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Use of Irradiation to Ensure the Hygienic Quality of Fresh, Pre-Cut Fruits and Vegetables and Other Minimally Processed Food of Plant Origin

Proceedings of a final research coordination meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and held in Islamabad, Pakistan, 22–30 July 2005

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FOREWORD

Sales and consumption of fresh pre-cut and minimally processed fruits and vegetables continue to grow. Changes occurring in life and eating styles, as well as demographic changes, have been cited as one of the reasons for the increasing demand for this type of produce.

Since fresh fruits and vegetables are grown, processed or packaged in areas that may be exposed to microbial pathogen contamination, there is an increasing concern that these products may harbour microbial pathogens. In fact, a number of outbreaks linked to the consumption of contaminated fresh pre-cut fruits and vegetables have been reported. Prior to this Coordinated Research Project (CRP), studies on various chemical and physical methods of decontamination for their efficacy in destroying pathogens have been made. The use of ionizing radiation seems to have several advantages in relation to other alternative treatments; however more research was needed in order to demonstrate its efficacy without producing negative effects in the physiological traits of the fruit, and thus the commercial quality of these products.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated in 2001 a Coordinated Research Project (CRP) on the Use of Irradiation to Ensure Hygienic Quality of Fresh, Pre-cut Fruits and Vegetables and other Minimally Processed Food of Plant Origin. This CRP included 15 participants from Argentina, Brazil, Canada, Chile, China, Egypt, Hungary, India, Malaysia, Pakistan, Portugal, Turkey, United Kingdom and the United States of America (2). Research coordinated meetings were held in Rio de Janeiro, Brazil (5–9 November 2001), Belfast, UK (14–18 April 2003) and Islamabad, Pakistan (25–29 July 2005).

This publication presents the research results presented in the final research coordination meeting, where the work completed during the last five years (2001–2005) was analysed.

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SUMMARY

1. INTRODUCTION

There is an increasing trend in advanced countries and many developing countries to centrally prepare and process fresh fruits and vegetables, properly packaged, for distribution and marketing. Changes occurring in demographics, lifestyles and eating habits have been cited as some of the reasons for the increasing demands for fresh cut or minimally processed fruits and vegetables. Sales of fresh-cut fruits and vegetables continue to flourish. The International Fresh-cut Produce Association (IFPA) estimates that the total retail sales in USA for the first quarter of 2006 were up 6.5 percent from this time last year. Total sales were more than $1.3 billion in the first quarter of 2006, with fresh-cut fruit generating $242 million of the total and fresh-cut vegetables (including salads) raking in US $1 billion. The fresh-cut fruit category is showing strong growth, with a 15.7% increase from first quarter 2005 sales.

Such a trend appears to result in spreading contamination by various pathogens from a central source. Indeed, a number of large foodborne disease outbreaks involving up to thousands of illnesses and many deaths attributable to the consumption of fresh, pre-cut and minimally processed produce occurred in the past decade. Several types of pathogenic bacteria and parasites, including *Escherichia coli* 0157:H7, *Listeria monocytogenes, Shigella, Aeromonas hydrophila, Yersinia enterocolitica,* and *Cyclospora cayetanensis*, some of which can proliferate at refrigeration temperature, were responsible for the outbreaks. Intestinal viruses are also not uncommon on fresh produce.

The potential sources of pathogenic microorganisms include the raw produce, contaminated water, plant workers, and the processing environment.

In many parts of the world, salad vegetables such as lettuce and carrots, and fruits such as cantaloupes and watermelons, are cut, sliced, and packaged in see-through containers that are stored at chilled temperatures, such that they are ready-to-use (RTU) upon purchase. Overall, RTU produce is by no means microbe-free. The minimally processed, chilled vegetables and fruits are usually carrying Pseudomonas, Enterobacteria, lactic acid bacteria and yeasts and moulds as natural microbiota. Since vegetables are often grown, processed or packed in areas that may be exposed to microbial pathogen contamination, there is an increasing concern that fresh, pre-cut fruits and vegetables may harbour microbial pathogens. In their preparation, intact vegetables are washed, typically with water that contains chlorine from 50 to 200 ppm, followed by cutting and packaging. While washing reduces microbial numbers, the cutting operation has the potential to recontaminate. Also, the fresh-cut produce provide a higher level of moisture, more simple nutrients, and a higher surface area, all of which make the RTU product more susceptible to microbial growth than the intact product.

The introduction of the Hazard Analysis Critical Control Point (HACCP) system does not appear to be completely effective. Several chemical and physical methods of decontamination have been investigated already for their efficacy in destroying pathogens. The fruit and vegetable surfaces are difficult to clean and a number of studies have demonstrated the capacity of certain foodborne pathogens to be present not only on outer surfaces but also in inner tissues since microorganisms are able to enter vegetable plants and their fruits from the time of seed germination or flowering. Thus, fresh, pre-cut and other minimally processed produce represent a health risk similar to food of animal origin.

Irradiation seems to be more efficient in the reduction of bacterial contamination than sanitizers. In general, doses of 2 kGy reduce the number of bacteria by 3 to 4 log cycles and yeasts by 1 or 2 log cycles. However, little was known about the effect of physiological tolerance as well as sensory attributes of fresh produce. The work under this CRP tried to resolve some research gaps.

In total, the participants of this CRP conducted research on more than 40 different produce and more than 12 pathogenic bacteria. This CRP demonstrated that in general, fruits can be exposed to doses between 1.0–2.0 kGy without affecting the sensory attributes. It was also demonstrated that most of the studied minimally processed vegetables could be irradiated with doses up to 2 kGy. These doses

were effective in reducing the initial microflora in 4–5 logs and at the same time extending the shelf-life of the products without adverse effect on their sensory characteristics.

On the other hand, doses of 2–2.5 kGy considerably reduced the microbial contamination of sprouts, and after seven days refrigerated storage they were still acceptable. Sensory analysis showed also no significant effect of irradiation up to 2.5 kGy. Irradiation of sprouts rather than seeds was recommended as a final treatment, as irradiation of the seeds was not sufficient to guarantee sufficient reduction of pathogens.

2. OBJECTIVES OF THE CRP

A. The overall objective of the CRP was to evaluate the effectiveness of irradiation as a method to ensure microbiological safety of fresh, pre-cut produce and other minimally processed food of plant origin and to appraise the quality of such products subject to radiation doses sufficient to control effectivity of these pathogens.

B. The specific objective of this CRP is to use validated methods for microbiological determination of food and validated procedures for irradiatino of food in controlling various foodbourne pathogens in fresh, pre-cut produce and other minimally processed food of plant origin.

3. ACHIEVEMENTS OF THE CRP

In total, the participants conducted research on more than 40 different produce and more than 12 pathogenic bacteria (see Tables 1 and 2).

The study of two viruses were also included.

TABLE 1. FRUITS AND VEGETABLES STUDIED

VEGETABLES AND SPROUTS	**COUNTRY (IES)**
Alfalfa (Seeds and sprouts)	Argentina, Hungary, USA, UK
Arugula (or rugola)	Argentina, Brazil
Bitter gourd	Pakistan
Broccoli (seeds or sprouts)	USA, UK
Cabbage (green and red)	Chile, Pakistan, USA
Carrot	Canada, Chile, China, Egypt, India, Pakistan, Turkey, USA
Cauliflower	Pakistan
Celery	Chile, Egypt, USA
Chickpea (seeds or sprouts)	India
Chicory	Argentina
Cilantro	USA
Clover sprouts	USA, UK
Coriander	Portugal
Cucumber	Egypt, India, Malaysia, Pakistan
Dew gram (seeds and sprouts)	India
Endive	USA
Garden pea (seeds and sprouts)	India
Green beans	Egypt
Green gram sprouts	India
Lettuce (green and/or red leaf, Iceberg and Romaine)	Brazil, Chile, Egypt, Portugal, USA
Mint	Portugal
Mixed vegetables	Argentina, Chile, China, Egypt, Turkey

Mixed lettuce	Brazil
Parsley	Portugal, USA
Onion	Malaysia
Onion (green)	USA
Radish (seeds and sprouts)	Hungary, UK, USA
Soy sprouts	Argentina, Turkey
Spinach	Chile, USA
Tomato	China, Hungary, Pakistan
Turnip	Portugal
Watercress	Brazil, Portugal
FRUITS	
Apples	Egypt, Pakistan
Jackfruit	Malaysia
Mango	Brazil
Melon	Hungary, Pakistan, Portugal, USA
Mixed fruit	Malaysia
Pears	Egypt
Pineapple	Brazil, India, Malaysia
Pomelo	Malaysia
Watermelon	Brazil, Hungary, Portugal
OTHER PRODUCTS	
Tofu	China
Blanched Vegetables	China

TABLE 2. BACTERIA STUDIED

BACTERIA	**COUNTRY (IES)**
Aeromonas hydrophila	Egypt
Bacillus cereus	Hungary
Enterococcus faecalis	Egypt
Escherichia coli	Canada, Chile, Egypt, Pakistan, Turkey
Escherichia coli 0157:H7	Brazil, Chile, China, Hungary, Malaysia, Portugal, USA, Turkey
Listeria innocua	Canada, Chile, China, Portugal, USA
Listeria monocytogenes	Argentina, Brazil, Canada, Egypt, Hungary, India, Malaysia, Turkey, USA
Salmonella Enteritidis	Argentina, China, Turkey
Salmonella Parathyphi A	Pakistan
Salmonella spp (S. Infantis, Typhimurium, Antum, Stanley, Newport)	Brazil, USA
Salmonella Typhimurium	India
Shigella sonnei	USA
Staphylococcus aureus	Argentina, Egypt

A radiation dose was recommended based on a 5 log reduction of the pathogenic micro-organism and the physical, chemical and sensorial changes observed during refrigerated storage.

Studies using Poliovirus Type 1 and bacteriophage MS2 were also performed (see report of S. Pillai)

3.1. Fruits

Minimally processed fruits have been involved in several food borne outbreaks due to the presence of pathogenic micro-organisms such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria*

monocytogenes. In order to improve the microbiological quality and to ensure the safety of these products, the feasibility for the use of irradiation was studied.

Studies were carried out with pre-cut apples, cantaloupes, jackfruits, mangoes, melon, pears, pineapples, pomelo, watermelon, and mixed fruits (pineapple and guava) to determine the appropriate dose necessary to improve their microbiological quality. The micro-organisms used for inoculation studies were *Listeria monocytogenes*, *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus* and *Aeromonas hydrophila*. The sensorial, physical and nutritional evaluations as well as the shelf life were also conducted.

Cantaloupes are of particular concern because a number of recent cases of gastroenteritis have been linked to this produce. Reports of the use of ionizing radiation for inactivation of enteric viruses are relatively limited. Historically it has been assumed that ionizing radiation is ineffective against viruses in foods because high doses must be applied. Researchers from the USA carried out studies to identify the D_{10} values of model enteric viruses, mainly MS2 bacteriophage and attenuate polio virus on cantaloupe surfaces using electron beam irradiation. The D_{10} value for MS2 bacteriophage was 4.54 kGy and for polio virus type 1 strain, the D_{10} value was 4.76 kGy. It is important to note that no sensory evaluations were conducted as part of these studies, since the primary focus was on identifying the dose that could inactivate the virus, which was proven to be very high.

In general, fruits can be exposed to doses between 1–2.0 kGy without affecting the sensory attributes. Exception shall be made to the mixed fruit (pineapple mixed with guava) samples that showed changes in sensory attributes when exposed to doses higher than 1.0 kGy. On the other hand, pineapple per se can tolerate doses higher than 2.0 kGy.

3.2. Vegetables

For fresh pre-cut vegetables that are eaten raw, there is no treatment that can be relied on to substantially reduce the numbers of contaminating micro-organisms. Washing with antimicrobial compounds, while important, often brings about only a relatively small reduction. This fact was also demonstrated under this CRP.

Eliminating the risks is difficult. Management of them is based on identifying and controlling those factors that are important in preventing contamination or limiting growth of pathogenic micro-organisms between the farm and consumer. Considering this fact and the relatively small reduction of the microflora when using antimicrobial compounds, irradiation could be a feasible alternative treatment to ensure the safety of these vegetables.

The radiation sensitivity of different micro-organism strains (wild types and collection types) was determined in a wide variety of minimally processed vegetables. Green vegetables (lettuce, endive, cabbage, spinach, chicory, celery, rugola and watercress), small leaf vegetables (mint, coriander and parsley), and five different types of mixed salads and other kinds of products (onion, cucumber, carrot, turnip and bitter gourd) were also studied.

The results showed that there was a wide variation in the radiation sensitivity of the tested micro-organisms (*L. monocytogenes*, *L. innocua, E. coli* O157:H7, *Salmonella* spp., *Salmonella* Enteritidis, *S. aureus, S.* Typhimurium, *S.* Parathyphi A, *Shigella sonnei* and *A. hydrophila)*. Small variations in the D_{10} values were found between strains of the same micro-organism and, in general, these doses differ according to the product. In general, *Listeria monocytogenes* was the most radiation resistant among the tested micro-organisms, which ranged up to 0.66 kGy in carrot. A non-verotoxigenic strain of *Escherichia coli* O157:H7 was the most radio sensitive micro-organism, showing in two kinds of mixed salads a D_{10} value of 0.09 kGy.

Most of the studied minimally processed vegetables can be irradiated with doses up to 2 kGy. These doses are effective in reducing the initial microflora in 4–5 logs and at the same time extending the shelf-life of the products without adverse effect on their sensory characteristics.

Researchers from Canada showed that the use of modified atmosphere packaging (MAP) could produce a sinergistic effect on the radiosensitivity of the pathogenic bacteria studied and could produce also an extension of the shelf-life of peeled carrots. It was showed that the D_{10} value for *L. monocytogenes* in carrots packaged under air condition was 0.36 kGy; this value was reduced to 0.17 kGy under MAP (60% O_2, 30% CO_2, 10% N). Therefore, the recommended dose to be applied (taking into account the 5 log reduction) would be 1.8 kGy under air condition and only 0.85 kGy under MAP. On the other hand the addition of antimicrobial compounds also affected the radiosensitivity of bacteria. In fact, the D_{10} value of 0.36 kGy for *L. monocytogenes* under air condition was reduced from 0.09 kGy when trans-cinnamaldehyde or Chinese cinnamon and MAP conditions were used; so in this case the minimum dose would be only 0.45 kGy.

On the other hand, a combination of MAP (5/5/90 or 10/10/80% CO_2, O_2 and N_2) and low dose irradiation (up to 0.6 kGy) proved to prevent regrowth of *L. monocytogenes* and background microflora during refrigerated storage of endive, thereby improving product safety. However, loss of texture and color resulting from anaerobic conditions was observed. The use of gas permeable plastics to control gas exchange, or the application of acidified rinses, to control color loss, are tools to be explored further in achieving balance in the preservation of quality while preventing the regrowth of *L. monocytogenes* following low-dose irradiation plus MAP.

To evaluate the radiation sensitivity of fresh-cut vegetables, a novel parameter of dose threshold was introduced by USA researchers. Dose threshold was defined as radiation dose at which electrolyte leakage was significantly ($P<0.05$) increased. The rate of increase in electrolyte leakage and dose threshold was negatively correlated. There was a large variation in dose thresholds ranging from 2.44 kGy for broccoli to 0.60 kGy for carrots. The dose thresholds for most of the fresh-cut vegetables were between 1 and 2 kGy. The endogenous antioxidant capacity and total phenolic content of the vegetables also varied significantly among the tested vegetables. The antioxidants, however, did not correlate well with the radiation thresholds.

In green vegetables such as endive, chicory, lettuce, arugula, spinach, cabbage and watercress, irradiation with a dose up to 2 kGy would be recommended.

In view of the higher microbial population detected in the organic products when compared to the conventional products, more research on organic products is needed.

3.3. Seeds and sprouts

Sprouted seeds are a frequent cause of food-borne outbreaks. The producing conditions (high temperature/humidity) are ideal for microbial growth. There is a need for a method to improve the microbiological safety of these products without affecting their sensory quality and nutritional values.

Literature data shows that existing decontamination methods, such as calcium hypochlorite washes, are ineffective. The 5 log reduction of micro-organisms cannot be achieved by them.

The effect of radiation on killing pathogens such as *L. monocytogenes*, *L. innocua, E. coli* O157:H7, *Salmonella* Enteritidis and *S.* Typhmurium, *Staphylococcus aureus*, and *Bacillus cereus* spores was investigated on seeds (green pea, dew pea, chick pea, garden pea, alfalfa, radish, broccoli, clover) and on sprouts (green pea, dew pea, chick pea, garden pea, alfalfa, radish, broccoli, clover, soy). The effect on the microbiological shelf-life was examined together with sensory analysis.

Combining the irradiation of seeds with treatments such as hypochlorite, eugenol or oregano oil did not significantly improve the microbiological quality during sprouting. Irradiating seeds with doses of 1-3 kGy reduced the initial microflora to an acceptable level; however, the sprouting efficiency was

significantly reduced. The remaining microbial load including pathogenic bacteria was able to reach very high levels during sprouting.

Among vegetative cells, *Listeria monocytogenes* proved to be the most radiation resistant organism (D_{10} = 0.28 - 0.58). The high variability of values observed can be due to the different inoculation methodology, sanitizing methods before inoculation (chemical and physical methods) and packaging conditions.

The initial microbiological contamination of commercially available sprouts was very high in all the cases, and in some products pathogenic bacteria (e.g. *Salmonella, Staph. aureus*) were also detected.

Doses of 2-2.5 kGy reduced the microbial contamination considerably, and after seven days refrigerated storage it was still acceptable. Sensory analysis showed no significant effect of irradiation up to 2.5 kGy.

Irradiation of sprouts rather than seeds is recommended as a final treatment, as irradiation of the seeds is not sufficient to guarantee sufficient reduction of pathogens. Based on D_{10} values observed for the most resistant organism studied (*L. monocytogenes*), irradiation with 2.5 kGy is recommended to ensure the microbiological safety and inactivate vegetative pathogenic bacteria by 5 log-cycles.

The combination of low-dose gamma irradiation with modified atmosphere packaging (2% O_2, 4% CO_2, 96% N_2 and 3-5% O_2, 10-15% CO_2 balanced with N_2) and refrigerated storage can improve the microbiological safety and shelf-life of alfalfa and radish sprouts. Irradiation at 2kGy reduced the initial levels of *L. monocytogenes* and total microflora under the applied atmospheres, but the microbiota regrew during storage on both the irradiated and the control samples.

Further investigations are necessary to develop the composition of head-space in MAP necessaryto prevent regrowth of surviving pathogens during storage.

A summary of the results under this CRP is presented in Table 3.

TABLE 1. SUMMARY OF THE EFFECTO OF IRRADIATON ON THE SAFETY, SHELF-LIFE AND QUALITY OF PRODUCTS STUDIED (EFFECTIVE IRRADIATION DOSES ARE CALCULATED BASED ON TEH 5 LOG REDUCTION OF THE MOST RADIO-RESISTANT PATHOGENIC MICROORGANISM

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Argentina	Chicory	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.24 kGy) S. aureus (D_{10}=0.18 kGy) *Salmonella* Enteritidis (D_{10}=0.12 kGy)	Pathogens eliminated, acceptable organoleptic characteristic
	Soy sprouts	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.4 kGy) *S. aureus* (D_{10}=0.2 kGy) *Salmonella* Enteritidis (D_{10}=0.24 kGy)	Pathogens eliminated, acceptable organoleptic characteristic
	Alfalfa sprouts	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.37 kGy) *S. aureus* (D_{10}=0.21 kGy) *Salmonella* Enteritidis (D_{10}=0.24 kGy)	Pathogens eliminated, acceptable organoleptic characteristic
	Mix salad (cherry tomatoes, carrots, lettuce and cabbage)	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.23 kGy) *S. aureus* (D_{10}=0.24 kGy) *Salmonella* Enteritidis (D_{10}=0.14 kGy)	Pathogens eliminated, acceptable organoleptic characteristic
	Organic chicory	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.26 kGy) *S. aureus* (D_{10}=0.18 kGy)	Pathogens eliminated, acceptable organoleptic characteristic

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Argentina (cont.)	Organic rugola	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.28 kGy) *S. aureus* (D_{10}=0.19 kGy)	Pathogens eliminated, acceptable organoleptic characteristic
Brazil	Mango (cultivars Haden and Tommy-Atkins)	Aerobic	7ºC	*Salmonella* spp. (D_{10}=0.52-0.62 kGy)	Cubes of cultivar Tommy Atkins were sensorially accepted until day 4 when exposed to 1 kGy; control samples rejected at 4 days of storage
	Watermelon	Aerobic	7ºC		Sensorially acceptable after irradiation with 1 and 2.5 kGy
	Pineapple				Sensorially acceptable after irradiation with 1 and 2.5 kGy Consumers showed some resistance in acquiring irradiated Pineapple & watermelon due to lack of information about irradiation process
	Iceberg lettuce	Aerobic	7ºC	*Salmonella* spp. (D_{10}=0.16 – 0.23 kGy) *E. coli* O 157:H7 (D_{10}=0.11-0.12 kGy)	Irradiation up to 0.9 kGy had no adverse effect on sensory attributes
	Watercress	Aerobic	7ºC	*Listeria monocytogenes* (D_{10}=0.37-0.48 kGy) *Salmonella* spp. (D_{10}=0.29-0.43 kGy)	No recovery of microorganisms was observed when doses equivalent to 5 and 8 D_{10} were applied. These dosesd did not have a significant impact on sensorial characteristics.
	Arugula	Aerobic	7ºC	*Listeria monocytogenes* (D_{10}=0.37-0.48 kGy)	D_{10} for *L. Monocytogene* in arugal varied from 0.37 to 0.48 kgy.
Canada	Mini carrot	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.36 kGy)	Shelf-life at least 21 days after irradiation with 0.5 kGy
	Mini carrot	MAP (60% O_2; 30% CO_2; 10% N_2)	4ºC	*Listeria monocytogenes* (D_{10}=0.17 kGy)	Irradiation (0.5 kGy) and MAP inhibited growth of *L. innocua* during 21 days storage

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Canada (cont.)	Mini carrot coated with *trans*-cinnamaldehyde	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.10 kGy)	Shelf-life at least 21 days after irradiation with 0.5 kGy
	Mini carrot coated with *trans*-cinnamaldehyde	MAP (60% O_2; 30% CO_2; 10% N_2)	4ºC	*Listeria monocytogenes* (D_{10}=0.09 kGy)	Irradiation (0.5 kGy) and MAP inhibited growth of *L. innocua* during 21 days storage
	Mini carrot coated with Spanish oregano essential oil	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.13 kGy)	
	Mini carrot coated with Spanish oregano essential oil	MAP (60% O_2; 30% CO_2; 10% N_2)	4ºC	*Listeria monocytogenes* (D_{10}=0.12 kGy)	
	Mini carrot coated with winter savory essential oil	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.14 kGy)	
	Mini carrot coated with winter savory essential oil	MAP (60%; 30% CO_2; 10% N_2)	4ºC	*Listeria monocytogenes* (D_{10}=0.10 kGy)	
	Mini carrot coated with Chinese cinnamon essential oil	Aerobic	4ºC	*Listeria monocytogenes* (D_{10}=0.12 kGy)	

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Canada (cont.)	Mini carrot coated with Chinese cinnamon essential oil	MAP (60%; 30% CO_2; 10% N_2)	4ºC	*Listeria monocytogenes* (D_{10}=0.09 kGy)	
Chile	Celery	Aerobic	4ºC	*E. coli* ATCC (D_{10}=0.18 kGy) *E. coli* wild strain (D_{10}=0.22 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
	Cabbage	Aerobic	4ºC	*E. coli* ATCC (D_{10}=0.22 kGy) *E. coli* wild strain (D_{10}=0.23 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
	Iceberg lettuce	Aerobic	4ºC	*Listeria innocua* (D_{10}=0.22 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
	Carrots	Aerobic	4ºC	*Listeria innocua* (D_{10}=0.20 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
	Spinach	Aerobic	4ºC	*Listeria innocua* (D_{10}=0.32 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
	Toscana mixed salad (containing chopped iceberg lettuce, red cabbage and shredded carrot	Aerobic	4ºC	*Listeria innocua* (D_{10}=0.19 kGy) *E. coli* O157:H7 (D_{10}=0.09 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Chile (cont.)	Four Seasons salad (mixture of chopped romaine lettuce, iceberg lettuce, Lollo Rossa lettuce and spinach)	Aerobic	4ºC	*Listeria innocua* (D_{10}=0.21 kGy) *E. coli* O157:H7 (D_{10}=0.09 kGy)	Elimination of test pathogens, no significant changes in the sensory parameters after irradiation and during 7 days storage
China	Cherry tomato	Aerobic	4-7ºC 16-18ºC	*E. coli* O157:H7 (D_{10}=0.08 kGy) *Salmonella* Enteritidis (D_{10}=0.24 kGy)	Sensorial parameters of products irradiated by doses lower than 2.0 kGy were not significantly reduced
	Carrot	Aerobic	4-7ºC 16-18ºC	*E. coli* O157:H7 (D_{10}=0.13 kGy) *Salmonella* Enteritidis (D_{10}=0.33 kGy)	Sensorial parameters of products irradiated by doses lower than 2.0 kGy were not significantly reduced Irradiation with doses lower than 2.0 kGy had no significant effect on sensory quality, shelf life at least 10 days
	Packaged tofu	Aerobic	No data	*Salmonella* Enteritidis (D_{10}=0.24 kGy) *L. innocua* (D_{10}=0.22 kGy)	Irradiation with doses lower than 2.0 kGy had no significant effect on sensory quality, shelf life at least 5 days
	Blanched vegetables (celery and peanut)	Aerobic	No data	*Salmonella* Enteritidis (D_{10}=0.28 kGy) *L. innocua* (D_{10}=0.29 kGy)	Irradiation with doses lower than 2.0 kGy had no significant effect on sensory quality, shelf life at least 5 days
	Blanched vegetables (mustard and soybeen)	Aerobic	No data	*L. innocua* (D_{10}=0.26 kGy)	Irradiation with doses lower than 2.0 kGy had no significant effect on sensory quality, shelf life at least 5 days
	Blanched almond	Aerobic	No data	*L. innocua* (D_{10}=0.24 kGy)	

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Egypt	Carrots	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.66 kGy) *S. aureus* (D_{10}=0.54 kGy) *E. coli* (D_{10}=0.21 kGy) *A.. hydrophila* (D_{10}=0.18 kGy)	Pathogens eliminated, shelf-life extended from 1 week (control) to at least 3 weeks at 4ºC with acceptable sensory quality.
	Cucumber	Aerobic	4ºC		Pathogens eliminated, shelf-life extended from 5 days (control) to at least 14 days at 4ºC with acceptable sensory quality at irradiation dose of 3 kGy.
	Lettuce	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.54 kGy) *S. aureus* (D_{10}=0.44 kGy) *E. coli* (D_{10}=0.31 kGy) *A.. hydrophila* (D_{10}=0.23 kGy)	Pathogens eliminated, shelf-life extended from 4 days (control) to at least 14 days at 4ºC with acceptable sensory quality at irradiation dose of 2 kGy.
	Mixed vegetable salad	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.53 kGy) *S. aureus* (D_{10}=0.46 kGy) *E. coli* (D_{10}=0.18 kGy) *A.. hydrophila* (D_{10}=0.16 kGy)	At irradiation dose of 3kGy eliminated pathogens, extended shelf-life from 4 days (control) to at least 14 days at 4ºC with acceptable sensory quality.
	Green beans	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.52 kGy) *S. aureus* (D_{10}=0.42 kGy) *E. coli* (D_{10}=0.11 kGy)	2 kGy dose eliminated pathogens, extended shelf-life from 4 days (control) to at least 14 days at 4ºC with acceptable sensory quality.

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Egypt (cont.)	Celery	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.55 kGy) *S. aureus* (D_{10}=0.44 kGy) *E. coli* (D_{10}=0.31 kGy) *A.. hydrophila* (D_{10}=0.25 kGy)	2 kGy dose eliminated pathogens, extended shelf-life from 6 days (control) to at least 14 days at 4ºC with acceptable sensory quality.
	Mixed peas with diced carrots	Aerobic	4ºC	*L. monocytogenes* (D_{10}=0.56 kGy) *S. aureus* (D_{10}=0.44 kGy) *E. coli* (D_{10}=0.31 kGy) *A.. hydrophila* (D_{10}=0.26 kGy)	3 kGy dose eliminated pathogens, extended shelf-life from 1 week (control) to at least 3 weeks at 4ºC with acceptable sensory quality.
	Pears	Aerobic Before packaging: immersed in 2% ascorbic acid and 1% calcium lactate for 3 min	4ºC 4ºC	*L. monocytogenes* (D_{10}=0.6 kGy) *S. aureus* (D_{10}=0.46 kGy) *E. coli* (D_{10}=0.25 kGy) *A.. hydrophila* (D_{10}=0.23 kGy)	Irradiation dose of 2 kGy reduced the initial microbial load, eliminated pathogens and extended shelf-life from 7 days (control) to at least 14 days at 4ºC with acceptable sensory quality.
	Apples	Aerobic dipped in distilled water containing ascorbic acid 2%, citric acid 0.2%, calcium chloride 0.1% and sodium chloride 0.05% for 5 min.	4ºC		Pathogens eliminated, shelf-life extended from 10 days (control) to at least 20 days at 4ºC with acceptable sensory quality at 2kGy.

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Hungary	Tomato	Aerobic	5ºC, 15ºC	*L. monocytogenes* 4ab *E. coli* O157:H7	No significant changes in sensory properties up to 2 kGy, inoculated pathogens able to grow at 15ºC
	Cantaloupe	Aerobic	5ºC, 15ºC	*L. monocytogenes* 4ab *E. coli* O157:H7	No significant changes in sensory properties up to 2 kGy, inoculated pathogens able to grow at both 15ºC and 5 ºC
	Watermelon	Aerobic	5ºC, 15ºC	*L. monocytogenes* 4ab *E. coli* O157:H7	Above 1.5 kGy significant changes in sensory properties, inoculated pathogens able to grow at both 15 ºC and 5 ºC
	Alfalfa sprouts	Aerobic	5ºC	*L. monocytogenes* 4ab (D_{10}=0.46 kGy)	No significant changes in sensory properties up to 2 kGy, Shelf-life of 2 kGy irradiated sprouts at least 10 days
	Alfalfa sprouts	MAP (2% O_2, 4% CO_2, 94% N_2)	5ºC	*L. monocytogenes* 4ab (D_{10}=0.58 kGy) *Bacillus cereus* spores (D_{10}=2.66 kGy, L= 1 kGy)	No significant changes in sensory properties up to 2 kGy, Survivors of *L. monocytogenes* able to grow in MAP at 5ºC, Combination of MAP with 2 kGy was able to reduce the natural microbiota to acceptable low level and there was no further increase detected in 10 days storage at 5 °C.
	Alfalfa sprouts	MAP (3-5% O_2, 10-15% CO_2 balanced with N_2)	5ºC	*L. monocytogenes* 4ab (D_{10}=0.45 kGy)	No significant changes in sensory properties up to 2 kGy Survivors of *L. monocytogenes* able to grow in MAP at 5 ºC, Combination of MAP with 2 kGy was able to reduce the natural microbiota to acceptable low level and there was no further increase detected in 10 days storage at 5°C.
	Radish sprouts	Aerobic	5ºC		No significant changes in sensory properties up to 2 kGy
	Radish sprouts	MAP (2% O_2, 4% CO_2, 94% N_2)	5ºC		No significant changes in sensory properties up to 2 kGy, Combination of MAP with 2 kGy was able to reduce the natural microbiota to acceptable low level and there was no further increase detected in 10 days storage at 5 °C.

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
India	Carrot	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.19 kGy) *L. monocytogenes* 4ab (D_{10}=0.31 kGy)	Irradiation up to 2 kGy did not significantly affect nutritional, organoleptic and textural properties of produce studied
	Cucumber	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.19 kGy) *L. monocytogenes* 4ab (D_{10}=0.35 kGy)	
	Pineapple	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.24 kGy)	
	Green gram sprouts	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.20 kGy) *L. monocytogenes* 4ab (D_{10}=0.58 kGy)	
	Green gram seeds	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.19 kGy) *L. monocytogenes* 4ab (D_{10}=0.30 kGy)	
	Dew gram sprouts	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.19 kGy) *L. monocytogenes* 4ab (D_{10}=0.53 kGy)	
	Dew gram seeds	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.22 kGy) *L. monocytogenes* 4ab (D_{10}=0.32 kGy)	
	Chick pea sprouts	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.21 kGy) *L. monocytogenes* 4ab (D_{10}=0.54 kGy)	

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
India	Chick pea seeds	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.29 kGy) *L. monocytogenes* 4ab (D_{10}=0.34 kGy)	
	Garden pea sprouts	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.20 kGy) *L. monocytogenes* 4ab (D_{10}=0.54 kGy)	
	Garden pea seeds	Aerobic	4ºC 10ºC	S. Typhimurium (D_{10}=0.30 kGy) *L. monocytogenes* 4ab (D_{10}=0.32 kGy)	
Malaysia	Jackfruit	Aerobic	5ºC ± 2 ºC	*Listeria monocytogenes* (D_{10}=0.4 kGy) *E. coli* O157 (D_{10}=0.16 kGy)	Shelf-life at least 8 days
	Pineapple	Aerobic	5ºC ± 2 ºC	*Listeria monocytogenes* (D_{10}=0.15 kGy) *E. coli* O157 (D_{10}=0.08 kGy)	Shelf-life at least 8 days
	Mixed fruit	Aerobic	5ºC ± 2 ºC	*E. coli* O157 (D_{10}=0.14 kGy)	Shelf-life at least 8 days
	Onion	Aerobic	5ºC ± 2 ºC	*Listeria monocytogenes* (D_{10}=0.23 kGy) *E. coli* O157 (D_{10}=0.11 kGy)	Shelf-life at least 14 days, Hardness not affected by doses up to 3 kGy
	Cucumber	Aerobic	5ºC ± 2 ºC	*Listeria monocytogenes* (D_{10}=0.23 kGy) *E. coli* O157 (D_{10}=0.06 kGy)	Shelf-life 8 days, Hardness not affected by doses up to 3 kGy

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Pakistan	Cucumber	Aerobic	5°C	*Salmonella* Parathyphi A (D_{10}=0.25 kGy) *E. coli* (D_{10}=0.19 kGy)	Shelf-life from one week (control)to two weeks
	Cabbage	Aerobic	5°C	*Salmonella* Parathyphi A (D_{10}=0.29 kGy) *E. coli* (D_{10}=0.17 kGy)	Shelf-life at least two weeks
	Cauliflower	Aerobic	5°C	*Salmonella* Parathyphi A (D_{10}=0.24 kGy) *E. coli* (D_{10}=0.20 kGy)	Shelf-life at least two weeks
	Bitter gourd	Aerobic	5°C	*Salmonella* Parathyphi A (D_{10}=0.28 kGy) *E. coli* (D_{10}=0.23 kGy)	Microbiologically acceptable for one week
	Tomato	Aerobic	5°C		Shelf-life two weeks
	Carrot	Aerobic	5°C		Shelf-life two weeks
	Apple	Aerobic	5°C		Shelf-life at least two weeks
	Musk melon	Aerobic	5°C		Shelf-life one week
Portugal	Coriander	Aerobic	5°C	*Listeria innocua* (D_{10}=0.27 kGy) *E. coli* (D_{10}=0.15 kGy)	Irradiation at 0.5 and 1 kGy did not affect the firmness, shelf-life 9 days
	Lettuce	Aerobic	5°C	*Listeria innocua* (D_{10}=0.19 kGy) *E. coli* (D_{10}=0.14 kGy)	Significant decrease in texture when irradiated with doses above 0.5 kGy, shelf-life 15 days

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Portugal (cont.)	Mint	Aerobic	5°C	*Listeria innocua* (D_{10}=0.29 kGy) *E. coli* (D_{10}=0.15 kGy)	Irradiation at 0.5 and 1 kGy did not affect the firmness, shelf-life 7 days
	Parsley	Aerobic	5°C	*Listeria innocua* (D_{10}=0.23 kGy) *E. coli* (D_{10}=0.16 kGy)	Irradiation at 1 kGy showed a significant decrease in texture, shelf-life 20 days
	Turnip			*Listeria innocua* (D_{10}=0.25 kGy) *E. coli* (D_{10}=0.11 kGy)	Shelf-life 16 days
	Watercress			*Listeria innocua* (D_{10}=0.16 kGy) *E. coli* (D_{10}=0.15 kGy)	Irradiated samples (0.5 and 1 kGy) showed a significant increase in texture, shelf-life 8 days
	Melon			*Listeria innocua* (D_{10}=0.26 kGy) *E. coli* (D_{10}=0.11 kGy)	Irradiation at 0.5 and 1 kGy did not affect the firmness, shelf-life 4 days
	Watermelon				Irradiation at 0.5 and 1 kGy did not affect the firmness, shelf-life 11 days
Turkey	Carrot	Aerobic	5°C	*Listeria monocytogenes* (D_{10}=0.29 kGy) *E. coli* (D_{10}=0.29 kGy)	1 kGy dose extended shelf-life from <5 days (control) to at least 10 days with acceptable sensory quality.
	Mixed salad (Polorosso, Lolorosso, red lettuce & green lettuce)	Aerobic	5°C	*Listeria monocytogenes* (D_{10}=0.26 kGy) *Salmonella* Enteritidis (D_{10}=0.19 kGy)	1 kGy dose extended shelf-life from <5 days (control) to 13 days, 1.5 kGy dose of irradiation did not cause softening of mixed salad and sensory attributes were not adversely affected

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
Turkey (cont.)	Soybean sprouts	Aerobic	5°C	*Listeria monocytogenes* (D_{10}=0.27 kGy) *E. coli* O157:H7 (D_{10}=0.16 kGy) *Salmonella* Enteritidis (D_{10}=0.14 kGy)	Irradiation doses up to 2.5 kGy had no effect on the sensory properties, 1.5 kGy dose extended shelf-life from <5 days (control) to 13 days
U.K.	Alfalfa seeds	Aerobic	5°C	*Listeria innocua* (D_{10}=0.45 kGy) *E. coli* (D_{10}=0.35 kGy)	Irradiation (2 kGy) reduced the initial microbial load to below the level of detection (< 10 cells). However, surviving microorganisms did grow rapidly during the sprouting process
USA	Broccoli Cilantro Red cabbage Endive Parsley Green and red leaf lettuce Iceberg lettuce Romaine lettuce Spinach Carrots Green onions Celery Alfalfa sprouts	Aerobic	4 °C		No deterioration of visual quality or membrane damage for all samples that received doses of 0.5 or 1.0 kGy compared to non-irradiated controls after 14 days post-irradiation storage. Irradiation induced increases in electrolyte leakage in all vegetables when measured immediately after irradiation. Therefore it is possible to use electrolyte leakage measurement to assess membrane damage and radiation sensitivity of various vegetables.
USA	Endive (cut leaf)	Aerobic	4 °C	*Listeria monocytogenes* (D_{10}=0.21 kGy) *Listeria innocua* (D_{10}=0.22 kGy)	Treatment with 0.84 kGy suppressed *L. monocytogenes* throughout the course of refrigerated storage. Doses up to 1.0 kGy had no significant effect on color of endive leaf material.
		MAP (5% CO_2, 5% O_2, 90% N_2)		*Listeria monocytogenes* *Listeria innocua*	Combination of irradiation and MAP prevents the regrowth of *L. monocytogenes* during post-irradiation refrigerated storage.
		MAP (10% CO_2, 10%O_2, 80% N_2)		*Listeria monocytogenes* *Listeria innocua*	Combination of irradiation and MAP prevents the regrowth of *L. monocytogenes* during post-irradiation refrigerated storage.

Country	Produce studied	Packaging conditions	Temperature of storage	Inoculated pathogens / Surrogates	Improvement in safety/shelf-life
USA	Lettuce extract broth	Aerobic	4 °C	*Shigella* sonnei F6129 (D_{10}=0.26 kGy) *Shigella* sonnei 10304-98 (D_{10}=0.30 kGy)	
	Lettuce pieces	Aerobic	4 °C	*Shigella* sonnei F6129 (D_{10}=0.26 kGy) *Shigella* sonnei 10304-98 (D_{10}=0.30 kGy)	Microbiological shelf-life up to two weeks.
	Alfalfa sprouts	Aerobic		*Shigella* sonnei F6129 (D_{10}=0.26 kGy) *Shigella* sonnei 10304-98 (D_{10}=0.30 kGy) Coctail of *Salmonella* Enteritidis Antum F4317, Stanley H0558, Newport H1275 and Infantis F4319 (D_{10}=0.46 kGy) Coctail of *Escherichia coli* O157:H7 C7927, Sea13B88 and F4546 (D_{10}=0.30 kGy)	The 2 kGy dose reduced the initial microbial background counts (total aerobic and coliform) and did not cause any undesirable nutrient loss.
USA	Cantaloupe			Poliovirus Type 1 (D_{10}=4.76 kGy) MS2 bacteriophage (D_{10}=4.54 kGy)	

4. CONCLUSIONS AND RECOMMENDATIONS

General conclusions

The microbial load of conventional and organic minimally processed fruits and vegetables from different participant countries were very high and in some cases pathogenic bacteria were found. Therefore, there is a need to apply a technology to improve their microbiological safety.

Irradiation proved to be an excellent process to improve the hygienic conditions and safety of fresh, pre-cut fruits and vegetables and other minimally processed food of plant origin. The doses applied for these purposes also extended the shelf-life of most of the produce studied.

There is a need to continue studying the effect of irradiation on this type of product since necessary doses to achieve their microbiological safety vary with the type and cultivar of fruits and vegetables and the type of packaging. In some cases, combined treatments should be considered.

Participants realized the lack of legislation in most of the countries about safety standards, especially on microbiological limits of fresh pre-cut minimally processed fruits and vegetables.

Irradiation is a process that can be used to ensure the microbiological safety of most of the fresh pre-cut and minimally processed products studied. Irradiation should not replace Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) and must be practiced together with Good Irradiation Practices (GIP) to enhance the efficiency of the irradiation treatment.

General recommendations

There is a real need to continue this kind of CRP to enhance knowledge on these types of products. There are still several gaps that could be filled by another CRP organized in conjunction with WHO.

There are two recommendations to governments:

1. To organize surveillance programmes on the quality and safety of fresh pre-cut minimally processed fruits and vegetables; and

2. To establish legislation about safety standards, especially on microbiological limits of fresh pre-cut minimally processed fruits and vegetables.

IMPROVEMENT OF HYGIENIC QUALITY OF FRESH, PRE-CUT, READY-TO-EAT VEGETABLES USING GAMMA IRRADIATION

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Abstract

In the last years, the consumer trends in Argentina have increased for fresh-like products and with reduced or no chemical preservatives. Minimally processed foods like fresh pre-cut vegetables and fruits have limited shelf life and mainly rely on Good Manufacturing Practices for preservation and safety. The incidence of outbreaks declared for fresh vegetables in the last 10 years in Argentina is 3% (54 affected). In this project, minimally processed conventional and organic vegetables of the local market were studied: conventional and organic chicory (*Chicorium endive*), organic rugola (*Eruca sativa* Mill), soy sprouts (*Glycine max*), alfalfa sprouts (*Medicago sativa*) and a mixed salad composed of cherry tomatoes (*Solanum lycopersicum)*, carrots (*Daucus carota L.*), lettuce (*Lactuca sativa)* and cabbage *(Brassica oleracea*). The analyses were carried out in order to determine the irradiation process needed to ensure the hygienic quality of these kinds of products, with minimum organoleptic modification. The investigated microorganisms were *Listeria monocytogenes* ATCC 15313*, Salmonella* Enteritidis ATCC 13076 and *Staphylococcus aureus* ATCC 6538P. From the radiation resistance studies of the pathogenic bacteria, *Listeria monocytogenes* was the most radiation resistant microorganism in conventional and organic chicory, organic rugola, soy sprouts, alfalfa sprouts and *Staphylococcus aureus* for the mixed salad. The minimum disinfection doses proposed for these products are 1.2 kGy for conventional chicory and mixed salad, 1.3 kGy for organic chicory, 1.4 kGy for organic rucula, and about 2.0 kGy for soy and alfalfa sprouts. The disinfection doses were effective in the conventional products even during storage time and they did not affect the sensorial properties. The main objective of this work, to find the adequate dose to assure the hygienic quality of fresh pre-cut vegetables, was achieved with acceptable organoleptic characteristics. This work contributes with information about these kinds of products, which have quite recently appeared in the market. It was noted that there was a lack of information about their production and specific controls. Minimally processed vegetables require legislation stricter than the existing one for fresh vegetables. Although producers apply good manufacturing practices, sometimes this was not enough to ensure the absence of pathogens in these products.

1. INTRODUCTION

In the last years, the consumer trends in Argentina have increased for fresh-like products and with reduced or no chemical preservatives. Furthermore, consumers require meal preparation that takes shorter time; food consumption is being altered by the need for speed. There is no doubt consumers are demanding convenience in all aspects of food, including fresh-like, ready to eat, healthy and safe products.

Minimally processed foods like fresh pre-cut vegetables and fruits, both conventional and organics have limited shelf life and mainly rely on Good Manufacturing Practices for preservation and safety. They also provide excellent conditions for microorganisms' growth, because of their high moisture content and cut surface exposed [1]. The microorganisms that can be present in this produce are the natural microflora (lactic acid bacteria, *Pseudomonas* spp., yeast and molds) or the bacteria incorporated in these products during their cultivation (irrigation, fertilization) and processing (harvest, post-harvest handling, processing, distribution) [1-5].

Fresh vegetables and fruits (conventional and organic indistinctly) have been regulated by MERCOSUR Resolution No. GMC 59/93, and incorporated into the "Código Alimentario Argentino", in 1995 (Food Argentine Code). It is also mentioned in these documents that the methodology for the microbiological analysis should be established on the basis of Codex Alimentarius, ICMSF [6] and other recognized international organizations.

In the case of Argentina's organic agricultural production, in 1992 the National Government established guidelines for the "*National System of Control for Organic Products* [7]. These national rules were based on existing guidelines (by the International Federation of Organic Agricultural Movements (IFOAM) and the European Community (EC). During the following years, adjustments to this initial legislation have been made, as international standards have also developed. Also for conventional vegetables, the national standards were improved, by the issue of the Resolution 71/99: Guidance of Good Agriculture and Hygienic Practices for Primary Production (culture and harvest), Packaging, Storage and Transportation [8].

The competent authority for the inspection system of conventional and organic vegetables production in Argentina is the *Secretaría de Agricultura, Ganadería, Pesca y Alimentación* (Ministry of Agriculture, Animal Husbandry, Fisheries and Nutrition), through the *"Servicio Nacional de Sanidad y Calidad Agroalimentaria*" (SENASA) (National Agrifood Health and Quality Service).

It has been determined that the incidence of outbreaks declared for vegetables in the last 10 years in Argentina is 3% (54 affected). The etiological agents found were *E. coli* and *Salmonella* sp. [9].Despite the possible contamination that these kinds of products can carry, their label rarely mentions the recommendation to wash the products before consumption [10]. Taking this into account, an inquiry was made, asking different groups of people if they washed the product or not before consumption. In a total of 30 persons (students, professionals of different areas, assistants, administrative people), 67% usually wash the products and 33% do not, so these types of products are a potential source of microbial contamination and foodborne diseases.

Approximately 15 main companies elaborate different kinds of *conventional* fresh precut vegetables around Buenos Aires City. The vegetables more frequently combined are: chicory, rugola, carrot, lettuce (several species), tomato (several species, including cherry), cabbage, soy and alfalfa sprout. About eight producers elaborate *organic* ready-to-eat vegetables all around the country (most of them produce onion, garlic and beans) [11].

In the present work, selected pathogenic bacteria (*Listeria monocytogenes* ATCC 15.313*, Staphylococcus aureus* ATCC 6538P and Salmonella *Enteritidis* ATCC 13076*)* were studied in order to determine the D_{10} value, and then the 5-log cycle reduction was applied. As agreed in the first Research Coordination Meeting (RCM) [12], the disinfection doses for each product were defined on the basis of the most radio-resistant microorganism studied. Sensorial and mechanical evaluations were also carried out.

2. MATERIAL AND METHODS

2.1. Raw material

In Argentina, producers have a standardized production system from cultivation, harvest, post-harvest, handling and transportation (refrigerated tracks), processing (clean processing area, chlorinated water, washing line equipment, centrifugation to eliminate residual water, clean packaging area, clean refrigerated storage area) and distribution (refrigerated tracks).

The produce from three companies (A, B and C) were purchased in a retail store, and the samples were taken directly from their commercial presentation.

The most frequently found and consumed vegetables in the local market were chosen for these studies: chicory, soy sprouts, alfalfa sprouts and mixed salad (cherry tomatoes, carrots, lettuce and cabbage).

2.1.1. Conventional products

(a) Chicory (*Chicorium endive*) (Company A): whole leaves (100 g) in polypropylene tray covered with polyvinylidene chloride (PVDC) film.
(b) Soy sprout (*Glycine max*) (Company B): 300 g in polypropylene tray covered with PVDC film.

(c) Mixed salad (Company A): 300 g of a mix of cherry tomatoes (*Solanum lycopersicum)*, lettuce leaves (*Lactuca sativa)*, cabbage *(Brassica oleracea*) and grated carrots (*Daucus carota*) in polypropylene tray covered with PVDC film.
(d) Alfalfa sprout (*Medicago sativa*)(Company B): 300 g in a polypropylene tray covered with PVDC film.

The four commercial presentations had an absorbent pad at the bottom of the tray to capture residual water.

2.1.2. Organic products

(a) Chicory (*Chicorium endive*) (Company C): whole leaves (200 g) in polypropylene bag.
(b) Rucula (*Eruca sativa* Mill) (Company C): whole leaves (200g) in polypropylene bag.

2.2 Irradiation

For each experiment, the irradiation process was performed in a Semi-Industrial Facility, Activity: 430 kCi (^{60}Co). Dosimetric measurements were carried out by means of silver dichromate [13] (ISO/ASTM 51401-2003) and Fricke dosimeters [14] (ASTM E1026-95).

2.3. Hygienic quality determination of the four conventional and two organics products

Microbiological analyses were performed by triplicate according to ICMSF [6] and FDA-Bacteriological Analytical Manual [15]. All samples were taken from the commercial trays.

(a) **Total Bacteria Count (TBC) (ICMSF):** Samples of 10 g of produce were homogenized with 90 mL of peptone water (0.1%) in a stomacher. Serial dilutions were made in tubes with 9 mL of the same diluent, spread on triptic soy agar plates (Difco), and incubated at 32ºC for five days.

(b) **Molds and YeastsCcount (M & Y) (ICMSF):** From the same dilutions used for TBC, spread plate on potato dextrose agar (Difco) and incubated at 25ºC, 5 days.

(c) ***Salmonella spp*. (ICMSF):** 25 g of fresh vegetables were blended with 225 mL of lactose broth (incubation: 32ºC, 18 h). 1 mL was taken from the enrichment broth and inoculated into two enrichment selective broths: selenite cistine broth and thetrationate broth (incubation: 32ºC, 24 h). Aliquots from each broth were spread on three different isolation media: bismute sulfite agar (Merck), Hektoen enteric agar (Difco) and xylose Lisine deoxycholate agar (Merck) (incubation: 32ºC, 24 h). Isolated suspicious colonies were tested in triple sugar iron (TSI) (Merck) and lysine iron agar (LIA) (Merck).

(d) ***Shigella spp*. (ICMSF):** 25 g of fresh vegetables were blended with 225 mL of Gram negative broth (incubation: 32ºC, 18 h). Aliquots were spread on three different isolation media: xylose lysine deoxycholate agar (Merck), Salmonella-Shigella agar (Merck) and Mac Conkey agar (Difco) (incubation: 32ºC, 24 h). Isolated suspicious colonies were tested in TSI (Merck).

(e) ***Staphylococcus aureus* (ICMSF):** From the same dilutions used for TBC, 0.2 ml aliquots were spread on five plates of Baird Parker agar plates with egg yolk tellurite (incubation: 32ºC, 48 h). Suspicious colonies were tested for coagulase.

(f) ***Bacillus cereus* (ICMSF):** From the same dilutions used for TBC, 0.2 mL aliquots were spread on mannitol polymixine yolk agar (Merck) (incubation: 30ºC, 24 h).

(g) ***Vibrio cholerae* (ICMSF):** 25 g of fresh vegetables were blended with 225 mL of Alcaline peptone water (incubation: 32ºC, 4-8 h). Aliquots were spread onto one selective isolation medium: sacharose bilis citrate thyosulfate agar (Merck) and one general isolation medium: nutritive agar (incubation: 32ºC, 24 h).

(h) ***Escherichia coli* 0157 (FDA-BAM):** 25 g of fresh vegetables were blended with 225 mL of Mac Conkey broth (incubation: 20 h, 32ºC). One mL was taken from the enrichment both and inoculated into the enrichment selective broth: lauril sulfate broth (incubation: 44.5ºC, 24 h). Aliquots were spread on two different isolation media: EMB (Merck), Mac Conkey agar (Difco) (incubation: 32ºC, 24 h).

a) ***Listeria monocytogenes* (FDA-BAM):** 25 g of fresh vegetables were blended with 225 mL of Listeria enrichment broth (incubation: 32ºC, 24-48 h). One mL was taken from the enrichment both at 24 and 48 h of incubation and spread onto selective isolation medium: Oxford medium (incubation: 32ºC, 24-48 h). Isolated colonies were cultured in triptic soy agar with yeast extract (6%). Isolated suspicious colonies were tested in purple carbohydrates broth with 0.5% of glucose, salicine, manitol, mannose, ramnose, trehalose, xylose, in nitrate broth, and for catalase reaction.

2.4. Packaging materials studies

The packaging materials chosen were those habitually used by the companies A and B. The maximum dose used in the vegetable treatment was applied to the packaging materials, in their final shape. Several studies were performed.

(a) **Materials:** Oval shaped plastic trays of polypropylene of two different shapes, dimensions and colours: one oval shaped, measuring 20 cm width, 25 cm length, and 5 cm height (Company A) and one rectangular shaped, measuring 10 cm width, 10 cm length, and 5 cm height (Company B). Covering film: polyvinylidene chloride film (PVDC), 100-micron thickness. Labels: paper coloured labels.

(b) **Irradiation:** Dose: Min: 5,23 – Max: 5,69 kGy. Dosimeter: Silver dichromate [13]. Dose rate: 0,167 kGy/min.

(c) **Tests**:

- Visual observation for colour;
- Fourier Transformed Infrared spectrophotometry (FTIR): transmittance spectra were used to examine the PVDC films to evaluate chemical modifications (IMPACT – 410 (NICOLET)).
- Mechanical tests: Punctures to rupture tests were performed on the covering film and the plastic trays as a measure of mechanical strength (INSTRON materials testing machine, model 1122, software Series IX., Load range: 0,1 kN, Diameter of penetrometer: 1 mm).

2.5. Determination of D_{10} values

2.5.1. Bacterial strains studied

Listeria monocytogenes ATCC 15313,

Salmonella Enteritidis ATCC 13076 in conventional and organic products,

Staphylococcus aureus ATCC 6538P in conventional products.

2.5.2. Inoculation procedure

Inoculation was carried out based on what was agreed at the first Research Coordination Meeting (RCM) in Rio de Janeiro [12].

Briefly, a single colony was used to inoculate a fresh brain heart infusion broth (Difco) for each experiment, which was incubated for 18 h at 32ºC with rotary agitation. Cell density was typically 10^8 CFU/mL. One hundred mL of this solution was used to inoculate 900 mL of phosphate buffer.

The products were fractionated and conditioned in a basket of adequate size. The material was sanitized by immersion in a solution of 300 ppm sodium hypochlorite at room temperature, gently agitating for three minutes, rinsing with adequate volume of sterile distilled water, and letting it dry in the laminar flow cabinet for 10 minutes, with intermittent manual shaking to remove excess surface water. The basket with the sanitized material was submerged in the inoculum solution for five minutes with gently agitation and then it was dried in the biological flow cabinet with manual shaking for 10 minutes. Samples (30 g) were fractionated in sterile bag and refrigerated until irradiation (30-60 minutes).

2.5.3. Irradiation

Products were irradiated in triplicate with doses of 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 kGy (dose rate: 0.1722 kGy/min (conventional products) and 0,156 kGy/min (organic products)) for L. *monocytogenes*, and 0.0, 0.3, 0.6, 0.9, 1.2, and 1.5 kGy (Dose rate: 0.1588 kGy/min (conventional products) and 0.150 kGy/min (organic products)) for *S. aureus* and *S.* Enteritidis. The samples were conditioned in an adequate box with wet ice, to maintain an internal temperature of 4ºC. Analyses were carried out 30 – 60 minutes after irradiation.

2.6. Evaluation of the effect of doses up to 2 kGy on the microbial contamination of fresh pre-cut vegetables

The material was bought from the local retail and analysed for total bacterial counts and pathogenic bacteria according to ICMSF [6] and FDA-BAM [14] methodologies. The irradiation was carried out the next day after elaboration at 4ºC and the control sample was also refrigerated. The analyses were carried out the first and fifth days after irradiation. The commercial storage time was five days after elaboration at 4ºC.

2.7. Evaluation of the disinfection doses effects on inoculated samples of the conventional products, during storage time

Inoculation was carried out based on the D_{10} determination protocol. Calculations were made in order to obtain a sample of the product (25 g) with a microorganism load of 10^4–10^5 CFU/sample. Samples were packed in polyethylene bags and refrigerated until irradiation. For each product the applied disinfection dose was calculated on the basis of the most radiation resistant microorganism studied.

After irradiation, the presence and bioburden of *L. monocytogenes*, *S. aureus* and S. Enteritidis was investigated twice during storage at 4ºC, after irradiation: day 1 and day 6. The bioburden determination was carried out by filtration, and then the membrane was put in the specific medium for each microorganism. The investigation was carried out as mentioned in the hygienic quality determination.

2.8. Determination of the firmness of cherry tomatoes

The firmness was determined by an INSTRON apparatus, using a 1 mm diameter penetrometer and a load of 5 kN. The determination was made on 20 samples and the data was statistically analysed by the t student test. The irradiation doses were 0.0, 1.5 and 3.0 kGy, based on the determined *L. monocytogenes* disinfection dose (5 D_{10}) for mixed vegetables and the possible dispersion of the dose in a commercial irradiation facility [15].

2.9. Sensorial evaluation of irradiated samples

2.9.1. Sensory pre-evaluation

Sensory pre-evaluation of irradiated samples was carried out in order to evaluate the compatibility of the products with irradiation before continuing with further research (previous to the determination of the irradiation dose). Depending on the results, the option of applying edible coatings and/or antioxidants prior to irradiation was evaluated. This study was performed at the same time when the TBC and mold and yeasts counts were determined. A test-trained panel made up of a group of five persons evaluated the general overall quality of the samples, initially and during storage.

Samples of the conventional products were irradiated at the Irradiation Facility (Activity: 450 kCi). The minimum irradiation dose was calculated on the basis of bibliographic radiation resistance of *L. monocytogenes* in fresh products, at 4ºC (average D_{10} value = 0.5 kGy, according to some research carried out in our laboratory on fresh meat and fish; not published results) and applying 5 log cycles reduction as the minimum disinfection dose (2.5 kGy). The maximum dose (5 kGy) was applied considering the possible dispersion dose in an industrial scale irradiation [15].

Samples of the organic vegetables were also pre-evaluated, but the irradiation doses were the determined ones for the conventional vegetables, on the basis of the most radiation resistant microorganism (*L. monocytogenes*). The doses used were 1.2 kGy and 2.4 kGy.

2.9.2. General acceptability

The sensorial evaluation for general acceptability was carried out using a nine-point hedonic scale with 22 panelists (10 male and 12 female) between ages 25 to 60.

The vegetables analysed were soy sprout and chicory. The material was obtained from the producer facility the same day of irradiation. The vegetables were washed in water twice and then one portion immersed in an antioxidant solution for five minutes (ascorbic acid 1.5%, citric acid 1.5%, calcium chloride 0.1%, sodium chloride 0.05% in distilled water). The excess water was removed by spinning. The vegetables were fractionated in small trays of the same material than the commercial ones (polypropylene) and covered with adherent film of PVDC. The irradiation doses delivered were 1.2 and 2.4 kGy for chicory and 2 and 4 kGy for soy sprout according to the determined disinfection dose for each product.

The minimum dose was calculated on the basis of the experimentally determined D_{10} value of *L. monocytogenes* in the fresh products (the most radiation resistant pathogenic bacteria analysed in this project), at 4ºC and applying 5 log cycles reduction; the maximum dose was applied considering the possible dispersion dose in an industrial scale irradiation (about 100%). In the irradiation facility, the minimum dose is guaranteed. The statistics employed was variance analysis, Dunnett test, $p< 0.05$.

3. RESULTS AND DISCUSSION

3.1. Hygienic quality determination of the products

In Tables 1 and 2 the hygienic quality results of the conventional and organic vegetables are shown.

TABLE 1. HYGIENIC QUALITY OF THE FOUR CONVENTIONAL PRODUCTS (AVERAGE OF THREE REPLICATES)

Investigation	Soy sprouts	Alfalfa sprouts	Mix salad	Chicory
TBC (CFU/g)	$3,6 \times 10^7$	$1,1 \times 10^8$	$5,6 \times 10^7$	$2,8 \times 10^7$
Y & M (CFU/g)	$6,5 \times 10^5$	$9,4 \times 10^4$	$1,1 \times 10^6$	$7,5 \times 10^5$
Salmonella spp. (25 g)	Absence	Absence	Absence	Absence
Shigella spp. (25 g)	Absence	Absence	Absence	Absence
B. cereus (1 g)	Absence	Absence	Absence	Absence
S. aureus (1 g)	Presence (3/3)	Presence (1/3)	Absence	Absence
V. cholerae (25 g)	Absence	Absence	Absence	Absence
L. monocytogenes (25 g)	Absence	Absence	Absence	Absence
E. coli (25 g)	Absence	Presence [a]	Absence	Absence

[a] Presence of *E. coli*, non enteropathogenic

TABLE 2. HYGIENIC QUALITY OF THE TWO ORGANIC PRODUCTS (AVERAGE OF THREE REPLICATES)

Investigation	Chicory	Rugola
TBC (CFU/g)	$8,2 \times 10^7$	$5,2 \times 10^7$
Y & M (CFU/g)	$7,6 \times 10^5$	$4,6 \times 10^4$
Salmonella spp. (25 g)	Absence	Absence
Shigella spp. (25 g)	Absence	Absence
B. cereus (1 g)	Absence	Absence
S. aureus (1 g)	Absence	Presence (1/3)
V. cholerae (25 g)	Absence	Absence
Listeria sp. (25 g)	Absence [a]	Absence
E. coli (25 g)	Absence	Presence[b] (2/3)

[a] Presence of *Listeria sp*. non monocytogenes

[b] Presence of *E. coli*, non enterotoxygenic

3.2. Evaluation of the compatibility of the package with irradiation

All tests were performed 30 days after the irradiation and in comparison with non-irradiated materials. Results are the averages of ten determinations.

TABLE 3. MECHANICAL STRENGTH IN IRRADIATED AND NON-IRRADIATED PACKAGING MATERIALS

PUNCTURE STRENGTH		Load to rupture (kN) ± Standard Deviation; n = 10
Film	Control	0,0035 ± 0,0005
	Irradiated	0,0037 ± 0,0004
Plastic tray (A)	Control	0,0397 ± 0,0036
	Irradiated	0,0488 ± 0,0024
Plastic tray (B)	Control	0,0202 ± 0,0015
	Irradiated	0,0244 ± 0,0038

A = oval shaped, measuring 20 cm width, 25 cm length, and 5 cm height
B = one rectangular shaped, measuring 10 cm width, 10 cm length, and 5 cm height

It can be said that the applied dose (5,23 kGy min – 5,69 kgy max) did not cause a loss of strength in the packaging materials used.

No colour changes were noticed in labels, plastic trays or covering films.

FTIR spectra showed no difference between irradiated and non-irradiated PVDC film. No chemical alterations such as oxidation could be detected by this sensitive technique.

3.3. D_{10} values of pathogenic bacteria in conventional and organic products

Figures 1 to 4 show survival curves of the microorganisms studied in conventional vegetables and Figures 5 and 6 show the survival curves in organic vegetables. The microorganisms were irradiated under the same conditions, so different survival curves show differences in radiation sensitivity.

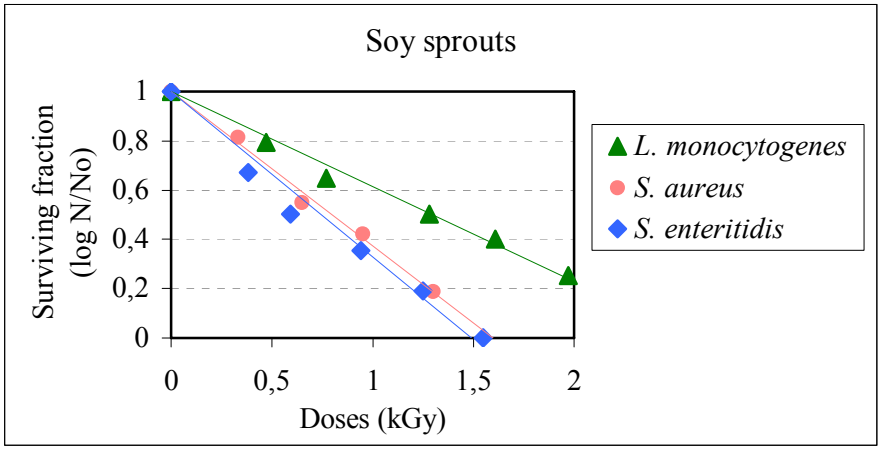

FIG. 1. Survival curves of the studied microorganisms on conventional soy sprouts.

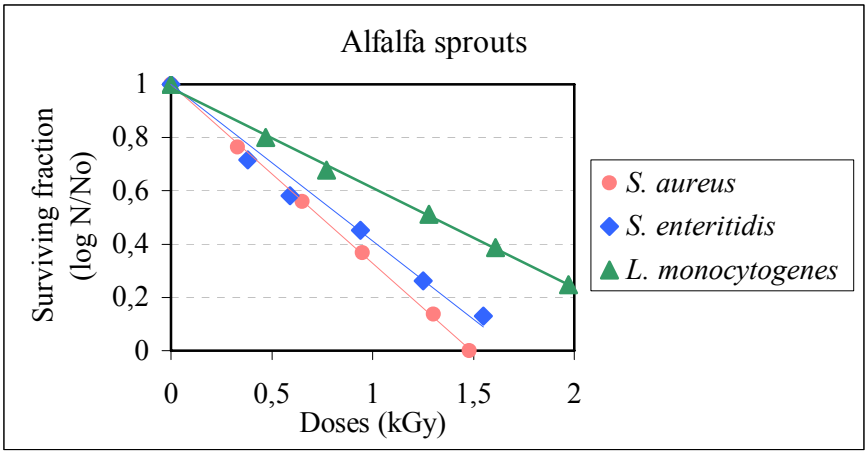

FIG. 2. Survival curves of the studied microorganisms on conventional alfalfa sprouts.

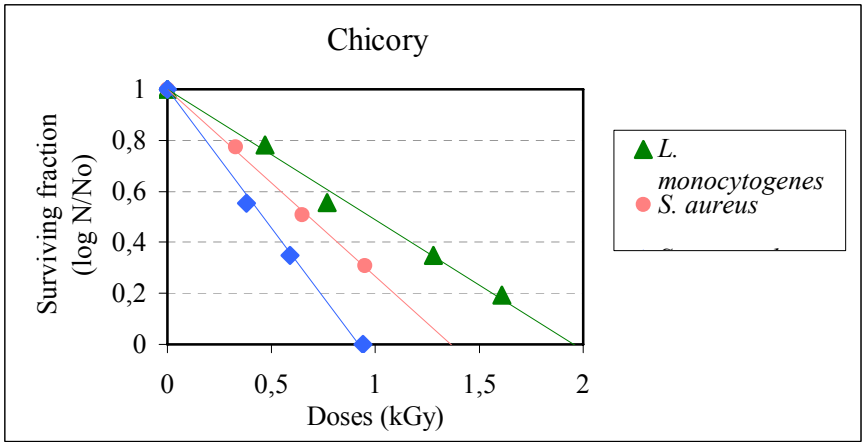

FIG. 3. Survival curves of the studied microorganisms on conventional chicory.

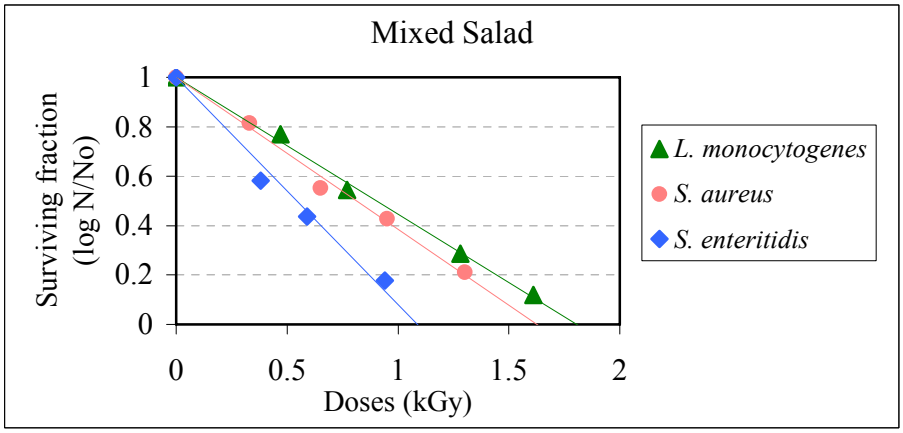

FIG. 4. Survival curves of the studied microorganisms on conventional mixed salad (cherry tomatoes, cut-lettuce leaves, cut-cabbage and grated carrots).

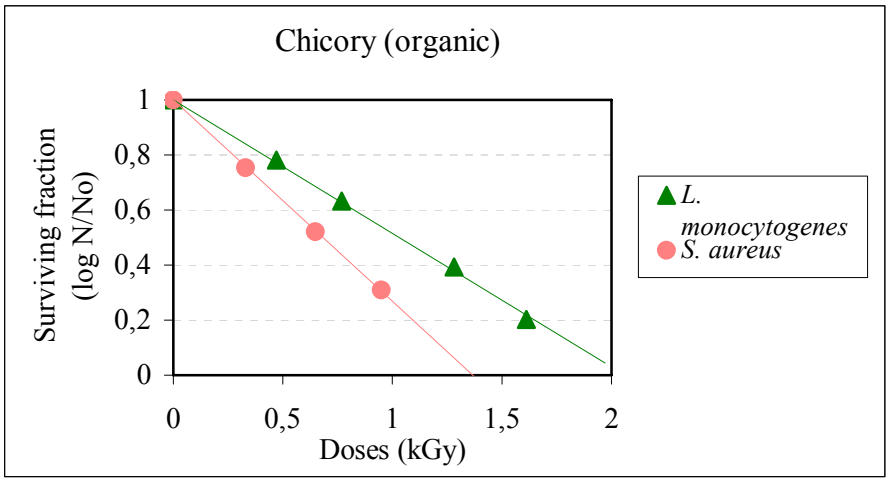

FIG. 5. Survival curves of the studied microorganisms on the organic chicory.

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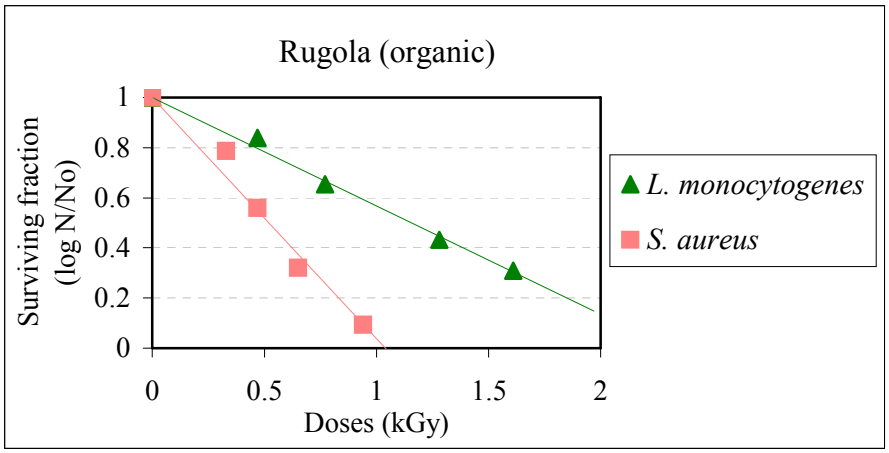

FIG. 6. Survival curves of the studied microorganisms on the organic rucula.

It can be seen in all products that *L. monocytogenes* proved to be the most radiation resistant microorganism (Fig. 1 to 6).

The results of D_{10} values are summarized in Table 4. *Salmonella* Enteritidis was not determined in the organic chicory and rugola, because it seems to be more sensitive in this type of vegetable (based on the results of conventional chicory).

In organic chicory D_{10} value of *L. monocytogenes* was somewhat higher compared to that obtained in conventional produce. This may be due to the commercial form of the organic vegetables where whole leaves are used. Pre-cut products possibly present a high moisture level that increases the sensibility of the microorganisms to irradiation.

TABLE 4. D10 VALUES OF THE PATHOGENIC MICROORGANISMS ON THE STUDIED PRODUCTS

Products	*Listeria monocytogenes*		*Staphylococcus aureus*		*Salmonella* Enteritidis	
Conventional	D_{10} (kGy)	Linear R	D_{10} (kGy)	Linear R	D_{10} (kGy)	Linear R
Soy sprouts	0.40	0.989	0.20	0.989	0.24	0.975
Alfalfa sprouts	0.37	0.995	0.21	0.995	0.24	0.987
Chicory	0.24	0.995	0.18	0.997	0.12	0.988
Mixed vegetables	0.23	0.991	0.24	0.999	0.14	0.997
Organic	D_{10} (kGy)	Linear R	D_{10} (kGy)	Linear R	D_{10} (kGy)	Linear R
Chicory	0.26	0.998	0.18	0.989	----------	----------
Rugola	0.28	0.994	0.19	0.998	----------	----------

3.4. Determination of the firmness of cherry tomatoes

Table 5 shows the averages of 20 individual determinations in irradiated and non-irradiated cherry tomatoes.

TABLE 5. FIRMNESS OF IRRADIATED AND NON-IRRADIATED SAMPLES OF CHERRY TOMATOES

	Day 1: Load to rupture (kN); n = 20	Day 5: Load to rupture (kN); n = 20
0.0 kGy	0.0043 ± 0.0006	0.0036 ± 0.0008
1.5 kGy	0.0031 ± 0.0007	0.0027 ± 0.0004
3.0 kGy	0.0025 ± 0.0005	0.0022 ± 0.0004

According to the t student test there are significant differences in tomatoes firmness between irradiated and untreated samples. On day 1, there was a difference of 28% between 0 and 1.5 kGy and 42% between 0 and 3.0 kGy. On day 5, there was a difference of 25% between 0 and 1.5 kGy and 39% between 0 and 3.0 kGy. However, it is important to note that the trained panel of five persons determined that all samples were considered acceptable, including the cherry tomatoes irradiated with 3 kGy.

3.5. Determination of the effect of the disinfection dose on the microbial contamination

Figures 7 to 10 show the influence of the disinfection dose (five times the D_{10} value) on the hygienic quality of the conventional vegetables.

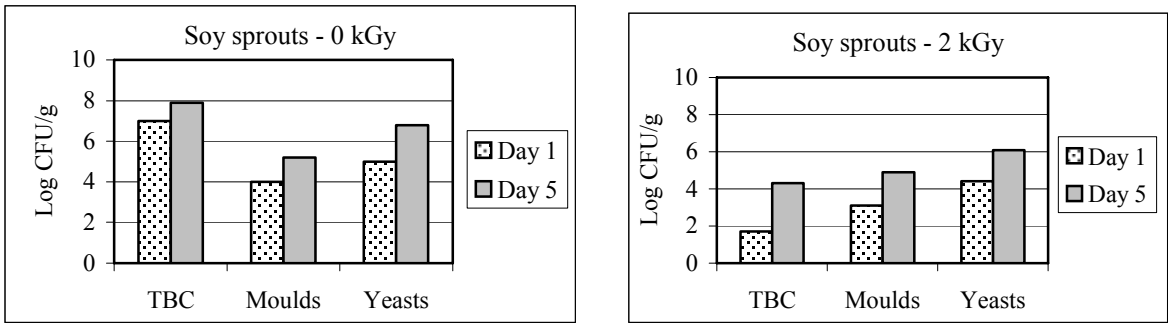

FIG. 7. *Comparison between control and irradiated soy sprout samples with the disinfection dose (2 kGY*).

