

Thesis No. 12403; Master of Science in Food Hygiene

Title: Antibacterial and anti-biofilm effects of thymol nanoemulsion prepared with monolaurin against *Staphylococcus aureus* and *Salmonella Enteritidis*

Abstract

Nanoemulsions are a type of fat carrier that is used as an ideal method to deliver these materials. They are simply made, have small size and improve access, biological effectiveness and sustainability. Nanoemulsions can be used as antimicrobial and disinfectant agents in various food equipment and surfaces. The aim of this study was to investigate the antibacterial and antibiofilm effects of thymol nanoemulsion prepared with monolaurin against *Staphylococcus aureus* and *Salmonella enteritidis* under laboratory conditions and in sterilized milk stored at 7°C. The nanoemulsion was prepared using the phase inversion method and stored at room temperature. The results showed that the average particle size of thymol nanoemulsion was 49.7 nm, and its zeta potential was -1.2 mV. The minimum inhibitory concentration (MIC) of thymol nanoemulsion against both bacteria was determined to be 0.14 mg/mL using the microdilution method. The growth curves of bacteria in the presence of thymol, monolaurin, the mixture of thymol and monolaurin, and thymol nanoemulsion were then examined for 48 hours. The mixed treatment and nanoemulsion showed the highest inhibitory effect. Furthermore, the effects of the treatments on biofilm formation and removal were investigated using two assays. In the biofilm inhibition assay, the 1×MIC (0.14 mg/mL) and 2×MIC (0.285 mg/mL) concentrations of thymol nanoemulsion were examined for their ability to prevent the formation of 48 hour biofilms of *S. aureus* and 72 hour biofilms of *Salmonella enteritidis* at 37°C. The nanoemulsion at a concentration of 0.14 mg/mL reduced the bacterial count of *Salmonella enteritidis* by 1.72 log cycles, and at a concentration of 0.285 mg/mL, it reduced the count by 2.26 log cycles. The biofilm of *S. aureus* was reduced by 2.02 and 2.3 log cycles at concentrations of 0.14 and 0.285 mg/mL, respectively. In the biofilm removal assay, the effect of the 10×MIC concentration of thymol nanoemulsion at 37°C and three time intervals (10, 20, and 30 minutes) on the 48 hour biofilm of *S. aureus* and the 72 hour biofilm of *Salmonella enteritidis* was examined. The results showed that increasing the contact time led to a significant increase in the antibiofilm effect of the nanoemulsion. At a contact time of 30 min, of *S. aureus* biofilm was reduced by 4.91 log cycles, and *Salmonella enteritidis* biofilm was reduced by 5.17 log cycles. In sterilized milk, the

nanoemulsion at 4×MIC resulted in a reduction of 1.09 log cycles in the bacterial count of *Salmonella enteritidis* and 0.83 log cycles in the count of *S. aureus* at the end of day 8. Overall, the results of this study demonstrated that thymol nanoemulsion exhibited suitable antibacterial and antibiofilm effects against both *Salmonella enteritidis* and *S. aureus*.

Keywords: antibiofilm, Thymol, monolaurin, nanoemulsion, *Staphylococcus aureus*, *Salmonella Enteritidis*