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# Effect of intervention strategies on the risk of infection from *Listeria monocytogenes* due to consumption of fresh baby spinach leaves: A quantitative approach

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ABSTRACT

The purpose of this study was to conduct a quantitative microbial risk assessment (QMRA) for *Listeria monocytogenes* infection from consumption of fresh baby spinach leaves, and provide recommendations on intervention strategies including water washing, use of sanitizers (peracetic acid (PAA), chlorine dioxide ($ClO_2$) and chlorine), irradiation, and irradiation-Modified Atmosphere Packaging (MAP) combinations, based on different scenarios. The food chain of fresh baby spinach from processing facility up to consumption was simulated employing @Risk software. The QMRA models predict that the mean annual number of *L. monocytogenes* infection cases per year for the U.S. population due to consumption of spinach leaves ranged from 115 to 536 cases – meaning that the incidence of *L. monocytogenes* infection ranged from 1.2 to 5.5 cases per hundred thousand people. Findings strongly encourage the application of irradiation combined with MAP since this intervention strategy reduced the mean risk of listeriosis per random serving by 65.6%. The results of this study suggest that intervention methods could reduce the concentration of *L. monocytogenes* on spinach leaves under the detectable limit (0.04 CFU $g^{-1}$) at the processing facility, though this pathogen could reach unacceptable levels at the time of consumption.



## 1. Introduction

In recent years, *L. monocytogenes* has been of interest to researchers because of the increased susceptibility to contamination of RTE (ready-to-eat) products by this pathogen (Todd & Notermans, 2011). This pathogen, causing listeriosis in humans with diverse symptoms including mild diarrhea, meningitis, and septicemia, is a widely seen foodborne pathogen in several foods, such as milk, vegetables, and meat (Painter et al., 2013). It causes approximately 1591 cases of foodborne illness annually and of these cases, there are 1455 hospitalizations and 255 deaths in the United States (Hoffmann, Batz, & Morris, 2012; Scallan et al., 2011). This microorganism has been isolated from a wide range of raw and RTE products as well as various food processing environments (Gombas, Chen, Clavero, & Scott, 2003; Lianou & Sofos, 2007; Park et al., 2012a; Pradhan et al., 2010; Prazak, Murano, Mercado, & Acuff, 2002; Sant'Ana, Igarashi, Landgraf, Destro, & Franco, 2012). Furthermore, *L. monocytogenes* is psychrotrophic and can grow under refrigeration temperatures (−0.5–9.3 °C) (Walker, Archer, & Banks, 1990). Tian et al. (2012) reported that the concentration of *E. coli O157:H7* and *Salmonella* reduced almost by 1.0 log $CFUg^{-1}$ whereas the concentration of *L. monocytogenes* almost grew 1.0 log $CFUg^{-1}$ in shredded romaine lettuce stored for 10 days at 5 °C.

Other studies have shown that the presence of *L. monocytogenes* in vegetables should be a concern because although Gombas et al. (2003) reported relatively low presence (0.74%) of *L. monocytogenes* on bagged salads, the lethality ratio of this pathogen is higher than for the other foodborne pathogens (Koseki, Mizuno, Kawasaki, & Yamamoto, 2011; Sant'Ana et al., 2012). For instance, *L. monocytogenes* is responsible for the largest number of deaths from foodborne disease in the UK (FSA, 2015). In addition, lower doses of *L. monocytogenes* can still cause infection in immunocompromised populations (Lianou & Sofos, 2007). Therefore, despite the 'zero tolerance' to *Listeria* policy (no detectable level of viable organisms permitted) in the U.S., the presence of the assessment of the risk posed by the pathogen is of high relevance due to the high mortality rate of the illness (20–40%), and how widespread the pathogen is in foods and the environment (Andersen & Nørrung, 2011). The Center for Disease Control (CDC)

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states that the occurrence of listeriosis in the United States was 0.26 cases per 100,000 individuals throughout 2012 (CDC, 2014) and product recalls and processors must face the challenge to control contamination with this pathogen and means to understand outcomes before the actual implementation of decontamination steps are critically needed.

Risk assessment principles with scenario analysis and predictive microbiology can provide an objective evaluation of the safety features of the fresh produce production process and allow the manufacturer to predict outcomes before actual implementation (Puerta-Gomez, Kim, Moreira, Klutke, & Castell-Perez, 2013). Therefore, quantitative microbiological risk assessment (QMRA) enhances food safety by evaluating the effects of intervention measures in food production processes because it helps to appreciate the impact of alleviation strategies on the number of pathogens present in leafy vegetables. In 2003, a QMRA of listeriosis for 23 categories of RTE products – some containing vegetables – was carried out by the Food and Drug Administration (FDA). In that study, vegetables were categorized as relatively low risk groups (<1 case/year); however, the study suggested that additional investigations for the subdivision of the vegetables category into several different groups are needed because of high uncertainty caused by the diversity of the products. So far, several studies have been published on QMRA for *E. coli* 0157:H7, *Salmonella* and L. *monocytogenes* in leafy green vegetables (Carrasco, Perez-Rodriguez, Valero, Garcia-Gimeno, & Zurera, 2010; Danyluk & Schaffner, 2011; Ding et al., 2013; Pielaat, Leusden, & Wijnands, 2014; Puerta-Gomez et al., 2013; Sant'Ana, Franco, & Schaffner, 2014; Tromp, Rijgersberg, & Franz, 2010), but more information is still needed.