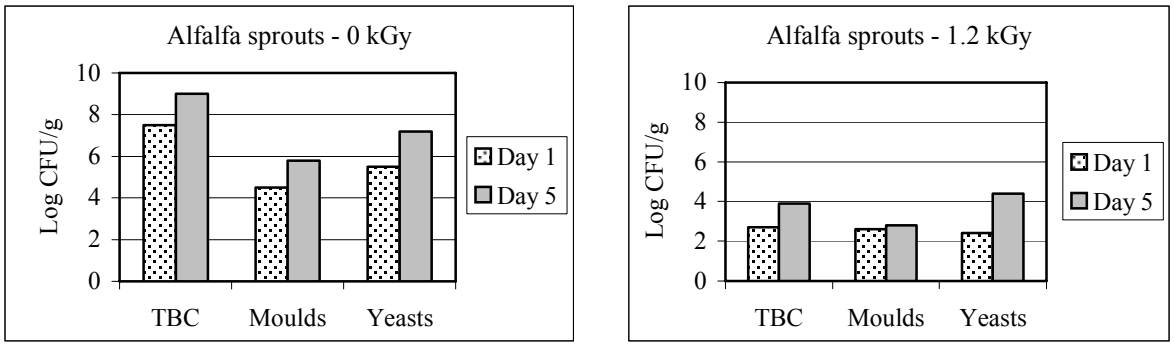

Fig 8. Comparison between control and irradiated alfalfa sprout samples with the disinfection dose (2 kGY).

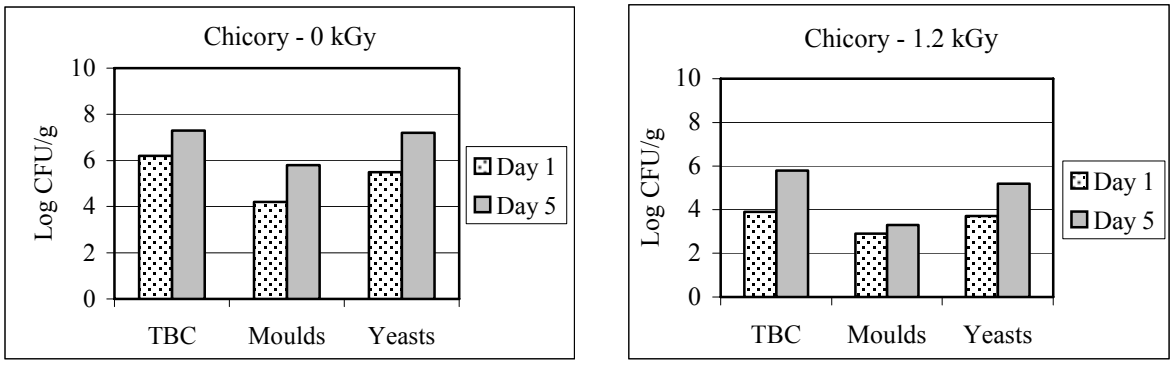

FIG. 9. Comparison between control and irradiated chicory samples with the disinfection dose (2 kGY).

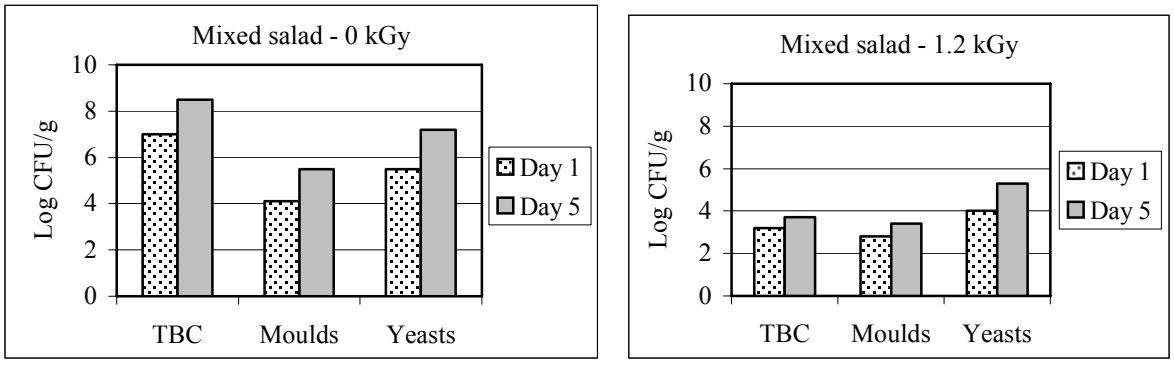

FIG. 10. Comparison between control and irradiated mixed salad samples with the disinfection dose (2 kGY).

No pathogenic bacteria were found in the irradiated samples.

In all cases, the disinfection dose produced a reduction of total bacterial, mold and yeast counts in more than four orders of magnitude for soy sprout, alfalfa sprout, mixed salad and almost two for chicory. After irradiation, the increment of each microorganism studied did not reach the level of 10^6 CFU/g during the storage period.

In untreated samples, macroscopic development of colonies was seen after five days of storage, probably corresponding to yeasts. The results showed that the microflora increased in two to three orders from the initial counts. A final visual observation of the studied products was made eight days after elaboration; a significant spoilage in the untreated samples was observed, while in the irradiated ones there was not.

3.6. Evaluation of the disinfection doses effects on inoculated samples of the conventional products

Results are summarized in Table 6. Effects of the following disinfection doses (to reduce 5 log cycles) in different vegetables were investigated.

Soy sprouts: 2 kGy (*L. monocytogenes*)
Alfalfa sprouts: 1.85 kGy (*L. monocytogenes*)
Chicory: 1.2 kGy (*L. monocytogenes*)
Mixed salad: 1.2 kGy (*S. aureus*)

TABLE 6. SURVIVAL OF THE INOCULATED MICROORGANISM DURING STORAGE TIME

Samples of 25 g	Inoculated microorganisms	Initial count (non-irradiated sample)	Day	Investigation[a]	Filtration[a]
Soy sprout: 2 kGy	*Listeria monocytogenes*	7.4×10^4 CFU/ 25 g	1	Absence	0 CFU
			6	Absence	0 CFU
Alfalfa sprout: 1,85 kGy	*Listeria monocytogenes*	8.3×10^4 CFU/ 25 g	1	Absence	0 CFU
			6	Absence	0 CFU
Chicory: 1,2 kGy	*Listeria monocytogenes*	1.5×10^5 CFU/ 25 g	1	Absence	0 CFU
			6	Absence	0 CFU
Mixed salad: 1,2 kGy	*Staphylococcus aureus*	1.2×10^5 CFU/ 25 g	1	Absence	0 CFU
			6	Absence	0 CFU

[a] The results are an average of three replicates

The disinfection dose was effective in all the products to reduce at least a 5-log cycle of the more resistant microorganisms studied, during storage time.

3.7. Sensorial evaluation of irradiated products

The results of the pre-evaluation carried out on the conventional vegetables, showed that the most sensible products were soy sprout and chicory. That is the reason why they were chosen for further evaluation.

Results of sensorial evaluations are shown in Figures 11 to 14. The characteristics evaluated on the basis of a 9-point hedonic scale were aroma, color, flavor, texture, taste and general acceptability.

Irradiated soy sprout samples presented a light browning with 2.5 and 5 kGy in comparison with the control, immediately after irradiation, although they were still considered acceptable (upper acceptability threshold). For this reason, the sensorial evaluation with more panelists was performed with the incorporation of the antioxidant solution (AO).

The panelists were given the option to add the following dressings or seasonings to the products: sunflower oil, lemon, salt and wine vinegar. This was to make the evaluation similar to what each panelist is used to, considering these products are not generally consumed without seasoning.

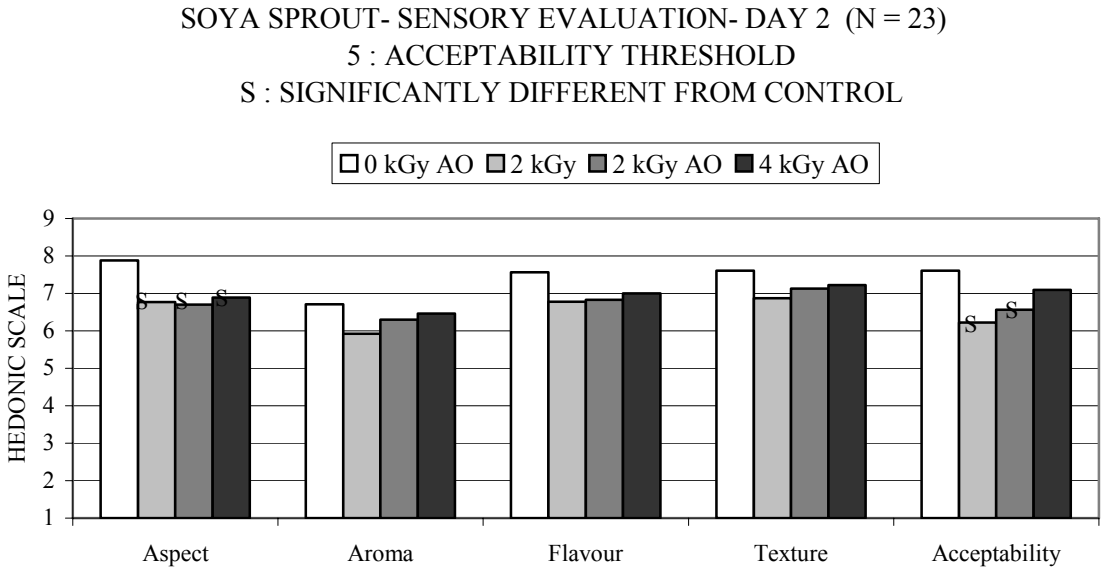

FIG. 11. Day 2 after irradiation. Analyses of variance, p=95% confidence.

The irradiation process reduced the appearance and general acceptability of soy sprout samples significantly; however, all samples were above the acceptability threshold after two days of storage.

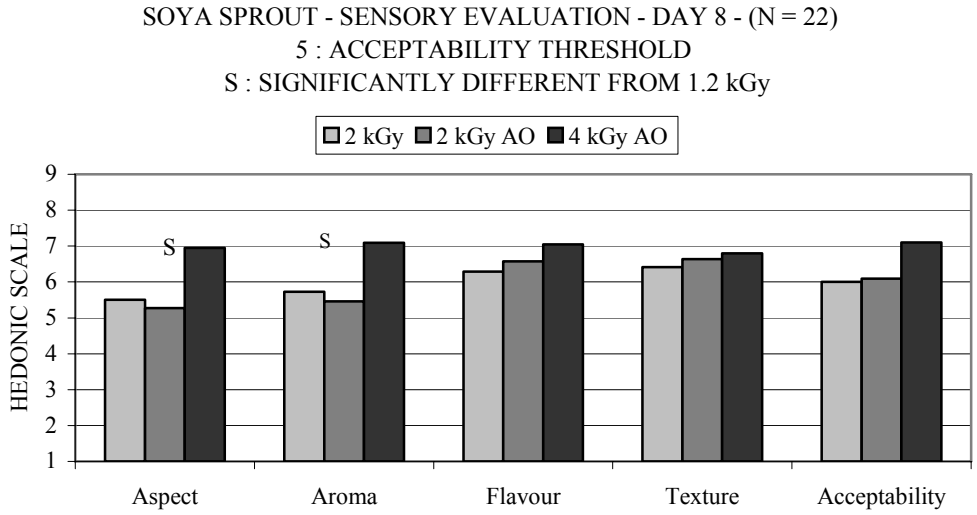

FIG. 12. Day 8 after irradiation. Analyses of variance, p=95% confidence.

The aspect and aroma of the soy sprout samples irradiated at 4 kGy were significantly better than the ones receiving 2 kGy. All samples were above the acceptability threshold. The antioxidant solution did not improve the aspect in the samples irradiated with 2 kGy. The texture of the soy sprouts did not change either at incremental doses or during storage.

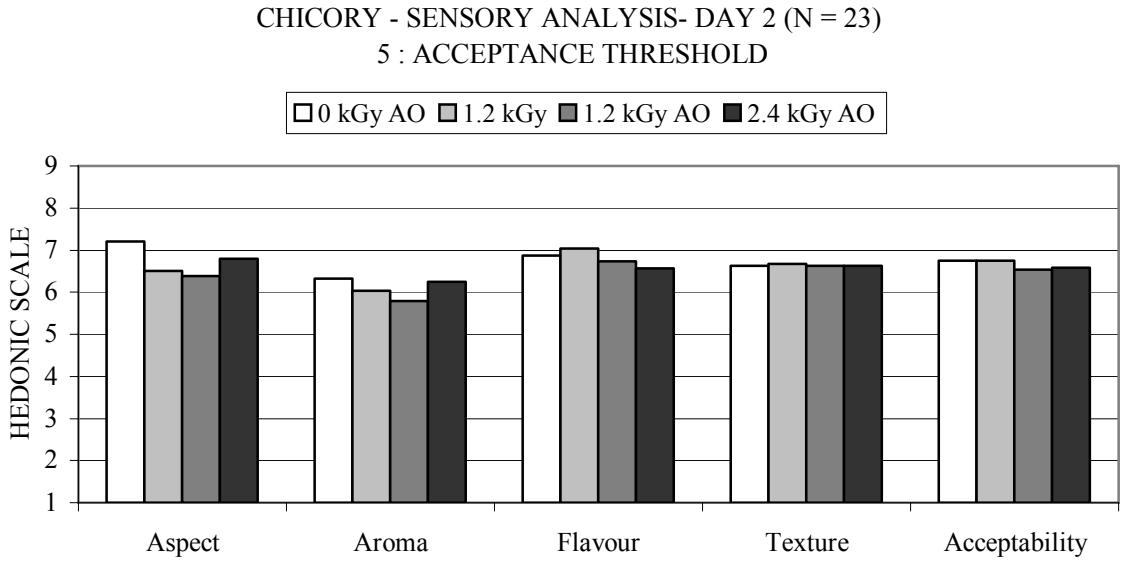

FIG. 13. Day 2 after irradiation. Analyses of variance, p=95% confidence.

In all attributes there were no significant differences between irradiated and control chicory samples. All samples were above the acceptability threshold on the second day of the storage period. The samples irradiated with 2.4 kGy looked better than those irradiated with 1.2 kGy.

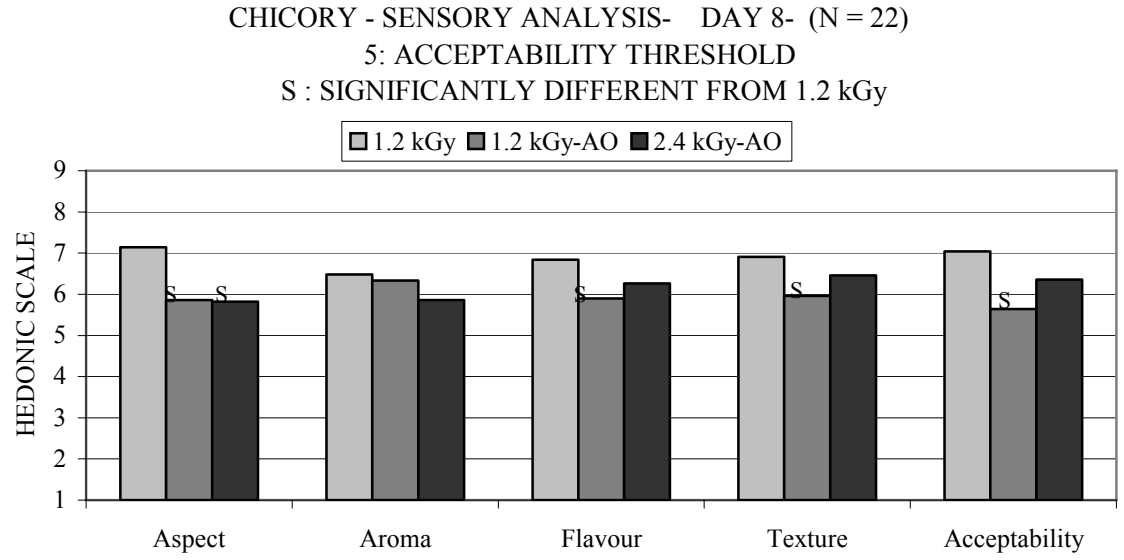

FIG. 14. Day 8 after irradiation. Analyses of variance, p=95% confidence.

The antioxidant solution reduced the appearance, flavor, texture and general acceptability significantly in chicory samples treated with 2 kGy and sensorially evaluated at the eighth day of storage. However, all samples were above the acceptability threshold. The flavor, texture and acceptability in samples irradiated with 2.4 kGy looked better than those irradiated with 1.2 kGy.

On the eighth day, all control samples presented a deteriorated aspect, in comparison with the irradiated samples, that is the reason why they were not considered for the sensorial evaluation.

The general acceptability of the organic products, evaluated by a trained panel (five persons), showed better results than conventional. This may be due to the commercial presentation of the organic product studied that was in whole leaves. Pre-cut product presents possibly a high moisture level that increases the sensibility of the products to irradiation.

4. CONCLUSIONS

1) The Argentinean producers of pre-cut and minimally processed vegetables have a standardized production applying good manufacturing practices. However, sometimes this is not enough to ensure the absence of pathogens during storage time.

2) The existing legislation in Argentina covers fresh vegetables, not specifically for minimally processed ones, which would have stricter requirements.

3) Among the microorganisms investigated, the most radiation resistant one in the products was *Listeria monocytogenes* in organic chicory and rugola, conventional chicory, alfalfa and soy sprouts, and *Staphylococcus aureus* in mixed salad.

4) Based on the application of five times the D_{10} value results, the minimum disinfection doses proposed for the products are 1.2 kGy for chicory and mixed salad**,** 1.3 kGy for organic chicory, 1.4 kGy for rucula, and about 2 kGy for soy and alfalfa sprouts.

5) In the case of conventional chicory and soy sprouts, the sensorial evaluation showed that these studied products had a better general acceptability when were irradiated with at least twice the disinfection dose. This dose seems to improve the shelf life of these products.

6) The sensorial evaluation of alfalfa sprouts, mixed salad, organic chicory and organic rugola has been evaluated by a trained panel of five persons, and no significant modifications in all the characteristics evaluated were found.

7) Further research is needed to evaluate the effect of irradiation and use of an edible coating and /or antioxidant.

ACKNOWLEDGEMENTS

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REFERENCES

[1] WILEY, R., Frutas y hortalizas minimamente procesadas. Ed. Acribia (1997).
[2] CORPORACIÓN DEL MERCADO CENTRAL DE BUENOS AIRES, Calidad higiénico sanitaria en productos hortícolas frescos, TECDOC (1997).
[3] CORPORACIÓN DEL MERCADO CENTRAL DE BUENOS AIRES, Uso de envases y embalajes en la comercialización de productos frutihortícolas frescos, TECDOC (1997).
[4] BARON, C, MORADEI, A., Control de frutas y hortalizas en el comercio minorista, TECDOC Corporación del Mercado Central de Buenos Aires (1997).
[5] BARON, C, Cloración y control de enfermedades poscosecha en frutas y hortalizas frescas, TECDOC Corporación del Mercado Central de Buenos Aires (1997).
[6] INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS (ICMSF) – Microorganismos de los alimentos 1: Su significado y métodos de enumeración (2da edición).

[7] SECRETARÍA DE AGRICULTURA, GANADERÍA, PESCA Y ALIMENTACIÓN (SAGPyA) Decree No. 423, 3 June 1992 (http://senasa.mecon.ar/calidad.htm).
[8] CAMPANA, B, DEMARCO, MF. SANTOS, S, Guía de buenas practicas para la producción primaria, empaque, almacenamiento y transporte de productos frutihorticolas, Dirección de Calidad Agroalimentaria, SENASA (1999).
[9] SIRVETA (Sistema de información para la vigilancia de las enfermedades transmitidas por los alimentos), Instituto Panamericano de Protección de Alimentos y Zoonosis, Pan American Health Organization / World Health Organization. (www.panalimentos.org/sirveta/e/index.htm).
[10] SECRETARÍA DE AGRICULTURA, GANADERÍA, PESCA Y ALIMENTACIÓN (SAGPyA) Normas de tipificación, empaque y fiscalización de frutas y hortalizas.
[11] SENASA, Productos orgánicos (SAGPyA) - Dirección de Industria Alimentaria (www.senasa.gov.ar).
[12] INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA), Report of 1st FAO/IAEA Research Coordination Meeting on the use of Irradiation to Ensure Hygienic Quality of fresh, Pre-cut Fruits and Vegetables and Other Minimally Processed Food of Plant Origin, 5-9 November 2001, Río de Janeiro, Brazil.
[13] AMERICAN SOCIETY FOR TESTING MATERIALS, E1401-1996 and 51401-2003 (E), Standard Practice for use of a dichromate dosimetry system.
[14] AMERICAN SOCIETY FOR TESTING MATERIALS, E1026-1995, Practice for using the Fricke reference standard dosimetry system.
[15] FOOD & DRUG ADMINISTRATION (FDA), Bacteriological Analytical Manual (8th edition) (www.cfsan.fda.gov/~ebam/bam-toc.html.
[16] AMERICAN SOCIETY FOR TESTING MATERIALS, E2303-2003, Standard guide for absorbed-dose mapping in Radiation Processing Facilities.

USE OF IRRADIATION TO IMPROVE THE MICROBIOLOGICAL SAFETY OF MINIMALLY PROCESSED FRUITS AND VEGETABLES

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Abstract

Minimally processed fruits and vegetables are a rich source of nutrients and they have become a growing market worldwide because of the new lifestyle. However, they have been involved in food borne disease outbreaks due to the presence of bacteria such as *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Irradiation is a non-thermal process that can be used to improve the microbiological safety of these foods. In the present research, the most consumed Brazilian minimally processed fruits such as mango (*Mangifera indica*), watermelon (*Citrullus vulgaris*) and pineapple (*Ananas comosus*), and vegetables such as iceberg lettuce (*Lactuca sativa L.*), watercress (*Nasturtium officinale*) and arugula (*Eruca sativa*) were used. The studies were carried out in order to determine the dose necessary to ensure the safety of those foods. Possible changes in sensory attributes were also studied as well as their shelf life. The irradiation showed to be a feasible process because doses necessary to ensure good microbiological quality did not change the overall quality of the vegetables and fruits tested. In some instances, there was an increase in the shelf life of the irradiated food when compared to the non-irradiated.

1. INTRODUCTION

Minimally processed fruits and vegetables (MPFV) are a rich source of nutrients and they have become a growing market worldwide because of the new lifestyle. They are foods that have fresh like characteristics and also provide the convenience demanded by consumers.

Preservation of fresh produce is limited to refrigeration and use of modified atmosphere packaging. However, even applying refrigeration and modified atmosphere packaging, these products have a short shelf life. Besides, sanitation with chlorine is not sufficient to ensure their safety.

Vegetables have a varied microflora. Minimally processed vegetables harbor a variety of microorganisms, most of them related to the spoilage of the product. These include bacteria such as *Pseudomonas spp.* and *Erwinia* spp. In terms of microbial safety, psychrotrophic pathogens, such as *Listeria monocytogens*, *Aeromonas hydrophila* and *Yersinia enterocolitica*, and mesophiles such as *Salmonella*, *Escherichia coli* O157:H7 and *Clostridium botulinum* are of particular concern [1] [2] [3] [4]. Research carried out in our laboratory has shown the presence of *Salmonella* spp and *Listeria monocytogenes* in MP watercress and spinach, respectively. According to Bean et al. [5], the number of documented outbreaks of human diseases associated with the consumption of raw and MP fruits and vegetables has considerably increased in recent decades. Therefore, technologies are being tested that besides improving their safety and increasing their shelf life, they should not alter their natural characteristics. Irradiation is one of these technologies.

In the present work, three microorganisms (*Escherichia coli, Listeria monocytogenes* and *Salmonella* spp*)* were studied in order to determine the D_{10} value, and then the 5-log cycle reduction was applied. As agreed in a previous meeting [6], a disinfection dose is achieved when a 5 log cycle reduction of a determined pathogenic microorganism is obtained. The disinfection dose for each product was defined on the basis of the more radiation resistant microorganism studied. Sensorial and mechanical evaluations were also carried out.

In Brazil, conventional and organic minimally processed vegetables are on the market. There are in Sao Paulo State, approximately 150 producers of organic foods such as vegetables including coffee, sugar, Brazilian drink, and broiler chicken.

Iceberg lettuce, watercress and arugula are the most consumed minimally processed vegetables in Brazil and mango, pineapple and watermelon are among the fruits most consumed.

The aims of this research were:

1. Determining the effect of irradiation on minimally processed iceberg lettuce spiked with *Salmonella* spp. and *Escherichia coli* O157:H7. The effect of irradiation on sensory attributes and the shelf-life of the product were also studied.

2. Determining the radiation D_{10} value of a cocktail of *Salmonella* strains inoculated on minimally processed watercress. The shelf life of the irradiated product was also studied.

3. Studying the behavior of *Listeria monocytogenes* and *Salmonella* spp. inoculated in organic watercress exposed to 1, 2 and 3 kGy during 16 days at 7ºC.

4. Determining the effect of low dose irradiation treatment on the populations of *L. monocytogenes* inoculated on arugula.

5. Determining the effect of low dose irradiation treatment on the population of *Salmonella* spp. in mango cubes as well as on sensory characteristics.

6. Determining the acceptance of irradiated minimally processed fruits, pineapple and watermelon, among Brazilian consumers.

2. MATERIAL AND METHODS

2.1. Substrates

The iceberg lettuce (*Lactuca sativa* L.) heads and watercress (*Nasturtium officinale*) were purchased from local markets on the day of experiment. They arrived at the market within 24 h after harvest. Arugula (*Eruca sativa*) was kindly provided by a producer.

The damaged and outer leaves of iceberg lettuce were discarded and the inside ones were removed and shredded (ca. 2 x 5 cm). The shredded iceberg lettuce was mixed and 800 – 900 g was used for each experiment.

The same procedure was used for watercress and arugula. Stalks and leaves were mixed and 800-900 g were used for each experiment.

Minimally processed (cubes) watermelon (*Citrullus vulgaris* Schrad), pineapple (cubes) (*Ananas comosus* L. Merril), and mango (cubes) (*Mangifera indica*, L.), cultivars Haden and Tommy-Atkins, were kindly provided by a plant located in Sao Paulo city.

2.2. Microorganisms

Three strains of *Escherichia coli* O157:H7 were used during the experiments with cut iceberg lettuce: EDL933 obtained from Dr T. Gomes (Universidade Federal de Sao Paulo) was isolated from hamburger [7]. Strains C7927 and E0019 were from Dr M. P. Doyle (Georgia University). The former was isolated from a patient with characteristic symptoms of infection by *E. coli* O157:H7 and the latter from bovine rectal swab.

Three strains of *Salmonella* (*S.* Infantis and *S.* Enteritidis were obtained from Instituto Adolfo Lutz and *S.* Thyphimurium belongs to Food Microbiology Laboratory) were used during experiments with shredded iceberg lettuce and watercress.

Listeria monocytogenes (strains isolated from spinach and ground beef, ATCC 7644 and ATCC 19115) were used for watercress and arugula experiments.

The strains were maintained at 4°C on tryptic soy agar (TSA, Difco, USA) slants.

2.3. Vegetable Sanitation

When 200 ppm sodium hypochlorite was applied for vegetable sanitation, the methodology by Niemira et al. [8] was used. Vegetable leaves were rinsed thoroughly in drinking water (0.3 – 0.45 mg Cl/L) and dried in a previously sanitized domestic salad spinner-type centrifuge. The leafy material was submerged in a solution of 200 ppm sodium hypochlorite for 15 min. Then, the leaves were thoroughly rinsed under running distilled water and spun in the sanitized salad spinner-type centrifuge to remove excess surface water. The processed vegetable leaves were kept under refrigeration overnight.

Mango, pineapple and watermelon: fruits were immersed for 5 min in ozonized water [ozone generator Ecozon Plus, Empresa Ecozon Ltda, São Paulo, S.P., Brasil, generating 4.58 g/h ozone (0.30 ppm ozone in water solution)], without rinsing. Then, the fruits were peeled and cut into cubes of 1 cm. Two hundred g were packed in plastic containers and kept refrigerated (7°C ± 1°C).

2.4. Preparation of the inoculum

To prepare the inoculum, the methodology developed by Niemira et al [25] was applied.

Each strain of *E. coli* or *Salmonella* was inoculated into tryptic soy broth (TSB, Oxoid, Basingstoke, UK) and incubated for 18 - 20 h at 37°C. A loopful was inoculated into 100 mL of TSB (Oxoid) and incubated at 37°C for 20 - 24 h. Two aliquots of 15 mL of each culture were added to two centrifuge tubes comprising 45 mL of culture per tube (this was considered the pool of bacteria). The cultures were centrifuged (centrifuge Hettich, Tuttlingen, Germany) at 700 g/30 min and the pellets were re-suspended in 45 mL of 0.85% (w/v) NaCl (LabSynth, Diadema, Brazil). *L. monocytogenes* strains were submitted to the same treatment but TSB was supplemented with 0.6% yeast extract (Difco).

2.5. Inoculation of vegetables and mango

To do the evaluation of vegetables and mango, the methodology developed by Niemira et al [25] was applied.

Iceberg lettuce: the pool of bacteria (*E. coli* or *Salmonella* spp) (90 mL) was mixed with 6 L of cold (2-5°C) distilled water in order to have ca 10^7–10^8 CFU/mL. The minimally processed vegetables were immersed into this suspension for 5 min. Then, the vegetable was spun in a sanitized salad spinner-type centrifuge to remove excess surface water. The vegetable showed a contamination of ca 10^6 CFU/g. The vegetable was then divided into 25 g and packed into polyethylene bags.

Watercress: the same procedure described above using *L. monocytogenes* and *Salmonella*.

Arugula: the same procedure described above using *L. monocytogenes*.

Mango: 200g of mango were transferred to plastic bags (Nasco - Wisconsin, EUA) and 5 mL (10^9 CFU/mL) of the pool of *Salmonella* were spiked on the surface. Afterwards, they were gently massaged for even distribution of the inoculum.

2.6. Microbiological analysis

The following analysis were done: enumeration of aerobic microorganisms [9], psychrotophic count [10], *Enterobacteriaceae*, coliforms, fecal coliforms and *E. coli* [11], yeasts and molds [12], lactic acid bacteria [13], *Pseudomonas* [14], *Salmonella* spp [15], and *L. monocytogenes* [16].

2.7. Irradiation Process

2.7.1. D_{10} values of different microorganisms inoculated on different vegetables

Iceberg lettuce samples spiked with *Salmonella* spp received absorbed doses of 0.0, 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0 kGy while the ones spiked with *E. coli* O157:H7 were exposed to doses of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 kGy. Samples of arugula spiked with *L. monocytogenes* were exposed to doses of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 kGy. Watercress samples spiked with *Salmonella* spp received absorbed doses of 0.0, 0.2, 0.5, 0.7, 1.0, 1.2 and 1.5 kGy. Mango samples spiked with *Salmonella* spp. were exposed to doses of 0.5, 1.0, 1.5, and 2.0 kGy.

2.7.2. Behavior of Salmonella spp. and L. monocytogenes during shelf life of irradiated watercress

Watercress samples spiked with *Salmonella* spp. and *L. monocytogenes* were exposed to doses of 1, 2 and 3 kGy in order to study the behavior of these microorganisms during the shelf life.

Each study included three samples per dose and was repeated three times, except for sensory evaluation.

Irradiated and non-irradiated samples were maintained under refrigeration (7^{o}C).

All samples were irradiated using a gamma radiation ^{60}Co source (JS 7500 MDS Nordion, Kanata, Canada) with 25,5 PBq (690 kCi). The dosimetric system used was the Harwell red perspex (United Kingdom) dosimeter. The accuracy is ±5% and the precision ±2%.

D_{10} values: the slopes of the individual survivor curves were calculated with linear regression using a computer graphic program (Microsoft® Excel 97 SR-2, Redmond, WA, USA). The ionizing radiation D-value (the radiation dose necessary to inactivate 90% of the population) was calculated by taking the negative reciprocal of the survivor curve slope.

2.8. Enumeration of *Salmonella* spp. and *L. monocytogenes* after irradiation

The enumeration of *Salmonella* spp. and *L. monocytogenes* was carried out using the method developed by Kang and Fung [17].

Each portion of 25 g was homogenized with 225 mL of 0.1% peptone water using a stomacher 400 for 2 min and serial diluted with 0.1% buffered peptone water. One mL of each dilution was pour plated using TSA (Oxoid). After solidification, 5-7 mL of MLCB agar (Oxoid) or Oxford agar (Oxoid) for *Salmonella* spp or *L. monocytogenes,* respectively, was overlaid on the TSA to determine the population of surviving bacteria. Two pour plates per dilution were incubated at 37^{o}C for 48 h. After incubation period, 3-5 colonies from each plate were tested with antisera (Difco) for *Salmonella* and with appropriate biochemical tests related to *L. monocytogenes* [16].

2.9. Shelf life of irradiated minimally processed vegetables and fruits

Sensory evaluation and microbial analyses were performed for shelf life studies.

2.9.1. Sensory evaluation of irradiated iceberg lettuce

Fifty packages (500 g each) of minimally processed iceberg lettuce (shredded) were acquired from a processing plant. They were air packed in polyethylene bags, processed and tested in the same day of harvesting. These packages were exposed to radiation dose of 0.0, 0.7, 0.9 and 1.1 kGy, corresponding to at least four times the highest D_{10} value for *Salmonella*. For sensory evaluation the Quantitative Descriptive Analysis was used according to Stone et al. [18]. Sensory panel was composed of six people that evaluated loss of exudates, texture and color. A non-structured 9-point scale was used. The results were compared among doses (0.0, 0.7, 0.9 and 1.2 kGy). Data were analysed using analysis of variance (SAS Institute Inc. Cary, N.C.) and mean separation was conducted using Tukey test ($P \leq 0.05$).

2.9.2. Sensory evaluation of irradiated watercress

Irradiated (1, 3 and 4 kGy) and non-irradiated packages (400 g) of non-inoculated, minimally processed watercress were subject to sensory evaluation [19] for shelf life determination. A panel composed of 25 – 30 non-trained members, aged 20 – 55 years, evaluated the samples on days 0, 2, 5, 7, 9 and 12 after treatment. Watercress was maintained under refrigeration (ca. 7°C) and served (30g) in white plastic dishes coded with 3-digit random numbers. The samples were presented one at time, following a completely randomized blocks design. Panelists were asked to rate each sample for overall liking on a hybrid 10-cm hedonic scale (0 = extremely dislike; 10 = extremely like) [20]. Data were submitted to a two-way ANOVA followed by the Tukey's means comparison test ($p < 0.05$).

2.9.3. Sensory evaluation of irradiated mango

The sensory panel was composed as describe above (2.9.2). They were asked to evaluate the overall liking as well as exudation, flavor, texture and color. A 10-point hedonic scale was used. The results were compared among doses (0.0, 1.0 and 2.0 kGy). Data were analysed using analysis of variance (SAS Institute, Inc. Cary, N.C.) and mean comparisons were conducted using the Tukey´s means comparison test (HSD) ($p \leq 0.05$).

2.9.4. Sensory evaluation of irradiated pineapple and watermelon in cubes

Panel for sensory evaluation [19] of irradiated pineapple and watermelon in cubes was composed of 60 members. They were asked to rate each sample for overall quality on a hybrid 10-cm hedonic scale. Sweetness (watermelon) and sourness (pineapple) were also rated using the just-about-right scale. In a second experiment, purchase intention was assessed using a 10-point numerical scale (1 = certainly would not buy, 10 = certainly would buy). Subjects were divided into two groups: a control group did not receive any information about irradiation, while the test group was informed about the irradiation process.

The irradiated and non-irradiated samples (10 g) were served on disposable white plates coded with a 3-digit number. Forks and water, to minimize the remaining flavor between samples, were also offered to the panelists. Samples were evaluated under white light in individual booths in the Sensory Analysis Lab.

2.9.5. Microbial analysis

It was performed as described in item 2.6.

3. RESULTS AND DISCUSSION

Sanitizing with 200 ppm sodium hypoclorite did not improve the microbiological quality of arugula as already reported by Goularte et al. [21] and Martins et al. [22] among other authors. Figure 1 shows the populations of mesophilic, *Pseudomonas*, psychrotrophic and lactic acid bacteria in unwashed (not sanitized) and washed (sanitized) arugula. Fecal coliforms as well as *E. coli*, when detected, were reduced by ca 1 log CFU/g, reaching undetected levels. *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* were not detected in any of the analysed products.

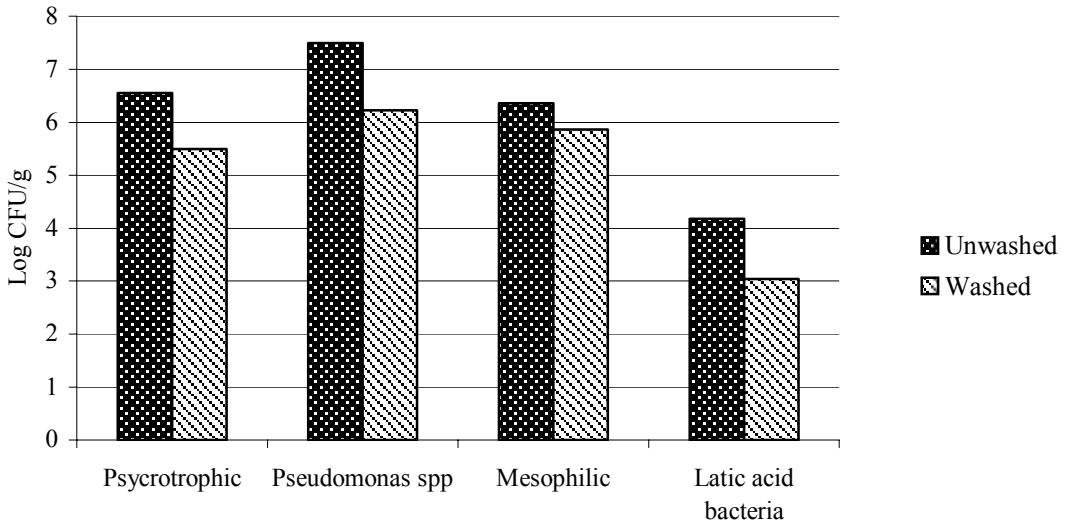

FIG. 1 Microbial populations of unwashed and washed arugula.

3.1. Determination of D_{10} values for microorganisms inoculated on vegetables and fruits

Table 1 shows the D_{10} values for *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 in different foods. *E. coli* O157:H7 was the most sensitive bacterium among the three tested microorganisms while *Salmonella* spp. in mango cubes presented the highest values. The radio-resistance of microorganisms (D_{10} values) varies with the chemical composition of the food, the presence of oxygen, the water activity of the food, among other factors. The lipid content of mango probably increased the resistance of *Salmonella* to radiation when compared to the vegetables.

L. monocytogenes and *Salmonella* spp. showed similar results for leafy vegetables, in general.

D_{10} for *L. monocytogenes* inoculated in minimally processed arugula ranged from 0.37 kGy to 0.48 kGy (Table 1). These results are higher than those reported by Niemira et al. [23] and by Tsuhako [24] in a mixture of four different cultivar of lettuce, and by Niemira et al. [25] working with endives. They are also higher than the results obtained by Martins et al. [26] for *L. monocytogenes* inoculated on watercress.

TABLE 1. D^{10} VALUES FOR *Listeria monocytogenes, Escherichia coli* O157:H7 AND *Salmonella* spp IN DIFFERENT LEAFY VEGETABLES AND MANGO

Products	*Listeria monocytogenes*	*Escherichia coli* O157:H7	*Salmonella* spp.
	D_{10} value (kGy)	D_{10} value (kGy)	D_{10} value (kGy)
Shredded iceberg lettuce	-----	0.11 – 0.12	0.16 – 0.23
Watercress	0.26 – 0.36	-----	0.29 - 0.43
Arugula	0.37 - 0.48	-----	-----
Mango	-----	-----	0.52 - 0.62

3.2. Behavior of *L. monocytogenes* and *Salmonella* inoculated on organic watercress exposed to 1, 2 and 3 kGy during 16 days at 7ºC

Storage at refrigeration temperature was not sufficient to control the growth of survived cells of *L. monocytogenes* on irradiated organic watercress. Doses of 1, 2 and 3 kGy reduced the population by ca. 4, 5 and 6 logs, respectively (Figure 2). The non-irradiated samples showed increasing counts during storage time at 7°C. The same behavior was presented by sample exposed to 1 kGy. Samples irradiated with 2 kGy and 3 kGy maintained the same population throughout their shelf-life. These results agree with those reported by Niemira et al. [27] with endive packed in normal atmosphere.

Salmonella population showed the same pattern as did *L. monocytogenes* when inoculated on organic watercress and exposed to 1, 2 and 3 kGy. The population of *Salmonella* on the non-irradiated sample presented a small increase between 14 and 16 days of storage at 7°C but the population was already very high (Figure 3).

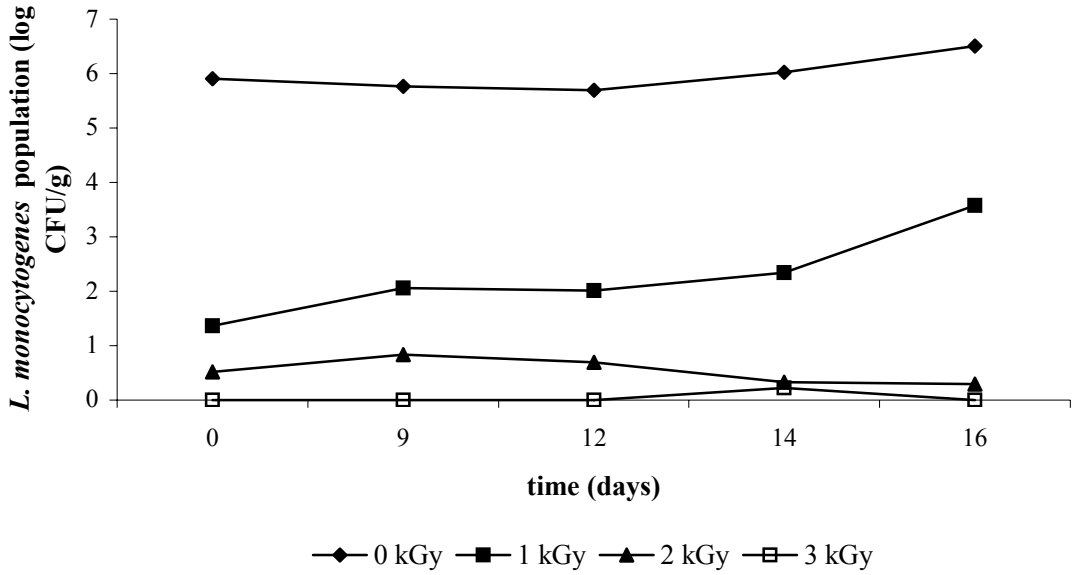

FIG.2 Behavior of L. monocytogenes inoculated on organic watercress exposed to 1, 2 and 3 kGy during 16 days at 7ºC (mean of two plates per dose).

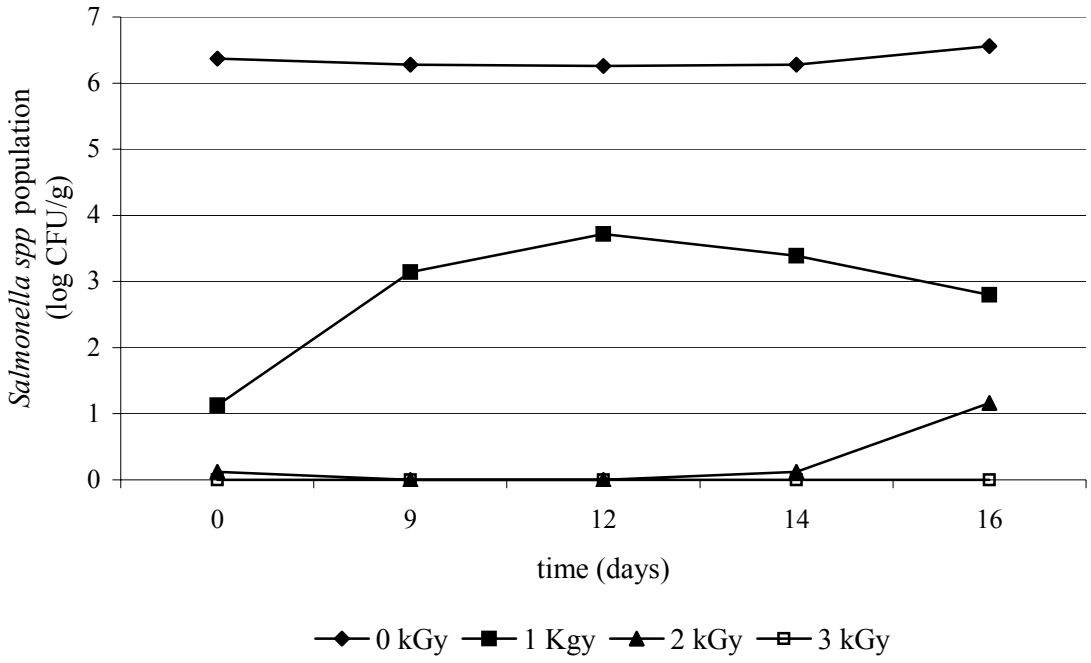

FIG. 3 Behavior of Salmonella spp. inoculated on organic watercress exposed to 1, 2 and 3 kGy during 16 days at 7ºC (mean of two plates per dose).

3.3. Sensory evaluation of irradiated vegetables and fruits

Although improving the microbiological safety of foods and sometimes increasing their shelf-lives, irradiation can cause changes on sensory quality of the product. Texture is the most affected attribute.

3.3.1. Sensory evaluation of irradiated iceberg lettuce

Table 2 shows the mean scores for exudate, odor, texture and color for minimally processed iceberg lettuce aerobically packed exposed to radiation doses of 0.0 (control), 0.7, 0.9 and 1.1 kGy. These data show that doses of 0.7 and 0.9 kGy did not affect the iceberg lettuce attributes. However, a significant difference in the texture with the dose of 1.1 kGy was observed. Therefore, above this dose irradiation treatment would not be suitable for iceberg lettuce.

Niemira et al. [8] observed that treatment up to 0.5 kGy did not change the texture of different types of iceberg lettuce. Studies by Foley et al. [28] and Hagenmaier and Baker [29] demonstrated that doses of 0.5 kGy did not induce alterations on visual attributes or softening in iceberg lettuce. Nevertheless, Hagenmaier and Baker [29] observed changes in the texture of iceberg lettuce exposed to 0.81 kGy.

TABLE 2. MEAN SCORE OF SENSORY ATTRIBUTES OF MINIMALLY PROCESSED ICEBERG LETTUCE PACKED UNDER AEROBIC CONDITIONS AND EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION

Attribute	Irradiation dose (kGy)			
	0.0	0.7	0.9	1.1
Exudate	0.00*[a]	0.04[a]	0.04[a]	0.02[a]
Odor	1.20[a]	1.38[a]	1.14[a]	1.32[a]
Texture	5.86[a]	4.56[a]	5.38[a]	3.30[b]
Color	0.02[a]	0.14[a]	1.34[a]	0.22[a]

*values are expressed as mean score of six judgements.
[a, b] mean within lines followed by same letters are not significantly different at 95% confidence levels.

3.3.2. Sensory evaluation of irradiated watercress

Table 3 shows that on day 0 and 2 the irradiated and non-irradiated watercress were equally appreciated even for samples exposed to 3 and 4 kGy. This feature was maintained up to day 5, when the 4 kGy sample obtained the lowest acceptability ($p<0.05$), under the neutral point (5 = "neither like, nor dislike") on the hedonic scale. From the seventh day on, the acceptability of all samples decreased, although only on days 9 and 12 significant differences ($p<0.05$) were observed with respect to 3 and 4 kGy samples. The acceptability of non-irradiated and 1 kGy irradiated watercress was higher - comparing to higher doses - and did not differ significantly ($p<0.05$) over all the days of the study. Based on these data, linear regressions of the acceptability means, as a function of storage time, were calculated (Figure 4). The shelf-life of each sample was obtained making y = 5.0 as the limiting acceptability. Non-irradiated watercress achieved a shelf-life of 14.5 days, while the product irradiated with a 1kGy dose reached 16 days. Higher doses of 3 and 4 kGy remarkably reduced shelf-life to 9 and 6 days, respectively (Figure 4).

These results evidence that irradiation of watercress is highly dependent on the applied radiation dose. An exposition to a 1kGy dose can even extend shelf-life of the product without changing its sensory characteristics negatively.

TABLE 3. ACCEPTANCE OF IRRADIATED WATERCRESS DURING SHELF-LIFE

Samples	Time† (days)						r‡	Estimated shelf-life (days)
	0	2	5	7	9	12		
0	6.9a	7.0a	6.8a	5.8a	5.6a	5.5a	-0.91	14.5
1 kGy	6.9a	7.1a	6.7a	6.3a	6.1a	5.3a	-0.94	16
3 kGy	6.2a	6.5a	6.7a	6.1a	4.8b	3.7b	-0.83	9
4 kGy	5.8a	6.3a	4.9b	5.1a	5.0a	3.2b	-0.88	6

† Means of acceptance in a day with the same letter do not differ significantly ($p<0.05$), according to Tukey's means comparison test.
‡ Linear regression coefficients are all significant ($p<0.05$)

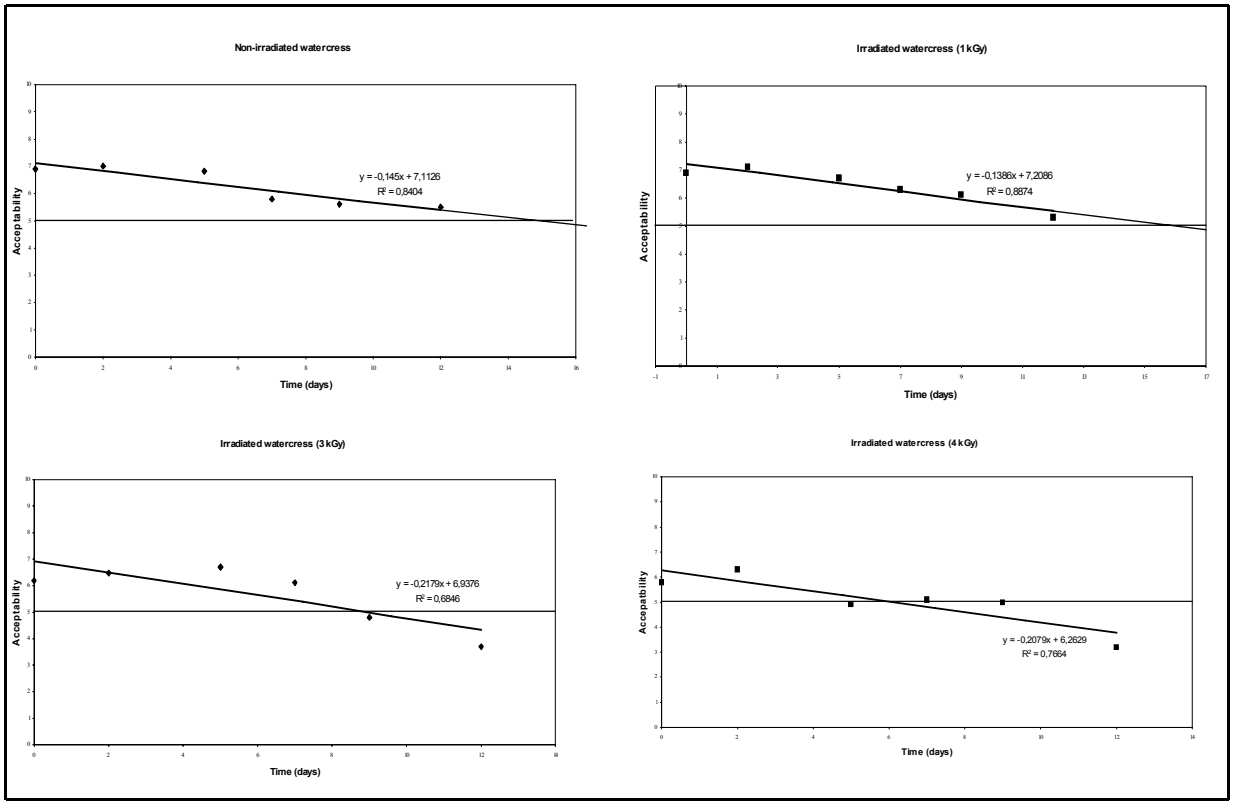

FIG. 4. Shelf life curves of non-irradiated and irradiated watercress showing the decrease of acceptability over time.

3.3.3. Sensory shelf life evaluation of minimally processed irradiated mango

Results presented on Table 4 (mango cultivar Tommy Atkins) did not show a significant difference ($p>0.05$) on the overall quality during the first two days of storage. However, afterwards, the three samples showed a reduction in the overall quality with the non-irradiated sample showing a significant difference. Samples exposed to 1 kGy and 2 kGy also presented the same tendency but without significant difference throughout the period. From day 4 on, however, the non-irradiated sample differed significantly from the irradiated ones.

Non-irradiated samples of mango cultivar Tommy Atkins cultivar showed a decline in texture scores during the storage period (Table 4) that was significant ($p<0.05$) from day 4 on when it was rejected by consumers. Samples exposed to 1 kGy presented better results than the 2 kGy sample agreeing with results obtained for overall quality (Table 4).

Tasting the mango on day 7 was not possible due to deterioration. However, it was possible to test for overall quality with results showing the acceptability of MP mango exposed to 1 kGy at least until day 4.

Regarding the overall quality of Haden cultivar (Table 5), there was no statistically significant difference ($p<0.05$) among the non-irradiated and irradiated samples during the storage period. There was a significant reduction ($p<0.05$) on the overall quality on day 7 that was observed for all samples. Both irradiated samples showed similar tendencies with reduction more evident after day 4.

Texture of these samples presented a reduction throughout the time that was significant ($p<0.05$) for non-irradiated sample on day 7 of storage (Table 5). Irradiated samples also showed the same

tendency with reduction being significant from day 4 on showing a negative effect of irradiation on the texture of the fruit.

There was not a significant difference ($p<0.05$) among the irradiated and non-irradiated samples when comparing samples within each day of shelf life.

TABLE 4. OVERALL APPEARANCE* AND TEXTURE** AVERAGE SCORES FOR IRRADIATED AND NON-IRRADIATED MINIMALLY PROCESSED TOMMY-ATKINS MANGO DURING SHELF LIFE AT 7°C.

Treatments	Storage time (days)				
Global Appearance					
Dose (kGy)	1	2	4	7	MSD
0	6.1a	5.9a	4.4bB	2.0cB	1.4
1	5.2a	6.2a	6.4aA	5.3aA	1.7
2	6.0a	6.1a	5.0aAB	4.7aA	1.6
MSD***	-	-	1.5	1.4	
Texture					
0	6.7a	6.5a	4.6b	-	1.5
1	6.2a	6.4a	6.1a	-	1.3
2	5.6a	5.2a	4.4a	-	1.5

*Values in rows not sharing common lower case letters are significantly different ($P<0.05$) according to the Tukey test.
**Values in columns not sharing common capital letters are significantly different ($P<0.05$) according to the Tukey test.
*** MSD – minimal significant difference ($p<0,05$).

Differentiation among fruits and vegetables by consumers is influenced primarily by appearance [30]. Our results suggested that a dose of 1 kGy maintained the appearance and texture scores of irradiated mango of both cultivars, Tommy Atkins and Haden, within acceptable limit (Table 4 and 5), agreeing with Khattak et al. [31] who worked with bitter gourd but disagreeing with those by Gunes et al. [32] who observed changes in the texture of minimally processed apples irradiated with doses of 0.34 kGy and higher.

TABLE 5. OVERALL QUALITY* AND TEXTURE** AVERAGE SCORES FOR IRRADIATED AND NON-IRRADIATED MINIMALLY PROCESSED HADEN MANGO DURING SHELF LIFE AT 7°C

Treatments	Storage time (days)				
Global appearance (0 to 10)	1	2	4	7	MSD**
Control	7.0a	6.8a	6.3a	3.8b	1.7
1 kGy	6.2a	7.0a	5.6ab	4.5b	1.6
2 kGy	7.3a	6.3ab	5.9ab	4.8b	1.6
Texture (0 to 10)					
0	7.3a	7.0a	6.5a	4.8b	1.6
1	6.4ab	6.9a	5.3b	4.4b	1.6
2	7.4a	6.7ab	5.4b	4.b	1.6

* Values in rows not sharing common letters are significantly different ($P<0.05$) according to the Tukey test.
** MSD – Minimal significant difference ($p<0.05$).

Microbial analyses showed growth of groups of tested microorganisms during the storage period, independent of the applied dose for both cultivars of mango (Tables 6 and 7). The cultivar Tommy Atkins showed higher contamination than Haden cultivar.

TABLE 6. POPULATIONS (Log CFU/g) OF MESOPHILIC, PSYCHROTROPHIC, FUNGI, LACTIC ACID BACTERIA AND Pseudomonas spp IN IRRADIATED MINIMALLY PROCESSED MANGO IN CUBES CULTIVAR HADEN DURING SHELF LIFE AT 7°C±1°C

		Doses		
Microorganisms	Time*(days)	Control (0 kGy)	1 kGy	2 kGy
Mesophilic	1	2.0	2.0	2.0
	2	2.0	2.0	2.0
	4	2.0	2.0	2.0
	7	5.36	2.6	1.77
Psychrotrophic	1	4.97	3.39	2.70
	2	6.05	4.29	2.86
	4	6.95	4.37	3.60
	7	7.15	5.8	3.70
Fungi	1	5.17	3.74	3.11
	2	5.59	4.27	3.23
	4	6.54	4.59	3.81
	7	6.92	5.02	3.96
Lactic acid bacteria	1	2.0	2.0	2.0
	2	2.0	2.0	2.0
	4	2.0	2.0	2.0
	7	5.63	2.0	2.0
Pseudomonas spp	1	3.47	1.74	1.30
	2	4.39	3.0	1.48
	4	5.0	3.90	1.73
	7	5.95	4.20	1.90

TABLE 7. POPULATIONS (Log CFU/g) OF MESOPHILIC, PSYCHROTROPHIC, FUNGI, LACTIC ACID BACTERIA AND and Pseudomonas spp. IN IRRADIATED MINIMALLY PROCESSED MANGO IN CUBES CULTIVAR TOMMY ATKINS DURING SHELF LIFE AT $7^{o}C \pm 1^{o}C$

		Doses		
Microorganisms	Time*(days)	Control (0 kGy)	1 kGy	2 kGy
Mesophilic	1	6.1	2.90	2.38
	2	6.15	5.23	2.58
	4	6.81	6.40	3.33
	7	7.02	7.14	3.94
Psychrotrophic	1	3.51	1.47	2.0
	2	3.58	3.55	2.0
	4	3.81	4.79	1.77
	7	7.55	7.13	6.38
Fungi	1	5.3	4.08	2.39
	2	6.12	4.26	2.80
	4	7.09	4.79	3.47
	7	7.14	5.81	3.86
Lactic acid bacteria	1	6.07	4.07	2.27
	2	6.91	5.69	4.53
	4	7.02	6.13	4.40
	7	7.87	6.68	6.40
Pseudomonas spp.	1	4.98	3.49	1.47
	2	6.36	3.79	1.53
	4	7.59	4.56	1.54
	7	7.91	5.30	3.94

3.3.4. Acceptance of irradiated pineapple and watermelon

The results obtained for irradiated cubes of watermelon and pineapple (Table 8) agree with the ones reported by Bandekar et al. [33] who did not observe changes in appearance, color, texture, odor, flavor and overall quality of irradiated pineapple. Furthermore, the process did not interfere with the sweetness of watermelon (Figure 5) or sourness of pineapple (Figure 6) [28].

TABLE 8. MEANS OF ACCPETANCE OF IRRADIATED MINIMALLY PROCESSED WATERMELON (*Citrulus vulgaris,* Schrad) AND PINEAPPLE (*Ananas comosus,* L. Merril)

Fruits	Control (0 kGy)	1 kGy	2.5 kGy
Watermelon	7.3	7.3	6.8
Pineapple	7.6	7.5	7.1

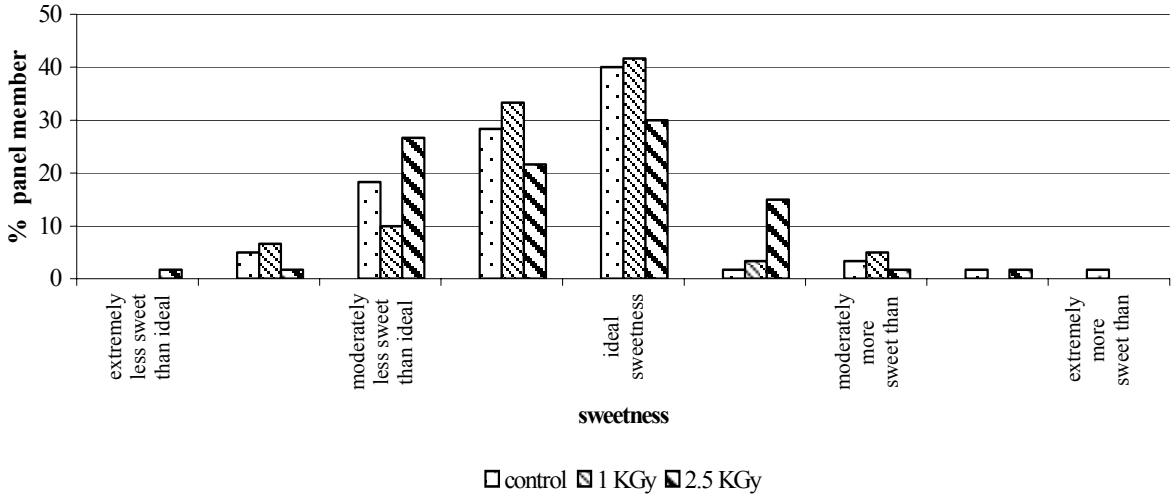

FIG. 5. Frequencies of responses on the just-about-right-scale in the evaluation of sweetness of irradiated and non-irradiated minimally processed watermelon (Citrulus vulgaris, Schrad).

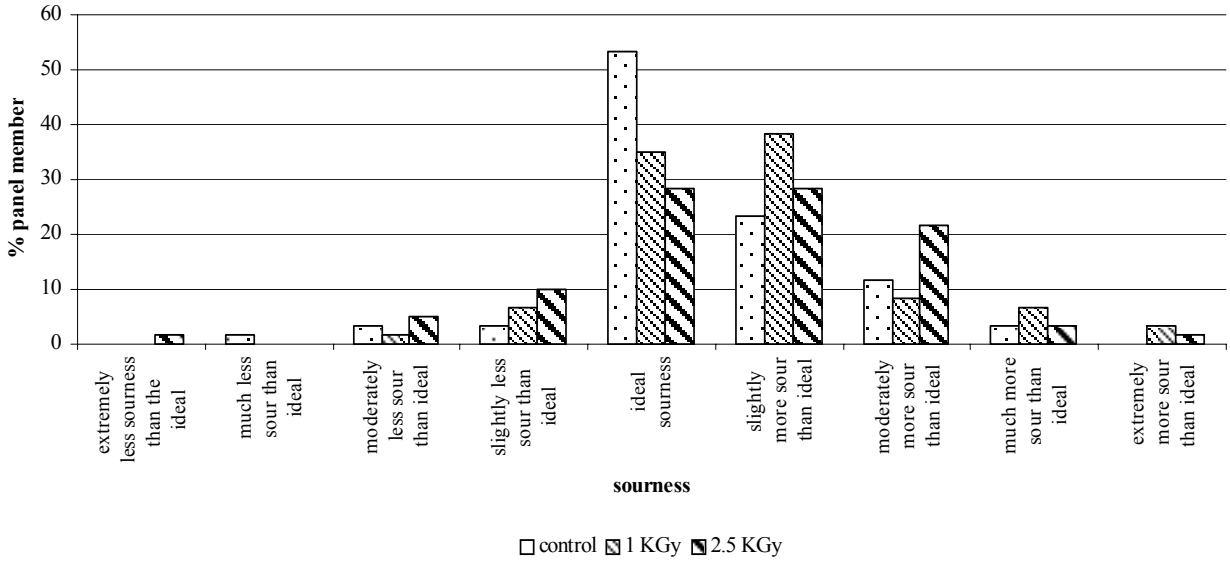

FIG. 6. Frequency of responses on the just-about-right-scale in the evaluation of sourness of irradiated and non-irradiated minimally processed pineapple (Ananas comosus, L. Merril).

The evaluation of purchase intention showed that consumers, in general, were prone to acquire irradiated watermelon in cubes as shown in Figure 7.

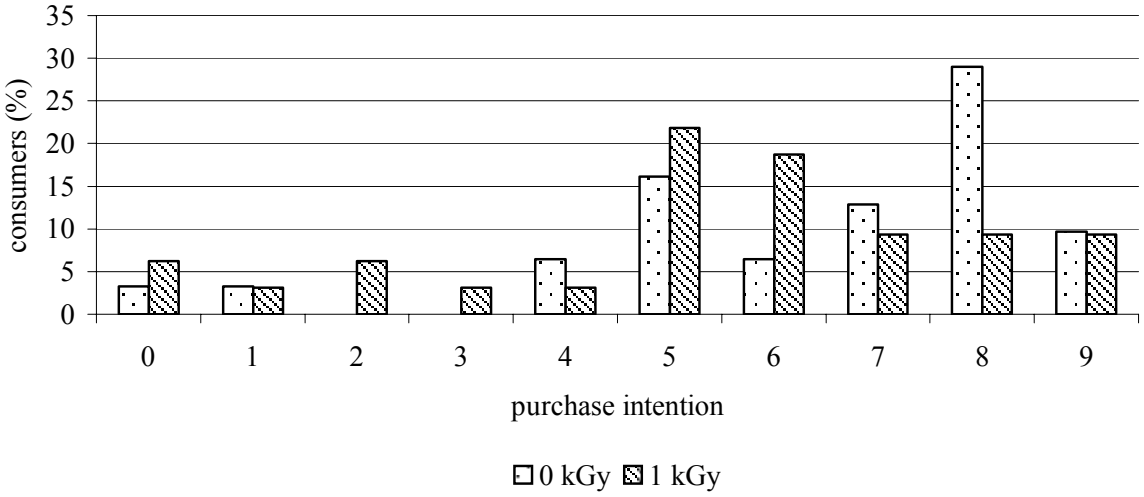

FIG. 7. Purchase intention for irradiated and non-irradiated watermelon.

4. CONCLUSIONS

1. *L. monocytogenes* population in watercress was fully recovered following exposition to a dose equivalent to 3 D_{10} when stored at $7^{O}C$ during 16 days. However, when higher doses were applied (equivalent to 5 and 8 D_{10}) no recovery was observed. These doses did not have a significant impact on sensorial characteristics.

2. Doses of 1, 3 and 4 kGy, equivalent to $2D_{10}$, $7D_{10}$ and $10D_{10}$ for *Salmonella* spp, did not impair sensorial characteristics of watercress up to day 4. However, the longest shelf life was achieved with 1 kGy (16 days) while the non-irradiated sample showed a shelf life of 14.5 days.

3. D_{10} for *L. monocytogenes* in arugala varied from 0.37 to 0.48 kGy.

4. Cubes of mango cultivar Tommy Atkins were sensory accepted until day 4 when exposed to 1 kGy. This cultivar showed a better response to irradiation than did Haden cultivar.

5. Pineapple and watermelon in cubes exposed to 1 and 2.5 kGy were sensory accepted. Irradiation did not influence the watermelon sweetness or pineapple sourness.

6. Consumers showed some resistance in acquiring irradiated pineapple and watermelon due to the lack of information about the irradiation process.

7. Irradiation proved to be a feasible process improving the hygienic conditions and safety of shredded lettuce, watercress, arugula and cubes of mango, pineapple and watermelon. In some cases, it also increased the shelf life of the product.

REFERENCES

[1] BEUCHAT L. R., Pathogenic microorganisms associated with fresh produce. J. Food Prot. **59** (1996) 204-216.

[2] BEUCHAT L. R., Ecological factors influencing survival and growth of human on raw fruits and vegetables. Microbes and Infect. **4** (2002) 413-423.

[3] BREIDT F., FLEMING H. P., Sing lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. Food. Technol. **51** (1997) 44-51.

[4] THAYER, D.W., RAJKOWSKI, K.T., Development in irradiation of fresh fruits and vegetables. Food Technol., **53** (1999) 62-65.

[5] BEAN N. H., GOULDING J. S., DANIELS M. T., ANGELO F. J. Surveillance for foodborne disease outbreaks - United States, 1988-1992. J. Food Prot **60** (1997) 1265-1286.

[6] INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA), Report of the first FAO/IAEA Research Coordination Meeting on the Use of Irradiation to Ensure Hygienic Quality of Fresh, Pre-cut, Fruits and Vegetables and other Minimally Processed Food of Plant Origin, 5-9 November 2001, Rio de Janiero, Brazil.

[7] RILEY, L.V., REMIS, R.S., HELGERSON, S.O., MCGEE, H.B., WELLS, J.G., DAVIS, B.R., HERBERT, R.J., OLCOTT, E.S., JOHNSON, L.M, Hemorrhagic colitis associated with a rare Escherichia coli serotype. N. Engl. J. Med., **308** (1983) 681-685.

[8] NIEMIRA, B.A., SOMMERS, C.H., FAN, X., Suspending iceberg lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157:H7. J. Food Prot., **65** (2002) 1388-1393.

[9] MORTON, D.R., "Aerobic plate count". Compendium of Methods for the Microbiological Examination of Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed., American Public Health Association, Washington, DC (2001).63-67.

[10] COUSIN, M.A., JAY, J.M., VASAVADA, P.C., Psychrotrophic Microorganisms", Compendium of Methods for the Microbiological Examination of Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed. American Public Health Association, Washington, DC (2001). 159-166.

[11] KORNACHI, J.L., JOHNSON, J.L., "*Enterobacteriaceae*, Coliforms, and *Escherichia coli* as quality and safety indicators". Compendium of Methods for the Microbiological Examination of

Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed., American Public Health Association, Washington, DC (2001) 69-82.
[12] BEUCHAT L. R., COUSIN, M.A. "Yeasts and Molds", Compendium of Methods for the Microbiological Examination of Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed., American Public Health Association, Washington, DC (2001) 209-215.
[13] HALL, P.A., LEDENBACH, L., FLOWERS, R.S., "Acid-producing microorganisms". Compendium of Methods for the Microbiological Examination of Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed. American Public Health Association Washington, DC (2001) 201-207.
[14] MEAD, G.C., ADAMS,.B.W., A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. Brit. Poultry Sci. **18** (1977) 661-667.
[15] ANDREWS W.H. FLOWERS, R.S., SILLIKER, J. BAILEY, J.S. "*Salmonella*", Compendium of Methods for the Microbiological Examination of Foods (DOWNES, F.P., ITO, K., Eds.), 4.ed. American Public Health Association Washington, DC (2001) 357-380.
[16] PAGOTTO, F., DALEY, E., FARBER, J., WARBURTON, D., Isolation of *Listeria monocytogenes* from all food and environmental samples. Available at: http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhee-dme/compendium/volume_2/e_mfhpb3001.html. Accessed 1January 2001.
[17] KANG, D., FUNG, D.Y.C., Thin-layer method for recovery of heat-injured *Listeria monocytogenes*.J. Food Prot. **62** (1999) 1346-1349.
[18] STONE, H., SIDEL, J., OLIVER, S., WOOSLEY, A., SINGLETON, N.C., Sensory evaluation by quantitative descriptive analysis. Food Technol., **28** (1974) 24-34.
[19] MEILGAARD, M., VIVILLE, G.V., CARR, B.T., Sensory evaluation techniques. 3rd ed. Boca Raton, CRC, 387p. (1999).
[20] VILLANUEVA N. D. M., PETENATE A. J., SILVA M. A. A. P., Performance of three affective methods and diagnosis of the ANOVA model. Food Quality and Preference. **11**(2000) 363-370.
[21] GOULARTE, L., MARTINS, C.G., MORALES-AIZPÚRUA, I.C., DESTRO, M.T., FRANCO, B.D.G.M., VIZEU, D.M., HUTZLER, B., LANDGRAF, M. Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.). Radiat. Phys. Chem. **71** (2004) 155-159.
[22] MARTINS, C.G., BEHRENS, J.H., DESTRO, M.T., FRANCO, B.D.G.M., VIZEU, D.M., HUTZLER, B., LANDGRAF, M. Gamma irradiation in the reduction of *Salmonella* spp. inoculated on minimally processed watercress (*Nasturtium officinalis*). Radiat. Phys. Chem. **71** (2004a) 87-91.
[23] NIEMIRA, B.A., Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on 4 iceberg lettuce types. J. Food Sci. **68** (2003a) 2784-2787.
[24] TSUHAKO, V.P. Irradiação de alface (*Lactuca sativa*): aspectos microbiológicos e sensoriais. Ms Dissertation presented at Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brazil. (2005) 53.
[25] NIEMIRA, B.A., FAN, X., SOKORAI, K.J.B., SOMMERS, C.H., Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria inoccua* ATCC 51742 inoculated on endive (*Chicorium endiva*). J. Food Prot. **66** (2003b) 993-998.
[26] MARTINS, C.G., BEHRENS, J.H., VIZEU, D.M., HUTZLER, B., DESTRO, M.T., FRANCO, B.D.G.M., LANDGRAF, M. Gamma irradiation to improve the microbiological safety of minimally processed watercress. Abstract published at the FOODMICRO Portoroz, Slovenia (2004b) 288.
[27] NIEMIRA, B.A., FAN, X., SOKORAI, K.J.B., Irradiation and modified atmosphere packaging of endive influences survival and regrowth of *Listeria monocytogenes* and product sensory qualities. Rad. Phys. Chem. **72** (2005) 41-48.
[28] FOLEY D. M., DUFOUR A., RODRIGUEZ L., CAPORASO F., PRAKASH A., Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. *Radiat. Phys. Chem*. **63** (2002) 391-396.
[29] HAGENMAIER R. D., BAKER R. A., Microbial population of shredded carrot in modified atmosphere packaging as related to irradiation treatment. J. Food Sci. 63 (1997) 162-164.

[30] GNANASEKHARAN, V., SCHEWFELT, R.I., CHINAN, M.S., Detection of color changes in green vegetables. J. Food Sci. **57** (1992) 149-154.
[31] KATTHAK, M.K., BIBI, N., KATTHAK, M.K., CHAUDRY, M.A., Effect of irradiation on microbial safety and nutritional quality of minimally processed bitter gourd (*Mormodica charantia*). J. Food Sci. **70** (2005) M255-M259.
[32] GUNES, G., HOTCHKISS, J.H., WATKINS, C.B., Effects of gamma irradiation on the texture of minimally processed apple slices. J. Food Sci. **66** (2001) 63-67.
[33] BANDEKAR, J. R., JADHAV, S.S., SHASHIDHAR, R., HAJARE, S., SHARMA, A., Use of irradiation to ensure hygienic quality of fresh, pre-cut fruits and vegetables and other minimally processed food of plant origin. Paper presented at the Second FAO/IAEA Research Coordination Meeting (RCM) of the Coordinated Research Project on "Use of irradiation to ensure hygienic quality of fresh, pre-cut fruits and vegetables and other minimally processed food of plant origin". Belfast, Ireland, (2003) 53-61.

