International Journal of Food Microbiology 263 (2017) 9–16

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Antimicrobial edible coatings and films from micro-emulsions and their food applications

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ARTICLE INFO

Keywords:
Micro-emulsion
Bio-emulsifier
High pressure homogenization
Pathogens
Food
Antimicrobial packaging

ABSTRACT

This study focused on the use of antimicrobial edible coatings and films from micro-emulsions to reduce populations of foodborne pathogens in foods. Corn-Bio-fiber gum (C-BFG) was used as an emulsifier with chitosan. Allyl isothiocyanate (AIT) and lauric arginate ester (LAE) served as antimicrobials. Micro-emulsions were obtained from a solution consisting of 1% chitosan, 0.5% C-BFG, and 1–4% AIT or LAE which was subject to high pressure homogenization (HPH) processing at 138 MPa for 3 cycles. Coatings and films produced from the micro-emulsions had micro-pores with sizes ranging from 100 to 300 nm and micro-channels that hold antimicrobials effectively and facilitate the release of antimicrobials from the center to the surface of the films or coatings, thus enhancing their antimicrobial efficacy. The coatings and films with 1% AIT reduced populations of *Listeria innocua* by over 5, 2, and 3 log CFU in culture medium (Tryptic soy broth, TSB), ready-to-eat meat, and strawberries, respectively. The coatings and films with 1% LAE reduced populations of *Escherichia coli* O157:H7 and *Salmonella* spp. by over 5 and 2 log CFU in TSB and strawberries, respectively. This study provides an innovative approach for the development of effective antimicrobial materials to reduce food borne pathogenic contaminants on ready-to-eat meat, strawberries, or other food.

1. Introduction

Fresh foods such as produce and meat are highly perishable products due to their biological composition. The cost of illness caused by 14 foodborne pathogens is over $14 billion; amongst them, that caused by *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 is over $6 billion. When food categories are taken into consideration, the cost of illness caused by contaminated meat (poultry, pork, beef, deli and other meats) and produce is $6.65 billion, and $1.44 billion, respectively (Batz et al., 2012).

Food deterioration and pathogen contamination usually starts at the food surface. Therefore, food surface treatments and packaging after the treatments are critical for protecting food quality and safety (Malhotra et al., 2015). One way to achieve this goal is the application of edible coatings or films on food surfaces. Edible coatings and films are composed of natural polymers, and can improve food quality and safety by providing selective barriers to moisture transfer, oxygen uptake, and lipid oxidation (Cerqueira et al., 2009) and by acting as carriers of antimicrobial agents, allowing for the films and coatings to have antimicrobial activity (Martins et al., 2010; Rhim et al., 2006). Those films and coatings have recently gained more interest in the field of food preservation and have been recently reviewed (Galus and Kadzinska, 2015; Salgado et al., 2015). Chitosan based edible films and coatings have shown great promise for their application in food preservation (Elsabee and Abdou, 2013; Kerch, 2015). Yuan et al. (2016) reviewed applications of chitosan films and coatings containing essential oils in food systems.

Nanoemulsions are described as nano-sized delivery systems of nanoencapsulated lipophilic ingredients in an oil matrix, with an extremely small droplet size (Mason et al., 2006; McClements, 2011; Solans et al., 2005). In pharmaceutical science, extensive research has been conducted on a variety of nanoemulsion-based drug delivery systems (Puri et al., 2009). High pressure homogenization (HPH) processing, or microfluidization, can produce dispersions or emulsions with narrower particle distributions due to the high shear stresses developed in the micro-channels of the interaction chamber (Sherwin et al., 1998; Strawbridge et al., 1995). Microfluidization has led to good nanoemulsion based systems in several research studies, with droplet sizes ranging from 60 to 600 nm (Hatanaka et al., 2010, 2008). Microfluidization can be used to produce porous polymers that provide the foundation for a new generation of food coatings. Porous polymers are a subset of porous materials that take advantage of the ease of

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http://dx.doi.org/10.1016/j.ijfoodmicro.2017.10.002
Received 10 April 2017; Received in revised form 31 August 2017; Accepted 1 October 2017
Available online 02 October 2017
0168-1605/ Published by Elsevier B.V.

processability associated with polymers to generate monoliths, films, and beads, often with well-defined porosities and high specific surface areas (SSAs) (Gokmen and Du Prez, 2012; Wu et al., 2012). The properties of porous polymers could allow for the development of films with appropriate pore sizes, adequate mechanical properties, highly porous and interconnected pore structures, and high surface area to volume ratios.

A surfactant or emulsifier plays a key role in an emulsion system. It reduces the interfacial tension by reducing the droplet size, increasing the number of droplets, and reducing the wall thickness. This reduction in wall thickness allows for the formation of interconnecting holes during polymerization and/or processing. Depending on the nature of the colloidal system employed (emulsions, micro-emulsions, solid particles, or breath figure droplets), the characteristic droplet or pore size can range from a few nanometers to hundreds of micrometers. Guo et al. (2015) developed new edible antimicrobial coatings and films which utilized barley straw arabinoxylan (BSAX) as a bio-emulsifier for micro-emulsions and allyl isothiocyanate (AIT) as an antimicrobial agent. BSAX was isolated from barley straw while corn fiber gum (C-BFG) was isolated from "corn fiber," a by-product of the corn wet and/or dry milling process. Although both of them are from plant by-products, they differ drastically in their structure and in their functionalities. There were no studies published in the literature which focused on their performance in micro-emulsions. Furthermore, Guo et al. (2015) did not focus their study on the microstructures (micro pores and channels) of films and coating prepared from micro-emulsions with bio emulsifiers, and did not discuss a possible relationship between the microstructures and antimicrobial efficacy as well. Therefore, the first objective of this study was to investigate the microstructures of the coatings and films from the micro-emulsions with bio fiber gum from corn (C-BFG), and the second objective was to compare the antimicrobial efficacy of coatings and films with micro-emulsions with AIT and lauric arginate ester (LAE) in culture media and real foods. Gram positive bacteria (*Listeria innocua*, a model for *Listeria monocytogenes*) and Gram negative bacteria (*Escherichia coli* O157:H7 and *Salmonella* spp.) were used in a bacterial growth medium (TSB) and foods, using ready-to-eat (RTE) deli meat and strawberries as models.

