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RADIOSENSITIVITY OF *E. coli* O157:H7 AND *Salmonella typhimurium* ON SWISS CHARD

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ABSTRACT

Swiss Chard is a beet (*Beta vulgaris* cicla) producing large yellowish green leaves with thick succulent stalks and often cooked as a potherb, called also seakale beet or chard. It is a nutritive vegetable rich in potassium, calcium, magnesium, sodium, phosphorus and vitamin C. Ionising radiation is an effective method to reduce pathogens. Radiation sensitivity of bacteria, however, depends on several factors. Particularly, few data are available on the ability of low-dose ionizing radiation to inactivate pathogenic bacteria on ready to eat vegetables. The aim of this study was the evaluation of the radiation sensitivity of pathogens experimentally contaminating the mentioned vegetable. Swiss chard leaves minimally processed were inoculated separately either with *E. coli* O157:H7 or *Salmonella typhimurium* by immersion to contain 6 log CFU/g and 1h later gamma-irradiated with 0.25kGy, 0.5kGy, 1kGy and 1.5kGy, dose rate of 2.94kGy/h. The assay of pathogen survivors was made by direct plating. After applying a radiation dose of 0.5kGy reductions of at least 3 log were achieved for both bacteria. The average D_{10} values, the radiation dose needed to inactivate 1 log of pathogen were 0.12 and 0.10 for *E.coli* O157:H7 and *S.typhimurium* respectively. These results indicate that irradiation may be an effective means for inactivating common foodborne pathogens that can eventually contaminate ready to eat vegetables.

1. INTRODUCTION

With trade increasing between countries, food safety risks are no longer concentrated on domestic markets only. Sharing knowledge and best practices produces the corollary of improved food safety around the world [1].

Marketing of fresh fruits and vegetables with minimal processing is gaining impetus due to its convenience, freshness and human health benefits not only in the developed world but also in developing countries [1, 2]. Minimal processing includes washing, peeling, slicing or shredding of fresh vegetables or fruits for sale within 7-8 days after preparation, and storage at low temperatures [3, 4].

Escherichia coli O157:H7 cause haemorrhagic colitis, and life-threatening complications like haemolytic uremic syndrome and trombotic thrombocytopenic purpura may occur in haemorragic colitis patients [5].

Salmonellosis is a foodborne disease caused by *Salmonella spp.* Despite global improvement in public health facilities, the disease remains a major problem in many parts of the world. Recently, human salmonellosis has been associated with fresh fruits and vegetables [1, 6].

Swiss Chard is a beet (*Beta vulgaris* cicla) producing large yellowish green leaves, called also seakale beet or Chard. It is a nutritive vegetable rich in potassium, calcium, magnesium, sodium, phosphorus and vitamin C [7].

Among food preservation methods, food irradiation is considered the most versatile treatment nowadays. Microorganisms can be inactivated by impairment of important molecules or organelles, such as DNA and the cytoplasmatic membrane [8]. Then, ionizing radiation became an important tool to be used by the food industry not only as a method of preserving food but also to improve food safety [8, 9].

The aim of this study was the evaluation of the radiation sensitivity of two important human pathogens, *E. coli* O157:H7 and *Salmonella typhimurium* on experimentally contaminated Swiss chard minimally processed.

2. MATERIALS AND METHODS

2.1 Material

Escherichia coli O157:H7 Can 55/82 and *Salmonella typhimurium* ATCC 14028 obtained from the *Seção de coleção de culturas, Instituto Adolfo Lutz* were used in this work. The isolates were grown in 5ml Brain Heart Infusion Broth (BHI) (Difco, Detroit, MI) at 35ºC for 24h. After this period, the cultures were spread plated onto Sorbitol MacConkey agar (Difco, Detroit, MI) and Salmonella-Shigella agar (Difco, Detroit, MI), and incubated at the same conditions to form single colonies. These colonies were used to inoculate fresh BHI for each experiment and grown for 18h at 35ºC. Aliquots of 100ml of the starting culture from each of the 2 isolates were mixed with 1800ml of sterile cold distilled water to make the working inoculum. The cell density of the starting inoculum was determined by serial dilution with sterile 1% (w/v) NaCl solution (Miletto, São Paulo, Brazil) and pour plating on MacConkey agar and SS agar for the respective microorganisms. The cell density was about 10^6 colony forming units-CFU/ml.

Samples of Swiss Chard minimally processed were provided as a courtesy from Refricon Alimentos on the day of each experiment. They arrived at the industry within 24h after harvest. Samples of the minimally processed vegetable were inoculated with either *Escherichia coli* O157:H7 or *Salmonella typhimurium* in separate experiments. Approximately 300g were transferred to a sterile inoculation tube and the working inoculum was added. The material was agitated gently with a sterile spoon for 60s to submerge completely the sample. The inoculum excess was removed by centrifugation and sub samples of 45g were placed in sterile stomacher bags. The samples were refrigerated (6ºC) until irradiation.

2.2 Irradiation

The Swiss chard samples contaminated with *E. coli* O157: H7 and *S. typhimurium* were exposed to the following radiation doses: 0.0, 0.25, 0.5, 1.0 and 1.5kGy, at initially 4ºC, in a ^{60}Co source Gammacell 220 (AECL) with dose rate of 2.94 kGy/h and Harwell dosimeters Amber 3042 for dose monitoring.

2.3 Microbiological quality of Swiss Chard

Samples of Swiss chard minimally processed were analyzed for the presence of *Escherichia coli* before the experiments to know the microbiological quality of the products commercialized in the local markets. The Most Probable Number (MPN) method with LST-MUG was used according to the procedures described by Silva *et al* [10].