THE INFLUENCE OF ANTIMICROBIAL COMPOUNDS OR COATING AND MODIFIED ATMOSPHERE PACKAGING ON RADIATION SENSITIVITY OF *LISTERIA MONOCYTOGENES* AND *LISTERIA INNOCUA* ON QUALITY MAINTENANCE OF READY-TO-USE CARROTS (*DAUCUS CAROTA*)

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Abstract

An edible coating based on caseinate and whey protein combined with modified atmosphere packaging (MAP): (60% O_2; 30% CO_2; 10% N_2) and gamma irradiation on the microbiological stability and physico-chemical quality of minimally processed carrots *(Daucus carota* L.) was evaluated. Carrots were irradiated at 0.5 or 1 kGy and stored at 4 ± 1ºC for 21 days. Results showed that gamma irradiation did not affect significantly ($p > 0.05$) the physico-chemical properties of the carrots. Microbiological analysis showed that for uncoated carrots a dose of 0.5 and 1 kGy applied under air and MAP reduced, respectively, by 3.5 and 4 log CFU/g and by 4 and 4.5 log CFU/g the content in aerobic plate count (APC). For coated carrots a dose of 0.5 and 1 kGy applied under air and MAP reduced, respectively, by 4 and 4.5 log CFU/g and by 3 and 4.25 log CFU/g the content of APC. The coating was able to protect carrots against dehydration during storage under air. Coating and irradiation treatment at 1 kGy were also able to protect the firmness during storage under air. MAP retarded whitening of uncoated carrots, but this treatment had a detrimental effect on the firmness. Processed carrots were also coated with caseinate based coating containing spice extracts or of antimicrobial compounds present in spices and irradiated at doses from 0.07 to 2.4 kGy to evaluate the radiosensitization of *Listeria monocytogenes* HPB 2812 serovar ½a (10^6 CFU/g). The dose (D_{10}) required to reduce *L. monocytogenes* population by 1 log was 0.36 kGy for samples packed under air and 0.17 kGy for those packed under MAP. In presence of *trans*-cinnamaldehyde, Spanish oregano, winter savory and Chinese cinnamon, the results showed that these active compounds had an effect on the irradiation sensitivity of bacterium in reducing *L. monocytogenes* population in carrots. The most efficient compound was *trans*-cinnamaldehyde where a mean increase of the relative radiation sensitivity of 3.8 times was observed for both atmospheres, as compared to the control. The effect of an antimicrobial edible coating containing *Trans*-cinnamaldehyde combined with MAP and gamma irradiation showed that the coating was able to reduce by 1.29 logs CFU/g the content of *L. innocua* in carrots packed under air after 21 days of storage whereas when packed under MAP, a 1.08 log CFU/g reduction was observed only after seven days of storage. Also, after seven days of storage, any *L. innocua* was detected in samples treated at 0.5 kGy under air or in samples treated at 0.25 kGy under MAP. A complete inhibition of *L. innocua* was also observed during all storage period in uncoated and coated samples treated at 0.5 kGy under MAP. These results revealed that the combination of irradiation, antimicrobial coating and MAP conditions played an important role in the bacterial radiosensitization.

1. INTRODUCTION

Minimally processed vegetables have gained considerable popularity in recent years due to their convenience, their human health benefits and the access for consumers of a wide variety of imported produce all year. Fresh-cut carrots (*Daucus carota* L.) represent an important component of the pre-cut vegetable production. Quality losses of these products related to cell breakage during transformation are nutrient loss, enzymatic reactions, mold growth, lactic acid fermentation, texture-loss, development of off-flavours and off-odours, and appearance defects [1]. The shelf life and wholesomeness of peeled mini carrots are principally influenced by microbiological changes caused by spoilage bacteria during refrigerated storage, but the contamination with pathogens as *Listeria* is also a frequent problem for ready-to-eat vegetables [1]. The microbial contamination potential of carrots with pathogens as well as other vegetables is high because of the wide variety of conditions to which the product is exposed during growth and processing [2]. Raw vegetables are already heavily contaminated when they enter in the processing chain [3]. The use of irradiation treatment and storage under modified atmosphere packaging (MAP) conditions are suggested to control or eliminate spoilage bacteria or/and foodborne pathogens in food and improve the shelf life of minimally processed vegetables [4, 5]. To control food contamination and quality loss, edible coating or biodegradable packaging has been recently introduced in food processing. Several applications for

meat, poultry, and seafood have been reviewed by Gennadios, et al [6]. The benefits of applying edible coatings to minimally processed carrots have been also demonstrated in our laboratories [5].

The objective of our study was to determine the radiosensitization of *L. monocytogenes* in peeled mini carrots packaged under air or under MAP conditions in presence of *trans*-cinnamaldehyde, Spanish oregano, winter savory or Chinese cinnamon. The combined effect of coating, MAP packaging and gamma irradiation on the content on *L. innocua* and APC and on the physico-chemical properties of peeled mini carrots stored at 4 ºC for 21 days was also evaluated.

2. MATERIALS AND METHODS

2.1. Transformation of peeled mini carrots

Peeled mini carrots (*Daucus carota* L.*)* samples were purchased from a local supermarket, transported to the Canadian Irradiation Centre (CIC) and were kept at 4 ± 1ºC. An inoculation bath was prepared by adding a microbial sample of *L. monocytogenes* to 3 l of sterile NaCl 0.85% (w/v). The pouches containing the carrots were opened under sterile conditions and each carrot was dipped to the inoculation bath and gently stirred for five minutes in order to obtain a contamination of 10^3 or 10^6 CFU/g depending of the experiment. After inoculation the carrots were allowed to dry 15 minutes in a sterile sieve before addition of the active compounds.

2.2. Materials for coating

Caseinate based coating was prepared according to Lafortune et al. [5]. *Trans*-cinnamaldehyde was purchased from Sigma-Aldrich (Oakville, ON, Canada). Spanish oregano (*Corydothymus capitatus*), winter savory (*Satureja montana*) and Chinese cinnamon (*Cinnamum cassia*) essential oils were provided by Robert et Fils (Montréal, QC, Canada). Concentration of the antimicrobial compounds used was 0.5% (wt/wt).

2.3. Treatment of peeled mini carrots

For each treatment, peeled mini carrots inoculated with *L. monocytogenes* were treated by spraying the corresponding antimicrobial compound solution (1 mL/carrot) under a biological containment hood. A dry period of 30 min was allowed before being transferred into the bags. Samples (3 carrots/package) were placed in 0.5 mm metallized polyester/2 mm EVA copolymer sterile bag (305 mm x 210 mm, Winpak, St-Leonard, QC, Canada) and packages were sealed under the appropriate atmosphere as under air or MAP (60%; 30% CO_2; 10% N_2). All bags were stored at 4 ± 1°C overnight prior to irradiation treatment.

2.4. Relative radiation sensitivity calculation

Relative radiation sensitivity was calculated using the following equation: relative radiation sensitivity = radiation D_{10} (kGy) control samples/radiation D_{10} (kGy) samples treated in presence of antimicrobial compounds. The irradiation treatment for D_{10} determination was done using a Gammacell 220 Excel equipment (MDS Nordion, Kanata, ON, Canada) at a dose rate of 0.686 kGy/h. D_{10}-value was determined by calculating the reciprocal of the slope provided by the log CFU/g versus irradiation dose. A value of D_{10} was calculated for each treatment lot using 9 irradiation doses ranged from to 0.1 - 2.4 kGy in samples without antimicrobial compound or 0.1 - 0.8 kGy in samples in presence of antimicrobial compounds. For each dose, three bags were irradiated. Samples were analysed immediately after irradiation to determine the microbial count. The effect of irradiation on the microbial count during storage was also determined. The irradiation treatment applied to determine the quality of peeled mini carrots during storage was done at doses of 0.25, 0.50 and 1 kGy in a Gammacell 220 Excel equipment (MDS Nordion, Kanata, On, Canada).

2.5. Microbiological analysis

Each carrot sample was weighed (ca. 10 ± 0.3 g) and homogenized 2 min in 90 mL of sterile peptone water (0.1% w/v) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this mixture, serial dilutions were prepared and appropriate ones were pour-plated in tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) and incubated 24 h at 35°C for bacteria enumeration.

2.6. Physico-chemical determination

A colorimeter based on the standard colour system CIELAB (Instrumar limited, St-John, NF, Canada) was used to evaluate the colour. Colour measurements evaluate the spectrum of the reflected light and convert it to a set of colour coordinates, L*, a* and b* which are coordinated in a three dimensional space containing all colours [5]. The carrots' firmness was assessed by measuring the force required to break the tissue, using a texture analyser Voland Stevens-LFRA (model TA-100, Texture technology corp., Scarsdale, NY, USA).

3. RESULTS

When non-irradiated and uncoated carrots were packed under air or MAP, the microbial level increased gradually to reach 5.54 and 5.80 log CFU/g at day 21. Irradiation treatment drastically reduced the level of microorganisms at day 1 (Fig.1A, B, C and D). Our data showed that packaging under MAP and irradiation has a synergistic effect for the control of microbial growth (Fig. 1 B and D). A 2.33 and 1.80 log CFU/g of APC were noted after seven days of storage for carrots treated with 0.5 and 1 kGy wich were stored under air (Fig.1 A), while a 3.81 and 1.65 log CFU/g of APC were observed after 14 days of storage in carrots treated with 0.5 and 1 kGy stored under MAP (Fig.1 B). The better combination of treatments allowing the smallest APCs results (2.40 log CFU/g) at the end of the storage period, was a dose of 1 kGy applied on uncoated carrots stored under MAP conditions (Fig. 1B). No synergistic effect was observed ($p > 0.05$) between MAP treatment and irradiation on coated samples.

Results on white discoloration showed that irradiation increased the whiteness index of uncoated carrots at day 1. However, coating applied before irradiation protected the carrots against whitening. Best results were obtained for coated carrots irradiated under air. An increase of the whiteness was also observed during storage. The most significant increase ($p \leq 0.05$) of whitening was observed with uncoated samples stored under air. Whiteness index values of 32.24, 34.74 and 34.00 were noted on unirradiated, 0.5 and 1 kGy samples respectively after 21 days of storage as compared to 18.93, 20.73 and 21.16 at day 1 of storage.

A mean loss of firmness of 17% during storage was observed for coated and irradiated samples stored under air. The coating and the irradiation treatment seem to have no influence on the firmness of the samples stored under air condition. However an important loss of firmness was observed on samples packed under MAP conditions. The most significant ($p \leq 0.05$) decrease of firmness during the storage was observed for the coated and unirradiated samples packed under MAP (47.26%).

Table 1 shows the radiosensitization of *L. monocytogenes* in peeled mini carrots packed under various atmosphere conditions, in absence and in presence of antimicrobial compounds. The atmosphere conditions and the presence of antimicrobial compounds affected the radiosensitivity of *L. monocytogenes*. A radiation D_{10} of 0.36 kGy was observed for control samples treated under air. The addition of antimicrobial compounds packaged under air increased significantly ($p \leq 0.05$) the radiosensitization of *L. monocytogenes*. The D_{10}-value in presence of *trans*-cinnamaldehyde was 0.10 kGy, which represented a 3.66-fold increase in the relative radiation sensitivity. In the presence of Spanish oregano essential oil, the D_{10}-value was 0.13 kGy and a 2.75-fold increase in the relative radiation sensitivity was observed. In presence of winter savory or Chinese cinnamon essential oil, the irradiation dose necessary to eliminate 90% of the *L. monocytogenes* population in carrots was reduced from 0.36 kGy for the control to 0.14 kGy for samples treated in presence of winter savory

and to 0.12 kGy in samples treated in presence of Chinese cinnamon, representing a 2.48-fold increase and a 2.9-fold increase in the relative radiation sensitivity, respectively.

The results obtained under MAP showed that the radiosensitization of *L. monocytogenes* was significantly higher ($p \leq 0.05$) under modified atmosphere, regardless the presence or the absence of antimicrobial compounds. Without antimicrobial compound, the D_{10}-value was reduced from 0.36 kGy under air to 0.17 kGy under MAP. This reduction represents a 2.06-fold increase in the relative radiation sensitivity. Under MAP and in presence of antimicrobial compounds, the radiosensitization of *L. monocytogenes* increased significantly ($p \leq 0.05$) when compared with the control under the same atmosphere. The D_{10}-values of *L. monocytogenes* in presence of *trans*-cinnamaldehyde, Spanish oregano, winter savory or Chinese cinnamon were 0.09, 0.12, 0.10 and 0.09 kGy, respectively. These D_{10}-values represent an increase of the relative radiation sensitivity of 3.95, 3.05, 3.74 and 3.86 times, respectively.

The addition of antimicrobial compounds and MAP had a marked effect on the radiation dose necessary to eliminate *L. monocytogenes* in mini carrots. The radiation dose necessary to eliminate the bacteria in mini carrots was 2.4 kGy under air and without antimicrobial compounds, but the lethal dose was reduce to 1.2 kGy under MAP. When mini carrots were packed under air, the lethal dose was 0.7 kGy in the presence of *trans*-cinnamaldehyde, 0.8 kGy in the presence of Spanish oregano or Chinese cinnamon, and 1 kGy in the presence of winter savory. When mini carrots were packed under MAP, the radiation dose necessary to eliminate *L. monocytogenes* in mini carrots was 0.6 kGy in the presence of antimicrobial compounds. The results indicated that compounds evaluated in this study increased the radiosensitivity of *L. monocytogenes* in carrots. The most efficient compound was *trans*-cinnamaldehyde under both atmospheres. A relative sensitivity of 3.66 and 3.95 was observed under air and under MAP respectively. However, for the control and samples in presence of essential oils, a higher relative sensitivity was observed under MAP. The addition of winter savory and Chinese cinnamon essential oils, showed an increase of the relative radiation sensitivity from 2.48 to 3.74 for winter savory and from 2.90 to 3.86 for Chinese cinnamon. These observations are supported by Mahrour et al. [7] who combined irradiation with marinating in rosemary, thyme and lemon juice, and obtained a significant bacterial radiosensitization in poultry. According to Chiasson et al. [8], the antimicrobial properties of essential oils components are responsible for this increased radiosensitivity. The compounds present in essential oils are responsible for outer membrane disintegration, disruption of the cytoplasmic membrane permeability and helped to reduce the intracellular ATP (the energy level) making it nearly impossible for the cell to make the necessary repairs of the damages caused by the increase of radiation [9, 10]. Also, the radiosensitivity of bacteria varies depending on the packaging atmosphere used. Bacteria are very sensitive to irradiation in the presence of oxygen [11]. The presence of 60% oxygen under MAP might be expected to enhance the lethal effect of radiation, due to oxygen radical and ozone formation during the treatment [12]. Oxygen has been implicated in the creation of free radicals during irradiation, which affects the DNA and hence the reproduction of bacteria. In general, the most common free radicals created following irradiation treatment stem from oxygen and water.

Table 2 shows the effects of MAP and gamma irradiation on *L. innocua* in mini carrots coated in the presence of *trans*-cinnamaldehyde. Radiation treatment significantly reduced ($p \leq 0.05$) the concentration of microorganisms. The combined treatments used (coating and/or MAP packaging) also have an important effect in decreasing the concentration of *L. innocua*. The better combinations of treatments allowing the complete inhibition of *L. innocua* during all storage period was obtained on samples treated at a dose of 0.5 kGy applied on coated or uncoated carrots and packed under MAP conditions. A synergistic antimicrobial effect was observed ($p \leq 0.05$) between MAP and irradiation on microbial growth of coated or uncoated carrots. Moreover, the antimicrobial coating had a significant ($p \leq 0.05$) antimicrobial effect against *L. innocua* for the unirradiated or irradiated samples stored under both atmospheres but the antimicrobial effect was observed during storage.

On the other hand, non-irradiated carrots stored under air had the highest concentrations of *L. innocua* after 21 days of storage with 2.23 log CFU/g for the uncoated samples and 2.26 log CFU/g for the

samples coated with the inactive coating (coating without *trans*-cinnamaldehyde). These results showed that the inactive coating did not have any influence on the *L. innocua* content**.** However, some samples coated with the inactive coating had a concentration of *L. innocua* significantly ($p \leq 0.05$) higher as compared to the respective uncoated samples. For example, *L. innocua* concentrations of 0.5-kGy samples coated with the inactive coating and packed under air were 1.73 and 1.07 log CFU/g after 7 and 14 days of storage, respectively, as compared with 1.15 and < 1 log CFU/g in 0.5-kGy uncoated samples and packed under air at the same days. These data suggest that bacteria under certain conditions seem to use milk protein-based coating in absence of *trans*-cinnamaldehyde as a substrate to sustain their growth. The results of the present study indicate that the incorporation of *trans*-cinnamaldehyde in coating applied on peeled mini carrots stored under air had an appreciable effect in reducing microbial growth. Baranowski and Nagel [13] reported that allylhydroxycinnamates, which are quite similar to cinnamaldehyde inhibited bacteria by a specific mode of action related to cellular energy depletion. Moleyar and Narasimham [14] proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity, suggesting an increase in electronegativity increases the antibacterial activity [14]. Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms.

4. CONCLUSIONS

Results based on mesophilic counts showed that a combination of irradiation treatment and MAP played a role in bacteria radiosensibilization and have a synergistic effect on the growth of bacteria during storage period. The data suggests that MAP allows for the conservation of an excess of surface moisture humidity on vegetables, thus increasing the time before the appearance of white discoloration. However, the wetting of the surface produced by MAP coupled with coating affected the firmness of carrots.

The combination of antimicrobial compounds with the irradiation treatment had a synergistic effect on the irradiation sensitivity of *L. monocytogenes* in peeled mini carrots. *Trans*-cinnamaldehyde for both atmospheres and winter savory or Chinese cinnamon for samples packed under MAP were the most effective compounds in increasing the relative sensitivity of *Listeria monocytogenes* in carrots. The radiosensitization of *L. monocytogenes* was significantly higher ($p \leq 0.05$) under MAP, regardless of the presence or absence of antimicrobial compounds.

A complete inhibition of *Listeria innocua* was obtained with the combination of low-dose irradiation (0.5 kGy) and MAP. Results also revealed that the combination of irradiation and MAP played a role in radiosensibilization of *Listeria innocua*, producing a synergistic antimicrobial effect on the growth of bacteria in peeled mini carrots during storage period. However, the antimicrobial coating treatment was necessary to reduce the *Listeria innocua* growth when carrots were packed under air during storage whereas for MAP, the coating treatment was not necessary to inhibit bacterial growth.

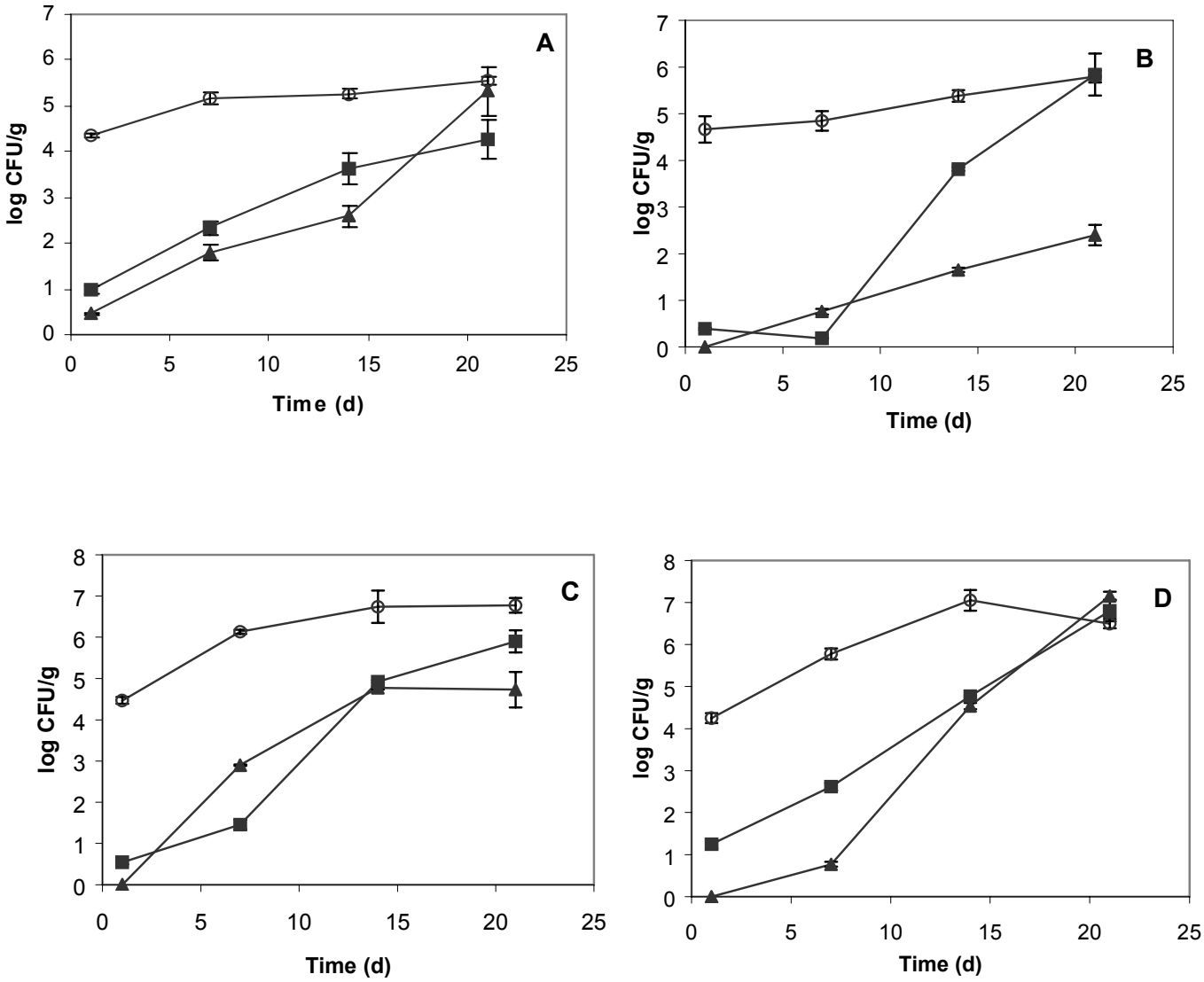

FIG. 1. Aerobic plate counts (APCs) of uncoated (A and B) and coated (C and D) mini-peeled C) and MAP: 60% O_2 ; 30% CO_2 ;10% N_2 (B and D).

∗ = Unirradiated; ■ = Irradiated 0.5 kGy; ↖ = Irradiated 1 kGy; ----- = Threshold of detection (< 10 CFU/g)

TABLE 1. RADIATION SENSITIVITY OF *Listeria monocytogenes* HPB 2812 SEROVAR ½ IN PEELED MINI CARROTS (DAUCUS CAROTA) PACKED UNDER AIR OF MAP IN PRESENCE OF VARIOUS ACTIVE COMPOUNDS

Active compounds[a]	Atmosphere[b]	Slope	D_{10} (kGy)	Relative Sensitivy
Control	Air	2.79 ± 0.17	0.36 ± 0.02	1.00 ± 0.00a
	MAP	5.72 ± 0.30	0.17 ± 0.01	2.06 ± 0.11b
Trans-cinnamaldehyde	Air	10.19 ± 1.22	0.10 ± 0.01	3.66 ± 0.44d
	MAP	10.99 ± 0.96	0.09 ± 0.01	3.95 ± 0.35d
Winter savory	Air	6.91 ± 0.19	0.14 ± 0.00	2.48 ± 0.07bc
	MAP	10.40 ± 0.88	0.10 ± 0.01	3.74 ± 0.32d
Chinese cinnamon	Air	8.05 ± 0.53	0.12 ± 0.01	2.90 ± 0.19c
	MAP	10.73 ± 1.36	0.09 ± 0.01	3.86 ± 0.49d
Spanish Oregano	Air	7.64 ± 0.52	0.13 ± 0.01	2.75 ± 0.19c
	MAP	10.99 ± 0.96	0.09 ± 0.01	3.95 ± 0.35d

[a] *Trans*-cinnamaldehyde is a synthetic compound, the other compounds are essential oils.
[b] Air: 78.1% N_2, 20.9% O_2 and 0.036% CO_2;
[b] MAP, modified atmosphere packaging: 60% O_2, 30% CO_2 and 10% N_2.
[c] data are presented as mean ± standard deviation.
[d] relative radiation sensitivity = radiation D_{10} (kGy) control samples / radiation D_{10} (kGy) samples treated in presence of antimicrobial compounds.

TABLE 2. EFFECT OF COATING APPLICATION, MAP PACKAGING AND IRRADIATION TREATMENT ON *Listeria innocua*[a] LSPQ 3285 IN PEELED MINI CARROTS, DURING STORAGE AT 4ºC

TREATMENTS[B]	**log CFU/g**[c] **(mean ± SD)**			
	Day 1	**Day 7**	**Day 14**	**Day 21**
Uncoated carrots- Air				
Control	3.22 ± 0.04eA	2.95 ± 0.03gB	2.60 ± 0.03fC	2.23 ± 0.08dD
Irradiated (0.25 kGy)	2.66 ± 0.02dA	2.39 ± 0.06defB	2.03 ± 0.07dC	1.49 ± 0.14bD
Irradiated (0.50 kGy)	1.91 ±0.07abA	1.15 ± 0.21aB	< 1.0[d]	< 1.0
MAP				
Control	3.17 ± 0.13eA	2.34 ± 0.14defB	< 1.0	< 1.0
Irradiated (0.25 kGy)	1.75 ± 0.17aA	1.38 ± 0.82abA	< 1.0	< 1.0
Irradiated (0.50 kGy)	< 1.0	< 1.0	< 1.0	< 1.0
Coated carrots with I[e] **Air**				
Control	3.03 ± 0.02eA	2.83 ± 0.02fgB	2.57 ± 0.03fC	2.26 ± 0.06dD
Irradiated (0.25 kGy)	2.59 ± 0.05dA	2.31 ± 0.04deB	1.80 ± 0.12cC	1.07 ± 0.15aD
Irradiated (0.50 kGy)	2.04± 0.07bcA	1.73 ± 0.11bcB	1.07 ± 0.15aC	< 1.0
MAP				
Control	3.24 ± 0.10eA	2.09 ± 0.29cdB	< 1.0	< 1.0
Irradiated (0.25 kGy)	2.14 ± 0.26cA	1.29 ± 0.53abB	< 1.0	< 1.0
Irradiated (0.50 kGy)	< 1.0	< 1.0	< 1.0	< 1.0
Coated carrots with A[e] **-Air**				
Control	3.04 ± 0.01eA	2.69 ± 0.02efgB	2.34 ± 0.03eC	1.75 ± 0.11cD
Irradiated (0.25 kGy)	2.55 ± 0.07dA	1.72 ± 0.36bcB	1.30 ± 0.01bC	< 1.0
Irradiated (0.50 kGy)	1.73 ± 0.11aA	< 1.0	< 1.0	< 1.0
MAP				
Control	3.13 ± 0.10eA	2.05 ± 0.13cdB	< 1.0	< 1.0
Irradiated (0.25 kGy)	2.09± 0.25bcA	< 1.0	< 1.0	< 1.0
Irradiated (0.50 kGy)	< 1.0	< 1.0	< 1.0	< 1.0

[a] Initial inoculation: 10^3 CFU/g.
[b] Air: 78.1% N_2, 20.9% O_2 and 0.036% CO_2; MAP, modified atmosphere packaging: 60% O_2, 30% CO_2 and 10% N_2; I, inactive coating; A, antimicrobial coating containing.
[c] Means within a same column which are bearing different lowercase letters are significantly different ($P \leq 0.05$) as determined by the Duncan's test. Means of each treatment lot with different uppercase letters are significantly different ($P \leq 0.05$) from other means at a different sampling period.
[d] No colony observed (counts under the detection threshold of 10 CFU/g).
[e] I: inactive coating without antimicrobial compound and A: antimicrobial coating in presence of *Trans*-cinnamaldehyde.

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REFERENCES

[1] CARLIN, F., NGUYEN-THE, C., HILBERT, G., CHAMBROY, Y., Modified atmosphere packaging of fresh ready-to-use grated carrots in polymeric films. J. Food Sci. **55** (1990) 1033-1038.

[2] HARRISON, W. A., PETERS, A. C., FIELDING, L. M., Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* colonies under modified atmospheres at 4 and 8°C using a model food system. J. Appl. Microbiol. **88** (2000) 38-43.

[3] NGUYEN-THE, C., CARLIN, F., The microbiology of minimally processed fresh fruits and vegetables. Cri. Rev. Food Sci. **34** (1994) 371–401.

[4] FARBER, J. M. Microbiological aspects of modified-atmosphere packaging technology - a review. *J. Food Sci*. **54** (1991) 58-70.

[5] LAFORTUNE, R., CAILLET, S., LACROIX, M., Combined effects of coating, modified atmosphere packaging and gamma irradiation on quality maintenance of ready-to-use carrots (*Daucus carota*). J. Food Prot. **68** (2005) 353-359.

[6] GENNADIOS, A., HANNA, M. A., KURTH, L. B., Application of edible coating on meats, poultry and seafoods: a review. Lebens-wiss Technol. **30** (1997) 337-3350.

[7] MAHROUR, A., CAILLET, S., NKETSIA-TABIRI, J., LACROIX, M., Microbial and sensorial quality of marinated and irradiated chicken. J. Food Prot. **66** (2003) 2156–2159.

[8] CHIASSON, F., BORSA, J., OUTTARA, B., LACROIX, M., Radiosensitization of *Escherichia coli* and *Salmonella Typhi* in ground beef. J. Food Prot. **67** (2004) 1157-1162.

[9] HELANDER, I. M., ALAKOMI, H.-L., LATVA-KALA, K., MATTILA-SANDHOLM, T., POL, I., SMID, E., GORRIS, L. G. M., VON WRIGHT, A., Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem. **46** (1998) 3590–3595.

[10] ULTEE, A., LETSAND, E. P. W., SMID, E. J., Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Appl. Environ. Microbiol. **65** (1999) 4606–4610.

[11] THAKUR, B. R., SINGH. R. K., Food irradiation—chemistry and applications. Food Res. Int. **10** (1994) 437–473.

[12] PATTERSON, M., Sensitivity of bacteria to irradiation on poultry meat under various atmosphere. Lett. Appl. Microbiol. **7** (1988) 55–58.

[13] BARANOWSKI, J. D., NAGEL, C. W., Inhibition of *Pseudomonas fluorescens* by hydroxycinnamic acids and their alkyl esters. J. Food Sci. **47** (1982) 1587-1589.

[14] MOLEYAR, V., NARASIMHAM, P., Antifungal activity of some essential oils components. Food Microbiol. **3** (1986) 331-336.

USE OF IONIZING RADIATION TO ENSURE THE SAFETY OF PRE-CUT FRESH VEGETABLES

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Abstract

The determination of D_{10} values for two *Escherichia coli* strains (an ATTC and a wild type), *Listeria innocua*, and a non toxigenic strain of *Escherichia coli* O157:H7 was carried out in different vegetal products. The studied products -celery (*Apium graveolens*), cabbage (*Lactuca sativa var. capitata*), iceberg lettuce (*Lactuca sativa* var. *capitata*), carrots (*Daucus carota L.*), spinach (*Spinacia oleracea*) and two mixed salads (Toscana, containing chopped iceberg lettuce (*Lactuca sativa* var. *capitata*), red cabbage (*Brassica oleraceae* var. *rubra*) and shredded carrot (*Daucus carota L.*) and Four Seasons, a mixture of chopped romaine lettuce (*Lactuca sativa var. longifolia*), iceberg lettuce (*Lactuca sativa var. capitata*), Butterhead lettuce (*Lactuca sativa* var. *acephala*) and spinach (*Spinacia oleraceae*))- were minimally processed. The effect of irradiation with 5 D_{10} doses on the natural microflora and on the sensory characteristics, during refrigerated storage, of the non inoculated products, was also studied. After irradiating with 5 D_{10} doses, a considerable reduction in the initial contamination of the studied vegetables was obtained. No significant variation of the microbiological counts of the irradiated products was observed during the refrigerated storage, while in the non-irradiated samples a progressive increase up to 3.5 logs in some vegetables was observed. In general, no significant changes in the sensory parameters were observed after irradiation and during seven days storage at refrigeration temperature (4º C).

1. INTRODUCTION

Consumers are increasingly demanding convenient, ready-to-use and ready-to-eat fruits and vegetables with a fresh-like quality, and containing only natural ingredients. For reasons of expense, labor and hygiene, the catering industry also aims to purchase vegetables and fruits that are already peeled and possibly also sliced, grated or shredded, that is, minimally processed or lightly processed. Some of the microbial pathogens associated with fresh vegetables include *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., enteropathogenic strains of *Escherichia coli*, hepatitis A, etc. The possible sources of contamination in these products involve the incoming raw vegetables, the plant workers, and the processing environment. When vegetables are chopped or shredded, the release of plant cellular fluids provides a nutritive medium in which microorganisms are able to grow. The high moisture content of fresh vegetables, the lack of lethal process to eliminate microbial pathogens, and the potential for temperature abuse during preparation, distribution, storage and handling further intensify the risk of food-borne illness [1].

For fresh-cut vegetables that are eaten raw, there is no treatment that can be relied on to substantially reduce the numbers of contaminating micro-organisms. Washing with antimicrobial compounds, while important, often brings about only a relatively small reduction. Eliminating the risks is difficult. Management of them is based on identifying and controlling those factors that are important in preventing contamination or limiting growth of pathogenic microorganisms between the farm and plate [2].

In Chile the market trend is to offer a great variety of pre-cut vegetables products, with a label specifying that they can be consumed without a previous treatment (washing, disinfecting, or cooking). Considering this fact and the relatively small reduction of the microflora when using

antimicrobial compounds, irradiation could be a feasible alternative treatment to ensure the safety of these vegetables.

The objectives of the present study were 1) to determine the D_{10} values for two *Escherichia coli* strains (an ATTC and a wild type), for *Listeria innocua* (a non-pathogenic surrogate for *Listeria monocytogenes*) and for a non-toxigenic strain of *Escherichia coli* O157:H7, some of them inoculated in minimally processed celery, cabbage, iceberg lettuce, carrots, spinach, and two mixed salads (Toscana and Four Seasons), 2) to evaluate the effect of irradiation on the natural microflora and on the sensory characteristics, during refrigerated storage, of the non- inoculated products irradiated with 5 D_{10} doses.

2. MATERIALS AND METHODS

2.1. Samples

Ready-to-eat diced celery (*Apium graveolens*), shredded cabbage (*Brassica oleraceae*), pre-cut iceberg lettuce (*Lactuca sativa var. capitata*), shredded carrot (*Daucus carota L.*), whole spinach leaves (*Spinacia oleracea*), pre-cut Toscana mixed salad containing chopped iceberg lettuce (*Lactuca sativa* var. *capitata*), red cabbage (*Brassica oleraceae* var. *rubra*) and shredded carrot (*Daucus carota L.*) and Four Seasons salad, a mixture of chopped romaine lettuce (*Lactuca sativa var. longifolia*), iceberg lettuce (*Lactuca sativa var. capitata*), Butterhead lettuce (*Lactuca sativa* var. *acephala*) and spinach (*Spinacia oleraceae*).

All these minimally processed vegetables were obtained from the local market. They came from the same producer and were harvested from a single source. The vegetables were sanitized using hypochlorite and packaged in polyethylene bags under nitrogen atmosphere. Date of purchase was the same day the products were delivered to the market. Shelf life data printed on the package was eight days, kept at 2ºC - 4ºC.

2.2. Bacterial strains

Escherichia coli ATCC 8739

*Escherichia coli (*wild type, isolated from a vegetable sample in the Laboratory)

Listeria innocua ATCC 33090

Escherichia coli O157:H7-VT(N) NCTC 12900

2.3. Bacterial strain-vegetable combinations

In the studies carried out, the strain - vegetable combinations were the following:

- *E. coli* ATCC 8739 and *E. coli* wild type were tested in celery and cabbage;
- *L. innocua* ATCC 33090 was tested in iceberg lettuce, carrots, spinach and Toscana and Four Seasons mixed salads; and
- *E. coli* O157:H7 was tested in Toscana and Four Seasons mixed salads.

2.4. Determination of D_{10} values

2.4.1. Sanitation of the samples

The samples were sanitized according to the studied strain, as follows:

- Celery and cabbage were irradiated overnight (25 kGy).

- Iceberg lettuce, carrots, spinach and Toscana/Four Seasons mixed salads for the study with *L. innocua* were sanitized using a solution of 300 ppm sodium hypochlorite at room temperature. The products were submerged and gently agitated for 3 min. Then the products were rinsed with sterile distilled water, and spun in a sterile salad spinner-type centrifuge to remove excess surface water.

- For the study with *E. coli* O157:H7, the Toscana and Four Seasons mixed salads were irradiated with a 2.0 kGy dose, in order to eliminate the indigenous microflora, mainly the coliform group, that could interfere with the *E. coli* count.

2.4.2. Preparation of the inocula

All strains were grown for 24 h at 35ºC, in tryptic soy broth (Oxoid) to a concentration of 10^9 colony forming units (cfu) /ml, then diluted with 0.1% peptone water (pH 7) and used to contaminate the vegetables so as to obtain $10^5 – 10^6$ cfu/g.

2.4.3. Inoculation

To inoculate the samples, ca. 100 g of each vegetable were introduced in a sterile flask containing 1 liter of the diluted culture suspension and shaken for 10 min. The liquid was withdrawn by spinning in a sterile salad spinner-type centrifuge. Aseptically, 10 g of each vegetable were weighed, packaged in sterilized bags and sealed.

2.4.4. Irradiation

The vegetables inoculated with the two strains of *E. coli*, were irradiated with doses of 0.5, 1.0 and 1.5 kGy in an experimental irradiator BPCDI Nº1 MK3 provided with a ^{137}Cs source (dose rate = 26.4 Gy /min). For *L. innocua* and *E. coli* O157:H7*,* the irradiation was carried out with doses of 0.1, 0.2, 0.3 and 0.4 kGy in an experimental irradiator Gamma cell 220-R, provided with a ^{60}Co source. Dose rates fluctuated between 59.18 and 67.51 Gy/min, for the different strain - vegetable combinations. In all cases, inoculated but not irradiated samples were used as control.

2.4.5. Determination of the surviving microorganisms

After the irradiation, 10 g samples were homogenized with 90 ml of 0.1% peptone water in a Stomacher for 1 min at normal speed. *E. coli* counts were carried out plating 1 ml decimal aliquots into Petri dishes and pouring tryptic soy agar (Oxoid). *Listeria* spp. counts were carried out spreading 0.1 ml aliquots of different decimal dilutions, on the surface of Oxford agar (Oxoid) and *E. coli* O157:H7 was counted by pouring 1.0 ml aliquots in Petri dishes and mixed with violet red bile agar (Oxoid). Plates were incubated at 35ºC for 24 – 48 h and counted. D_{10} value was calculated using the mean of 3 replicates of the experience for each vegetable.

2.5. Irradiation with 5 D_{10} doses

2.5.1. Evaluation of the radiation effect on inoculated samples

Aseptically, 20 g of each vegetable inoculated with *L. innocua* and *E. coli* O157:H7, following the same method used for the D_{10} value determination, were weighed, packaged in sterilized bags and sealed. Each vegetable was irradiated with the calculated 5 D_{10} dose in an experimental irradiator provided with a ^{137}Cs source. Non-irradiated samples were used as control. The effect of the irradiation was determined by counting the viable microorganisms remaining after irradiation. For *L. innocua*, aliquots of 1 ml per dilution were spread on the surface of three plates containing Oxford agar and for *E. coli* O157:H7, 1.0 ml aliquots were poured in Petri dishes and mixed with violet red bile agar. The experiment was repeated three times for each vegetable.

2.5.2. Evaluation of the radiation effect on non inoculated samples

2.5.2.1. Microbiological analysis

Each vegetable was irradiated with the calculated 5 D_{10} dose in an experimental irradiator provided with a ^{60}Co or a ^{137}Cs source, according to the studied product. Non-irradiated samples were used as a control. The products were assessed during storage at 4ºC for up to seven days, controlling at days 0, 2, 5 and 7. The assayed microbiological parameters, according to the different vegetables and studied strains were, total plate count (TPC) [3], *Enterobacteriaceae* count (Ent) [4], MPN of *E. coli* (Ec) [5], presence/absence of *Salmonella, Listeria* spp and *E. coli* O157:H7 [5]. The experiment was repeated two times for each vegetable.

2.5.2.2. Sensory evaluation

Due to the capacity of the irradiator, whole sealed packages of the samples (ca. 300 g) were irradiated by using a ^{137}Cs source. Non-irradiated samples were used as control. All samples were stored at 4ºC. It is necessary to consider that all samples were tested by the judges without salt, salad dressing or spices.

Celery and cabbage: Sensory evaluation was carried out by a trained panel consisting of six staff members of the Chilean Commission of Nuclear Energy, on days 1, 4 and 7 after irradiation. The quality of vegetables was measured using parameters such as appearance, color, aroma, sweetness, acidity, bitterness, texture, flavor and total quality. The Scoring method and a scale of 1 to 9 points were used. Data was analysed by ANOVA and Duncan Test (5% level).

Iceberg lettuce, carrots, spinach and Toscana and Four Seasons mixed salads: Sensory evaluation was carried out by a trained panel consisting of ten judges of the Department of Food Science, Universidad de Chile, on days 0, 3 and 7 after irradiation. In order to determine differences between the control and the irradiated samples, triangular tests at day 0 and day 7 were carried out**.** Sensory parameters were assayed using a 10-cm structured scale, anchored in the center and the extremes, the central anchor of the scale corresponding to the non-irradiated sample (control). A score of 5.00 was assigned to the control, which was stored seven days under the same conditions as the irradiated samples. The evaluation of the different sensory parameters was carried out by comparing the non-irradiated sample with the irradiated ones. Assayed parameters were: appearance, colour, aroma, flavour, sweetness, bitterness, texture and total quality. The experience was replicated two times for each vegetable. Data were analysed by ANOVA and Fisher's least significant difference (LSD) procedure ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Determination of D_{10} values

Results for the different combinations between strains and vegetables are shown in Table 1.

TABLE 1. D10 VALUES (kGy)[1] FOR THE DIFFERENT STRAINS INOCULATED IN THE STUDIED VEGETABLES

Vegetable	*E. coli* ATCC	*E. coli* wild strain	*L. innocua*	*E. coli* O157:H7
Celery	0.18 ± 0.01	0.22 ± 0.03		
Cabbage	0.22 ± 0.03	0.23 ± 0.01		
Iceberg lettuce			0.22 ± 0.03	
Carrots			0.20 ± 0.02	
Spinah			0.32 ± 0.01	
Toscana Salad			0.19 ± 0.01	0.09 ± 0.01
Four Seasons Salad			0.21 ± 0.03	0.09 ± 0.01

[1] Data shown is the mean from three trials ± standard deviation.

Celery and cabbage: In this case *E. coli* was chosen as the indicator microorganism in order to know the behavior of the natural microflora of this kind of vegetables. The determination of the D_{10} values for two different strains of the microorganism (a wild and an ATCC strain) was carried out to treat the samples later with 5 D_{10} doses. In both vegetables, the D_{10} values calculated for the ATCC strain are lightly lower than the obtained for the wild strain. For cabbage, the values obtained for both strains were lightly higher than those calculated for celery. However, no statistically significant differences between both strains and vegetables were found ($p \geq 0.05$).

Although no literal data about D_{10} values was found for minimally processed vegetables inoculated with this microorganism, a comparison with results in other matrices will be considered. D_{10} values of 0.2 kGy and 0.25 - 0.5 kGy for E. coli were reported by Garbutt [6] and Adams & Moss [7], respectively, although no further details on food matrices and bacterial strain are given. Doyle et al. [8] presents values of 0.23 – 0.35 kGy when inoculating in fresh foods of animal origin. According to this literal data, the results found in this study could be considered within the range of reported D_{10} for *E. coli*.

Iceberg lettuce, carrots, spinach, Toscana, and Four Seasons mixed salads: in general, the obtained values for *Listeria innocua* inoculated in iceberg lettuce, carrots and the two mixed salads agreed with results obtained by Niemira et al. [9], for the same microorganism in cut leaf pieces of endive (*Cichorium endiva*), where a D_{10} value of 0.22 ± 0.01 was reported. No significant differences ($p \geq 0.05$) between these vegetables were observed. Results obtained for *Listeria* inoculated in spinach showed a greater resistance to radiation.

No literal data of *E. coli* O157:H7 inoculated in vegetables, was found. In general, data from studies with this microorganism inoculated in poultry, fresh foods and meat, showed results of 0.27 kGy, 0.24 – 0.27 kGy and 0.24 kGy, respectively [10, 8, 11]. ICMSF [12] reports for ground poultry and ground meat, different values that range between 0.241 and 0.39 kGy, using wild and O157:H7 strains. The radiation source, gaseous phase and temperature are detailed. Nevertheless, low D_{10} values for this microorganism ranging between 0.05 to 0.18 kGy; have been reported by Buchanan et al. [13] when irradiating the strain in a Brain Heart Infusion Broth, adjusted to pH between 5.0 and 5.5. According to the literature the pH of vegetable tissue is in the range 5 – 7 [14].

3.2. Evaluation of the effect of irradiation with 5 D_{10} doses

3.2.1. Radiation effect on inoculated samples

The experiment was carried out in samples where *L. innocua* and *E. coli* O157:H7 were tested. Results are shown in Table 2.

TABLE 2. REDUCTION OF L. INNOCUA AND E. COLI O157:H7 BY IRRADIATION WITH 5 D10 DOSES

Vegetable	*L. innocua* (log cfu)[1]		*E. coli* O157:H7 (log cfu/g)	
	O kGy	5 D_{10} dose	0 kGy	5 D_{10} dose
Iceberg lettuce	6.10 ±0.12	<1.00 ± 0.00		
Carrots	6.20 ± 0.21	<1.00 ± 0.00		
Spinach	6.20 ± 0.15	<1.00 ± 0.00		
Toscana Salad	6.51 ± 0.11	1.15 ± 0.21	6.12 ± 0.05	1.00 ± 0.00
Four Seasons Salad	6.51 ± 0.11	1.15 ± 0.21	6.12 ± 0.05	1.00 ± 0.00

[1] data shown is the mean from 3 trials ± standard deviation

In all cases after irradiating with a 5 D_{10} dose, a reduction in 5 or more logs was observed. The obtained results validated the determined D_{10} values, for each of the strains inoculated in the studied vegetables.

3.2.2. Evaluation of the radiation effect and storage on the microbiological quality of non inoculated samples

It is necessary to consider, that the food legislation in Chile [15] has no established criteria for the contamination of minimally processed foods. For vegetables similar to ready-to-eat products specified in the Chilean legislation and in order to compare the results of the counts obtained in the present study, the following limits are considered. For TPC, n=5, c=1, m= 4.7 log cfu/g and M=5.7 log cfu/g and n=5, c=1, m=3.0 log cfu/g and M=4.0 log cfu/g for Ent. For a three-class attributes plan, the definition of the cited parameters are: “n” number of sample units drawn; “c” maximum allowable of positive results between “m” and “M”; “m” limit is used to separate acceptable from marginally acceptable quality and “M” is used to separate the marginally acceptable quality from defective quality.

3.2.2.1. Celery and cabbage

Figures 1 and 2 show the results obtained in the reduction of the initial microflora of celery and cabbage, after irradiating with the 5 D_{10} dose determined for the two *E. coli* strains and the changes during storage.

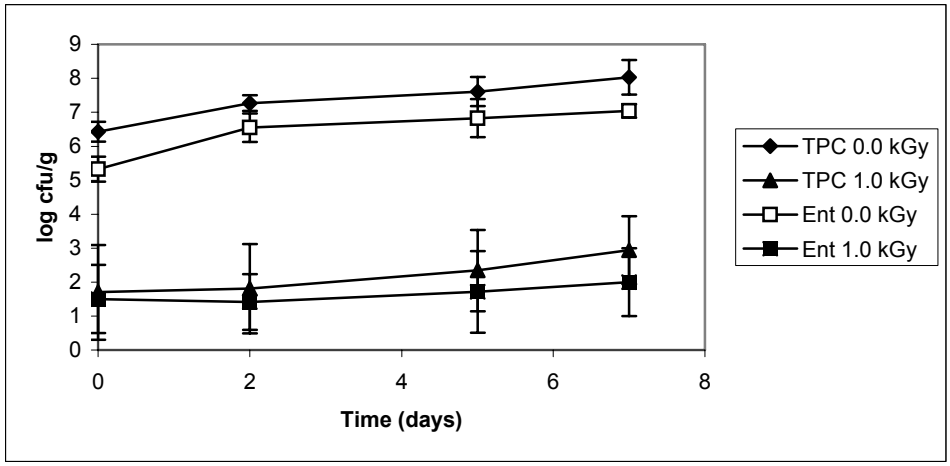

FIG. 1. Effect of irradiation and storage time on the TPC and Ent in celery.

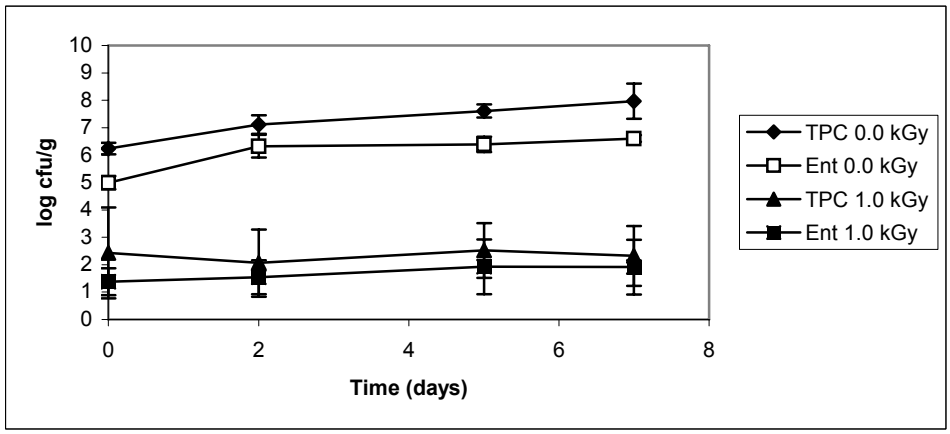

FIG. 2. Effect of irradiation and storage time on the TPC and Ent in cabbage.

In the control samples studied the initial total plate and *Enterobacteriaceae* counts are above the maximal limits established in the Chilean legislation. No *E. coli* or *Salmonella* were detected.

After irradiating celery with 1 kGy, a reduction of 4.7 and 3.8 logs in TPC and Ent, respectively, was observed. For TPC this result agreed with Prakash et al. [16], who reported a reduction of 4.6 logs in the aerobic microorganisms count, after irradiating diced celery with 1 kGy. The effect of the 1 kGy dose was lower in cabbage; the reduction was of 3.8 and 3.6 logs in TPC and Ent, respectively. A 3.5 and 4.0 log reduction for the same microbiological parameters, in carrots cubes irradiated with the same dose was achieved by Farkas et al. [17]. The population of TPC on the non-irradiated samples increased during storage. At the end of the study, changes of 1.6 and 1.7 logs were observed in celery and cabbage, respectively. Other authors reported an increase of 2.4 logs in diced celery after 8 storage days; 2.5 logs in fresh pre-cut sliced bell peppers at day 7, and 2.0 logs in cut romaine lettuce packaged under modified atmosphere, at day 11 [16, 17, 18]. At day 7, *Enterobacteriaceae* counts increased in 1.7 and 1.6 logs in control samples of celery and cabbage, respectively. Values informed by Farkas et al. [17] in sliced bell peppers, showed an increase of almost 3 logs. Statistically significant differences ($p \leq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters in the studied vegetables.

During the storage of the irradiated samples, the increase of the surviving flora was lower than that obtained in the non-irradiated products. In general, TPC and Ent counts remain practically stable during the storage in both products. The higher count at day 7 was observed in celery, where TPC was 1.2 logs higher than the result obtained at the initial day. Prakash et al. [16] reported in diced celery irradiated with 1 kGy, a variation in TPC of 1.9 logs after eight days at 5ºC. No statistically significant differences ($p \geq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters. The colony's characteristics observed in the plates of the counts belonging to the survivors of the irradiated samples, presented a great variety between the replicate samples. These can be due to a non homogeneous microflora of the initial products, depending on non standardized processes or treatments during the elaboration of the minimally processed products and/or different culture conditions of the vegetable. It is possible to emphasize that when irradiating with 1 kGy dose, an important reduction of the initial microflora can be achieved.

3.2.2.2. Iceberg lettuce, carrots and spinach

Figures 3, 4 and 5 show the results obtained in the reduction of the initial microflora, after irradiating with the 5 D_{10} dose determined for *Listeria innocua* and the changes during storage.

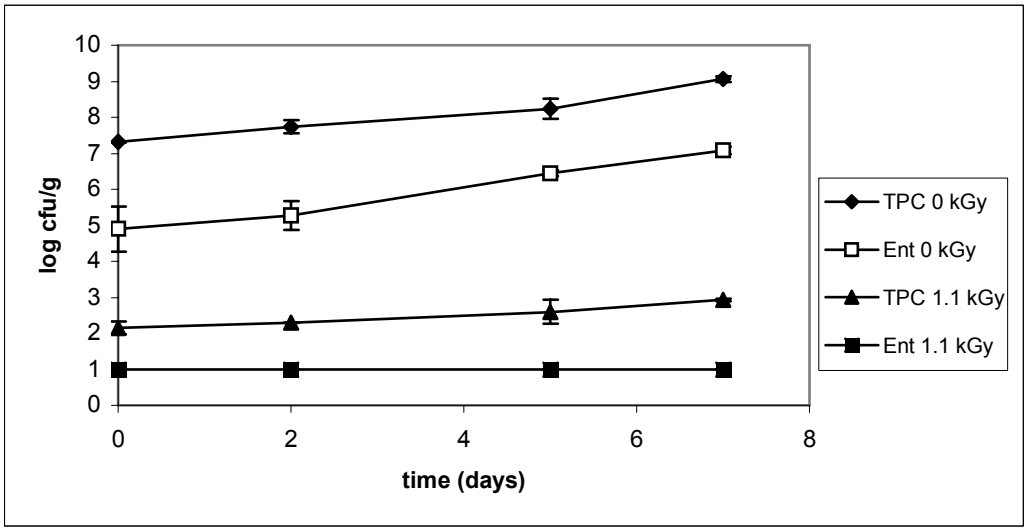

FIG. 3. Effect of irradiation and storage time on the TPC and Ent in iceberg lettuce.

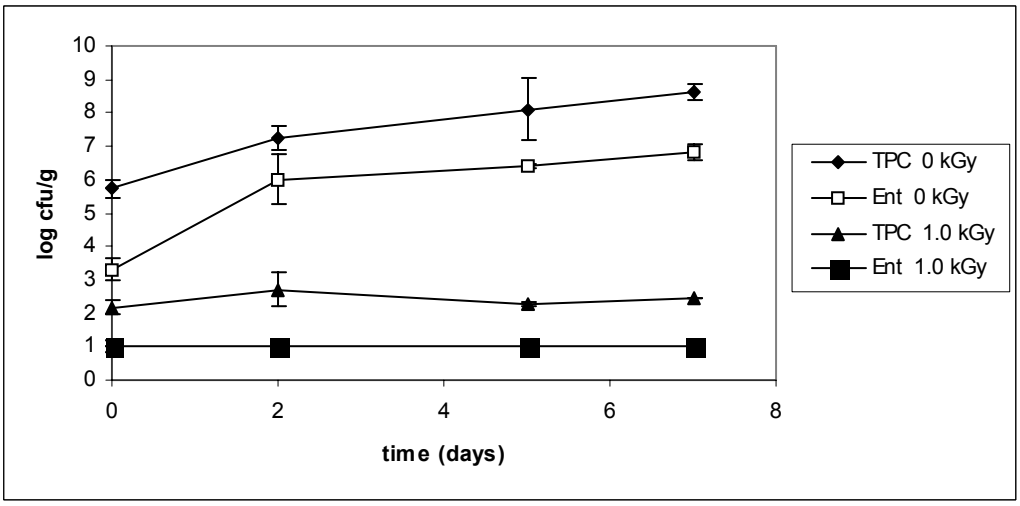

FIG. 4. Effect of irradiation and storage time on the TPC and Ent in carrots.

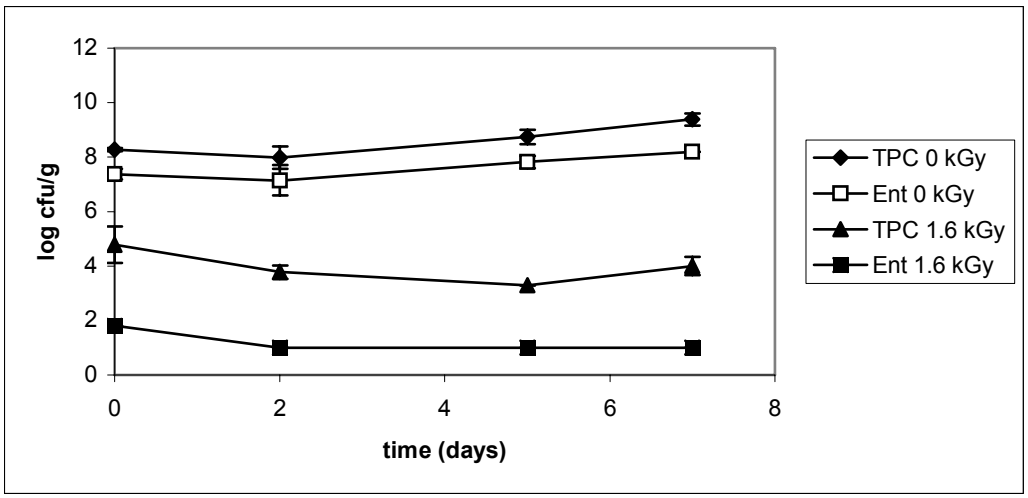

FIG. 5. Effect of irradiation and storage time on the TPC and Ent in spinach.

Initial total plate counts were high, reaching the highest counts in spinach, 8.3 log cfu/g. In general, the same was observed for the *Enterobacteriaceae* counts in this kind of vegetable. Only the Ent count in carrots is within the limits "m" and "M" established in the Chilean legislation. In all studied samples, no *Listeria spp.* in 25 g was detected.

When irradiating with a 5 D_{10} dose, the highest reduction was observed in iceberg lettuces for TPC and in spinach for *Enterobacteriaceae*, where a decrease of 5.2 and 5.6 logs, respectively, was obtained. For Ent in carrots, only a 2.3 logs reduction was observed, while in the other vegetables the decrease was an average of 3.7 logs.

During the storage at 4ºC of the non-irradiated samples, the highest variation in the microflora was observed in carrots, where the *Enterobacteriaceae* count increased in 3.5 logs in seven days. In the rest of the studied samples, variations in TPC and *Enterobacteriaceae* counts between 1.0 and 2.9 logs were observed. Similar variations have been reported by Prakash et al. [18] in cut romaine lettuce. Fan et al. [19] observed an increase of almost 1.5 logs in TPC after seven days storage at 3°C, samples of fresh-cut green onions where irradiated previously with 1 kGy. Statistically significant differences ($p \leq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters in the studied vegetables.

In the irradiated samples, the increase in time was not more than 0.8 logs in iceberg lettuce TPC and the Ent were reduced in 0.8 logs in spinach. In the other cases no variation over time was observed. No statistically significant differences ($p \geq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters.

3.2.2.2. Toscana and Four Seasons salads

Samples of the same day of production of Toscana and Four Seasons salad were used in order to know the initial TPC and Ent counts. Using samples of the same batch for both salads, the effect of the different 5 D_{10} doses calculated for each strain, was determined.

Figures 6 and 7 show the results obtained in the reduction of the initial microflora, after irradiating with the 5 D_{10} dose determined for *Listeria innocua* and the changes during storage.

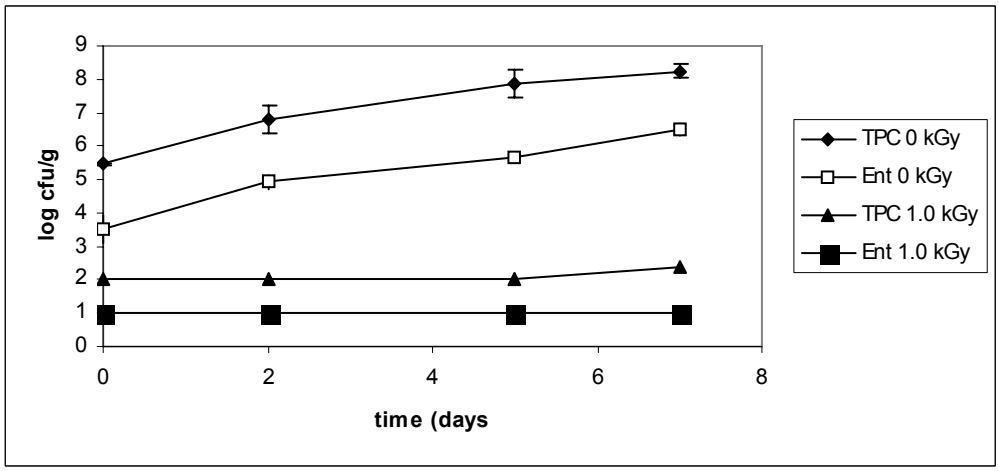

FIG. 6. Effect of irradiation and storage time on the TPC and Ent in Toscana salad.

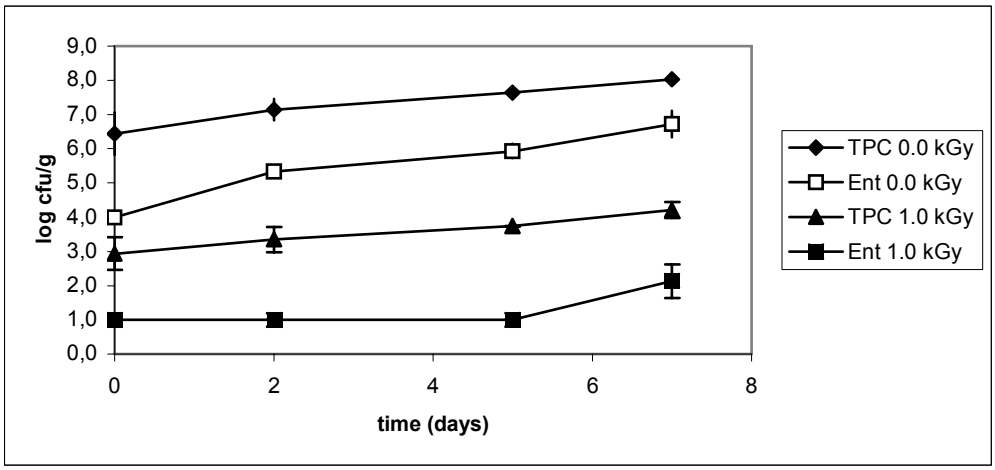

FIG. 7. Effect of irradiation and storage time on the TPC and Ent in Four Seasons salad.

The initial TPC in both salads are above the limits established in the Chilean legislation. The *Enterobacteriaceae* counts in both salads reached the specified limits. In all samples, no *Listeria spp.* in 25 g was detected.

Irradiation with a 1.0 kGy dose decreases in 3.5 logs the TPC in both salads. A difference of 0.5 logs in the reduction of the Ent count was observed between the Toscana and Four Seasons salad samples. The lower value was found in Toscana salad (2.5 logs were reduced).

During the storage at 4ºC of the non-irradiated samples, the higher increase at day 7 was observed in the Ent count of the Toscana salad, reaching a variation in 3.0 logs. In the Four Seasons salad an increase of only 1.6 logs in the TPC was the lowest observed variation. Statistically significant differences ($p \leq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters in the studied vegetables.

In the irradiated samples, the increase of the TPC after the refrigerated storage was only of 1.3 logs in the Four Seasons salad, while in the Toscana salad a 0.4 logs variation was observed. No changes in the *Enterobacteriaceae* count and only an increase of 1.1 logs in the Toscana salad and Four Seasons salads, respectively, was observed. No statistically significant differences ($p \geq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters.

Although *Listeria* was not initially present in the assayed samples, the irradiation with 1.0 kGy could destroy up to 5 logs of the possible presence of the microorganism. This fact is important, considering that such a high contamination is not supposed to be present in this kind of product.

Figures 8 and 9 show the results obtained in the reduction of the initial microflora, after irradiating with the 5 D_{10} dose determined for *E. coli* O157:H7 and the changes during storage.

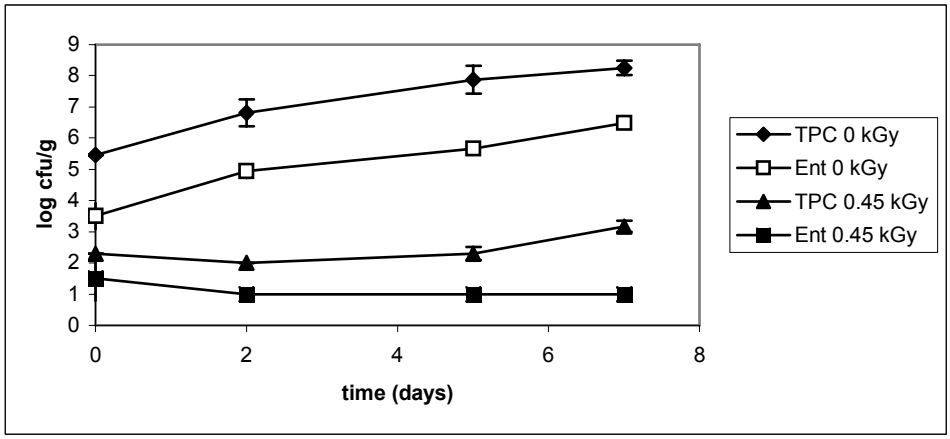

FIG. 8. Effect of irradiation and storage time on the TPC and Ent in Toscana salad.

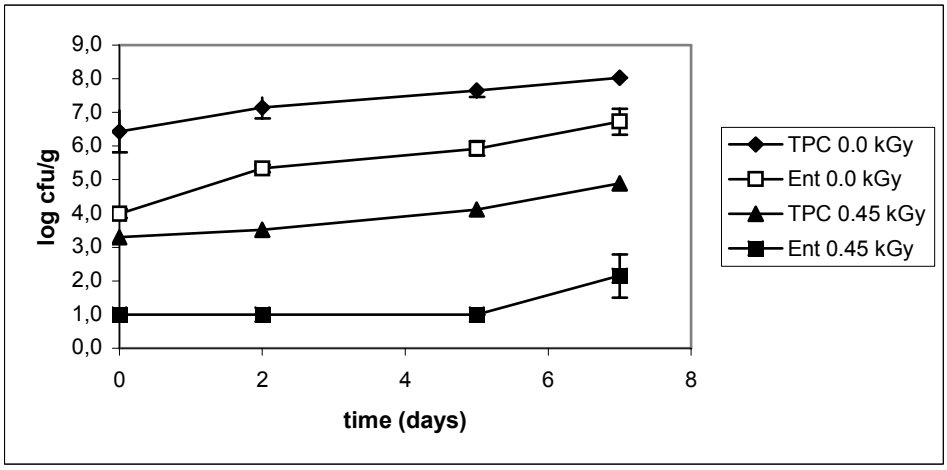

FIG. 9. Effect of irradiation and storage time on the TPC and Ent in Four Seasons salad.

The initial TPC and Ent counts were the same as in the samples used in the above study concerning *Listeria*. In this case, no *E. coli* O157:H7 in 25 g was detected.

Irradiation with a 0.45 kGy dose decreases in 3.2 the initial TPC in both salads. The Ent counts, were reduced in 2.0 and 3.0 logs in the Toscana and Four Seasons salads, respectively.

The variation over time, of the initial contamination of the non-irradiated samples was the same cited in the *Listeria* study.

In the Toscana salad, an increase of 0.9 logs for a total plate count in the irradiated samples at day 7 was observed. However, at the end of the storage the *Enterobacteriaceae* count decreased to 0.5 logs.

A higher variation in time was observed in Four Seasons salad, where an increase of 1.6 and 1.2 logs for TPC and Ent counts at day 7, was observed. No statistically significant differences ($p \geq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters.

The irradiation of all studied vegetables with the 5 D_{10} doses calculated for the studied strains, reduced to levels within the limits established of the Chilean legislation and maintained these levels during the seven days of refrigerated storage. This fact was not achieved in the case of the non-irradiated samples.

3.2.3. Evaluation of the radiation effect and storage on the sensory quality of non inoculated samples.

3.2.3.1. Cabbage and Celery

Table 3 shows the effect of irradiation (5 D_{10} dose) in the sensorial parameters of celery and cabbage.

In cabbage no significant differences were detected comparing the control with the results obtained for the irradiated samples, in the same day. Total quality of the irradiated cabbage was acceptable. Only sweetness presented lower scores than score 5 considered to be normal.

Comparing the non-irradiated control with the results obtained for celery, in the same day, no significant differences were detected. In general, total quality of the irradiated samples was acceptable, considering that the score between 5 and 6 means "normal". Only aroma and sweetness presented scores under 5, considered as normal.

In general, cabbage presented better total quality than celery, which could be explained with the cut uniformity of the former. Celery presented irregular pieces and no uniformity in peeling. It is necessary to consider that the samples were tested by the judges without salt, salad dressing or spices, which could have affected the obtained scores.

The irradiation of both vegetables with 5 D_{10} dose for *E. coli*, did not produce significant changes in their sensory characteristics. Total quality of the irradiated samples was acceptable, considering that the score between 5 and 6 means "normal".

TABLE 3. EFFECT OF 5 D10 VALUE ON THE SENSORY PARAMETERS OF CABBAGE AND CELERY

Parameter	Day	Cabbage		Celery	
		0.0 kGy	1.0 kGy	0.0 kGy	1.0 kGy
Color	1	5.0 ± 0.0[1]	4.8 ± 0.4	3.8 ± 0.8	5.3 ± 0.5
	4	4.7 ± 0.8	4.2 ± 1.2	4.2 ± 1.3	4.7 ± 1.6
	7	5.2 ± 0.4	5.0 ± 0.6	5.2 ± 1.5	5.0 ± 0.9
Aroma	1	4.7 ± 1.4	4.5 ± 0.5	3.3 ± 2.0	3.8 ± 1.9
	4	4.7 ± 1.7	5.2 ± 1.7	4.7 ± 1.6	4.7 ± 1.0
	7	4.8 ± 1.5	5.2 ± 1.0	4.2 ± 2.2	4.5 ± 1.4
Sweetness	1	4.2 ± 1.6	4.2 ± 2.0	4.0 ± 1.5	3.8 ± 1.8
	4	4.7 ± 1.0	4.3 ± 1.6	3.8 ± 2.4	3.0 ± 2.3
	7	4.0 ± 1.9	5.2 ± 1.3	2.2 ± 1.5	2.5 ± 1.6
Acidity	1	1.5 ± 1.2	1.7 ± 1.2	1.7 ± 0.8	2.2 ± 1.0
	4	2.2 ± 1.5	2.2 ± 1.0	2.3 ± 2.0	2.2 ± 1.3
	7	2.3 ± 1.2	1.8 ± 1.0	2.2 ± 1.2	2.3 ± 1.6
Bitterness	1	1.5 ± 0.8	1.3 ± 0.5	2.7 ± 2.0	2.3 ± 1.8
	4	2.3 ± 1.8	2.3 ± 1.4	2.2 ± 1.9	1.8 ± 1.2
	7	3.0 ± 2.1	2.0 ± 1.3	2.2 ± 1.5	2.3 ± 1.4
Flavour	1	5.0 ± 0.6	4.5 ± 0.8	4.0 ± 1.1	5.0 ± 1.3
	4	4.5 ± 1.0	5.0 ± 1.1	4.3 ± 1.2	4.5 ± 1.0
	7	4.8 ± 0.8	5.5 ± 0.8	4.7 ± 1.7	5.0 ± 1.4
Texture	1	6.7 ± 1.2	6.7 ± 1.4	6.5 ± 1.0	6.8 ± 1.5
	4	6.5 ± 1.5	6.7 ± 1.5	5.3 ± 1.4	5.7 ± 1.6
	7	6.2 ± 1.2	7.0 ± 0.6	5.3 ± 1.8	6.5 ± 1.0
Appearance	1	7.5 ± 0.8	7.2 ± 1.2	6.5 ± 0.8	6.3 ± 1.0
	4	6.5 ± 1.4	6.8 ± 1.2	5.7 ± 2.2	5.5 ± 2.2
	7	6.5 ± 1.4	7.2 ± 1.3	6.0 ± 1.5	5.7 ± 2.0
Total Quality	1	7.0 ± 1.1	6.7 ± 1.5	6.2 ± 1.9	6.7 ± 1.8
	4	6.5 ± 1.4	5.2 ± 1.9	6.0 ± 1.4	5.7 ± 1.8
	7	5.7 ± 1.9	6.8 ± 1.2	4.5 ± 2.0	5.3 ± 1.9

[1] data shown is the mean from 2 trials ± standard deviation.

3.2.3.2. Iceberg lettuce, carrots and spinach

In all cases, no significant differences (p=1/3) were found in the results obtained for the triangular test neither at day 0 nor day 7. This fact means that at the beginning of the study, the panelists were not able to differentiate between the non-irradiated and the irradiated vegetable samples.

Table 4 shows the effect of irradiation (5 D_{10} dose) in the sensorial parameters of iceberg lettuce, carrots, spinach, and mixed vegetable salads.

The central anchor of the 10-cm structured scale corresponded to the non-irradiated sample (control). A score of 5.0 was assigned to this control, which was stored during 7 days, under the same conditions as the irradiated samples. The evaluation of the different sensory parameters was carried out by comparing the irradiated with the non-irradiated samples.

At the end of the study, better scores than the control were achieved for appearance, color, aroma, texture and total quality in carrots. Lower scores than the control were obtained for bitterness, which is considered to be good. Only flavor and sweetness were slightly diminished in respect to the non-irradiated sample. The method used to discriminate among the means was Fisher's least significant difference procedure. According to this, only the parameter appearance did not show significant differences during day 0 and the other days (3 and 7). The rest of the sensory attributes differs significantly on different days.

In iceberg lettuce, better scores than the control were observed for appearance, color, aroma, flavor and total quality. Sweetness was found slightly increased over the control score. For texture, the achieved value was slightly below the score 5.0. By using Fisher's least significant difference procedure, in all the assayed parameters significant differences amongst days were found.

The results for the irradiated samples of spinach, obtained from the trained panel, were generally close to the control. Considering that the spinach leaves were irradiated with a higher dose than that used for lettuce and carrots, no damage in the sensory parameters was observed.

Results obtained from Fisher's least significant difference procedure, showed that in all the assayed parameters significant differences amongst days were found.

When comparing the results obtained for the three vegetables, it can be observed that in most cases lettuce presented better scores than carrots and spinach. The 5 D_{10} doses used to irradiate did not produce significant changes that could be considered as spoilage of their sensory characteristics, neither at day 0 nor at the end of the refrigerated storage.

