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Inactivation of natural microflora and inoculated *Listeria innocua* on whole raw shrimp by ozonated water, antimicrobial coatings, and cryogenic freezing

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ABSTRACT

Shrimp have been associated with outbreaks of foodborne illnesses. A survey was conducted to determine the microbiological safety and quality of frozen whole raw shrimp, purchased at markets in the Northeast United States. Shrimp were composed of 32 brands from 9 different countries of origin. The average aerobic, psychrotrophic, coliform, and *Enterobacteriaceae* microbial counts were 3.64, 4.06, 0.02 and 1.43 log CFU/g, respectively. Of shrimp samples that were *Listeria* spp. positive (21.9%), 12.5% were identified as *Listeria monocytogenes*. No *Salmonella* spp., *Escherichia coli* O157:H7, *Vibrio* spp., or *Staphylococcus aureus* were detected. The survival of natural microflora and inoculated *Listeria innocua* on shrimp were investigated after treatments with ozonated water, antimicrobial coating and cryogenic freezing, alone or in combination. Ozonated washes, cryogenic freezing and antimicrobial coating treatments, applied singly, reduced the natural bacteria or *L. innocua* by <2 log CFU/g; however, in combination, treatments provided additive or synergistic reductions of the natural bacteria and *L. innocua*. The chitosan coating with allyl isothiocyanate, in combination with cryogenic freezing, inactivated more than 5 log of *L. innocua* and natural microorganisms. These information may be valuable for seafood processors and distributors to adopt intervention strategies to enhance the safety and extend the microbial shelf-life of shrimp.

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1. Introduction

According to Centers for Disease Control and Prevention (CDC), from 2005 to 2010, 39 outbreaks and 2348 illnesses were linked to imported food from 15 countries. Of those outbreaks, seafood (17 outbreaks) was the most common source of implicated imported foodborne disease outbreaks, and nearly 45 percent of the imported foods causing outbreaks came from Asia (CDC, 2012).

The United States imported *ca.* 1.27 billion pounds of shrimp in 2011, at a value of $5.16 billion (USDA, 2012). Additionally, there was *ca.* 3 million pounds of shrimp produced domestically in 2010 (Texas Aquaculture Association [TAA], 2012). The per capita consumption of shrimp in the U.S. is estimated to be 4.1 lbs. *Listeria monocytogenes* has been identified as a pathogen of concern for shrimp, based on product detentions and recalls (Ababouch, Gandini, & Ryder, 2005; Wan Norhana, Poole, Deeth, & Dykes, 2010). Contamination of seafood processing plants and the seafood itself is problematic as the incidence of *Listeria* contamination can be high, and seafood is often consumed raw (Chen, Pyla, Kim, Silva, & Jung, 2010; Gurtler & Kornacki, 2007, chap. 17; Pagadala et al., 2012). Most shrimp imported into the U.S. comes from developing countries, where an appropriate HACCP system may not be applied. In addition, according to the U.S. Government Accountability Office (GAO), only about 2% of imported seafood is inspected (Gilbert, 2011).

Nonthermal processing and chemical preservation are used to treat heat-sensitive food to avoid quality loss. Shrimp is one such food when the product is sold raw. Ozone in both gaseous and dissolved-aqueous forms was reported to inactivate microorganisms on foods, including fungi, yeast, parasites and viruses (Guzel-

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Seydim, Greene, & Seydim, 2004), and bacteria, such as *L. monocytogenes* (Paranjpye, Peterson, Poysky, & Eklund, 2008) and the tetracycline-resistant *Listeria innocua* (Vaz-Velho, Silva, Pessoa, & Gibbs, 2006). However, there have been conflicting results, as reported by Kim, Silva, Chamul, and Chen (2000) and Crapo, Himelbloom, Vitt, and Pedersen (2004), that ozone washing or spraying treatments had very limited antimicrobial efficacy for seafood.

Cryogenic freezing, which directly exposes food to subfreezing temperatures, has been used for the preservation of shrimp quality. Boonsumrej, Chaiwanichsiri, Tantratian, Suzuki, and Takai (2007) reported that cryogenic freezing caused less quality changes of tiger shrimp than air-blast freezing; and Lopkulkiaert, Prapatsornwattana, and Rungsardthong (2009) also found that cryogenic freezing combined with sodium bicarbonate significantly improved yield, freezing time, freezing rate, cutting force and color of white shrimp, when compared with other freezing methods. Recently, edible antimicrobial coatings and packaging have been developed and several applications for meat, poultry, and seafood have been reviewed (Campos, Gerschenson, & Flores, 2011; Gennadios, Hanna, & Kurth, 1997). Some studies showed that the antimicrobial coating alone or when combined with other techniques, such as gamma irradiation and modified atmosphere packaging, could inactivate or retard microbial growth and prolong the shelf-life of shrimp (Jiang, Liu, & Wang, 2011; Mastromatteo, Danza, Conte, Muratore, & Del Nobile, 2010; Ouattara, Sabato, & Lacroix, 2001). However, there is very limited information regarding the combination of these treatments; therefore, there is a need to develop nonthermal technologies, which can reduce microbial contamination and levels of pathogens on shrimp.

