Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.17622

Gamma radiation effects on the survival and reduction of *Listeria monocytogenes* on carrot and tomato

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Abstract

Assessment of pathogen survival is important for food safety. *Listeria monocytogenes* causes significant produce and food contamination worldwide. The objective of this research was to assess survival and reduction of *L. monocytogenes* on post-harvest carrot and tomato subjected to low-dose radiation at different storage temperatures and times. Radiation levels of 0, 0.25, 0.50, 0.75, and 1 kGy were applied on produce inoculated with *L. monocytogenes*. Gamma radiation reduced *Listeria* populations by 5.9 logs on carrot and 3.9 logs on tomato at 3 days of storage (5 °C), while reductions were 4.4 and 4.0 logs on carrot and tomato, respectively; at 7 storage days (5 °C). At 20 °C, *Listeria* reductions were 2.8 logs on carrot and 4.2 logs on tomato (3 storage days) and pathogen decreases were 2.2 logs on carrot and 2.9 logs on tomato (7 storage days). Although pathogen reductions by radiation treatment varied with storage temperatures and days, the linear decreases were significant as the dosage increased from 0.25 to 1.0 kGy, implying that treatments were efficacious for pathogen inactivation. As low dose gamma radiation (1 kGy) showed substantial reduction of *Listeria monocytogenes* on fresh carrot and tomato, thus, use of low dose low dose gamma radiation can improve the post-harvest safety of carrot and tomato.

Keywords: *Listeria monocytogenes*; gamma radiation; inactivation; carrot; tomato; post-harvest.

Practical Application: *Listeria* control on produce by low-dose gamma radiation.

1 Introduction

The recent trends in consumer's attitudes to foods and produce have documented increases in the utilization of minimally processed fruits and vegetables in many parts of the world. Among the ready-to-eat vegetables that are routinely consumed with minimal preparation include carrot and tomato (United States Department of Agriculture, 2021). These are important vegetables due to their nutritional value and commercial importance as the two produce accounts for over 16.8 metric tons of total world production (United States Department of Agriculture, 2021; Food and Agriculture Organization, 2021). In the United States, annual consumption of carrots and tomatoes are approximated at over 6.2 metric tons. Although carrot and tomato have been processed by produce industry and utilized as processed vegetables, the bulk of produce may be consumed with minimal preparation (United States Department of Agriculture, 2021).

Pathogen occurrence and contamination of carrot and tomato have been documented in United States and various parts of the world (Ajayeoba et al., 2016; Arumugaswamy et al., 1994; Ponniah et al., 2010). For example, *Listeria monocytogenes*, *Salmonella enterica* or *Escherichia coli* O157:H7 and other pathogens have been associated with post-harvest contamination of ready-to-eat produce and other foods (Callejón et al., 2015; Carstens et al., 2019; Buchanan et al., 2017; Castro-Ibáñez et al., 2017). The above foodborne pathogens have drawn attention, due to their impacts on food safety and consumer health (Scallan et al., 2011). Therefore, to mitigate the deleterious health effects to consumers, post-harvest interventions to enhance produce safety should be diversified and show effective controls (Scharff, 2015; Olanya et al., 2019).

Listeria monocytogenes is one of the pathogens that pose consistent risks to food safety and consumer's health (Vahidy et al., 1992; Costa et al., 2022). The bacterium has a peculiar life-cycle adaptation in which it is capable of saprophytic survival in the soil, but can transition to pathogenic life cycle upon entry in human or animal cells (Freitag et al., 2009). *L. monocytogenes* is a psychrophilic foodborne bacterium that can survive and sustain growth at temperatures below 1 °C; making its occurrence problematic under refrigerated conditions and in food-manufacturing environments (Buchanan et al., 2017). Additionally, *L. monocytogenes* can also multiply in diverse pH and osmotic conditions, under both aerobic and anaerobic situations (Buchanan et al., 2017; Välimaa et al., 2015). Furthermore, the bacterium could adhere to food contact surfaces or occur in biofilms for longer durations and has tolerance for high concentrations of decontaminant agents, enhancing its persistent survival (Ferreira et al., 2014; Kara & Aslan, 2021; Murray et al., 2017).

Received 18 Mar., 2022
Accepted 15 July, 2022
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Contaminated carrots and tomatoes have been associated with various foodborne pathogens including *L. monocytogenes* and other pathogens (Ponniah et al., 2010; Marik et al., 2020). A common approach for decontamination of fresh-cut fruits and vegetables in the produce industry is by the application of sanitizing agents (Ukuku et al., 2012). Chemical sanitizers (chlorine-based compounds and oxidizing agents) have been used, however; these have resulted in some toxic by-products on fresh produce (Alvarado-Casillas et al., 2007). Non-thermal interventions have impacted microbial growth and pathogen survival on produce following its application (Julien-Javaux et al., 2019). Other compound such as phloretin (polyphenol) obtained from produce has also shown good potential for in-vitro inhibition of *L. monocytogenes* (Zhao et al., 2022).

Gamma radiation is one of the non-thermal measures that have shown efficacy in decontamination of produce. Gamma radiation intervention has been utilized to inhibit sprout development in tuberous crops and regulate fruit ripening in many commodities (Ramos et al., 2013). It has also been approved for use in extending the shelf life of produce and for insect disinfestation at the dosage level of approximately 1 kGy (Pinela & Ferreira, 2017). Gamma radiation has a rapid decontamination process in which the application is quick and non-time consuming, with less toxic by-products and lower impact on sensory properties of foods (Prakash & Foley, 2004). The justification for low-dose gamma irradiation (GR) in this research is the continuous occurrence of post-harvest produce contamination despite advances in food safety measures and low-dose GR presents a viable technology to decontaminate produce.

Data on low-dose GR levels that could be utilized for *Listeria* inactivation as well as generate knowledge on injury and recovery or re-growth of *Listeria* under such conditions are lacking (Morehouse & Komolprasert, 2004). Similarly, data on pathogen survival would also be useful for designing optimum inactivation measures for *Listeria* on produce. It was anticipated that knowledge on radiation efficacy would promote the safe consumption of baby carrot (*Daucus carota*) and tomato (*Lycopersicon esculentum*). Due to GR benefits in regulation of fruit maturation and preventing insect infestation, FDA has approved ionizing radiation as a food additive for processing of fresh produce for radiation dosage up to 1 kGy level (Food and Drug Administration, 2019). Additionally, World Health Organization and Food and Agriculture Organization endorsed gamma irradiation use for eliminating harmful micro-organisms based on its food safety applications (Barkai-Golan & Follett, 2017). Therefore, the objective of this research was to assess the survival and reduction of *L. monocytogenes* on post-harvest carrot and tomato when subjected to low-dose radiation at different storage temperatures and times.