TABLE 4. EFFECT OF A 5 D^{10} DOSE ON THE SENSORY PARAMETERS OF THE VEGETABLES

Parameter	Day	Iceberg lettuce	Carrots	Spinach	Toscana Salad		Four Seasons Salad	
		1.1 kGy	1.0 kGy	1.6 kGy	0.45 kGy	1.0 kGy	0.45 kGy	1.0 kGy
Color	0	5.0 ± 0.9[1]	4.6 ± 1.3	4.4 ± 1.7	4.7 ± 0.8	4.5 ± 1.1	5.5 ± 1.8	4.8 ± 2.1
	3	4.7 ± 1.1	5.5 ± 1.3	4.9 ± 0.4	5.0 ± 1.5	5.2 ± 0.6	4.9 ± 0.6	4.7 ± 1.5
	7	6.2 ± 1.0	5.4 ± 1.8	4.3 ± 1.6	5.5 ± 1.5	5.8 ± 1.1	5.3 ± 1.8	5.3 ± 2.0
Aroma	0	4.9 ± 1.3	5.8 ± 1.1	4.8 ± 1.9	4.7 ± 1.1	5.0 ± 1.6	4.3 ± 1.4	4.2 ± 2.0
	3	5.0 ± 1.1	4.6 ± 1.9	5.2 ± 1.3	4.4 ± 1.7	4.1 ± 1.2	4.4 ± 1.2	4.9 ± 1.6
	7	5.4 ± 0.7	5.6 ± 1.5	4.6 ± 1.1	3.9 ± 1.6	4.4 ± 1.5	4.8 ± 1.6	5.2 ± 2.1
Sweetness	0	4.9 ± 0.5	5.1 ± 1.3	4.3 ± 2.2	5.3 ± 1.2	4.3 ± 1.6	5.0 ± 1.9[a]	4.7 ± 2.2
	3	4.8 ± 1.2	4.8 ± 1.2	4.7 ± 0.9	4.9 ± 0.9	4.6 ± 2.0	4.6 ± 1.2[a]	4.4 ± 1.1
	7	5.1 ± 1.3	4.7 ± 1.4	4.9 ± 0.9	4.4 ± 1.2	4.7 ± 1.3	4.6 ± 1.2[a]	5.3 ± 1.6
Bitterness	0	4.5 ± 1.2	2.2 ± 2.3	4.4 ± 2.1	3.6 ± 2.1[a]	3.7 ± 1.8[a]	4.1 ± 2.1	3.7 ± 2.0
	3	4.7 ± 1.6	4.3 ± 1.2	5.0 ± 1.1	4.2 ± 1.5[a]	3.7 ± 1.8[a]	4.4 ± 1.3	4.9 ± 1.6
	7	4.8 ± 0.7	3.7 ± 1.8	5.1 ± 0.9	3.6 ± 1.8[a]	3.7 ± 2.1[a]	5.3 ± 1.3	5.0 ± 1.4
Flavor	0	5.0 ± 0.7	5.4 ± 1.3	4.7 ± 1.4	4.9 ± 1.0[a]	4.6 ± 1.3	5.1 ± 1.3[a]	4.7 ± 1.9
	3	4.7 ± 1.2	4.7 ± 1.2	4.9 ± 0.7	4.9 ± 1.7[a]	4.9 ± 1.0	5.1 ± 1.1[a]	5.0 ± 1.2
	7	5.1 ± 0.8	4.5 ± 1.7	5.0 ± 1.4	5.2 ± 1.4[a]	4.8 ± 1.5	5.4 ± 1.0[a]	5.5 ± 1.6
Texture	0	5.0 ± 0.5	6.0 ± 1.2	4.4 ± 1.2	4.8 ± 0.8	4.9 ± 0.9[a]	5.1 ± 1.3	4.4 ± 1.2
	3	4.8 ± 1.2	5.0 ± 0.8	4.3 ± 1.2	5.0 ± 1.6	5.7 ± 1.3[a]	4.9 ± 1.2	4.0 ± 1.5
	7	4.9 ± 0.6	5.2 ± 1.1	4.7 ± 1.4	5.7 ± 1.2	5.7 ± 0.9[a]	5.2 ± 1.5	5.2 ± 1.7
Appearance	0	5.1 ± 0.8	5.3 ± 1.3[a]	4.6 ± 1.0	5.1 ± 1.1	4.8 ± 1.6	5.6 ± 1.8	4.6 ± 1.9
	3	4.7 ± 1.1	5.7 ± 1.2[a]	5.2 ± 0.7	4.9 ± 1.3	5.2 ± 0.6	5.0 ± 1.0	4.4 ± 1.5
	7	6.2 ± 1.0	5.3 ± 1.4[a]	4.2 ± 1.5	5.2 ± 1.7	6.0 ± 1.5	5.4 ± 1.8	5.5 ± 2.1
Total Quality	0	5.1 ± 0.7	5.6 ± 1.3	4.9 ± 1.3	5.0 ± 0.8	4.9 ± 1.2	4.9 ± 1.6[a]	4.6 ± 1.5
	3	4.8 ± 1.2	5.3 ± 1.2	4.7 ± 0.6	5.2 ± 1.2	5.2 ± 0.9	4.9 ± 0.7[a]	4.3 ± 1.4
	7	5.6 ± 0.9	5.1 ± 1.4	4.7 ± 1.6	5.9 ± 1.1	5.9 ± 0.8	5.2 ± 1.1[a]	4.9 ± 1.5

* means within a column bearing the same letter are not significantly different ($p \geq 0.05$)

[1] data shown is the mean from 2 trials ± standard deviation.

3.2.3.3. Toscana and Four Seasons salads

In both cases, no significant differences (p=1/3) were found in the results obtained from the triangular test neither at day 0 nor 7. This means that at the start of the study, the panelists were not able to differentiate between the non-irradiated and the irradiated vegetal samples.

As in the case of the other vegetables, the central anchor of the 10-cm structured scale corresponded to the non-irradiated sample (control). A score of 5.0 was assigned to this control, which was stored during seven days, under the same conditions as the irradiated samples.

At the end of the study, the Toscana salad irradiated with a 5 D_{10} dose for *Listeria*, scored better than the control that was achieved for color, flavor, sweetness, bitterness, texture, appearance and total quality. A slightly lower score than the control was obtained for aroma. It must be considered that in the case of bitterness and sweetness, lower scores than those obtained for the control mean better characteristics. The method used to discriminate among the means was Fisher's least significant difference procedure. According to this, no significant differences were observed for bitterness and texture. The rest of the evaluated parameters showed significant differences amongst day 0 and the other days (3 and 7).

After the seven days of storage of the Four Seasons salad irradiated with a 5 D_{10} dose for *Listeria*, better scores than the control were achieved for color, flavor, aroma, texture, appearance and total quality. Bitterness was evaluated with the same score as the control and sweetness presented a slightly stronger intensity than the non-irradiated salad. All the evaluated parameters showed significant differences amongst day 0 and the other days (3 and 7).

When irradiating Toscana salad with the 5 D_{10} dose for *E. coli* O157:H7, better scores than the control were observed for color, flavor, sweetness, bitterness, texture, appearance and total quality at the end of the refrigerated storage. A lower score than the control was obtained for aroma. No significant differences were observed for bitterness and flavor. The rest of the evaluated parameters showed significant differences amongst day 0 and the other days (3 and 7).

On day 7 of refrigerated storage of the Four Seasons salad irradiated with the 5 D_{10} dose for *E. coli* O157:H7, better sensory characteristics than the control were achieved for color, flavor, sweetness, texture, appearance and total quality. A slightly lower score than the control was obtained for aroma. Bitterness presented a slightly stronger intensity than the non-irradiated salad. No significant differences were observed for sweetness, flavour and total quality. The rest of the evaluated parameters showed significant differences amongst day 0 and the other days (3 and 7).

Irradiating the Toscana and Four Seasons salads with the 5 D_{10} doses, calculated for both strains, did not significantly modify the sensory parameters of the studied vegetables with respect to the control, after the seven days of refrigerated storage. Most of the evaluated parameters presented better scores than the control.

Control samples of the minimally processed vegetables and mixed salads studied presented high levels of total plate and *Enterobacteriaceae* counts. In most cases, they did not meet the specifications established in the Chilean legislation. Nevertheless, no presence of *E. coli, Salmonella* spp., *Listeria* spp. and *E. coli* O157:H7 was detected in the corresponding assayed samples.

Similar D_{10} values for two different strains of *E. coli* were obtained in celery and cabbage (0,18 - 0,23 kGy). The same situation was observed for *Listeria innocua* in carrots, iceberg lettuce, Toscana and Four Seasons salads (0,19 0,22 kGy), but a higher D_{10} was obtained for the same microorganism, when inoculated in spinach (0,32 kGy). *E. coli* O157:H7 showed a greater radio sensibility, presenting in both mixed salads a D_{10} value of 0.09±0.01 kGy.

A reduction in 5 logs was obtained, when irradiating the vegetables (iceberg lettuce, carrots, spinach, and mixed vegetable salad) inoculated with the target microorganism (*Listeria innocua* and *E. coli* O157:H7) with 5 D_{10} doses.

At the end of the seven days of refrigerated storage of the non-irradiated vegetables, an increase in TPC and *Enterobacteriaceae* counts, was observed. Refrigeration did not prevent the increase of these microbiological parameters.

However, the irradiated vegetables maintained the level of the microflora within the limits established by the Chilean legislation, during the same period of storage.

Irradiation of the studied vegetables with 5 D_{10} dose, practically did not modify the sensory characteristics of the products. In most cases an improvement of the sensory parameters compared with the non-irradiated samples, was observed.

4. CONCLUSIONS

1. Control samples of minimally processed vegetables and mixed salads studied, presented high levels of total plate and *Enterobacteriaceae* counts. In most cases, they did not meet the specifications established in the Chilean legislation. Nevertheless, no presence of *E. coli, Salmonella* spp., *Listeria* spp. and *E. coli* O157:H7 was detected in the corresponding assayed samples.

2. Similar D_{10} values for two different strains of *E. coli* were obtained in celery and cabbage (0,18 - 0,23 kGy). The same situation was observed for *Listeria innocua* in carrots, iceberg lettuce, Toscana and Four Seasons salads (0,19 - 0,22 kGy), but a higher D_{10} was obtained for the same microorganism, when inoculated in spinach (0,32 kGy). *E. coli* O157:H7 showed a greater radio sensibility, presenting in both mixed salads a D_{10} value of 0.09±0.01 kGy.

3. A reduction in 5 logs was obtained, when irradiating the vegetables (iceberg lettuce, carrots, spinach, and mixed vegetable salad) inoculated with the target microorganism (*Listeria innocua* and *E. coli* O157:H7) with 5 D_{10} doses.

4. At the end of the seven days of refrigerated storage of the non irradiated vegetables, an increase in TPC and *Enterobacteriaceae* counts, was observed. Refrigeration did not prevent the increase of these microbiological parameters. However, the irradiated vegetables maintained the level of the microflora within the limits established by the Chilean legislation, during the same period of storage

5. Irradiation of the studied vegetables with 5 D_{10} dose, practically did not modify the sensory characteristics of the products. In most cases an improvement of the sensory parameters compared with the non irradiated samples, was observed.

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REFERENCES

[1] CANADIAN FOOD INSPECTION AGENCY FOOD OF PLANT ORIGIN DIVISION. Code of Practice for Minimally Processed Ready-to-Eat Vegetables. http://www.inspection.gc.ca/english/plaveg/fresh/read-eat_e.shtml. Access in June, 2005.

[2] FOOD SAFETY AND HYGIENE. A bulletin for the Australian Food Industry, 2000.

[3] ICMSF (International Commission on Microbiological Specifications for Foods), Microorganisms in foods. 1. Their significance and methods of enumeration. 2nd Ed. University of Toronto Press, 1978 p. 118.

[4] ISO 7402:1993 (E), Microbiology – General guidance for the enumeration of Enterobacteriaceae without resuscitation – MPN technique and colony-count technique.

[5] BACTERIOLOGICAL ANALYTICAL MANUAL (BAM) on line (2001). Food and Drug Administration. http://www.cfsan.fda.gov/~ebam/bam-toc.html. Access in June 2005.

[6] GARBUTT J., Essentials of Food Microbiology. London: Ed. Arnold; 1997. p. 113.

[7] ADAMS, M.R., MOSS, M.O. Food Microbiology. Cambridge: The Royal Society of Chemistry; 1995. p. 76.

[8] DOYLE, M., BEUCHAT, L., MONTVILLE, T. Microbiología de los Alimentos. Fundamentos y Fronteras. Zaragoza: Ed. Acribia; 1997. p. 533.

[9] NIEMIRA, B., FAN, X., SOKORAI, K., SOMMERS, C. Ionizing Radiation Sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria innocua* ATCC 51742 Inoculated on Endive (*Cichorium endiva*), J. Food Prot. **66** (2003) 993-998.

[10] FEDERIGHI M. AND THOLOZAN J.L. Traitments ionisants et hautes pressions des aliments. Paris: Ed. Economica; 2001. p. 59.

[11] OLSSON D.G. Irradiation of food. IFT Expert Panel on Food Safety and Nutrition, Food Techn **52(1)** (1998) 56-62.

[12] ICMSF (International Commission on Microbiological Specifications for Foods), Microbiological Specifications of Food Pathogens. London: Published by Blackie Academic & Professional; 1996. p. 135.

[13] BUCHANAN, R.L., EDELSON, S.G., BOYD, G. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic Escherichia coli, J. Food Prot **62(3)** (1999) 219-228.

[14] ICMSF (International Commission on Microbiological Specifications for Foods), Microbial Ecology of Foods. Volume 2. Food Commodities. Academic Press, New York. 1980 p. 606.

[15] MINISTRY OF HEALTH. Nuevo Reglamento Sanitario de los Alimentos. Santiago de Chile: Ediciones Publiley; 2000. p. 89.

[16] PRAKASH, A., INTHAJAK, P., HUIBREGTSE, F., CAPORASO, F., FOLEY D.M. Effects of Low-dose Gamma Irradiation and Conventional Treatments on Shelf Life and Quality Characteristics of Diced Celery, J. Food Sci. **65(6)** (2000a) 1070-1075.

[17] FARKAS, J., SÁRAY, T., MOHÁCSI-FARKAS, C., HORTI, K., ANDRÁSSY E. Effects of Low-dose Gamma Radiation on Shelf-life and Microbiological Safety of Pre-cut/Prepared Vegetables, Advances in Food Science **19(3/4)** (1997) 111-119.

[18] PRAKASH, A., GUNER, A., CAPORASO, F., FOLEY, D. Effects of Low-dose Gamma Irradiation on the Shelf Life and Quality Characteristics of cut Romaine Lettuce Packaged under Modified Atmosphere. J. Food Sci **65(3)** (2000b) 549-553.

[19] FAN, X., NIEMIRA, B.A., SOKORAI, K.J.B. Use of ionizing radiation to improve sensory and microbiological quality of fresh-cut green onion leaves, J. Food Sci. **68(4)** (2003) 1478 – 1483.

USE OF IRRADIATION TO ENSURE HYGIENIC QUALITY OF FRESH PRE-CUT AND BLANCHED VEGETABLES AND TOFU

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Abstract

Cherry tomato (*Lycopersicon esculentum* var. cerasiform Dun) and fresh pre-cut carrot (*Daucus carota* L.), were selected as experiment materials. Additionally, packaged tofu and a mixture of blanched vegetables were also studied. D_{10}-values of selected pathogens – *E. coli* 0157:H7, *Listeria innocua* and *Salmonella* Enteritidis - and also, the effects of irradiation on microbiological, nutrient and sensory qualities of the products mentioned were studied. D_{10}-values of *E. coli* O157:H7 inoculated in cherry tomato and fresh pre-cut carrot were 0.08 kGy and 0.13 kGy, respectively; D_{10}-values of *Salmonella* Enteritidis inoculated in cherry tomato, fresh pre-cut carrot, a mixture of blanched celery and peanut and packaged tofu were in the range of 0.24 kGy to 0.33 kGy. D_{10} -values of *Listeria innocua* inoculated into a mixture of blanched mustard and soybean, a mixture of blanched almond and carrot, a mixture of blanched celery and peanut and packaged tofu were between 0.22 kGy and 0.29 kGy. Irradiation could control the growth of pathogens during storage period. Irradiation at doses lower than 2.0 kGy did not have an important effect on colour (Hunter values), nutritional quality (Soluble Solids Content (SSC), Cell Membrane Permeability (CMP) and sensory quality of pre-cut carrot and cherry tomato stored under room and refrigerated temperatures. Sensory quality, content of crude protein and amino acid in the mixture of blanched celery and peanut, and content of crude protein, amino acid, crude fat in packaged tofu was not significantly affected by doses lower than 2 kGy. Treatment with doses less than 2.0 kGy might be applied to ensure the hygienic quality of the products studied.

1. INTRODUCTION

There is a highly popular trend in advanced and many developing countries to consume fresh pre-cut vegetables and fruits and other minimally processed food of plant origin. In the past decades, a number of large food-borne disease outbreaks involving up to thousands of cases and many deaths attributable to consumption of fresh, pre-cut and minimally processed produce were reported in several countries [1][2]. Several types of pathogenic bacteria were responsible for these outbreaks; these pathogens widely exist in many produces.

With the development of the Chinese economy and increase in the living standard of the Chinese people, the consumption of fresh pre-cut vegetables and other minimally processed food of plant origin and ready-to-eat foods has become more and more common and popular. These foods usually contain large numbers of spoilage microorganisms as well as possible pathogens.

Ionizing radiation is increasingly recognized as an effective method to ensure the microbiological safety of food. As one of the cold physical sterilization methods, irradiation can play an important role in case of heat sensitive food products [3, 4, 5]. However, little is known about the effect of irradiation on the sensory quality of fresh pre-cut vegetables, packaged tofu and a mixture of blanched vegetables.

Cherry tomatoes and fresh, pre-cut carrots were selected as the research materials. Additionally, a mixture of blanched vegetables and packaged tofu were included in the study. *Listeria innocua*, *Salmonella* Enteritidis and *E. coli* O157:H7 were selected as representatives of pathogens in this project.

The objective of this project was to investigate the use of the irradiation technology to ensure the microbiological safety and acceptability in the sensory and/or nutrient quality of fresh pre-cut vegetables, packaged tofu and the mixture of blanched vegetables.

2. MATERIALS AND METHODS

2.1. Materials

Cherry tomato, pre-cut carrot, packaged tofu and a mixture of blanched vegetables (called Chinese salad) were obtained from local supermarkets. Cherry tomato, pre-cut carrot and the mixture of blanched vegetables in polypropylene tray were covered by PVDC film. Each sample was about 230-250g.

The Chinese mixture of blanched vegetables contains different kinds of vegetables. A mixture of blanched almond, a mixture of blanched mustard and soybean, and a mixture of blanched celery and peanut were selected as experiment material. Soybean and mustard are usually macerated for about 1-2 hours in water at 50ºC, than boiled in water for about 20-30 min at 100ºC. The green vegetables were boiled for 3-5 min at 100ºC, and then cooled in tap water. Finally, blanched vegetables were salted, mixed with spices and sent to the supermarket.

Listeria innocua L83 and Salmonella *Enteritidis* 500041 were brought from the Sanitation and Antiepidemic Station, the Ministry of Public Health, China. *E. coli* O157:H7 882364 was obtained from Institute for Epidemic Disease, the Ministry of Public Health, China.

2.2. Sample preparation

2.2.1. D_{10} value measurement

Samples of cherry tomato and pre-cut carrot were sanitized by immersion in a solution of 300 ppm sodium hypochlorite for three minutes, rinsing with sterile distilled water. Samples of packaged tofu and a mixture of blanched vegetables were sanitized with irradiation (14 kGy). The pure cultures of pathogens were inoculated into 250 ml nutrient broth and grown at 37°C for about 12 to18 hours. Cell density was typically 10^8 cfu/ml.

Selected products were submerged in the solution of pathogens (containing 10^8 cfu/ml bacteria) for 10 minutes and then dried under laminar flow cabinet. The samples of cherry tomato, pre-cut carrot, and the mixture of blanched vegetables were fractionated (25g) and packaged in sterile bags and irradiated.

Portions of packaged tofu, 10 g each, were placed in sterile bags, then 1 ml of pathogen inoculum was injected into the sample and blended together, and then immediately irradiated.

2.2.2. Microbiological, nutrient and sensory analysis

Cherry tomato, pre-cut carrot, and packaged tofu were analysed microbiologically,sensorially and for nutrient content. Blanched vegetables were analysed sensorially and microbiologically except for the blanched celery and peanut (mixture of 100 g celery and 40 g peanut) which were analysed for nutrient content.

2.3. Methods

2.3.1. Irradiation

The irradiation process for pathogens was applied using a research Co-60 irradiator at the Institute for Application of Atomic Energy, Chinese Academy of Agricultural Sciences.

The irradiation doses applied to cherry tomato and pre-cut carrot samples artificially contaminated with *E. coli* 0157:H7 were 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 kGy.

The irradiation doses applied to cherry tomato and pre-cut carrot samples artificially contaminated with *S*. Enteritidis were 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 kGy.

The irradiation doses applied to tofu and blanched vegetables artificially contaminated with *Listeria innocua* and *S*. Enteritidis were 0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy.

All quality analysis and sensory evaluation of tomato and pre-cut carrot were conducted at the Institute of Nuclear-Agricultural, Chinese Academy Sciences. The irradiation doses were o.0, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy.

For storage, quality analysis and sensory evaluation of packaged tofu and the mixture of blanched vegetables the irradiation doses were 0.0, 1.0, 2.0 and 3.0 kGy.

2.3.1. Microbiological analysis

Total Bacterial Count (TBC), Coliform Bacteria, Yeasts and Molds, and pathogens (*Listeria* spp. and *Salmonella* spp.) were detected according to the microbiological analysis methods BAM (2001) [6].

2.3.2. Sensory Evaluation

Sensory properties of selected products were evaluated through a panel of nine trained persons using the scoring method, and scale of 1 to 9 (1=dislike extremely and 9=like extremely, above score of 5 was acceptability), evaluated parameters were color, odor, sweetness, flavor, test and acceptability [8, 9].

2.3.4. Analysis of quality of tomato and pre-cut carrot

After irradiation, the samples were stored at room temperature (16-18ºC) and at refrigerated temperature (4-7ºC). Samples from the various treatments were selected at random each time and analysed as follows:

Color measurement: Hunter Lab colorimeter was used for color evaluation on L-value (lightness, 100=white, 0=black), a-value (redness (+), greenness (-)), and b-value (yellowness (+) or blueness (-)).

Soluble Solids Content (SSC): Hand refractometer was used for determination soluble solids content.

Cell Membrane Permeability (CMP): Cell Membrane Permeability was measured by relative conductivity.

2.3.5. Protein and fat content of tofu and the mixture of blanched celery and peanut

Protein and fat content of the tofu and the mixture of blanched vegetables were analysed by the Feed and Food Analysis Center of Institute of Animal Science, Chinese Academy of Agricultural Science according to GB/T9695.11-1988, GB/T9595.7-1988.

2.4. Statistical Analysis

Data were processed by Statistical Analysis System (SAS, 2000). Mean values were reported, and the differences in the mean values were compared by ANOVA procedure and Duncan's Multiple Range Test, and differences were declared significant when $P \leq 0.05$.

3. RESULTS

3.1. The initial hygienic quality of selected products

The results of the initial quality of the selected products are presented in Table 1. Cherry tomato samples were most seriously contaminated, and packaged tofu had the best microbiological quality. *Listeria* spp. and *Salmonella* spp. were not detected in any of the selected samples.

TABLE 1. INITIAL HYGIENIC QUALITY OF VEGETABLE PRODUCTS (N=10)

Product	TBC (cfu/g)	Coliform count (MPN/100g)	Yeasts and Molds (cfu/g)	*Listeria* spp. in 25g	*Salmonella* spp. in 25g
Cherry tomato	7.6×10^{6}	2.8×10^{3}	3.2×10^{2}	absence	absence
Pre-cut carrot	3.8×10^{4}	6.4×10^{2}	1.1×10^{2}	absence	absence
Tofu	7.2×10^{3}	$\leq 4.0\times10^{1}$	<10	absence	absence
Blanched mustard and soybean	7.1×10^{5}	3.7×10^{2}	2.7×10^{1}	absence	absence
Blanched almond	4.8×10^{5}	3.1×10^{2}	<10	absence	absence
Blanched celery and soybean	5.0×10^{5}	6.0×10^{2}	2.3×10^{1}	absence	absence

The high contamination of "blanched vegetables" may be the result of the addition of spices (non-treated) after the treatment and before the packaging.

3.2. Determination of D_{10}-values of pathogens in selected products

Results of experimental D_{10}-values are summarized in Table 2.

TABLE 2. D10-VALUES OF EXAMINED PATHOGENIC BACTERIA IN PRODUCTS STUDIED

Pathogens	Medium	Average of D_{10} values (kGy)	R^2
E. coli O157H:7	Tomato.	0.08	0.9695
	Carrot	0.13	0.9701
Salmonella Enteritidis	Tomato	0.24	0.9784
	Carrot	0.33	0.9697
	Blanched vegetables (celery and peanut)	0.28	0.9891
	Packaged tofu	0.24	0.9636
Listeria innocua	Blanched vegetables (blanched mustard green and soybean)	0.26	0.9919
	Blanched vegetables (blanched almond)	0.24	0.9682
	Blanched vegetables (celery and peanut)	0.29	0.9887
	Packaged tofu	0.22	0.9938

Irradiation with doses less than 2.0 kGy were sufficient to improve the safety of the products studied. These results agree with other data published by other authors [1, 7, 10, 11].

3.3. Effect of irradiation on the quality of pre-cut carrot stored at different conditions

3.3.1. Effect of irradiation on color (Hunter values)

L-value (lightness in color), a-value (color hue, red arising at a > 0) and b-value (chroma in color) were determined in samples stored at room temperature (0 day, 3 days and 7 days after irradiation) and at refrigerated temperature (0 day, 4 days and 10 days after irradiation). The results are presented in Tables 3 and 4.

TABLE 3. EFFECT OF IRRADIATION ON THE HUNTER VALUES OF CARROT STORED AT ROOM TEMPERATURE

Treatment	Hunter Values								
	0 day			3 days			7 days		
kGy	L	a	b	L	a	b	L	a	b
0.0	61.75^{b*}	38.94^{a}	36.71^{a}	62.55^{d}	29.89^{c}	35.57^{a}	64.37^{a}	32.36^{c}	36.29^{a}
1.0	63.20^{ab}	36.02^{abc}	35.13^{a}	64.12^{c}	32.55^{a}	33.32^{a}	65.85^{a}	37.15^{a}	37.15^{a}
1.5	64.61^{a}	37.14^{ab}	35.49^{a}	64.96^{ab}	30.16^{bc}	34.33^{a}	65.81^{a}	33.44^{bc}	36.14^{a}
2.0	65.25^{a}	33.83^{bcd}	36.06^{a}	65.65^{a}	32.89^{a}	34.98^{a}	69.23^{a}	32.75^{c}	35.44^{a}
2.5	64.81^{a}	34.49^{bcd}	35.24^{a}	65.63^{a}	32.48^{a}	33.28^{a}	66.22^{a}	36.98^{a}	36.03^{a}
3.0	65.16^{a}	33.57^{cd}	35.59^{a}	64.66^{bc}	32.46^{ab}	34.35^{a}	66.27^{a}	33.67^{ab}	36.51^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

Immediately after irradiation and at room temperature storage, the L-values of irradiated carrot samples were significantly higher than that of control samples, but there was no significant difference between the irradiated samples. The results were consistent with previously reported results [8]. The a-values decreased with increase of irradiation doses, and irradiation doses had significant effect on it. The b-values were not significantly different.

TABLE 4. EFFECT OF IRRADIATION ON HUNTER VALUES OF CARROT STORED AT REFRIGERATED TEMPERATURE (4-5°C)

Treatment	Hunter Values								
	0 day			4 days			10 days		
kGy	L	a	b	L	a	b	L	a	b
0.0	61.75^{b*}	38.94^{a}	36.71^{a}	66.23^{a}	33.04^{a}	36.79^{a}	63.52^{b}	33.98^{a}	34.47^{a}
1.0	63.20^{ab}	36.02^{abc}	35.13^{a}	66.93^{a}	33.15^{a}	34.53^{a}	64.9^{ab}	35.18^{a}	33.67^{a}
1.5	64.61^{a}	37.14^{ab}	35.49^{a}	66.93^{a}	33.07^{a}	34.98^{a}	64.43^{ab}	34.41^{a}	34.43^{a}
2.0	65.25^{a}	32.83^{d}	36.06^{a}	66.87^{a}	32.8^{a}	35.39^{a}	64.29^{ab}	35.95^{a}	34.62^{a}
2.5	64.81^{a}	34.49^{bcd}	35.24^{a}	67.26^{a}	32.21^{a}	35.37^{a}	65.61^{a}	34.57^{a}	33.66^{a}
3.0	65.16^{a}	33.57^{cd}	35.59^{a}	67.38^{a}	32.08^{a}	35.23^{a}	64.99^{ab}	33.55^{a}	34.21^{a}

*Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

After four days of storage at refrigerated temperature, the L-values of both the control and the irradiated samples were significantly higher than that of the unirradiated control on the day of treatment (0 day). There was no significant difference in a-values and b-values compared to the unirradiated samples during 10 days of refrigerated storage.

3.3.2. Effect of irradiation on soluble solids content (SSC)

The SSC of the samples tested are shown in Table 5. There was no significant difference of SCC of the samples under any conditions, except after three days of storage at room temperature. The SSC of samples irradiated with doses of 1.5 kGy and 2.0 kGy were significantly lower than in the case of control samples. The SSC of samples at refrigerated temperature was higher than at room temperature.

TABLE 5. EFFECT OF IRRADIATION ON THE SOLUBLE SOLIDS CONTENT (%) OF CARROT AT DIFFERENT TEMPERATURE

Treatment	Room temperature			Refrigerated temperature	
(kGy)	0 day	3rd day	7th day	4th day	10th day
0.0	7.64[a*]	7.83[a]	7.75[a]	10.00[a]	9.10[a]
1.0	7.48[a]	7.57[ab]	7.75[a]	8.93[a]	10.10[a]
1.5	7.53[a]	6.53[b]	7.56[a]	8.90[a]	10.60[a]
2.0	7.59[a]	6.50[b]	7.50[a]	9.83[a]	10.00[a]
2.5	7.47[a]	7.77[a]	8.00[a]	10.20[a]	10.30[a]
3.0	7.63[a]	7.23[ab]	7.47[a]	10.00[a]	10.40[a]

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

3.3.3. Effect of irradiation on Cell Membrane Permeability (CMP)

The results of cell membrane permeability at ambient and refrigerated temperatures, respectively, are given in Figures1 and 2.

The results in Fig.1 shows that CMP of samples, especially at the third day of storage decreased with increasing irradiation.

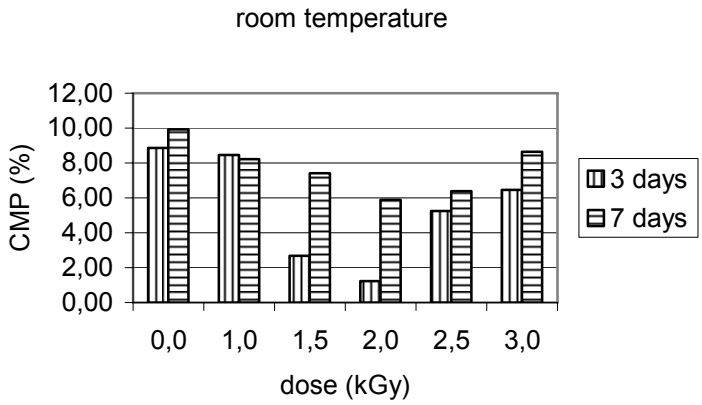

FIG. 1. Cell membrane permeability of carrot stored at room temperature after irradiation.

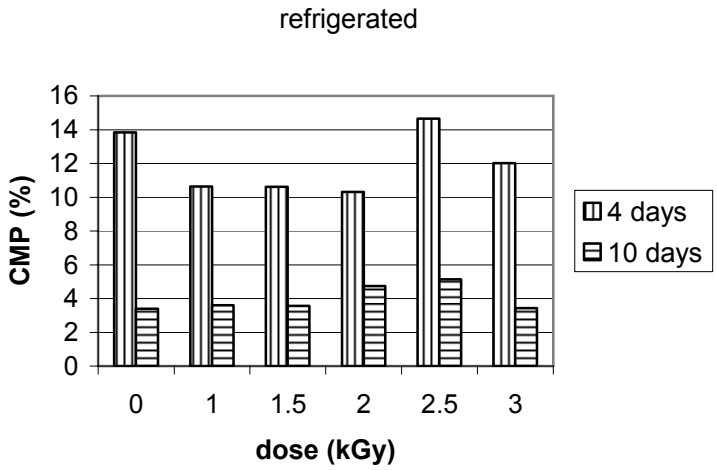

FIG. 2. Cell membrane permeability of carrot stored at refrigerated temperature after irradiation.

The results from Fig. 2 indicated that CMP of irradiated samples were lower than control samples (except for 2.5 kGy samples). No significant effect on CMP was observed in samples stored for 10 days.

3.3.4. Effect of irradiation on sensory attributes

The results of the sensory evaluation of carrot stored at room temperature for 3 days and 7 days indicated that irradiation with lower than 2.0 kGy had no significant effect on color, lightness, flavor, sweetness, odor, taste. During storage, overall acceptability of control samples was not higher than samples irradiated with doses below 2.0 kGy (data not shown).

The results of the sensory evaluation of carrot stored at refrigerated temperature for 4 days and 10 days indicated that color, lightness, odor, taste, sweetness and flavor of samples irradiated with lower than 2.0 kGy (included 2.0 kGy) were not significantly different from that of non-irradiated ones. Overall acceptability of samples irradiated with doses lower than 2.0 kGy was higher than that of non-irradiated samples (data not shown).

3.4. Effect of irradiation on quality parameters of cherry tomato stored at different temperatures

3.4.1. Effect of irradiation on the Hunter values

The Hunter colour values of cherry tomatoe samples stored at room temperature are presented in Tables 6-8.

TABLE 6. L-VALUE OF TOMATO STORED AT ROOM TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 days	2 days	4 days	6 days	8 days	10 days
0.0	31.52^{b*}	34.57^{a}	34.44^{b}	34.88^{a}	35.38^{a}	33.91^{a}
0.5	31.58^{b}	34.29^{a}	34.86^{b}	34.87^{a}	34.84^{a}	34.25^{a}
1.0	31.44^{b}	34.95^{a}	34.94^{b}	34.64^{a}	34.72^{a}	34.37^{a}
1.5	32.28^{ab}	34.23^{a}	34.68^{b}	34.79^{a}	34.76^{a}	33.66^{a}
2.0	32.08^{ab}	34.16^{a}	35.14^{a}	34.92^{a}	34.32^{a}	33.89^{a}
2.5	32.85^{a}	34.16^{a}	34.53^{b}	34.96^{a}	34.70^{a}	33.95^{a}
3.0	33.01^{a}	34.28^{a}	34.20^{b}	34.48^{a}	33.91^{a}	33.70^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

With regard to lightness (L-value) of cherry tomato (Table 6), an increase was observed in control samples during the storage at room temperature. Irradiation above 1.5 kGy caused significant increase in lightness, but there were no differences after six days of storage between control and irradiated samples.

The a-values of irradiated samples (above 0.5 kGy) were significantly higher than that of control samples (Table 7). Increase of redness of all samples was observed during 2-4 days storage at room temperature.

Irradiation with 0.5-3.0 kGy did not affect the b-value of tomato samples (Table 8). During storage, significant increase of b-value was observed both in irradiated and control samples.

TABLE 7. a-VALUE OF TOMATO STORED AT THE ROOM TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 days	2 days	4 days	6 days	8 days	10 days
0.0	24.91^{b*}	26.8^{c}	31.23^{a}	30.48^{a}	29.99^{a}	30.60^{a}
0.5	24.92^{b}	27.5^{bc}	30.86^{a}	30.80^{a}	31.13^{a}	29.22^{ab}
1.0	27.70^{ab}	27.53^{bc}	31.44^{a}	30.11^{a}	30.84^{a}	28.81^{b}
1.5	27.90^{ab}	30.33^{a}	28.92^{a}	30.81^{a}	30.81^{a}	28.51^{b}
2.0	28.84^{a}	29.37^{ab}	29.93^{a}	30.40^{a}	30.67^{a}	29.09^{ab}
2.5	27.68^{ab}	30.09^{a}	27.61^{b}	30.07^{a}	29.66^{a}	29.10^{ab}
3.0	27.68^{ab}	29.03^{ab}	28.20^{b}	29.22^{a}	29.67^{a}	28.53^{b}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

TABLE 8. b-VALUE OF TOMATO STORED AT THE ROOM TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 day	2 days	4 days	6 days	8 days	10 days
0.0	21.42^{a*}	21.51^{c}	24.00^{ab}	26.61^{a}	26.94^{b}	37.53^{ab}
0.5	20.68^{a}	22.46^{c}	24.68^{a}	26.64^{a}	29.25^{a}	35.85^{b}
1.0	23.43^{a}	24.37^{b}	24.98^{a}	26.61^{a}	26.14^{b}	37.62^{ab}
1.5	22.98^{a}	24.97^{ab}	23.30^{ab}	26.49^{a}	27.35^{ab}	36.97^{ab}
2.0	22.02^{a}	25.13^{ab}	23.69^{ab}	26.86^{a}	26.07^{b}	39.04^{a}
2.5	23.05^{a}	27.15^{a}	22.90^{b}	27.23^{a}	26.41^{b}	37.82^{ab}
3.0	23.20^{a}	27.66^{a}	22.81^{b}	26.26^{a}	25.81^{b}	38.17^{ab}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

Hunter color values of irradiated tomato samples stored at refrigerated temperature for 0, 2, 4, 6, 8, and 10 days are reported in Tables 9-11.

In general, lightness (L-value) and redness (a-value) of irradiated samples and control were not significantly different during storage at refrigerated temperature. The b-value of irradiated and control samples increased during 2-8 days of refrigerated storage.

TABLE 9. L-VALUE OF TOMATO STORED AT REFRIGERATED TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 days	2 days	4 days	6 days	8 days	10 days
0.0	31.52^{a*}	34.51^{a}	34.91^{ab}	33.99^{a}	34.14^{a}	34.76^{a}
0.5	31.58^{a}	34.39^{a}	34.70^{ab}	34.09^{a}	33.95^{a}	35.46^{a}
1.0	31.44^{a}	34.80^{a}	34.19^{b}	34.36^{a}	34.40^{a}	35.13^{a}
1.5	32.23^{a}	34.00^{a}	34.72^{ab}	33.95^{a}	34.40^{a}	35.01^{a}
2.0	32.08^{a}	34.27^{a}	35.14^{a}	34.45^{a}	35.12^{a}	34.80^{a}
2.5	32.58^{a}	34.12^{a}	34.27^{b}	34.38^{a}	34.13^{a}	34.43^{a}
3.0	32.01^{a}	34.34^{a}	34.55^{ab}	34.59^{a}	34.06^{a}	34.07^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

TABLE 10. a-VALUE OF TOMATO STORED AT REFRIGERATED TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 days	2 days	4 days	6 days	8 days	10 days
0.0	24.91^{b*}	31.38^{a}	31.59^{ab}	28.59^{a}	30.86^{b}	29.08^{a}
0.5	24.92^{b}	31.11^{a}	30.66^{ab}	27.85^{a}	30.61^{b}	29.33^{a}
1.0	27.70^{ab}	30.77^{a}	29.23^{b}	29.25^{a}	30.86^{b}	28.97^{a}
1.5	27.90^{ab}	30.29^{a}	31.05^{ab}	28.66^{a}	31.60^{a}	29.90^{a}
2.0	28.84^{a}	29.71^{a}	32.65^{a}	27.89^{a}	31.99^{a}	28.80^{a}
2.5	27.68^{ab}	29.61^{a}	32.94^{a}	29.46^{a}	30.93^{b}	28.77^{a}
3.0	27.68a^{b}	29.83^{a}	32.00^{a}	29.48^{a}	31.55^{a}	27.96^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

TABLE 11. b-VALUE OF TOMATO STORED AT REFRIGERATED TEMPERATURE AFTER IRRADIATION

Dose (kGy)	0 days	2 days	4 days	6 days	8 days	10 days
0.0	21.42^{a*}	26.11^{b}	28.63^{a}	24.30^{b}	28.29^{a}	25.73^{ab}
0.5	20.68^{a}	27.39^{ab}	27.11^{ab}	23.32^{b}	27.92^{a}	26.90^{a}
1.0	23.43^{a}	28.32^{a}	28.38^{a}	24.94^{b}	27.19^{a}	26.34^{ab}
1.5	22.98^{a}	27.07^{ab}	26.50^{b}	24.63^{b}	28.20^{a}	27.41^{a}
2.0	22.02^{a}	26.08^{b}	26.61^{b}	29.81^{a}	28.61^{a}	26.57^{ab}
2.5	23.05^{a}	27.13^{ab}	27.94^{ab}	25.55^{b}	27.06^{a}	26.70^{ab}
3.0	23.20^{a}	26.70^{ab}	27.20^{ab}	24.55^{b}	27.09^{b}	25.10^{b}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

Despite the fact that irradiated and control samples presented significant differences in L, a, and b values during the storage at room temperature, these differences were not detected by the sensorial panel. The overall acceptability of control samples was not higher than samples irradiated with doses below 2kGy. The samples irradiated with doses lower than 2 kGy were even better than the control samples

3.4.2. Effect of irradiation on the SSC of tomato at different temperatures

The results of SSC of tomato stored at different temperatures for 0, 2, 4, 6, 8 and 10 days after irradiation are given in Tables 12 and 13.

TABLE 12. EFFECT OF IRRADIATION ON THE SOLUBLE SOLID CONTENT (%) OF TOMATO STORED AT ROOM TEMPERATURE

Doses (kGy)	Storage (days)					
	0	2	4	6	8	10
0.0	7.34^{b*}	7.10^{a}	7.14^{a}	6.79^{a}	7.70^{a}	6.89^{a}
0.5	7.47^{ab}	6.83^{b}	6.77^{a}	6.66^{a}	7.80^{a}	6.99^{a}
1.0	7.40^{b}	6.99^{b}	6.80^{a}	6.51^{a}	7.66^{a}	7.2^{a}
1.5	7.45^{ab}	6.80^{b}	6.97^{a}	6.63^{a}	7.49^{ab}	6.86^{a}
2.0	7.27^{b}	6.96^{b}	6.75^{a}	6.53^{a}	7.40^{ab}	7.04^{a}
2.5	7.45^{ab}	7.15^{b}	6.78^{a}	6.59^{a}	7.23^{b}	6.95^{a}
3.0	7.64^{a}	7.02^{b}	7.10^{a}	6.32^{b}	7.30^{b}	6.85^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

Results of the samples stored at room temperature indicated that SSC of non-irradiated samples was similar compared with that of the irradiated ones.

TABLE 13. EFFECT OF IRRADIATION ON THE SOLUBLE SOLID CONTENT (%) OF TOMATO STORED AT REFRIGERATED TEMPERATURE

Dose (kGy)	Storage (days)					
	0	2	4	6	8	10
0.0	7.34^{b*}	6.98^{a}	7.06^{ab}	6.83^{b}	7.37^{b}	7.33^{b}
0.5	7.47^{ab}	7.04^{a}	6.65^{b}	7.07^{b}	7.61^{b}	7.44^{b}
1.0	7.40^{b}	7.07^{a}	7.00^{ab}	7.09^{b}	7.62^{b}	7.20^{b}
1.5	7.45^{ab}	7.02^{a}	6.59^{b}	6.93^{b}	7.79^{ab}	7.49^{b}
2.0	7.27^{b}	6.98^{a}	6.68^{b}	7.20^{b}	7.58^{b}	7.31^{b}
2.5	7.45^{ab}	7.02^{a}	6.70^{b}	7.54^{a}	7.97^{a}	7.83^{a}
3.0	7.64^{a}	7.02^{a}	7.24^{a}	7.84^{a}	7.90^{a}	7.68^{a}

*Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

Results of samples stored at refrigerated temperature after irradiation indicated that after six days storage the SSC of irradiated samples (with doses higher than 2.0 kGy) was significantly higher from that of the non-irradiated samples.

3.4.3. Effect of irradiation on the sensory quality of tomato stored at different temperatures

Color, taste, sweetness and flavor of tomato irradiated with doses higher than 1.0 kGy were significantly lower than control samples, especially, taste and flavor. Overall acceptability of non-irradiated samples was higher than that of irradiated samples. The panel members did not accept samples irradiated with doses above 2.0 kGy (data not shown).

The results of the sensory evaluation of tomato stored at refrigerated temperature indicated that irradiation with doses above 1.5 kGy had negative effect on color and lightness, and with doses above 1.0 kGy on the taste, sweetness and flavor. Overall acceptability indicated that control samples were more acceptable than irradiated ones, and panels rejected samples irradiated at doses above 2.0 kGy (data not shown).

The sensory evaluation showed that the selected dose for cherry tomatoes is between 1-1.5 kGy.

3.5. Effect of irradiation on the quality of the mixture of blanched vegetables

3.5.1. Effect of irradiation on the microbiological quality of the mixture of blanched vegetables during storage period

Microbiological quality of irradiated (1, 2, and 3 kGy) and control samples of blanched vegetables during storage period was determined. The results are given in Tables 14-16.

TABLE 14. EFFECT OF IRRADIATION ON THE TBC (CFU/g) OF THE MIXTURE OF BLANCHED MUSTARD AND SOYBEAN DURING STORAGE PERIOD

Dose (kGy)	0 day (cfu/g)	3 days (cfu/g)	5 days (cfu/g)
0	1.5×10^{5}	2.3×10^{6}	6.3×10^{6}
1.0	2.8×10^{3}	4.4×10^{4}	9.4×10^{4}
2.0	2.5×10^{2}	9.4×10^{2}	7.8×10^{3}
3.0	1.0×10^{2}	1.2×10^{2}	2.0×10^{2}

TABLE 15. EFFECT OF IRRADIATION ON THE TBC (CFU/g) OF BLANCHED ALMOND DURING STORAGE PERIOD

Dose (kGy)	0 day (cfu/g)	3 days (cfu/g)	5 days (cfu/g)
0	3.1×10^{5}	3.8×10^{6}	1.8×10^{7}
1.0	2.2×10^{3}	4.2×10^{3}	3.1×10^{4}
2.0	2.2×10^{2}	6.7×10^{2}	1.8×10^{3}
3.0	4.5×10^{1}	4.0×10^{1}	3.5×10^{1}

TABLE 16. EFFECT OF IRRADIATION ON THE TBC (CFU/g) OF THE MIXTURE OF BLANCHED PEANUT AND CELERY DURING STORAGE PERIOD

Dose (kGy)	0 day (cfu/g)	3 days (cfu/g)	5 days (cfu/g)
0	5.8×10^{4}	1.2×10^{6}	6.1×10^{7}
1.0	1.2×10^{2}	3.1×10^{4}	2.4×10^{5}
2.0	6.2×10^{1}	1.1×10^{2}	5.0×10^{3}
3.0	2.0×10^{1}	1.3×10^{1}	3.5×10^{2}

The results indicated that irradiation with 1.0-3.0 kGy produced a reduction of 2-4 log in Total Bacterial Counts on blanched vegetables and a delay in the growth of TBC during storage period. These results were consistent with some literature data [1, 5, 7, 9].

3.5.2. Effect of irradiation on sensory quality of blanched vegetables

The sensory quality (color, flavor, taste and acceptability) of the mixture of blanched vegetables was analysed. The results are presented in Tables 17 to 19. A nine-point hedonic scale was used, scores above 5 were acceptable.

TABLE 17. EFFECTS OF IRRADIATION ON THE SENSORY QUALITY OF THE MIXTURE OF BLANCHED MUSTARD AND SOYBEAN

Dose (kGy)	Color	Flavor	Taste	Acceptability
0.0	7.38	7.23	6.74	7.00
1.0	7.29	6.75	6.57	6.69
2.0	7.16	7.38	7.00	6.92
3.0	6.86	5.38	5.00	4.57

Sensory evaluation of the mixture of blanched mustard and soybean indicated that significant differences were not observed in color, flavor, taste and acceptability scores of samples irradiated at dose lower than 2.0 kGy.

TABLE 18. EFFECT OF IRRADIATION ON SENSORY QUALITY OF THE MIXTURE OF BLANCHED ALMONDS

Dose (kGy)	Color	Flavor	Taste	Acceptability
0.0	6.89	6.16	6.57	6.43
1.0	7.03	5.91	6.57	6.86
2.0	5.75	5.83	7.29	6.44
3.0	4.11	5.57	5.03	4.57

Sensory evaluation of the mixture of blanched almonds showed that scores of samples irradiated with 1.0 kGy were not significantly different with that of non-irradiated samples. However, irradiation with 3 kGy resulted in decrease in all tested sensory properties.

Sensory analysis of the mixture of blanched celery and peanut (Table 19) showed significant difference between samples irradiated with 3 kGy and the controls. After five days storage, sensory quality of control samples became unacceptable (limit of acceptance was score 5), but in case of samples irradiated with 1 and 2 kGy, the sensorial changes were not significant.

TABLE 19. EFFECT OF IRRADIATION ON SENSORY QUALITY OF THE MIXTURE OF BLANCHED CELERY AND PEANUT

day	Dose (kGy)	Color	Flavor	Taste	Acceptability
1	0	7.83	7.71	7.55	7.89
	1	7.57	7.64	7.86	8.01
	2	6.94	7.57	7.17	7.29
	3	6.53	6.68	5.61	6.17
3	0	7.01	6.33	5.85	5.78
	1	7.46	7.01	7.69	7.23
	2	7.22	6.95	6.98	6.88
	3	6.62	5.88	5.75	5.63
5	0	6.13	4.81	------	3.13
	1	6.97	6.63	6.87	6.81
	2	6.91	6.78	6.96	6.82
	3	6.58	6.75	6.03	6.55

3.5.3. Effect of irradiation on fat and protein content of blanched celery and peanut

Crude fat and crude protein were analysed in order to know the effect of irradiation on the nutritional quality of blanched celery and peanut. The results did not show a clear tendency, so more research is needed. On the other hand, it was demonstrated that irradiaton did not affect the amino acid content.

3.6. Effect of irradiation on quality of packaged tofu

3.6.1. Effect of irradiation on microbiological quality of packaged tofu

Samples of commercial packages were irradiated at 1.0-3.0 kGy, the results are given in Table 20.

TABLE 20. EFFECT OF IRRADIATION ON TBC (CFU/g) OF TOFU DURING STORAGE PERIOD

Dose (kGy)	0 day (cfu/g)	5 days (cfu/g)	10 days (cfu/g)
0	7.1×10^{3}	1.9×10^{6}	8.7×10^{7}
1.0	6.5×10^{1}	2.6×10^{3}	7.4×10^{4}
2.0	<10	7.6×10^{1}	5.8×10^{2}
3.0	<10	<10	2.0×10^{1}

Irradiation at 1.0-3.0 kGy caused a reduction of 2-3 logs in Total Bacterial Counts on packaged tofu. Furthermore, irradiation delayed the growth of TBC during 10 days storage period.

3.6.2. Effect of irradiation on sensory quality of packaged tofu

The color, flavor, taste and acceptability scores of packaged tofu samples are shown in Table 21.

TABLE 21. EFFECT OF IRRADIATION ON SENSORY QUALITY OF PACKAGED TOFU DURING STORAGE PERIOD

Day	Dose (kGy)	Color	Flavor	Taste	Acceptability
1	0	8.58	8.23	8.27	8.31
	1	8.66	8.04	8.18	8.26
	2	8.65	6.88	6.29	6.84
	3	8.51	5.75	4.85	4.72
5	0	8.27	8.08	7.77	8.17
	1	8.31	7.98	7.83	8.05
	2	8.34	7.14	6.86	7.21
	3	8.09	5.22	5.13	5.31
10	0	7.48	4.85	-------	4.36
	1	7.55	6.93	7.13	7.11
	2	7.57	6.35	6.76	6.63
	3	6.85	5.63	5.48	5.47

The effect of irradiation was significant in samples irradiated with 2 kGy and 3 kGy (color, taste, general acceptability). Other sensorial properties of control samples significantly decreased to the end of storage.

3.6.3. Effect on the content of fat and protein of packaged tofu

Crude fat and crude protein were analysed as values of nutritional quality of the mixture of packaged tofu. The results did not show a clear tendency as and effect of irradiation and a storage period, so more studies are needed. The amino acid content was not affected by irradiation treatment.

4. CONCLUSIONS

1. D_{10} of different pathogens studied were in the range of 0,08-0,33 kGy. *E. coli* O157:H7 was the most sensitive bacteria to irradiation (0,08 kGy). Irradiation with doses less than 2.0 kGy dose could ensure a 5 log reduction of the most resistant examined pathogen, *Salmonella* Enteritidis.

2. Quality parameters (sensorial) of carrot and tomato irradiated by doses lower than 2.0 kGy were not significantly reduced.

3. Irradiation with doses lower than 2.0 kGy had no significant effect on sensory quality of blanched vegetables. The effects of irradiation and storage on the content of crude protein in blanched celery and peanut were also not significant ($p>0.05$). However, significant effect of irradiation and storage on content of crude fat was obtained. Effect of irradiation doses on amino acids was not significant, and effect of storage time on the most amino acid was significant ($p<0.05$).

4. Sensory quality of the packaged tofu irradiated at lower than 2 kGy had no significant effect during the storage. The effects of irradiation dose on crude fat, crude protein and amino acid were also not significant. However, a significant effect of storage time on crude fat was observed. Most amino acids showed a significant ($p<0.05$) decease with extention of storage time**.**

REFERENCES

[1] VISWANATHAN P., KAUR R., Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. Int. J. Hyg. Environ. Health, **203** (2001) 205-213.

[2] BEUCHAT, L. R. *Listeria monocytogenes*: incidence in vegetables. Food Control. **7** (1996) 223-228.

[3] SAMELIS, J., KAKOURIS, A. et al., Use of ionizing radiation dose of 2 kGy and 4 kGy to control *Listeria* spp. and *E.coli* 0157:H7 on frozen meat trimmings used for dry fermented sausage product, Meat science. **70** (2005) 189-195.

[4] THAYER, D.W., RAJKOWSKI, K.T., Developments in irradiation of fresh fruits and vegetables, Food Technol., **53** (1999) 62-65.

[5] FARKAS, J. Irradiation as method for decontaminating food. Int. J. Food Microbiol, **44** (1998) 189-204.

[6] BAM, Bacteriological Analytical Manual, USA, 2001.

[7] CHAUDRY, M. A., BIBI, N., et al. Irradiation treatment of minimally processed carrots for ensuring microbiological safety. Radiation Physics and Chemistry **71** (2004) 169-173.

[8] CHERVIN C., BOISSEAU P., Quality maintenance of ready-to-eat shredded carrots by gamma irradiation, J. Food Sci, **59** (1994) 359-361.

[9] FARKAS, J., SARAY, T. et al. Effect of low dose gamma radiation on shelf life and microbiological safety of precut/prepared vegetables. Adv. Food Sci. **19** (1997) 111-119.

[10] NIEMIRA, B. A., FAN, X., et al. Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC49594 and *L. innocua* ATCC 51742 inoculated on endive (*Cichorium endive*), J. Food Prot., **66** (2003) 993-998.

[11] FOLEY, D. M., DUFOUR, A., et al. Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. Radiation Physics and Chemistry, **71** (2004) 155-159

[12] XUETONG FAN, KIMBERLY J. B. SOKORA, Assessment of radiation sensitivity of fresh-cut vegetables using electrolyte leakage measurement, Postharvest Biology and Technology, **36** (2005) 191-197.

USE OF IRRADIATION TO ENSURE HYGIENIC QUALITY OF MINIMALLY PROCESSED VEGETABLES AND FRUITS

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Abstract

In the first part of this work, a limited survey (15 samples of each product) of minimally processed vegetables: fresh-cut carrots (*Daucus carota* L.), fresh-cut cucumbers *(Cucumis sativus* L*.)* fresh-cut lettuce (*Lactuca sativa*), mixed vegetables salad, fresh-cut green beans (*Phaseolus vulgaris* L*.)* fresh-cut celery *(Apium gravelones L.)* and mixed peas *(Pisum sativum)* with diced carrots and fruits (fresh-cut pears (*Pyrus communis*) and fresh cut apples (*Malus domestica*)) was conducted to evaluate their microbiological quality. In the second part of the work, each fresh produce mentioned above was inoculated with *Listeria monocytogenes, Staphylococcus aureus, E. coli* and *Aeromonas hydrophila*, then exposed to incremental doses to determine their D_{10}-values. In the third part of this work, the packaged minimally processed vegetables and fruits under investigation were gamma irradiated in the dose range of 1 to 6 kGy, then stored at refrigeration temperature. The effect of different irradiation doses used on the microbiological, physical and sensorial quality of these products was studied during refrigeration storage. The microbiological analysis showed that the studied minimally processed products had high microbial counts and harbor some food-borne pathogenic bacteria. There was a wide variation in the radiation sensitivity of the studied pathogens, and there was a small difference in the D_{10}-value of the same microbe according to the product. *L. monocytogene*s was the most radioresistant with D_{10}-value ranging from 0.52 to 0.66 kGy, while *A. hydrophila* was the most radiosensitive with D_{10}-value ranged from 0.16 to 0.26 kGy. The great reduction in microbial counts, shelf-life extension and elimination of food borne pathogens present without impairing quality was fulfilled using the following doses: 1.5 kGy for fresh-cut celery (minimum 14 days of storage); 2 kGy for fresh-cut lettuce, fresh-cut green beans, fresh-cut apples, fresh-cut pears (15 days of storage); 3 kGy for fresh-cut cucumbers, mixed vegetables salad, mixed peas with diced carrots (15 days of storage); and 4 kGy for fresh-cut carrots (21 days of storage).

1. INTRODUCTION

Increasing demand for ready-to-eat foods has encouraged the production and sale of minimally processed vegetables and fruits where the processor does all the preparation such as trimming, cutting and washing, thereby saving labor and time for the purchaser. It also reduces transport costs since waste materials are removed prior to shipment [1]. Although minimally processed fruits and vegetables are convenient and nutritious, at the same time they are highly perishable and have a limited shelf-life, usually 5-7 days at refrigeration temperature. This short shelf-life resulting from microbial deterioration during cutting, slicing and shredding as well as native enzymatic activity [2, 3]. Moreover, the tendency of fresh-cut fruits such as apples and pears to rapid enzymatic browning and loss of firmness restricted the production and development of minimally processed fruits [4, 5]. Some chemicals such as isoascorbic acid, citric acid, cysteine and 4-hydroxylresorcinol, etc are used as browning inhibitors [6]. Loss of firmness can be prevented by using firmness agents such as calcium lactate [7].

The susceptibility of these products to be contaminated by many pathogenic bacteria such as enteropathogenic *E. coli, E. coli* 0157;H7, *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Vibrio cholerae, Yersinia enterocolitica, Aeromonas hydrophila* and *Campylobacter jejuni* is linked to several foodborne-illness outbreaks [8, 9, 10]. Prolonging shelf-life and elimination of foodborne pathogens is of great importance for marketing these products.

The conventional commercial methods usually used for shelf-life extension and for microorganisms inhibition of minimally processed fruits and vegetables are washing with chlorinated water, modified atmosphere packaging and refrigeration [11, 12]. Recent studies have demonstrated the inability of these methods in controlling all pathogens contaminating these products [13, 14].

Irradiation is an alternative method that has shown to be very effective and safe in reducing spoilage microorganisms (increasing shelf-life) and in eliminating pathogenic bacteria (ensuring safety) in many foodstuffs including fruits, vegetables and their minimally processed products [15, 16, 17, 18]. Little information on the effects of irradiation on fresh-cut fruits is available in the literature, and the application of irradiation to fresh-cut vegetables has been the subject of recent investigation to improve their quality and safety. The effect of irradiation on microbiological and sensorial quality of minimally processed vegetables during refrigeration storage has been reported by some investigators [18, 19, 20]. They found that low irradiation doses greatly reduced their initial microbial counts and eliminated non-spore forming pathogens without adverse effect on sensory quality.

In Egypt, whole fresh fruits and vegetables are usually consumed raw after only washing with tap water. However, many minimally processed vegetables are also commercially produced and becoming available in certain local supermarkets, such as fresh-cut carrots (rings), shredded carrots, fresh-cut lettuce, shredded cabbage, mixed vegetables salad, mixed peas with diced carrots, fresh-cut celery, fresh-cut parsley, fresh-cut mafa spinach, and trimmed okra. On the other hand, there is increasing interest to offer some other minimally processed vegetables as well as some types of fresh-cut fruits that can be used as ready-to-eat products such as fresh-cut apples and fresh-cut pears.

Fresh-cut carrots, fresh-cut lettuce, mixed vegetables salad, fresh-cut celery and mixed peas with diced carrots are popular ready-to-use products commercially available in certain local supermarkets. Trimming and cutting during preparation steps led to increasing microbial contamination and brown discoloration. Thus, these products have a short shelf-life when kept at refrigeration. However, information on the microbiological quality of local minimally processed vegetables, especially with respect to the incidence of human foodborne pathogens, is not available in Egypt. Therefore, a small survey was conducted to evaluate the microbiological quality of these fresh produce. Fresh-cut fruits are not commercially available in local markets. But, there is interest to offer some fresh-cut fruits, as ready-to-eat, such as fresh-cut pear and fresh-cut apple. Thus, fresh-cut pears and fresh-cut apples were manually prepared at the laboratory and treated with antibrowning and firmness agents.

The main objectives of these studies were:

- Microbiological quality assessment of fresh-cut carrots, fresh-cut cucumbers, fresh-cut lettuce, mixed vegetables salad, fresh-cut green beans, fresh-cut celery, mixed peas with diced carrots, fresh-cut pears and fresh-cut apples;

- Determination of D_{10}-values of *Listeria monocytogenes, Staphylococcus aureus, E. coli* and *Aeromonas hydrophila* artificially inoculated in the above mentioned products to identify irradiation dose for 5 log reduction;

- Use of different irradiation doses for refrigerated shelf-life extension of the above products and for inactivation of pathogenic bacterial contaminants;

- Evaluation the sensorial and physical changes in the irradiated products during refrigerated storage; and

- Identify the optimum irradiation dose for each product that reduce microbial load and inactivate pathogens with minimum changes in sensory and physical quality attributes.

2. MATERIALS AND METHODS

2.1. Survey samples for evaluating microbiological quality

In this small survey fifteen packages of each commercially available minimally processed vegetables, namely fresh-cut carrots (250 gm), fresh-cut lettuce (250 gm), mixed vegetables salad (300 gm), fresh-cut green beans (300 gm), fresh-cut celery (250 gm) and mixed peas with diced carrots (450 gm), were

purchased from different local supermarkets at Great Cairo. The minimally processed vegetables were produced according to local commercial preparation, and then packaged in polyethylene with thickness of 0.02 mm after preparation. Fifteen samples of whole cucumbers, apples and pears were purchased from different market.

2.2. Storage experiments samples

Sixty packages of each commercially available minimally processed vegetable were purchased from local supermarket (at the same day of receiving) and used to study the effect of irradiation and refrigeration storage on their microbiological and sensorial quality. Sixty packages of fresh-cut cucumbers, fresh-cut apples and fresh-cut pears were manually prepared as follows:

Intact and fresh whole cucumbers (15 kg) were purchased from the farmers. Whole cucumbers were selected for uniform size. After removing a piece of 1cm from the top of cucumbers, they were manually sliced (2cm x 4cm) using a sharp knife and then washed with tape water. The slices were left to drip dry for 15 minutes at filter papers. Fresh-cut cucumbers were then packaged (in approximately 200 gm) in plastic bags and wrapped with thin film of polyvinyl chloride (non-perforated, permeable, self-clinging).

Whole fresh pears of uniform size (130-140 g) at commercially maturity stage (based on external color and firmness) were purchased from the farmer and brought to the laboratory. The fruits were manually processed immediately. The processing includes rinsing the whole fruits with 0.02% sodium hypochlorite to reduce the surface microbial load. The intact pears were washed with tap water and manually peeled and cut into quarters using sharp stainless steel knife. Part of the pear quarters were cored and dipped in distilled water for 5 min (served as control). The other parts were immersed in tap water containing 2% ascorbic acid and 1% calcium lactate for 3 min. After immersing, the fresh-cut pear quarters were left to drain for 15 min in a perforated cage, and then air dried at ambient temperature (about 27°C) for 1 hr. The air dried pear quarters were packaged in foam bags and wrapped with thin film (thickness 10 μm) of polyvinyl chloride (non-perforated, permeable, and self-clinging). Each bag contained four quarters (approx. 100 g) may be from different fruits.

Fresh apples (15 kg) were purchased from the farmer. After reaching to the laboratory they were washed with tap water, cored and cut into eight slices (wedges). The apple slices were divided into small part and large part. The small part slices were dipped in distilled water for 5 min (served as control). The slices of the large part were dipped in distilled water containing ascorbic acid 2%, citric acid 0.2%, calcium chloride 0.1% and sodium chloride 0.05% for also 5 min. After dipping time, the apple slices were removed and left to drainage on filter paper. The drained slices were packaged in plastic packs (approx. 250 gm) and wrapped with thin film of PVC.

2.3. Irradiation process

Irradiation doses used were: 0, 2, 4, and 6 kGy for fresh-cut carrots; 0, 2, 3, and 4 kGy for fresh-cut cucumbers; 0, 1, 2, and 3 kGy for fresh-cut lettuce, mixed vegetable salad, mixed peas with diced carrots and fresh-cut pears; 0, 1, and 2, kGy for fresh-cut green beans; 0, 1. 1.5, and 2 kGy for fresh-cut celery; 0, 2, and 3 kGy for fresh-cut apples. The samples were irradiated in the Russian Facility Irradiator Model ISSLEDOVATED Co-60, located at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate of that source was 6.66 kGy at the time of irradiating the first experiment (Sept. 2001) and decreased to 4.27 kGy at the time of irradiating the last experiment (Feb. 2005). The dose rate was established using alanine transfer dosimeter. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field.

2.4. Storage

All irradiated and non irradiated samples were kept at 4°C ± 1 for the length of the study.

2.5. Methods of analysis

All analysis was carried out on three packages (triplicate) each sampling day.

2.5.1. Microbiological analysis

Microbiological determination was performed using 25 gm of chopped sample. The samples were homogenated with 225 ml of 0.85% NaCl physiological solution in a stomacher pouch (Stomacher 400, Seward Medical, London). Tenfold dilution was made with the same solution.

Total aerobic bacterial count (TABC) was counted on plate count agar (Oxoid) at 30°C for 48 h according to [21]. Lactic acid bacteria (LAB) were counted on de Man, Rogosa and Sharp agar plates (MRS agar, Oxoid). The inoculum's was plated between two layers of the medium. Colonies were counted after incubation at 30°C for 48 h. Total mold and yeast (M & Y) were count on Czapek's yeast extract agar medium. Chloramphenicol (100mg/L) as an antibiotic was added to prevent bacterial growth. The poured plates were incubated at 25°C for 48-72 h. Coliforms were determined according to [22] with the most probable number (MPN) technique using Mac Conkey broth. Three series of three tubes were incubated at 35°C for 48h. The tubes that showed yellow tint (acid production) and gas was considered positive. For enumeration of *E. coli*, a loopful of each positive culture broth was transferred to another tube of MacConkey broth, and then incubated at 44.4°C for 24-48 h. Confirmation of *E. coli* was carried out by IMViC tests according to [21].

Staphylococcus aureus was enumerated on Baird-Parker agar medium at 37°C according to [23]. Suspected colonies were submitted to coagulase activity and biochemical reactions. *Enterococcus faecalis* was enumerated by plate counts using kanamycin aesculin azid agar medium at 37°C as recommended by [24]**.** *Aeromonas hydrophila* was detected and counted using starch ampicillin agar (SAA) medium at 37°C [25]. Suspected colonies were confirmed by biochemical tests. *Listeria monocytogenes* was detected and enumerated using Oxford *Listeria* agar (Oxoid, CM856) at 37°C as recommended by [26]. Biochemical tests confirmed suspected colonies. *Salmonella* was detected in accordance with ISO 6579:1993 using buffered peptone water, selenite cysteine broth and brilliant green phenol red agar medium.

2.5.2. Determination of D_{10}-values

The D_{10}-values of *L. monocytogenes, S. aureus, E. coli* and *A. hydrophila* (mixed vegetable salad isolates) on commercially available fresh-cut carrots, fresh-cut cucumbers, fresh-cut lettuce, mixed vegetable salad, fresh-cut green beans, fresh-cut celery and mixed peas with diced carrots and manually prepared fresh cucumber slices and fresh-cut pears were determined.

2.5.2.1. Preparation of inocula

The stock cultures of the tested pathogenic bacteria were separately activated by growing each in 100 ml tryptic soy broth (TSB) at 37°C for 24h to obtain suspension of approximately $10^7 – 10^8$ cfu/ml. The cultures broth were diluted with sterilize 1% peptone water and used for inoculation of the samples (work cultures suspension). To know the concentration of the work culture suspension, 1 ml of the appropriate dilutions were spread onto the surface of prepared plates of tryptic soy agar (TSA) and incubated at 37°C for 24 h and counted.

2.5.2.2. Inoculation

Aseptic techniques were used throughout the inoculation procedure. Five hundred grams of each product in sealed polyethylene bags were exposed to 20 kGy of gamma radiation for sterilization. They were placed in 3 L sterile flasks, each containing 1 L of work culture suspension and gently shaken for 3 min. The liquid was withdrawn and the samples were drained and kept at 25°C for at least 12 h for equilibrium.

2.5.2.3. Irradiation

After inoculation, 25 g of each product samples were packed in sterilized polyethylene bags and sealed. They were exposed to different irradiation doses (from 0.25 to 3.0 kGy). Three replicates were used in each dose.

2.5.2.4. Plating

After irradiation, the samples were homogenized with 225 ml of 1% peptone water in stomacher for 1 min and decimal dilutions were done. Selected dilutions (1 ml) of each tested microorganism were transferred in duplicates onto Petri dishes and poured by TSA medium. The plates were incubated at 37°C for 24 h and counted.

2.5.2.5. D_{10} value calculation

The D_{10} value for each tested pathogen was determined from its dose-response curve, which was constructed by plotting log survival counts against irradiation doses used.

2.5.3. Sensory evaluation

General visual appearance, odor, taste and texture are the main sensory attributes used for evaluating minimally processed vegetables and fruits quality. Ten untrained persons from NCRRT were used for judging. The panel testers used scoring scale of 1 to 9 where 1 = very poor; 5 = fair; 9 = excellent [18]. A score of 4 was regarded as unacceptable. Fresh celery, peas and carrots and pear samples recently harvested (only one day after harvesting) were used as reference samples.

2.5.4. Firmness

Firmness measurement was carried out on 1 cm^3 sample of fresh-cut carrots, fresh cucumber slices, fresh-cut pears and fresh-cut apples using a Model 1140 Instron Universal Testing Machine (Instron, High Wycombe, UK). The weight on the load cell was taken as the force (kg/cm^3) and converted to pressure [28].

3. RESULTS

3.1. Microbiological quality of collected samples

The range of microbial counts (range of 15 samples) of minimally processed vegetables (fresh-cut carrots, fresh-cut cucumbers, fresh-cut lettuce, mixed vegetable salad, fresh-cut green beans, fresh-cut celery, mixed peas with diced carrots) and fruits (fresh-cut pears and fresh-cut apples) samples collected from different markets at Cairo are shown in (Table 1). It is evident from the table that the microbiological quality varied from product to another. The collected minimally processed vegetable and fruit samples had in general high levels of microbial population (TABC, LAB, M&Y), The microbial counts of fresh-cut vegetables were higher than that of fresh-cut fruits. The highest TABC was found in fresh-cut green beans (1.8×10^6 – 3.2×10^8 cfu/g) and the lowest TABC was found in fresh-cut pears (7.5×10^1 – 3.5×10^4 cfu/g). The same table shows that coliforms and *E. coli* were found in all minimally processed vegetable samples and found in 40% of fresh-cut apple samples and in 13.3% of fresh-cut pear samples. *Staph. aureus* and *Ent. fecalis* were found in most samples. *A. hydrophila* was detected in all mixed vegetables salad samples and in 6.7, 33.3, 46.6 and 66.6% of fresh-cut cucumber, fresh-cut carrot, fresh-cut lettuce, and fresh-cut green beans samples, respectively. *L. monocytogenes* was detected in two samples (13.3%) of mixed vegetable salad. *Salmonella* was detected in one sample (6.7%) of fresh-cut cucumber and mixed vegetable salad.

3.2. Radiation sensitivity (D_{10}-values) for pathogens

Radiation dose response curves for *L. monocytogenes* in different artificially contaminated minimally processed vegetables and fruits are shown in Fig. 1. The D_{10}-values obtained ranged from 0.52 to 0.66 kGy in different fresh produce. These D_{10}-values lead to a prediction that radiation doses of about 2.6–3.3 kGy should inactivate 5-log cycles of *L. monocytogenes* in different minimally processed fruits and vegetables under investigation.

The radiation dose response curves of *Staphylococcus aureus* in different fresh produce under investigation are shown in (Fig. 2). The D_{10}-values obtained ranged from 0.42 to 0.54 kGy. The 5-log reduction of *S. aureus* in fresh produce could be achieved by about 2.1 – 2.7 kGy.

Radiation dose response curves for *E. coli* in different minimally processed fruits and vegetables are illustrated in (Fig. 3). The D_{10}-value ranged from 0.11 to 0.31 kGy. The 5-log reduction of *E. coli* in fresh produce could be achieved by about 0.55 – 1.55 kGy.

Radiation dose response curves for *Aeromonas hydrophila* in different minimally processed fruits and vegetables are shown in (Fig. 4). The D_{10}-value ranged from 0.16 to 0.26 kGy. The 5-log reduction of *A. hydrophila* in fresh produce could be achieved by about 0.80 – 1.50 kGy.

TABLE 1. MICROBIOLOGICAL QUALITY OF MINIMALLY PROCESSED VEGETABLES AND FRUITS

Products	Range of microbial counts (cfu/g)									
	TABC	*LAB*	M & Y	Colif.	E. coli	*S. aur.*	*E. FEC.*	*A. hyd.*	*L. mono.*	*Salm.*
Fresh-cut carrots	1.4×10^{4} to 2.4×10^{6}	5.0×10^{2} to 2.4×10^{5}	<10 to 5.5×10^{2}	43 to 1100	9 to 20	<100 to 2.3×10^{3}	<100	<100 to 4.0×10^{2}	<100	—
Fresh cucumber slices	8.5×10^{2} to 2.5×10^{6}	<10 to 2.5×10^{4}	4.5×10^{1} to 1.0×10^{4}	3 to 43	3 to 20	<100 to 8.5×10^{2}	<100 to 6.9×10^{3}	<100 to 2.0×10^{2}	<100	+
Fresh-cut lettuce	4.0×10^{5} to 9.9×10^{6}	2.0×10^{3} to 2.6×10^{5}	1.0×10^{2} to 5.5×10^{4}	23 to >2400	9 to >2400	1.2×10^{2} to 1.5×10^{4}	4.0×10^{2} to 6.8×10^{4}	3.0×10^{2} to 5.0×10^{3}	<100	—
Mixed vegetable salad	2.5×10^{5} to 7.0×10^{7}	2.0×10^{3} to 7.1×10^{6}	1.3×10^{2} to 4.5×10^{3}	210 to >2400	4 to >2400	1.0×10^{2} to 9.0×10^{4}	1.5×10^{2} to 3.3×10^{4}	1.7×10^{2} to 6.0×10^{3}	2.0×10^{2} to 7.0×10^{2}	+
Fresh-cut green beans	1.8×10^{6} to 3.2×10^{8}	8.3×10^{3} to 3.3×10^{7}	1.0×10^{2} to 4.1×10^{5}	>2400	210 — >2400	<100 to 1.0×10^{2}	<100 to 1.0×10^{2}	<100 to 1.0×10^{2}	<100	—
Fresh-cut celery	9.5×10^{5} to 4.6×10^{7}	2.7×10^{4} to 1.8×10^{5}	1.8×10^{4} to 1.5×10^{5}	150 to >2400	93 — >2400	<100 to 3.0×10^{2}	4.5×10^{2} to 8.5×10^{3}	<100	<100	—
Mixed peas with diced carrots	9.0×10^{5} to 1.1×10^{7}	3.4×10^{4} to 3.1×10^{5}	5.3×10^{3} to 1.4×10^{5}	150 to >2400	93 to >2400	<100 to 2.8×10^{2}	1.4×10^{2} to 1.5×10^{4}	<100	<100	—
Fresh cut pears	7.5×10^{1} to 3.7×10^{4}	<10 to 3.2×10^{8}	<10 to 5.3×10^{3}	<3 to 43	<3 to 9	<100 to 5.0×10^{2}	<100	<100	<100	—
Fresh-cut apples	7.5×10^{3} to 5.0×10^{5}	1.7×10^{3} to 3.0×10^{4}	5.0×10^{2} to 1.4×10^{5}	23 to 1100	4 to 1100	2.0×10^{2} to 7.9×10^{2}	<100	<100	<100	—

— = Not present
+ = Present in only one sample of fresh-cucumber slices and mixed vegetable salad
< 100 & < 10 = Below detectable level
<3 = No positive tubes have been shown in the first three dilutions
>2400 = The first three dilutions had all their tubes positive

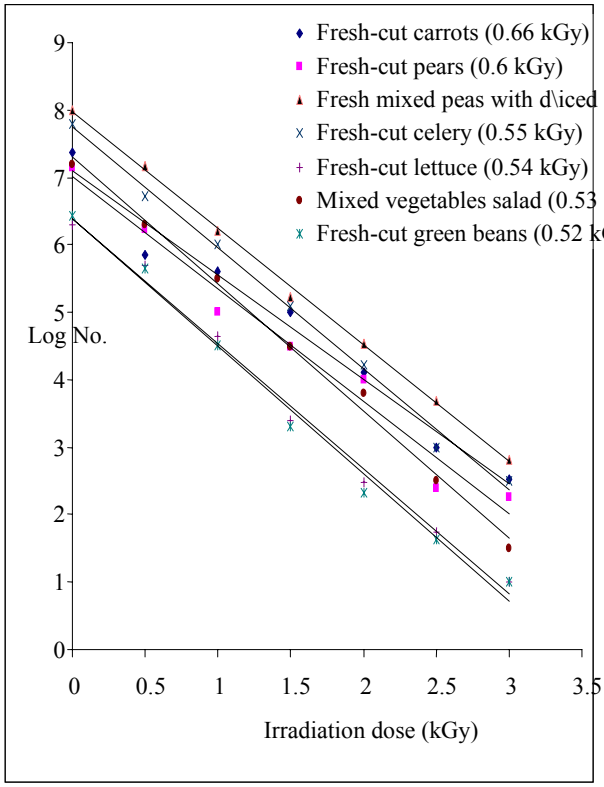

FIG. 1. Survival curves of Listeria monocytogenes in artificially contaminated minimally processed vegetables and fruits.