The objectives of this study were to: 1) conduct a survey to assess microbiological safety and quality of shrimp from local markets, including the incidence of *Listeria*, and 2) evaluate the effect of ozone washing, antimicrobial coating, and cryogenic freezing, when applied alone or in combination on the survival of background microflora and inoculated *L. innocua* on shrimp surfaces.

2. Materials and methods

2.1. Background microflora and foodborne pathogens

The U.S. Food and Drug Administration (FDA) standard methods described in the FDA's Bacteriological Analytical Manual (BAM) were used (FDA, 2012). Approximately 375 g of frozen raw shrimp were removed from 5 lb boxes, and blended (Waring Laboratory, Torrington, CT) with buffered peptone water (BBL-Difco Laboratories, Sparks, MD) at a 1:10 dilution for 2 min. One mL of diluted shrimp sample was then pipetted onto aerobic plate count (APC), coliform and *Enterobacteriaceae* Petrifilms™ (3M, St. Paul, MN, USA) in duplicate. The films were incubated according to the manufacturer's specifications. In addition, a second set of APC films were inoculated and incubated at 22 °C to obtain psychrotrophic counts. A BAX Polymerase Chain Reaction (PCR) test was then conducted using the BAX® System Q7 (Dupont Qualicon, Wilmington, DE, USA) to determine the presence of *Listeria* spp., *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Vibrio* spp. If a positive PCR result was obtained, FDA's approved methods were used to recover viable foodborne pathogens (FDA, 2012). The *Listeria* species that were recovered (Table 2) were identified using API test strips (BioMeroiux, Marcy l'Etoile, France).

2.2. Antimicrobials

Chitosan (Low Molecular Weight, 150 kDa, 75–85% deacetylation) and allyl isothiocyanate (AIT) (95% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetic acid, lactic acid and levulinic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). Lauric arginate ester (LAE) solution (CytoGuard®) containing 20% LAE was obtained from A&B Ingredients (Fairfield, NJ).

2.3. Sample preparation for coating treatment

After thawing overnight in a refrigerator (4 °C), shrimp were divided into three groups: the first group was used to test background microflora in shrimp without temperature abuse, the second group was used to test background microflora in shrimp at an abused temperature (room temperature [22 °C] for 12 h) before any treatments, and the third group was inoculated with *L. innocua*.

2.4. Inoculum and inoculation

Three *L. innocua* strains (ATCC 51742, 33090, and 33091) obtained from the American Type Culture Collection (Manassas, Va., U.S.A.) were reported to have similar inactivation kinetics as pathogenic *L. monocytogenes* used in previously reported studies for both thermal and nonthermal processing technologies (Sommers, Geveke, & Fan, 2008; Sommers, Kozempel, Fan, & Radewonuk, 2002; Sommers, Scullen, & Sites, 2010). Each strain was maintained on Tryptic soy agar (BD-Difco) at 0–2 °C. Prior to experiments, each strain was cultured independently in 30 mL Tryptic soy broth (BBL/Difco) on a rotary shaker (150 RPM) at 37 °C for 18 h. The aliquot (30 mL) of fresh culture from each strain was combined. This inoculum (90 mL) was used to inoculate shrimp samples. The concentration of the inoculum was *ca.* 9.0–9.5 log CFU/mL.

The prepared inoculum (90 mL) was added to 5 L of sterile deionized water mixed with 200 pieces of shrimp, and kept in a refrigerator (4 °C) overnight to allow bacterial attachment. The inoculums were then decanted and the shrimp were allowed to drip dry, before being placed in a sterile polypropylene pan. The population of *Listeria* on the shrimp after inoculation was *ca.* 8.2 log CFU/g.

2.5. Antimicrobial treatment

2.5.1. Ozone wash treatment

An aluminum alloy basket, 15.2 × 15.2 × 15.2 cm ($L \times W \times H$), with perforated openings of 0.8 cm × 2.5 cm ($L \times H$) was specifically constructed for this study. Shrimp were placed in the basket and manually agitated, while 9.5 L/min of ozonated water, generated on site with OSW-3 ozonated water generator (Ozone Solutions, Inc., Hull, IA, USA), at 20 kPa with a temperature of 19–22 °C and an ozone concentration of 1.6–1.9 ppm, was immediately sprayed directly on the shrimp for 55–65 s, using a size 20 nozzle spray head (Loc-Line #51845, Lake Oswego, OR).

2.5.2. Coating solution preparation and coating treatment of shrimp

Three coating solutions were prepared for this study. Chitosan coating solution #1 (CC1) included 200 mg chitosan in 10 mL of an acid solution, containing 2% each of acetic, lactic and levulinic acids. Chitosan coating solution #2 (CC2) included chitosan coating solution #1 (CC1) + 6% (v/v) AIT; and Chitosan coating solution #3 (CC3) included chitosan coating solution #1 (CC1) + 5% (v/v) LAE. Each mixture was stirred with a magnetic stir bar on a stir plate until the polymer was completely dissolved. Shrimp were dipped in coating solutions for 1 min. The non-coated (control) and coated shrimp were placed in a bio-safety cabinet to dry at room temperature for 2 h, prior to other treatments or microbial analysis.

2.5.3. Cryogenic freezing treatments

Shrimp were placed in a perforated basket (40 × 40 × 15 cm [$L \times W \times H$]) built in Cryo-Test Chamber (Air Products and