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Opinion Article

Impact of Cold Storage on the Growth Dynamics of *Salmonella enterica* in Leafy Greens

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DESCRIPTION

The consumption of fresh leafy greens has increased globally due to rising health awareness and dietary preferences favoring raw vegetables. However, the microbiological safety of these products has become a concern, particularly with the increasing number of foodborne outbreaks linked to contaminated produce. Among various pathogens, *Salmonella enterica* is frequently associated with foodborne illnesses linked to leafy greens such as lettuce, spinach and arugula. Although refrigeration is widely used as a key preservation method, the ability of *Salmonella enterica* to survive or even slowly proliferate under cold storage conditions poses a challenge to food safety management. This study aimed to investigate the growth dynamics and survival behavior of S. *enterica* in leafy greens stored at refrigeration temperatures over a period of 14 days, using both microbiological enumeration and molecular techniques to gain insights into pathogen persistence.

Fresh samples of romaine lettuce, spinach, and arugula were purchased from local agricultural markets in Taichung, Taiwan. The samples were confirmed to be initially free from *Salmonella* through pre-screening. Each type of leafy green was then artificially inoculated with a standardized cocktail of three S. *enterica* serovars (Enteritidis, Typhimurium, and Newport) to achieve a final concentration of approximately 10^4 CFU/g. The samples were packaged in perforated polyethylene bags to mimic commercial storage and were stored at 4°C and 8°C, representing ideal and slightly abusive refrigeration conditions, respectively. Enumeration of Salmonella was performed at 0, 3, 7, 10 and 14 days using XLD agar, while the expression of cold-shock-related virulence genes (cspA, invA) was analyzed via RT-qPCR to monitor sublethal responses under cold stress.

Results showed that while cold storage limited the exponential growth of S. *enterica*, the pathogen demonstrated significant survival capabilities over the 14-day period. At 4°C, bacterial counts slightly decreased in the first three days but remained stable thereafter, with only a 0.5 log–1 log reduction by day 14. In contrast, at 8°C, a slight increase in population (0.5 log–0.8 log) was observed in some samples, particularly in spinach, which has higher moisture and surface complexity. Spinach appeared to provide a more conducive environment for bacterial persistence compared to romaine or arugula, likely due to its larger surface area and higher chlorophyll and water content. These micro-environmental factors can protect pathogens from cold-induced stress and allow them to remain metabolically active.

Gene expression analysis revealed upregulation of the cspA gene, especially at 4°C during early storage (days 3–7), suggesting an adaptive stress response to cold conditions. The invA gene, a known virulence marker, remained detectable throughout the storage period, indicating that S. *enterica* maintained its pathogenic potential even under refrigeration. These findings suggest that refrigeration may not be entirely effective in eliminating the risk posed by this pathogen in leafy greens, especially if there is pre-harvest or post-harvest contamination. Moreover, low temperatures may trigger physiological adaptations that help S. *enterica* persist in hostile environments, raising concerns about its role in cold chain-associated outbreaks.

The study also considered the packaging conditions and their impact on bacterial survival. Samples stored in oxygen-permeable packaging showed greater reductions in *Salmonella* levels compared to those stored in low-permeability packaging, possibly due to oxidative stress. This observation underscores the importance of packaging material selection as part of a comprehensive food safety strategy. Additionally, residual moisture in the packaging was found to correlate with higher bacterial survival rates, highlighting the need for proper drying and moisture control during pre-packaging.

The implications of these findings are critical for both producers and consumers. From a public health standpoint, reliance on cold storage alone may create a false sense of safety, as *Salmonella* can persist without significant die-off during refrigeration. The results emphasize the need for stringent hygienic practices throughout the leafy green supply chain, from

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farm to retail. Pre-harvest interventions such as the use of clean irrigation water, post-harvest washing with antimicrobial solutions, and consumer education on proper handling are all essential to minimize risk.