

Survival of pathogens on work-in-process fresh-cut produce ingredients



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Summary

Work-in-process (WIP) fresh-cut produce ingredients, often serving as minor components of finished products, are held in containers for various lengths of times before being utilized. The survival of cold-adapted *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella* will be evaluated on WIP ingredients as a function of holding time, temperature, pathogen inoculation level, and washing with sanitizers. Also to be determined are innate natural antimicrobials and background microbiota, which may serve as indicators for the presumed variation in pathogen survival on WIP ingredients. The transfer probability of pathogens from WIP ingredients to holding containers will be calculated. Models will be developed to predict the survival of pathogens on WIP ingredients and containers. Preliminary results indicate the importance of maintaining low refrigerated temperature (4°C) for WIP ingredients.

Objectives

1. Evaluate the survival of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on the surface of WIP ingredients during storage, as affected by time, temperature, pre- and post-sanitization, pathogen populations, background microbiota, and innate natural antimicrobials.
2. Study the transfer of pathogens from WIP ingredients to holding containers and suitability of reusing holding containers.
3. Model factors contributing to the survival and growth of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* on WIP ingredients and the transfer between pathogens and holding containers.

Methods

While waiting for onboarding of the postdoc, preliminary experiments were conducted. *Listeria* and *E. coli*, inoculated into liquid media, were incubated for 12 days to assess growth at 4 and 12°C. Green cabbage was sliced, inoculated with the pathogens, and populations determined over 6 days at 4 and 12°C. WIP ingredients (carrot, red cabbage, kale, Brussels sprouts, onion, broccoli stalk, and baby spinach) inoculated with cold-adapted *Listeria*, *E. coli* O157:H7 and *Salmonella*, will be stored at 4, 8, 12 and 16°C for 6 days. Populations of pathogens, aerobic bacteria, *Enterobacteriaceae*, lactic acid bacteria, yeast and mold will be monitored. Pathogen transfer to holding containers will be determined by storing pathogen-inoculated WIP, and sampling over time. Statistical models will be developed to predict pathogen survival.

Results to Date

Preliminary results indicate that both *E. coli* and *Listeria* multiplied in liquid media, even at 4°C (Figure 1), although growth of *E. coli* was minimal. These results indicate that these genera of bacteria will multiply at 4°C, when nutrients are available, and that we can produce, in the laboratory, cold-adapted isolates for future studies. *Listeria* cells grew at 12°C on WIP cabbage (Figure 2), but not at 4°C during the 6-day holding time, although they survived at that temperature. *E. coli* did not growth at 12°C on WIP cabbage (Figure 3), while at 4°C, populations decreased over time. These results suggest that carefully controlling refrigeration temperature (4°C) during the holding time is critical for inhibiting growth of *Listeria* and *E. coli* on WIP cabbage.

Benefits to the Industry

The proposed research will provide a scientific basis for standardizing the handling and processing of WIP ingredients, including holding time, temperature, sanitization of WIP fresh-cut produce, and holding container cleaning for each individual common WIP ingredient. Conditions (time and temperature) not supporting the growth of pathogens will be predicted for each WIP ingredient. Based on these predicted conditions, the industry can monitor and document the temperature and time during WIP storage, to prevent pathogen multiplication. It is likely that a separate storage protocol will be needed for each individual WIP ingredient studied.