2 Materials and methods

2.1 Bacterial strains and microbial propagation

Listeria monocytogenes used in this experiment consisted of a cocktail of strains 008, 2625, and 2634. The pathogen strains were from the culture collections at USDA-ARS, Wyndmoor, PA. The bacterial stock cultures were maintained in Tryptic Soy broth (TSB; BBL, Sparks, MD, USA) in 20% glycerol, stored at −80 °C. The cultures were revived by transfer of a loopful (~ 0.05 mL) to 9 mL TSB and incubated at 37 °C for *L. monocytogenes* for 24 h (Olanya et al., 2019; Berrios-Rodriguez et al., 2020a). The bacterial cells were centrifuged (10,000 × g, 5 min, 5 °C) and cell pellets were washed twice with 0.1% BPW (BBL, Sparks, MD, USA). Bacterial cell pellets were re-suspended in 10 mL of phosphate buffer saline to concentrations of 6-7 log CFU/mL (Berrios-Rodriguez et al., 2017).

2.2 Bacteriological media

Total bacteria were quantified on Trypticase Soy Agar (TSA). *L. monocytogenes* was quantified on a modified Oxoid agar (MOX) selective agar (BBL, Sparks, MD, USA) by spread-plate method. The media and plating methods were previously used (Berrios-Rodriguez et al., 2020a).

2.3 Produce and pathogen inoculations

Carrots (*Daucus carota*) and tomatoes (*Solanum lycopersicum*) were purchased from a local grocery store in Wyndmoor, PA and refrigerated at 5 °C. Prior to use, produce types were rinsed in sterile de-ionized water, sanitized in 200 ppm sodium hypochlorite, and then rinsed with sterile de-ionized water (Berrios-Rodriguez et al., 2020b; Olanya et al., 2018). Produce (10 g) was weighed for the total number of treatments. Tomato and carrot (10 g each) were inoculated with 6-7 log of *Listeria monocytogenes* by dipping in 20 mL inocula suspension in a biosafety cabinet (LABGARD ES Class II) for 5 min and air-dried for 30 min (Olanya et al., 2018).

2.4 Radiation treatments on Listeria monocytogenes populations

The effects of gamma radiation treatments on the survival of *Listeria populations* on produce were investigated in randomized and replicated experiments. Radiation treatments were as follows: 1) Control (0), 2) 0.25 kGy, 3) 0.50 (kGy), 4) 0.75 (kGy), 5) 1 kGy and applied on 2 produce types (carrot and tomato) following *Listeria* inoculations and then the produce was subjected to 3 sampling times (0, 3, and 7 days). Treatments were applied on produce types and, then stored at 5 °C (refrigerated temperature) and 20 °C (room temperature) in order to assess if produce storage temperatures after gamma radiation treatment of pathogens could affect bacterial survival. Treatments (5 radiation dosages and 2 produce types) were arranged in a completely randomized design with 3 replications and the experiment was conducted twice.

2.5 Gamma radiation effects on L. monocytogenes survival on carrots and tomatoes

After pathogen inoculations, produce was irradiated at 4 °C using a Lockheed-Georgia (Marietta, GA) cesium-137 self-contained gamma radiation source as previously described (Niemira et al., 2005; Niemira & Boyd, 2013). Produce in stomacher bags with designated treatment was irradiated at the dosages of 0.25 to 1.0 kGy using methodology from previous research, but different pathogen/host combinations (Berrios-Rodriguez et al., 2022; Olanya et al., 2015).

2.6 Assessment of injury and recovery of L. monocytogenes on carrots and tomatoes

Injury to *L. monocytogenes* cells was quantified by the overlay culture procedure (Lee & Kang, 2001). The non-selective medium (TSA) was used to facilitate repair of injury of *Listeria* cells. Dilutions of 100 µL obtained from radiated produce samples that had been stomached (containing *L. monocytogenes*) were pipetted and spread-plated onto TSA medium and plates were air-dried at 25 °C for 2 h. This was done to promote the resuscitation of injured cells (Kang & Siragusa, 1999). Then, TSA plates were overlaid with MOX (BBL/Difco, Sparks, MD) selective medium. These were air-dried for 2 h at 25 °C and incubated at 37 °C for 2 days. Typical tiny white, pin-point colonies of *L. monocytogenes* were enumerated from the randomized and replicated plates. The injured *Listeria* were computed from the overlay medium as a proportion of the pathogen counts (CFU/g) on the selective medium. Recovery of injured populations were quantified on MOX selective medium as a proportion of the cells on the non-selective (TSA) medium.

2.7 Microbiological evaluations

Irradiated carrot and tomato samples were subjected to microbiological analysis by adding 40 mL of buffer (BPW) to produce samples (previously inoculated with *L. monocytogenes* and then treated with gamma radiation at different doses) when compared to untreated control. The produce samples were stomached for 2 min at 230 rpm (Stomacher 400 Circulator, Seward 400, UK) and serially diluted. *L. monocytogenes* populations were enumerated on modified oxoid agar (MOX) selective medium (BBL) modified with supplements and incubated at 37 °C. The colony forming units from the plating media were recorded (Berrios-Rodriguez et al., 2022).

2.8 Data analysis

L. monocytogenes populations from produce that were irradiated and from the untreated (control) were plated on MOX medium and quantified as colony forming units. The counts of *Listeria* populations from pathogen-inoculated carrot and tomato were converted to Log CFU/g of produce. Data were analyzed by general linear models (PROC GLM) of the Statistical Analysis System, SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). The effects of treatments (radiation dosages, produce, storage days and temperatures) on mean *Listeria* populations were computed by Analysis of Variance (PROC GLM) of the statistical analysis system (SAS, Statistical Analysis System, Cary, NC). Significant differences among treatment means were computed by using Least Significant Difference (LSD) statistics at $P < 0.05$.

