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Quantitative assessment of the effectiveness of intervention steps to reduce the risk of contamination of ready-to-eat baby spinach with *Salmonella*

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ABSTRACT

The objective of this study was to develop a quantitative risk assessment model to evaluate the microbial hazards during processing of baby spinach leaves using scenario analysis and predictive microbiology. The effectiveness of intervention strategies (temperature control during harvest, washing, and irradiation) was also integrated into the risk assessment model. Monte Carlo simulation was used to take into account the variability of the model parameters.

Cross-contamination seems the most probable scenario for prevalence of contamination on an entire lot of daily production. If the cross-contamination level of bacteria was low ($\sim$1 $\log_{10}$ CFU/g, normal distribution) either on the field or after the washing treatments, the percentage of samples over the safety limit (1.33 $\log_{10}$ CFU/g of sample) increased from 16.8% to 84% for a highly cross-contaminated lot ($\sim$3 $\log_{10}$ CFU/g). The risk assessment revealed that exposure of the leafy greens to irradiation (1 kGy) reduces the number of tainted samples from 84% to 0.1%, for highly cross-contaminated lots (3 $\log_{10}$ CFU/g).

This study shows that the spinach processor can deliver a highly safe product in a cross-contamination scenario (on the field or packing shed) if the produce is harvested at 20 °C, stored for at least 5 h, washed with water and chlorine (220 ppm), and exposed to irradiation treatment with a dose of 1 kGy.

1. Introduction

Leafy greens are responsible for 30% of produce related bacterial outbreaks in the United States. These produce can support the growth of many different pathogenic bacteria, including *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, and *Listeria*, because of their large surface area and potential for pathogen internalization in structures such as the stomata. Once internalized, pathogens are difficult to remove using surface treatments such as washing (Buchanan, 2006). Furthermore, most commercial interventions employ chemical agents, which are not effective in reducing microorganisms to a safe level for consumption (Puerta-Gomez, 2004). Since thermal processing is not a viable option, non-thermal interventions such as irradiation are an attractive option as a lethality step in the handling and processing of fresh leafy greens.

On August 22, 2008, the Food and Drug Administration (FDA) published a final rule that allows the use of ionizing radiation to make fresh iceberg lettuce and fresh spinach safer and last longer without spoiling. Many studies show that irradiation, when performed correctly, kills pathogens or markedly reduces pathogen counts (Castell-Perez & Moreira, 2010; Gomes, Moreira, & Castell-Perez, 2011; Gomes et al., 2007; Moreira & Castell-Perez, 2011, chap. 7). However, to date, a quantitative risk assessment for the processing of ready-to-eat leafy vegetables has not yet been developed. Such an assessment is crucial to support management decisions regarding process design and selection of appropriate technologies to ensure delivery of safe and healthy fresh produce to the consumer.

Salmonella sickens about 40,000 people and kills approximately 600 in the U.S. each year (Sivapalasingam, Freidman, Cohen, & Tauxe, 2004). Although most cases of *Salmonella* poisoning are caused by undercooked eggs and chicken, in recent years, several U.S. spinach producers have had bagged baby spinach recalled for potential *Salmonella* contamination (Buchanan, 2006). In 2011, a producer in California announced a recall of bagged spinach, following positive results for *Salmonella* in the product headed for

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the market. Although still relatively uncommon, the frequency of these outbreaks is increasing, and tracing back to the source of contamination is practically impossible (Danyluk & Schaffner, 2011).

Risk assessment principles with scenario analysis and predictive microbiology can provide an objective evaluation of the safety features of the production process and allow the manufacturer to predict outcomes before actual implementation. Thus, the objective of this study was to develop a quantitative risk assessment model for estimating the likelihood of acquiring *Salmonella* Enteritidis infection from consumption of ready-to-eat baby spinach under various processing scenarios, using data obtained with a surrogate, *Salmonella* Typhimurium LT2 (temperature and washing strategies) and *Salmonella* spp. (irradiation). The model was then used to determine the impact (if any) of different combinations of mitigation strategies (temperature control during harvest, washing liquid, and irradiation) on the number of pathogens present in the produce.

2. Materials and methods

2.1. Background and model development

A thorough literature search was conducted to determine the potential gaps and critical points to be addressed with experimental trials. Information was gathered on models for bacterial growth that could be implemented in a quantitative risk assessment framework. This step was critical since, for instance, most models do not address the changes in temperature during storage.

