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# Control of *Listeria monocytogenes* on skinless frankfurters by coating with phytochemicals

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ABSTRACT

The efficacy of four phytochemicals, namely *trans*-cinnamaldehyde (0.1, 0.2%), carvacrol (0.1, 0.2%), thymol (0.1, 0.2%) and eugenol (0.3, 0.4%) incorporated in a chitosan-based coating for inactivating *Listeria monocytogenes* (LM) on frankfurters was investigated. Frankfurters were inoculated with a 5-strain LM mixture (~$10^6$/frankfurter, either prior to or after coating with phytochemicals), vacuum-packed, and stored at 4 °C for 42 days. Representative samples were analyzed on days 0, 1, 3, 7, 14, 28, and 42 for surviving LM. Moreover, the effect of phytochemicals on lipid oxidation, meat pH and expression of LM genes critical for attachment to meat was studied. On day zero, all phytochemical coatings reduced LM counts by >2.5 log CFU/frankfurter compared to controls ($P < 0.05$). From days 1 to 42, LM counts on phytochemical-coated frankfurters were consistently lower, and on day 42, the coatings reduced LM by ~5.0 log CFU/frankfurter. The phytochemical coatings also significantly reduced lipid oxidation and expression of LM meat attachment genes ($P < 0.05$). No change in pH of frankfurters was observed ($P > 0.05$). Results suggest that the aforementioned phytochemicals could potentially be used as an effective antilisterial coating, however, follow up studies on the sensory and quality characteristics of phytochemical-coated frankfurters are necessary.



## 1. Introduction

*Listeria monocytogenes* (LM) is a major foodborne pathogen in the United States that causes life-threatening infections in susceptible populations (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). *L. monocytogenes* is of significant concern in ready-to-eat (RTE) meats since the pathogen can be continuously introduced in food-processing plants from surrounding environment (Chaturongakul, Raengpradub, Wiedmann, & Boor, 2008) as well as through the hide and meat of slaughtered animals (Dmowska, Wieczorek, Lynch, & Osek, 2013). In addition, the ability of LM to form biofilms facilitates its long-term survival in food-processing environment (Fatemi & Frank, 1999). The heat treatment received by frankfurters generally eliminates LM; however, the products are re-contaminated during post-processing steps such as peeling, slicing or packaging (Tompkin, 2002). Multiple listeriosis outbreaks have been linked to the consumption of commercially prepared, ready-to-eat (RTE) meats, including frankfurters, which were contaminated during post-processing operations (Scallan et al., 2011). Because of the severity of illness and high case fatality rates (~30%) associated with listeriosis, the United States Department of Agriculture (USDA) has adopted a zero tolerance policy for the presence of LM in RTE meat products (USFSIS, 1989). The USDA-FSIS alternatives 1 and 2 of the interim final rule for the control of LM (9 CFR 430) mandate that all RTE meat-processing plants implement post-lethality interventions on RTE food products, which may include the use of antimicrobials to inactivate LM, and suppress its growth during refrigerated storage (USFSIS, 2012).

The efficacy of various thermal interventions such as hot water dips, infrared radiation, steam inactivation (Huang & Sites, 2008; Muriana, Quimby, Davidson, & Grooms, 2002) and non-thermal processing such as application of high pressure (Chen, 2007) and irradiation (Zhu, Mendonca, Ismail, Du, & Ahn, 2005) to control post-processing LM contamination of RTE meat products has been

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investigated with varying degrees of success. In addition, the efficacy of modified atmosphere packaging (MAP), either alone or in combination with other technologies such as irradiation (Thayer & Boyd, 1999; Zhu et al., 2005) or antimicrobials (Fang & Lin, 1994) to inhibit LM growth has been studied. The majority of the aforementioned approaches aim to reduce post-processing contamination of frankfurters during their production, and do not exert significant antimicrobial effect during post-processing refrigerated storage. Since LM is a psychrotrophic pathogen that can grow during refrigerated storage of foods, there is a need to develop intervention strategies that can effectively reduce LM post-processing contamination and control subsequent growth during refrigerated storage.

Plant extracts have been used as natural preservatives, and flavor enhancers in foods around the world. Extensive research in the past decade has identified a plethora of active components present in plant extracts with potent antimicrobial activity (Burt, 2004). *Trans*-cinnamaldehyde (TC) is an aromatic aldehyde obtained from cinnamon tree (*Cinnamomum zeylandicum*). Carvacrol [CR; 2-methyl-5-(1-methylethyl) phenol] and thymol (TH; 2-isopropyl-5-methylphenol), are monoterpenoid phenolic compounds present in oregano oil obtained from *Origanum glandulosum*. Eugenol (EG; 4-allyl-2-methoxyphenol) is a plant-derived antimicrobial present as a major component of clove oil (*Eugenia caryophyllus*) (Leriche & Carpentier, 1995). All the aforementioned compounds are classified as generally recognized as safe (GRAS) by the FDA (Adams et al.,2004; Adams et al., 2005; U.S. FDA, 2012, 2013). The multifold antimicrobial activity and high margin of safety of these phytochemicals make them ideal candidates for use against pathogenic microorganisms in food products. Chitosan is a natural, GRAS-status biopolymer obtained by the deacetylation of chitin, which is the major component of the exoskeleton of crustaceans (Jeon, Park, & Kim, 2001). Due to its low cost and potent film forming property, chitosan has previously been used as an antimicrobial film or coating to increase the shelf life and microbial safety of food products (Belalia, Grelier, Benaissa, & Coma, 2008). Previous studies have investigated the potential TC, CR, and EG as antimicrobial dip treatments for inactivating foodborne pathogens (Mattson et al., 2011; Upadhyaya et al., 2013; Upadhyay, Upadhyaya, Kollanoor-Johny, & Ananda Baskaran, et al., 2013), however there are no reports on their antimicrobial efficacy when used in edible coating on frankfurters. The objective of this study was to investigate the efficacy of chitosan-based edible coating incorporating TC, CR, TH or EG in reducing LM on skinless frankfurters during refrigerated storage.

