



Prediction of targeted *Salmonella enterica* serovar typhimurium inactivation in fresh cut cantaloupe (*Cucumis melo* L.) using electron beam irradiation

Ezekiel Chimbombi¹, Rosana G. Moreira^{*}, Jongsoon Kim, Elena M. Castell-Perez

Department of Biological and Agricultural Engineering, Texas A&M University, College Station, TX 77843-2117, United States

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ABSTRACT

Pathogens internalize in fresh produce. When using e-beam accelerators to treat the produce, it must be designed to ensure that the entrance dose will translate into the effective dose delivered at the interior of the food. The low penetration capacity of e-beam accelerators makes this a difficult task. If dose is non-uniform at different locations within the product, under-or-over-dosage could compromise safety and quality. We calculated the dose required for “targeted” inactivation of *S. typhimurium* LT2 internalized in fresh cantaloupe flesh using e-beam irradiation. Target inactivation is the estimation of the dose required to inactivate bacteria to the target level at a certain depth in the product. Growth and mobility data were used for simulation of irradiation protocols in a commercial facility (10 MeV e-beam linear accelerator) using radiation theory. Bacterial infestation and internalization into cantaloupe would require more than 1.0 kGy and compensators should be used to reduce the dose required at a certain depth in the produce.

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1. Introduction

Irradiation of foods for microbial inactivation requires quantification of both the microbial load and the desired optimal dose to ensure safety while maintaining product quality. The mobility of bacteria within the host (the fresh produce) needs to be fully understood and quantified as a function of time and space so that fresh produce inactivation interventions can be properly guided and targeted. Maintaining organoleptic and nutritional quality and keeping costs down are important factors, making it desirable to use the lowest possible doses necessary to achieve desired levels of microbiological control on a commercial scale. This requires establishment of the efficacy of the radiation treatment and threshold doses for quality changes (Farkas, 1998).

Increased microbial resistance has been observed in food-borne pathogens such as enterohemorrhagic *Escherichia coli* O157:H7 inoculated in ground beef and repeatedly subjected to electron beam irradiation, resulting in a significant increase ($P < 0.05$) of the D_{10} -value from 0.24 ± 0.03 to 0.63 ± 0.02 kGy for ATCC strain 35150 and isolate L3, following four cycles of e-beam processing with the microorganism being able to resist doses as high as 3.0 kGy (Levanduski and Jaczynski, 2008). The practical implications of this increased microbial resistance to e-beam processing

for the food industry and general public health is that the minimum level of radiation for several relevant foods may need to be revised.

Fan and Sokorai (2008) reported that the appearance, texture, and aroma of most of the fresh-cut vegetables (iceberg, romaine, green and red leaf lettuce, spinach, tomato, cilantro, parsley, green onion, carrot, broccoli, red cabbage, and celery) were not negatively affected by 1.0 kGy gamma irradiation when stored in air or MAP (modified atmospheric packaging). The authors further observed that the appearance and aroma of many irradiated vegetables were better than that of the corresponding controls after 14-day storage at 4 °C, probably due to the reduction of decay and browning. However, vitamin C content was reduced by irradiation in some vegetables, particularly green and red leaf lettuce.

Gomes et al. (2008) observed that broccoli heads irradiated up to a dose of 3.0 kGy using electron beam showed same quality attributes as the non-irradiated samples. High irradiation doses may have undesirable effects on quality and sensorial attributes of the produce; therefore it is crucial to minimize the dose. Sensory quality of fresh cut lettuce was preserved by an irradiation dose of 1.0 kGy for 8 days at a storage temperature of 4 °C, while a 1.5 kGy dose resulted in a decrease in sensory quality, possibly due to damage to tissue caused by the high dose (Zhang et al., 2006). Rodriguez et al. (2006) assessed the radiation sensitivity of bacteria such as *Listeria monocytogenes*, and the surrogate *E. coli* K-12 MG1655 by comparing their D_{10} -value in cantaloupe with that in model foods. They found that *E. coli* K-12 MG1655 had higher D_{10} -values in cantaloupe (~ 0.45 kGy) than in the model food

^{*} Corresponding author. Tel.: +1 979 847 8794.

E-mail address: rmoreira@tamu.edu (R.G. Moreira).

¹ Present address: Department of Agricultural Engineering and Land Planning, Botswana College of Agriculture, Gaborone, Botswana.

(gelatin system) (~ 0.18 kGy) while *Listeria monocytogenes* had similar D_{10} -values in both cantaloupe (~ 0.15 kGy) and the gelatin system (~ 0.15 kGy). These findings show that irradiation sensitivity is food product specific, therefore it should not be generalized for all fresh produce.

In addition, the surface of fruits and vegetables may be curved or irregular in shape, requiring corrections for contour irregularities. Compensators are generally used to match doses at a target to an iso-dose distribution produced on a flat phantom (Podgorsak, 2005). The thickness of the compensator is based on the reduction of the dose that is required at a certain depth of interest in the target.

Research on targeted irradiation inactivation (the dose required to inactivate bacteria to the target level at a certain depth in the product) in terms of the level of bacterial contamination and location of the pathogens (space dimension) is deficient, resulting in either an under-dose or over-dose of some parts of the produce. The need for targeted irradiation is even more relevant due to the ability of pathogens such as *S. typhimurium* to internalize in fresh produce to depths up to 50 mm (Chimbombi et al., 2009; Gomes et al., 2009).

The objective of this study was to determine the optimum set up to ensure that the microorganism, in this case *S. typhimurium* LT2 internalized in fresh cantaloupe flesh, is inactivated using electron beam irradiation.