3 Results and discussion

The survival of *L. monocytogenes* populations on carrot and tomato were significantly ($P < 0.05$) impacted by gamma radiation treatment. When data were averaged across days for each inoculated produce, pathogen populations at either 5 °C (refrigerated temperature) or 20 °C (room temperature) varied with each radiation dosage (Table 1). In the un-treated produce (control), the average population ranged from 6.67 ± 0.23 to 7.43 ± 0.12 log CFU/g of produce. The mean populations of *Listeria* on carrot were 6.67 logs (0 kGy) and 1.39 (1 kGy) at 5 °C, and at 20 °C, these were 6.98 (0 kGy) and 4.25 (1 kGy) (Table 1). On tomato, the *Listeria* populations were 7.18 (0 kGy) and 3.16 (1 kGy) at 5 °C, while bacterial numbers were 7.43 (0 kGy) and 3.63 (1 kGy) of treatment following storage at 20 °C (Table 1).

The mean occurrence of *L. monocytogenes* populations on non-irradiated samples (0 kGy) after pathogen inoculations of produce were not significantly ($P > 0.05$) different on carrot and tomato (Table 1). Therefore, this indicated similar levels of pathogen presence on the surfaces of both carrot and tomato following produce inoculations, perhaps due to similar bacterial attachment and retention. Our findings contrast with a previous study in which occurrence of *L. monocytogenes* was noted to be greater on carrots than on tomato by about 2.3% (Zhu et al., 2017). However, we hypothesize that the frequency of occurrence of *L. monocytogenes* on minimally processed fresh produce or foods may vary, due to persistence of *Listeria* in different environmental conditions (Buchanan et al., 2017; Olanya et al., 2018). It has been documented that *L. monocytogenes* can grow in diverse environments such as 4.3 to 9.3 pH values (Ferreira et al., 2014; Zhu et al., 2017). The range of pH values cited above have been described to coincide with those found in many fruits and vegetables, thereby facilitating pathogen occurrence or growth (Zhu et al., 2017).

The radiation treatments of pathogen-inoculated produce resulted in significant ($P < 0.05$) reductions of *L. monocytogenes* (Figure 1). Linear decreases in pathogen populations were similar on carrot and tomato at subsequent radiation dosages on the untreated produce at 0 kGy to 1 kGy of gamma radiation. A 4.6 log reduction of *L. monocytogenes* populations on carrot from 7.37 ± 0.29 (control) to 2.78 ± 0.20 (1 kGy) log CFU/g of produce were recorded for samples processed immediately after irradiation treatment (Figure 1). On tomato, a 4.5 log reduction

Table 1. Effects of gamma radiation on *Listeria monocytogenes* populations on post-harvest carrot and tomato.

Temperature (°C)[w]	Dosage (kGy)	Carrot (Log CFU/g)[w]	Tomato (Log CFU/g)[w]
5	0	6.67 ± 0.23a	7.18 ± 0.18a
	0.25	5.75 ± 0.33b	6.65 ± 0.16b
	0.50	4.85 ± 0.20c	5.60 ± 0.15c
	0.75	3.79 ± 0.16d	4.05 ± 0.07d
	1.00	1.39 ± 0.53e	3.16 ± 0.17e
Means	-	4.49	5.33
$LSD_{(0.05)}$	-	0.88	0.53
20	0	6.98 ± 0.09a	7.43 ± 0.12a
	0.25	6.08 ± 0.18a	6.75 ± 0.07b
	0.50	5.39 ± 0.15b	5.73 ± 0.11c
	0.75	4.73 ± 0.13bc	5.01 ± 0.20d
	1.00	4.25 ± 0.23c	3.63 ± 0.32e
Means	-	5.49	5.71
$LSD_{(0.05)}$	-	1.1	0.68

[w]Bacteria populations were averaged at radiation treatment across days with 3 replicates per inoculated produce stored at either 5 °C or 20 °C, subsequent to radiation treatments and plated on MOX selective medium. Data represents means and associated standard errors for *Listeria monocytogenes* populations at radiation dosage. The same letters within columns indicate non-significant differences ($P > 0.05$) in mean *Listeria* populations. LSD = least significant difference statistics

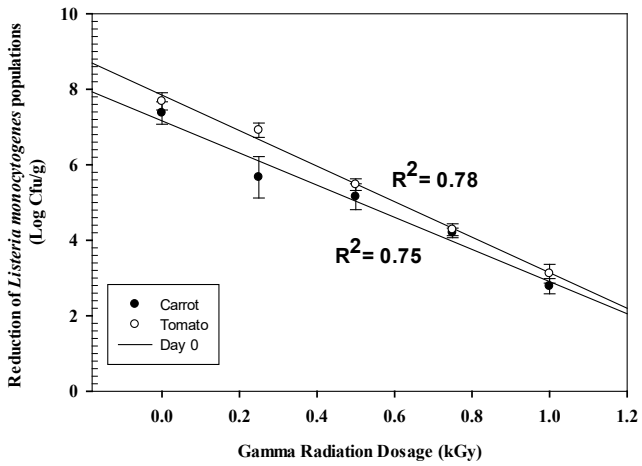

Figure 1. Reduction of *L. monocytogenes* populations on post-harvest carrot and tomato following pathogen inoculations on produce (7-8 logs). Low-dose gamma radiation (0-1.0 kGy) was applied to pathogen-inoculated produce processed after irradiation treatment. The graph represents pathogen reduction with associated standard errors at various radiation doses.

of *L. monocytogenes* (from 7.68 ± 0.22 (control) to 3.11 ± 0.25) at 1 kGy was recorded on day 0 of storage.

When produce was stored at 5 °C for 3 days after gamma radiation treatment, significant linear decreases in *Listeria* populations were observed on both carrot and tomato (Figure 2). On carrot, 5.9 log reductions (from 7.04 ± 0.15 to 1.09 ± 0.25 log CFU/g) and on tomato, 3.9 log reductions (7.31 ± 0.11 to 3.34 ± 0.26 log CFU/g) were recorded (Figure 2). Pathogen reductions were significantly ($P < 0.05$) greater on carrot than on tomato. The reduction in *L. monocytogenes* on pathogen-treated tomato from radiation treatment was also recorded at 20 °C. *Listeria* reductions on carrot and tomato were from 6.77 ± 0.17 to 3.95 ± 0.24 (2.8 log CFU/g) and from 7.21 ± 0.22 to 2.98 ± 0.20 (4.2 log CFU/g), respectively (Figure 2).