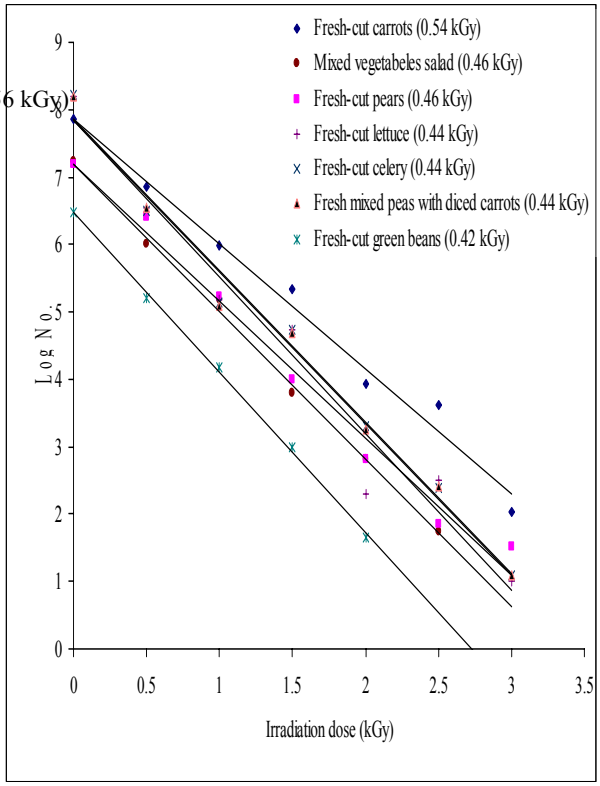

FIG. 2. Survival curves of Staphylococcus aureus in artificially contaminated minimally processed vegetables and fruits.

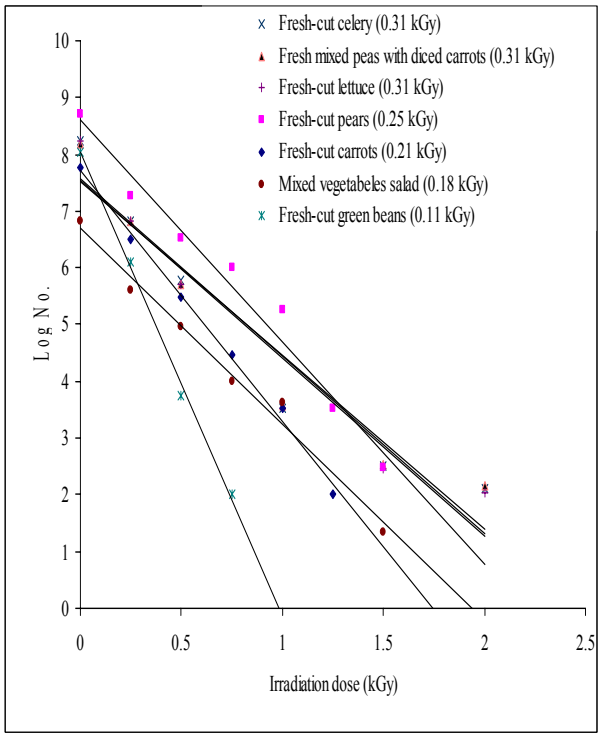

FIG. 3. Survival curves of E. coli in artificially contaminated minimally

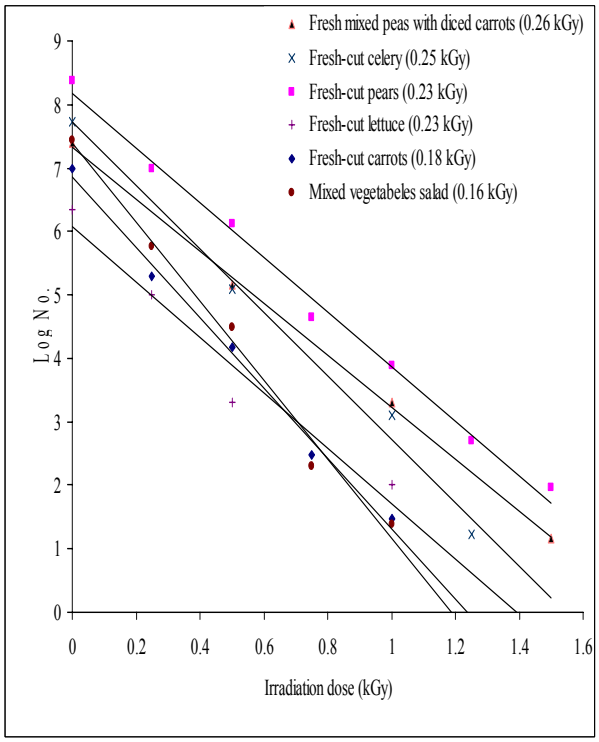

FIG. 4. Survival curves of A. hydrophila in artificially contaminated minimally processed vegetables and fruits.

3.3. Effect of irradiation

3.3.1. Effect of irradiation on microbial load

The effect of different irradiation doses on the microbial load (TABC, LAB, M&Y) of minimally processed fruits and vegetables are presented in Tables (2 – 10) according to type of fresh produce. It is necessary to note that the fresh pre-cut fruits were immersed in 2% ascorbic acid (browning inhibition) and 1% calcium lactate (firmness agent) (Tables 9 – 10). It can be seen that irradiation caused great reduction in all microbial load of all minimally processed products. Higher irradiation doses used for any fresh produce were found to be better for controlling microbial counts than lower doses. During refrigeration storage, all microbial counts of each minimally processed type increased with increasing storage time, but the rate of increase was lower in irradiated samples in comparison with that of non-irradiated ones.

TABLE 2. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT CARROTS DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	2	4	6
Total aerobic bacterial count (TABC)	0	1.2×10^{5}	1.7×10^{2}	6.0×10	4.8×10
	7	2.3×10^{6}	3.0×10^{3}	7.0×10	2.5×10
	14	"R"	2.5×10^{6}	9.0×10^{2}	2.5×10^{3}
	21	"R"	"R"	1.4×10^{4}	3.1×10^{2}
Lactic acid bacteria (LAB)	0	7.5×10^{3}	2.0×10	<10	<10
	7	1.0×10^{1}	<10	<10	<10
	14	"R"	6.4×10^{2}	<10	<10
	21	"R"	"R"	<10	<10
Total mold and yeast (M&Y)	0	2.0×10^{3}	2.5×10^{2}	<10	<10
	7	3.2×10^{4}	2.0×10^{2}	1.0×10^{1}	<10
	14	"R"	2.0×10^{3}	2.5×10^{2}	1.0×10^{2}
	21	"R"	"R"	7.0×10^{2}	<10

R = Rejected by sensory evaluation

TABLE 3. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH CUCUMBER SLICES DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	2	3	4
Total aerobic bacterial count (TABC)	0	3.0×10^{4}	2.2×10^{3}	7.3×10^{2}	2.7×10^{2}
	5	2.4×10^{6}	1.1×10^{4}	6.9×10^{2}	3.4×10^{2}
	10	"R"	6.0×10^{6}	9.0×10^{2}	7.0×10^{2}
	15	"R"	"R"	8.1×10^{3}	1.4×10^{3}
Lactic acid bacteria (LAB)	0	2.5×10^{2}	<10	<10	<10
	5	5.0×10^{3}	<10	<10	<10
	10	"R"	<10	<10	<10
	15	"R"	"R"	<10	<10
Total mold and yeast (M&Y)	0	3.9×10^{2}	3.0×10	2.0×10	<10
	5	4.6×10^{4}	1.1×10^{2}	7.1×10^{2}	4.3×10
	10	"R"	5.0×10^{4}	4.1×10^{3}	1.1×10^{2}
	15	"R"	"R"	9.3×10^{3}	4.5×10^{3}

R = Rejected by sensory evaluation

TABLE 4. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT LETTUCE DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	1	2	3
Total aerobic bacterial count (TABC)	0	7.0×10^{6}	3.0×10^{-4}	3.0×10^{3}	2.0×10^{2}
	5	1.3×10^{7} "R"	2.5×10^{5}	1.0×10^{4}	3.0×10^{3}
	10	"R"	3.0×10^{5}	3.0×10^{4}	3.0×10^{3}
	15	"R"	"R"	5.0×10^{4}	4.0×10^{3}
	20	"R"	"R"	"R"	2.0×10^{3} "R"
Lactic acid bacteria (LAB)	0	3.0×10^{3}	3.3×10^{3}	3.0×10^{2}	1.7×10^{2}
	5	1.1×10^{3}	<10	<10	<10
	10	"R"	<10	<10	<10
	15	"R"	"R"	"R"	<10
	20	"R"	"R"	"R"	<10 "R"
Total mold and yeast (M&Y)	0	1.2×10^{4}	1.4×10^{3}	2.5×10^{2}	3.0×10^{3}
	5	2.1×10^{4}	3.2×10^{3}	1.5×10^{3}	2.5×10^{2}
	10	"R"	2.2×10^{4}	5.0×10^{3}	5.0×10^{3}
	15	"R"	"R"	2.0×10^{3}	3.5×10^{3}
	20	"R"	"R"	"R"	3.5×10^{3} "R"

R = Rejected by sensory evaluation

TABLE 5. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF MIXED VEGETABLE SALAD DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	1	2	3
Total aerobic bacterial count (TABC)	0	5.9×10^{5}	6.8×10^{4}	6.2×10^{3}	4.3×10^{1}
	4	3.9×10^{6}	4.0×10^{4}	2.0×10^{3}	9.0×10^{1}
	7	5.6×10^{7} "R"	4.0×10^{5}	2.0×10^{4}	1.0×10^{2}
	12	"R"	3.0×10^{6}	7.0×10^{5}	3.1×10^{2}
	15	"R"	"R"	"R"	1.1×10^{5}
Lactic acid bacteria (LAB)	0	1.8×10^{4}	2.0×10^{2}	3.0×10^{1}	1.0×10^{1}
	4	7.9×10^{4}	7.0×10^{2}	5.0×10^{1}	3.0×10^{1}
	7	1.5×10^{7}	5.0×10^{3}	7.2×10^{1}	5.5×10^{1}
	12	"R"	5.0×10^{3} "R"	2.0×10^{3}	8.0×10^{2}
	15	"R"	"R"	"R"	8.9×10^{3}
Total mold and yeast (M&Y)	0	1.1×10^{4}	1.1×10^{2}	2.0×10^{1}	1.0×10^{1}
	4	4.4×10^{4}	2.0×10^{2}	2.0×10^{1}	1.0×10^{1}
	7	1.5×10^{5}	5.1×10^{2}	7.0×10^{1}	<10
	12	"R"	7.0×10^{2}	9.0×10^{1}	<10
	15	"R"	"R"	"R"	<10

"R" = Rejected by sensory evaluation

TABLE 6. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT GREEN BEANS DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)		
		Control	1	2
Total aerobic bacterial count (TABC)	0	2.9×10^{6}	5.8×10^{4}	3.6×10^{3}
	5	1.5×10^{7} "R"	3.3×10^{5}	8.6×10^{3}
	10	3.0×10^{8} "R"	5.1×10^{6}	2.0×10^{4}
	15	"R"	8.3×10^{6}	9.0×10^{5}
Lactic acid bacteria (LAB)	0	2.0×10^{4}	1.2×10^{2}	3.0×10^{1}
	5	7.2×10^{4}	5.0×10^{2}	4.2×10^{1}
	10	1.2×10^{5}	2.0×10^{3}	3.3×10^{2}
	15	"R"	2.6×10^{4}	1.1×10^{4}
Total mold and yeast (M&Y)	0	1.0×10^{2}	5.7×10^{1}	1.5×10^{1}
	5	9.5×10^{2}	1.0×10^{2}	6.3×10^{1}
	10	4.3×10^{3}	7.9×10^{3}	3.7×10^{2}
	15	"R"	3.6×10^{3}	6.2×10^{2}

"R" = Rejected by sensory evaluation

TABLE 7. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT CELERY DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	1	1.5	2
Total aerobic bacterial count (TABC)	0	8.0×10^{6}	2.0×10^{5}	3.8×10^{4}	1.3×10^{4}
	7	1.5×10^{7} "R"	2.8×10^{5}	5.3×10^{4}	1.8×10^{4}
	14	"R"	5.1×10^{6}	3.1×10^{5}	6.0×10^{4}
Lactic acid bacteria (LAB)	0	1.7×10^{5}	1.3×10^{3}	2.8×10^{2}	1.5×10^{2}
	7	8.7×10^{5}	6.1×10^{3}	2.7×10^{3}	2.0×10^{3}
	14	"R"	5.4×10^{4}	2.7×10^{4}	6.5×10^{3}
Total mold and yeast (M&Y)	0	$1.1x10^{5}$	1.0×10^{4}	3.9×10^{3}	1.9×10^{3}
	7	$1.9x10^{5}$	1.7×10^{4}	4.5×10^{3}	2.2×10^{3}
	14	"R"	"R"	1.7×10^{4}	5.0×10^{3}

"R" = Rejected by sensory evaluation

TABLE 8. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH MIXED PEAS WITH DICED CARROTS DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Irradiation dose (kGy)			
		Control	1	2	3
Total aerobic bacterial count (TABC)	0	5.5×10^{6}	2.6×10^{5}	2.5×10^{4}	3.1×10^{3}
	7	1.9×10^{7}	6.9×10^{5}	5.6×10^{4}	4.8×10^{3}
	14	7.0×10^{7} "R"	8.2×10^{6}	2.9×10^{5}	2.4×10^{4}
	21	"R"	"R"	4.2×10^{5}	3.3×10^{4}
Lactic acid bacteria (LAB)	0	5.5×10^{4}	8.7×10^{3}	2.3×10^{3}	3.1×10^{2}
	7	1.9×10^{5}	2.1×10^{4}	4.1×10^{3}	2.2×10^{3}
	14	5.0×10^{5}	3.6×10^{4}	2.5×10^{4}	1.9×10^{4}
	21	"R"	"R"	4.3×10^{4}	2.7×10^{4}
Total mold and yeast (M&Y)	0	1.2×10^{5}	2.2×10^{3}	3.7×10^{2}	4.6×10
	7	4.0×10^{5}	5.1×10^{3}	2.9×10^{3}	1.7×10^{2}
	14	7.7×10^{5}	7.3×10^{3}	3.5×10^{3}	6.1×10^{2}
	21	"R"	"R"	4.5×10^{4}	2.9×10^{3}

"R" = Rejected by sensory evaluation

TABLE 9. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT PEARS DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
			Control	1	2	3
Total aerobic bacterial count (TABC)	0	6.4×10^3	6.0×10^3	2.8×10^2	2.5×10	1.3×10
	7	3.0×10^4	1.9×10^4	8.0×10^3	1.5×10^2	2.5×10
	14	2.8×10^5	1.7×10^5	1.5×10^4	6.6×10^2	3.8×10
Lactic acid bacteria (LAB)	0	3.0×10^2	1.8×10^2	7.0×10	< 10	< 10
	7	5.5×10^3	3.1×10^3	8.1×10	< 10	< 10
	14	8.7×10^3	7.5×10^3	9.0×10	2.0×10	< 10
Total mold and yeast (M&Y)	0	8.5×10^2	6.5×10^2	1.0×10^2	< 10	< 10
	7	9.8×10^3	5.0×10^3	1.2×10^3	1.7×10	< 10
	14	3.5×10^4	3.5×10^4	6.7×10^3	5.0×10	< 10

TABLE 10. EFFECT OF IRRADIATION ON THE MICROBIAL COUNTS (CFU/g) OF FRESH-CUT APPLES DURING REFRIGERATED STORAGE

Microorganisms	Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)		
			Control	2	3
Total aerobic bacterial count (TABC)	0	6.5×10^3	1.4×10^3	1.9×10^2	1.6×10^2
	5	8.4×10^3	1.8×10^3	3.2×10^2	1.9×10^2
	10	"R"	1.6×10^3	5.2×10^2	1.7×10^2
	15	"R"	"R"	7.7×10^2	3.6×10^2
	20	"R"	"R"	4.0×10^2	4.0×10^2
Lactic acid bacteria (LAB)	0	4.0×10^3	3.0×10^3	2.2×10	3.0×10
	5	5.3×10^3	4.7×10^2	4.1×10	3.6×10
	10	"R"	6.0×10^2	5.0×10	3.0×10
	15	"R"	"R"	3.7×10	2.0×10
	20	"R"	"R"	7.7×10	5.0×10
Total mold and yeast (M&Y)	0	8.2×10^3	7.0×10^3	5.0×10	8.0×10
	5	9.1×10^3	5.2×10^3	8.3×10	1.0×10^2
	10	"R"	6.4×10^3	3.8×10^2	2.1×10^2
	15	"R"	"R"	7.2×10^2	4.1×10^2
	20	"R"	"R"	2.1×10^3	7.6×10^3

"R" = Rejected for sensory evaluation

3.3.2. Effect of irradiation on bacteria of particular concern

Coliform bacteria are considered of particular importance because its presence implies the possible occurrence of similar but pathogenic organism. *E. coli* is becoming increasingly important from the view point of public health, particularly psychrotrophic strains. The effect of irradiation on the coliforms as well as on the pathogenic bacteria present in different types of minimally processed fruits and vegetables were determined. The obtained results showed that the lowest irradiation dose used (1 kGy) was efficient and sufficient in eliminating coliforms, *E. coli and A. hydrophila* found in fresh produce under investigation. While, irradiation dose of 2 or 3 kGy was very effective in controlling the other pathogens present. Minimally processed products receiving these irradiation doses were free from coliforms and pathogens throughout their storage period. Neither coliforms nor pathogen have been detected in non-irradiated fresh-cut pears.

3.3.3. Effect of irradiation on sensory quality attributes

Sensory quality attributes including visual appearance, odor, taste and texture of different minimally processed vegetable and fruit products were evaluated immediately after irradiation and during refrigeration storage. The panelists scores are shown in Tables (11 – 19) Irradiation doses up to 2 kGy had no adverse effect on the sensory quality of the studied fresh-produce. Fresh-cut irradiated carrots presented scores higher than control samples after 7 and 14 days of storage. At the end of the storage period (21 days) all control and irradiated samples with 2 kGy were rejected. At the same time, the samples treated with higher doses (4 and 6 kGy) showed a deleterious effect in all parameters studied. Fresh cucumber slices irradiated at doses of 3-4 kGy presented good sensorial characteristics up to 10 days of the storage period. Control and irradiated samples with 2 kGy for the same period of time were rejected. Irradiation doses of 2-3 kGy slightly affected the sensory quality attributes of fresh-cut lettuce after 15 days of storage. The sensory quality of non-irradiated and irradiated samples of all fresh produce samples decreased with increasing refrigeration storage time; irradiated samples were sensorially superior to their non-irradiated counterparts throughout their storage periods.

Mixed vegetable salad, fresh-cut celery, mixed peas with diced carrots, fresh-cut apples and fresh-cut pears samples irradiated with 2 or 3 kGy presented good sensorial characteristics after 12-21 days of storage (double the time in comparison with control and irradiated samples) at 2 kGy. Fresh-cut carrots and fresh-cut green beans were prolonged to 2- to 3-fold by using 2 and 4 kGy irradiation doses, respectively, as compared to control samples.

TABLE 11. SENSORY EVALUATION OF IRRADIATED FRESH-CUT CARROTS DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose kGy			
		Control	2	4	6
Color	0	8.6	8.4	8.7	8.4
	7	4.9	6.5	7.5	7.3
	14	"R"	4.3	6.0	6.2
	21	"R"	"R"	4.5	"R"(4.0)
Texture	0	8.7	8.5	8.0	6.1
	7	4.1	6.8	6.5	5.7
	14	"R"	"R" (3.8)	5.7	4.3
	21	"R"	"R"	4.9	"R" (3.8)
Odor	0	8.3	8.3	8.4	7.2
	7	6.3	6.2	6.0	6.3
	14	"R"	"R" (3.5)	4.9	5.5
	21	"R"	"R"	5.5	4.4
Taste	0	8.8	8.6	8.6	7.0
	7	"R" (3.8)	6.5	6.5	6.3
	14	"R"	"R" (3.8)	5.8	5.5
	21	"R"	"R"	4.7	"R" (3.8)

"R" = Rejected by sensory evaluation

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 12. SENSORY EVALUATION OF IRRADIATED FRESH CUCUMBER SLICES DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose kGy			
		Control	2	3	4
Color	0	8.0	7.8	8.0	7.9
	5	5.1	6.5	8.5	8.3
	10	"R" (2.1)	"R" (4.0)	5.9	6.5
	15	"R"	"R"	4.9	4.5
Texture	0	8.6	8.5	8.5	7.3
	5	5.8	6.3	7.0	6.2
	10	"R" (2.3)	"R" (4.3)	5.8	5.2
	15	"R"	"R"	"R" (4.5)	"R" (4.0)
Odor	0	8.8	8.8	8.9	8.8
	5	4.8	6.8	7.0	7.4
	10	"R" (2.2)	4.3	6.4	6.3
	15	"R"	"R"	"R" (4.3)	"R" (4.5)
Taste	0	8.0	8.1	8.1	7.9
	5	4.6	6.5	7.3	7.5
	10	"R" (1.3)	4.1	6.3	5.6
	15	"R"	"R"	"R" (4.2)	"R" (4.0)

"R" = Rejected by sensory evaluation

TABLE 13. SENSORY EVALUATION OF IRRADIATED FRESH-CUT LETTUCE DURING REGFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose kGy			
		Control	1	2	3
Appearance	0	9.0	8.6	8.7	8.6
	5	8.0	8.0	8.2	8.6
	10	“R” (3.0)	“R” (4.0)	7.0	7.0
	15	“R”	“R”	5.6	5.6
	20	“R”	“R”	“R”	4.4
Texture	0	8.6	9.0	8.7	7.0
	5	8.0	8.0	7.6	6.6
	10	“R” (3.9)	“R” (4.0)	5.5	5.5
	15	“R”	“R”	5.0	5.0
	20	“R”	“R”	“R”	4.8
Odor	0	8.6	8.5	8.4	8.4
	5	8.2	8.2	8.4	8.4
	10	“R” “4.0”	“R” (4.0)	6.6	6.6
	15	“R”	“R”	5.0	5.4
	20	“R”	“R”	“R”	4.2
Taste	0	9.0	8.8	8.8	8.6
	5	8.0	8.2	8.0	8.0
	10	“R” (4.0)	“R” (4.0)	5.5	6.0
	15	“R”	“R”	4.8	5.6
	20	“R”	“R”	“R”	4.3
Wilting	0	8.0	8.0	7.8	7.6
	5	7.0	7.2	7.0	7.0
	10	“R” (4.0)	6.0	6.5	6.5
	15	“R”	“R”	6.5	6.5
	20	“R”	“R”	“R”	4.5

“R” = Rejected by sensory evaluation

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 14. SENSORY EVALUATION OF IRRADIATED MIXED VEGETABLE SALAD DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose kGy			
		Control	1	2	3
Appearance	0	8.4	8.2	8.3	8.2
	4	6.5	7.0	7.8	7.5
	7	4.8	5.9	6.5	6.8
	12	"R"	4.8	5.1	5.8
	15	"R"	"R"	"R" (3.0)	5.3
Odor	0	8.2	8.2	8.0	8.0
	4	6.2	6.7	7.2	7.2
	7	4.4	5.8	6.5	6.5
	12	"R"	"R" (4.5)	5.3	5.6
	15	"R"	"R"	"R" (3.7)	5.0
Taste	0	8.6	8.4	8.6	8.2
	4	6.0	6.5	7.6	7.4
	7	"R" (4.0)	5.6	6.3	6.8
	12	"R"	"R" (4.3)	5.0	5.8
	15	"R"	"R"	"R" (3.6)	5.0

"R" = Rejected by sensory evaluation

Scores on the basis of 9 point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 15. SENSORY EVALUATION OF IRRADIATED FRESH-CUT GREEN BEANS DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose kGy		
		Control	1	2
Appearance	0	8.7	8.5	8.7
	5	6.1	7.5	8.0
	10	"R" (3.0)	6.6	7.1
	15	"R"	6.0	6.2
Odor	0	8.5	8.5	8.3
	5	6.4	7.7	7.9
	10	"R" (2.7)	6.6	7.0
	15	"R"	6.1	6.4
Texture	0	8.6	8.7	8.4
	5	6.0	7.8	7.8
	10	"R" (2.5)	7.0	7.2
	15	"R"	6.0	6.0

"R" = Rejected by sensory evaluation

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 16. EFFECT OF IRRADIATION ON THE SENSORY EVALUATION OF FRESH-CUT CELERY DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose (kGy)			
		Control	1	1.5	2
Appearance	0	8.8	8.8	8.5	8.5
	7	7.5	8.0	8.4	8.3
	14	"R" (4.0)	6.2	6.5	7.0
Odor	0	8.8	8.5	8.7	8.4
	7	7.6	7.5	8.0	8.2
	14	"R" (4.0)	5.5	6.0	6.6
Wilting	0	9.0	8.5	8.3	8.4
	7	7.2	7.8	8.0	7.5
	14	"R" (4.0)	5.8	6.2	6.5

"R" = Rejected by sensory evaluation

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 17. EFFECT OF IRRADIATION ON THE SENSORY EVALUATION OF FRESH MIXED PEAS WITH DICED CARROTS DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Irradiation dose (kGy)			
		Control	1	2	3
Appearance	0	8.8	8.7	8.5	8.5
	7	8.0	8.4	8.0	8.0
	14	"R" (4.0)	7.2	7.4	7.5
	21	"R" (2.8)	"R" (4.0)	5.5	6.5
Texture	0	8.7	8.5	8.0	8.0
	7	7.2	7.6	7.5	7.5
	14	"R" (4.0)	6.2	7.0	7.0
	21	"R" (2.5)	4.5	5.8	6.2
Odor	0	8.6	8.5	8.3	8.3
	7	6.8	7.5	7.8	7.4
	14	4.2	5.6	6.5	7.0
	21	"R" (3.3)	4.5	5.2	6.6

"R" = Rejected by sensory evaluation

TABLE 18. EFFECT OF COMBINATION TREATMENT WITH IRRADIATION ON THE SENSORY QUALITY ATTRIBUTES OF FRESH-CUT PEARS DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
			Control	1	2	3
Color	0	8.8	8.8	8.6	8.6	8.3
	7	"R" (3.0)	6.3	6.5	6.3	6.0
	14	"R" (1.0)	"R" (4.0)	4.5	4.3	"R" (4.0)
Taste	0	8.9	8.7	8.7	8.5	8.5
	7	"R" (3.0)	6.0	5.8	6.2	5.8
	14	Not tested	4.2	4.8	4.8	4.1
Odor	0	8.8	8.6	8.6	8.5	8.1
	7	"R" (3.3)	5.7	6.0	6.2	5.5
	14	"R" (2.0)	4.5	4.5	5.1	4.3
Texture	0	8.6	8.8	8.8	8.6	8.0
	7	6.3	6.5	6.5	6.0	5.5
	14	"R" (3.3)	5.0	5.3	5.1	4.9

"R" = Rejected

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

TABLE 19. EFFECT OF COMBINATION TREATMENT WITH IRRADIATION ON THE SENSORY QUALITY ATTRIBUTES OF FRESH-CUT APPLES DURING REFRIGERATED STORAGE

Parameters	Storage period (days)	Control immersed in water only	Immersion in water containing antibrowning and firmness agents and irradiated with different doses (kGy)		
			Control	2	3
Color	0	8.8	8.6	8.6	8.4
	5	"R" (2.4)	7.3	8.0	8.0
	10	"R"	"R" (3.3)	6.3	7.5
	15	"R"	"R"	5.5	4.5
	20	"R"	"R"	4.4	"R" (2.6)
Taste	0	8.5	8.1	8.0	8.2
	5	5.4	7.0	8.0	8.0
	10	"R"	5.3	8.0	8.0
	15	"R"	"R"	6.5	6.3
	20	"R"	"R"	5.1	5.5
Odor	0	8.3	8.0	8.1	8.0
	5	5.8	6.8	7.7	8.0
	10	"R"	6.6	7.1	7.5
	15	"R"	"R"	6.6	6.6
	20	"R"	"R"	6.0	5.8
Texture	0	8.4	8.6	8.0	8.0
	5	5.7	7.5	7.0	7.5
	10	"R"	"R" (3.6)	8.0	7.2
	15	"R"	"R"	6.5	6.3
	20	"R"	"R"	5.0	4.7

"R" = Rejected sensory evaluation

Scores on the basis of 9-point scale where 1 = very poor; 5 = fair; 9 = excellent

3.3.4. Effect of irradiation on firmness

The changes occurred in firmness of fresh-cut carrots, fresh cucumber slices, fresh-cut pears and fresh-cut apples are shown in Tables 20 – 23. It was found that irradiation doses reduced firmness of the above fresh produce and this reduction was proportional with irradiation dose. The mean values of firmness were also decreased when the storage period increased.

TABLE 20. FIRMNESS CHANGES (KG/CM2) IN FRESH-CUT CARROTS AS A RESULT OF IRRADIATION AND REFRIGERATED STORAGE

Storage period (days)	Irradiation dose (kGy)			
	Control	2	4	6
0	12.5	8.8	6.8	5.4
7	7.6	8.8	6.7	5.2
14	"R"	8.6	6.4	4.8
21	"R"	"R"	4.8	4.0

"R" = Rejected sensory evaluation

TABLE 21. FIRMNESS CHANGES (KG/CM2) IN FRESH CUCUMBER SLICES AS A RESULT OF IRRADIATION AND REFRIGERATED STORAGE

Storage period (days)	Irradiation dose (kGy)			
	Control	2	3	4
0	4.4	3.3	2.4	1.2
5	3.8	2.6	2.0	0.8
10	"R"	2.2	1.6	0.6
15	"R"	"R"	1.4	0.4

"R" = Rejected sensory evaluation

TABLE 22. EFFECT OF COMBINATION TREATMENT WITH IRRADIATION ON THE FIRMNESS (KG/CM2) OF FRESH-CUT PEARS DURING REFRIGERATED STORAGE

Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
		Control	1	2	3
0	4.80	5.32	4.30	3.50	3.00
7	4.53	5.10	4.10	3.00	3.23
14	3.50	4.80	3.82	3.00	2.12

TABLE 23. FIRMNESS CHANGES (KG/CM2) IN FRESH-CUT APPLES AS A RESULT OF IRRADIATION AND REFRIGERATED STORAGE

Storage period (days)	Control immersed in water only	Immersion in water containing antibrowning and firmness agents and irradiated with different doses (kGy)		
		Control	2	3
0	3.7	3.8	3.1	2.9
5	3.2	3.3	2.9	2.2
10	"R"	3.0	2.5	2.2
15	"R"	"R"	2.2	1.8
20	"R"	"R"	2.0	1.4

"R" = Rejected

4. CONCLUSIONS

It can be concluded that minimally processed vegetables commercially available in local markets in Egypt had in general high microbial counts and harbor some foodborne pathogens such as *E. coli, S. aureus, L. monocytogenes, A. hydrophila* and *Salmonella* spp.

The D_{10}-values of *L. monocytogenes, S. aureus, E. coli,* and *A. hydrophila* in the minimally processed vegetables and fruits under investigation ranged from 0.52 - 0.66, 0.42 - 0.54, 0.11 - 0.31 and 0.16-0.26 kGy, respectively.

Irradiation dose of 1.5 kGy can be used to extend the refrigeration shelf-life of fresh-cut celery without changes in their sensorial quality attributes. An irradiation dose of 2 kGy can be used to extend the refrigeration shelf-life of fresh-cut lettuce and fresh-cut green beans. A 2 kGy irradiation dose, in combination with anti-browning and firmness agents can be used to extend the refrigeration storage life (15 days) of fresh-cut pears and apples with acceptable sensory quality. Irradiation dose of 3 kGy can be used to extend the refrigeration storage life (15 days) of fresh cucumber slices, mixed vegetable salad and mixed peas with diced carrots without changes in their sensory quality attributes. Irradiation dose of 4 kGy can be used to extend the refrigeration storage life of fresh-cut carrots (21 days) without changes in their sensory quality. Irradiation at these dose levels resulted in obtaining minimally processed vegetables and fruits that are microbiologically safe and acceptable sensory quality.

REFERENCES

[1] BALDWIN, E. A., NISPEROS-CARRIED, M.O., BAKER, R.A., Use of edible coatings to preserve quality of lightly (one slightly) processed products. Crit. Rev. Food Sci. Nutr. **35** (1995) 509-534.

[2] CALIN, F., et al., Modified atmospheres packaging of fresh-ready-to-use grated carrots in polymeric film. J. Food Sci. **55** (1990) 1033-1038.

[3] BRACKETT, R.E., "Minimally Processed Refrigerated Fruits and Vegetables", Microbiological Spoilage and Pathogen in Minimally Processed Refrigerated Fruits and Vegetables (WEILY R. C. Ed) New York, Chapman and Hall, (1994) 260-312.

[4] MACHEIX, J.J., FLEURIET, A., BILLOR, J., Fruit phenolics. Boca Raton, F. L. CRC Press. (1990) p.1.

[5] WALKER, J.R.L., "Enzymatic Browning and its Prevention", Enzymatic Browning in Fruits: its Biochemistry and Control (LEE, C.Y. AND WHITAKER, J.R. Ed), ACS Symposium Series 600. Washington D.C. American Chemicals Society (1995) 8-22.

[6] ABBOTT, J.A., BUTA, J.G., Effect of antibrowning treatment on color and firmness of fresh-cut pears. J. Food Quality **25** (2002) 333-341.

[7] DONG, X., WROLSTAD, R.E., SUGAR, D., Extending shelf-life of fresh-cut pears. J. Food Sci. **65** (2000) 181-186.

[8] BEUCHAT, L.R., Pathogenic microorganisms associated with fresh produce. J. Food Prot. **59** (1996) 204-216.

[9] FOLEY, D.M., et al., Reduction of *Escherichia coli* 0157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. Radiation Physics and Chemistry. **63** (2002) 391-396.

[10] GOMBAS, D.E., et al., Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. **66** (2003) 55-569.

[11] WILEY, R.C., Minimally processed refrigerated fruits and vegetables. New-York Chapman and Hall, (1994) p. 366.

[12] BARRY-RYAN, C, PACUSSI, J.M., BEIRNE, D.O., Quality of shredded carrots as affected by packaging film and storage temperature. J. Food Sci. **65** (2000) 726-730.

[13] BERRANG, M.E., BRACKETT, R.E., BEUCHAT, L.R., Growth of *Listeria monocytogens* on fresh vegetables stored under controlled atmosphere. J. Food Prot. **52** (1989) 702-705.

[14] BEUCHAT, L.R., Surface disinfection of raw produce. Dairy Food Environ. Samit. **12** (1992) 6-9.

[15] THAYER, D.W., et al., Radiation pasteurization of food issue pepper No.7 (1996) Ames, IOWA: Council of Agricultural Science and Technology.

[16] FARKAS, J., Irradiation as a method for decontaminating food: a review. Int. J. Food Microbiol. **44** (1998) 189-204.

[17] THAYER, D.W., RAJKOWSKI K., T., Developments in irradiation of fresh fruits and vegetables. J. Food Sci. Techno. **53** (**11**) (1999) 62-65.

[18] LU, Z., et al., Preservation effects of gamma irradiation on fresh-cut celery. J. Food Engin. **67** (2005) 347-351.

[19] CHERVIN, C., BOISSEAU, P., Quality maintenance of ready-to-eat shredded carrots by gamma irradiation. J. Food Sci. **59** (1994) 359-361.

[20] FAN, X., SOKORAI, K., Sensorial and chemical quality of gamma-irradiated fresh-cut iceberg lettuce in modified atmosphere packages. J. Food Prot. **65** (2002) 1760-1765.

[21] American Public Health Association (APHA), Standard Methods for the Examination of Dairy Products. American Public Health Association, Washington, D.C. (1992).

[22] World Health Organization (WHO), Guidelines for drinking water quality, Vol.1. Recommendations, 2nd Edn. Geneva, World Health Organization (1993).

[23] International Commission on Microbiological Specifications for Food (ICMSF), "Microorganisms in Foods" 1, Their Significance and Methods of Enumeration, 2nd ed. Toronto, University of Toronto Press (1978).

[24] MOSSEL, D. A., "Streptococci", Streptococci in Food (SKINNER F.A., QUESNEL L.B. Ed) London: Academic Press (Society for Applied Bacteriology, Symposium Series No. 7) (1978) p. 392.

[25] PALUMBO, S.A., et al., Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microbiol. **50** (1985) 1027-1030.
[26] Food and Agricultural Organization of the United Nations (FAO), Manual of Food Quality Control. 12 Qualities Assurance in the Food Control Microbiological Laboratory, Rome, Food and Agriculture Organization of the United Nation (1991).
[27] International Organization for Standardization (ISO) , Microbiology- General Guidance on methods for the detection of Salmonella. ISO 6579:1993, (1993).
[28] AINA, J.O., OLADUNJOYE, O.O., Respiration, pectolytic activity and textural changes in ripening African mango (*Irvingia gabonensis)* fruits. J. Sci. Food Agric. **63** (1993) 451-454.

IMPROVING THE MICROBIOLOGICAL SAFETY OF SOME FRESH PRE-CUT AND PRE-PACKAGED CHILLED PRODUCE BY LOW-DOSE GAMMA IRRADIATION

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Abstract

Minimally processed (MP) ready-to-eat vegetables are increasingly consumed as a part of healthy diets. The aim of our studies was to determine radiation doses improving the microbial safety of MP vegetables and fruits without diminishing quality parameters of these produce. Effects of low dose irradiation on the microbiota, antioxidant vitamin contents and sensory properties of pre-cut tomato (*lycopersicon syn. L. esculentum)*, cantaloupe (*Cucumis melo*), watermelon (*Citrullus lanatus)* and sprouted seeds (alfalfa (*Medicago sativa)* and radish (*Raphanus sativus))* were investigated. Challenge testing with pathogens such as E. coli O157:H7 and *L. monocytogenes* were also carried out. Doses of 1-3 kGy are able to reduce considerably the microbiological contamination of fruits, vegetables, radish and alfalfa sprouts, but microorganisms surviving the irradiation are able to regrow during refrigerated storage. 1 kGy was acceptable radiation dose for the treatment of these products, having no significant effect on sensory properties, firmness and antioxidant vitamins. The effect of combination of low dose gamma-irradiation and modified atmosphere packaging on alfalfa and radish sprouts during storage was also investigated. Two different gas composition were applied in MAP: 2% O_2, 4% CO_2, 96% N_2 (1) and 3-5% O_2, 10-15% CO_2 balanced with N_2 (2). In inoculation studies, the behaviour of *Bacillus cereus* (1) and *Listeria monocytogenes* (1; 2) on alfalfa sprouts was studied. Experiments were carried out to adapt a new, rapid impedimetric method for potential detection of *L. monocytogenes* on treated sprouts. Furthermore, another aim was to examine possible relationship between electronic nose results and irradiation doses. Irradiation at 2 kGy reduced the initial levels of the examined pathogens and total microflora under the applied atmospheres, but the microbiota regrew during storage on the irradiated and the control samples too. The sensory analysis showed no significant differences between treated samples. Results of electronic nose investigations indicated significant difference between control (samples packed in air) and treated samples (MAP and MAP+ irradiation) at the day of irradiation treatment. Combination of low-dose gamma irradiation with modified atmosphere packaging and refrigerated storage can improve the microbiological safety and shelf-life of alfalfa sprouts. Further investigations are necessary to develop the composition of head-space in MAP able to prevent regrowth of surviving pathogens during storage.

1. INTRODUCTION

There is an increasing consumer demand on producing minimally processed vegetables and fruits without any preservatives. This produce is perceived as fresh, healthy and convenient. Minimal processing (MP) covers a wide range of technologies that aim to preserve food during transport from farm to fork, changing the inherent fresh-like attributes as little as possible [1]. Sales of MP (minimally processed) ready-to-eat fruits and vegetables have grown rapidly in developed countries in the last decade.

Minimally processed, chilled vegetables and fruits usually carry pseudomonads, enterobacteria, lactic acid bacteria and yeasts and molds as natural microbiota. The high moisture content and damaged plant tissues surfaces provide excellent conditions for the growth of microorganisms in these pre-cut/prepared products. There are several reports of outbreaks of enteric pathogens due to consumption of fresh fruits and vegetables (Table 1) [2, 3]. The potential sources of pathogenic bacteria include the raw produce, plant workers, and processing environment [4].

TABLE 1. EXAMPLES OF FRESH PRODUCE AND JUICE FROM WHICH BACTERIAL PATHOGENS HAVE BEEN ISOLATED [3]

Pathogen	**Product**
Aeromonas	alfalfa sprouts, asparagus, broccoli, cauliflower, celery, lettuce, pepper, spinach
Bacillus cereus	alfalfa sprouts, cress sprouts, cucumbers, mustard sprouts, soybean sprouts
Campylobacter jejuni	green onions, lettuce, mushroom, potato, parsley, pepper, spinach
Clostridium botulinum	cabbage, mushrooms, pepper
E. coli O157:H7	alfalfa sprouts, apple juice, cabbage, celery, cilantro, coriander, cress sprouts, lettuce
Listeria monocytogenes	bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushrooms, potatoes, radish, salad vegetables, tomato
Salmonella	alfalfa sprouts, artichokes, beet leaves, celery, cabbage, cantaloupe, cauliflower, chili, cilantro, eggplant, endive, fennel, green onions, lettuce, mungbean sprouts, mustard cress, orange juice, parsley, pepper, salad greens, spinach, strawberries, tomato, watermelon
Shigella	celery, cantaloupe, lettuce, parsley, scallions
Staphylococcus	alfalfa sprouts, carrot, lettuce, onions sprouts, parsley, radish
Vibrio cholerae	cabbage, coconut milk, lettuce

Sprouted seeds are also increasingly consumed as a part of health diets. Sprouts represent a specific issue because the sprouting procedure (conducted under high humidity at higher/elevated temperatures) is extremely favourable to growth of bacterial pathogens. The first reported outbreak of human illness associated with seed sprouts was in 1973 [5]. Vegetable sprouts produced using a home sprouting kit contained large numbers of *Bacillus cereus*. Raw alfalfa and clover sprouts have emerged as recognized sources of foodborne illness in the United States. The National Advisory Committee on Microbiological Criteria for Foods [6] reviewed the literature of sprout-associated outbreaks and identified the organisms and production practices of greatest public health concern. *Salmonella* or *Escherichia coli* O157:H7 infections are the most common illnesses associated with sprout consumption. Some publications reported, however, the presence of *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae* and *Aeromonas hydrophila* in sprouts [2]. Sprout-associated outbreaks have become a world-wide problem [6]. Although contamination of the sprouts could occur from seeds, contaminated equipments, contaminated water or poor hygienic handling, seed appears to be the most likely source of contamination in sprouts associated outbreaks [6]. Several chemical methods to decontaminate alfalfa seed have been investigated, such as rinsing with calcium hypochlorite, acidified sodium chlorite, acidified chlorine dioxide, trisodium phosphate, peracetic acid and ethanol [7; 8a,b]. The combination of a hot water treatment and a rinse in solutions of various chlorine-containing compounds has also been investigated [9]. Nevertheless, until now, no treatment has been found which is capable of completely eliminating *E. coli* O157:H7 or *Salmonella* spp. from alfalfa seeds destined for sprouting. The surface of fresh sprouts is difficult to clean, since the pathogen can be present not only on outer surfaces but also in inner tissues and in stomata when radish sprouts were raised from *E. coli* O157:H7-contaminated seeds [10].

Irradiation seems more efficient in reduction of bacterial contamination than sanitizers [11]. Literature reviews [12, 13, 14] show that irradiation with doses ranging from 0.5 to 2 kGy had no adverse effect on fresh produce stored a few days under refrigeration as minimally processed fresh fruits and vegetables. At 2 kGy, number of bacteria was usually reduced by 3 to 4 log cycles and yeasts by 1 or 2 log cycles. Limiting factors in irradiation of horticultural products are, however, sensorial changes, particularly softening of fruit and vegetable tissues, and vitamin losses.

Modified atmosphere packaging (MAP) is commonly applied to various fresh products to extend the shelf-life and maintain high quality of minimally processed fruits and vegetables. These conditions reduce deterioration by limiting product respiration and maturation as well as by slowing down the proliferation of aerobic spoilage organisms. MAP may be passive, in which packages are sealed in air, or active, in which a defined mixture of gases are used to flush the package, typically with reduced O_2 (2-3%) and increased CO_2 (5-20%), with the balance composed of N_2. For vegetables packaged under either system, there is no single ideal or standard gas mixture; the mixture of gases within the package changes over time in response to the respiration of the produce and the gas permeability of the packaging material, and the specific vegetable under consideration [15].

Chemosensor arrays, the so called electronic noses have been used in the last 20 years. An electronic nose is a system originally created to mimic the function of an animal nose. It involves various types of electronic chemical gas sensors with partial specificity, as well as suitable statistical methods enabling the recognition of complex odours. The raw responses generated by the sensors are analysed using various statistical methods. There is a wide range of applications on the field of food industry. Different applications have been published covering meat, poultry, cheese, fish, grain, beverages etc. [16, 17, 18, 19, 20]. Pathogenic strains sometimes found in meat products were detected and correctly classified [21, 22].

The aim of our studies was to determine radiation doses which do not diminish the quality parameters of pre-cut vegetables and fruits and sprouted seeds, and to investigate the effect of low dose irradiation of these products inoculated with food-borne pathogens, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7. Since traditional methods to detect pathogenic organisms are very labour- and time-consuming, there is a continuous need to develop and adapt new, rapid microbiological methods. Impedance microbiology is a rapid method that enables qualitative and quantitative tracing of microorganisms by measuring the change in the electrical conductivity. With direct impedance technology, the change in the conductivity of a liquid culture medium serves as a measuring parameter, whereas with indirect impedimetry, the change in the electrical conductivity of a reaction solution, which occurs through the absorption of gases from the inoculated bacterial culture, is measured. Most investigations concerning the applicability of impedimetry in food microbiology deal with the impedimetric detection or enumeration of *Enterobacteriaceae,* especially the detection of *Salmonella.* Furthermore, a great number of published findings concern the impedimetric determination of the total bacterial count. The successful application of this fast method on further areas of food hygiene, such as tracing antibiotics and testing additives for their antimicrobial effect, has also been described. In general the use of impedimetry for the application areas stated has been judged positively. However, the time and expense required by the user to optimize the method, the deficits when testing slightly contaminated sample material or determining the bacterial count in those cases in which the microorganisms are sublethally damaged, and the necessity of performing individual calibration for each food category limit the applicability of impedimetry [23].

The behaviour of vegetable-associated pathogens after combination of irradiation and MAP treatment will help to determine the applicability of these preservation treatments to these products as an antimicrobial intervention. The aim of our further studies was to investigate the effect of the combination of low-dose gamma irradiation and modified atmosphere packaging on alfalfa and radish sprouts. Examination of the effect of irradiation and MAP on survival of food-borne pathogens, such as *L. monocytogenes* and *Bacillus cereus* strains inoculated on sprouts to improve the microbiological safety of these products has been also carried out. Furthermore, another aim was to examine possible relationship between electronic nose results and irradiation doses. Similar relationship was studied

between the electronic nose responses and the observed microbiological or sensory quality of MAP packaged sprouts.

2. MATERIALS AND METHODS

2.1. Bacterial strains used in the inoculation studies

- *Escherichia coli* O157 ATCC 43 888, an avirulent strain, obtained from Dr. H. Niessen, Matforsk, Norway
- *Listeria monocytogenes* 4ab No. 10, an avirulent strain, obtained from Dr. B. Ralovich, Hungarian Meat Research Institute
- *Bacillus cereus* F4.26.90, psychrotrophic strain, ATO-DLO, Wageningen

2.2. Examinations on pre-cut vegetables and fruits

2.2.1. Source and preparation of fruits

Tomato (*Lycopersicon esculentum*), cantaloupe melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) were purchased from the local market. Tomatoes ("Precisa" cultivar) were washed with tap water, wiped with a dry cloth and were sliced or chopped (quartered). Cantaloupe and watermelons were washed with tap water and dried. After removing the rind, melons were cut with knives and the flesh was cut into approximately 2 cm cubes.

2.2.2. Radiation treatment

Approx. 100 g portions of pre-cut fruits were placed in covered plastic containers. Samples were irradiated with doses of 0.5 kGy, 1 kGy, 1.5 kGy and 2 kGy at a dose rate of 2.9 kGy/h at room temperature by a self-shielded ^{60}Co irradiator at the Central Food Research Institute, Budapest.

2.2.3. Estimation of sensorically acceptable radiation dose

Unirradiated control and irradiated batches were analysed sensorically by a sensory panel of 15 people, directly after the treatment on the basis of hedonic scores on colour, odour, taste and texture ranging from score (9) as excellent to score (1) as non-marketable. The firmness of the samples was instrumentally measured by using INSTRON Universal Texture Analyser.

2.2.4. Determination of antioxidant vitamins of radiation processed tomatoes

Samples of sliced tomatoes were irradiated with 1 kGy dose and stored in a refrigerator for three days. Directly after irradiation and after three days of storage antioxidant vitamins of irradiated and untreated samples were determined by *HPLC* in triplicates.

The level of ascorbic acid, carotenoids and tocopherols was measured by using a Beckman liquid chromatograph equipped with a Model 114 M pump, a Model 421 controller and UV detector (Model 165 variable wavelength UV/visible detector (for carotenoid and organic acid) and Shimadzu fluorescence detector (for tocopherols). The detector signals were recorded by a Model C-R2A Shimadzu Integrator. For photodiode-array detection a Waters Model 990 liquid chromatograph was used [24].

The method described by Daood and co-workers [25] was used for carotenoid and tocopherol extraction. Ascorbic acid was extracted with 2% metaphosphoric acid, and then filtered.

Quantitative determination and peak identification: The peaks of β-carotene were identified by computing their retention times and spectra with that of a pure standard (Sigma, St. Louis, USA).

Identification of cis isomers of carotenoids was based on the appearance of extra maxima between 320 and 360 nm in the absorption spectrum of the individual peaks [26]. For identification of peaks the retention times and maximum absorption spectra of tocopherols were compared with those of standard materials (Sigma, St. Louis, USA) which were also used for quantification. Chromatographic peaks of ascorbic acid were identified by comparing both retention time and absorbance spectra with those of standards (Fluka, Switzerland).

2.2.5. E. coli and Listeria monocytogenes inoculum, inoculating and packaging procedures

The stock cultures of the test organisms were activated by culturing in brain heart infusion broth (BHI, OXOID CM 225) at 30°C for 24 hours in a shaking waterbath. These cultures were diluted with sterile water to yield a suspension of approx. 10^7 CFU/ml. Five kg each of pre-cut fruits were dipped in 3 liter volumes of suspensions of the test strains for 10 minutes, resulting in an initial contamination level of 10^4-10^5 CFU/g. Samples were drained and approx. 100 g portions of pre-cut inoculated vegetables were placed in approx. 200 ml volume, covered plastic containers.

2.2.6. Radiation treatment of inoculated samples and storage

Inoculated samples were irradiated with 1 kGy of dose. Unirradiated control and irradiated batches were stored at 5 and 15°C, respectively, for seven days. Directly after irradiation and on 3rd and 7th day of storage, duplicate samples from both untreated and irradiated batches were analysed at each sampling time.

2.2.7. Microbiological analysis

2.2.7.1. Effect of the irradiation on the natural microbiota of pre-cut tomatoes

Stock suspensions of pre-cut tomatoes were prepared by combining 100 g samples with 100 ml of sterile aqueous diluent containing 0.1% peptone and 0.9% NaCl. Total aerobic microorganisms populations were estimated on plate count agar (PCA, MERCK 1.05463), 48 h at 30°C. Presumptive lactic acid bacteria were estimated using double layers of MRS agar (MERCK, 1.10660, 30°C for five days), whereas the numbers of yeasts and molds were followed by surface spreading of 0.1 ml aliquots on glucose yeast agar (MERCK 1.10877) supplemented with chloramphenicol in 0.1 g/l concentration (25°C, five days).

2.2.7.2. Microbiology of inoculated samples

At each sampling time, duplicates of approx. 100 g of samples were homogenized with the same amount of buffered peptone water in a stomacher blender for 1 min. Serial dilutions were made in saline.

Total plate counts were determined on plate count agar (MERCK 1.05463), plates were incubated at 30°C for 48 h.

L. monocytogenes: For selective estimation of *Listeria* 0.1 ml of the stock suspensions and dilution levels were investigated by spread-plate on Listeria Selective Agar (OXOID CM 856) supplemented with *Listeria* selective supplement (Oxford formulation, OXOID SR 140 E). The plates were incubated for 24 h at 30°C before characteristic presumptive *L. monocytogenes* colonies were investigated with catalase and oxidase tests.

E. coli O157: Serial dilutions of the samples (1 ml) were transferred to sterile Eppendorf tubes containing 0.02 ml of Dynabeads® anti-E.coli O157 immonomagnetic beads (Dynal, UK). According to the manufacturers instructions, the Eppendorf tubes were placed in a MPC®-M rack, mixed for 30 min, before discarding the supernatant fluid and re-suspending the beads (without the magnet) in wash buffer (PBS + 0.05% Tween 20). Two further washing stages were performed prior to transferring the beads (in 0.1 ml buffer) onto CT-SMAC selective agar plates (sorbitol MacConkey agar supplemented

with cefixime 0.05 mg/l and potassium tellurite 2.5 mg/l, SIFIN, TN 1813) and incubated at 37°C for 18-24 h.

2.3. Effect of irradiation on the safety of seeds and sprouts

2.3.1. Radiation treatment of seeds and sprouts

Alfalfa (*Medicago sativa*) and radish (*Raphanus sativus*) seeds were obtained from local producer. Seeds were irradiated in plastic bags with doses of 1 kGy, 2 kGy and 3 kGy at a dose rate of 2.42 kGy/h at room temperature by a self-shielded ^{60}Co irradiator at the Central Food Research Institute, Budapest. Unirradiated control and irradiated batches were stored at room temperature until microbiological analysis and germination studies. Sprouts were stored in a refrigerator 4°C until analysis.

2.3.2. Effect of irradiation on the natural microbiota of alfalfa and radish seeds

Removing cells from seeds: 2 g of seeds were combined with 18 ml of sterile dilution fluid (containing 1 g/l Tween 80, 8.5 g/l NaCl, 1 g/l peptone). The mixture was agitated in a shaker at room temperature for 1 hour. Further serial dilutions were made in saline.

Total plate counts were determined on plate count agar (MERCK 1.05463) by spread-plating of 0.1 ml aliquots, plates were incubated at 30°C for 48 h.

Enterobacteriaceae: double layers of VRBD Agar (MERCK 1.10275), incubation at 37°C for 24 h.

Yeast and mold count: surface spreading on Rose-bengal chloramphenicol agar (MERCK 1.00467), incubation at 25°C for 3-5 days.

All the microbiological investigations have been carried out in triplicates.

2.3.3. Effect of irradiation on the microbiota of alfalfa and radish sprouts

Sprouting procedure: 100-100 seeds (irradiated and untreated) were counted onto sterile paper waddings covered with filter paper and placed in Petri-dishes. Sprouts were grown at room temperature and watered twice daily by spraying with water. After five days sprouts were removed from the Petri-dishes and placed in a sterile stomacher bag. Sprouts were diluted five-times with saline and pummelled for 1 min in a stomacher. Total aerobic plate count, Enterobacteriaceae and yeast- and mold count were determined as described in 2.3.2.

2.3.4. Estimation of radiation dosage effects on seed germination and yield ratio

Five-day old sprouts grown from 100 irradiated or untreated seeds were weighed and the number of sprouted seeds was calculated.

2.3.5. Inoculation studies

2.3.5.1. *E. coli* and *Listeria monocytogenes* inoculums, inoculating procedures

Inoculum was prepared as described in 2.2.5. 150 g of seed were submerged in 300 ml cell suspension for 1 min with constant gently agitation. The cell suspension was decanted, and seeds were placed on sterile paper waddings covered by filter paper in large Petri-dishes. Inoculated seeds were dried under laminar air-flow cabinet at room temperature for 24 hours.

2.3.5.2. Radiation treatment of inoculated seeds

After 24 hours drying, inoculated seeds were packed in plastic bags and irradiated with 1, 2 and 3 kGy of doses at the Central Food Research Institute, Budapest.

2.3.5.3. Examination the effect of irradiation on survival of pathogenic microorganisms on seeds

Listeria monocytogenes: Stock suspensions and selective estimation was made as described in section 2.2.7. Thin agar layer (TAL) method [27] was also performed to estimate the number of sublethally damaged cells. Double thin layer of plate count agar were poured on the surface of Listeria selective agar, and 0.1 ml of suspensions were spreaded. The plates were incubated for 24 h at 30°C.

E. coli O157: Method described in section 2.2.7. was performed. In case of seeds irradiated with 2 kGy, before immunomagnetic separation, selective enrichment was performed in mEC Broth with Novobiocin (MERCK 1.14582) at 37°C for 20 h.

2.3.5.4. Experiments on sprouts produced from inoculated seeds

Sprouts were grown as in section 2.3.3., using seeds inoculated with *E. coli* O157 or *L. monocytogenes*. Sprouts were also produced from seeds after one week storage at room temperature subsequent to inoculation with *L. monocytogenes* and irradiation with 1, 2 and 3 kGy. The numbers of *E. coli* and *L. monocytogenes* were determined on 5 days old sprouts. Sprouts grown from inoculated seeds were irradiated with 1, 2 and 3 kGy. Survival of pathogenic microorganisms was detected by the method 2.2.7**.**

2.3.5.5. Determination of radiation D_{10}-value of Listeria monocytogenes on alfalfa sprouts

The inoculation and sprouting procedure was conducted the same way as described in section 2.3.5.1. and 2.3.3., respectively.

Sprouts were irradiated in plastic bags with doses of 0.5 kGy, 1 kGy, 1.5 kGy and 2 kGy at room temperature. Unirradiated control and irradiated batches were stored in a refrigerator until microbiological analysis.

Irradiations of the samples in all experiments in this project were carried out by a self-shielded ^{60}Co irradiator at the Central Food Research Institute, Budapest.

For determination of radiation survivors of *L. monocytogenes*, Thin agar layer (TAL) method [27] was performed to promote the recovery of the sublethally damaged cells (2.3.5.3.). The microbiological investigations have been carried out in triplicates.

2.3.6. Effect of irradiation on the sensorial properties of alfalfa and radish sprouts

Commercial batches of fresh alfalfa and radish sprouts were obtained from local Bio shop. Samples were irradiated with 1 kGy and 2 kGy doses at room temperature. Unirradiated control and irradiated batches were analysed sensorically by a sensory panel of 15 people after the treatment on the basis of hedonic scores on colour, odour, taste and texture ranging from score (9) as excellent to score (1) as non-marketable. The colour of the samples was determined instrumentally with a Minolta CR200 type colorimeter to estimate the tristimulus colour values of the sprouts.

2.3.7 Determination of antioxidant vitamins of radiation processed alfalfa and radish sprouts

Samples of fresh commercial alfalfa and radish sprouts were irradiated with 1 and 2 kGy of doses. After irradiation antioxidant vitamins (as ascorbic acid and tocopherols) of irradiated and untreated samples were determined by HPLC in duplicates by the same method described in 2.2.4.

2.3.8. Effect of irradiation on the microbiological shelf-life of alfalfa and radish sprouts

Commercial samples of fresh alfalfa and radish sprouts were irradiated with 1 kGy and 2 kGy doses. After irradiation samples were stored in a refrigerator at 5°C for 10 days. Irradiation of the samples was carried out at the second day of the declared shelf-life (10 days at a refrigeration temperature) given by the producer on the packaging material.

Microbiological analysis was carried out periodically in duplicates. Microbiological counts were statistically analysed by Microsoft Office Excel 97.

Sprouts were diluted five times with pepton-saline and pummelled for 1 min in a Stomacher.

Total aerobic plate counts were determined on plate count agar (MERCK 1.05463) by spread-plating of 0.1 ml aliquots, plates were incubated at 30°C for 48 h.

Enterobacteriaceae: double layers of VRBD agar (MERCK 1.10275), incubation at 37°C for 24 h.

Lactic acid bacteria (LAB): de Man-Rogosa-Sharp medium (MERCK 1.10660), overlaid with the same medium and incubated aerobically at 30°C for three days.

Yeast and mold count: surface spreading on Rose-bengal Chloramphenicol Agar (MERCK 1.00467), incubation at 25°C for 3-5 days.

Salmonella: Presence-absence test (EN-ISO 6579:2002) [28].

L. monocytogenes: Presence-absence test (EN-ISO 11290-1:1996) [29].

2.4. Combination of irradiation and MAP to improve safety of sprouts

2.4.1. Source and preparation of sprouts

Fresh alfalfa and radish sprouts were obtained from local Bio shop. Declared shelf-life of these products given by the supplier was 10 days at 5°C.

2.4.2. MAP studies

From alfalfa and radish sprouts 5-5 g were placed in CombiTherm 80 bags flushed with two different gas mixtures containing 2% O_2, 4% CO_2 and 94% N_2 (1), and 3-5% O_2, 10-15% CO_2 balanced with N_2 (2) before sealing with MULTIVAC packaging equipment. Gas mixture (2) was used only in case of alfalfa sprouts.

2.4.3. Radiation treatment

The samples were irradiated in a NORATOM Co^{60} gamma irradiator of the Institute for Radiobiology, Budapest, at room temperature and at a dose rate of 6.47 kGy/h.

2.4.4. Microbiological analysis

After irradiation, samples were stored in a refrigerator at 5°C for 10 days. Microbiological analysis was carried out periodically (0, 2, 6, 10 days) in triplicates. Microbiological counts were statistically analysed by Microsoft Office Excel 97. Sprouts were diluted five-times with peptone-saline and pummelled for 1 min in a Stomacher.

Total aerobic plate counts, *Enterobacteriaceae*, Lactic acid bacteria (LAB), Yeast and mold count were determined as in 2.3.8.

Gas composition in the head-space of the sample bags were analysed periodically by CombiCheck 9800-1 apparatus (PBI Dansensor, Denmark) in triplicates.

2.4.5. Estimation of survival and growth of pathogenic bacteria on modified atmosphere packaged alfalfa sprouts after gamma irradiation

2.4.5.1. Inoculation studies with *Listeria monocytogenes*

The stock culture of the test organism were activated by culturing in brain heart infusion broth (BHI, OXOID CM 225) at 30°C for 24 hours in a shaking waterbath. The culture was diluted with sterile water to yield a suspension of approx. 10^7 CFU/ml.150 g of alfalfa sprouts were dipped in 500 ml volume of suspensions of the test strain for one minute with constant gentle agitation. The cell suspension was decanted, and sprouts were placed on sterile filter papers.

For determination of radiation survivors of *L. monocytogenes***,** parallel with selective plating on Palcam agar, TAL method was also performed (2.3.5.3.)

2.4.5.2. Estimation of survival and growth of *L. monocytogenes* by rapid method

For rapid, instrumental detection of *L. monocytogenes* RABIT impedimetric equipment (Don Whitley Scientific, U.K.) was used to adapt the method developed in our laboratory [30]. Half ml of diluted suspensions were pipetted into 4,5 ml selective conductimetric broth (Whitley Impedance broth + glucose (2 g/l) + lithium-cloride (15 g/l) + aesculin (1 g/l) + Fe(III)-ammonium-citrate (1 g/l) + FRASER Selective Supplement "half" concentration, MERCK 1.10399) in triplicate and incubated at 30°C in the RABIT equipment for 24 h. "Indirect measurement" was carried out [31], the TTD-values were recorded automatically.

2.4.5.3. Inoculation studies with Bacillus cereus spores

The spore suspension was prepared on nutrient agar plates fortified with minerals according to composition of FNA medium described by Johnson et al. [32]. FNA plates were surface inoculated with an overnight culture of *B. cereus* grown in trypticase soy broth (TSB, Oxoid CM 129) at 30°C for three days, then the plates were held at 5°C for 24 hr and the extent of sporulation was checked by phase contrast microscopy.

Spores developed on the FNA plates were suspended in cold sterile distilled water by scraping the surface with a bent glass rod. To purify spores from vegetative debris, the suspension was treated with membrane sterilized lysosyme (concentration of Lyosyme C: 2100 U/ml) for 24 hr at 5°C. Following the lysosyme treatment, the suspension was centrifuged five times for 20 min with 8000 rpm. Between each centrifugation the supernatant fluid was discarded and pellets were re-suspended in cold sterile distilled water. The final stock suspension containing 98,8% refractive spores were stored at a concentration of ca. 10^9 spores per ml in sterile distilled water in a refrigerator.

The spore suspension was heat activated at 60°C for 30 min and 1 ml aliquot was diluted with 1 litter of sterile peptone saline. 260 g of alfalfa sprouts were dipped into the suspension with gentle agitation for 5 min. The cell suspension was decanted, and sprouts were placed on sterile filter papers.

For determination of radiation survivors of *B. cereus***,** parallel with selective plating on MYP agar (Cereus selective agar acc. to Mossel, MERCK 1.05267 with Polymyxin B supplement Nr. 1.09875 and egg yolk emulsion). From serial dilutions of the samples 0.1 ml of suspensions were spread onto the agar plates. The plates were incubated for 48 h at 30°C. All the microbiological investigations have been carried out in triplicates.

2.4.5.4. Radiation treatment of inoculated MAP samples

After drying, inoculated samples were packaged and irradiated as described in 2.4.3. Survival curves were estimated from radiation doses of 0, 0.5, 1.0, 1.5, and 2.0 kGy for *L. monocytogenes* 4ab, and doses of 0, 1.0, 2.0 and 3.0 for *B. cereus*. Samples were stored at 5°C for 10 days and analysed periodically.

2.4.6. Determination of sensorial quality of irradiated sprouts in modified atmosphere packages

Non-inoculates samples were used for sensory testing one day after the combined treatment (1) and after the combined treatment and the end of the storage in case of (2) by panels of ten judges rating samples on the basis of hedonic scores on colour, odour, taste and texture ranging from score (9) as excellent to score (1) as non-marketable. Samples packaged in commercial plastic containers (without MAP) served as absolute controls. Hedonic scores were transformed to rank-sums and evaluated statistically by Kramer's method.

2.4.7. Electronic nose analysis

Electronic nose determinations were performed with an NST 3320 instrument (Applied Sensor, Sweden), with a built-in headspace autosampler for 12 samples, a detector unit containing 24 different sensors and software for collecting and processing data from the sensors. The NST 3320 is equipped with 10 MOSFET (metal oxide semiconductor field effect transistor) sensors, 12 MOS (metal oxide semiconductor) sensors and a selective infra-red absorption sensor for carbon dioxide and a humidity sensor for measuring relative humidity. Temperature control range was 8-65°C. The sample amount was 1-3 g, and the measuring temperature 25°C. The software NST Senstool includes the following tools: principal component analysis (PCA), partial least squares regression (PLS) and artificial neural network (ANN).

3. RESULTS AND DISCUSSION

3.1. Studies on pre-cut vegetables and fruits

3.1.1. Selection of acceptable radiation dose

Sensory testing of pre-cut, irradiated tomatoes, cantaloupe melon and watermelon showed that according to Kramer's rank test, statistically significant differences in organoleptic properties (colour, odour, taste and texture) were determined only in case of watermelon, at doses higher than 1.5 kGy (Table 2). Rank sums of pre-cut tomato and cantaloupe cubes were not significantly different neither at $\alpha \leq 0.01$ nor at $\alpha \leq 0.05$ probability level (data not shown).

TABLE 2. SENSORY TESTING OF WATERMELON CUBES

Radiation dose (kGy)	Score means				Rank sums			
	Colour	Odour	Taste	Texture	Colour	Odour	Taste	Texture
0	6,47	6,4	6,60	7,13	40,5	46	35	34,5
0,5	6,2	6,87	5,8	6,73	41,5	36	49,5	38
1	6,67	6	5,06	6,07	39,5	53,5	53,5	55
1,5	7,13	7,73	7,33	7,13	33,5	24,5**	27,5**	35,5
2	4,93	5,13	4,87	5	70,0*	65	59,5*	62,0**

** rank sums within the range 30-60 are not significantly different at $\alpha \leq 0.01$ probability level
* rank sums within the range 32-58 are not significantly different at $\alpha \leq 0.05$ probability level

Firmness of the samples measured by INSTRON equipment showed statistically significant softening (tendering) only of watermelon cubes at radiation dose of 2 kGy (Fig.1).

On the basis of the sensory panel, a dose of 1 kGy has been selected for radiation treatment. According to the regulation of US Food and Drug Administration, published in 1993, the word "fresh" can be used in labelling if the treatment of raw foods with ionizing radiation not to exceed the maximum dose of 1 kGy [33].

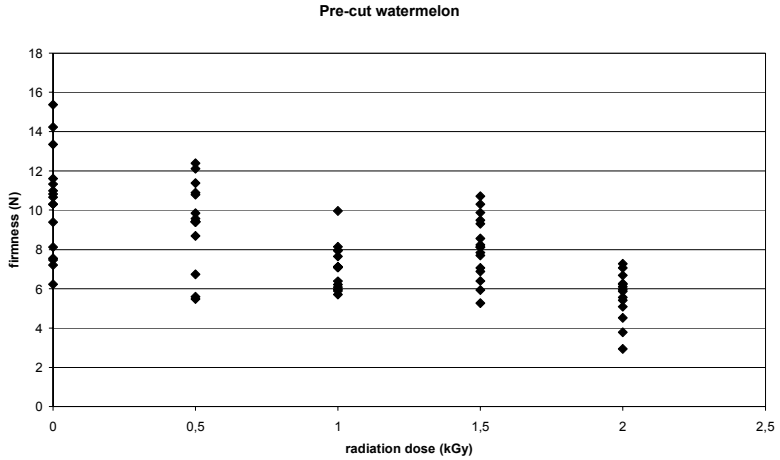


FIG. 1. Firmness of pre-cut watermelon measured by INSTRON equipment.

3.1.2. Assessment of maintenance of antioxidant vitamins of radiation processed pre-cut tomato

HPLC-patterns of carotenoids extracted from 1 kGy irradiated and untreated tomatoes are shown in Fig. 2. Radiation dose of 1 kGy had no significant effect on total carotenoid and vitamin C content of sliced tomatoes, however, it caused approx. 40% decrease in α-tocopherol (Fig. 3).

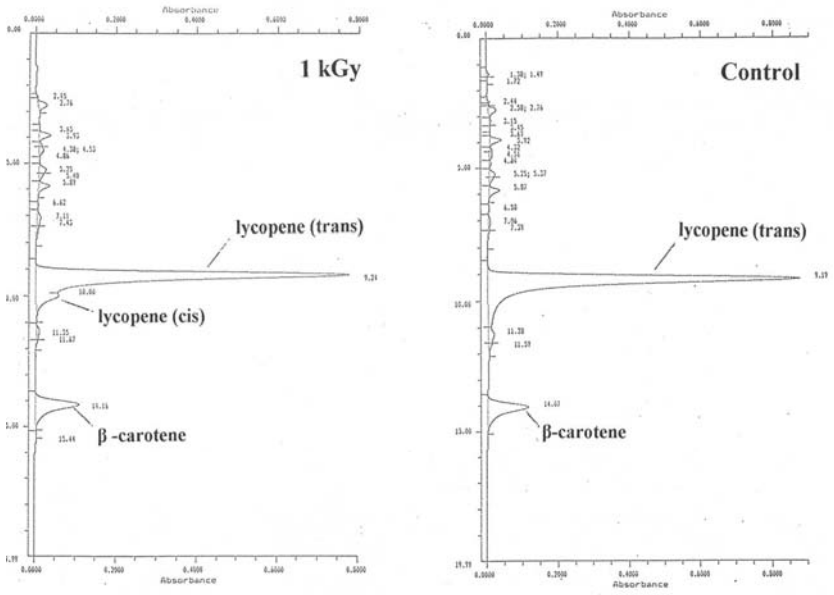


FIG. 2. HPLC patterns of carotenoids extracted from 1 kGy irradiated and untreated pre-cut tomatoes.

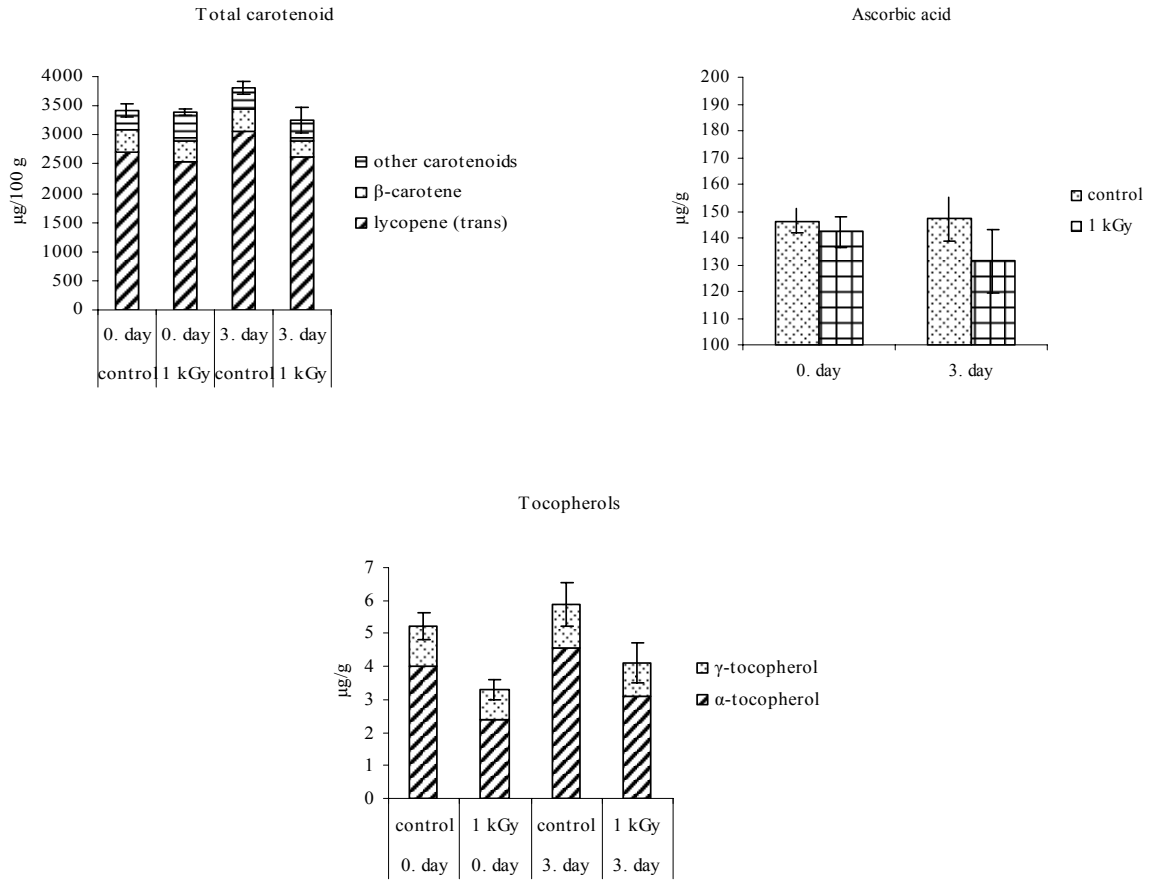


FIG. 3 Effect of the irradiation on the carotenoid content of pre-cut tomatoes (mean values of three samples with their standard deviations).