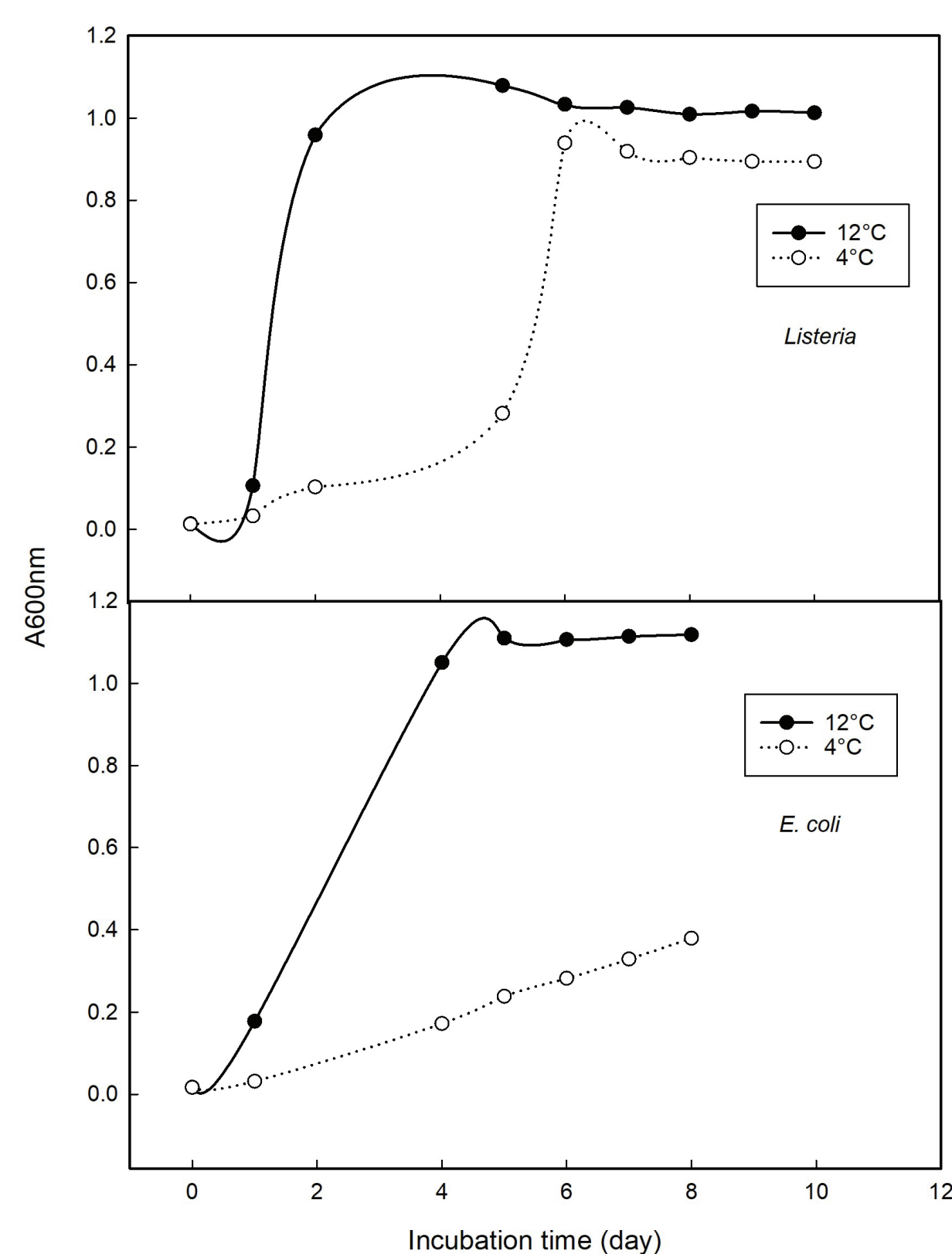


Figure 1. Growth of *Listeria* and *E. coli* in Tryptic Soy Broth during incubation at 4 and 12 °C