At 7 days of produce storage at 5 °C after pathogen inoculations and radiation treatment, the reduction of *Listeria* populations was similar to that stored at 5 °C for 3 days. A 4.4 log reduction of *Listeria* (from 6.21 ± 0.41 to 1.76 ± 0.17 log CFU/g) was recorded on carrot, while a 4.0 log pathogen reduction (from 6.97 ± 0.44 to 2.94 ± 0.19 log CFU/g) was obtained on tomato stored at 5 °C (Figure 3).

The survival of *L. monocytogenes* populations on carrot and tomato progressively decreased as radiation dosage applied on produce increased from 0.25 kGy to 1.0 kGy. The surviving *Listeria* populations differed and was significantly ($P < 0.05$) lower at 5 °C than at 20 °C when data were averaged across produce and storage days. This suggests that *L. monocytogenes* survival may be adversely impacted by low-dose gamma radiation treatments at the storage temperatures investigated. Gamma radiation application for inactivation of bacterial pathogens is not unexpected as radiation treatments have previously resulted in bacterial DNA breakage and cell damage, depending on radiation dosage and application duration (Prakash & Ornelas-Paz, 2019; Niemira & Boyd, 2013). Damage to other bacteria cellular components may also occur, leading to injury and possible cell

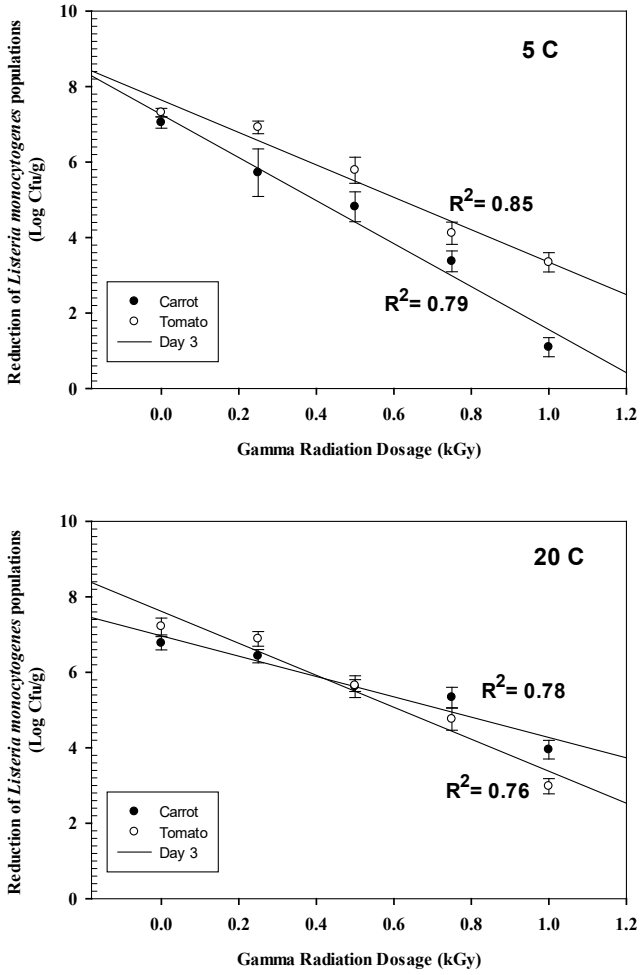

Figure 2. Reduction of *L. monocytogenes* populations on post-harvest carrot and tomato subsequent to pathogen inoculations on produce (8-9 logs). Low-dose gamma radiation (0-1.0 kGy) was applied to pathogen-inoculated produce stored at 5 C and 20 C for 3 days, then processed. The graph represents pathogen reduction with associated standard errors at various radiation doses.

death. The radiation sensitivity of *L. monocytogenes* in turkey nuggets was recorded at 0.70 kGy (Sommers & Thayer, 2000), implying that dosage at similar values would be sufficient for pathogen inactivation at the storage temperatures indicated.

The significant ($P < 0.05$) *L. monocytogenes* reductions on carrot and tomato by low-dose gamma radiation, demonstrated the efficacy of radiation treatment on both produce types. For example, *Listeria* reductions on carrot and tomato were > 4.5 when produce samples were processed immediately. At 5 °C (3 days), reductions of *Listeria* were 5.9 logs (carrot) and 3.9 logs (tomato). The pathogen reductions were 4.4 logs (carrot) and 4.0 logs (tomato) at 7 storage days. At 20 °C (3 days), pathogen reductions were 2.8 logs (carrot) and 4.2 logs (tomato), while *Listeria* decreases were 2.2 logs (carrot) and 2.9 logs (tomato) at 7 days of storage. Therefore, these findings imply that low-dose radiation as an established but under-utilized technology, can be an effective and efficacious intervention tool for *L. monocytogenes* reductions on post-harvest carrot and tomato. Similar or identical research methods may be used for a different

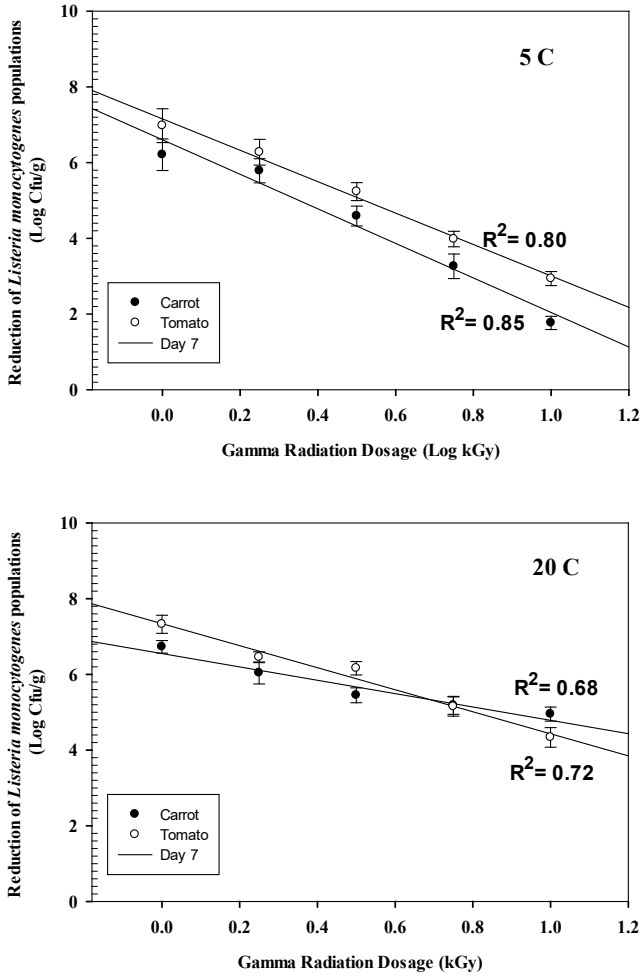

Figure 3. Reduction of *L. monocytogenes* populations on post-harvest carrot and tomato subsequent to pathogen inoculations on produce (8-9 logs). Low-dose gamma radiation (0-1.0 kGy) was applied to pathogen-inoculated produce that was stored at 5 °C and 20 °C for 7 days, then processed. The graph represents pathogen reduction with associated standard errors at various radiation doses.