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight, 150 kDa, 75–85% deacetylation), allyl isothiocyanate (AIT, 95% purity), and Tween 80 (T80) were purchased from Sigma Aldrich (St. Louis, MO, USA). Gum Arabic (GA) was obtained from TIC Gums Inc. (Belcamp, MD, USA). Lauric arginate ester (LAE) was purchased from A & B Ingredients (Fairfield, NJ, USA). Food grade acetic, lactic, and levulinic acids were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Isolation of corn-bio-fiber gum (C-BFG) and barley straw arabinoxylan (BSAX)

Corn-bio-fiber gum (C-BFG) and barley straw arabinoxylan (BSAX) were isolated from corn fiber and barley straw respectively in our laboratory (Wyndmoor, PA), following the procedures described by Guo et al. (2015).

2.3. Preparation of micro-emulsions and casting of composite films

Predetermined amounts of chitosan, AIT, LAE, and emulsifier (C-BFG, T80, or GA) were dispersed in an acid solution containing 1% (v/v) lactic acid and levulinic acid, under constant agitation using stir plates at ambient temperature (approximately 22 °C) for 12 h to make coarse emulsions. The coarse emulsions were subject to high pressure homogenization (HPH) treatment by passing the emulsion through an EmulsiFlex-B3 high-pressure homogenizer (Avestin Inc., Ottawa, Canada) at 138 MPa (20,000 psi) pressure for 3 cycles to produce micro-emulsions.

Ten milliliters of coarse emulsions (non-HPH) and micro-emulsions (film forming solutions) were casted in 57-mm-diameter aluminum petri plates, and vacuum dried at 35 °C for 24 h. The films were peeled off from the aluminum petri plates before use. The average film thickness was 0.22 mm.

2.4. Determination of AIT residues in emulsions and films

AIT residues in emulsions and films were determined using a spectrophotometer method described by Li et al. (2007) with some modifications. Sample preparation before measurement in a spectrophotometer: (1) for AIT concentration in coarse emulsion and micro-emulsion, one ml of sample was mixed well with 9 ml 1% acid solution, and the mixed solution was used for AIT determination; (2) for AIT concentration in films, one film was placed in 25 ml acid solution, ground in a blender (Bullet Blended Gold, Next Advance Inc., Averill Park, NY. USA) for 8 min to obtain a homogeneous solution that was used for AIT determination; (3) for AIT on film surface, one film was placed in 10 ml hexane, shaken for 20 min and the hexane solution was used for AIT determination; (4) for AIT releasing to water, one film was placed in 25 distill water (DW) and gently shaken for 1 h at room temperature, allowing the film sample swell but not dissolve. The film-DW mixture was centrifuged (5702R, Eppendorf, Hauppauge, NY. USA) at 2000 × *g* for 2 min, and the supernatant was used for measuring AIT concentration in DW. AIT concentrations in all prepared samples were determined using spectrophotometer (Genesys 10 UV, Thermo Electron Corp., Madison, WI, USA), and the UV absorbance at 248 nm was used to calculated the AIT concentration for each sample based on pre-established equations from AIT standard curves. The determinations were carried out in triplicate for one sample with three samples from each emulsion and film.

2.5. Inoculum preparation

Three strains of *Listeria innocua* (ATCC 33090, 33091, 51742) from the American Type Culture Collection (Manassas, VA., USA) were used for TSB and RTE meat inoculation; the *Listeria* cocktail was prepared according to the method of Guo et al. (2015). Two strains of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) (*E. coli* ATCC 43895 and *E. coli* C9490), as well as four serovars of *Salmonella enterica* (*Salmonella newport* H1275 (ERRC culture collection), *St. Paul* 02-517-1 (cantaloupe outbreak), *stanley* H0558 (CDC stool sample, 1995 sprout outbreak), and *montevideo* G4639 (1993 tomato outbreak)) were used for TSB and strawberry inoculation. The six strain *E. coli/Salmonella* composite was prepared according to the method of Gurtler et al. (2014). All isolates were selected for spontaneous mutants resistant to 100 ppmof nalidixic acid and were from Dr. Gurtler's lab (Wyndmoor, PA, USA).

2.6. Determination of morphology of emulsions and films

Scanning electron microscopy (SEM) was used to determine the morphology of films and emulsion solutions. Emulsion solution samples were diluted 100 times and coated on stubs and sputter gold coated 70s × 2 (Edwards Scancoat 6, West Sussex, UK). They were then observed with a scanning electron microscope, FEI Quanta 200 F (Hillsboro, OR, USA) with an accelerating voltage of 10 KV in high vacuum mode.

Film samples were dry-fractured with scalpel blades into 3 × 5 mm^2 pieces, and cross-sectioned fragments were mounted vertically on specimen stubs with glue (Duco Cement, ITW Performance Polymers, Riviera, FL, USA). Prior to viewing, the samples were sputter coated with a thin layer of gold using a model Scancoat SixSputter