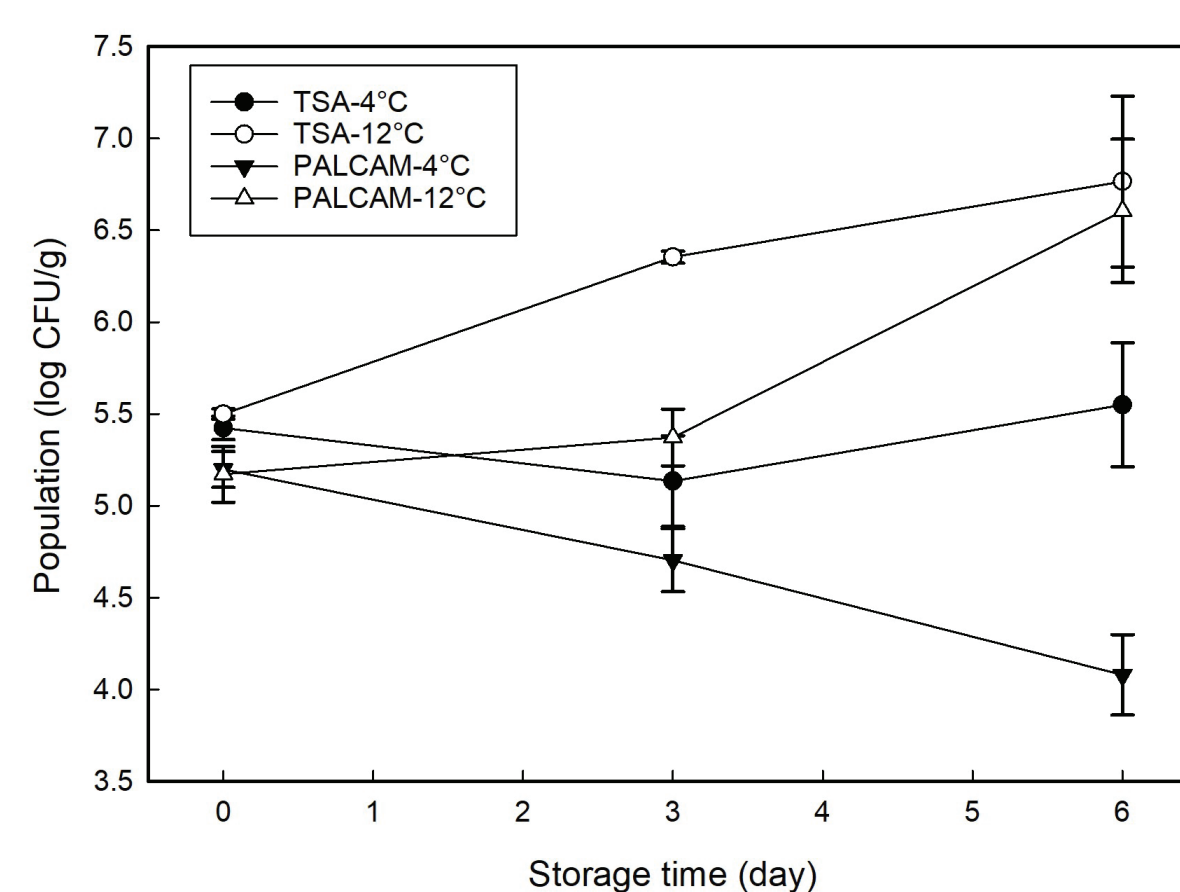


Figure 2. Growth of *Listeria innocua* on sliced cabbages during storage at 4 and 12°C. Bacteria were enumerated on non-selective (TSA) and selective (PALCAM) media. Vertical bars represent standard deviations.

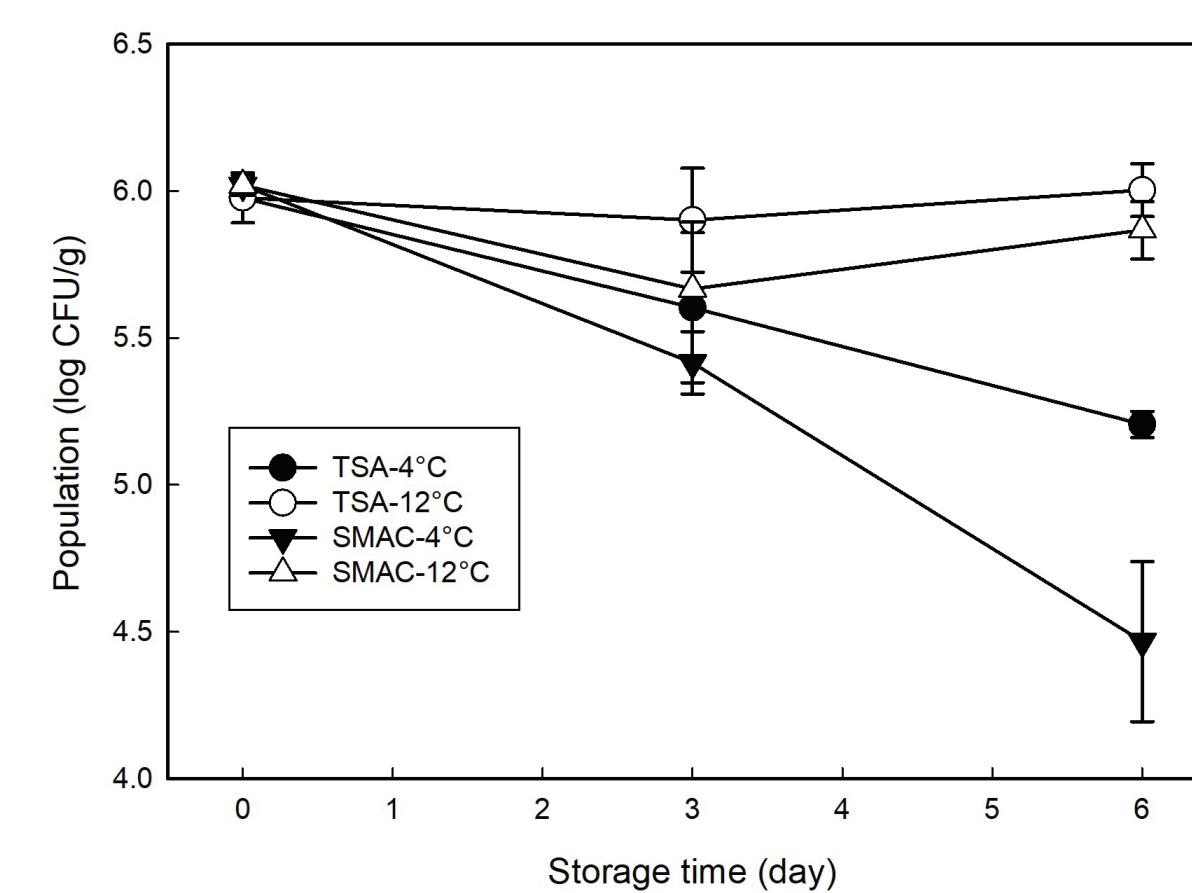


Figure 3. Growth of *E. coli* on sliced cabbage during storage at 4 and 12°C. Bacteria were enumerated on non-selective (TSA) and selective (SMAC) media. Vertical bars represent standard deviations.