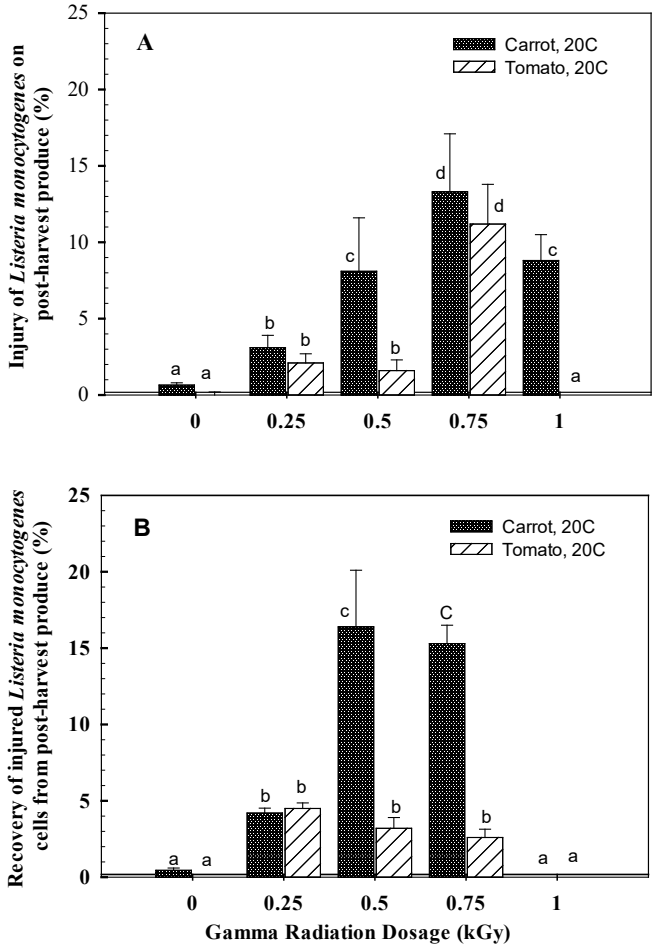

Figure 4. Radiation treatment effects on injury and recovery of *L. monocytogenes* inoculated on post-harvest carrot and tomato. Data indicates % of injured (A) and recovered populations (B). The injured populations were computed from the overlay medium as a proportion of the pathogen counts (CFU/g) on selective medium. Recovery of injured populations were quantified on MOX selective medium as a proportion of the cells on non-selective (TSA) medium. Bars with the same letters on the graph are not significantly different ($P>0.05$).

bacterial-host combination since pathogen inactivation results on one post-harvest produce may not always be directly applied to another produce without adequate data or validation.

In previous studies, irradiation treatments showed reductions of *L. monocytogenes* on various food substrates with results similar to this study. At 1 kGy of irradiation dose, 4.14 log CFU/g reductions of *Listeria* were recorded on tomato (Bari et al., 2005). Similarly, a 4.0 log reduction of *L. monocytogenes* on carrot was documented in published research (Dhokane et al., 2006). Therefore, our results are consistent with previous studies and is indicative of the efficacious nature of gamma radiation applications for *Listeria* inactivation, even though the *Listeria* strains used in the cited studies were different from those used in this research. A 1 kGy dosage of gamma radiation application has been designated by FDA, WHO and FAO as suitable for food applications for control of pathogenic bacteria (Barkai-Golan & Follett, 2017; Food and Drug Administration, 2019).

In this research, pathogen reductions showed significant ($P < 0.05$) linear decreases (Figures 1-3) and the average *L. monocytogenes* reductions on produce was slightly better at 5 °C (4.5 logs) than at 20 °C (3.0 logs), when data were averaged across storage days regardless of produce types. Our results are consistent with previous studies in which radiation treatment of produce and low temperature storage resulted in greater reductions of *L. monocytogenes* (Arvanitoyannis et al., 2009). For example, when shredded carrots packed in polyethylene bags and inoculated with *L. monocytogenes* and *E. coli* were subjected to a 1 kGy of radiation treatment and stored at 5 °C, *L. monocytogenes* reduction in excess of 4 logs was recorded (Arvanitoyannis et al., 2009). Similarly, the effect of radiation treatment and low temperature exposure reduced *L. monocytogenes* populations in sliced cabbage and radish by 4-5 logs in comparison to non-irradiated produce (Prakash & Foley, 2004).

Injury associated with *Listeria* populations following radiation treatment varied on carrot and tomato, for samples processed immediately after treatment (Figure 4). The mean populations of injured cells were either below detection to low values on

samples in the control (un-treated) or exposed to 0.25 kGy. The populations of injured cells ranged from 2.1-13.3 logs on carrot and tomato. The average % of injured *Listeria* cells from produce stored for 3 days were lower at 5 °C than at 20 °C (Table 2). On carrot and tomato stored at 5 °C, injured cells were 7.0% and 3.3%; respectively, whereas at 20 °C, these were 16.1% and 3.6%, on carrot and tomato, respectively (Table 2).

After 7 days of produce storage at 5 °C, % injured cells on carrot and tomato were 8.9% and 5.2%, respectively. At 20 °C storage, the mean values were 6.9% and 3.3% on carrot and tomato, respectively (Table 3). The mean recovery (%) of injured *Listeria* populations on produce varied and was comparatively greater on carrot (11.9%) than on tomato (3.4%), for samples processed immediately (Figure 4). The mean % of recovery of injured *Listeria* cells on produce after 3 days of storage were similar on both carrot and tomato (1.8-8.9%) and at 5 °C and 20 °C (Table 2). Similarly, the average % of injured cells recovered from produce following storage for 7 days ranged from 1.5 to 9.6% (Table 3). This implies that less injury of bacterial cells may be expected at lower storage temperatures at 3 days of storage for the *Listeria* strains investigated.