2. Materials and methods

Ripe cantaloupes (golden/orange in color underneath and within the outer rind) were randomly selected and purchased from the local fresh produce market on the day of the experiment. Measured quality attributes of cantaloupe included the pH 6.68 ± 0.06 ; $a_w = 0.91 \pm 0.01$; °Brix = 11 ± 0.31 ; and moisture content = 0.89 ± 0.04 g/g product (Chimbombi et al., 2009).

2.1. D_{10} -value determination for *S. typhimurium* LT2 in fresh cut cantaloupe flesh

Nalidixic acid and novobiocin resistant *S. typhimurium* LT2 (ATCC 700720) was obtained from Dr. James Q.A. Bryd (USDA-ARS, College Station, TX), and maintained and grown in tryptic soy broth (TSB) supplemented with 20 $\mu\text{g}/\text{ml}$ nalidixic acid (Sigma, St. Louis, MO, USA) and 25 $\mu\text{g}/\text{ml}$ novobiocin (Sigma, St. Louis, MO, USA). A minimum of two transfers with a transfer after every instance of incubation of the culture media for 18–24 h and subsequent serial dilutions up to a yield of 3–4.11 \log_{10} CFU/g were done to produce the inoculums ready for the challenge test.

Fifteen (15) cylindrical fresh cantaloupe samples (50 mm long and 19.05 mm diameter) were placed in a test tube rack and point inoculated with 8.01 logs CFU/g (50 μL) of the surrogate at the top end. The non-pathogenic surrogate bacteria *S. typhimurium* LT2 was used because of the practical limitations of using the pathogenic bacteria in the Van de Graaff irradiation facility.

Three samples each were irradiated with average electron beam doses (penetration up to 5 mm) of 0.2 (± 0.08), 0.4 (± 0.16), 0.5 (± 0.20), 0.6 (± 0.23), and 0.8 (± 0.31) kGy using a 1.35 MeV Van de Graaff accelerator (High Voltage Engineering Corp., Cambridge, MA) at room temperature (23 °C), with the beam targeted at the point of bacterial inoculation. The radiation set up is shown in Fig. 1. The delivered dose by the Van de Graaff accelerator was measured using a Farmer-type ion chamber (Markus chamber, Type 23343). The Markus® chamber is a high-performance ionization chamber for precise dose measurement of high-energy electron beams in radiation therapy. The ion chamber measures the dose resulting from electrons passing through the irradiation

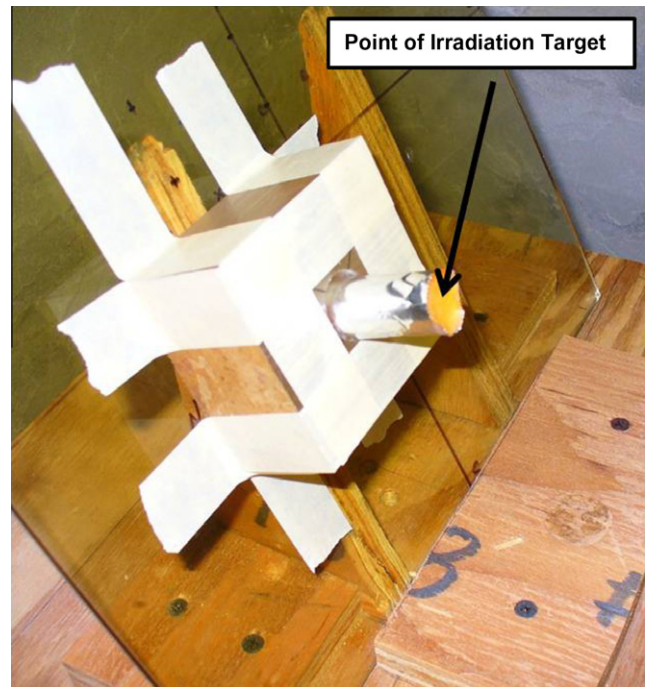


Fig. 1. Sample placement for irradiation using the 1.35 MeV Van de Graaff accelerator.

target area. The radiation dose measured from the Markus® chamber was matched against a counter measuring the transmission ion chamber (beam monitoring apparatus), directly attached to the exit beam window. Generally, the ionization chamber is the most widely used type of dosimeter for precise measurements, such as those required in radiotherapy. Such chambers use the quantity 'exposure' to determine absorbed dose in certain materials. They are also one of the absolute dosimeters, which can be assembled and used to measure the absorbed dose deposited in its own sensitive volume without requiring calibration in a known field of radiation. However, our Farmer-type ion chamber has been periodically calibrated with beta particle sources (90Sr/90Y) following German National Standard Laboratory Procedure (PTB: Physikalisch-Technische Bundesanstalt) at the Nuclear Science Center, Texas A&M University, College Station, TX.

Microbiological analysis was done on the 5-mm thick top end by the standard plate count method using Tryptic Soy Agar (TSA) medium supplemented with 1 mL/L nalidixic acid (Sigma, St. Louis, MO, USA) to determine total colony counts. The samples were cut 50 mm-long for ease of handling, placement and bacterial inoculation despite that the analysis was done on the top 5-mm length, i.e., the penetration depth of the 1.35 MeV electron beam accelerator. The D_{10} -value was determined according by computing the slope of the survivors against the dose. Each dose treatment was replicated at least three times.

2.2. Growth duration dependent *S. typhimurium* inactivation in fresh-cut cantaloupe flesh

Twenty-one (21) cantaloupe flesh (50 mm long by 19.05 mm diameter) were wrapped in an aluminum foil, placed in a test tube rack with the top end open and inoculated with 50 μL volume of 3.77–4.11 logs CFU/g concentration of *S. typhimurium* LT2 inoculums and allowed variable time intervals of 0.5, 5, 10, 15, 20, 25 and 30 h for growth. The cantaloupe samples were inoculated at the top end and allowed 7–10 min in the undisturbed normal

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