3.1.3. Native microbiota of pre-cut tomatoes

Change in natural microbiota of sliced tomatoes (initial pH: 4.0) are shown in Fig. 4. It can be seen that the radiation treatment reduced the relatively low initial total aerobic plate count (TPC) by more than one log-cycle. After seven days of storage at 5°C, the total aerobic plate counts of the unirradiated samples were around 10^7 CFU/g while those of irradiated samples were 10^4 CFU/g. As compared to the total aerobic plate counts, the presumptive lactic acid bacteria (LAB) were dominant in the initial microbiota of the sliced tomato, but under this refrigeration conditions lactic acid bacteria could not grow rapidly. Yeasts and molds were initially relatively in low numbers present on the sliced tomatoes, but at the end of the storage period they formed a dominant part of the microbiota.

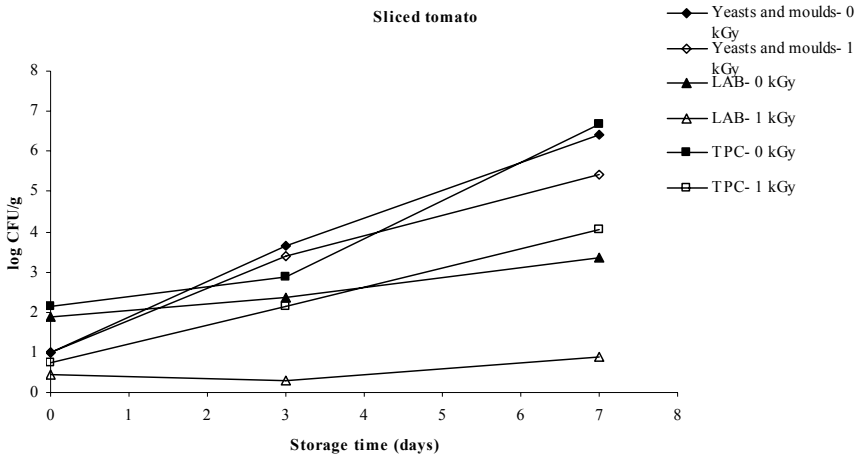


FIG. 4. Native microbiota of pre-cut, irradiated tomato.

3.1.4 Inoculation studies

The fate of microbiota on untreated and irradiated inoculated tomatoes (initial pH: 4.1) are shown by the Figures 5 and 6. The low-dose irradiation reduced the viable cell count of *Listeria monocytogenes* by two log-cycles. At 15°C recovery of surviving *L. monocytogenes* was noted, but the number of CFU of irradiated samples did not exceed the initial inoculation level. Growth of *Listeria* was not detected in samples stored at 5°C. Beuchat and Brackett [34] investigated the growth of *L. monocytogenes* on raw tomatoes, and found that this pathogenic microorganism was able to grow at refrigeration temperatures, but tomatoes were not a good substrate for the organism. *L. monocytogenes* can remain viable on raw whole and chopped tomatoes for periods extending beyond their normal shelf-life expectancy. *E. coli* O157 strain proved to be more sensitive to radiation treatment. A dose of 1 kGy reduced the viable cell number by more than 5 log-cycles. *E. coli* was able to grow on tomatoes only at 15°C, even survivors of radiation treatment, after three days of storage. At refrigeration temperature the number of *E. coli* remained stable during seven days of storage.

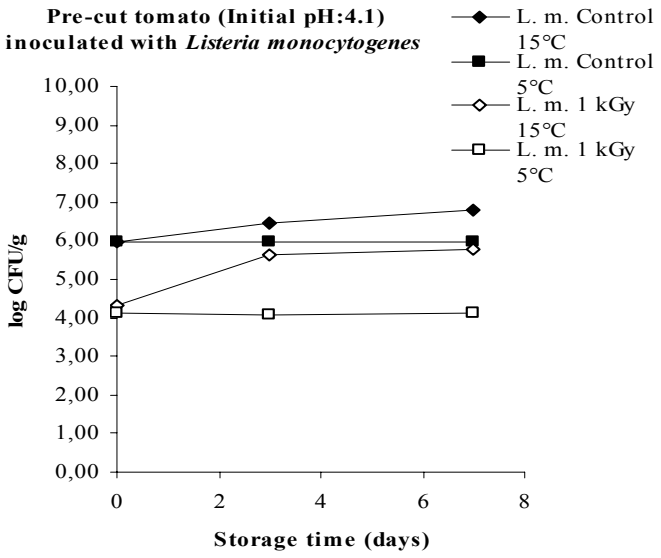


FIG. 5. Effect of low-dose irradiation on viable cell counts of pre-cut tomato inoculated with Listeria monocytogenes.

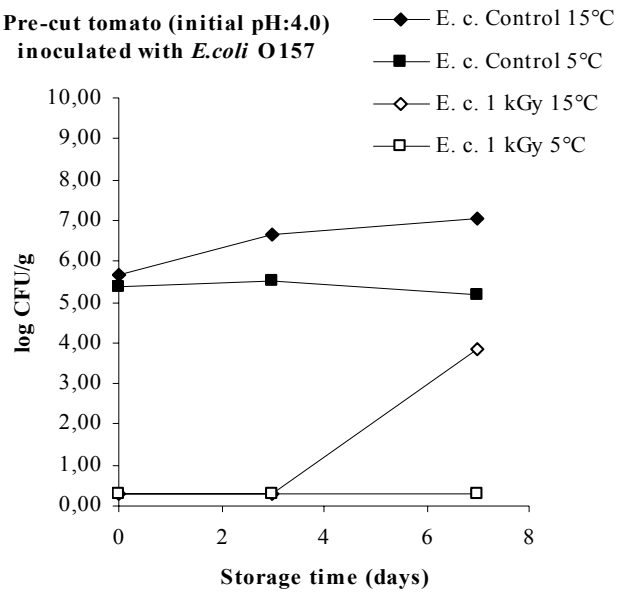


FIG. 6. Effect of low-dose irradiation on viable cell counts of pre-cut tomato inoculated with E. coli O157.

In the case of pre-cut cantaloup samples, 1 kGy irradiation caused 2 log cycles reduction of *L. monocytogenes*, and about 5 log-cycles reduction of inoculated *E. coli* O157 (Figures 7 and 8). The relatively high initial pH of cantaloup melon allowed the growth both of *L. monocytogenes* and *E. coli* 157 cells even at 5°C storage temperature.

Results of inoculation studies of watermelon are shown at Figures 9 and 10. Low-dose irradiation had the same effect in case of pre-cut watermelon, with initial pH of 5.5. After irradiation, the surviving cells of both pathogens examined were able to grow at 15°C, and *L. monocytogenes* at 5°C temperatures. Del Rosario and Beuchat [35] demonstrated that *E. coli* O157:H7 was able to grow on cubes of cantaloupe and watermelon stored at 25°C but remained constant at 5°C over a 34-h storage period.

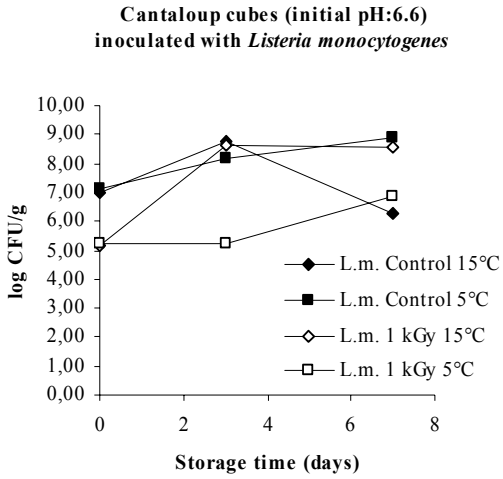


FIG. 7. Effect of low-dose irradiation on cell counts of L. monocytogenes inoculated on pre-cut cantaloupe.

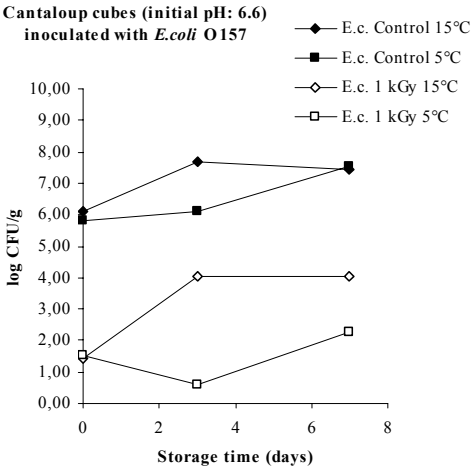


FIG. 8. Effect of low-dose irradiation on cell counts of E. coli 0157 inoculated on pre-cut cantaloupe.

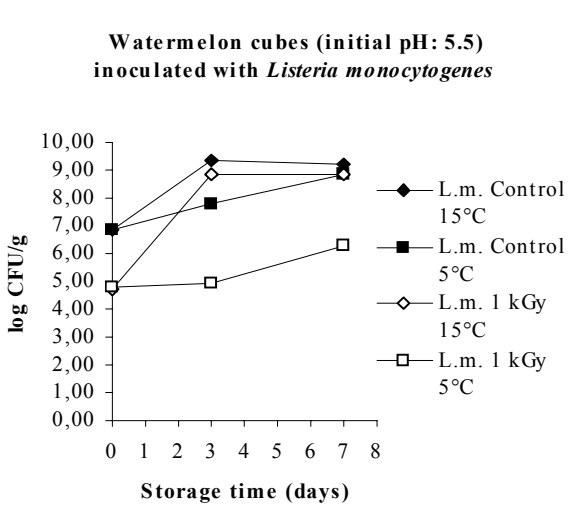


FIG. 9. Effect of low-dose irradiation on cell counts of L. monocytogenes inoculated on pre-cut watermelon.

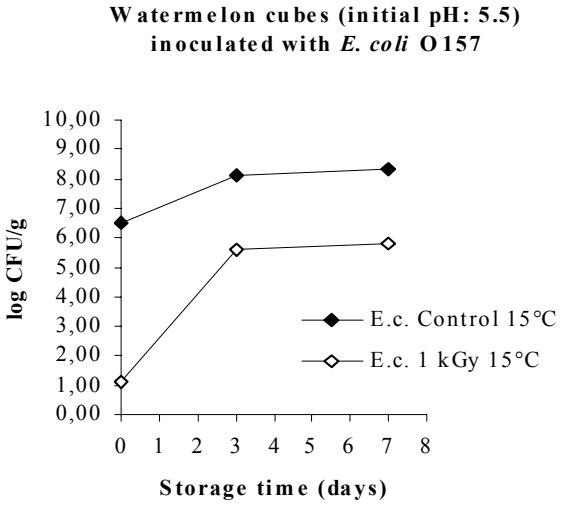


FIG. 10. Effect of low-dose irradiation on cell counts of E. coli 0157 inoculated on pre-cut cantaloupe.

3.2. Effect of irradiation on the safety of seeds and sprouts

3.2.1. Effect of irradiation on the natural microbiota of alfalfa and radish seeds

The changes in microbiota of radish and alfalfa seeds after irradiation are shown in Figures 11-13. The initial total aerobic plate counts of these seeds were around 10^4 CFU/g, which is in accordance with literature data [36, 37]. A radiation dose of 1 kGy resulted in about 1 log-cycle reduction of total aerobic plate count of radish seeds, whereas on alfalfa seeds 2 kGy dose caused about 1.5 log-cycle reduction. *Enterobacteriaceae* did not represent the dominant part in the microbiota. The number of *Enterobacteriaceae* was under detection limit (10/g) on all radish seed samples. On untreated alfalfa seeds about 10^3/g *Enterobacteriaceae* were detected, but after irradiation with 2 kGy, their number

was under detection limit. Molds were detected only on the untreated radish seeds, their number was under 100 CFU/g.

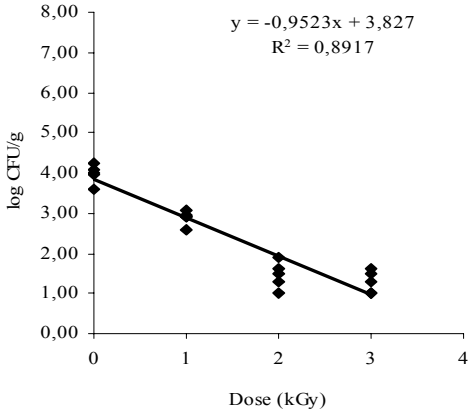


FIG. 11. Effect of irradiation on total plate count of radish seed.

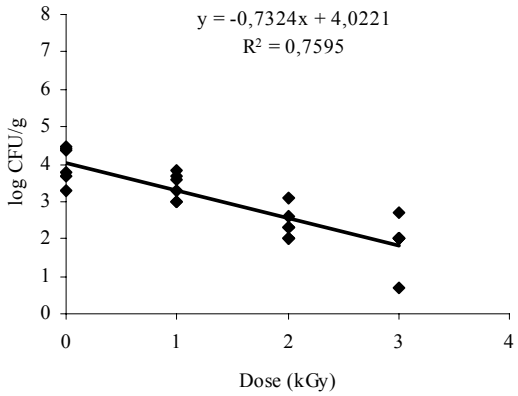


FIG. 12. Effect of irradiation on total plate count of alfalfa seed.

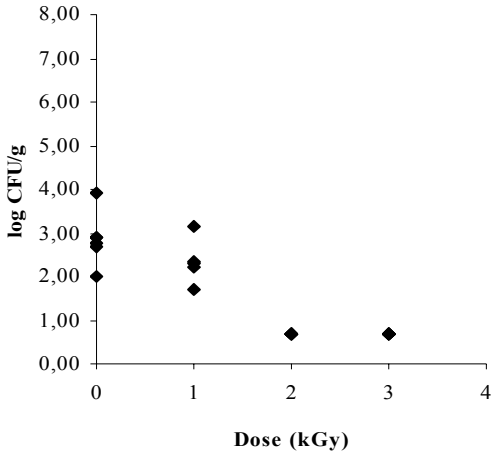


FIG. 13. Effect of irradiation on Entobacteriaceae-count of alfalfa seed.

3.2.2. Microbiota of vegetable sprouts grown from irradiated seeds

The evenly high total plate counts of radish and alfalfa sprouts (Figures 14 and 16) demonstrated that the sprouting conditions (high humidity, room temperature) are ideal for bacterial growth. The microbiota surviving the low-dose radiation treatment is able to grow and reach the same level as on the control samples after five days. *Enterobacteriaceae* were present in all samples (Figures 15 and 17), but did not represent the dominant part of the microbiota. Yeasts or molds were not detected.

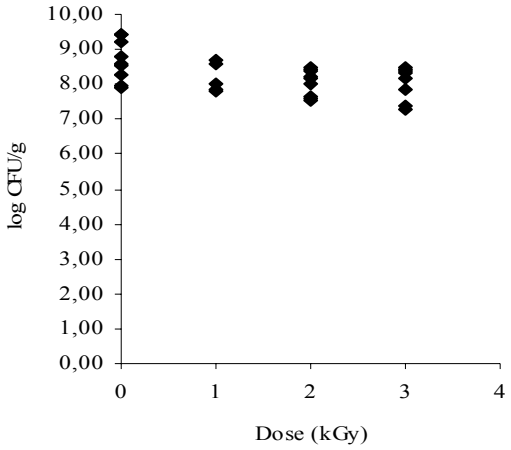


FIG. 14. Total plate count of radish sprouts grown from irradiated seeds.

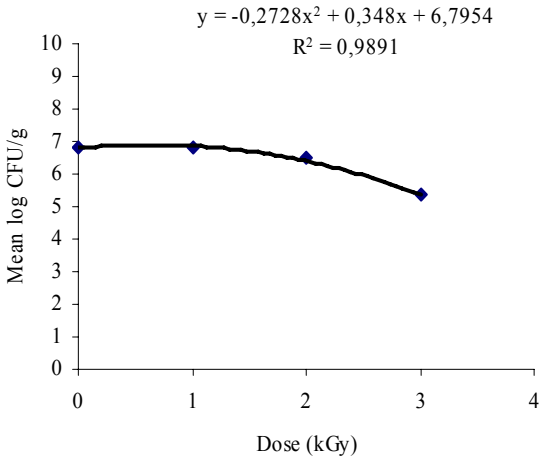


FIG. 15. Entererobacteriaceae-count of radish sprouts grown from irradiated seeds.

3.2.3. Estimation of radiation dosage effects on seed germination and yield ratio

The effects of the low-dose gamma irradiation on the germination ratio of radish and alfalfa seeds are shown on the Figures 18-19. Radiation doses in the range of 1-3 kGy, resulted in different reduction in germination capacity of the radish and alfalfa seeds. Radish seeds were more sensitive for the irradiation, 1 kGy radiation dose decreased the germination ratio by about 18%. The same dose in case of alfalfa seeds caused only about 7% reduction in germination. These experiments demonstrated that the effect of selected processing dose on the germination of the seeds has to be studied item by item.

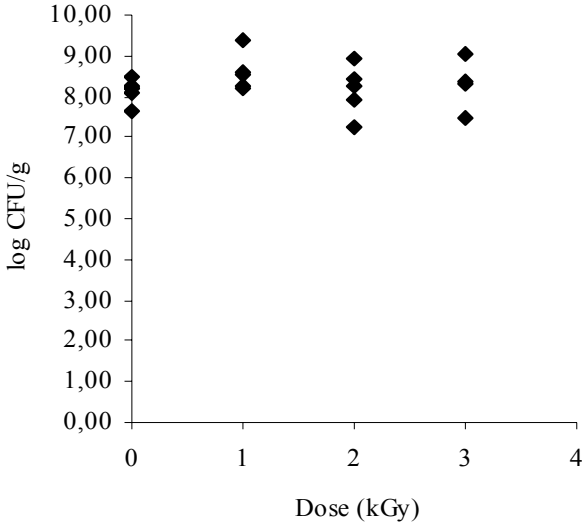


FIG. 16. total plate count of alfalfa sprouts grown from irradiated seeds.

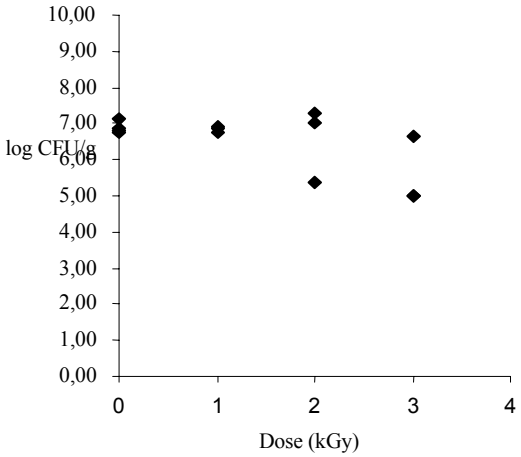


FIG. 17. Enterobacteriaceae-count of alfalfa sprouts grown from irradiated seeds.

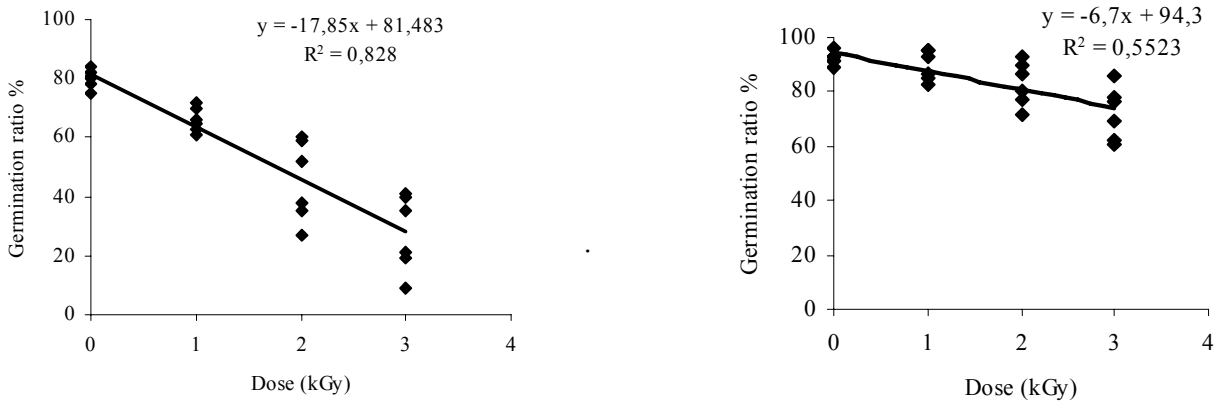


FIG. 18-19. Effect of the irradiation on the germination of radish seeds and of alfalfa seeds

3.2.4. Examination of the effect of low-dose irradiation on survival of pathogenic microorganisms inoculated on seeds

The number of *L. monocytogenes* in the suspension used for the inoculation of the seeds amounted $1.4x10^7$ CFU/ml. After 1 min duration of inoculation about 10^5 cells/g were adhered on the surface of the seeds. Irradiation with 1 kGy dose reduced their number by about 1 log-cycle on seeds examined (Figures. 20 and 21). A sublethal damage of *L. monocytogenes* cells was demonstrated by the TAL-method, however, its degree was low.

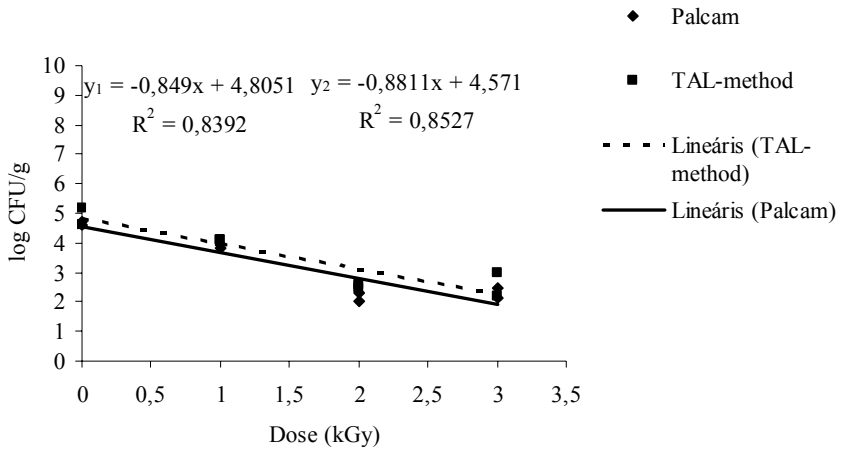


FIG. 20. Effect of irradiation on survival of Listeria monocytogenes on radish seeds.

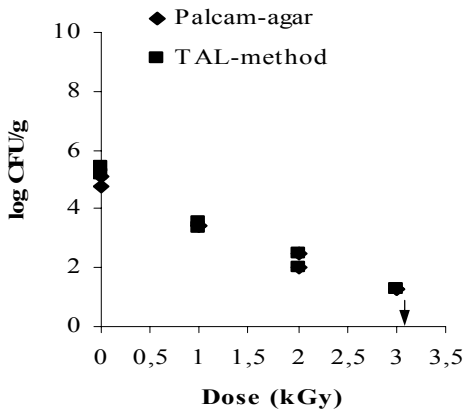


FIG. 21. Effect of irradiation on survival of Listeria monocytogenes on alfalfa seeds.

The *E. coli* O157 strain proved to be more sensitive for low-dose irradiation: 1 kGy dose reduced their initial number (10^4 CFU/g) by about 2-3 log-cycles both on radish or alfalfa seeds (Figures 22 and 23).

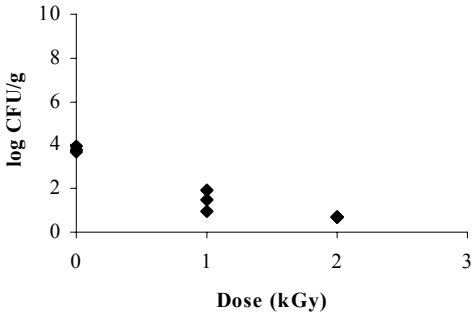


FIG. 22. Effect of irradiation on survival of E. coli O157 on radish seeds.

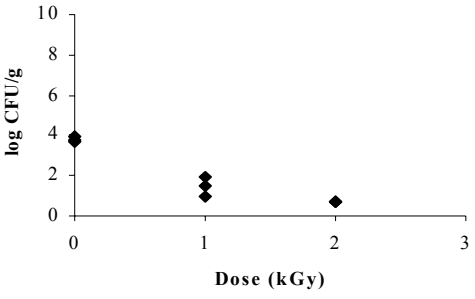


FIG. 23. Effect of irradiation on survival of E. coli O157 on alfalfa seeds.

3.2.5. Growth of L. monocytogenes during sprouting

The radiation survivors of *L. monocytogenes* were able to grow during sprouting procedure (Figures 24 and 25) and reached the same order of magnitude in all samples. The background microbiota had no effect on the growth of this pathogen. Pálmay and Buchanan [38] showed the potential of *L. monocytogenes* to proliferate rapidly on germinating alfalfa sprouts. The pathogen was able to reach relatively high levels (about 10^9 CFU/g) in the first 24 h of sprouting, and maintained those levels throughout the rest of sprouting and subsequent refrigerated storage.

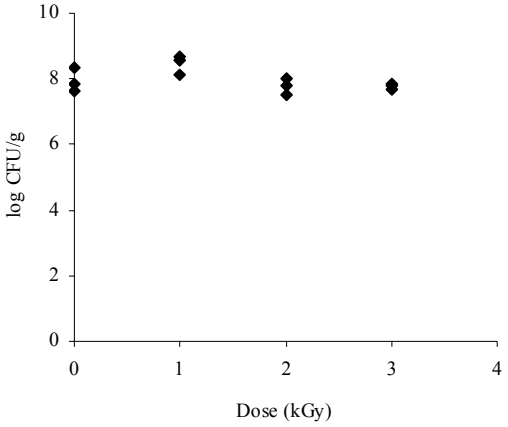


FIG. 24. Growth of Listeria monocytogenes on sprouts produced from inoculated and irradiated radish seeds.

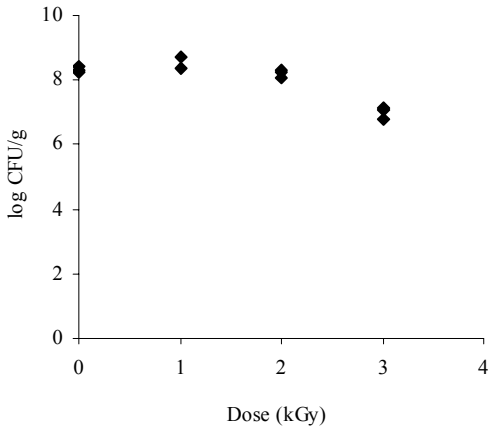


FIG. 25 Growth of Listeria monocytogenes on sprouts produced from inoculated and irradiated alfalfa seeds.

3.2.6. Effect of low-dose gamma irradiation on survival of pathogenic microorganisms on sprouts

Listeria monocytogenes grown on radish and alfalfa sprouts were more sensitive to low-dose irradiation than in radish and alfalfa seeds (Figures 26 and 27): 1 kGy dose reduced their number by about 2 log-cycles. The same radiation treatment was able to diminish the number of *E. coli* O157 by about 5 log-cycles (Figures 28 and 29); while after 2 kGy radiation treatment no survivors could be detected. These results are in accordance with the available literature data. Rajkowski and Thayer [39] determined the radiation D value of *E. coli* O157:H7 strains inoculated on alfalfa, broccoli and radish sprouts. The radiation D values were the same for both alfalfa and broccoli (0.27 ± 0.02 kGy), but significantly higher for the radish (0.34 ± 0.01 kGy for meat isolates, and 0.30 ± 0.02 kGy for vegetable isolates).

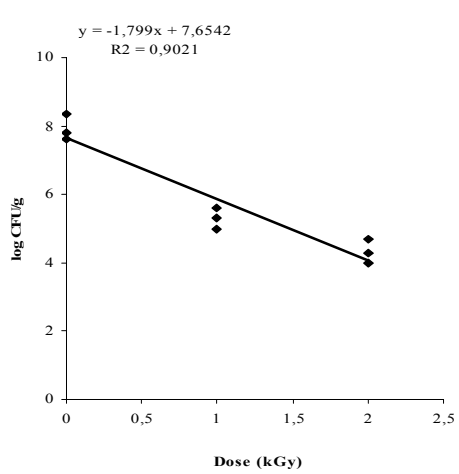


FIG. 26. Effect of irradiation on the survival of L. monocytogenes radish sprouts.

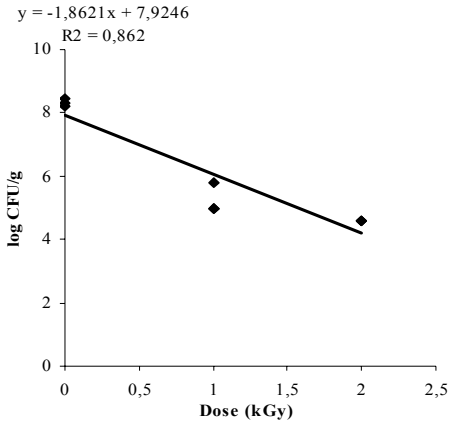


FIG. 27. Effect of irradiation on the survival of L. monocytogenes on alfalfa sprouts.

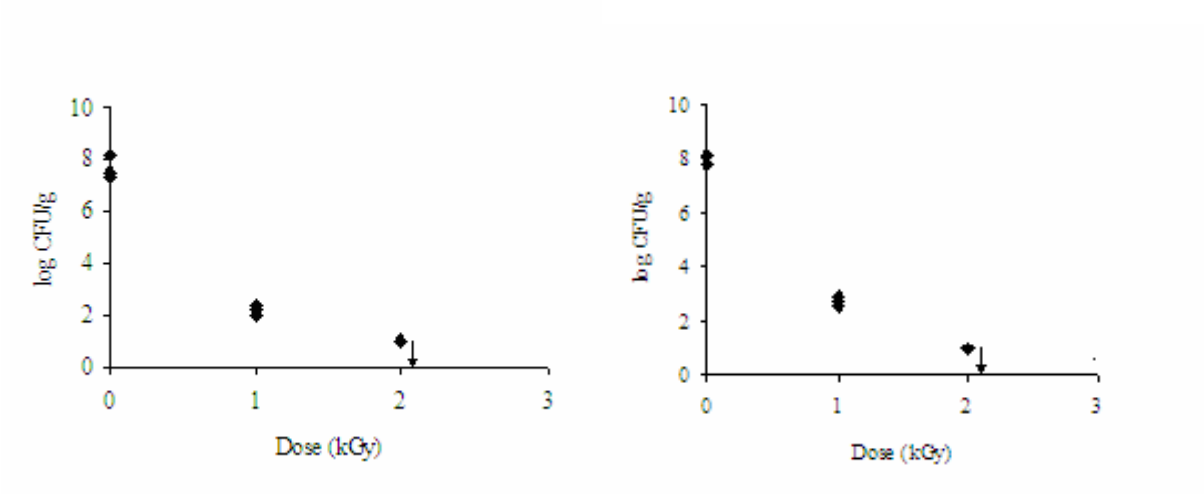


FIG. 28. Effect of irradiation on the survival of E. coli O157 on radish sprouts.

FIG. 29. Effect of irradiation on the survival of E. coli O157 on alfalfa sprouts.

3.2.7. Determination of radiation D_{10}-value of Listeria monocytogenes on alfalfa sprouts

Survival of *L. monocytogenes* after irradiation with 0.5 to 2 kGy is shown on the Fig. 32. On alfalfa sprouts the estimated D_{10}-value for the avirulent 4ab strain of *L. monocytogenes* is 0.46 kGy (R^2= 0.9677). In our previous studies D_{10}-value of 0.4 kGy was found for the same test strain in pH 7.0 phosphate buffer [40]. In phosphate buffered saline Patterson [41] found D-values of different strains of *L. monocytogenes* 0.32 to 0.49 kGy.

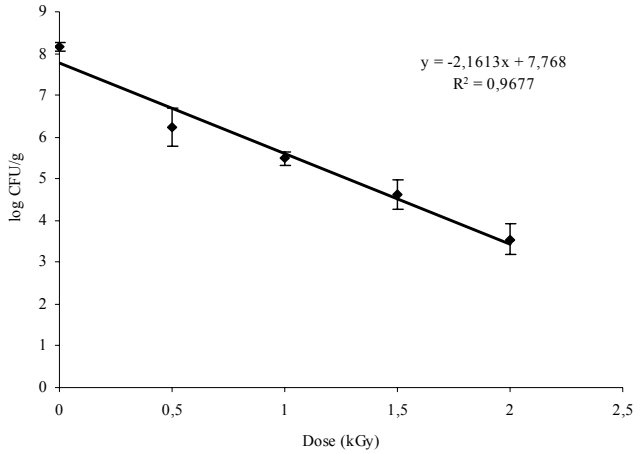


FIG. 30. Radiation survival of Listeria monocytogenes 4ab on alfalfa sprouts.

3.2.8. Effect of irradiation on the sensorial properties of alfalfa and radish sprouts

Sensory testing of alfalfa and radish sprouts showed that when applying Kramer's rank test, statistically significant differences in organoleptic properties (colour, odour, taste and texture) were not found in any case with doses up to the 2 kGy dose tested. Rank sums of both alfalfa sprouts and radish sprouts were not significantly different neither at $\alpha \leq 0.01$ nor at $\alpha \leq 0.05$ probability level (data not shown). Irradiation up to 2 kGy had no significant effect of the color measured instrumentally (figure not shown).

3.2.9. Determination of antioxidant vitamins of radiation processed alfalfa and radish sprouts

Ascorbic acid and tocopherol content of irradiated sprouts are summarized in Table 3.

TABLE 3. ASCORBIC ACID AND TOCOPHEROL CONTENT (MG/G FRESH WT) OF ALFALFA AND RADISH SPROUTS (VALUES ARE MEANS OF TWO REPLICATES)

Sample	Dose (kGy)	Ascorbic acid		α-tocopherol		β-tocopherol		γ-tocopherol	
Alfalfa sprout	0	Nd	-	13,7	± 1,27	0,85	± 0,07	12,75	± 0,49
	1	Nd	-	26,25	± 1,34	1,5	± 0,14	29,7	± 1,27
	2	Nd	-	22	± 0,99	1,65	± 0,07	22	± 0,99
Radish sprout	0	1,55	± 0,21	3,4	± 0,14	0,1	± 0,01	0,75	± 0,07
	1	4,05	± 0,35	1,2	± 0,14	0,05	± 0,01	0,65	± 0,07
	2	2,25	± 0,21	0,65	± 0,07	0,02	± 0,00	0,8	± 0,14

Nd: not detectable

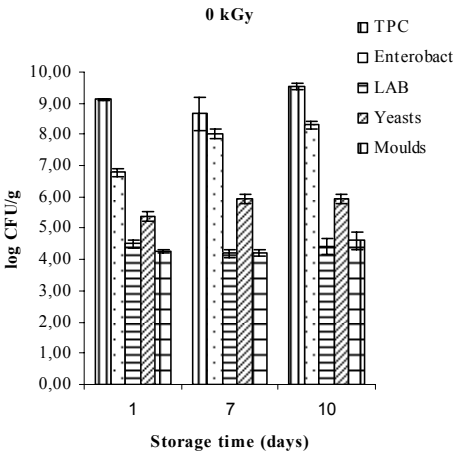
Surprisingly, ascorbic acid was not detected in alfalfa sprouts, and ascorbic acid content of radish sprout samples was also very low. Radiation dose of 2 kGy had no negative effect on the tocopherol content of alfalfa sprouts examined, however, it caused decrease in α- and β-tocopehrols in radish sprouts. Fan and Thayer [42] examined the quality of irradiated alfalfa sprouts during refrigerated storage. They observed that in the dose range of 0 to 2.57 kGy, storage duration had much larger effect on total ascorbic acid (TAA) content than irradiation. Non irradiated alfalfa sprouts lost two-third of TAA at seven days of storage.

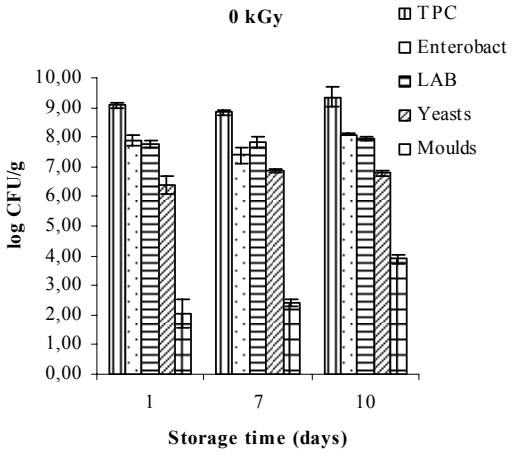
3.2.10. Effect of irradiation on the microbiological shelf-life of alfalfa and radish sprouts

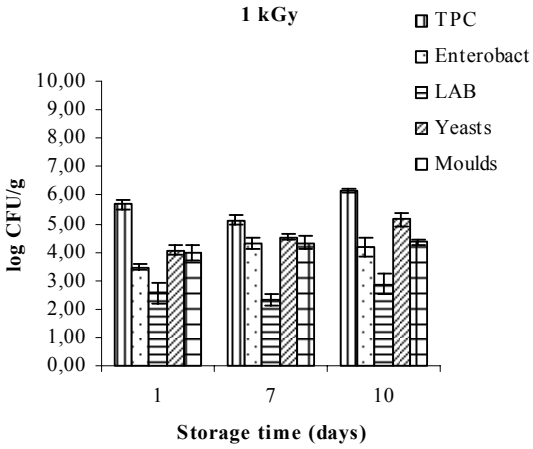
The effects of the irradiation on the natural microbiota of alfalfa and radish sprouts are shown on the Figures 31 and 32. Total aerobic plate counts of both sprouts were about 10^9 cfu/g already at the beginning of the shelf-life. *Enterobacteriaceae* proved to be the considerable group in the microbiota, ranging from 5 x 10^6 to 1.1 x 10^8 cfu/g. Number of LAB were much higher on radish sprouts (5.0 x 10^7 to 7.5 x 10^7 cfu/g) than on alfalfa sprouts (2.3 x 10^4 to 3.9 x 10^4 cfu/g). Yeasts were also present on both type of sprouts (10^5 to 10^6 cfu/g), while molds were found in wider range (~10^4 cfu/g on alfalfa, and ~10^6 cfu/g on radish sprouts). Pathogenic bacteria such as *Salmonella* or *Listeria monocytogenes* were not detected in any of the initial samples. During 10 days of storage at 5°C, counts of *Enterobacteriaceae* on alfalfa sprouts, and both *Enterobacteriaceae* and LAB counts could grow and reach 10^8 cfu/g level.

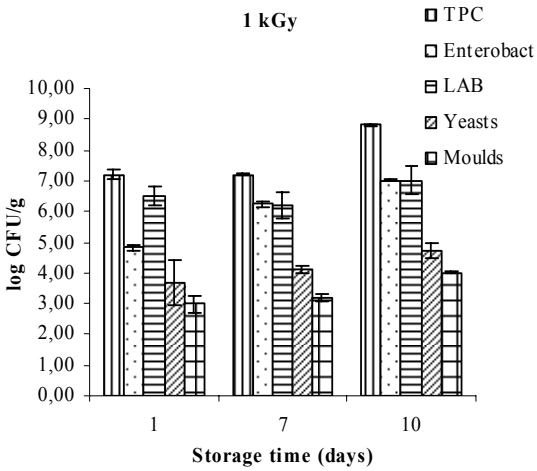
Irradiation with 2 kGy reduced the number of both total aerobic bacteria and *Enterobacteriaceae* by 3-4 log-cycles. Molds were relatively resistant to irradiation, 2 kGy dose reduced their number with max. 1 log-cycle. After 10 days of refrigerated storage the number of total aerobic bacteria on alfalfa and radish sprouts irradiated with 2 kGy were about 10^5 and 10^7 cfu/g, respectively.

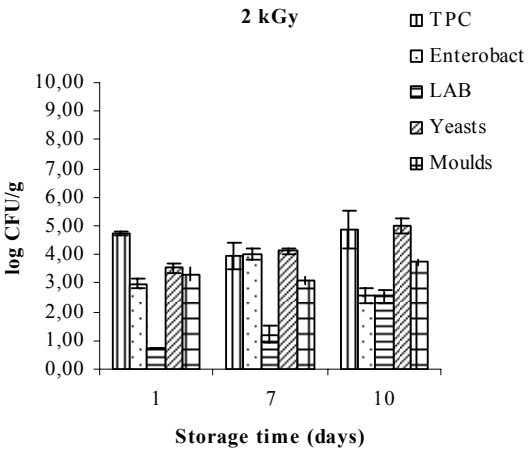
The microbial flora of sprouts from retail outlets were identified by Patterson and Woodburn [43]. Total aerobic plate counts were 10^8 cells/g, psychrotrophic counts were 10^7 cells/g, total coliforms were 10^6 cells/g, lactobacilli and faecal streptococci counts were low. Faecal coliforms appeared to be part of the normal flora of sprouts. Becker and Holzapfel [44] investigated the microbiological quality of commercial prepackaged sprouts. Total aerobic plate counts of more than 80% of samples ranged between 10^8 and 10^9 microorganisms/g. *Enterobacteriaceae* and pseudomonads were present with counts between 1.0 x 10^4 and 5.0 x 10^7 cfu/g. The LAB, yeasts and molds counts varied in a wide range between 10^2 to 10^7 cfu/g. *Bacillus cereus* and *Listeria innocua* was also detected in some cases. They found that thorough washing of the sprouts in water was not sufficient for decontamination.











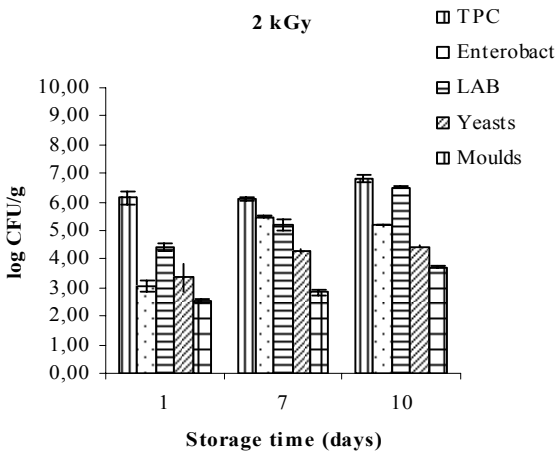


FIG. 31. Effect of irradiation on the microbiota of alfalfa sprouts.

FIG.32. Effect of irradiation on the microbiota of radish sprouts.

The microbial load of our commercial Bio sprouts examined were much higher already at the beginning of the shelf-life than it is recommended by Belgian authors (Table 4) for ready-to-eat vegetables.

TABLE 4. MICROBIOLOGICAL CRITERIA FOR READY-TO USE VEGETABLES [45]

Organism	Goal[a] (CFU/g)	Tolerance[a] (CFU/g)	Limit[b] (CFU/g)
Total aerobic count	10^6	10^7	10^8
LAB	10^3	10^4	10^7
Yeasts	10^3	10^4	10^5
L. monocytogenes	Absence in 0,01g	Absence in 0,01g	Absence in 0,01g

[a] Goal and tolerance criteria (CFU/g) on the day of production (day 0).
[b] Limit criteria (CFU/g) on the last day of shelf life.

3.3. Combination of MAP and irradiation

3.3.1. Effect of MAP and irradiation on the microbiological shelf-life of alfalfa and radish sprouts

Changes in head-space gas composition in MAP samples are shown in Figures 33-35. Respiration of radish samples was more intensive than that of alfalfa sprouts in the (1) gas composition. CO_2 content of head-space reached an equilibrium level (15 and 10%, respectively) after about seven days of refrigerated storage. In the (2) case, CO_2 content reached an equilibrium level (approx. 15%) at the sixth day when the O_2 concentration reduced to zero.

The effect of the modified atmosphere packaging and irradiation on the natural microbiota of radish and alfalfa sprouts is shown on the Figures 36-38.

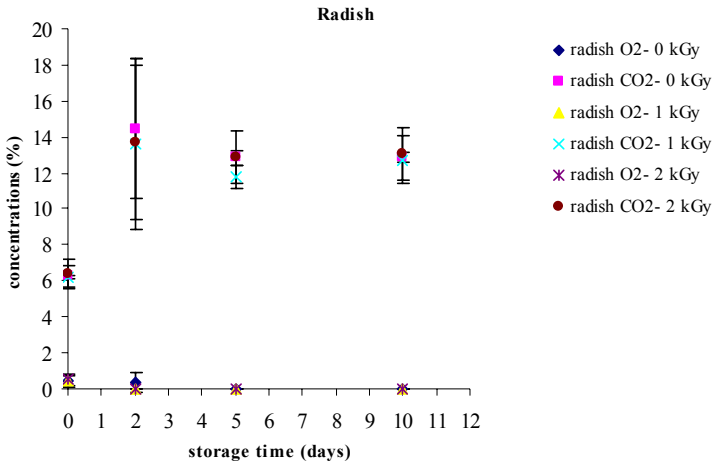


FIG. 33. Changes in head-space gas composition (1) of MAP and irradiated radish sprouts during storage at 5 °C.

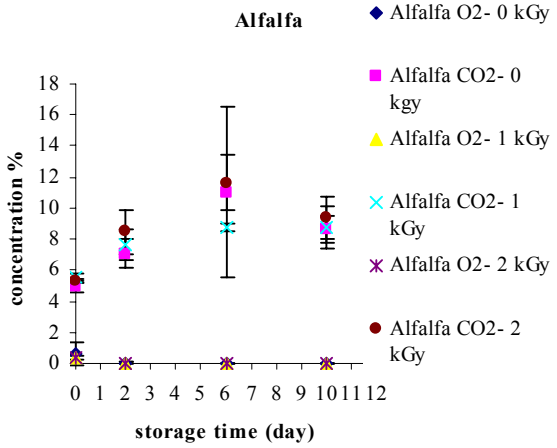


FIG. 34. Changes in head-space gas composition (1) of MAP and irradiated sprouts (storage at 5°C).

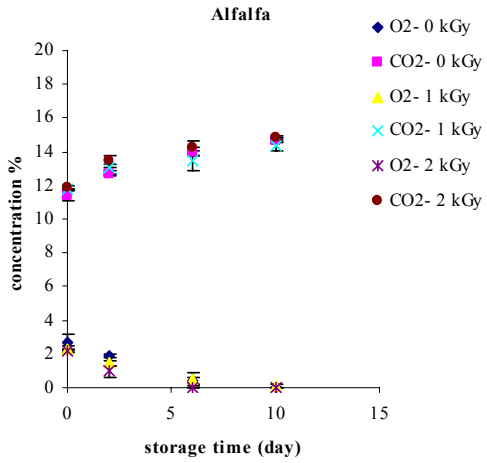


FIG. 35. Changes in head-space gas composition (1) of MAP and irradiated radish alfalfa sprouts (storage at 5°C).

Total plate counts (TPC) of both sprouts packaged in the (1) gas concentration were very high, about 10^9 cfu/g at the beginning of the shelf-life. *Enterobacteriaceae* proved to be the dominating group in the microbiota, in slightly lower number than the TPC. Number of LAB was two log cycles higher on radish sprouts. Yeasts and molds were present on both types of sprouts in relatively low number (below 10^2 CFU/g level - not shown in Figures). During ten days of storage at 5°C, due to elevated CO_2 content of packages LAB were able to grow with one log unit, while number of *Enterobacteriaceae* remained steady. Because there was no difference in the effect of MAP between alfalfa and radish sprouts, the (2) gas composition was investigated only in case of alfalfa sprouts. Total aerobic plate counts (TPC) were about 10^9 CFU/g at the beginning of the shelf-life too. Two dominant groups were TPC and *Enterobacteriaceae* in the microbiota. The (2) gas composition did not reduce the initial level of microorganisms, but strong increase was not observed during the storage. The (1) gas composition seems to be more effective to reduce the number of microorganisms. Results

compared to our previous observations shows that the total number of bacteria on MA-packaged sprouts was one log-cycle lower than that of untreated samples.

Irradiation with 1 and 2 kGy of MAP sprouts reduced the number of both total aerobic bacteria and *Enterobacteriaceae* by 3 and 4 log-cycles, respectively.

Zagory [46] reported that an elevated CO_2 condition extends the lag phase of the bacterial growth and can slow the propagation of the bacteria. Irradiation effects, which reduced the levels of aerobic bacteria in salted Chinese cabbage, were maintained, irrespective of its packaging condition, during storage for three weeks at 4°C. At three weeks, aerobic bacteria were not detected in the samples irradiated at 2 kGy. After three weeks of storage, coliform bacteria in the Chinese cabbage treated with both irradiation and MA packaging were not detected, while the samples under aerobic conditions showed a 2–4 log10 CFU/g. Their results indicated that the combined treatment of irradiation and MA packaging is more useful for inhibiting the growth of the aerobic and coliform bacteria than when these treatments are used alone. However, Lactic acid bacteria increased substantially during storage, coinciding with the higher levels of the CO_2 packaging conditions. After storage, a higher CO_2 condition showed significantly higher levels of lactic acid bacteria than the aerobic ones, but irradiation reduced the lactic acid bacteria in the salted Chinese cabbage even at a higher CO_2.

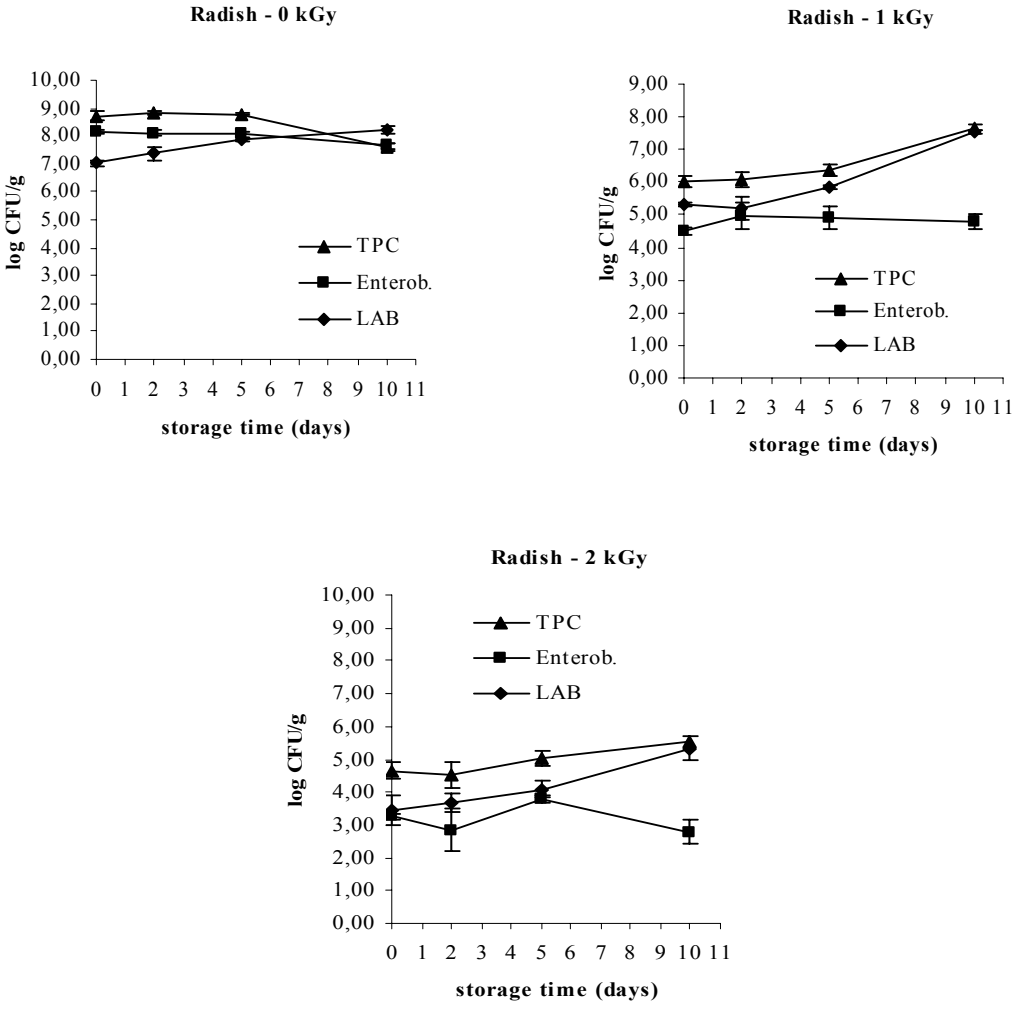
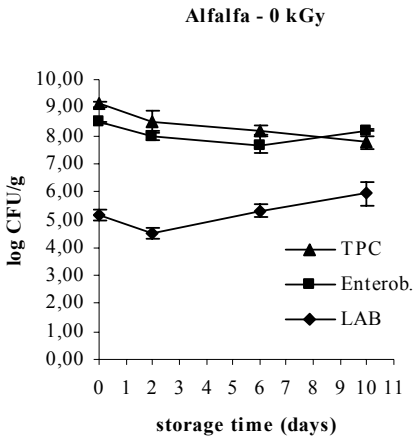
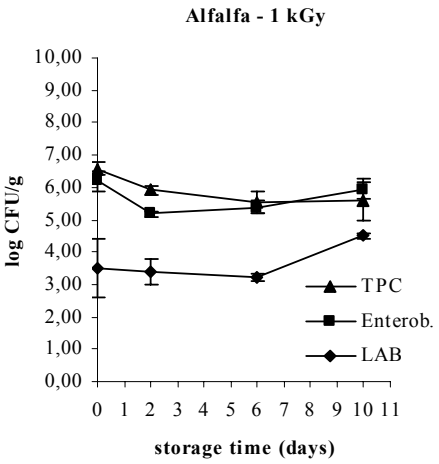


FIG. 36. The effect of the modified atmosphere packaging (1) and irradiation on the natural microbiota of radish sprouts.





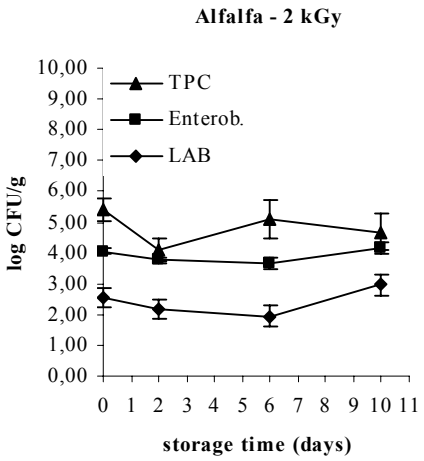
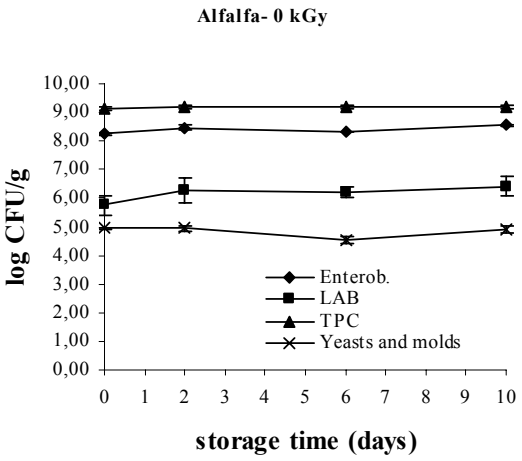
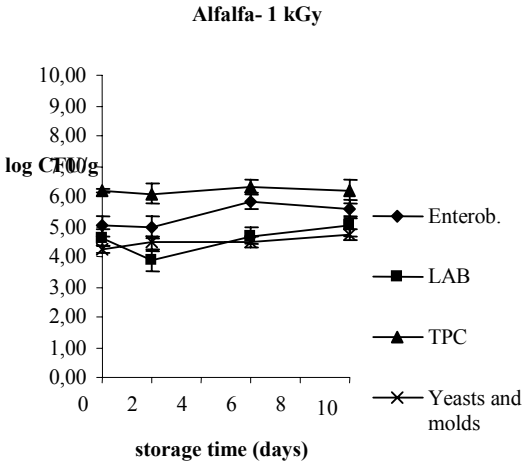


FIG. 37. The effect of the modified atmosphere packaging (1) and irradiation on the natural microbiota of alfalfa sprouts.





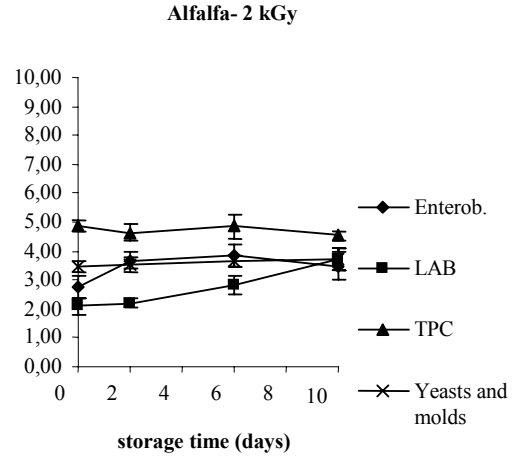


FIG. 38. The effect of the modified atmosphere packaging (2) and irradiation on the natural microbiota of alfalfa sprouts.

3.3.2. Estimation of survival and growth of pathogenic bacteria on modified atmosphere packaged alfalfa sprouts after gamma irradiation

Estimation of radiation D-value of spores of *Bacillus cereus* psychrotrophic strain is shown in Fig. 41. The survival curve of the spores of our test organism probably had a shoulder of L = 1.0 kGy, and a $D_{exp.}$ value of ca. 2.66 kGy. For the same test strain D value of 2.0 kGy and similar shoulder was found by Farkas [47] on sous-vide treated ready-to-eat meals. Thayer and Boyd [48] estimated D-value for a mixture of endospores of six *Bacillus cereus* strains in different meat products 1.91-2.78 kGy. Neither the presence nor absence of air during irradiation significantly affected radiation resistance of endospores of *B. cereus* when present on mechanically deboned chicken meat.

Growth of *Bacillus cereus* spores on MAP and irradiated alfalfa sprouts is shown in Fig. 40. There was no change observed in the number of spores during 10 days refrigerated storage in modified atmosphere packaging.

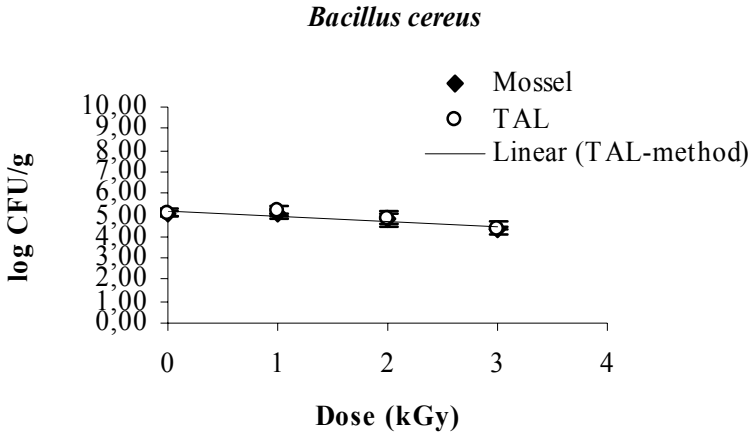


FIG. 39. Radiation survival of Bacillus cereus spores on alfalfa sprouts in modified atmosphere (1) packaging (y = -0.375x + 5.5267, R^2 = 0.9901).

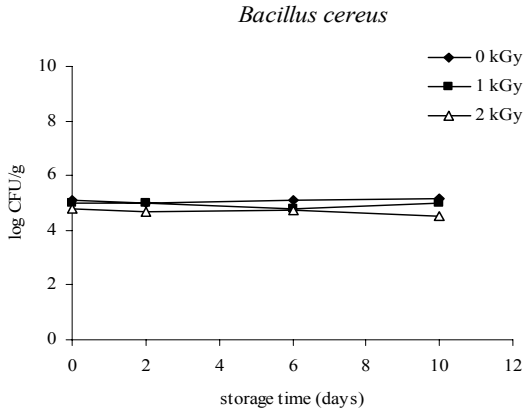


FIG. 40. Growth of Bacillus cereus spores on irradiated alfalfa sprouts. in modified atmosphere (1) packaging stored at 5°C

Survival of *L. monocytogenes* after irradiation with 0.5 to 2 kGy is shown on the Figure 41. The estimated D_{10}-value for the avirulent 4ab strain of *L. monocytogenes* is 0.58 kGy (R^2= 0.9796) in case (1), and D_{10}- value is 0.45 kGy (R^2=0,9534) at gas composition (2). The irradiation was less effective on the test strain in case of MA (1) packaged samples compared to air packaged sprouts. In our previous experiments D_{10}-value for the same *L. monocytogenes* 4ab strain was 0.46 kGy. This could be explained by the reduced O_2 content of the bags.

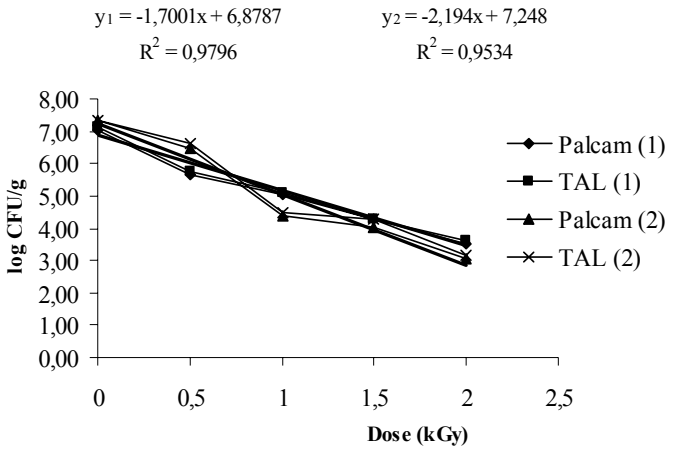


FIG. 41. Radiation survival of Listeria monocytogenes 4ab on alfalfa sprouts in modified atmosphere packaging (1) and (2).

After irradiation with 1 kGy, during refrigerated storage survivals of *L. monocytogenes* were able to grow in MAP (Figures 42 and 44) and the number of CFU increased by two log-cycles. There was no difference between selective plating and TAL method.

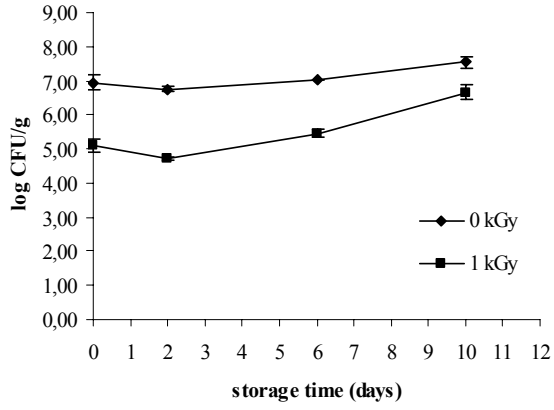


FIG. 42. Growth of Listeria monocytogenes 4ab on irradiated alfalfa sprouts in modified atmosphere packaging (1) stored at 5°C.

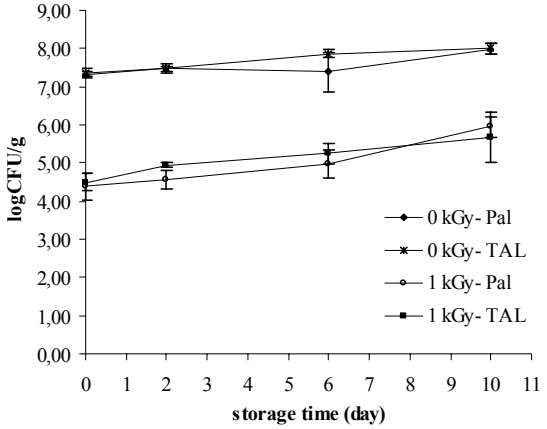


FIG. 43. Growth of Listeria monocytogenes 4ab on irradiated alfalfa sprouts in modified atmosphere packaging (2) stored at 5°C.

Extension of the lag phase and reduction of the growth rate is often considered to be a prominent effect of CO_2 [49]. Bennik et al. [50] have shown that this effect is consistant only at very high CO_2 concentrations but CO_2-enriched Modified atmospheres are not reliable way to control the fate of *Listeria monocytogenes* in vegetable products. Concerning product safety, psychrotrophic pathogens such as *Listeria monocytogenes* and psychrotrophic strains of *Bacillus cereus* are not suppressed under MA conditions that are optimal for respiring produce [51].

The potential for combining MAP with low-dose irradiation has been explored in a variety of foods, including lettuce [52, 53]. However, the extent to which headspace gas composition influences the regrowth of irradiated *L. monocytogenes* on vegetables is poorly understood, particularly with regard to the bacteriostatic effects of elevated CO_2 levels on spoilage and pathogenic bacteria [54].

Niemira and co-workers [55] inoculated cut pieces of endive with *Listeria monocytogenes*, packaged in gas-impermeable bags in air, 5/5/90% or 10/10/80% CO_2, O_2 and N_2 ("Air-0", "5/5" and "10/10", respectively) and irradiated to 0.0 (control), 0.3 or 0.6 kGy. Irradiation significantly reduced initial levels of *L. monocytogenes* and total microbiota under each of the three atmospheres examined (Air-0, 5/5 and 10/10 O_2/CO_2). During storage, *L. monocytogenes* and total microbiota regrew on the irradiated Air-0 samples. In contrast, the *L. monocytogenes* and total microbial populations on the irradiated 5/5 and 10/10 samples remained at or close to the initial reduced levels. In each of the three atmospheres, O_2 declined and CO_2 increased, irrespective of radiation dose.

3.3.3. Estimation survival and growth of Listeria monocytogenes on modified atmosphere packaged alfalfa sprouts after gamma irradiation by RABIT instrument

Strong correlation was found between the impedimetric TTD (detection time: time elapsed until certain change in conductivity is observed, considered as microbial growth) and the plate counts of *Listeria monocytogenes* (Fig. 44) in the log CFU/g range of 3 to 8.

In case of presence-absence test or samples having lower numbers of *Listeria*, an additional 24 hours enrichment step is needed before impedimetric investigation. Samples showing positive electrical response within 20 hours require further step to confirm the presence of *L. monocytogenes.*

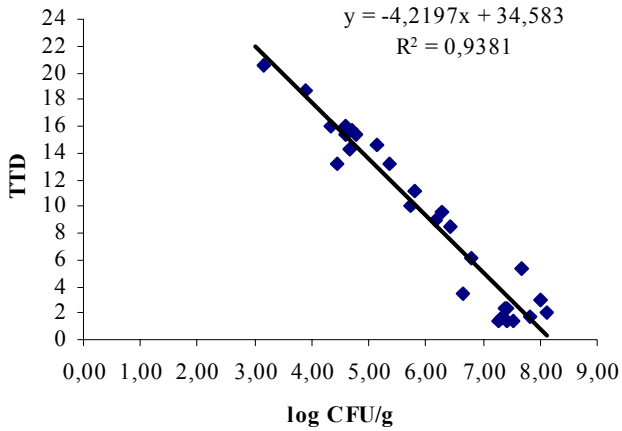


FIG. 44. Correlation between log CFU/g and TTD in detection of L. monocytogenes.

3.3.4. Determination of sensorial quality of irradiated sprouts in modified atmosphere packages

Sensory testing of alfalfa and radish sprouts showed that according to Kramer's rank test, statistically significant difference only in odour (at $\alpha \leq 0.05$ probability level) was determined in case of untreated (Control) alfalfa sprouts (1) and in color in case of 1 kGy treated alfalfa sprouts (2) at the end of 10 days refrigerated storage (Tables 5-6). Rank sums of radish sprouts) and alfalfa sprouts (2), were not significantly different neither at $\alpha \leq 0.01$ nor at $\alpha \leq 0.05$ probability level (data not shown).

The sensory panel could not detect any significant differences in color, odour, taste and texture from all irradiated and/or MAP treated samples on the day of treatment.

TABLE 5. SENSORY TESTING OF ALFALFA SPROUTS IN MAP (1). (Control: samples packaged in air; 0-2 kGy: samples packaged in MAP and irradiated)

Radiation dose (kGy)	Score means								Rank sums			
	Colour		Odour		Taste		Texture		Colour	Odour	Taste	Texture
Control	7,8±	1,03	7,50±	1,65	6,90±	1,29	7,80±	1,03	31	**22.00***	28,5	27,5
0	7,6±	1,26	6,70±	1,89	6,50±	1,72	7,30±	1,49	35,5	35,5	36,5	37
0,5	7,80±	1,14	6,60±	1,71	6,60±	1,26	7,60±	1	30	40	32,5	30
1	8,00±	0,82	7,30±	0,95	7,10±	0,74	7,60±	1,07	26	32	26,5	30
1,5	7,30±	1,16	6,20±	1,75	6,30±	1,16	7,00±	1,49	41,5	42	42	43
2	7,10±	1,37	6,50±	1,58	6,20±	1,14	7,00±	1,7	46	38,5	44	42,5

** rank sums within the range 20-50 are not significantly different at $\alpha \leq 0.01$ probability level
* rank sums within the range 22-48 are not significantly different at $\alpha \leq 0.05$ probability level

TABLE 6. SENSORY TESTING OF ALFALFA SPROUTS IN MAP (2) AT THE END OF THE STORAGE (Control: samples packaged in air; 0-2 kGy: samples packaged in MAP and irradiated)

Radiation dose (kGy)	Score means								Rank sums			
	Colour		Odour		Taste		Texture		Colour	Odour	Taste	Texture
Control	6,92±	1,98	6,08±	2,27	6,00±	1,65	6,42±	1,73	30	31,5	34,5	31
0	6,92±	1,24	5,42±	1,83	6,00±	1,41	6,25±	1,36	34,5	38	29,5	36
1	7,92±	1	6,83±	1,7	6,25±	1,86	7,00±	1,21	21,00*	23	29	22
2	6,58±	1,51	6,42±	2,27	5,92±	2,15	6,50±	1,45	34,5	27,5	27	31

** rank sums within the range 19-41 are not significantly different at $\alpha \leq 0.01$ probability level
* rank sums within the range 21-39 are not significantly different at $\alpha \leq 0.05$ probability level

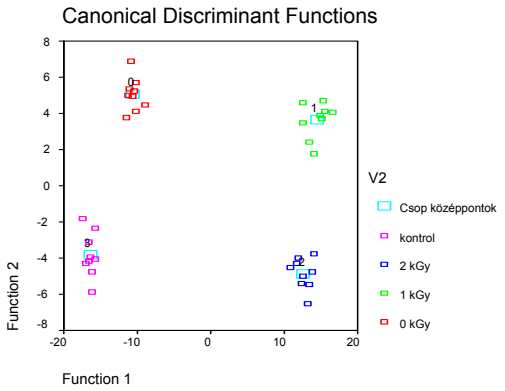
3.3.5. Electronic nose analysis

Results of electronic nose investigations were different in case of the two types of sprouts examined. Data analysis was performed using multivariate methods including PCA and discriminant analysis.

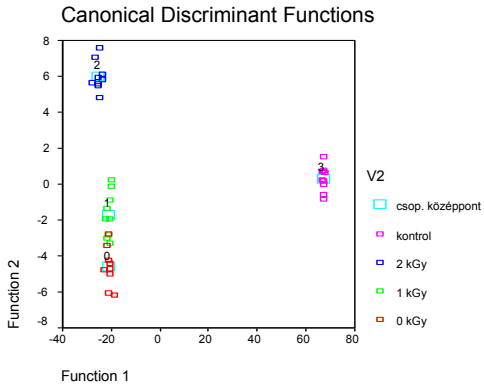
In case of alfalfa sprouts (Fig. 45), significant differences between control (samples packed in air) and treated samples (MAP and MAP + irradiation) were already observed on the day of radiation treatment. Electronic nose could clearly distinguish sample groups with different radiation doses. Some overlapping of samples with 1 and 2 kGy irradiation could be seen at the following sampling days. Concerning storage time good discrimination could be observed between the treated sample groups until the 6th day. Samples taken on the 6th and 10th storage days could not be separated. Deviation between sprouts irradiated with 2 kGy and control samples could be observed consequently, possibly because of higher amount of volatile compounds produced in the untreated control sample due to microbiological activity.

Electronic nose could clearly distinguish sample groups of radish sprouts with different radiation doses with the exception of some overlapping of samples with 1 and 2 kGy irradiation on day 0 and 10.

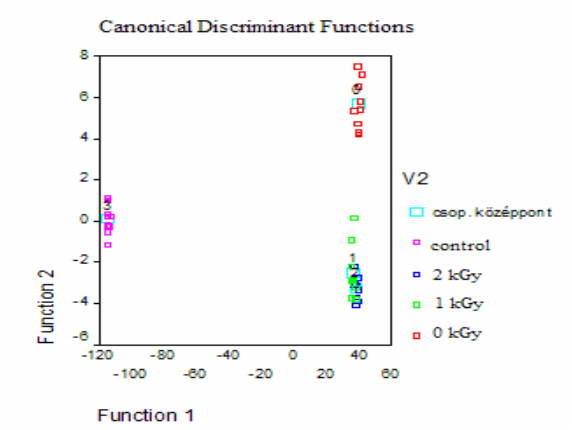
Chemosensor array could not separate samples taken on different days of storage.



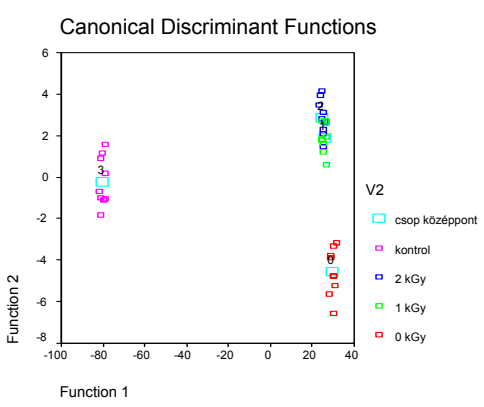
0 days



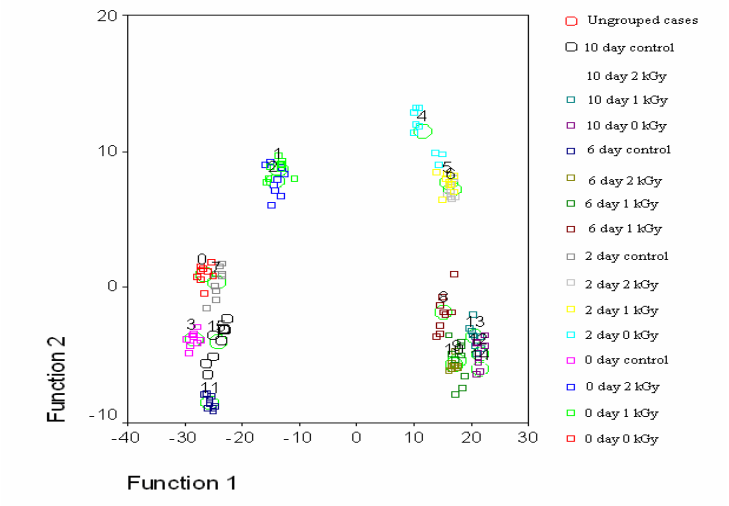
2 days



6 days



10 days



Summary

FIG. 45. Discriminate analysis of electronic nose results of alfalfa sprouts.

4. CONCLUSIONS

Pre-cut vegetable and fruits

1. Both *Listeria monocytogenes* and *E. coli* O157 were able to survive and grow at the abusive storage temperature (15°C) and at 5°C, inoculated onto pre-cut tomatoes, cantaloupes and watermelon cubes.
2. Radiation dose of 1 kGy is sensorially acceptable for the treatment of these pre-cut fruits without significant effect on total carotenoid and vitamin C content of sliced tomatoes.
3. Low-dose irradiation is able to improve the microbiological safety of the selected fruits. Radiation treatment diminished very much the viable cell count of *L. monocytogenes* and *E. coli* O157. Considering that these microorganisms occur most probably in low contamination level, and the extent of the decrease of the number of their colony forming units as an effect of 1 kGy irradiation, under good manufacturing practices this low-dose treatment may practically eliminate both pathogenic bacteria from the pre-cut fruit.