## 2. Materials and methods

### 2.1. Bacterial strains and culture conditions

All culture media were procured from Difco (Becton Dickinson, Sparks, MD). Five strains of LM, including ATCC Scott A, ATCC 19115, 101, 1 and Presque-598 were used in this study. Each LM strain was cultured separately in 10 ml of sterile tryptic soy broth with 0.6% yeast extract (TSBYE) in 30 ml sterile tubes, and incubated at 37 °C for 24 h. Following incubation, the cultures were centrifuged (3600× *g* for 15 min) at 4 °C. The pellet was washed twice, resuspended in 10 ml of sterile phosphate buffered saline (PBS, pH 7.0), and serial ten-fold dilutions were cultured on duplicate tryptic soy agar (TSA) and oxford agar plates, followed by incubation at 37 °C for 24 h. Equal quantities of the five strains were combined, diluted appropriately, and the resulting suspension was used as the inoculum (7 log CFU/ml). The bacterial concentration in the five-strain cocktail of LM was enumerated by plating 100 μl of culture suspension from appropriate dilutions on TSA and oxford agar plates followed by incubation at 37 °C for 24 h.

### 2.2. Determination of SIC and MBC of phytochemicals

The sub-inhibitory concentration (SIC) and minimum bactericidal concentration (MBC) of each phytochemical and chitosan against LM were determined, as described previously (Upadhyay, Upadhyaya, Kollanoor-Johny, & Venkitanarayanan, 2013). Briefly, sterile 24-well polystyrene tissue culture plates (Costar, Corning Incorporated, Corning, NY) containing TSBYE (1 ml/well) were inoculated separately with ~6.0 log CFU of LM, followed by the addition of 0.1–10 μl of TC, CR, TH, EG, or chitosan (Sigma–Aldrich Corp, St. Louis, MO) with an increment of 0.5 μl. The plates were incubated for 24 h at 37 °C followed by bacterial enumeration on TSA plates. The highest concentration of each phytochemical or chitosan that did not significantly inhibit the growth of LM after 24 h of incubation was selected as its respective SIC for the study. The lowest concentration of phytochemicals that reduced LM population by ~5.0 log CFU/ml after incubation at 37 °C for 24 h was selected as its MBC.

### 2.3. Preparation and inoculation of frankfurters

Fresh, skinless, pork-beef frankfurters (20% fat; ~50 g) were procured from a local manufacturer, and equilibrated to room temperature (23 ± 2 °C). To rule out any inherent LM on the frankfurters, representative samples were placed in a Whirl-Pak™ bag (one frankfurter/bag) (Nasco, Fisher Scientific Co LLC, Hanover Park, IL) containing 50 ml of PBS, homogenized for 2 min, and streaked on duplicate oxford agar plates (limit of detection 500 cells/frankfurter). In addition, the meat homogenate from the Whirl-Pak™ bag was enriched by transferring 1 ml of the sample to 20 ml of TSBYE (limit of detection 50 cells/frankfurter), and incubating at 37 °C for 24 h. The culture was then streaked on oxford agar, incubated the plates at 37 °C for 48 h and observed for typical LM colonies.

The frankfurters were inoculated with the five-strain cocktail of LM, as described previously (Jiang, Neetoo, & Chen, 2011). Briefly, 100 μl of a 5-strain cocktail of LM (~9.0 log CFU/ml) was appropriately diluted and spot inoculated on frankfurters and spread uniformly to obtain 6.0 log CFU per frankfurter. After inoculation, the frankfurters were placed in a sterile container for 30 min at 4 °C to facilitate bacterial attachment.

### 2.4. Preparation of chitosan-based phytochemical coating

Chitosan-based phytochemical coating was prepared as described previously (Chen et al., 2012). Briefly, 2 g of low molecular weight chitosan (~5–15 kDa) (Sigma Aldrich) was dissolved in sterile deionized water containing 1% acetic acid (Sigma Aldrich) and stirred at room temperature for 12 h to obtain a final chitosan concentration of 2% (pH 4.6). Subsequently, each phytochemical was added to the chitosan solution at the desired concentration (TC, CR, TH, 0.1, 0.2%; EG, 0.3, 0.4%), and the solution was stirred for another 6 h to facilitate proper mixing of the compounds.

### 2.5. Inoculation and treatments

Skinless frankfurters were divided into pre-inoculated and pre-coated groups. The frankfurters from the pre-inoculated group were first inoculated with LM (6 log CFU/frankfurter) as previously described followed by phytochemical coating treatments, whereas the frankfurters in the pre-coated group were first coated with the phytochemical and subsequently subjected to LM inoculation.