

Title In-vitro and microscopic analysis of ozone (O₃) fumigation effects on *Salmonella typhimurium* and *Listeria monocytogenes*

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Abstract

Food safety has caught the world's attention after a series of deadly outbreaks such as an outbreak of *Salmonella* in Britain that sickened 169 people and an outbreak of *Listeria monocytogenes* in the United States which caused 29 deaths. These fatal outbreaks forced the development of a standard sanitization method that is able to eliminate the pathogenic bacteria before fresh produce is distributed to consumers. This has led to the objective of this study which is to evaluate the effectiveness of ozone fumigation as a potential sanitization method in eliminating common foodborne pathogens; *Salmonella typhimurium* and *Listeria monocytogenes*. In-vitro analysis in this study shows that ozone concentration significantly ($P < 0.05$) affects bacterial inhibition percentage where the inhibition percentage increases as the ozone concentration increases. Exposure time also significantly ($P < 0.05$) influences the inhibition percentage with a positive pattern but showed a plateau response after 6 hours exposure. The in-vitro analysis also showed that *S. typhimurium* is more resistant towards ozone oxidation than *L. monocytogenes*. This resistance is clearly illustrated as exposure to 0.1 ppm of ozone for 2 hours only eliminated 10.8% of *S. typhimurium* CFU but destroyed 85.9% of *L. monocytogenes* CFU. The differences in the susceptibility of bacterial species towards ozone oxidation may be due to the differences in their cell wall composition and structure where ozone may react more progressively against gram positive bacteria compared to gram negative bacteria. This hypothesis is confirmed from scanning electron microscopy analysis as ozone produced more damaging effects on *L. monocytogenes* cell wall compared to the other tested pathogens. The in-vitro analysis also shows that exposure to 1 ppm of ozone for 2 hours could achieve nearly complete death of the tested pathogens hence implying that ozone fumigation can be a very effective sanitization method in destroying pathogenic bacteria. An in-vivo study is however required to be performed in order to determine the effects of ozone towards the tested pathogen on contaminated fresh produce.