The quantification of radiation effects on pathogen injury and recovery is important for documenting its sensitivity to gamma radiation, particularly as they pertain to post-harvest produce. This is important to ascertain if radiation treatment would be sufficient to minimize resuscitation of *L. monocytogenes*. In this research, the mean % of recovery of injured *Listeria* cells on produce at 3 days of storage were similar on both carrot and tomato (3.6-5.8%) and at 5 °C and 20 °C storage; while % recovery of injured *Listeria* cells from both produce were 3.3 to 8.3%, following 7 days of storage. Therefore, we may expect low levels of recovery of *L. monocytogenes* cells from produce that has been gamma-irradiated. The high levels of pathogen reductions suggest that gamma radiation treatment can be utilized to mitigate *Listeria* contamination of post-harvest carrot and tomato. In a commodity such as rice grains, gamma radiation dose of < 5kGy had no significant effects on sensory quality of rice protein (Yao et al., 2022).

4 Conclusion

The mitigation of pathogen contamination on fresh produce is a significant food safety issue. Low-dose gamma radiation significantly reduced *Listeria* populations at 5 °C and 20 °C storage across days. Linear decreases were significant as radiation dosage increased from 0.25 to 1.0 kGy, implying that the treatments were efficacious for *Listeria* control. The food applications of low-dose radiation for control of pathogenic bacteria can improve post-harvest safety of carrot and tomato.

Table 2. Injury and recovery of *Listeria monocytogenes* populations from post-harvest carrot and tomato after low-dose gamma radiation treatments[w].

Temp	Dosage (kGy)	Carrot		Tomato	
		Injury (%)[x]	Recovery (%)[y]	Injury (%)[x]	Recovery (%)[y]
Day 3 5 °C	0	1.2c	0.6e	1.7c	0.8c
	0.25	2.7c	1.7d	3.2b	3.3b
	0.50	6.9b	0.9e	3.4a	0.6c
	0.75	7.2b	1.8d	4.8b	2.3b
	1.00	16.8a	4.6c	ND….	ND….
Means	-	7.0	1.9	3.3	1.8
$LSD_{(0.05)}$	-	4.2	0.8	1.4	1.5
Day 3 20 °C	0	4.5c	3.9c	0.8c	3.9c
	0.25	17.4b	9.3b	1.2c	13.6a
	0.50	17.8b	15.5a	3.7bc	6.8b
	0.75	18.6b	7.8b	5.5ab	1.7d
	1.00	22.0a	7.9b	6.8a	ND…..
Means	-	16.1	8.9	3.6	6.5
$LSD_{(0.05)}$	-	3.3	5.2	3.0	2.2

[w]Bacteria populations were averaged across gamma radiation treatment for each produce stored for 3 days either 5 °C or 20 °C subsequent to radiation treatments; [x]Injured populations (%) was computed as: 1– (*Listeria* populations in overlay/*Listeria* populations in TSA) × 100%. Means followed by the same letters are not significantly different ($P > 0.05$); [y]Pathogen populations recovered from injury (%) was computed as: 1– (*Listeria* populations in MOX selective media/*Listeria* populations in TSA) × 100%. Means followed by the same letters are not significantly different ($P > 0.05$). ND = below detection.

Table 3. Injury and recovery of *Listeria monocytogenes* populations from post-harvest carrot and tomato after low-dose gamma radiation treatments[w].

Temp	Dosage (kGy)	Carrot		Tomato	
		Injury (%)[x]	Recovery (%)[y]	Injury (%)[x]	Recovery (%)[y]
Day 7 5 °C	0	4.2c	2.3c	2.6bc	2.2b
	0.25	5.8bc	4.2b	3.9b	4.5a
	0.50	6.7bc	16.4a	5.2b	3.2b
	0.75	7.9b	15.3a	8.6a	2.6b
	1.00	18.3a	ND….	8.8a	ND….
Means	-	7.0	9.6	5.2	3.1
$LSD_{(0.05)}$	-	3.7	1.8	1.5	1.2
Day 7 20 °C	0	2.3c	0.5e	2.2c	10.4b
	0.25	5.1c	1.7d	7.8c	17.8a
	0.50	5.3c	0.9e	12.3b	4.6c
	0.75	9.7b	1.8d	16.2ab	3.2c
	1.00	20.3a	2.6c	18.6a	2.3c
Means	-	8.5	1.5	11.4	7.6
$LSD_{(0.05)}$	-	4.2	0.5	4.5	5.6

[w]Bacteria populations were averaged across gamma radiation treatment for each produce stored at either 5 °C or 20 °C subsequent to radiation treatments; [x]Injured populations (%) was computed as: 1– (*Listeria* populations in overlay media/*Listeria* populations in TSA) x 100%; [y]Pathogens recovered (%) was computed as: 1– (*Listeria* populations in MOX media/*Listeria* populations in TSA) x 100%. Means followed by the same letters in columns are not significantly different (P>0.05). ND = below detection level.

References

Ajayeoba, T. A., Atanda, O. O., Obadina, A. O., Bankole, M. O., & Adelowo, O. O. (2016). The incidence and distribution of *Listeria monocytogenes* in ready-to-eat vegetables in south-western Nigeria. *Food Science & Nutrition*, 4, 59-66. http://dx.doi.org/10.1002/fsn3.263. PMid:26788311.

Alvarado-Casillas, S., Ibarra-Sánchez, S., Rodríguez-García, O., Martínez-Gonzáles, N., & Castillo, A. (2007). Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *Journal of Food Protection*, 70(3), 655-660. http://dx.doi.org/10.4315/0362-028X-70.3.655. PMid:17388055.

Arumugaswamy, R. K., Ali, G. R. R., & Hamid, S. N. B. A. (1994). Prevalence of *Listeria monocytogenes* in foods in Malaysia. *International Journal of Food Microbiology*, 23(1), 117-121. http://dx.doi.org/10.1016/0168-1605(94)90227-5. PMid:7811569.

Arvanitoyannis, I. S., Stratakos, A., & Tsarouhas, P. (2009). Irradiation applications in vegetables and fruits: a review. *Critical Reviews in Food Science and Nutrition*, 49(5), 427-462. http://dx.doi.org/10.1080/10408390802067936. PMid:19399670.

