

Title Understanding *E. coli* internalization in lettuce leaves for optimization of irradiation treatment

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Abstract

Irradiation penetrates food tissues and effectively reduces the number of food microorganisms in fresh produce, but the efficacy of the process against internalized bacteria is unknown. The objective of this study was to understand the mechanisms of pathogen colonization of plants relative to lettuce leaf structures so that radiation treatment of fresh leafy vegetables can be optimized. Leaves of iceberg, Boston, green leaf, and red leaf lettuces were cut into pieces, submerged in a cocktail mixture of two isolates of *Escherichia coli* (Rifampicin resistant), and subjected to a vacuum perfusion process to force the bacterial cells into the intercellular spaces in the leaves. Sixty bags containing 20 g of lettuce each were tested. The inoculated leaves were gamma irradiated (Lanthanum-140, 0.16 kGy/h) at 0.25–1.0-kGy (surface dose values), with increments of 0.25 kGy at 15 °C. Microbial analysis was performed right after irradiation, including non-irradiated leaf pieces (controls). A dose uniformity ratio (max/min dose) of 2.8 was set to confirm the effect of non-uniform dose distribution. Calculated D_{10} -values varied between 48 and 62% based on the dose distribution from the entrance dose. However, despite the subtle differences in composition and structure among the four lettuce varieties, the D_{10} - values were not significantly different. Irradiation up to 1.0-kGy resulted in 3–4-log reduction of internalized *E. coli* on the lettuce leaves. The SEM images suggest that the contamination sites of pathogens in leafy vegetables are mainly localized on crevices and into the stomata. This study shows that irradiation effectively reduces viable *E. coli* cells internalized in lettuce, and decontamination is not influenced by lettuce variety. Ionizing irradiation effectively reduced the population of internalized pathogen in a dose-dependent manner and could be used as an effective killing step to mitigate the risk of foodborne disease outbreaks.