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Evaluation of corn oil as an additive in the pre-enrichment step to increase recovery of *Salmonella enterica* from oregano

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ABSTRACT

Phenolic compounds associated with essential oils of spices and herbs possess a variety of antioxidant and antimicrobial properties that interfere with *Salmonella* detection from fresh and dried products. Finding a compound to neutralize the effect of these antimicrobial compounds, while allowing *Salmonella* growth during pre-enrichment, is a crucial step in both traditional pathogen isolation and molecular detection from these foods. This study evaluated the effectiveness of corn oil as a component of the pre-enrichment broth to counteract antimicrobial compounds properties and increase the recovery of *Salmonella* from spices. Oregano samples artificially contaminated with *Salmonella enterica* were pre-enriched in modified Buffered Peptone Water (mBPW) supplemented with and without 2% (vol/vol) corn oil respectively. Samples were incubated overnight at 37 °C. The results showed that recovery of *Salmonella* from oregano samples was increased by ≥50% when pre-enriched with corn oil. Serovars were confirmed using a PCR serotyping method. In addition, shot-gun metagenomics analyses demonstrated bacterial diversity and the effect of corn oil on the relative prevalence of *Salmonella* in the oregano samples. Modifying pre-enrichment broths with corn oil improved the detection and isolation of *Salmonella* from oregano, and may provide an alternative method for pathogen detection in dried food matrices such as spices.

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1. Introduction

Foodborne outbreaks of *Salmonella* have increasingly been associated with the consumption of contaminated spices, underscoring the need for increased surveillance of products in which spices are incorporated (Schnirring, 2013; Van Doren et al., 2013; CFSAN, http://www.fda.-gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm367339.htm). Between 1973 and 2010, fourteen reported outbreaks were attributed to consumption of pathogen-contaminated spices around the world (Canada, Denmark, France, Germany, New Zealand, Norway, Serbia, the United Kingdom, and the United States) (CFSAN, http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm367339.htm). These outbreaks accounted for 1946 reported human illnesses, 128 hospitalizations and two deaths. *Salmonella enterica* subspecies *enterica* was identified as the causative agent in ten of the fourteen outbreaks accounting for 87% of reported illnesses (CFSAN, http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm367339.htm). In the United States, both *Salmonella* serovars Montevideo and Senftenberg have been linked to outbreaks involving cracked pepper coatings on salami products, which sickened 272 people in 44 states (Schnirring, 2013; CDC, http://www.cdc.gov/salmonella/outbreaks.html).

Spices are commonly used as food adjuncts for flavoring, seasoning, and coloring agents. Many spices have medicinal properties and possess beneficial effects, such as antioxidant activity, digestive stimulant action, as well as anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, and anticarcinogenic potential (Shan et al., 2005). Spices or spice extracts contain phenolic compounds that are often used as additives to extend the shelf life

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of foods. Phenolic compounds from spices essential oils are associated with the antimicrobial activities of spices (Verluyten et al., 2004). Phenolic compounds are composed of phenols, aldehydes and alcohols, such as carvacrol [2-methyl-5-(1-methylethyl) phenol] and thymol (2-isopropyl-5-methylphenol) (Baratta et al., 2010; Shan et al., 2005; Tabanca et al., 2004; Ben Hamida-Ben Ezzeddinea et al., 2000; Palumbo and Harris, 2011). There are various reports on the mechanisms of action of the phenolic compounds on bacterial cell membranes and bacterial inactivation (Espina et al., 2013; Helander et al., 1998; Ultee et al., 1999, 2002). These antimicrobial compounds are soluble lipophiles, compounds that combine or dissolve lipids or fats. Phenolic compounds cross through the cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids, and permeabilize the cells (Stefanakis et al., 2013). Espina et al. (2013) described carvacrol as a compound that causes destabilization of bacterial membrane, decrease in the membrane potential, dissipation of pH gradients, and perturbation of lipid fractions of bacterial cytoplasmatic membranes, which lead to bacterial cell death.

Even with the presence of these antimicrobial properties, spices have been implicated in *Salmonella* outbreaks (Van Doren et al., 2013). There are methods for isolating *Salmonella* from many dried foods (Sperber and Deibel, 1969) that are effective. The current detection approach for spices are based on the FDA Bacteriological Analytical Manual (BAM) method for *Salmonella* (Andrews et al., 2014), and it has limited success with a number of spices, creating a need for an improved method for *Salmonella* detection in spices. The BAM states that at this time there are no known methods for neutralizing the toxicity of oregano and recommends diluting at 1:100 sample/broth ratio beyond the toxic levels. In many cases even when the samples are diluted as directed in the BAM, *Salmonella* was not recovered from the implicated spices. This could possibly be due to the inhibitory effects of the antimicrobial compounds, like carvacrol, that may not be fully diluted below the toxic levels. Although the essential oils may be beneficial, they can interfere with methods for the detection of pathogens associated with spices and spice containing commodities.

