Title Comparison of spot, spray, and dip inoculation methods to determine efficacy of chlorine dioxide gas and chlorinated water treatments in reducing *Salmonella* spp. on green peppers

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Abstract

To successfully evaluate efficacy of antimicrobial agents, it is necessary to develop a standardized inoculation method that best represents naturally existing bacteria on produce. Our objective was to compare spot, spray, and dip inoculation to determine efficacy of chlorine dioxide (ClO_2) gas and chlorinated water treatments in reducing *Salmonella* spp. on green peppers using a bacterial enumeration technique, cryo-scanning electronic microscopy (CSEM) and fluorescence microscopy (FM). A mixture of five strains of *Salmonella* spp. was used for inoculation while a green fluorescence protein-labeled *Salmonella enteritidis* was used for FM. Peppers were spot-inoculated or spray-inoculated with 7-8 log cfu/cm^2 *Salmonella,* or dip-inoculated in 9 log cfu/ml bacterial suspension for 1 min, air-dried for 2 hrs or stored for 1 and 3 days at 4°C, and then treated with 0.3-0.6 mg/l ClO_2 gas for 5-10 min or 200 ppm chlorinated water for 10 min, respectively. Bacterial populations were enumerated using tryptic soy agar followed by membrane-transferring plating on bismuth sulphite or xylose lysine desoxycholate agars. After one day storage, recovered populations among three inoculation methods were not significantly different ($P>0.05$). After 3 day storage, the population for dip inoculation was the lowest compared to other methods. After 0.6 mg/1 ClO_2 gas treatment for 10 min. the ranges of log reductions were 4-6, 3.5-4, and 2-3 log cfu for spot, spray, and dip inoculation, respectively. FM and CLSM studies demonstrated different bacterial distribution and viability after inoculation and ClO_2 gas treatment. For 3-day stored samples, log reductions after 200ppm chlorinated water treatment were 3.9±0.3 for spot, 29±0.3 for spray, and 2.8±0.5 for dip. Important factors affecting antimicrobial efficacy of these treatments were identified as inoculation method, storage condition, and time. Results will help improve and ensure the safety of produce by using precisely defined and effective sanitation technologies, such as ClO_2 gas treatment.