

Susceptibility of Pathogenic Nontyphoidal *Salmonella* Serovars and Avirulent *Salmonella* LT2 to Elevated Hydrostatic Pressure and CitricidalTM

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Abstract—Nontyphoidal *Salmonella enterica* serovars are the leading cause of foodborne hospitalization and death episodes in the United States. In a typical year, it is estimated that >1 million Americans experience illness due to foodborne nontyphoidal Salmonellosis. While the private industry and manufacturing rely heavily on results of validation studies for inactivation and decontamination of this prevalent and opportunistic pathogen of public health concern, availability of such studies conducted in realistic environments of manufacturing and processing is limited due to pathogenic nature of the organism. The current study investigated effects of elevated hydrostatic pressure and a bioactive product from grapefruit seed extract (CitricidalTM) for elimination of nontyphoidal *Salmonella* serovars. Additionally, sensitivity of an avirulent strain of the bacterium (*Salmonella* LT2) investigated relative to the pathogenic *Salmonella* serovars. Our results show that the avirulent strain utilized in the current study, has similar sensitivity to the treatments compared to nontyphoidal pathogenic *Salmonella* serovars. Thus, this non-pathogenic strain could be used interchangeably for public health microbiology hurdle validation studies as a surrogate for pathogenic *Salmonella* serovars. Additionally, we observed >5 log reduction of the pathogen (*i.e.* > 99.999%) due to treatments with elevated hydrostatic pressure at 500 MPa and at 300 MPa with presence of CitricidalTM. Our result additionally exhibits that combination of mild hydrostatic pressure and bioactive compounds such as CitricidalTM could be utilized to ensure safe and efficacious elimination of this prevalent pathogen of public health concern from common commodities.

Introduction

Various serovars of nontyphoidal *Salmonella enterica* continue to be a major global, national, and regional public health challenges (Kumar et al., 2019; Majowicz et al., 2010). The pathogen is particularly a concern for foodborne and waterborne infectious diseases as nontyphoidal *Salmonella* serovars could survive for several weeks in low-nutrient environments (Kabir et al., 2020).

In the United States, it is estimated that in a typical year over 1 million Americans contract nontyphoidal *Salmonella*, a disease that has estimated 27.2% and 0.5% hospitalization and death rates, respectively (Scallan et al., 2011). From 2009 to 2019 there had been at least 53 outbreaks associated with nontyphoidal *Salmonella* in State of Tennessee with vast majority of the outbreaks associated with contaminated food (CDC, 2020). Government of Tennessee Interactive Data additionally indicate that Salmonellosis could be zoonotic in nature as well since over 19,000 samples of Tennessee animals were tested positive for this pathogen in 2020 (Tennessee Department of Agriculture, 2021).

Considering current epidemiological burden of the pathogen in the country and in Tennessee, emerging technologies are needed to further eliminate this pathogen of public health

concern from the food chain. Utilization of elevated hydrostatic pressure is an emerging technology that is gaining increasing popularity in private manufacturing and the pharmaceutical industry. This technology exposes the packaged commodities to elevated levels of hydrostatic pressure, typically several times higher than atmospheric pressure in the deepest part of the oceans (Mariana Trench), to eliminate the pathogenic microorganisms from the product (Kabir et al., 2021; Aras et al., 2020a). The technology exposes the product to pressure intensity levels of typically up to 650 MPa, however, application of lower pressure intensity levels augmented with natural antimicrobials could benefit the practitioners for optimizing cost and improving sustainability of their products (Allison et al., 2018a; Allison, et al., 2018b; Aras, et al., 2020b; George et al., 2020; Kabir et al., 2019). CitricidalTM is an antimicrobial extracted from grapefruit seed that has been associated with elimination of foodborne pathogens (Valenzuela-Melendres et al., 2016). Although this plant-based bioactive compound has been investigated against an array of Gram-positive and Gram-negative bacterial pathogens of public health concern (Castro-Rosas et al., 2002), use of this bactericidal agent has not been investigated under the elevated hydrostatic pressure. Thus, the current study investigated effects of elevated hydrostatic pressure for elimination of nontyphoidal *Salmonella* serovars at 500 MPa and 300 MPa and additionally investigated 300 MPa treatments as augmented by CitricidalTM. The comparison of pathogenic *Salmonella* serovars with a non-pathogenic strain

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