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 (Puerta-Gomez et al., 2013).

The main objective of this study was to conduct a QMRA (1) to evaluate the risks of infection from *L. monocytogenes* contamination on fresh baby spinach leaves in the United States from the processing facility up to consumption; and (2) to determine the effectiveness of different intervention methods in reducing listeriosis cases. Such methods include as water washing, use of sanitizers (aerosolized peracetic acid, chlorine dioxide, and chlorine), irradiation, and irradiation-modified atmosphere packaging (MAP) combination.

## 2. Materials and methods

### 2.1. Risk assessment methodology and data sources

The different unit operations for processing of the raw material at the factory up to the table and related pathogen events containing initial concentration in raw fresh baby spinach, growth patterns and cross contamination before packaging were considered and defined. The contamination levels of *L. monocytogenes* in fresh baby spinach at time of consumption were used to predict the likelihood of infection resulting from a single exposure. For this aim, data were calculated or taken from the literature.

### 2.2. Hazard characterization

Hazard characterization, known as the dose-response assessment, defines the association between amount of a pathogen consumed (dose) and the likelihood of development and severity of illness or other adverse health outcomes (response) (Barraj & Petersen, 2004). The exponential dose-response non-threshold and single hit model (Eq. (1)), was employed to estimate the probability of infection with *L. monocytogenes*, *P(D),* linked to fresh baby spinach, due to exposure to a consumed single portion with a certain pathogen dose,

$$P(D) = 1 - e^{-r \times D} \quad [1]$$

where, *P (D)* is the probability of illness for individuals exposed to a certain dose *D*, *D* is the number of pathogen cells consumed (CFU/serving); and *r* is the probability of host-pathogen interaction. The *r*-value used for evaluating risks of consumption of *L. monocytogenes* cells, independent of subtype, ranges from $1.58 \times 10^{-10}$ (5% percentile) to $2.24 \times 10^{-10}$ (95% percentile) with mean value of $1.91 \times 10^{-10}$ (Chen et al., 2006). We used a pert distribution with minimum, most likely, and maximum values of $1.58 \times 10^{-10}$, $2.24 \times 10^{-10}$, and $1.91 \times 10^{-10}$ (Sant'Ana et al., 2014). Different subpopulations with different levels of vulnerability to illness following exposure were not considered in this study.

### 2.3. Exposure assessment

Fig. 1 shows the scope of the exposure assessment for *L. monocytogenes* in fresh baby spinach leaves within the food chain. The model inputs in terms of (a) prevalence, (b) initial contamination level after harvest, (c) cross-contamination level before packaging, (d) temperature abuse at table, and (e) growth in retail and at home before consumption were collected based on available literature data.

#### 2.3.1. Prevalence and initial level of Listeria monocytogenes

The initial inputs of the QMRA model were the prevalence and the initial contamination level of *L. monocytogenes* in the spinach. Data on prevalence of *L. monocytogenes* on spinach was adopted from literature on microbial contamination of vegetables for raw consumption in the U.S. (Table 1). The beta distribution, $p = s + 1$, $n - s + 1$, where *s* refers to the number of positive samples and *n* refers to the total number of samples (Franz, Tromp, Rijgersberg, & Fels-Klerx, 2010), was used to simulate the variability about prevalence of *L. monocytogenes* in spinach leaves. In addition, the mean value of *L. monocytogenes* concentration (in $CFUg^{-1}$) of samples under detection limit was estimated by (Ding et al., 2013) as:

$$\text{Mean} = \frac{-2.303}{V} \cdot \log\left(\frac{Z}{E}\right) \quad [2]$$

where, *V* refers to the quantity of material tested, *Z* refers to the number of samples tested as negative, and *E* refers to the total number of samples analyzed. The quantity of samples (*V*) in the study used for prevalence of *L. monocytogenes* in spinach leaves was 25 g and there were 3347 negative samples (*Z*) collected in a total of 3390 (*E*). After calculation, the mean value of −3.29 log $CFUg^{-1}$ was assumed as the mean value of a left-hand-tailed cumulative distribution with maximum value of −1.40 log $CFUg^{-1}$ (99%) and minimum value of −5.18 log $CFUg^{-1}$ (1%).

The initial contamination level refers to the concentration of *L. monocytogenes* in the spinach brought to the manufacturing facility. Currently, there is no survey data available regarding concentration of the pathogen in spinach. We assumed the pathogen