Seeds and sprouts

1. Low-dose irradiation (1-3 kGy) is able to reduce considerably the microbial contamination of radish and alfalfa seeds. The effect of selected processing dose on the germination of the seeds has to be studied item by item.
2. Microorganisms surviving the low-dose irradiation are able to re-grow during sprouting of seeds and reach high numbers within few days.
3. Irradiation of sprouts seems to be the more effective way to improve microbiological safety of vegetable sprouts: 2 kGy dose reduced the number of *L. monocytogenes* by about 4 log-cycles, and the number of *E. coli* O157 by at least 8 log-cycles. The radiation survivors are able to re-grow during sprouting.
4. Irradiation up to 2 kGy dose did not cause any significant difference in organoleptic properties of alfalfa and radish sprouts.
5. Combination of low-dose gamma irradiation with modified atmosphere packaging and refrigerated storage can improve the microbiological safety and shelf-life of alfalfa and radish sprouts.
6. Combination of MAP with 2 kGy gamma irradiation was able to reduce the natural microbiota to acceptable low level and there was no further increase detected in 10 days storage at 5°C.
7. Estimated D_{10}-values of *L. monocytogenes* 4ab strain on alfalfa sprouts:
    a) 0.46 kGy, packaged in air
    b) 0.58 kGy, packaged in gas mixture containing 2 % O_2, 4 % CO_2 and 94 % N_2
    c) 0,45 kGy, packaged in gas mixture containing 3-5% O_2, 10-15% CO_2 balanced with N_2
8. Estimated D_{10}-value of spores of *Bacillus cereus* psychrotrophic strain on alfalfa sprouts ca. 2.66 kGy (with a shoulder of L = 1.0 kGy).
9. Further investigations are necessary to develop the composition of head-space in MAP able to prevent regrowth of surviving pathogens such as *Listeria monocytogenes* during storage.
10. Impedimetric method developed can be used to detect and enumerate *L. monocytogenes* present in numbers higher than 3 log CFU/g within 24 hours. For presence-absence test an enrichment step and confirmation is needed.
11. Further research is needed to prove the ability of electronic nose for prediction of microbiological quality and shelf-life of MAP/irradiated sprouts.

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REFERENCES

[1] NICHOLL, P., MCINEREY, S. AND PRENDERGAST, M., Growth dynamics of indigenous microbial populations on vegetables after decontamination and during refrigerated storage, J. Food Processing and Preservation, **28,** (2004) 442-459.

[2] BEUCHAT, L.R., Pathogenic microorganisms associated with fresh produce, J. Food Prot. **59**, (1996) 204-216.

[3] BUCK, J. W., WALCOTT, R. R. and BEUCHAT, L. R., Recent trends in microbiological safety of fruits and vegetables, APSnet, Plant Health Progress, Feature Story, January/February 2003

[4] ODUMERU, J.A., MITCHELL, S.J., ALVES, D.M., LYNCH, J.A., YEE, A.J., WANG, S.L., STYLIADIS, S. AND FARBER, J.M., Assessment of the microbiological quality of ready-to-use vegetables for health-care food services, J. Food Prot., **60,** (1997) 954-960.

[5] PORTNOY, B. L., GOEPFERT, J. M. and HARMON, S. M., An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts, Am. J. Epidemiol., **103,** (1976) 589-594.

[6] NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS, Microbiological safety evaluations and recommendations on sprouted seeds, Int. J. Food Microbiol., **52**, (1999) 123-153.

[7] BEUCHAT, L. R., Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production, Int. J. Food Microbiol., **34**, (1997) 329-333.

[8a] TAORMINA, P. J. and BEUCHAT, L. R., Comparison of chemical treatments to eliminate enterohaemorrhagic *E. coli* O157:H7 on alfalfa seeds, J. Food Prot., **62**, (1999) 318-324.

[8b] TAORMINA, P. J. and BEUCHAT, L. R., Behaviour of enterohaemmorhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatment with various chemicals, J. Food Prot., **62**, (1999) 850-856.

[9] JAQUETTE, C. B., BEUCHAT, L. R., MAHON, B. E., Efficiacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage, Appl. Environ. Microbiol., **62**, (1996) 2212-2215.

[10] ITOH, Y., SUGITA-KONISHI, Y., KASUGA, F., IWAKI, M., HARA-KUDO, Y., SAITO, N., NOGUCHI, Y., KONUMA, H. and KUMAGAI, S., Enterohemorrhagic *E. coli* O157:H7 present in radish sprouts, Appl. Environ. Microbiol., **64,** (1998) 1532-1535.

[11] NGUYEN-THE, C., CARLIN, F., Fresh and processed vegetables, In: B.M. Lund, T.C. Baird-Parker, G.W. Gould (eds.) The Microbiological Safety and Quality of Food,Vol. I., 620-684, Aspen Publishers, Inc., Gaithersburg, Maryland (2000).

[12] BRACKETT, R.E., Shelf stability and safety of fresh produce as influenced by sanitation and disinfection, J. Food Prot., **55**, (1992) 808-814.

[13] NGUYEN-THE, C., CARLIN, F., The microbiology of processed fresh fruits and vegetables, Crit. Rev. Food Sci. Nutr., **34**, (1994) 371-401.

[14] FARKAS, J., Irradiation of minimally processed foods. In: R. Molins (ed.) Food Irradiation Principles and Applications, John Wiley & Sons, Inc., New York, (2001) 273-290.

[15] AL-ATI, T., HOTCHKISS, J.H., Application of packaging and modified atmosphere to fresh-cut fruits and vegetables, In: Lamikanra, O. (Ed.), Fresh-Cut Fruits and Vegetables, Science, Technology and Market, CRC Press, Boca Raton, FL, (2002) 305–338.

[16] ANONYMUS, Elektronische Nasen, Die Ehrnahrungsindustrie, **6**, (1996) 54-55.

[17] BAZEMORE R., ROUSEFF, R. and SIMS, C., Comparison of fresh squeezed orange juice analyses by 'electronic nose', sensory taste panel and GC FID, Olfaction and Electronic Nose, 3rd Int. Symp., Toulouse (1996).
[18] ÓLAFSSON, R., MARTINSDOTTIR, E., ÓLAFSDOTTIR, G., SIGFUSSON, P.I. and GARDNER, J.W., Monitoring of fish freshness using tin oxide sensors, In: Gardner, J.W. and Bartlett, P.N. (eds.), Sensory Systems for an Electronic nose, Dordrecht, Kluwer Academic Publisher, (1992) 257-272.
[19] SIMON, J.E., HETZRONI, A., BORDELON, B, MILES, G.E. and CHARLES, D.J., Electronic sensing of aromatic volatiles for quality sorting of blueberries, J. Food Sci. (1996) 967-969.
[20] WANG H-H., Assessment of solid state fermentation by a bioelectronic artificial nose, Biotechnol. advances, **11**, (1993) 701-710.
[21] ROSSI, V., GARCIA, C., TALON, R., DENOYER, C. and BERDAGUÉ, J.-L., Rapid discrimination of meat products and bacterial strains using semiconductor gas sensors., In: Etiévant, P. and Schreier, P. (Eds.), Proc. of Bioflavour 95. Dijon: INRA éditions, (1995) 85-89.
[22] MOY, L., VASIC, G., BERDAGUÉ, J.-L. and ROSSI, V., Transient signal modeling for fast odour classification, In: Etiévant, P. and Schreier, P. (Eds.), Proc. of Bioflavour **95**. Dijon: INRA éditions, (1995) 55-58.
[23] WAWERLA, M., STOLLE, A., SCHALCH, B., EISGRUBER, H., Impedance Microbiology: Applications in Food Hygiene, J. of Food Protection, **62** (12), (1999) 1488-1496.
[24] DAOOD, H. G., BIACS, P. A., DAKAR, M. A. and HAJDU, F., Paired-ion chromatography and photodiode-array detection of vitamin C and organic acids, J. Chromatogr. Sci., **37**, (1994) 481-487.
[25] DAOOD, H. G., CZINKOTAI, B., HOSCHKE, Á. and BIACS, P., High-performance liquid chromatography of chlorophylls and carotenoids from vegetables, J. Chromatogr., 472, (1989) 296-302.
[26] CHANDLER, L. A. and SCHWARZ, S. J., Separation of cis-trans carotene isomers in fresh and processed fruits and vegetables, J. Food Sci., **52**, (1987) 669-672.
[27] KANG, D. and FUNG, D. Y. C., Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*, J. Food Prot., **62**, (1999) 1346-1349.
[28] EN ISO 6579:2002, Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp.
[29] EN ISO 11290-1:1996, Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method.
[30] KISKÓ, G., MOHÁCSI-FARKAS, CS., TACZMANN-BRÜCKNER, A., ROHONCZY, K., Impedimetric detection of *Listeria monocytogenes* from vegetable and fruit products, FoodMicro 2004, The 19th International ICFMH Symposium, New Tools for Improving Microbial Food Safety and Quality, 12-16 September 2204, Portoroz, Slovenia, Book of Abstracts, 334.
[31] BOLTON, F.J. & GIBSON, D.M., Automated electrical techniques in microbiological analysis, In: Patel, P.D. (Ed.): Rapid Analysis Techniques in Food Microbiology, Blackie Academic & Professional, London, (1994) 131-170.
[32] JOHNSON, K.M., NELSON, C.L., BUSTA, F.F., Germination and heat resistance of *Bacillus cereus* spores from strains associated with diarrheal and emetic food-borne illnesses, J. Food Sci., **47**, (1982) 1268-1271.
[33] MERMELSTEIN, N.H., Emerging technologies and „fresh" labelling, Food Technol., **55**, (2001) p.64-67.
[34] BEUCHAT, L.R., and BRACKETT, R.E., Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature, J. Food Sci. **55** (3) (1990) 755-758, 870.
[35] DEL ROSARIO, B.A. and BEUCHAT, L.R., Survival and growth of enterohaemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon, J. Food Prot., **58**, (1995) 105-107.
[36] ANDREWS, W. H., MILIVEC, P.B., WILSON, C.R., BRUCE, V.R., POELMA, P.L., GIBSON, R., TRUCKSESS M.W., and YOUNG K., Microbial hazards associated with bean sprouting, J. Assoc. Off. Anal. Chem. **65**, (1982).241-248.

[37] PROPOKOWICH, D. and BLANK, G., Microbiological evaluation of vegetable sprouts and seeds, J. Food Prot., **54**, (1991) 560-562.
[38] PÁLMAY, M. and BUCHANAN, R. L., Growth of *Listeria monocytogenes* during germination of alfalfa sprouts, Food Microbiology, **19**, (2002) 195-200.
[39] RAJKOWSKI, K. and THAYER, D. W., Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma radiation of inoculated sprouts, J. Food Prot., **63,** (2000) 871-875.
[40] FARKAS, J., ANDRÁSSY, É., MÉSZÁROS, L., BÁNÁTI, D., Growth of untreated and radiation-damaged *Listeria* as affected by environmental factors, Acta Microbiol. et Immunol. Hung., **42**(1), (1995) 19-28.
[41] PATTERSON, M. F., Sensitivity of *Listeria monocytogenes* to irradiation on poultrymeat and in phosphate-buffered saline, Lett. Appl. Microbiol. 8, (1989) 181-184.
[42] FAN, X. and THAYER, D., Quality of irradiated alfalfa sprouts, J. Food Prot, **64**, No.10, (2001) 1574-1578.
[43] PATTERSON, J. E. and WOODBURN, M. J., *Klebsiella* and other bacteria on alfalfa and bean sprouts at the retail level, J. Food Sci., **45**, (1980) 492-495.
[44] BECKER, B. and HOLZAPFEL, W. H., Mikrobiologisches Risiko von fertigferpackten Keimlingen und Massnahmen zur Reduzierung ihrer mikrobiellen Belastung, Archiv für Lebensmittelhyhiene, **48**, (1997) 73-96.
[45] JACXSENS, L., DEVLIEGHERE, F., FALCATO, P. and DEBEVERE, J., Behavior of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere, J. Food Prot, **62**, No. 10, (1999) 1128-1135.
[46] ZAGORY, D., Effects of post-processing handling and packaging on microbial populations, Postharvest Biology and Technology, **15**, (1999) 313–321.
[47] FARKAS, J., Developing some ready-to-eat meals by combination of radiation treatment and certain antimicrobial hurdles, Progress Report of IAEA 9603/R1 Co-ordinated Research Program on Production of Safe, Self-Stable and Ready-to-Eat Meals through Irradiation Processing (1999).
[48] THAYER, D.W., BOYD, G., Control of enterotoxic *Bacillus cereus* on poultry or red meats and in beef gravy by gamma irradiation, J. Food Prot., 57, (1994) 758-764.
[49] FARBER, J.M., Microbiological aspects of modified atmosphere packaging technology – a review, J. Food Prot., **54**, (1991) 58-70.
[50] BENNIK, M.H.J., SMID, E.J., ROMBOUTS, F.M. and GORRIS, L.G.M., Growth of psychrotrophic foodborne pathogens in solid surface model system under the influence of carbon dioxide and oxygen, Food Microbiol., **12**, (1995) 509-519.
[51] CARLIN, F., NGUYEN-THE, C., ABREU-DASILVA, A. and COCHET, C., Effects of carbon dioxide on the fate of L. monocytogenes, of aerobic bacteria and on the development of spoilage in minimally processed fresh endive, Int. J. Food Microbiol., **32**, (1996) 159-172.
[52] HAGENMAIER, R.D., BAKER, R.A., Low-dose irradiation of cut iceberg lettuce in modified atmosphere packaging, J. Agric. Food. Chem. **45**, (1997) 2864–2868.
[53] PRAKASH, A., GUNER, A.R., CAPORASO, F., FOLEY, D.M., Effects of low-dose gamma irradiation on the shelf life and quality characteristics ofcut Romaine lettuce packaged under modified atmosphere, J. Food Sci. **65** (3), (2000) 549–553.
[54] YUAN, J.T.C., Modified atmosphere packaging for shelflife extension, In: Novak, J.S., Sapers, G.M., Juneja, V.K. (Eds.), The Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL, (2003) 205–219.
[55] NIEMIRA, B.A., FAN, X., SOKORAI K.J.B., Irradiation and modified atmosphere packaging of endive influences survival and regrowth of Listeria monocytogenes and product sensory qualities, Rad. Phys. Chem. **72**, (2005) 41–48.

USE OF IRRADIATION TO ENSURE HYGIENIC QUALITY OF FRESH, PRE-CUT FRUITS AND VEGETABLES AND OTHER MINIMALLY PROCESSED FOODS OF PLANT ORIGIN

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Abstract

Minimally processed fruits and vegetables are in demand as they offer convenience to consumers. However, these products are often unsafe due to possibility of contamination with pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes,* etc. Therefore, a study was carried out to analyse microbiological quality of minimally processed carrot (*Daucus carota L.)*, cucumber (*Cucumis sativus L.)*, pineapple (*Ananas comosus)* and different sprouts: Green gram (*Phaseolus aureus)*, Dew gram (*Phaseolus aconitifolius)*, Chick pea (*Cicer arietinum)*, and Garden pea (*Pisum sativum),* and to optimise radiation dose necessary to ensure safety of these commodities. Microbiological quality of carrot was poor as compared to that of tomato and cucumber. Only one sample of carrot was contaminated with *L. monocytogenes.* Microbiological quality of pineapple was also found to be poor; all samples were positive for faecal coliforms and one sample was positive for *Salmonella*. Microbiological quality of sprout samples was poor with high aerobic plate count and yeast and mold count. A large percentage of samples were found contaminated with *E. coli,* coagulase positive *S. aureus* and *Salmonella.* D_{10} values of *Salmonella Typhimurium* and *Listeria monocytogenes* in these minimally processed foods (MPF) were in the range of 188 to 362 Gy, except in sprouts where, *L. monocytogenes* showed much higher D_{10} values (531-579 Gy). A dose of 2 kGy would be necessary for 5 log reduction of these pathogens. The MPF irradiated at 1 kGy/2 kGy was free from all pathogens up to 12 days of storage at 4ºC and 10ºC. This dose did not significantly affect nutritional, organoleptic and textural properties of MPF. These results suggest that radiation processing can ensure safety of minimally processed foods of plant origin.

1. INTRODUCTION

The consumption of raw, minimally processed foods (MPF) like vegetables, fruits and sprouts is on the increase in many parts of the world due to dietary trends and increased understanding of the nutritional value of these foods. In India fruits and vegetables are bought from local market, washed with water and either eaten raw or cooked depending on the type of vegetable. However, in a metropolis like Mumbai, a number of street vendors serve pre-cut fruits and salads that are cut in the morning and handled in unhygienic conditions before serving to consumers. Sprouts are being increasingly sold in Indian cities. Traditionally, seeds are soaked in water for 24 to 48 hours and sold in open baskets. However, in some of the super markets, sprouts are now available in packets. Though traditionally sprouts are consumed after cooking, due to increasing health consciousness there is an increase in the consumption of raw sprouts.

Contaminated irrigation water as well as faecal matter from grazing animals can result in contamination of vegetables, fruits and seeds which are used for preparing sprouts with pathogenic microorganisms. During harvesting, storage, transportation and marketing fruits and vegetables are handled in unhygienic condition and cross contamination is frequent.

Many large food-borne disease outbreaks have occurred in the recent past in several countries due to the consumption of fresh, pre-cut products of plant origin [1, 2]. Several types of pathogenic bacteria such as *E. coli* O157:H7*, Salmonella, Shigella, Listeria monocytogenes, Aeromonas hydrophila* were responsible for these outbreaks. It has been concluded by United States Centre for Disease Prevention and Control that fresh, pre-cut produce, as category, represent a health risk.

Previous studies on the microbial flora of salad vegetables and fruits from street vendors of Mumbai have shown that these products have very high microbial load and are also contaminated with food borne bacterial pathogens such as *Staphylococcus aureus, Salmonella*, and other pathogens. A report

of the Scientific Committee on Food of the European informs that the coliform counts were also found to be very high in this type of produce [3]. A study on the microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads in India showed that the microbiological quality of these fresh produce was very poor with high coliform counts. Tomato (7/62), coriander (5/10), spinach (2/4) and cabbage (1/4) samples were contaminated with *Listeria monocytogenes* [4]. Thus, there is a need to process MPF to ensure their safety.

The non-spore forming bacterial pathogens are sensitive to radiation and the effectiveness of radiation processing to ensure safety of foods of animal origin has been demonstrated [5]. Previous studies have shown that irradiation with doses ranging from 0.5 to 2 kGy had no adverse effect on fresh produce stored a few days under refrigeration as minimally processed fresh fruits and vegetables [6].

The aim of our studies was to:

1. Analyse microbiological quality of raw vegetables such as tomato, carrot, cucumber, minimally processed pineapple and sprouts sold in local market mainly with reference to bacterial pathogens namely, *Staphylococcus aureus, Salmonella*, *Listeria monocytogenes, Vibrio cholerae* and faecal coliforms.
2. To find decimal reduction dose for *Salmonella typhimurium* and *Listeria monocytogenes* inoculated in these minimally processed foods and based on D_{10}, to determine the radiation processing dose required to eliminate 10^5 cfu/g of these pathogens.
3. To study the effect of radiation processing on organoleptic, textural and nutritional quality of these products.

2. MATERIAL AND METHODS

2.1. Chemical and media

All bacteriological media were from Hi Media Ltd., Mumbai. The chemicals were from standard manufacturers.

2.2. Bacterial strains

S. Typhimurium MTCC 98 (IMTECH, Chandigarh) and avirulent *L. monocytogenes* 4ab NCAIM-B-01442 (Courtesy Dr. Cs. Mohacsi-Farkas, Szent Istvan University, Budapest, Hungary) were used for inoculated pack studies. The cultures were maintained on nutrient agar slants at 4ºC with regular sub-culturing.

2.3. Sampling

A total of 28 samples of fresh vegetable (twelve of tomato (*Lycopersicon esculentum*), eight of carrot (*Daucus carota)*, and eight of cucumber (*Cucumis sativus)*,) and 12 samples of cut pineapple (*Ananas comosus)* were procured from local markets. Vegetables were washed thoroughly with running tap water before carrying out the microbiological analysis.

124 sprout samples Green gram (*Phaseolus aureus)*, Dew gram (*Phaseolus aconitifolius)*, Chick pea (*Cicer arietinum)*, and Garden pea (*Pisum sativum)* were collected from different vendors in Mumbai and its suburbs, stored overnight in refrigerator and microbiological analysis was carried out on the next day.

2.4. Microbiological analysis

The microbiological analysis was done as per standard methods adopted from Bacteriological Analytical Manual, USFDA for detection, enumeration and identification to species level of individual pathogen (BAMO) [7]. Respective reference organisms were used for validation of the identification procedures employed.

2.4.1. Aerobic Plate Count (APC)

The samples were chopped into fine pieces and 25 g of the representative sample was suspended in 225 ml of sterile physiological saline. After serial dilutions, the appropriate dilutions were pour plated on nutrient agar. The colonies were counted after 48 h of incubation at room temperature (28°C).

2.4.2. Coliforms and faecal coliform counts

Saline dilutions were plated on violet red bile agar and typical colonies counted after 48 h incubation at 37ºC. The isolates from each plate were inoculated in EC broth and incubated at 44.5°C for 48 h. The positive cultures were streaked on eosin methylene blue agar (EMB) plates and typical colonies were tested for indole, methyl red, Voges Proskauer and citrate IMViC reactions. The positive isolates were streaked on sorbitol MUG medium for the detection of *E. coli* O157:H7.

2.4.3. Salmonella

Twenty five g of the samples were pre-enriched in lactose broth, enriched in selenite cystein and tetrathionate broth and streaked on bismuth sulphite agar, xylose lysine deoxycholate agar and Hektoen enteric agar. The typical colonies were purified and tested further biochemically. Serotyping of all presumptive positive isolates was carried out at Central Research Institute, Kasauli, India.

2.4.4. Vibrio cholerae

Twenty five g of the sample homogenized in 225 ml of alkaline peptone water (APW) and incubated overnight at 37ºC. After secondary enrichment in APW for 6 h, a loopful of the culture was streaked on thiosulphate citrate bile salt sucrose agar. Typical yellow, translucent colonies from each plate were tested further biochemically.

2.4.5. Staphylococcus aureus

Saline dilutions were spread plated on Baird Parker's agar and the characteristic black shining colonies were counted after 24 - 48 h incubation at 37°C. The colonies showing typical morphology were tested for coagulase production.

2.4.6. Listeria monocytogenes

Twenty five g sample was homogenized in 225 ml of the brain heart infusion broth (BHI) and incubated at 4°C for 48 h. Secondary enrichment was done in Listeria enrichment broth for 48 h at 4°C. A loopful of the culture was streaked on Listeria selective agar and characteristic positive colonies were picked up for further biochemical confirmation.

2.5. Inoculated pack studies

2.5.1. Preparation of inoculum

L. monocytogenes /*S*. Typhimurium was inoculated in 20 ml of brain heart infusion broth (BHI) and incubated at 37^{o}C for 20 h at 100 rpm. 2.4 ml of 20 h old culture was then diluted with 2400 ml of sterile tap water to obtain 10^{6} cfu / ml.

2.5.2. Inoculation of carrot, cucumber, pineapple and sprouts with the test organism

After washing and slicing, carrot and cucumber samples were packed in LDPE bags. The cut pineapple samples as well as sprout samples from market were also packed in LDPE bags. The packaged samples were exposed to 3 kGy (Sprout samples with 6 kGy) dose in Food Package Irradiator, Atomic Energy of Canada Ltd. at the dose rate of 40 Gy/min at melting ice temperature for the elimination of native microbial flora. The decontaminated samples were dipped in

S. Typhimurium/*L. monocytogenes* suspension (10^6 cfu/ml) for two minutes and dried on sterile blotting paper to remove excess suspension under aseptic conditions. Samples were then packed in LDPE bags (25 g each).

2.5.3. Irradiation

The carrot and cucumber samples were irradiated at 100, 200, 400, 600 and 800 Gy (at melting ice temperature 0 to 3ºC) in a cobalt-60 irradiator (Gamma cell 220, AECL, Canada) at the dose rate of 11 Gy/min. The sprouts and seeds inoculated with *S. Typhimurium* were irradiated at 100, 200, 400, 500 and 600 Gy, while seeds inoculated with *L. monocytogenes* were irradiated with 250, 500, 750, and 1000 Gy and sprouts inoculated with *L. monocytogenes* were irradiated at 500, 1000, 1500 and 2000 Gy. The dosimetry was done using Fricke's method. Each study included three samples per dose and was repeated three times.

2.5.4. Enumeration of microorganisms

Irradiated and non-irradiated samples were maintained at melting ice temperature. Both irradiated and non-irradiated samples were aseptically homogenized for 1 min in a sterile stomacher bag (Stomacher lab blender, model – 400, Seward, U.K.) containing 225 ml of sterile saline. Serial dilutions of the homogenate were prepared and appropriate dilutions were used to determine viable counts using plate count agar. The plates were incubated at 37°C for 48h and colony-forming units were counted. The average number of surviving viable cells (cfu/g) in the samples was plotted against irradiation dose. The slopes of the individual survivor curves were calculated with linear regression using a computer graphic programme (Origin 6.1). The D_{10} value was calculated by taking the negative reciprocal of the survival curve slope.

2.6. Storage studies of carrot, cucumber, pineapple and sprouts

The market samples in duplicate were irradiated at 2 kGy, stored at 4°C. The microbial quality of control and irradiated samples were determined by plating on PCA on day 0 and subsequently on the 4^{th} and 8^{th} day. Enrichment and selective plating was carried out to confirm the complete elimination of *Salmonella*, *Staph. aureus, L. monocytogenes* and faecal coliforms [7]. Each experiment was repeated at least three times.

2.7. Nutritional studies

2.7.1. Vitamin C extraction and analysis

Total Vitamin C content of the MPF samples was estimated according to standard AOAC official method [8]. 10 g of sample was ground in 50 ml of metaphosphoric acid- acetic acid solution. All the ascorbic acid present in the homogenate was converted to de-hydro ascorbic acid by activated charcoal. This total ascorbic acid was further reacted with o-phenylenediamine (Sigma Chemical Corp., St. Louis, Missouri, USA) to form fluorescence conjugate. Total conjugate formed was measured by CS-5000 fluorimeter (Shimadzu Corporation, Japan). The excitation and emission wavelengths were 350 nm and 430 nm, respectively. Concentration of total ascorbic acid in the samples was determined by comparing the fluorescence of conjugate formed by standard ascorbic acid.

2.7.2. Total carotenoids extraction and analysis

Estimation of total carotenoids content of MPF samples was done according to standard AOAC official method after slight modification [9]. A 10 g of sample was finely ground in acetone: hexane (4:6) containing 0.05% butylated hydroxy toluene (BHT) as an antioxidant. It was then filtered through sintered glass funnel. The residue was washed with 25 ml portions of acetone and then with 25 ml hexane, till the residue turned almost colourless. All washings were pooled and then acetone was removed from the extract by five 100 ml washings of distilled water, in a separating funnel. The

upper hexane layer was removed and passed over anhydrous sodium sulphate to remove any traces of water. The volume of this hexane layer was measured and absorbance was taken at 452 nm wavelength. A calibration curve was plotted using standard β- carotenes (Sigma Chemical Corp., St. Louis, Missouri, USA) with different concentrations ranging from 0.5 to 3.0 μg/ml in hexane. Absorbance of these samples was measured at 452 nm and a curve was plotted using standard concentrations versus absorbance value. The concentration of total carotenoids was determined in terms of β- carotenes, using slope value calculated from standard curve.

2.8. Sensory Evaluation

The sensory analysis was carried out for irradiated and non-irradiated samples after 24 h of storage at 10ºC. Sensory evaluation was done by an expert panel of researchers/ scientists (18 no.) from different sections of Food Technology Division, BARC, in a Taste Panel Laboratory in individual partitioned compartment of a controlled environment room. The sensory panel consisted of ten men and eight women (aged 25 to 50 years) and the members were trained to recognize and score different quality attributes including appearance, colour, texture, odour, taste and overall acceptability of minimally processed food samples before actual testing. Standard Triangular test was used for this assessment and the results obtained were analysed using statistical tools like mean and standard deviation. A seven-point hedonic scale was used for marking, ranging from very poor to excellent. For all samples, sensory analysis was done in three separate experiments in order to eliminate the bias emerging from biological variation in samples procured from various locations

2.9. Texture analysis

For textural studies, all the carrot, cucumber and pineapple samples were sliced with uniform thickness of about 1 cm. The texture of these samples was measured using Instron, Universal Testing Instruments (TM Model, Instron Engineering Corp., USA), which was equipped with a brass needle probe for penetration and a 100 kg load cell. Texture was calculated as maximum force (kg) measuring resistance to the penetration (5 mm fixed depth) imposed by the sample to the metal probe. The cross head and chart speed was fixed to 2 cm/min and 10 cm/min, respectively. The instrument was calibrated before each use. Texture of the irradiated and non-irradiated (control) salad pieces were determined after every fourth day of storage for 16 days.

In the case of sprouts, texture was determined with a texture analyser TA.HD plus (Stable Micro Systems, UK).

The different parameters set before taking texture were:

A. Pre test speed: 10 mm/sec
B. Post test speed: 10 mm/sec
C. Test speed: 0.50 mm/sec
D. Distance: 5.000 mm (Chick pea and Garden pea)
   2.000 mm (Green gram and Dew gram)
E. Hold time: 0. 01 sec
F. Trigger force: 10.0g

After setting the above parameters and attaching desired probe (75 mm compression platen), the texture was measured by compressing ten (green gram and dew gram) or five (chick pea and garden pea) seeds of sprouts. Maximum force (kg) of compression during the measurement was measured.

2.10. Effect of radiation processing on germination of seeds

After radiation processing at different doses, the seeds were allowed to germinate under humid conditions up to 48 h. After both 24 h and 48 h, 100 seeds were selected randomly for measuring the sprouting efficiency. For sprout length measurement, about ten seeds of each sprout were selected

randomly and the length of the sprout was measured in centimeters. Both the experiments were carried out at least thrice.

3. RESULTS AND DISCUSSION

3.1. Microbiological quality of MPF

3.1.1. Microbiological quality of carrot, cucumber, tomato and pineapple

The microbiological quality of carrots was of poorest grade among the vegetable samples analysed. The APC of carrot samples were in the range of 4 - 6 log cfu/g. Six out of eight samples were positive for faecal coliforms. Confirmed 33 *E. coli* isolates were obtained from all these samples, however, none of the isolates were *E. coli* O157:H7.

The APC of cucumber and tomato samples were < 4 log cfu/g and all the samples were free from faecal coliforms. All 40 samples of the fruits and vegetables tested were contaminated with *Staphylococcus* sp (Table 1).

Salmonella and *Vibrio cholerae* were not detected in any of the samples, and *Listeria monocytogenes* was isolated only from one carrot sample.

The microbiological quality of the minimally processed pineapple samples was not good. APC were in the range of 3.3 - 5.5 log CFU/g. All the samples were positive for faecal coliforms. *Staphylococcal* counts were in the range of 2.0 - 3.7 log CFU/g. One sample was positive for *Salmonella* (Table 1). These findings are consistent with the previously reported results. Vishwanathan et al. have reported that 37.5% of the screened pineapple samples were contaminated with *Salmonella* [2]. The harvesting, storage, transport and marketing need to be done in hygienic conditions. Further processing of the vegetables is necessary to improve their hygienic quality.

3.1.2. Microbiological quality of sprouts

A total of 124 sprout samples were analysed which included 46 Green gram, 27 Dew gram, 25 Chick pea and 26 Garden pea. The results of APC, Coliform count, YMC, Staphylococci counts of sprouts are shown in Table 2. There was no significant difference for the counts in all the four varieties of sprout samples. Very high bacterial load was found in all the samples analysed. APC ranged from 7.6-8.9 log CFU/g; such a high count is due to favourable conditions generated during the sprouting process. Analysis of the seeds showed that APC was $\leq$ 2 log CFU/g (data not shown). This naturally occurring population can rapidly increase during germination and sprouting due to nutrients released during germination, high moisture and favourable temperature. Coliforms constituted a large proportion of bacterial flora ranging from 5.4 – 7.9 log CFU/g; they might have come from a variety of sources like the soil in which the seeds were grown, the water used for washing the sprouts or people involved in the making and distribution of sprouts. YMC was high ranging from 3.6 - 7.3 log CFU/g. *Staphylococci* counts were also significantly high ranging from 3.3 - 6.6 log CFU/g indicating unhygienic handling.

E. coli, *Salmonella,* coagulase positive *Staph. aureus*, and *Listeria sp.* were detected in the samples analysed (Table 2). These pathogens may be introduced into sprouts from seeds, water used during production and improper sanitation during production and marketing [10]. 27% of *E. coli* and 5% of *Staph. aureus* isolates were found confirmed positive by biochemical analysis. 16%, *Salmonella* isolates were confirmed by serology.

For *E. coli,* 13% Green gram, 26% Dew gram, 40% Chick pea and 19% Garden pea samples were found positive, suggesting faecal contamination. *E. coli* 0157:H7 was not detected in any of the samples analysed.

The majority of the reported outbreaks due to the consumption of sprouts and other minimally processed foods were related to *Salmonella* [11]. There are more than 2300 serovars of *Salmonella* and all of them considered to be pathogenic. *Salmonella* can be introduced from seeds used for sprouting or during processing, it can survive and grow at storage temperatures greater than 8°C; also this pathogen had been shown to grow on surface of alfalfa sprouts [12]. Among the four different sprout samples tested *Salmonella* was detected in 22% Green gram, 30% Dew gram, 8% Chick pea and absent in Garden pea samples. All but one *Salmonella* isolates belonged to serovar Typhimurium; one isolate belonged to serovar Washington.

L. monocytogenes is present in the intestinal tract of many animals, including humans, so this organism can be found in the faeces of these animals, on the land they occupy, in sewage, and in soils [13]. *L. monocytogenes* is a psychrophile can grow at temperatures as low as 2°C [14]. Risk of listeriosis is increased when products are stored for longer periods before consumption. Thus, this pathogen is of concern in MPF; however, it was not detected in any of the samples analysed, other species of *Listeria* were detected of which *L. gryai* was found in 4% Green gram and 3% Dew gram, *L. innocua* in 7% Dew gram and 4% Chick pea, *L. welshimeri* in 2% Green gram samples.

Staph. aureus is known to be carried in the nasal passages of healthy food handlers, contamination by this can occur due to unhygienic conditions employed. Coagulase positive *Staph. aureus* was detected in 4% Green gram, 11% Dew gram and 4% Chick pea samples.

These results indicate that the sprout samples carried a very high bacterial and mold load. Moreover, if the seeds are contaminated with pathogens, the sprouting conditions are favourable for their growth and pathogens can reach very high numbers. Also, the pathogens like *S. aureus* can be introduced during processing and handling. Washing of sprouts can not ensure their safety and there is a need for processing of sprouts to ensure their safety.

3.2.1. Decimal reduction dose (D_{10}) for S. Typhimurium and L. monocytogenes

Before the inoculation of known pathogen to samples, all the samples were irradiated to eliminate background flora. A 3 kGy dose was sufficient for decontamination of carrot, cucumber and pineapple, but a 6 kGy dose was required to eliminate high background flora of sprouts. The D_{10} values of *S.* Typhimurium and *L. monocytogenes* in these commodities including the seeds of the different sprouts was in the range of 188 to 304 Gy and 296 to 579, respectively. The wide variation in D_{10} is probably due to intrinsic properties of the studied vegetables as well as irradiation conditions.

L. monocytogenes showed much higher D_{10} values (531-579 Gy) in all four different sprout samples as compared to those in seeds (Table 3). The higher radiation resistance might be due to the radioprotective substances produced during seed germination.

3.2.2. Radiation hygienization of minimally processed carrot, cucumber, pineapple and sprouts

The MPF samples treated with 1 kGy /2 kGy doses were free from *E. coli*, *Salmonella,* coagulase positive *Staph. aureus* and *L. monocytogenes* on 0 day and up to 12 days of storage at 4° and 10ºC (data not shown). The APC, YMC were significantly reduced and samples were in good conditions up to 12 days. The D_{10} values for *Salmonella* (200 Gy) suggested that 1 kGy dose could be sufficient for 5 log reduction while higher D_{10} for *L. monocytogenes* indicated that 2-2.8 kGy dose would be necessary for 5 log elimination of this pathogen.

In view of the non-linear nature of the survival curves, the D_{10} values can not be used for computing a dose for complete elimination. It has been well documented that though a linear decrease is observed in radiation survival studies, there is a trailing effect observed as the population decreases and for complete elimination of pathogens, a much higher dose is needed [15, 16].

Since the level of incidence and contamination of these pathogens reported in pre-packaged prepared vegetables was usually very low [17, 18, 19], radiation processing with 1.5 kGy could be sufficient to

improve the safety of minimally processed carrot, pineapple and cucumber. Treatment with 1 kGy dose of radiation along with good manufacturing practices has also been recommended by Farkas et al. [20] for hygienization of bell pepper and carrot based on their studies which showed 4 $\log_{10}$ reduction of *L. monocytogenes* at this dose. However, if elimination of 5 log CFU/g of these pathogens is the objective, treatment with 2-2.8 kGy will be necessary.

3.3. Effect of radiation processing on Vitamin C, total carotenoids, texture and organoleptic properties of carrot cucumber pineapple and sprouts

There was no significant difference ($p<0.05$) in the Vitamin C content and total carotenoids in the radiation processed (1 / 2 kGy) samples and control samples (Tables 4 and 5). Variation in the content of Vitamin C and carotenoids during storage also was not statistically significant from the control samples.

The qualified test panelist could not differentiate between irradiated and unirradiated samples.

The dose of 2 kGy did not change the organoleptic properties of any of these products (Table 6).

The radiation processing did not affect the textural properties of the above mentioned minimally processed produce (Table 7). There was significant reduction ($p<0.05$) in the firmness of the peripheral region of the carrot after exposure to gamma rays. However, the acceptability of radiation processed carrot was not affected. In fact, there was a slight increase in the sweetness after irradiation and the taste panelists preferred irradiated carrot over controls. During storage, there was significant increase ($p = 0.05$) in the firmness of the peripheral region of both control and irradiated samples.

3.4. Effect on germination of seeds

Radiation processing of seeds did affect the germination in the case of chick pea (Table 8). There was dose dependent decrease in sprouting efficiency up to 2 kGy. The sprouting efficiency at 2 kGy was 48% in Chick pea, 70% in Dew gram and 92% in Green gram after 48 h (Table 8). The D_{10} values of the *S.* Typhimurium and *L. monocytogenes* inoculated in seeds was in the range of 188 to 343 Gy. These values correspond to 1.5 kGy dose for 5 log cycle reduction. At this dose, germination percentage after 48 h was above 80% for all sprouts. However, the sprout length was affected significantly (Table 9). Radiation hygienization of seeds would not be completely effective to ensure their safety because of the possibility of pathogen contamination during sprouting.

4. CONCLUSIONS

1. Microbiological quality of carrot and pineapple was poor as compared to that of tomato and cucumber. Microbiological quality of sprout samples was poor with high APC and YMC. A large percentage of samples were found contaminated with *E. coli,* coagulase positive *S. aureus* and *Salmonella.* Only one sample of carrot was contaminated with *L. monocytogenes.*
2. D_{10} for *S. typhimurium* MTCC98 in MPF was around 200 Gy indicating that treatment with 1 kGy would achieve 5 log reductions. However, D_{10} for *L.* monocytogenes was found to vary for different commodities. Radiation processing with 2-2.8 kGy would be necessary for 5 log reduction in carrot and cucumber. However, a higher dose would be needed in the case of sprouts.
3. Radiation processing even at a dose of 2 kGy did not affect organoleptic quality of MPF.
4. There was no significant effect of radiation processing with 2 kGy dose of gamma radiation on Vitamin C content as well as total carotenoids of MPF.
5. Except for carrots, there was no significant effect of radiation processing on textural properties of all the MPF studied. Reduction in the firmness of radiation processed carrot did not affect its acceptability by the taste panellists.

TABLE 1. MICROBIOLOGICAL PROFILE OF CARROT, CUCUMBER, TOMATO AND PINEAPPLE

Microbiological parameter	Carrot (8)	Cucumber (8)	Tomato (12)	Pineapple(12)
APC (log cfu/g)	4.4 - 6.6	3.3 - 4.8	3.2 - 4.7	3.3-5.5
Faecal coliforms (log cfu/g)	+[a]	ND	ND	+[c]
E. coli in 25 g	+[b]	ND	ND	ND
Salmonella in 25 g	ND	ND	ND	1[f]
Staphylococcus sp. (log cfu/g)	2.6 – 4.5	2.7 – 3.3	1.0 – 4.0	2.0-3.7
Listeria monocytogenes in 25 g	1[e]	Nde[d]	ND	ND
Vibrio cholerae in 25 g	ND	ND	ND	ND

ND- Not detected
[a] six out of eight samples were positive.
[b] six out of eight samples were positive ; *E. coli* 0157:H 7 was absent
[c] all twelve samples were positive
[d] *L. monocytogenes* was absent in four samples and was not determined (Nde) in four samples
[e] isolate from carrot
[f] isolate from pineapple

TABLE 2. MICROBIOLOGICAL PROFILE OF SPROUTS

Microbial Profile	Green gram (46)	Dew gram (27)	Chick Pea (25)	Garden Pea (26)	All Sprouts (124)
APC (log cfu/g)	7.8 - 8.2	8.0 - 8.9	7.6 - 8.4	8.0 - 8.6	7.6 - 8.9
Coliform count (log cfu/g)	5.4 - 6.2	7.0 – 7.9	6.0 - 7.2	6.0 - 7.7	5.4 – 7.9
YMC (log cfu/g)	3.6 – 6.2	3.8 - 7.0	5.4 - 6.4	5.0 - 7.1	3.6 - 7.3
Staphylococci count (log cfu/g)	3.3 - 6.3	5.2 - 6.4	4.1 - 6.	4.5 - 5.1	3.3 - 6.6
E. coli (% sample positive)	13	26	40	19	27
Salmonella (% sample positive)	22	30	8	0	16
Coagulase positive *Staph. aureus* (% sample positive)	4	11	4	0	5
L. monocytogenes (% sample positive)	0	0	0	0	0

TABLE 3. D10 VALUES FOR S. TYPHIMURIUM AND L. MONOCYTOGENES

Sample		D_{10} Value (Gy)	
		S. Typhimurium	*L. monocytogenes*
Carrot		188.7 ± 13.6	307±10.1 Gy
Cucumber		189 ± 3.8	362±15.8
Pineapple		242 ± 5	ND*
Sprouts	Green gram	200 ± 5	579 ± 50
	Dew gram	193 ± 10	531 ± 31
	Chick Pea	206 ± 9	535 ± 15
	Garden Pea	200 ± 14	535 ± 4
Seeds	Green gram	188 ± 3	296 ± 30
	Dew gram	215 ± 8	319 ± 31
	Chick Pea	290 ± 9	343 ± 23
	Garden Pea	304 ± 8	316 ± 2

*ND – not done

TABLE 4. EFFECT OF RADIATION PROCESSING ON VITAMIN C CONTENT OF MPF

Sample	Carrot (mg/100g)		Cucumber (mg/100g)		Pineapple (mg/100g)	
	Control	2 kGy	Control	2 kGy	Control	2 kGy
0 Day	7.28± 0.47	7.63± 0.92	10.68± 0.38	8.99 ±0.77	31.55± 2.83	29.09 ±0.80
4 Day	6.74± 0.71	5.21± 0.33	8.72± 1.02	7.93± 0.39	23.46± 2.82	19.45± 1.46
8 Day	5.04± 0.23	4.84± 0.24	7.81± 0.99	7.19± 0.09	16.87±0.56	17.08± 0.61
12 Day	4.50± 0.23	4.77± 0.89	7.44± 0.59	7.89± 0.27	17.78± 0.42	14.66± 1.85
16 Day	4.25± 0.74	3.48± 0.19	8.02± 1.52	6.35±0.55	16.20± 0.07	12.70± 1.84

TABLE 4a. EFFECT OF RADIATION PROCESSING ON VITAMIN C CONTENT OF MPF

Sample	Chick pea (mg/100g)			Dew gram (mg/100g)		
4^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	30.56± 8.33	30.10±6.99	28.48±7.56	25.15±0.52	25.08±0.23	26.98± 0.03
4 Day	19.63± 0.48	24.44±4.21	21.39± 0.19	21.37±0.50	20.39±0.67	20.89± 0.67
8 Day	21.46± 3.79	24.24±7.15	21.58± 3.40	19.13±0.00	20.15±0.31	19.32± 0.48
12 Day	19.22± 1.57	22.92±1.79	18.85± 0.21	19.31±0.55	17.96±0.12	18.05± 0.05

TABLE 4b. EFFECT OF RADIATION PROCESSING ON VITAMIN C CONTENT OF MPF

Sample	Green gram (mg/100g)			Garden pea (mg/100g)		
4^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	13.53± 0.41	15.99± 0.63	17.32± 0.57	101.81± 11.12	90.58± 0.05	91.44± 6.65
4 Day	15.30± 0.31	15.72± 0.24	14.67± 0.03	87.82± 6.16	87.44± 4.14	95.13± 19.60
8 Day	15.45± 2.77	16.46± 1.07	15.31± 1.40	74.51± 9.04	78.55± 1.75	82.36± 0.49
12 Day	13.38±0.37	14.31± 1.08	14.01± 0.34	73.17± 8.00	68.95± 2.17	76.41± 4.45

TABLE 4c. EFFECT OF RADIATION PROCESSING ON VITAMIN C CONTENT OF MPF

Sample	Chick pea (mg/100g)			Dew gram (mg/100g)		
8^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	30.56± 8.33	30.10± 6.99	28.48± 7.56	25.15±0.52	25.08± 0.23	26.98±
4 Day	21.30± 0.48	22.01± 1.57	25.02± 1.46	25.06± 0.57	24.17± 0.02	21.67±
8 Day	19.07± 3.79	22.19± 1.00	25.80± 7.42	20.49± 0.77	19.33± 0.20	17.45±
12 Day	19.88± 1.57	19.35± 1.08	19.92± 3.92	19.18± 0.82	17.80± 0.11	17.92±

TABLE 4d. EFFECT OF RADIATION PROCESSING ON VITAMIN C CONTENT OF MPF

Sample	Green gram (mg/100g)			Garden pea (mg/100g)		
8^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	13.53± 0.41	15.99± 0.63	17.32± 0.57	101.81± 11.12	90.58± 0.05	91.44± 6.65
4 Day	16.86± 0.72	16.67± 0.69	16.69± 0.59	90.85± 2.85	79.50± 4.03	81.67± 7.68
8 Day	15.17± 1.73	16.48± 1.53	14.93± 1.12	84.31± 5.77	85.88± 7.87	85.81± 9.77
12 Day	13.60± 0.00	13.26± 0.44	12.37± 1.02	65.75± 3.35	76.27± 1.54	66.35± 0.53

TABLE 5. EFFECT OF RADIATION PROCESSING ON CAROTENOIDS OF MPF

Sample	Carrot (mg/ 100 g)		Pineapple (μg/ 100 g)	
	Control	2 kGy	Control	2 kGy
0 Day	15.06±0.39	12.50±0.87	711.73±54.16	711.05± 31.15
4 Day	12.22±1.30	12.30±0.20	778.96±46.61	698.48± 27.08
8 Day	14.61±0.89	14.11±2.44	658.85±9.83	667.67± 100.71
12 Day	14.16±1.47	14.23±1.38	732.80±36.89	620.94± 22.15
16 Day	13.55±0.93	13.88±2.05	711.00±48.56	659.87± 22.47

TABLE 5a. EFFECT OF RADIATION PROCESSING ON CAROTENOIDS OF MPF

Sample	Dew gram (μg/100g)			Green gram (mg/100g)		
4^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	289.34±6.56	332.74± 30.60	324.44± 0.88	1.47± 0.09	1.47± 0.13	1.34± 0.07
4 Day	280.98±2.29	272.36± 21.35	267.62± 14.80	1.51± 0.04	1.47± 0.11	1.63± 0.10
8 Day	282.53±9.44	271.29± 11.45	269.34± 9.19	1.67± 0.06	1.80± 0.02	1.80± 0.24
12 Day	269.75±12.10	285.65± 38.86	280.94± 20.48	1.70± 0.06	1.47± 0.15	1.41± 0.03

TABLE 5b. EFFECT OF RADIATION PROCESSING ON CAROTENOIDS OF MPF

Sample	Dew gram (μg /100g)			Green gram (mg/100g)		
8^0 C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	289.34±6.56	332.74± 30.60	324.44± 0.88	1.47± 0.08	1.47± 0.13	1.34± 0.07
4 Day	272.12± 1.06	259.09± 9.57	286.44± 9.63	1.63± 0.13	1.61± 0.07	1.79± 0.15
8 Day	243.88± 28.03	289.67± 16.33	286.14± 11.28	1.82± 0.06	1.55± 0.00	1.58± 0.07
12 Day	271.89± 19.09	244.86± 12.12	245.46± 23.49	1.39± 0.00	1.53± 0.09	1.60± 0.05

TABLE 6. EFFECT OF RADIATION PROCESSING ON ORGANOLEPTIC QUALITIES OF MPF

Sample	Carrot		Cucumber		Pineapple	
Parameter	Control	2 kGy	Control	2 kGy	Control	2 kGy
Appearance	5.7± 0.7	5.5± 0.9	5.8±0.7	5.5±0.8	5.5±1.0	5.3±1.0
Colour	5.5± 0.7	5.4± 0.8	5.6±0.7	5.5±0.8	5.6±0.9	5.5±0.8
Texture	5.3± 0.9	5.2± 0.9	5.5±0.8	5.0±0.8	5.4±0.9	5.1±0.8
Odour	5.2± 1.0	5.1± 0.9	5.3±0.8	5.0±0.9	5.2±1.1	5.0±0.9
Taste	5.3±1.1	5.4± 0.9	5.2±0.9	5.0±0.8	5.3±1.1	5.3±0.9
Overall Acceptability	5.4±0.9	5.4± 0.7	5.4±0.7	5.1±0.8	5.4±0.9	5.3±0.7

TABLE 6a. EFFECT OF RADIATION PROCESSING ON ORGANOLEPTIC QUALITIES OF MPF

Sample	Dew gram			Green gram		
Parameter	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
Appearance	6.0± 0.6	5.9± 0.6	5.8±0.6	5.4±0.9	5.5±0.8	5.7±0.7
Colour	5.7± 0.6	5.7± 0.6	5.8±0.7	5.4±0.9	5.5±0.9	5.5±0.8
Texture	5.8± 0.6	5.6± 0.7	5.5±0.7	5.4±0.8	5.4±0.9	5.2±0.7
Odour	5.6± 0.8	5.4± 0.9	5.5±0.7	5.0±1.0	5.2±0.9	5.2±0.7
Taste	5.5±0.9	5.5± 0.9	5.8±0.6	5.4±0.7	5.5±0.6	5.3±0.6
Overall Acceptability	5.6±0.8	5.7± 0.7	5.8±0.6	5.4±0.9	5.6±0.9	5.5±0.5

Hedonic scale
7 = Excellent, 6 = Very good, 5 = Good, 4 = Satisfactory, 3 = Fair, 2 = Poor, 1 = Very poor

TABLE 7. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Carrot central (kg)*		Carrot peripheral (kg)*		Pineapple (kg)*	
	Control	2 kGy	Control	2 kGy	Control	2 kGy
0 Day	7.25± 0.35	7.25± 0.21	5.15± 0.46	4.82 ±0.34	5.30±0.49	5.69±0.51
4 Day	8.45± 0.07	7.35± 0.92	5.76± 0.47	4.86± 0.65	5.32±0.65	5.70±0.44
8 Day	7.4± 0.36	7.00± 0.95	5.97± 0.41	5.20± 0.32	5.00±0.61	5.28±0.75
12 Day	6.9± 0.56	6.30± 0.66	5.36± 0.46	4.68± 0.28	4.97±0.53	5.08±0.50
16 Day	7.95± 1.34	7.56± 0.37	6.50± 0.47	5.17±0.32	4.77±0.22	4.91±0.69

TABLE 7a. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Cucumber central (kg)		Cucumber peripheral (kg)		Cucumber peripheral (kg)	
	Control	2 kGy	Control	2 kGy	Control	2 kGy
0 Day	1.02± 0.35	0.75± 0.18	2.34± 0.11	2.15 ±0.19	2.28± 0.11	2.30 ±0.36
4 Day	0.83± 0.05	1.04± 0.07	2.86± 0.49	2.19± 0.17	2.72± 0.40	2.19± 0.14
8 Day	0.91± 0.25	0.93± 0.06	2.62± 0.15	2.79± 0.47	2.54±0.17	2.76± 0.38
12 Day	0.98± 0.11	1.07± 0.10	2.93± 0.39	2.73± 0.49	2.94± 0.27	2.74± 0.41
16 Day	1.02± 0.11	1.12± 0.08	3.04± 0.49	2.95±0.52	2.94± 0.37	2.68± 0.27

TABLE 7b. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Chick pea (kg)			Dew gram (kg)		
4^0C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	34231± 5822	32801± 5301	33910±5206	16448± 1263	17559 ±2017	18544± 1015
4 Day	27073± 6555	32607± 2735	33530±6450	17145± 1530	17067± 1633	18158± 1134
8 Day	31573± 4288	30481± 4354	31651±4120	17296± 1577	17845± 1648	17490±1108
12 Day	31659± 7158	32597± 4938	28614±4725	17397± 889	17748± 1085	16681± 1333

TABLE 7c. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Chick pea (kg)			Dew gram (kg)		
8^0C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	34231± 5822	32801± 5301	33910±5206	16448± 1263	17559 ±2017	18544± 1015
4 Day	32691± 8942	30282± 5581	29959±7253	19432± 1851	18744± 1957	18787± 1259
8 Day	28312± 6411	29807± 6576	28606±6468	16046± 1738	17335± 1431	15560±1440
12 Day	28154± 5924	29683± 7695	30673±7185	15664± 1921	12818± 1754	13913± 1647

TABLE 7d. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Green gram (kg)			Garden pea (kg)		
4^0C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	17133± 1090	14081± 2040	13832±1414	38662± 7716	36873 ±5589	36114± 7028
4 Day	15265± 1511	13278± 1308	14368±1994	38983± 3445	39107± 7757	39534± 9173
8 Day	15887± 832	14517± 435	15712±1027	36714± 7637	38626± 4317	32807±4037
12 Day	15382± 808	12756± 1790	13726±1236	40369± 6216	36161± 2716	36629± 7702

TABLE 7e. EFFECT OF RADIATION PROCESSING ON TEXTURAL QUALITIES OF MPF

Sample	Green gram (kg)			Garden pea (kg)		
8^0C	Control	1 kGy	2 kGy	Control	1 kGy	2 kGy
0 Day	17133± 1090	14081± 2040	13832±1414	38662± 7716	36873 ±5589	18544± 1015
4 Day	14640± 1567	14636± 1845	14076±1904	39081± 6288	39586± 4578	18787± 1259
8 Day	12980± 862	13496± 1244	13848±946	37158± 6589	38756± 8920	15560±1440
12 Day	12197± 1273	12249± 1756	12945±1550	35374± 6692	37391± 3475	13913± 1647

* The force needed to compress MPF is expressed in kg units.

TABLE 8. EFFECT OF RADIATION PROCESSING ON % GERMINATION OF SEEDS

Sample	Chick pea (%)		Dew gram (%)		Green gram (%)	
Dose (kGy)	24 h	48 h	24 h	48 h	24 h	48 h
0	97	97	98	100	96	100
0.5	65	86	90	92	91	100
1.0	58	80	82	80	86	97
1.5	52	80	74	80	85	94
2.0	32	48	65	70	86	92

TABLE 9. EFFECT OF RADIATION PROCESSING ON SPROUT LENGTH

Sample	Chick pea (cm)		Dew gram (cm)		Green gram (cm)	
Dose (kGy)	24 h	48 h	24 h	48 h	24 h	48 h
0	2.34± 0.22	3.39± 0.28	2.4± 0.06	2.57± 0.14	2.68± 0.22	2.94± 0.16
0.5	1.50± 0.23	2.32± 0.17	2.17± 0.14	2.17± 0.16	2.23± 0.22	2.10± 0.29
1.0	1.39± 0.19	2.06± 0.25	1.9± 0.14	1.95± 0.15	1.76± 0.13	1.74± 0.08
1.5	1.05± 0.14	1.95± 0.13	1.81± 0.14	1.80± 0.17	1.59± 0.08	1.75± 0.08
2.0	1.01± 0.16	1.16± 0.19	1.49± 0.13	1.86± 0.13	1.46± 0.15	1. 68±0.10

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REFERENCES

[1] BEUCHAT, L. R. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59 (1996) 204-216.

[2] VISWANATHAN P., KAUR R. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. Int. J. Hyg. Environ. Health, **203** (2001) 205 – 213.

[3] EUROPEAN COMMISSION Risk profiles on microbiological contamination of fruits and vegetables eaten raw. Report of the Scientific Committee on Food, European Commission, Brussels (2002).

[4] PINGULKAR, K., KAMAT, A., BONGIRWAR, D. Microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads: an evidence of inhibition of Listeria monocytogenes in tomatoes. Int. J. Food Sci. Nutrition, **52** (2001) 15 – 23.

[5] FARKAS, J. Irradiation as method for decontaminating food. Int J. Food Microbiol. **44** (1998) 189-204.
[6] FARKAS, J. Irradiation of minimally processed food. In: R. Molins (ed.) Food Irradiation Principles and Applications. John Willey & Sons, Inc., New York (2001).
[7] BAMO, Bacteriological Analytical Manual Online, USA, http://cfsan.fda.gov/-ebam/bam_toc.html
[8] Official Methods of Analysis of AOAC International, USA, (1980) 738-739.
[9] Official Methods of Analysis of AOAC International, USA, (1980) 746.
[10] SOYLEMEZ, G., BRASHERARS, M.M. SMITH, D.A., CUPPETT, S.L., Microbial quality of alfalfa seeds and sprouts after a chlorine treatment and packaging modifications. Food Microbiology and Safety, **66** (2001) 153-157.
[11] SIVAPALASINGAM, S., FRIEDMAN, COHEN, C. R. L., TAUXE, R. V., Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. **67** (2004) 2342 – 2353.
[12] JAQUETTE C.B., BEUCHAT L.R., MAHON B.E., Efficacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl. Environ. Microbiol. **62** (1996) 2212 – 2215.
[13] BEUCHAT L. R., Listeria incidence on vegetables. Food Control, **7** (1996) 223 – 228.
[14] ROCOURT, C. P., *Listeria monocytogenes*. In: Doyle MP, Beuchat LR, Montville TJ (eds.) Food Microbiology: Fundamentals and Frontiers,. Washington, DC, American Society for Microbiology (1997). 337 – 352.
[15] QUINN, D.J., ANDERSON, A.W., DYER J.F. The inactivation of infection and intoxication of micro-organisms by irradiation of seafood. In Microbiological Problems in Food Preservation by Irradiation, IAEA Vienna (1967).
[16] ANDERSON A.W., Irradiation inactivation of food infection microorganisms in seafoods, In Freezing and Irradiation of Fish, Kreuzer R. (ed.) Fishing News (Books), London (1969).
[17] SIZMUR, K., WALKER, C.W. *Listeria* in pre-packed salads. Lancet I (1988)1167.
[18] FARKAS, J., ANDRASSY, E., MESZAROS, L., BANATI, D. Growth of untreated and radiation damaged *Listeria* as affected by environmental factors. Acta Aliment. Hung. **42** (1) (1995)19-28.
[19] BANDEKAR, J.R., DHOKANE, V.S., SHASHIDHAR, R., HAJARE, S., GHADGE, N., KAMAT, A.S., SHARMA, A. Microbiological quality of carrot, tomato and cucumber from Mumbai market. J. Food Sci. Technol. **42**(1) (2005) 99-101.
[20] FARKAS, J., SARAY, T., MOHACSI-FARKAS, CS., HORTI, K., ANDRASSY, E., Effects of low dose gamma irradiation on shelf-life and microbiological safety of pre-cut/prepared vegetables. Adv. Food Sci. (CTML), **19**(3/4) (1997) 111-119.

EFFECT OF GAMMA RADIATION ON THE SAFETY AND QUALITY OF SELECTED MINIMALLY PROCESSED FRUITS AND VEGETABLES

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Abstract

Microbiological status of selected commercial minimally processed fruits and vegetables were studied. Determination of D_{10} values of *L. monocytogenes* and *E. coli* 0157 in minimally processed fruits (pineapple (*Ananas comosus)*, jackfruit (*Artocarpus heterophyllus*) pomelo (*Citrus maxima)* and mixed fruits (pineapple and guava (psidium guajava)) and vegetables (onion (*Allium cepa*) and cucumber (*Cucumus sativus*) were carried out. The effect of various doses of gamma radiation (0.0, 0.5, 1.5 and 3.0 kGy) on the microbiological and sensory or physical properties of the fruits or vegetables stored for 15 days were also evaluated. Commercial samples were found to have Aerobic Plate Count (APC) range from 10^3 CFU/g to 10^6 cfu/g and *L. monocytogenes* and *E. coli* 0157 were not detected. Lower D_{10} values were calculated for both pathogens studied in pineapple (0.15, 0.08 kGy) compared to in jackfruit (0.4, 0.16 kGy). The D_{10} value for *E. coli* 0157 in mixed fruit was 0.14 kGy. However, the D_{10} values were the same (0.23 kGy) for *L. monocytogenes* in both onion and cucumber. Radiation was found to cause variable changes in the sensory quality of minimally processed fruit varieties but it generally improves the texture and color of the minimally processed vegetable.

1. INTRODUCTION

Demand for fresh-like products has increased among the consumers worldwide. Minimally processed foods (MPF) have fresh-like characteristics and also provide the convenience demanded by consumers [1]. 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 [2]. Biological processes like respiration, ripening and senescence continue in harvested fruits and vegetables. The rate of respiration determines how long the fruit will take to deteriorate. Minimal processing may induce or enhance changes in flavor, texture and appearance which occur during senescence [3].

The technique of minimal processing has actually been practiced widely for quite some time in the food service sector such as restaurants, hotels and food courts. However, it is only recently that the sale of minimally processed fruit and vegetable has made its way in the supermarkets. In Malaysia, the consumption of ready to eat minimally processed vegetables and fruits have become popular simply because of the convenience, fresh and health reasons.

Minimally processed vegetables and fruits are defined as fresh vegetables and fruits that have been processed to increase their functionality without greatly changing their fresh-like properties. The type of process is dependent on the type of produce but generally involves washing, cutting, mixing and packaging [4]. This produce is characterized by a good degree of freshness, convenience and lack of preservatives [5].

Minimal processing of fruit and vegetable was not preferred before because of the faster spoilage of the produce due to the undesirable changes in color, taste and appearance after it is cut. The changes due to the enzymatic and microbial growth are inevitable once the plant tissue is exposed to oxygen and the transfer of microbes from the skin to the flesh. With the basic knowledge in plant physiology and microbiology the spoilage can be delayed or even prevented.

Onions are among the daily taken vegetables by the Malaysian community. Onion or scientifically known as *Allium cepa* has been consumed mainly for its flavor and it was a very important ingredient in most of the Malay dishes. The *Allium* family has over 500 members each differing in appearance, color and taste but close in biochemical, phytochemical and nutraceutical content. *Alliums* are known to possess antibacterial and antifungal activities, and contain the powerful sulfur and other numerous

phenolic compounds which arouse great interest [6, 7]. Since onion bulbs grow in the soil, the microorganisms present may originate from the soil, water and it is further contaminated by the handlers during harvesting. Therefore, the safety of fresh onions has become very important especially if it is consumed raw.

Cucumber, scientifically known as *Cucumis sativus,* is one of the vegetables that is usually eaten raw.

Raw or fresh onions and cucumber are used in certain Malaysian dishes such as salad, satay and grilled chicken. In these dishes the minimally processed onions and cucumber are served together with the main. In a big restaurant where a large amount of onion and cucumber cuts need to be prepared instantly it is a cumbersome task. Often the customer is served bad quality onion and cucumber cuts and the safety is questioned.

Food irradiation has been approved by almost 60 countries and is being applied commercially in many countries. Irradiation is a well-known technology for the elimination of microbial contamination [3]. The effect of irradiation on the inactivation of food-borne microorganisms has been discussed by Monk et al. [8]. The survival of microbial cells upon irradiation was found to depend on the number of cells, pH, temperature and chemical composition of the food in which cells are suspended. The relative resistance of microorganisms to irradiation was summarized by Ingram and Farkas [9]. The D_{10}-values determined were mostly for food pathogens in animal products. Low-dose irradiation can reduce or totally eliminate the majority of spoilage organisms in foods such as vegetables and fruits and also other easily spoiling foods like meat and poultry. Irradiation treatment combined with proper refrigeration for storage can prolong the shelf-life of these food items without affecting the flavor and texture.

The aim of this research was to study the quality changes occurring in the irradiated minimally processed onion and cucumber as well as some fruits (pineapple, jackfruit and mixed fruits). The effect of gamma radiation on the destruction of pathogenic bacteria such as *L. monocytogenes* and *E. coli* 0157 was also studied.

2. MATERIALS AND METHODS

2.1. Experimental design

The minimally processed fruits (pineapple, jackfruit, mixed fruits) and vegetables (onion and cucumber) samples were irradiated with gamma radiation at the dose range between 0.0 to 3.0 kGy. The 0 kGy samples were considered as control. All samples were kept under refrigeration (5 ± 2^{o}C) - eight days for fruits and fifteen days for vegetables in a laboratory chiller. The samples were taken on appropriate day for microbiological, sensory, hardness and color intensity evaluation. The results were compared between doses and also between different storage periods. Data were analysed using ANOVA and Duncan of the Statistical Analysis System (SAS) programme.

2.2. Fruits

Minimally processed jackfruit, pineapple and pomello were bought from supermarkets, food courts and mobile food stalls. Whole fruits were bought from a wholesaler market in Selangor. The whole jackfruit was left at room temperature for two days to ensure ripening.

2.3. Vegetables

Onion and cucumber were bought from a wholesale market in Selangor and stored in a chiller before processing.

2.4. Minimal processing of fruits and vegetables

The whole fruits were first cleaned with brush and running water and then wiped dry. The skin of pineapple were peeled and then soaked in cold water for a few minutes and then drained in plastic basket. The pineapple was then cut into either longitudinal quarters, 1/8th or disc slices.

Onion and cucumber skins were peeled and washed manually under running tap water. After washing, the excessive water was drained to dry. The onion and cucumber were then cut into small slices.

2.5. Packaging

The slices of fruit or vegetable were packed into a polypropylene (PP) rigid container and covered with a lid. Each container contained vegetable cuts of 45 g for D_{10} determination and 150 g for quality evaluation. The packed minimally processed fruits or vegetables were stored in a laboratory chiller at $5^{o}C \pm 2^{o}C$.

2.6. Inoculation of fruits and vegetables

A 5 ml portion of a known concentration of *L. monocytogenes* or *E. coli* 0157 were poured onto each of the 45 g of pre weighed minimally processed fruits or vegetables in the polypropylene (PP) container. The PP containers were then arranged in the transportation box.

2.7. Transportation of packed fruits and vegetables

Prepackaged minimally processed vegetable were arranged inside an insulated box which was layered inside with frozen gels to prevent the sudden drop of temperature. The insulated boxes were then transported from the Malaysia Agricultural Research and Development Institute (MARDI) to the Sinagama Complex of Malaysian Institute for Nuclear Technology Research (MINT) and vice versa within 30 minutes.

2.8. Irradiation of fruits and vegetables

Samples were exposed to gamma radiation with doses between 0.5 to 3.0 kGy by calculating the exposure time which was based on the dosimetry experiment done earlier. All samples were irradiated inside a gamma chamber with Cobalt 60 source. After the irradiation treatment, all samples were transported back to microbiological laboratory within 30 minutes.

2.9. Determination of decimal reduction dose (D_{10})

D_{10} was calculated by plotting a graph between log cfu/g of organism studied against doses of gamma radiation used. A straight trend line was applied and the reciprocal of the slope was determined. This value is the D_{10} value of that organism in the fruit or vegetable.

2.10. Microbiological Analysis

All of the microbiological analyses were done using BAM methodologies [10]

Aerobic Plate Count (APC): A 10 gram sample of vegetable was homogenized for 30 second in a 90 ml single strength Ringers solution using a stomacher. The homogenate were then serially diluted with 9 ml Ringers. One ml-s of three selected dilutions (e.g. -1, -2 and -3) were pour plated using plate count agar (PCA) to determine the population of surviving microbes in the samples. The plates were incubated for 48 hours at $37^{o}C$. The colonies that grew were counted and results were reported as log 10 colony forming unit per gram sample (log cfu/g).

***Analysis of E. coli* 0157:** A 25 gram sample of jackfruit or mixed fruit was inoculated into a 225 ml of EC broth (Oxoid) supplemented with Novobiocin (Oxoid) and the mixture was incubated at $37^{o}C$.

After six hours and 24 hours of incubation, a loopful of the mixture was streaked onto CT SMAC agar (Sorbitol Mac Conkey agar supplemented with cefixime tellurite, Oxoid). The plates were incubated for 24/48 hr at 37^{o}C. Typical colonies of *E. coli* 0157 (neutral/gray with smoky centre, 1-2 mm diameter) were observed. A maximum of five typical colonies were picked and restreaked on nutrient agar. Colonies from nutrient agar were used for the serological test to confirm *E. coli serogroup* 0157 using test kit by Medvet (MEDVET Sci. Pty Ltd). Results were reported as presence in a 25 gram sample.

Analysis of Listeria monocytogenes: A 25 g sample was inoculated into 225 ml of Listeria enrichment broth (Oxoid) and then incubated at 35^{o}C for 4 hr. A selective agent was then added into the broth and incubation was continued. After 24 and 48 hr incubation, a loopful of the enrichment broth was streaked on to PALCAM agar (Oxoid). PALCAM agar plates were incubated at 35^{o}C. Typical colonies of *L.monocytogenes* on PALCAM agar will appear as gray colonies with black halo centre. A maximum of five typical colonies were picked and restreaked on Nutrient agar. Colonies from nutrient agar were used for serological test to confirm *Listeria monocytogenes* using test kit by Medvet (MEDVET Sci. Pty Ltd). Results were reported as presence in 25 gram sample.

2.11. Sensory evaluation

Assessments were made using the hedonic scale with a score of 1 to 9, where 1 represented 'dislike extremely' and 9 'like extremely'. Sensory attributes categories consisted of color, aroma, texture, taste and overall acceptability. Panelists were instructed to rate the intensity of each attribute using the score of 1 to 9 for three different samples which were pineapple, jackfruit and mixed fruit (pineapple and guava).

2.12. Color intensity analysis

The readings of the surface color intensity of each vegetable sample were taken four times using Minolta Chromameter (CR-200, Japan) based on the Hunter's system (L*, a* and b* values). The L* value denotes lightness on a 0-100 scale from black to white, a* value denotes redness (+) or greenness (-) and b* value denotes yellowness (+) or blueness (-).

2.13. Texture analysis (Hardness)

The texture analysis of the vegetables was done by measuring the hardness of the samples. The hardness was determined using a texture analyser (Steven Fernell model QTS25, UK) and the results recorded as g.

3. RESULTS AND DISCUSSION

3.1. Aerobic Plate Count (APC) of commercial minimally processed jackfruit, pineapple, pomello, and mixed fruits (pineapple and guava)

Aerobic Plate Count (APC) of jackfruit ranges from 10^4 CFU/g to 10^7 CFU/g in which most samples have the count of 10^6 CFU/g (Table 1). Aerobic plate counts of pineapple were in the range of 10^4 CFU/g to 10^6 CFU/g.

Aerobic plate counts of pomello were very much lower than that of jackfruit and pineapple (max. 10^3 CFU/g). The count of 10 CFU/g to 10^3 CFU/g is considered very low for a fresh produce. However, this is not surprising since the segments of pomello are still covered with a suberous layer which is dry and prevents the fruit flesh being exposed to the environment. The high count was also very much influenced by the place where the fruits were sold. Samples with high APC count were samples sold at the mobile stalls where contaminations were unavoidable whereas samples with low APC count were samples sold in supermarkets and food courts inside building.

TABLE 1. AEROBIC PLATE COUNT OF COMMERCIAL MINIMALLY PROCESSED JACKFRUIT, PINEAPPLE, POMELLO AND MIXED FRUIT

Sample No.	Jackfruit (CFU/g)	Pineapple (CFU/g)	Pomello (CFU/g)	Mixed fruit (CFU/g)
1	3.5×10^6	5.9×10^4	3.5 X 10	3.3×10^4
2	2.4×10^6	6.4×10^4	4.5 X 10	1.0×10^5
3	4.7×10^6	2.3 X 105	2.4×10^3	4.9×10^5
4	3.5×10^6	1.9×10^6	4.0×10^2	5.8×10^5
5	2.1×10^6	4.7×10^4	3.6×10^2	1.1×10^6
6	1.6×10^6	1.5×10^6	3.6 X 10	3.6×10^5
7	3.2×10^5	6.3×10^5	4.4×10^2	5.0×10^6
8	2.7×10^7	8.5×10^4	7.5 X 10	5.3×10^5
9	1.5×10^4	3.8×10^5	9.5×10^2	2.2×10^4
10	2.8×10^4	5.6×10^5	2.5 X 10	2.0×10^4

3.2. Presence of *E. coli* 0157 and *L. monocytogenes* in minimally processed jackfruit, pineapple, pomello and mixed fruit

E. coli 0157 and *L. monocytogenes* were not detected in jackfruit, pineapple, pomello and the mixed fruit samples analysed.

3.3. Effect of packaging on the Aerobic Plate Count (APC) of minimally processed jackfruit, pineapple and pomello

Two types of packaging used in this experiment gave a significant difference to the APC of fruits studied (Table 2).

TABLE 2. EFFECT OF PACKAGING ON THE AEROBIC PLATE COUNT (APC) OF MINIMALLY PROCESSED JACKFRUIT, PINEAPPLE AND POMELLO STORED AT 5±2°C

	Shrink wrap (Polyethylene) (CFU/g)			**Polypropylene rigid container** (CFU/g)		
Day of storage	Jackfruit	Pineapple	Pomello	Jackfruit	Pineapple	Pomello
0	4.7×10^5	2.7×10^5	9.7×10^2	4.7×10^6	4.7×10^6	9.7×10^2
2	2.3×10^5	9.4×10^4	1.1×10^2	3.6×10^5	3.6×10^5	2.1×10^2
4	2.1×10^5	5.2×10^5	3.8 x 10	4.1×10^5	4.1×10^5	4.0 x 10
6	8.9×10^5	7.9×10^5	1.0 x 10	1.7×10^5	1.7×10^5	1.0 x 10
8	6.3×10^4	4.8×10^5	1.8 x 10	3.2×10^6	5.6×10^6	1.0 x 10
12	4.1×10^5	1.4×10^5	1.8 x 10	7.1×10^6	2.8×10^6	2.8 x 10

Both jackfruit and pineapple cuts have APC values lower when they were packed with shrink wrapped polyethylene plastic than when they were packed in polypropylene container. However, at the end of storage period the APC of both fruits were 10^6 CFU/g. Since the fruits were still respiring, it resulted in water loss by the plant tissue and the fruits became soft.

3.4. D_{10}-values of *E. coli* 0157 and *L. monocytogenes* in minimally processed fruits and vegetables

D_{10}-value was not obtained for *L. monocytogenes* in mixed fruit because the organism was not able to grow in this produce. Since *L. monocytogenes* was able to grow in pineapple it is suspected that guava inhibited the growth of *L. monocytogenes.* This observation was proven with another experiment by inoculating a known concentration of *L. monocytogenes* in minimally processed guava and stored for one week. It was found that even at the day of inoculation, *L. monocytogenes* was not detectable.

Results show (Figs 1-5) that both *L. monocytogenes* and *E. coli* 0157 have different D_{10}-value in different fruits when irradiated with gamma rays. *L. monocytogenes* is proven to be more radiation resistant than *E. coli* O157. D_{10}-values of *L. monocytogenes* were 0.15 kGy in pineapple, but 0.4 kGy in jackfruit. D_{10}-values of *E. coli* 0157 were lower, 0.08 kGy in pineapple 0.16 kGy in jackfruit, and 0.14 kGy in mix fruit.

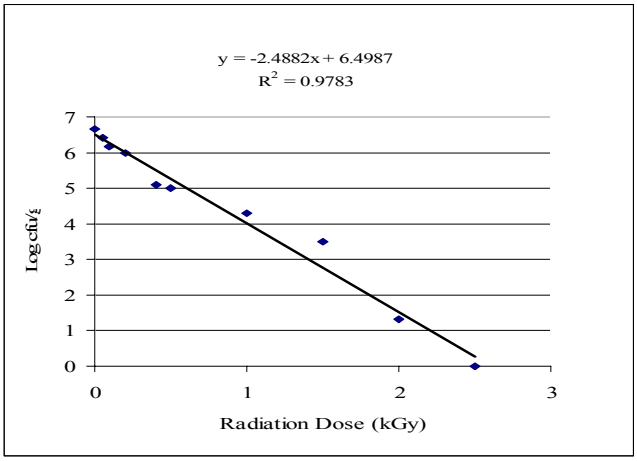

FIG. 1. Survival curve of L. monocytogenes in minimally processed jackfruit D_{10}=0.4 kGy.