Bari, M. L., Nakauma, M., Todoriki, S., Juneja, V. K., Isshiki, K., & Kawamoto, S. (2005). Effectiveness of irradiation treatments in inactivating *Listeria monocytogenes* on fresh vegetables at refrigeration temperature. *Journal of Food Protection*, 68(2), 318-323. http://dx.doi.org/10.4315/0362-028X-68.2.318. PMid:15726975.

Barkai-Golan, R., & Follett, P. A. (2017). *Irradiation for quality improvement, microbial safety and phytosanitation of fresh produce*. Amsterdam: Elsevier.

Berrios-Rodriguez, A., Olanya, O. M., Annous, B. A., Cassidy, J. M., Orellana, L., & Niemira, B. A. (2017). Survival of *Salmonella* Typhimurium on soybean sprouts following treatments with gaseous chlorine dioxide and biocontrol *Pseudomonas* bacteria. *Food Science and Biotechnology*, 26(2), 513-520. http://dx.doi.org/10.1007/s10068-017-0071-9. PMid:30263573.

Berrios-Rodriguez, A., Olanya, O. M., Ukuku, D. O., Niemira, B. A., Orellana, L. E., Mukhopadhyay, S., Cassidy, J. M., & Boyd, G. (2020a). Inactivation of *Listeria monocytogenes* on post-harvest carrot and tomato by gamma radiation, sanitizer, biocontrol treatments and their combinations. *Lebensmittel-Wissenschaft + Technologie*, 118, 1-8. http://dx.doi.org/10.1016/j.lwt.2019.108805.

Berrios-Rodriguez, A., Ukuku, D. O., Olanya, M., Cassidy, J., Orellana, L. E., Mukhopadhyay, S., & Niemira, B. A. (2020b). Nisin-based organic acid inactivation of Salmonella on grape tomatoes: efficacy of treatment with Bioluminescence ATP assay. *Journal of Food Protection*, 83(1), 68-74. http://dx.doi.org/10.4315/0362-028X.JFP-19-275. PMid:31845829.

Berrios-Rodriguez, A., Olanya, O. M., Niemira, B. A., Ukuku, D. O., Mukhopadhyay, S., & Orellana, L. E. (2022). Gamma radiation treatment of post-harvest produce for *Salmonella enterica* reduction on baby carrot and grape tomato. *Journal of Food Safety*, 42(1), e12951. http://dx.doi.org/10.1111/jfs.12951.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*, 75, 1-13. http://dx.doi.org/10.1016/j.foodcont.2016.12.016.

Callejón, R. M., Rodríguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M. C., & Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathogens and Disease*, 12(1), 32-38. http://dx.doi.org/10.1089/fpd.2014.1821. PMid:25587926.

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in Microbiology*, 10, 2667. http://dx.doi.org/10.3389/fmicb.2019.02667. PMid:31824454.

Castro-Ibáñez, I., Gil, M. I., & Allende, A. (2017). Ready-to-eat vegetables: current problems and potential solutions to reduce microbial risk in the production chain. *Lebensmittel-Wissenschaft + Technologie*, 85, 284-292. http://dx.doi.org/10.1016/j.lwt.2016.11.073.

Costa, P. V., Nascimento, J. S., Costa, L. E. O., Ferreira, P. B. M., & Brandão, M. L. L. (2022). Listeria monocytogenes: challenges of microbiological control of food in Brazil. *Food Science and Technology*, 42, e08322. http://dx.doi.org/10.1590/fst.08322.

Dhokane, V. S., Hajare, S., Shashidhar, R., Sharma, A., & Bandekar, J. R. (2006). Radiation processing to ensure safety of minimally processed carrot (*Daucus carota*) and cucumber (*Cucumis sativus*): optimization of dose for the elimination of *Salmonella* Typhimurium and *Listeria monocytogenes*. *Journal of Food Protection*, 69(2), 444-448. http://dx.doi.org/10.4315/0362-028X-69.2.444. PMid:16496592.

Ferreira, V., Wiedmann, M., Teixeira, P., & Stasiewicz, M. J. (2014). *Listeria monocytogenes* persistence in the food-associated environments: epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, 77(1), 150-170. http://dx.doi.org/10.4315/0362-028X.JFP-13-150. PMid:24406014.

Food and Agriculture Organization – FAO. (2021). *World food and agriculture: statistical yearbook 2021* (368 p.). Rome: FAO. https://doi.org/10.4060/cb4477en.

Food and Drug Administration – FDA. (2019). *Ionizing radiation for the treatment of food*. Retrieved from https://www.ecfr.gov/cgi-bin/text-idx?SID=c9b212d0ba92cf37273f8ecf1657093c&mc=true&node=se21.3.179_126&r gn=div8

Freitag, N. E., Port, G. C., & Miner, M. D. (2009). *Listeria monocytogenes* – from saprophyte to intracellular pathogen. *Nature Reviews. Microbiology*, 7(9), 623-628. http://dx.doi.org/10.1038/nrmicro2171. PMid:19648949.

Julien-Javaux, F., Gerard, C., Campagnoli, M., & Zuber, S. (2019). Strategies for the safety managemnent of fresh produce from farm to fork. *Current Opinion in Food Science*, 27(Suppl. 1), 145-152. http://dx.doi.org/10.1016/j.cofs.2019.01.004.

Kang, D. H., & Siragusa, G. R. (1999). Agar underlay method for recovery of sub-lethaly heat-injured bacteria. *Applied and Environmental Microbiology*, 65(12), 5334-5337. http://dx.doi.org/10.1128/AEM.65.12.5334-5337.1999. PMid:10583985.

Kara, R., & Aslan, S. (2021). Investigation of *Listeria monocytogenes* in workers, equipment and environments at Kaymak processing plants. *Food Science and Technology*, 41(2), 449-452. http://dx.doi.org/10.1590/fst.02620.

Lee, S.-Y., & Kang, D.-H. (2001). Suitability of overlay method for recovery of heat-injured *Listeria monocytogenes* and *Salmonella* Typhimurium. *Food Science and Biotechnology*, 10, 323-326.

Marik, C. M., Zuchel, J., Schaffner, D. W., & Strawn, L. K. (2020). Growth and survival of *Listeria monocytogenes* on intact fruit and vegetable surfaces during postharvest handling: a systematic literature review. *Journal of Food Protection*, 83(1), 108-128. http://dx.doi.org/10.4315/0362-028X.JFP-19-283. PMid:31855613.