The spice used for this study was oregano or *Origanum* spp. Oregano's essential oils manifest broad and high antimicrobial activity against most Gram positive and negative bacteria (Baratta et al., 2010). Stefanakis et al. (2013) reported that of essential oils in oregano, carvacrol is the most active. Oregano essential oils are effective against *Escherichia coli*, *Saccharomyces cerevisiae*, and *Listonella anguillarum*. Stefanakis et al. (2013) also showed that thymol and carvacrol, found in oregano, have high antimicrobial activity against *Vibrio* species. Gulten et al. (2010a, 2012) reported that oregano oils can effectively reduce the population of *Salmonella* spp. inoculated onto lettuce leaves. Other studies have demonstrated the effectiveness of phenolic compounds from oregano essential oils in inhibiting the growth of pathogenic microorganisms in fresh produce (Gulten et al., 2010a, 2010b, 2012), in meat (Tsigarida et al., 2000; Verluyten et al., 2004), bologna sausages (Viuda-Martos et al., 2010), tomatoes (Gulten et al., 2010b), and mayonnaise (Lima da Silva and Gombosy de Melo Franco, 2012). Oregano's essential oils have shown activity against a variety of bacteria, molds, and yeasts using a number of *in vitro* studies (Elgayyar et al., 2001; Viuda-Martos et al., 2007, 2010).

One approach in making these antimicrobial components of spices ineffective is to formulate the pre-enrichment broths to neutralize or partition antimicrobial compounds away from the bacteria thus enabling better isolation of foodborne pathogens. Since many of the antimicrobial compounds such as carvacrol are lipophilic, the approach in this study is to add a hydrophobic compound such as corn oil to the growth media that will partition the carvacrol away from the bacteria thereby allowing *Salmonella* to grow in the aqueous phase of the pre-enrichment broth. The choice of oil is essential; the oil cannot contain any antimicrobial components such as those described for olive oil (Palumbo and Harris, 2011). Holtman previously demonstrated the use of corn oil for increased mold recovery (Holtman, 1945).

The goal of this study was to evaluate the ability of the corn oil in the pre-enrichment growth media to increase recovery and detection of *Salmonella* from oregano. In this investigation 2% (vol/vol) corn oil was added to the pre-enrichment broth and evaluated for its ability to increase the recovery of *Salmonella* from oregano. Studies were conducted with commercially available oregano from a retail distributing center and oregano obtained from FDA field lab surveillance before processing at the distributing centers. The increased recovery of *Salmonella* due to corn oil was assessed using plating methods, and screening for positive oregano samples was conducted using molecular serotyping PCR. In the case of non-culturable *Salmonella*, shot-gun metagenomics was added as a molecular tool to detect low abundance of *Salmonella* in the oregano samples and genomic identification of non-culturable pathogens.

2. Methods

2.1. Bacterial strains

S. enterica serovar Montevideo (GenBank #AESL01) was used in this study to evaluate the effectiveness of corn oil to increase *Salmonella* recovery (Lienau et al., 2011). *Salmonella* Montevideo was obtained from FDA Center for Food Safety and Applied Nutrition (CFSAN), Office of Applied Research and Safety Assessment (OARSA) culture collection and were originally obtained from contaminated foods and stored at −80 °C.

2.2. Oregano

Two types of oregano were used: dry oregano from the retail distributors represented samples considered as low or reduced load bacterial flora (processed), and dry non-processed oregano obtained from the CFSAN spice sample collection were considered high load bacterial flora and not treated by any processing.

2.3. Enrichment broth

The method described in the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) method for the isolation of *Salmonella* (Andrews et al., 2014) was used for these studies with the modification of using modified Buffer Peptone Water [(mBPW), Buffered Peptone Water (BPW) (Difco-Becton Dickinson Co, Hunt Valley, MD) plus 3.5 g of disodium phosphate and 1.5 g of monopotassium phosphate per liter] as the base enrichment broth instead of Trypticase soy broth (TSB), similar to studies conducted for various food matrices described by Jean-Gilles Beaubrun et al. (2012, 2014). Filter sterilize food grade corn oil was added to the mBPW at a concentration of 2% (vol/vol) to partition the phenolic compounds away from the *Salmonella* in the pre-enrichment broth.

2.4. Salmonella isolation and identification

Each oregano sample was processed for the detection of *S. enterica* using a modified method described in this report based on the standard BAM method (Andrews et al., 2014). *Salmonella* from a frozen culture was plated onto Trypticase Soy Agar with 5% sheep blood agar (TSAB) and incubated at 37 °C for 18–24 h to