacteria was inoculated to 10 mL of TSB and incubated at 37 °C for 18 h. This culture served as an inoculum for an antimicrobial test.

2.5.2. Agar diffusion method

Qualitative antimicrobial activity of the films was evaluated by the agar diffusion method following the procedure recommended by Maizura et al. (2007). The inoculum (100 μL) of *S. aureus* containing approximately 10^6 CFU/mL was spread on the surface of Mueller Hinton agar plate. An edible film sample was cut into a 6-mm diameter disc and then placed on the agar plate. The plate was incubated at 37 °C for 24 h. The plate was then examined for a zone of inhibition of the film disc. The total diameter of the inhibition zone including the film disc was measured.

2.5.3. Viable cell count method

The biocide property of the antimicrobial films was evaluated by employing the macrodilution method recommended by the National Committee of Clinical Laboratory Standards (NCCLS, 1999). About 0.3 g of each film specimen was placed in a sterilized flask into which 10 mL of *S. aureus* culture containing approximately 10^7 CFU/mL was added. The suspension was incubated at 37 °C. 100 μL of the sample was taken at 0, 6, 12, 18 and 24 h and spread on a TSA agar plate, which was incubated at 37 °C for 24 h. The number of colonies was counted. The inhibition of bacteria growth was expressed as the reduction of cell number by $\log N/N_0$. The test was performed in triplicate.

2.6. Transmission electron microscopy (TEM)

S. aureus was grown in TSB at 37 °C for 18 h. One millilitre of the cell culture was centrifuged using a Biofuge 28 RS (Heraeus Sepatech GmbH, model 3654, Osterode, Germany) at 11,000 rpm for 10 min. The cell pellets were resuspended in 10 mL of TSB, which contained 0.3 g of antimicrobial film or 120 μL of a filter paper disc (6 mm in diameter) soaked with the galangal extract. After incubation at 37 °C for 6 h, the suspension was centrifuged at 11,000 rpm for 10 min. The cell pellets were washed twice with 0.1 M sodium phosphate buffer (pH 7.3) and were then fixed with 2.0% (w/v) paraformaldehyde, 2.5% (v/v) glutaraldehyde in 25 mM buffer.