An evaluation of the investigative report of the 2006 *E. coli* O157:H7 outbreak associated with Dole pre-packaged spinach (California Department of Health Services & USFDA, 2007, pp. 1–50) provided motivation and insight on which processing parameters to address, such as initial inoculums (pathogen population levels), temperature fluctuation during processing and storage, and sanitation procedures (e.g., water vs. chlorinated water washing). The study concluded that current practices were not sufficient to prevent the outbreak. This conclusion supports our central hypothesis that an additional mitigating step (irradiation or in combination with other pre-treatments) can aid in avoiding further outbreaks.

The quantitative risk assessment procedure described here utilizes the tools of probability to predict final pathogenic load using assumptions on the distribution of initial load, cross-contamination levels, and models of load growth/reduction through discrete processing steps. Instead of assuming normal distributions of load (based solely on mean and standard deviation) throughout the processing stages, the model uses available data to describe empirical distributions of load that may be skewed heavily in one direction (e.g., lognormal distribution). Since bacterial outbreaks are likely triggered by relatively infrequent instances of very high pathogenic loads, such an approach leads to more accurate predictions than when assuming a symmetric distribution for load. To standardize the scope, the model in this study includes all operations from field harvesting to shipment from the packinghouse.

2.2. Components of microbial risk assessment model for baby spinach

To characterize the production process for baby spinach, the microbial risk assessment model includes three main processing stages (Whiting & Buchanan, 1997): (1) micro-growth after harvesting, transportation and pre-processing conditions (20 °C for 5 h), (2) cleaning (water and chlorinated washing), and (3) post-packed intervention strategies (irradiation) (Fig. 1). The model uses validated data to simulate bacterial population through each

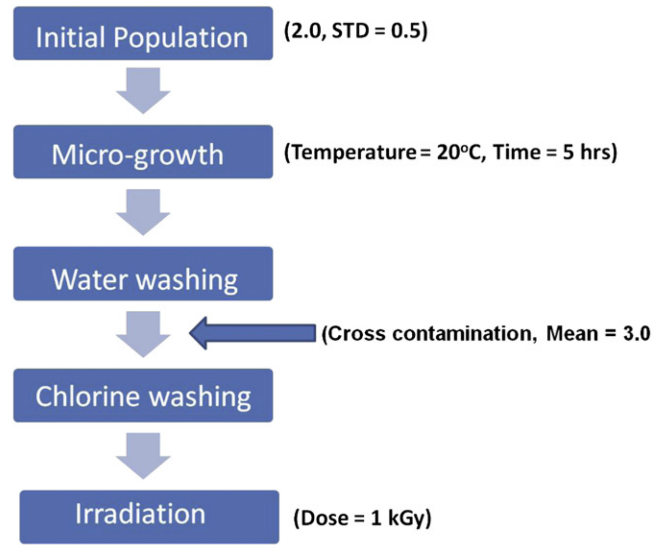

Fig. 1. Flow chart of baby spinach processing line for use in the risk assessment model.

of these stages to predict, based on various intervention strategies, the microbial load in a specified quantity of ready-to-eat baby spinach (the retail package). From this final load, we determined the probability that the retail package will contain enough pathogens to infect the consumer and to cause a widespread outbreak.

Simulation of pathogen population growth and reduction at each processing stage was driven by either experimental data (cleaning, effect of temperature on microbial growth) or information extracted from the literature (inactivation by irradiation, Gomes et al., 2011). Model requirements for each of these stages and the requirements for the dose–response relationship are described below.

2.2.1. Micro-growth after harvesting, transportation, and pre-processing conditions

In Texas, baby spinach is one of the first crops planted in spring in Uvalde County, located at the Southwest of Texas. The climate of this area has been described as continental, semi-arid, and subtropical–sub humid. The average rainfall is 23.22 inches annually. Temperatures range from an average low of 3 °C and average high of 17 °C in January to an average low of 22 °C and high of 37 °C in July. Harvesting of baby spinach starts on mid-November and finishes by the middle of February. Fecal contamination from wild animals (e.g., deer, hogs) is more likely to occur in this area than in Rio Grande Valley, where mature spinach is harvested (Anciso, 2011). Before harvesting, the field crop is sampled to check for possible pathogen contamination. Baby spinaches are harvested by machine and then placed in trays, which may have ice to cool them down from the field heat when the temperature is above 27 °C.

Input to the risk assessment model for this stage included quantitative information on the pathogenic load in field-harvested ingredients described by a probability density function (pdf) (Fig. 2). The pdf is presented in the form of a histogram that graphs levels of pathogen population (log CFU/g) against frequency (e.g., the percentage of samples having the given infectious pathogen population).

After field harvest, the spinaches are transported to the packing shed where they are cooled by forced air systems. The temperature of contaminated spinaches affects the numbers of *Salmonella* Typhimurium LT2 in the spinach leaves, and effective temperature control is critical to restricting the growth of the pathogen. At this