Morehouse, K. M., & Komolprasert, V. (2004). *Irradiation of food and packaging: recent developments*. Washington, D.C.: American Chemical Society. Irradiation of food and packaging: an overview, pp. 1-11. http://dx.doi.org/10.1021/bk-2004-0875.

Murray, K., Wu, F., Shi, J., Xue, S. J., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, 1(4), 289-301. http://dx.doi.org/10.1093/fqsafe/fyx027.

Niemira, B. A., & Boyd, G. (2013). Influence of modified atmosphere and varying time in storage on the irradiation sensitivity of *Salmonella* on sliced roma tomatoes. *Radiation Physics and Chemistry*, 90, 120-124. http://dx.doi.org/10.1016/j.radphyschem.2013.04.021.

Niemira, B. A., Fan, X., & Sokorai, K. J. B. (2005). Irradiation and modified atmosphere packaging of endive influences survival and re-growth of *Listeria monocytogenes* and product sensory qualities. *Radiation Physics and Chemistry*, 72(1), 41-48. http://dx.doi.org/10.1016/j.radphyschem.2004.01.005.

Olanya, O. M., Hoshide, A. K., Ijabadeniyi, O. A., Ukuku, D. O., Mukhopadhyay, S., Niemira, B. A., & Ayeni, O. (2019). Cost estimation of listeriosis (*Listeria monocytogenes*) occurrence in South Africa in 2017 and its food safety implications. *Food Control*, 102, 231-239. http://dx.doi.org/10.1016/j.foodcont.2019.02.007.

Olanya, O. M., Niemira, B. A., & Phillips, J. G. (2015). Effects of gamma irradiation on the survival of *Pseudomonas fluorescens* inoculated on romaine lettuce and spinach. *Lebensmittel-Wissenschaft + Technologie*, 62(1), 55-61. http://dx.doi.org/10.1016/j.lwt.2014.12.031.

Olanya, O. M., Ukuku, D. O., Solaiman, D. S. Y., Ashby, R. D., Niemira, B. A., & Mukhopadhyay, S. (2018). Reductionin *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* O157:H7 *in vitro* and on tomato by sophorolipid and sanitiser as affected by temperature and storage time. *International Journal of Food Science & Technology*, 53, 1303-1315. http://dx.doi.org/10.1111/ijfs.13711.

Pinela, J., & Ferreira, I. C. F. R. (2017). Nonthermal physical technologies to decontaminate and extend the shelf-life of fruits and vegetables: trends aiming at quality and safety. *Critical Reviews in Food Science and Nutrition*, 57(10), 2095-2111. http://dx.doi.org/10.1080/10408398.2015.1046547. PMid:26192014.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., Nishibuchi, M., Nakaguchi, Y., & Malakar, P. K. (2010). *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. *Food Control*, 21(5), 774-778. http://dx.doi.org/10.1016/j.foodcont.2009.09.008.

Prakash, A., & Foley, D. (2004). Improving safety and extending shelf life of fresh-cut fruits and vegetables using irradiation. In V. Komolprasert & K. M. Morehouse (Eds.), *Irradiation of food and packaging: recent developments* (pp. 90-106). Washington, D.C.: American Chemical Society. http://dx.doi.org/10.1021/bk-2004-0875.ch006.

Prakash, A., & Ornelas-Paz, J. J. (2019). Irradiation of fruits and vegetables. In E. M. Yahia (Ed.), *Postharvest technology of perishable horticultural commodities* (pp. 563-589). Chihuahua: Elsevier. http://dx.doi.org/10.1016/B978-0-12-813276-0.00017-1.

Ramos, B., Miller, F. A., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2013). Fresh fruits and vegetables – an overview of applied methodologies to improve its quality and safety. *Innovative Food Science & Emerging Technologies*, 20, 1-15. http://dx.doi.org/10.1016/j.ifset.2013.07.002.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Diseases*, 17(1), 7-15. http://dx.doi.org/10.3201/eid1701.P11101. PMid:21192848.

Scharff, R. L. (2015). State estimates of annual costs of foodborne illness. *Journal of Food Protection*, 78(6), 1064-1071. http://dx.doi.org/10.4315/0362-028X.JFP-14-505. PMid:26038894.

Sommers, C. H., & Thayer, D. W. (2000). Survival of surface-inoculated *Listeria monocytogenes* on commercially available franfurters following gamma radiation. *Journal of Food Safety*, 20(2), 127-137. http://dx.doi.org/10.1111/j.1745-4565.2000.tb00293.x.

Ukuku, D. O., Olanya, M., Geveke, D. J., & Sommers, C. H. (2012). Effect of native microflora, waiting period, and storage temperature on *Listeria monocytogenes* serovars transferred from cantaloupe rind to fresh-cut pieces during preparation. *Journal of Food Protection*, 75(11), 1912-1919. http://dx.doi.org/10.4315/0362-028X.JFP-12-191. PMid:23127699.

United States Department of Agriculture – USDA. (2021). *Vegetables 2020 summary*. Washington, D.C.: USDA.

Vahidy, R., Jahan, F., & Nasim, R. (1992). Isolation of *Listeria monocytogenes* from fresh fruits and vegetables. *HortScience*, 27(6), 628b. http://dx.doi.org/10.21273/HORTSCI.27.6.628b.

Välimaa, A.-L., Tilsala-Timisjärvi, A., & Virtanen, E. (2015). Rapid detection and identification methods for *Listeria monocytogenes* in the food chain: a review. *Food Control*, 55, 103-114. http://dx.doi.org/10.1016/j.foodcont.2015.02.037.

Yao, G., Guo, Y., Cheng, T., Wang, Z., Li, B., Xia, C., Jiang, J., Zhang, Y., Guo, Z., & Zhao, H. (2022). Effect of γ-irradiation on the physicochemical and functional properties of rice protein. *Food Science and Technology*, 42, e12422. http://dx.doi.org/10.1590/fst.12422.

Zhao, P., Zhang, Y., Deng, H., & Meng, Y. (2022). Antibacterial mechanism of apple phloretin on physiological and morphological properties of *Listeria monocytogenes*. *Food Science and Technology*, 42, e55120. http://dx.doi.org/10.1590/fst.55120.

Zhu, Q., Gooneratne, R., & Hussain, M. (2017). *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. *Foods*, 6(3), 21. http://dx.doi.org/10.3390/foods6030021. PMid:28282938.