2.4 Enumeration of *E. coli* O157:H7 and *S. typhimurium* after irradiation

After treatment, irradiated and non-irradiated samples were maintained under refrigeration (7º C). Portions of 25g of Swiss chard were suspended in 225ml of 0.1% peptone water (Difco) and serial decimal diluted in the same medium. One milliliter of each dilution was pour plated using Sorbitol MacConkey agar or SS agar (Difco) to determine the population of surviving bacteria as MPN. After an incubation period the plates were counted for the determination of D_{10} value.

2.5 Radiosensitivity Measurement

For each irradiation dose, the survival fraction was estimated dividing the number of viable cells after irradiation with dose D (N_D) by the initial viable cell number (N_O). D_{10} values were calculated according to the formula: $N_D = N_O^{-D/D10}$ where D is the applied dose and D_{10} is the radiation dose necessary for the destruction of 90% of microbial population. The formula can be expressed as a linear equation:
$\log N = \log N_O - (1/ D_{10})D$, being D_{10} the positive inverse of the slope estimated by linear regression of the overall survival curve [11].

3. RESULTS AND DISCUSSION

Microbiological evaluation of foods is of prime importance due to the presence of harmful human pathogens and their inactivation is essential to ensure the hygienic quality of food material [12]. *E. coli* is the microbiological indicator of direct fecal contamination, once is of easy isolation in conventional media culture and resistant for more time [10, 13].

Escherichia coli were detected in 8% of the assayed samples before the experiment. According to an ANVISA Resolution [14] the presence of *E. coli* in vegetables minimally processed is not permitted. The *E. coli* isolated from these samples most likely originates from contaminated water, soil or handling of the products [13, 15]. Other authors found even much higher percentages of *E. coli* contamination in diverse fresh minimally processed vegetables samples [2, 3, 12, 13, 16].

Survivor plots ($\log_{10} N_D$ of survivors vs dose) were determined by regression analysis of the data (Figure 1) for *E. coli* O157:H7 and *Salmonella typhimurium* experimentally inoculated in Swiss chard.

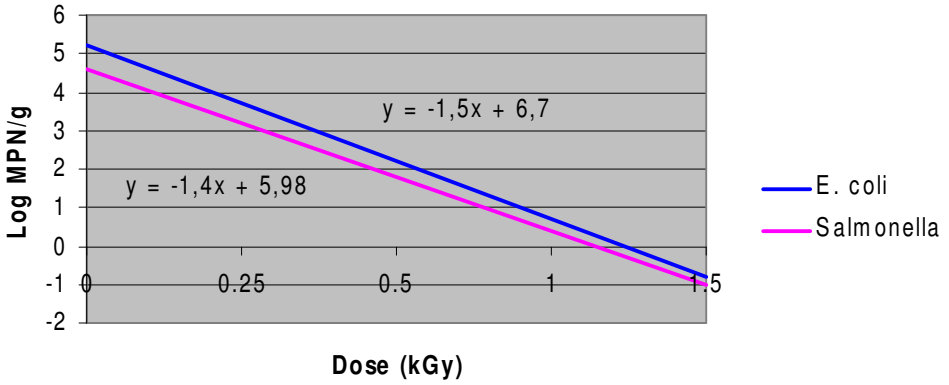

Fig 1. Survival populations (log MPN/ml) of *Escherichia coli* O157:H7 and *Salmonella typhimurium* inoculated on Swiss chard exposed to different doses of gamma radiation.

Irradiation effectively reduced the population of *E. coli* O157:H7 and *Salmonella typhimurium* on Swiss chard. *Salmonella typhimurium* was slightly more radiosensitive than *E.coli* O157:H7 (Table 1). A dose of 0.5 kGy would be sufficient to reduce pathogens contamination in 3 log cycles.

Table 1. D_{10} values for *Escherichia coli* O157:H7 and *Salmonella typhimurium* on Swiss chard minimally processed

Organism	D_{10} value (kGy)
Escherichia coli O157:H7	0.12
Salmonella typhimurium	0.10

When D_{10} values observed in this work are compared with those referred in the literature a similar level of resistance is found. Goulart *et al* [17] reported values ranging from 0.11 to 0.12 kGy for *E. coli* O157:H7 and 0.16 to 0.23 kGy for *Salmonella spp* on minimally processed lettuce. Despite of D_{10} value for *Salmonella* be higher than in the present study, the D_{10} for *E. coli* O157:H7 was very close.

Lee *et al* [18] reported D_{10} values between 0.28-0.42 kGy for four pathogens inoculated in ready-to-eat vegetables, demonstrating the diversity of radiosensitivity among the diverse foodborne pathogens. Similar results were reported by Martins *et al* [19] for *Salmonella spp* in watercress experimentally contaminated, where D_{10} values varied from 0.29 to 0.43kGy.

Niemira [6] obtained D_{10} values of 0.19 and 0.20kGy for *L. monocytogenes* and between 0.23 and 0.31 kGy for *Salmonella .spp* using different types of lettuce. These values are

higher than that obtained in the present research probably due to differential effect of diverse vegetables as well as radiation conditions [18, 21].

4. CONCLUSIONS

Based on the present results minimally processed chard had not satisfactory conditions for consumption as in 8% of the samples the presence of *E. coli* was found. D_{10} values of *E. coli* O157:H7 and *Salmonella typhimurium* inoculated in Swiss chard were 0.12 and 0.10kGy comparable to those of the literature. Proper radiation procedure together with good manufacturing practices (GMP) could significantly increase the microbiological safety of minimally processed Swiss chard.

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