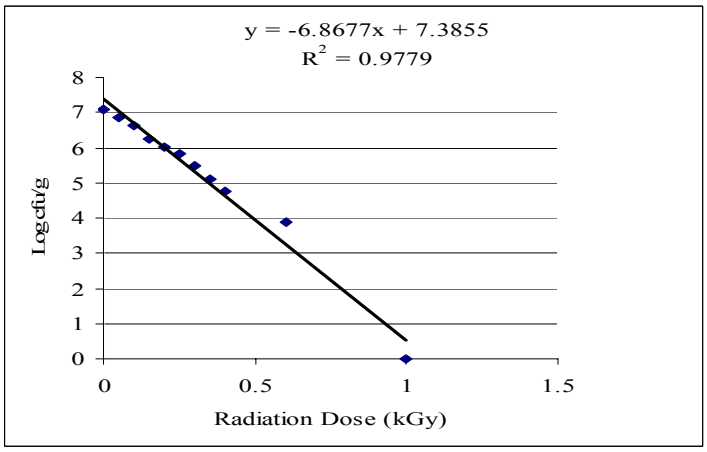

FIG. 2. Survival curve of L. monocytogenes in minimally processed pineapple D_{10}=0.15 kGy.

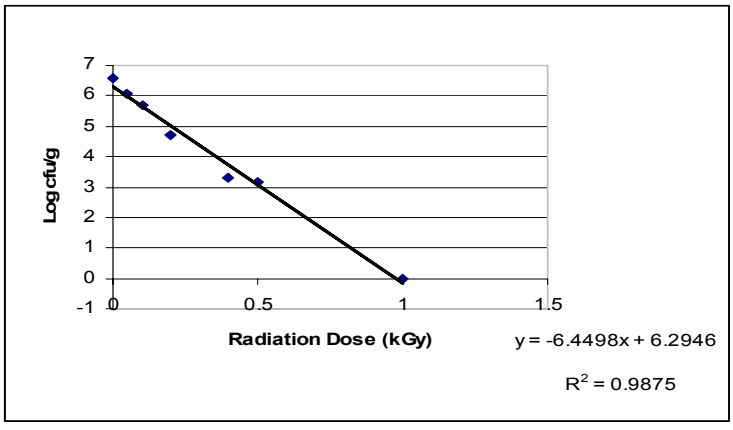

FIG. 3. Survival curve of E. coli 0157 in minimally processed jackfruit D_{10}=0.16 kGy.

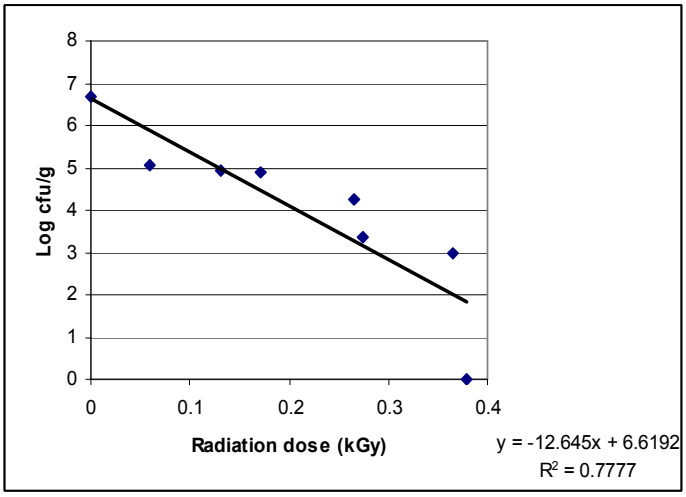

FIG. 4. Survival curve of E. coli 0157 in minimally processed pineappleD_{10}=0.08 kGy.

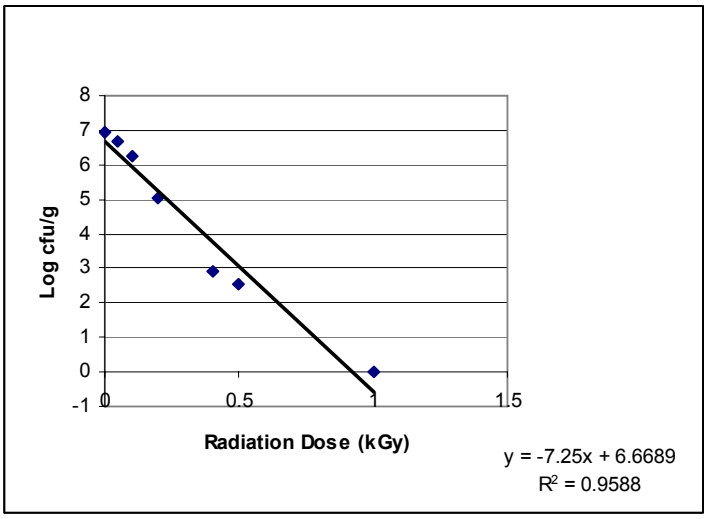

FIG. 5. Survival curve of E. coli 0157 in minimally processed mixed fruit D_{10}=0.14 kGy.

D_{10}-value of *L. monocytogenes* was 0.23 kGy both in onion and in cucumber (Figs 6-7). The nearest comparison for radiation resistance of *L. monocytogenes* found in literature is the D_{10} in cooked cauliflower which was determined as 0.564 kGy. This value is quite high if compared to the D_{10}-values determined in our experiments.

D_{10}-values of *E. coli* 0157 (Figs 8-9) were lower (0.11 kGy in onion and 0.06 in cucumber) compared to D_{10}-values of *L. monocytogenes* in these vegetables.

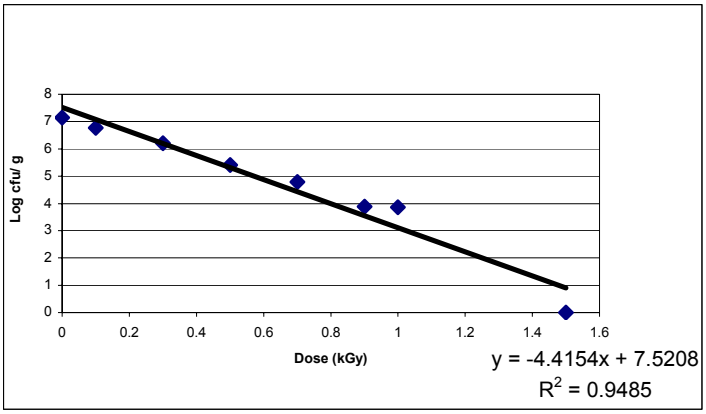

FIG 6. Survival curve of L. monocytogenes in minimally processed onion D_{10}=0.23 kGy.

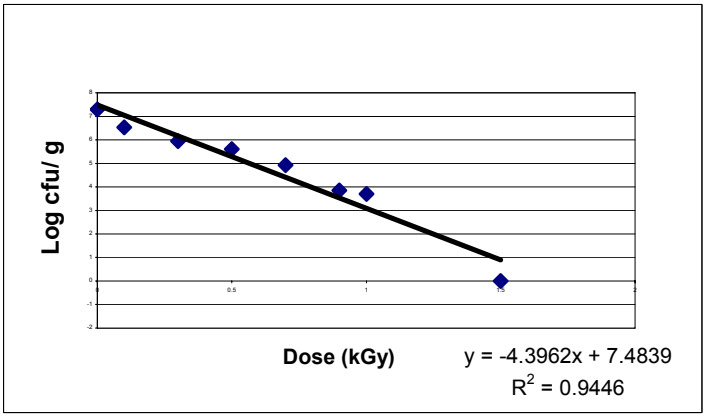

FIG 7. Survival curve of L. monocytogenes in minimally processed cucumber D_{10}=0.23 kGy.

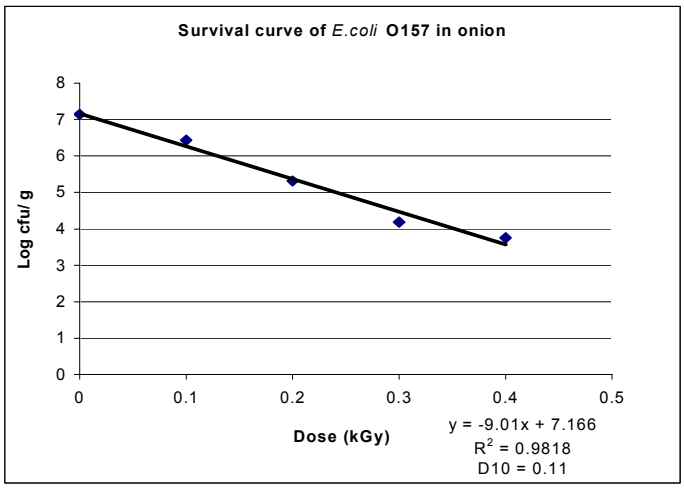

FIG 8. Survival curve of E. coli 0157 in minimally processed onion D_{10} =0.11 kGy.

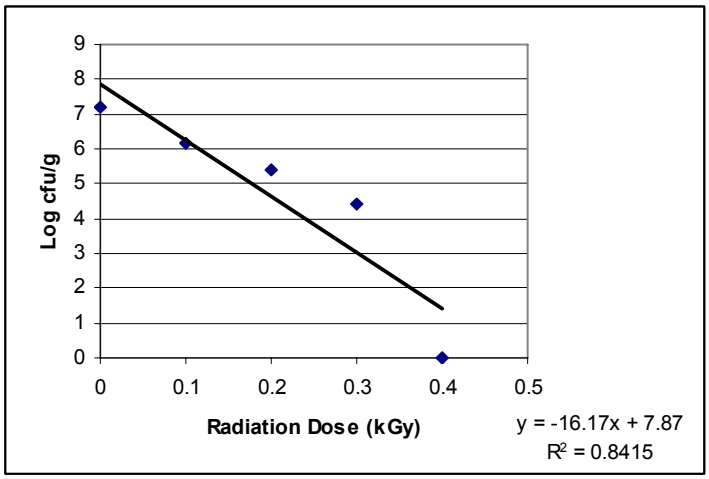

FIG. 9. Survival curve of E. coli 0157 in minimally processed cucumber D_{10} =0.06 kGy.

3.5. Effect of irradiation on Aerobic Plate Count in minimally processed fruits and vegetables

Irradiation with 0.5 to 3 kGy caused 1-3 log cycles reduction in the APC of the fruits. However, depending on the applied dose, growth of surviving microorganisms was observed on storage day 4 or 8. The total viable count was almost always lower when the dose of radiation was higher (Figures 10-12).

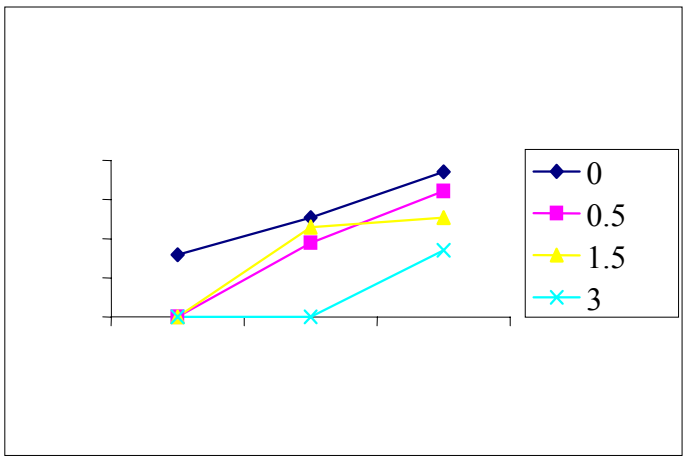

FIG. 10. Aerobic plate count (APC) changes in irradiated minimally processed jackfruit stored at 5 ± 2°C.

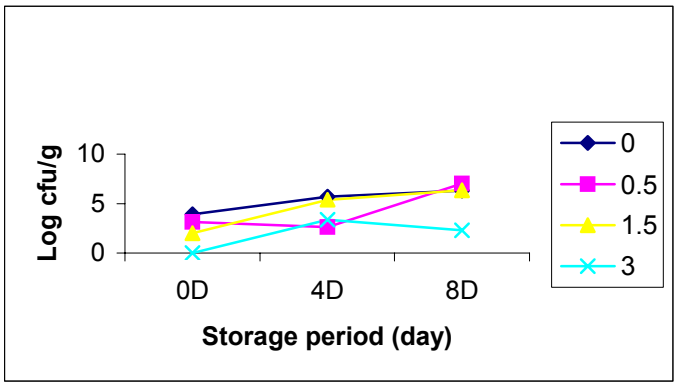

FIG. 11. Aerobic plate count (APC) changes in irradiated minimally processed pineapple stored at 5 ± 2°C.

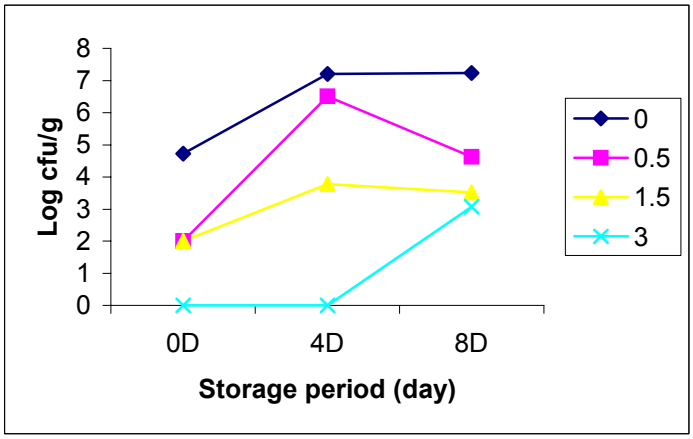

FIG. 12. Aerobic plate count (APC) changes in irradiated minimally processed mixfruit stored at 5 ± 2°C.

Generally, the total viable count in both onion and cucumber increased during storage (Figs 13 and 14). However, the microbial count was lower in the irradiated samples (0.5, 1.5, 3.0 kGy) compared to the non irradiated samples. The difference was statistically significant ($p < 0.05$).

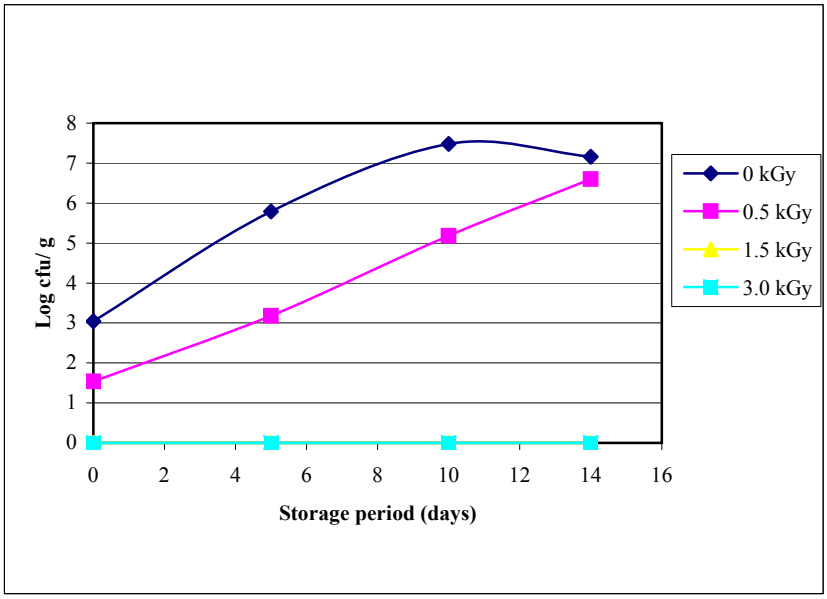

FIG. 13. APC changes in irradiated onion stored at 5 ± °C.

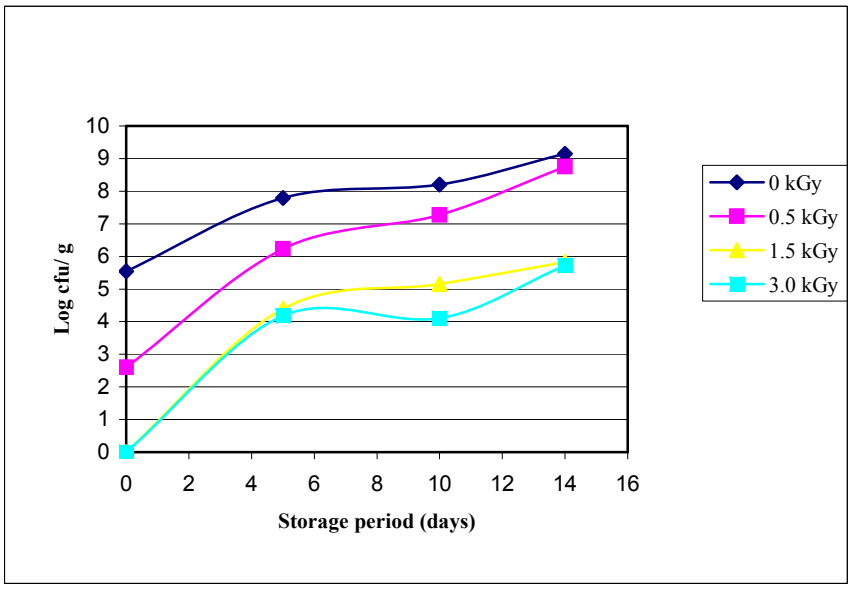

FIG. 14. APC changes in irradiated cucumber stored at 5 ± °C.

3.6. Effect of irradiation on sensory quality of minimally processed fruits

Panelists' acceptance of irradiated and non-irradiated fruits during eight days of storage is shown in Tables 3-5.

Different fruits received different pattern of scoring from the panelists. For jackfruit, all attributes shared the same scoring pattern from the panelists. For every storage period, non-irradiated sample consistently received highest score. The results showed that irradiation affected the sensorial quality of jackfruit, especially the higher doses 3 kGy.

TABLE 3. SENSORY QUALITIES OF IRRADIATED MINIMALLY PROCESSED JACKFRUIT REFRIGERATED STORED FOR 8 DAYS

Attribute	**Color**			**Aroma**			**Texture**			**Taste**		
Dose (kGy)	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8
0	7	7	7	7	7	7	7	7	7	7	7	7
0.5	7	7	7	7	7	7	6	6	6	7	6	6
1.5	7	7	6	7	6	6	5	5	5	6	5	5
3.0	7	7	6	7	5	6	4	4	4	4	4	4

TABLE 4. SENSORY QUALITIES OF IRRADIATED MINIMALLY PROCESSED PINEAPPLE REFRIGERATED STORED FOR 8 DAYS

Attribute	**Color**			**Aroma**			**Texture**			**Taste**		
Dose (kGy)	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8
0	7	7	6	7	6	5	8	7	7	6	6	5
0.5	7	7	6	7	6	5	7	7	6	6	6	4
1.5	7	7	6	6	6	5	7	7	6	6	6	6
3.0	7	6	5	6	6	5	7	6	6	5	5	5

Each attribute showed a different pattern of scoring from the panelists.

For pineapple, after three days of storage, panelists gave the highest score to the irradiated sample (1.5 kGy) and on the eighth day of storage, the highest score was shared by the non-irradiated and irradiated sample (1.5 kGy).

Overall acceptability for mixed fruit showed that on day 0, panelists had given the highest score to irradiated sample (0.5 kGy). However, this scoring pattern changed during storage. On the third and eighth day of storage, panelists gave the highest score to non-irradiated sample.

TABLE 5. SENSORY QUALITIES OF IRRADIATED MINIMALLY PROCESSED MIXED FRUIT REFRIGERATED STORED FOR EIGHT DAYS

Attribute	**Color**			**Aroma**			**Texture**			**Taste**		
Dose (kGy)	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8	Day 0	Day 3	Day 8
0	7	6	5	7	6	6	7	6	5	7	6	5
0.5	7	5	5	7	5	5	7	5	5	7	5	5
1.5	7	5	5	6	5	5	6	5	4	6	4	4
3.0	6	4	5	6	5	5	5	4	4	5	4	4

3.7. Effect of irradiation on color of minimally processed onion and cucumber

Color intensity analysis (L)*

Table 6 shows the results of colour intensity analysis in onions. There was no significant difference (P>0.05) for the L* value of most samples except samples on day 0 at different doses. This result shows that at day 0 the lightness of onions was affected by the irradiation with doses of 1.5 and 3.0 kGy.

Color intensity analysis (a*)

For onion samples all the values recorded were negative (-) which refer to greenness. After irradiation with 1.5 and 3.0 kGy, there were significant differences observed in a*-values of onion samples. However, during storage there were no significant differences found between any other samples.

Color intensity analysis (b*)

Results given for onion and cucumber were positive values which mean that these vegetables have more yellow color than blue (Tables 6 and 7). There was no significant difference (p>0.05) for the yellowness of the samples except on day 0, where there was a significant difference (P<0.05) when compared between samples exposed to different doses of irradiation. This result shows that at day 0 the yellowness of onion was affected by the irradiation with doses 0.5, 1.5 and 3.0 kGy.

Table 7 shows the results of colour intensity analysis in cucumber. The storage and irradiation did not affect the quality of lightness for minimally processed cucumber. There were no significant differences (P>0.05) in the L* values among all samples if compared between samples exposed to different doses of irradiation (0.0, 0.5, 1.5, 3.0 kGy) and also between different storage period (day 0, 5, 10 and 15).

For cucumber samples, all the values recorded were negative (-) which refer to greenness. There were no significant differences (p>0.05) in the greenness of the samples treated with different doses of irradiation during 15 days of storage.

For cucumber, there was no significant difference (p>0.05) for the yellowness of the samples treated with different doses of irradiation during 15 days of storage.

TABLE 6. COLOR INTENSITY IN ONION IRRADIATED WITH VARIOUS DOSES AND STORED AT 5±2^{O}C

Dose (kGy) / Storage Period (days)		Color intensity			
		0 kGy	0.5 kGy	1.5 kGy	3.0 kGy
L*	0	63.87$_{Aa}$	66.38$_{Aa}$	61.83$_{Ba}$	72.22$_{Ca}$
	5	73.04$_{Aa}$	64.67$_{Aa}$	67.34$_{Aa}$	69.27$_{Aa}$
	10	67.14$_{Aa}$	69.66$_{Aa}$	65.17$_{Aa}$	65.73$_{Aa}$
	15	71.08$_{Aa}$	68.93$_{Aa}$	68.94$_{Aa}$	66.73$_{Aa}$
a* (negative value)					
	0	13.71$_{Aa}$	14.20$_{Aa}$	18.44$_{Ba}$	10.44$_{Ca}$
	5	10.88$_{Aa}$	17.93$_{Aa}$	15.74$_{Aa}$	12.65$_{Aa}$
	10	14.93$_{Aa}$	13.56$_{Aa}$	9.95$_{Aa}$	14.69$_{Aa}$
	15	9.79$_{Aa}$	12.69$_{Aa}$	13.67$_{Aa}$	16.83$_{Aa}$
b* (positive value)					
	0	22.95$_{Aa}$	25.62$_{Ba}$	30.95$_{Ca}$	17.84$_{Da}$
	5	18.78$_{Aa}$	30.14$_{Aa}$	24.75$_{Aa}$	20.61$_{Aa}$
	10	27.17$_{Aa}$	23.63$_{Aa}$	19.82$_{Aa}$	26.98$_{Aa}$
	15	18.51$_{Aa}$	22.52$_{Aa}$	24.90$_{Aa}$	30.11$_{Aa}$

3.8. Effect of radiation on texture (hardness) of onion and cucumber

Results showed that there were no significant differences in the hardness between the onion samples (Fig. 15). Data indicate that hardness of onion was not affected by the irradiation in the range of 0.5 to 3.0 kGy. The results also indicate that storage of onion for 15 days did not affect this parameter.

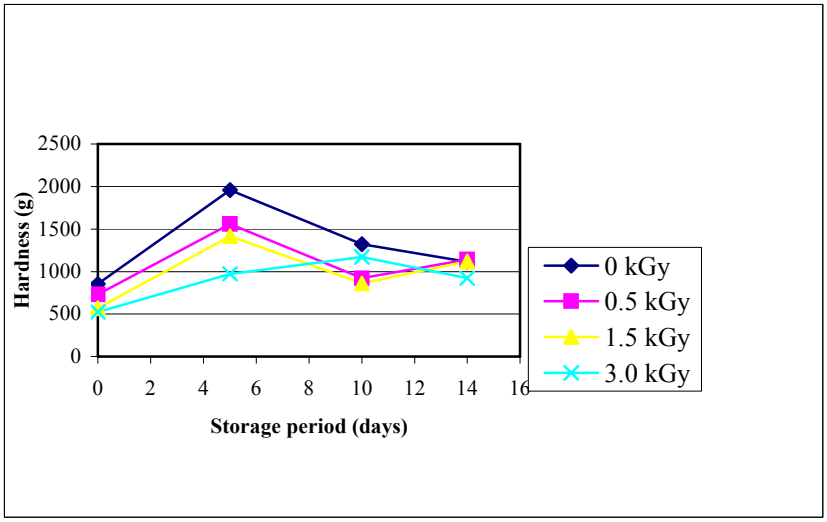

FIG .15. Texture (hardness) changes in irradiated onion stored at 5 ± ^{o}C.

Data indicate that hardness of cucumber was not affected by the irradiation in the range of 0.5 to 3.0 kGy (Fig 16). The results also indicate that storage of the cucumber for 15 days did not affect its hardness.

TABLE 7. COLOR INTENSITY IN CUCUMBER IRRADIATED WITH VARIOUS DOSES AND STORED AT 5 ±2^{O}C

Dose (kGy) / Storage period (days)		Color intensity			
		0 kGy	0.5 kGy	1.5 kGy	3.0 kGy
L*	0	58.96 $_{Aa}$	54.97 $_{Aa}$	51.26 $_{Aa}$	55.02 $_{Aa}$
	5	65.29 $_{Aa}$	59.74 $_{Aa}$	51.80 $_{Aa}$	55.36 $_{Aa}$
	10	52.98 $_{Aa}$	59.04 $_{Aa}$	55.34 $_{Aa}$	55.63 $_{Aa}$
	15	60.17 $_{Aa}$	51.87 $_{Aa}$	56.34 $_{Aa}$	56.83 $_{Aa}$
a* (negative value)					
	0	22.48 $_{Aa}$	23.19 $_{Aa}$	22.55 $_{Aa}$	23.34 $_{Aa}$
	5	19.87 $_{Aa}$	21.52 $_{Aa}$	21.54 $_{Aa}$	22.34 $_{Aa}$
	10	21.11 $_{Aa}$	21.89 $_{Aa}$	21.67 $_{Aa}$	20.60 $_{Aa}$
	15	21.77 $_{Aa}$	20.86 $_{Aa}$	20.00 $_{Aa}$	21.16 $_{Aa}$
b* (positive value)					
	0	34.24 $_{Aa}$	35.93 $_{Aa}$	32.90 $_{Aa}$	35.14 $_{Aa}$
	5	35.65 $_{Aa}$	35.97 $_{Aa}$	34.78 $_{Aa}$	37.53 $_{Aa}$
	10	33.44 $_{Aa}$	37.14 $_{Aa}$	35.97 $_{Aa}$	34.76 $_{Aa}$
	15	37.69 $_{Aa}$	34.89 $_{Aa}$	38.29 $_{Aa}$	40.99 $_{Aa}$

A, B, C: Means followed by a common letter within the same row are not significantly different ($p>0.05$) for the samples with different irradiation dose on the same day of storage

a: Means followed by a common letter within the same column are not significantly different ($p>0.05$) for the samples with same irradiation dose on different day of storage

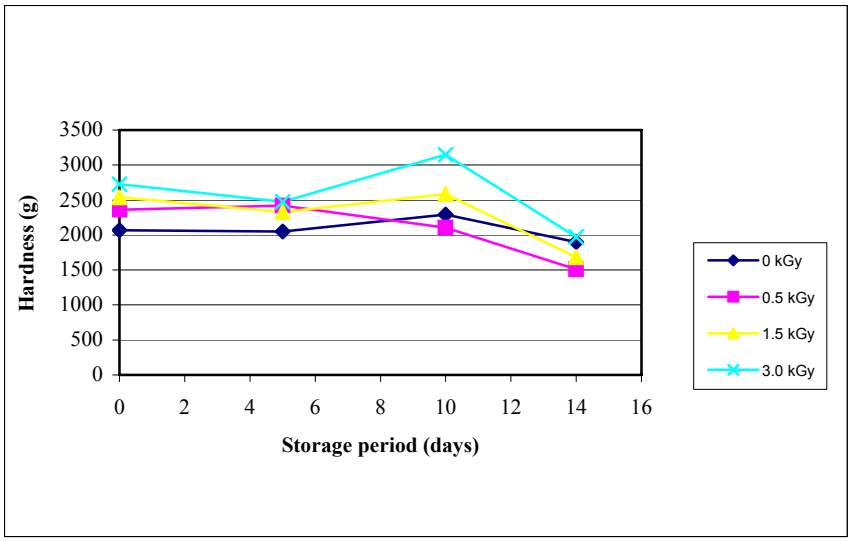

FIG. 16. Texture (hardness) changes in irradiated cucumber stored at 5 ± ^{o}C.

5. CONCLUSIONS

1. This study shows that irradiation treatment reduces the growth of APC in minimally processed pineapple, jackfruit, mixed fruits, onion and cucumber samples. The higher doses used resulted in a lower APC count.

2. Generally it can be concluded that a low dose of irradiation can eliminate or reduce the risk of food borne pathogens in the minimally processed fruits studied.

3. Irradiation did not affect the color (lightness, redness and greenness) of the minimally processed onion and cucumber.

ACKNOWLEDGEMENTS

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REFERENCES

[1] GOULARTE ,L., MARTIN ,C.G., MORALES-AIZPURUA, I.C., DESTRO,M.T., FRANCO B.D.G.M., VIZEU ,D.M., HUTZLER ,B.W. & LANDGRAF, M. (2004). Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L). Radiation Physics and Chemistry. **71**: 155-159.

[2] CARLIN, F., NGUYEN-THE, C., CUDENNEC, P., CHAMBROY, Y. (1990). Modified atmosphere packaging of fresh "ready to use" grated carrots in polymeric films. *J. Food Sci*. **55**: 1033-1038.

[3] MUHAMMAD, A. C., NIZAKAT, B., MISAl, K., MAAZULLAH, K., AMAL, B. & MUHAMMAD, J. Q. (2004). Irradiation treatment of minimally processed carrots for ensuring microbiological safety. *Radiation Physics and Chemistry*. **71**: 169–173.

[4] SALUNKHE, D.K., BOLIN, H.R. & REDDY, N.R. (1991). *Storage, processing and nutritional quality of fruits and vegetables volume 2*. Boca Raton : CRC Press.

[5] SHEWFELT (1990). Quality of fruit and vegetables. Food Technology. **44 (6)**: 99-106.

[6] RIVLIN, R.S. (2001). Historical perspective on the use of garlic. *Journal of Nutrition*. **131**: 951S-954S.

[7] BENKEBLIA, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic. *Lebensm-Wiss u- Technol*. **37**: 263-268.

[8] MONK, D. J; BEUCHAT, L.R; and DOYLE, M.P. (1995). Irradiation inactivation of food borne microorganisms. *J. Food Protect. 58: 197 – 207.*

[9] INGRAM, M. T; and J. FARKAS (1977). Microbiology of foods pasteurized by ionizing radiation. *Acta Aliment.6: 123 – 185*.

[10] Bacteriological Analytical Manual (BAM). U.S. Food and Drug Administration. Centre for Food Safety and Applied Nutrition. (2001).

RADIATION TREATMENT OF MINIMALLY PROCESSED FRUITS AND VEGETABLES FOR ENSURING HYGIENIC QUALITY

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Abstract

Fresh samples of cucumber (*Cucumis sativus*), tomato (*L. esculentum)*, carrots (*Daucus carota)*, cabbage (*Lactuca sativa var. capitata*), apples *(Pyrus malus)*, melon (*Cucumis melo)*, cauliflower (*Brassica oleracea botrytis)* and bitter gourd (*Momordica charantia)* were minimally processed, and packaged in polyethylene pouches followed by irradiation at different doses. The refrigerated samples were analysed weekly. The results revealed that a dose of 2 kGy for carrots, 1.5 kGy for cauliflower and 2.5 kGy for apples, cucumber and cabbage was sufficient to keep the minimally processed fruits/vegetables microbiologically and sensorially acceptable for two weeks at refrigerated temperature. Tomato, melon and bitter gourd due to the soft texture and microbial spoilage can not be stored up to two weeks, a dose of 2.5 kGy for tomato, 2 kGy for bitter gourd and 1 kGy for melon can be recommended for the refrigerated storage up to one week. According to the results, *Salmonella* and *E. coli* spiked on minimally processed cucumber, cabbage, cauliflower and bitter gourd showed D_{10}-values of 0.25 and 0.29, 0.24 and 0.28 kGy, respectively for *Salmonella* parathyphi. While the D_{10}-values for *E. coli* were 0.19, 0.17, 0.20 and 0.23 kGy in cucumber, cabbage, cauliflower and bitter gourd respectively.

1. INTRODUCTION

The increasing significance of human pathogens on fresh fruits, vegetables and juices has been recognized during the last years [1, 2 and 3]. Minimal processing may increase microbial spoilage of fruit through transfer of skin micro flora to fruit flesh where microorganisms can grow rapidly upon exposure to nutrient laden juices. Low temperature restricts the respiration and transpiration rate of plant tissues and microbes, thereby prolonging the shelf life of the material. Methods to control physiological and microbiological spoilage of whole fruit have been reported [4]. However, specific treatments to benefit from minimally processed fruits and vegetables with low dose radiation have been reported in very few cases so more research is needed.

Food irradiation is a viable technology for the reduction of post harvest losses, extension of shelf life of perishable commodities and improvement of hygienic and sensorial quality of foods [5, 6 and 7]. The present research was undertaken to study the radiation sensitivity and effect of different doses of radiation on the overall quality of minimally processed, (PE) packaged and irradiated fruits and vegetables stored at low temperature (5±1^{o}C).

2. MATERIALS AND METHODS

2. 1. Sample preparation

Fresh fruits/vegetables were procured from the local market. The samples were washed, and superficial moisture removed followed by cutting (using a vegetable cutter) and immediately packaged (100 to 150 g) in a polyethylene package (0.021mm thick).

2.2. Irradiation

The samples of fresh and minimally processed fruits and vegetables included in this study were irradiated at 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 kGy dose in a Co-60 gamma source. The irradiated samples were kept at refrigeration temperature (5±1^{O}C) for two weeks and analysed on the 1st, 7th and 14th day of storage.

2.3. Microbiological analysis

Total bacterial (TBC) and total fungi (TFC) counts were determined using the methods according to IAEA Technical Report Series [8] whereas total coliform were determined using BAM methods [9]. TBC and coliforms were determined by dilution plate method using nutrient agar media and Mac Conkey agar, respectively. A 10 g sample was taken in 90 ml sterilized peptone water and thoroughly mixed. This represented 1:10 dilution. Further dilutions were made in similar way. One ml of each sample (in triplicate) was poured in the sterilized Petri dishes using sterilized pipettes. The above mentioned sterilized media (15 - 20 ml) were added to plates followed by mixing through gentle motion of the dishes. The colonies were counted using a colony counter (Gallen Kamp, Model CNW-300-010M) after incubation at 30 ±1°C (TBC) and 36 ±1°C (coliform) for 24 - 48 hours.

For determination TFC, preparation of homogenates and dilution procedure was the same as described for assessment of total bacterial counts (TBC) except that potato dextrose agar was used as a growth media. Bacterial growth on the above media was retarded by the addition of 100 μg/ml of chlortetracycline. The developed colonies were counted with the help of a colony counter after 3-5 days of incubation at 28 ± 1^{o}C.

2.4. Sensory Evaluation

The sensory evaluation for appearance and taste was carried out using 10-point scale of Larmond [10]. Eight judges tested all the samples (coded) and mean of eight observations were taken. Each judge was free to taste any sample at any time. The maximum sensory score (10) indicated like extremely and minimum score (1) indicated dislike extremely.

2.5. Texture

The texture of the samples was determined using Universal Hardness Texture Meter having 1-5 kg-force scale. The plunger of the tester was forced against the samples to the full length and the readings were recorded in kg force. For hardness, mean of ten observations at different positions were taken.

2.6. D_{10}-value for *E. coli* and *Salmonella*

The D_{10}-values were calculated only in the vegetables selected.

The fresh vegetables (cauliflower, bitter gourd, cucumber and cabbage) were purchased from local market on the day of experiment, washed thoroughly with clean drinking water, peeled and cut into pieces and submerged in a solution of 300 ppm sodium hypochlorite for three minutes for surface sterilization [11]. Then the cut vegetables were rinsed thoroughly with sterile distilled water followed by removal of excess surface water.

2.6.1. Preparation of the inoculums

Pure cultures of *E. coli* (wild) and *Salmonella* Parathyphi A were obtained from Khyber Medical College, Peshawar, Pakistan. The cultures were grown in tryptic soy broth (TSB Difco) for 18 to 20 hours to a concentration of 10^9 TBC/ml. The cultures were diluted with 0.1% sterilized peptone water. The vegetables were packed in polyethylene bags (25 g) and then submerged into this diluted cultures of bacteria for five minutes to obtain 10^6–10^8 CFU/g. After removal of excess surface water, the bags were then sealed and kept at 5^{o}C before irradiation.

2.6.2. Irradiation doses

The cauliflower and bitter gourd samples inoculated with *E. coli* were irradiated at the doses of 0.0, 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 kGy while the samples contaminated with *Salmonella* Parathyphi A were exposed to irradiation doses of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 kGy.

2.6.3. Enumeration of Salmonella and E. coli

After irradiation, 25 g of each sample was homogenized with 225 ml of 0.1% peptone water in blender for 2-3 minutes and required serial dilutions were made in the same way as described for total bacterial counts. 1 ml of each dilution was pour plated using tryptic soy agar in duplicate. Plates were incubated at 35±1^{o}C for 24 - 48 hours and the colonies were counted. D_{10}-value was determined by taking the negative reciprocal of the survivor curve slope. On the basis of five D_{10}-values, the doses selected for the storage stability studies for two weeks at refrigerated conditions were 0.0, 0.5, 1.0, 1.5 and 2.0 kGy for both vegetables (cauliflower and bitter gourd).

2.7. Statistical Analysis

All data were analysed using analysis of variance (ANOVA) and the means were separated by Duncan Multiple Range (DMR) test [12].

3. RESULTS AND DISCUSSION

Table 1 shows that cucumber samples treated with 3.0 kGy had the minimum TBC after 7th (3.1 x 10^{1} CFU/g) as well as the 14th day (7.2 x 10^{2} CFU/g) day of storage. The fungal count also had similar trend. The control samples stored for 14 days showed visible fungal growth while the 3.0 kGy treated samples had 3.5 x 10^{2} CFU/g after 14 days of storage. The control cucumber samples had coliform count of 8.0 x 10^{3} (TCC/g) after 14 days of storage while the 2.0 kGy treated samples had no coliform after the same storage period.

The first and foremost parameter is appearance by which a product is purchased by the consumer. All the samples (irradiated and control) were subjected to sensory evaluation for appearance and flavor scores.

The overall appearance score of cucumber decreased with storage period while un-irradiated samples had the highest score (6.89) which decreased to 6.00 for 2.5 kGy treated samples. The appearance score on the 0, 7th and 14th day decreased with doses from 0 to 2.5 kGy followed by an increase in appearance score for samples treated with 3.0 kGy.

The decrease in firmness with increasing radiation dose was gradual; however, a sharp decrease occurred from 1.0 kGy (3.30 kg-force) to 2.0 kGy (2.58 kg-force). The mean firmness of control cucumber samples at the beginning of the storage was 2.86 kg-force, and after 14 days of storage was 2.83 kg-force, showing almost no significant change during the storage.

TABLE 1. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED CUCUMBERS DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (log CFU/g)			TFC (log CFU/g)			TCC (log CFU/g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	3.00	6.42	discarded	2.70	7.42	vg	1.00	1.88	3.90
0.5 kGy	2.40	6.54	discarded	2.6	7.51	vg	nd	1.40	2.96
1.0 kGy	1.50	3.34	6.80	1.18	5.80	vg	nd	nd	< 1.00
2.0 kGy	fc	3.11	4.18	fc	3.40	4.56	nd	nd	nd
2.5 kGy	fc	2.93	3.53	fc	2.53	4.72	nd	nd	nd
3.0 kGy	fc	1.49	2.86	fc	2.32	2.54	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	6.89	6.24	6.04	6.39^{B}	6.42	6.25	5.00	5.89^{F}
0.5 kGy	6.57	6.01	6.12	6.23^{D}	6.57	6.01	5.50	5.30^{E}
1.0 kGy	6.60	6.00	6.20	6.27^{C}	6.33	6.01	5.80	6.05^{D}
2.0 kGy	6.25	6.10	6.01	6.12^{E}	6.08	6.17	6.20	6.15^{A}
2.5 kGy	6.00	6.20	6.12	6.11^{E}	6.01	6.23	6.15	6.13^{B}
3.0 kGy	6.43	6.50	6.44	6.46^{A}	6.13	6.18	6.01	6.11^{C}
Mean	6.46^{A}	6.18^{B}	6.15^{B}		6.26^{A}	6.14^{B}	5.78^{C}	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	3.20	3.26	3.22	3.23B
0.5 kGy	3.24	3.14	3.28	3.22B
1.0 kGy	3.26	3.30	3.33	3.30A
2.0 kGy	2.60	3.10	2.05	2.58D
2.5 kGy	2.86	2.70	2.90	2.82C
3.0 kGy	2.01	2.00	2.22	2.08E
Mean	2.86ab	2.92a	2.83b	

TBC = Total bacterial counts, TFC = Total fungal counts, TCC = Total coliform counts, nd= Not detected in 10: 1dilution, vg= Visible growth, fc = Few colonies (less than 10) in 10: 1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

TABLE 2. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED TOMATOES DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC(CFU/g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	$6.0x10^3$	$8.5x10^4$	$2.5x10^6$	$4.3x10^2$	$2.3x10^4$	$2.5x10^7$	12	35	26
0.5 kGy	$1.2x10^2$	$2.6x10^3$	$3.5x10^5$	$6.5x10^1$	$4.3x10^3$	$2.4x10^6$	fc	13	37
1.0 kGy	$1.3x10^2$	$4.3x10^2$	$7.7x10^4$	$2.6x10^1$	$6.2x10^2$	$2.5x10^5$	nd	nd	nd
2.0 kGy	fc	12	$3.5x10^2$	fc	$1.4x10^2$	$7.3x10^3$	nd	nd	nd
2.5 kGy	fc	fc	$4.2x10^2$	nd	fc	$4.3x10^2$	nd	nd	nd
3.0 kGy	fc	fc	$1.0x10^2$	nd	nd	$2.4x10^2$	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Means	0	7	14	Mean
Control	7.56	6.02	4.00	6.19^F	7.12	5.98	5.48	6.20^D
0.5 kGy	7.26	6.13	5.49	6.29^E	6.84	6.00	5.13	6.16^E
1.0 kGy	7.43	6.43	6.01	6.26^D	6.70	6.43	6.00	6.38^C
2.0 kGy	7.14	6.87	6.22	6.74^C	6.84	6.33	6.64	6.61^B
2.5 kGy	7.29	6.88	6.63	6.93^B	6.7	6.25	6.82	6.59^B
3.0 kGy	7.27	7.01	6.87	7.05^A	6.72	6.28	6.93	6.66^A
Means	7.33^A	6.56^B	6.03^C		6.82^A	6.21^C	6.26^B	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	2.47	2.40	2.45	2.44^A
0.5 kGy	2.41	2.49	2.36	2.42^A
1.0 kGy	2.43	2.31	2.25	2.33^B
2.0 kGy	2.37	2.37	2.32	2.35^B
2.5 kGy	2.09	2.11	2.15	2.12^C
3.0 kGy	2.06	2.00	2.01	2.02^D
Mean	2.31^A	2.28^{AB}	2.26^B	

TBC = Total bacterial counts, TFC = Total fungal counts, TCC = Total coliform counts, nd= Not detected in 10: 1dilution, fc = Few colonies (less than 10) in 10: 1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

The data of Table 2 shows that a dose of 2.0 kGy in tomato samples was effective in lowering the TBC and fungal colony to safe limits even after 14 days of storage. Coliform were eliminated by the irradiation treatment with 1 kGy and above while few colonies were found in 0.5 kGy treated samples.

The appearance and flavor scores for tomatoes showed similar trends as that for cucumbers. The appearance score decreased from 7.33 for fresh samples to 6.03 after 14 days storage. The appearance enhanced from 6.19 for un-irradiated samples to 7.05 for 3.0 kGy treated samples. Flavor of tomatoes enhanced with irradiation. The un-irradiated samples had a mean score of 6.20 which increased to 6.66 for 3.0 kGy treated samples. The flavor although deteriorated during storage, the data did not indicate a clear trend. The trend of changes in firmness of tomatoes was also similar to that of cucumbers. Firmness of 0.5 kGy treated samples (2.42 kg-force) was similar to that of control samples (2.44 kg-force). The 3.0 kGy irradiated samples had the minimum firmness (2.02 kg-force). Firmness of fresh tomatoes was 2.31 kg-force and that of samples stored for 14 days at 5^{o}C was 2.25 kg-force. Although these two values are significantly different, the difference is very small as compared to the effect of irradiation.

TABLE 3. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED CARROTS DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)		
	Storage period-days					
	0	7	14	0	7	14
Control	$6.3x10^{2}$	$3.7x10^{4}$	$6.5x10^{5}$	$2.7x10^{1}$	$3.5x10^{2}$	1.2×10^{4}
0.5 kGy	$2.3x10^{1}$	nd	$3.0x10^{2}$	nd	$2.2x10^{2}$	$9.6x10^{2}$
1.0 kGy	12.0	nd	fc	nd	nd	fc
2.0 kGy	nd	nd	nd	nd	nd	nd
2.5 kGy	nd	nd	nd	nd	nd	nd
3.0 kGy	nd	nd	nd	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	7.5	7.4	8.0	7.6^{A}	7.8	7.8	7.0	7.53^{A}
0.5 kGy	7.4	7.1	7.3	7.3^{AB}	7.4	7.5	6.5	7.13^{AB}
1.0 kGy	6.4	7.1	6.7	6.7^{BC}	6.4	7.3	6.5	6.73^{ABC}
2.0 kGy	6.7	6.6	7.4	6.9^{BC}	6.8	6.6	6.5	6.63^{BC}
2.5 kGy	7.0	6.2	7.4	6.8^{BC}	6.3	6.1	6.5	6.30^{BC}
3.0 kGy	6.2	5.8	7.3	6.33^{C}	6.1	5.8	6.0	5.97^{C}
Mean	6.8^{B}	6.7^{B}	7.4^{A}		6.8^{A}	6.85^{A}	6.5^{A}	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	4.38	4.40	4.48	4.42^{A}
0.5 kGy	4.22	4.40	4.38	4.33^{AB}
1.0 kGy	4.26	4.26	4.40	4.31^{B}
2.0 kGy	4.20	4.46	4.36	4.34^{AB}
2.5 kGy	4.30	4.40	4.24	4.31^{B}
3.0 kGy	4.32	4.28	4.48	4.36^{AB}
Mean	4.28^{B}	4.37^{A}	4.39^{A}	

TBC = Total bacterial counts, TFC = Total fungal counts, TCC = Total coliform counts, nd= Not detected in 10 : 1dilution, fc = Few colonies (less than 10) in 10 : 1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

The initial bacterial load (Table 3) in control carrot samples was 6.3 x 10^2 CFU/g in control samples and reached 6.5 x 10^5 CFU/g after 14 days of storage. A dose of 1 kGy reduced the bioload to 12.0 CFU/g, which were few colonies after 14 days storage. The samples receiving 2 kGy or higher doses were completely free of bacteria during 14 days refrigerated storage. The control samples had 2.7 x 10^1 CFU/g fungal counts initially and increased to 1.2 x 10^4 CFU/g after 14 days of storage and the samples irradiated at a dose higher than 1 kGy were also completely free of any fungi during the two weeks storage at 5^0C.

The appearance scores of minimally processed carrots were affected by radiation doses ($p< 0.05$) and the mean scores decreased from 7.6 (control) to 6.33 (3.0 kGy treated). This suggested that minimally processed carrots should not be irradiated at more than 2.0 kGy dose for storage at low temperature. Contrary to this, storage period had no adverse effect on sensory scores, rather there was an improvement in the appearance scores 6.8 (0-day) to 7.4 (14 days) while the mean flavor scores significantly decreased from 7.53 to 5.97 with increasing dose of gamma radiation.

The changes in firmness of minimally processed carrots exposed to different doses of gamma radiation were not significant ($p< 0.05$) from each other. But the changes in control and all irradiated samples were significant.

TABLE 4. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED CABBAGE DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC (CFU/g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	$1.0x10^3$	$2.0x10^5$	$1.1x10^5$	$2.5x10^3$	$3.4x10^5$	$5.6x10^5$	15	360	$5.9x10^3$
0.5 kGy	$6.3x10^3$	$4.2x10^4$	$4.9x10^5$	$7.0x10^2$	$2.6x10^4$	$1.2x10^4$	30	93	$4.0x10^2$
1.0 kGy	$3.7x10^2$	$2.4x10^4$	$3.7x10^4$	$6.0x10^1$	$4.1x10^2$	$3.2x10^3$	nd	nd	fc
2.0 kGy	$6.0x10^1$	$8.1x10^2$	$2.6x10^3$	nd	nd	fc	nd	nd	nd
2.5 kGy	$7.0x10^1$	$1.5x10^2$	$8.5x10^2$	nd	nd	nd	nd	nd	nd
3.0 kGy	fc	$1.0x10^2$	$4.0x10^2$	nd	nd	nd	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	7.2	7.15	6.16	7.12^A	7.13	7.00	6.85	6.99^A
0.5 kGy	7.62	6.76	6.63	7.00^A	7.13	6.88	6.75	6.91^A
1.0 kGy	7.83	6.85	6.62	7.12^A	7.50	6.75	6.65	6.96^A
2.0 kGy	7.50	6.87	6.50	7.08^A	7.50	6.75	6.50	6.91^A
2.5 kGy	7.38	7.12	6.50	7.00^A	7.25	6.88	6.62	6.91^A
3.0 kGy	7.63	6.87	6.45	6.98^A	7.38	6.63	6.40	6.80^B
Mean	7.00^A	6.94^A	6.70^B		7.31^A	6.81^B	6.62^C	-

TBC = Total bacterial counts, TFC = Total fungal counts, TCC = Total coliform counts, nd= Not detected in 10:1dilution, fc = Few colonies (less than 10) in 10:1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

The initial TBC in cabbage control samples increased from $1.0x10^3$ to $1.0x10^5$ CFU/g after 14 days of storage (Table 4). In case of irradiated samples the counts ranged $7.1x10^1$ to $6.3x10^3$ CFU/g at 0-day and increased to $4.2x10^4$, $2.4x10^4$, $8.1x10^2$, $1.5x10^2$ CFU/g for 0.5, 1.0, 2.0, 2.5 and 3.0 kGy irradiated samples respectively, after 7 days of storage. The bacterial counts of 2 kGy irradiated samples were within the permissible limits. It was also noted that samples irradiated with a dose of more than 1 kGy were completely free of coliforms. Few colonies were detected after 14 days storage of 1 kGy treated samples. In case of fungal counts, a dose of 2.5 kGy completely left the samples free of viable fungal colonies up to 14 days of refrigerated storage.

The mean appearance scores for all treatments were almost the same, showing non-significant effect of radiation on appearance of minimally processed cabbage. However, the mean scores decreased significantly from 7.0 to 6.7 after 14 days refrigerated storage. It is clear from this data that the appearance of irradiated minimally processed cabbage was not affected significantly with the applied gamma radiation doses up to 3.0 kGy. The flavor scores of control and 0.5 kGy irradiated samples decreased from 7.1 to 6.8 and 7.1 to 7.5 after 14 days of storage. The scores of other irradiated samples decreased with increasing the radiation doses but remained within the acceptable limit.

Maximum decrease was noted in 3 kGy irradiated samples and the values decreased from 7.3 to 6.6 after 14 days of storage in a refrigerator. The overall treatment means ranged from 6.8 to 7.0, showing minimum effect of treatments on the flavor of cabbage.

Data on texture are not included in the table. For determining the texture of the samples, the plunger of the tester was forced against the samples to the full length and the readings were recorded in kg force. It was not possible to determine texture in case of cabbage only due to naturally soft texture of cabbage and because of very thin pieces (used commonly as salad) of prepared cabbage samples.

TABLE 5. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED CAULIFLOWER DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC(CFU /g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	2.0×10^{4}	3.0×10^{5}	6.0×10^{6}	3.5×103	4.5×10^{4}	6.5×10^{5}	4000	8600	6.5×10^{4}
0.5 kGy	4.8×10^{3}	3.5×10^{4}	2.3×10^{5}	8.5×102	1.6×103	4.2×10^{3}	25	350	8.5×10^{2}
1.0 kGy	5.5×10^{2}	7.4×10^{2}	1.5×10^{3}	2.6×102	8.4×102	3.8×10^{1}	nd	nd	fc
1.5 kGy	1.3×10^{2}	9.6×10^{2}	3.5×10^{2}	nd	nd	fc	nd	nd	nd
2.0 kGy	1.5×10^{1}	3.8×10^{1}	6.4×10^{1}	nd	nd	nd	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	7.8	7.8	7.3	7.63^{B}	7.6	7.4	6.5	7.17^{B}
0.5 kGy	8.0	8.0	7.8.	7.93^{A}	7.8	7.7	7.1	7.53^{A}
1.0 kGy	8.0	8.0	7.8	7.93^{A}	7.7	7.7	7.3	7.57^{A}
1.5 kGy	8.0	8.0	7.0	7.67^{B}	7.9	7.5	6.8	7.40^{AB}
2.0 kGy	8.0	7.8	7.0	7.6^{B}	7.9	7.4	6.8	7.37^{AB}
Mean	7.96^{A}	7.92^{A}	7.38^{B}		7.78^{A}	7.54^{B}	6.9^{C}	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	3.1	3.2	2.6	2.96^{B}
0.5 kGy	3.0	3.0	2.9	2.97^{A}
1.0 kGy	3.2	2.9	2.6	2.90^{C}
1.5 kGy	3.0	2.8	2.6	2.80^{D}
2.0 kGy	2.8	2.7	2.5	2.67^{E}
Mean	3.04^{A}	2.92^{B}	2.64^{C}	-

TBC = Total Bacterial counts, TFC = Total fungal counts, TCC = Total Coliform counts, nd= Not detected in 10: 1dilution, fc = Few colonies (less than 10) in 10:1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p\leq0.05$)

In the case of irradiated cauliflower samples (Table 5), a dose of 2.0 kGy completely left free of fungi and coliform bacteria up to 14 days of refrigerated storage. It was also found that samples irradiated

with more than 1 kGy radiation dose were completely free of coliform; only few colonies were detected after 14 days storage of 1 kGy treated samples. These results suggested that the cauliflower samples should be treated with a dose of 1.5 kGy to keep them microbiologically acceptable.

The data on sensory evaluation of cauliflower revealed that mean scores for appearance showed an increase from 7.63 (control) to 7.93 (0.5 and 1.0 kGy) for treated samples while the same score decreased during storage with mean values of 7.96 (0 day), 7.92 (7 days) and 7.38 (14 days). The flavor scores decreased for all the samples after 7 and 14 days storage but are within acceptable limits. The highest mean flavor scores were received by 1.0 kGy treated samples (7.57) followed by 0.5 kGy treated samples (7.53) and minimum by control samples (7.17).

The firmness decreased with increasing dose levels from 3.1 (control) to 2.8 (2.0 kGy) as well as during storage with mean values of 3.04, 2.92 and 2.64 for 0, 7^{th} and 14^{th} day of storage at refrigerated temperature ($5\pm1^{o}C$). The mean values of firmness showed minimum changes in the texture of fresh (2.96) and 0.5 kGy irradiated samples 2.97 and maximum changes in 2.0 kGy irradiated samples (2.67).

TABLE 6. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED BITTER GOURD DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC (CFU/g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	2.2×10^{5}	5.2×10^{7}		9.0×102	3.5×10^{4}		3.5×10^{2}	6.5×10^{4}	
0.5 kGy	1.3×10^{4}	1.7×10^{5}		1.8×10^{2}	2.9×10^{3}		1.0×10^{2}	3.5×10^{3}	
1.0 kGy	2.0×10^{3}	4.3×10^{4}	d	8.0×10^{1}	8.5×10^{2}	d	4.5×10^{1}	7.1×10^{2}	d
1.5 kGy	8.2×10^{2}	9.5×10^{3}		1.2×10^{1}	2.1×10^{2}		nd	fc	
2.0 kGy	1.5×10^{2}	1.1×10^{3}		fc	1.5×10^{1}		nd	fc	

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	6.5	5.7	5.5	5.90D	6.5	5.7	4.0	5.4^{D}
0.5 kGy	6.6	6.3	6.2	6.37^{C}	6.8	6.5	5.1	6.13^{C}
1.0 kGy	7.0	6.8	6.6	6.80B	7.0	6.9	6.9	6.93^{B}
1.5 kGy	7.1	7.0	7.0	7.03^{B}	7.6	7.4	7.3	7.43^{A}
2.0 kGy	7.8	7.5	7.4	7.57^{A}	7.9	7.6	7.4	7.63^{A}
Mean	7.0^{A}	6.66^{B}	6.54^{B}		7.16^{A}	6.82^{A}	6.14^{B}	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	3.15	3.08	2.00	2.74^{A}
0.5 kGy	3.13	2.90	1.80	2.61^{B}
1.0 kGy	2.95	2.78	1.55	2.43C
1.5 kGy	2.70	2.60	1.48	2.26D
2.0 kGy	2.70	2.50	1.43	2.21^{D}
Mean	2.92^{A}	2.77^{B}	1.65^{C}	-

TBC = Total Bacterial counts, TFC = Total fungal counts, TCC = Total Coliform counts, d= Discarded, nd= Not detected in 10: 1dilution, fc = Few colonies (less than 10) in 10 : 1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p\leq0.05$)

Results for bitter gourd summarized in Table 6 show that bitter gourd should be treated with a dose of 1.5-2.0 kGy to keep them microbiologically acceptable for seven days. After two weeks all the samples, irrespective of treatments were found almost spoiled and discarded.

The data on sensory evaluation showed that highest appearance scores of irradiation samples were obtained for samples treated with 2.0 kGy while control samples got the minimum score. Due to high microbial load, the samples were not tasted at the 14^{th} day of storage and were just evaluated from its external appearance and smell. The mean values showed that all the radiation doses can maintain the quality of minimally processed bitter gourd.

The data on firmness of bitter gourd showed that although the mean values of firmness showed continuous and significant decrease with increase in radiation dose but the changes remained within acceptable limits. Similarly the mean firmness of radiated minimally processed bitter gourd showed significant decrease during two weeks storage from 2.92 (0 day) to 2.77 (1^{st} week) and 1.65 (2^{nd} week).

TABLE 7. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED MELON DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC (CFU/g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	$4.8x\ 10^5$	$6.7x10^7$		$1.4x10^2$	$3.7x10^3$		$9.1x10^1$	$1.3x10^3$	
0.5 kGy	$8.8x\ 10^4$	$1.0x10^7$	And	fc	$2.5x10^2$	And	$2.0x10^1$	$7.5x10^1$	
1.0 kGy	$1.9x\ 10^4$	$3.8x10^5$		fc	fc		fc	fc	
2.0 kGy	$6.1x\ 10^2$	$5.3x10^4$		nd	fc		nd	fc	
2.5 kGy	80	920		nd	nd		nd	nd	
3.0 kGy	fc	390		nd	nd		nd	nd	

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	6.3	5.1	3.0	4.8^C	7.0	5.1	0.4*	4.2^C
0.5 kGy	6.5	5.5	3.1	5.0^C	6.8	5.0	0.9*	4.2^C
1.0 kGy	6.6	6.0	3.1	5.1^{BC}	6.3	5.1	1.0*	4.1^C
2.0 kGy	6.6	7.3	3.3	5.3^B	6.9	6.0	1.3*	4.7^B
2.5 kGy	6.8	6.9	4.5	6.2^A	6.6	6.5	3.8*	5.6^B
3.0 kGy	7	6.0	4.9	6.3^A	6.6	6.4	3.6*	5.5^a
Mean	6.6^A	6.0^C	3.6^C	-	6.7^A	5.7^B	1.8^C	-

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	1.6	1.0	0.5	1.0^b
0.5 kGy	1.5	0.9	0.5	1.0^c
1.0 kGy	1.3	0.9	0.6	0.9^c
2.0 kGy	1.3	1.2	0.6	1.0^b
2.5 kGy	1.3	1.3	0.8	1.1^a
3.0 kGy	0.8	1.2	0.7	0.9^d
Mean	1.3^A	1.1^B	0.6^C	-

TBC = Total Bacterial counts, TFC = Total fungal counts, TCC = Total Coliform counts, nd= Not detected in 10: 1dilution, fc = Few colonies (less than 10) in 10: 1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

Due to high bioload, samples were not tasted and marked visually.

The TBC (Table 7) for control samples of musk melons increased from 4.8 x 10^5 to 6.7 x 10^7 CFU/g after seven days storage at refrigeration temperature (5^{o}C). Very few colonies were recorded in the samples irradiated at the dose of 3.0 kGy, which increased to 3.9x10^2 CFU/g after seven days storage. A radiation dose of 1.0 kGy completely controlled fungal growth and coliform bacteria during seven days storage. After one week, all the samples, irrespective of treatment were spoiled and discarded. The data on sensory evaluation of musk melons revealed that a dose of 2.5 kGy and above can maintain the sensory qualities within acceptable limits during the seven days of storage. The firmness of irradiated sample for 2.5 and 3.0 kGy were 0.8 and 0.7 respectively at 14th day of storage and thus a dose of up to 2.5 kGy could maintain the texture to some extent.

Musk melons after radiation treatment (0-3.0 kGy) could not maintain its appearance and sensorial quality more than seven days at refrigerated temperature and were discarded due to soft texture and high microbial load.

TABLE 8. MICROBIOLOGICAL, SENSORY AND TEXTURAL CHANGES OF IRRADIATED APPLES DURING STORAGE AT $5^{O}C$

Microbiological changes

Treatments	TBC (CFU/g)			TFC (CFU/g)			TCC (CFU /g)		
	Storage period-days								
	0	7	14	0	7	14	0	7	14
Control	$9.0x10^3$	$2.5x10^5$	$5.5x10^5$	$3.2x10^2$	$1.0x10^3$	$8.0x10^3$	150	nd	440
0.5 kGy	$6.7x10^3$	$9.2x10^3$	$6.2x10^4$	$1.5x10^2$	$4.1x10^2$	$5.2x10^2$	20	nd	110
1.0 kGy	$4.5x10^2$	$1.6x10^3$	$1.5x10^4$	$2.0x10^1$	$1.2x10^2$	$7.4x10^2$	nd	nd	fc
2.0 kGy	$1.2x10^2$	$9.8x10^2$	$6.3x10^3$	fc	nd	fc	nd	nd	nd
2.5 kGy	$1.0x10^2$	$3.0x10^2$	$5.2x10^2$	nd	nd	nd	nd	nd	nd
3.0 kGy	$4.0x10^1$	$8.0x10^1$	$2.1x10^2$	nd	nd	nd	nd	nd	nd

Sensory changes

Treatments	Appearance				Flavor			
	Storage period-days							
	0	7	14	Mean	0	7	14	Mean
Control	7.57	7.0	6.57	7.04^{B}	7.29	6.57	6.57	6.81^{B}
0.5 kGy	7.14	6.42	6.28	6.62^{AB}	7.29	6.86	6.57	6.91^{B}
1.0 kGy	7.14	6.28	6.00	6.47^{A}	7.14	6.72	6.57	6.81^{B}
2.0 kGy	6.71	6.28	6.28	6.43^{AB}-	7.58	6.72	6.29	6.86^{B}
2.5 kGy	6.42	6.28	6.00	6.24^{AB}	7.14	6.57	6.14	6.61^{A}
3.0 kGy	6.28	6.14	6.0	6.14^{A}	7.0	6.57	6.14	6.57^{A}
Mean	6.88^{C}	6.40^{B}	6.19^{A}		7.24^{C}	6.67^{B}	6.38^{A}	

Textural changes (kg-force)

Treatments	Storage period-days			
	0	7	14	Mean
Control	3.68	3.50	3.32	3.50^{A}
0.5 kGy	3.36	3.30	3.12	3.26^{BC}
1.0 kGy	3.32	3.34	3.14	3.27^{C}
2.0 kGy	3.38	3.32	3.20	3.30^{B}
2.5 kGy	3.20	3.30	3.20	3.23^{C}
3.0 kGy	3.44	2.96	2.94	3.11^{D}
Mean	3.40^{A}	3.29^{B}	3.15^{C}	

TBC = Total bacterial counts, TFC = Total fungal counts, TCC = Total coliform counts, nd= Not detected in 10:1dilution, fc = Few colonies (less than 10) in 10:1dilution

ABCDEF of column and abc of row means, the values sharing common letters are not significantly different ($p \leq 0.05$)

Irradiation treatments of minimally processed apple samples lowered the bacterial load (Table 8) initially and at the end of the experiment and at the end of the 14 days storage, the TBC values increased to $3.7x10^4$, $2.6x10^3$, $8.5x10^2$ and $4.0x10^2$ for 1.0, 2.0, 2.5 and 3.0 kGy treated samples, respectively. Minimum TBC were recorded in 2.5 and 3.0 kGy treated minimally processed apples. In case of coliform and fungal load samples treated with 2.0 kGy or more remained completely free of coliform. These results suggested that the apples should be treated with a dose of 2.5 kGy to keep the minimally processed apples microbiologically acceptable.

Sensory evaluation of apples revealed that that fresh un-irradiated samples had the highest score (7.57) while samples irradiated at 3.0 kGy and stored for 14 days got the lowest scores (6.19).

The differences in mean flavor scores of irradiated samples (0.5-3.0 kGy) were non-significant and those for 2.5-3.0 kGy were significantly different from control and other irradiated samples. However, during storage the mean flavor scores decreased from 7.24 to 6.38 after the same period of storage. Hardness (kg-Force) of apples decreased with increasing dose levels as well as during storage. Hardness of fresh un-irradiated samples was 3.68 and decreased to 3.44 at 3.0 kGy irradiation. Hardness of fresh un-irradiated samples decreased to 3.32 at 14^{th} day of storage while that of 3.0 kGy irradiated samples decreased to 2.94 during the same storage period.

The D_{10} values for each of the tested pathogens (*E. coli* and *Salmonella* Parathyphi A*)* were determined from its dose-response curve, which was constructed by plotting survival counts against irradiation doses used (see Figs. 1-4)

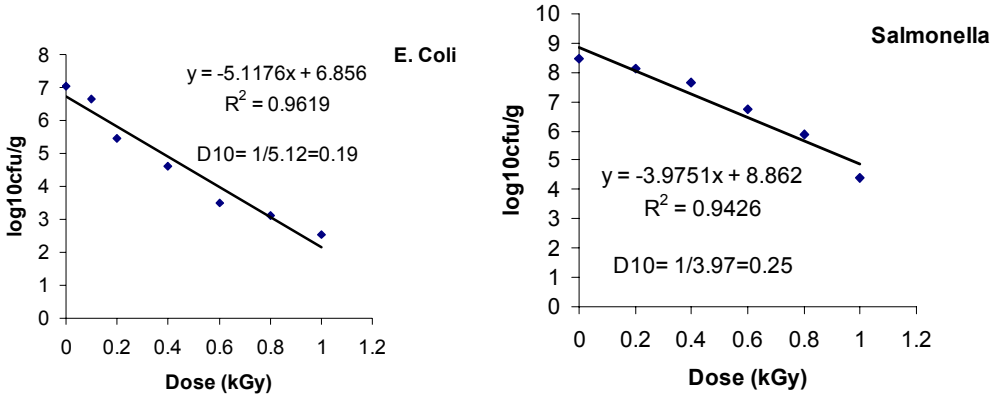

FIG. 1. D_{10} -values for E. coli and Salmonella Parathyphi A *for minimally processed cucumber.*

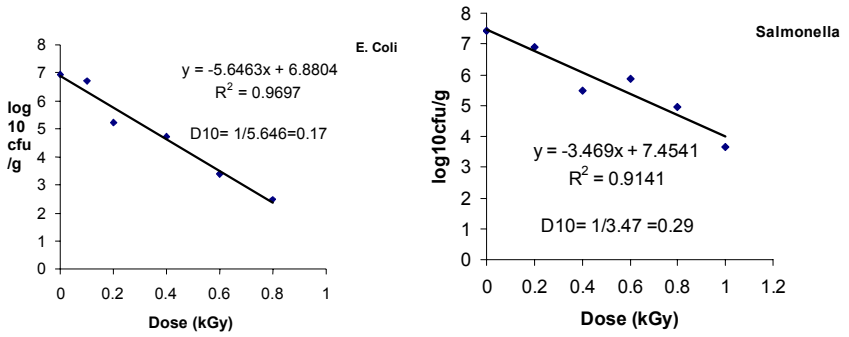

FIG. 2. D_{10} -values for E. coli and Salmonella Parathyphi A *spiked on minimally processed cabbage.*

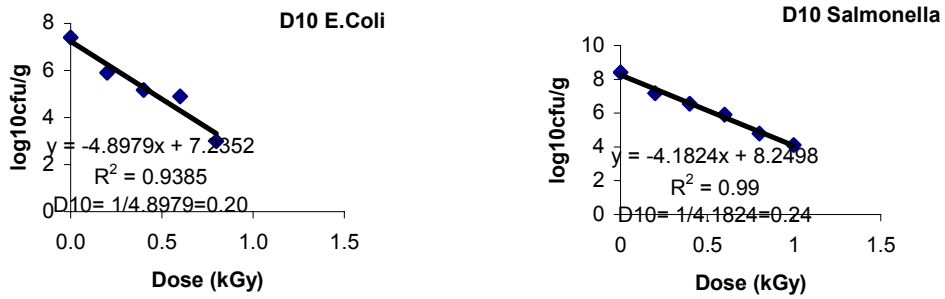

FIG. 3. D_{10} -values for E. coli and Salmonella Parathyphi A *spiked on minimally processed cauliflower.*

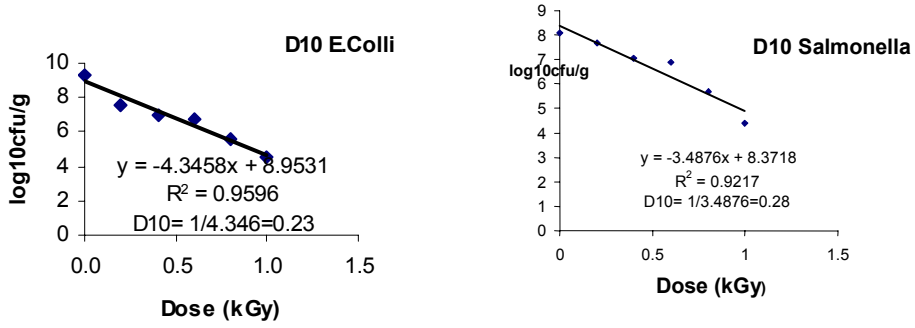

FIG. 4. D_{10} -values for E. coli and Salmonella Parathyphi A spiked on minimally processed bitter gourd.

4. CONCLUSIONS

The recommended dose to ensure microbial safety and retain sensory qualities for minimally processed cucumber, tomato, cabbage and apples (two weeks) was 2.5 kGy, carrots (two weeks) 2.00 kGy, melon (one week) 2.0 kGy, cauliflower (two weeks) 1.5 kGy and bitter gourd (one week) 2.0 kGy.

The D_{10} values for *E. coli* and *Salmonella* Parathyphi A spiked on minimally processed vegetables were 0.19 and 0.25 kGy (cucumber), 0.17 and 0.29 kGy (cabbage), 0.20 and 0.24 kGy (cauliflower) and 0.23 and 0.28 kGy (bitter gourd).

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REFERENCES

[1] BEUCHAT, L.R., Pathogenic micro-organisms associated with fresh produce. J Food Prot. **59** (1996) 204-216.

[2] TAUXER, R., KRUSE, H., MEDBERG, C., POTTER, M., MADDEN, J and WACHSMUTH, K., Microbial hazards and emerging issues associated with produce: a preliminary report to the national advisory committee on microbiological criteria for foods. J. Food Prot **60** (1997) 1400-1408.

[3] THAYER, D.W. and RAJKOWSKI, K.T. Developments in irradiation of fresh fruits and vegetables. Food Technol. **53** (1999) 62-65.

[4] SHEWFELT, R. L., Post-harvest treatment for extending the shelf life of fruits and vegetables. Food Technol. **40** (1986) 70-72, 76-78, 80, 89.

[5] FAN, X., NIEMIRA, B. A., SOKORAI, K. J. B., Sensorial, nutritional and microbiological quality of fresh cilantro leaves as influenced by ionizing radiation and storage. J. Food Res. Intern. **36** (2003) 713-719.

[6] KADER, A.A.A., summary of CA requirements of recommendations for fruits other than some fruits: Proceedings of the Fourth National Controlled Atmosphere Research Conference, 1985. Raleigh, NC, 445-470, Deptt. Horti.Sci, North Carolina State University USA (1985).

[7] LABUZA, T.P. and BREENE, W. M., Application of active packaging for improvement of shelf life and nutritional quality of fresh and extended shelf life foods. J. Food Process Preserv. **13** (1989) 1-69.

[8] ANON. Microbiological Specification and Testing Methods for Irradiated Foods. IAEA Technical Report Series Vienna, Austria No.104 (1970) 28-30.

[9] Bacteriological Analytical Manual (BAM) (on line) Food and Drug Administration. Chapter III. Center for Food Safety and Applied Nutrition, FDA, USA. (2001).

[10] LARMOND, E., Method for sensory evaluation of food. Canada Dept. of Agriculture, Public.No1286 (1977) 36-37.

[11] NEIMIRA B. A., Fan, X. and Cristophere, H. S., Irradiation temperature influences product quality factors of frozen vegetables and radiation sensitivity of inoculated Listeria monocytogenes. J. Food Prot. **65** (2002) 1406-1410.

[12] STEEL, R. G. D. and TORRIE, J.H. Principles and Procedures of Statistics. McGraw Hill Publ. Co. Inc. New York. (1984).

IMPROVING QUALITY AND SAFETY OF MINIMALLY PROCESSED FRUITS AND VEGETABLES BY GAMMA IRRADIATION

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Abstract

Fresh fruit and vegetables are an essential part of the diet of people all around the world. Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields, or during harvesting, processing and distribution. Raw materials used in the preparation of minimally processed food can be contaminated with pathogens like *Escherichia coli* 0157:H7 and *Listeria monocytogenes*. Ionising radiation as a non-thermal technology is a highly promising technology. The main objective of this study was to analyse the effect of gamma radiation on the quality and safety in ready-to-eat vegetables, namely coriander (*Coriandrum sativum* L.*)*, lettuce (*Lactuca sativa* L cv. Frisada.), mint (*Mentha spicata* L.), parsley (*Petroselinum crispum* M.), turnip (*Brassica campestris* L.)*)*, watercress (*Nasturtium officinale* L.*)*, melon (*Cucumis melo* L. cv. Piel de Sapo) and watermelon (*Citrullus lanatus* (thunb.) Mansfeld). Therefore studies on the physico–chemical, sensorial properties and inactivation of natural microbiota after applying several doses were carried out. Shelf life of the irradiated and non-irradiated produce was compared. The dose required for inactivation 90% (D_{10}) of *Escherichia coli* 0157:H7 and *Listeria innocua* on artificial contaminated vegetables were also determined. No important differences were verified in the overall of the sensorial and the physico-chemical properties after irradiation up to 1 kGy, although a considerable decrease of natural microbiota was noticed (>2 log). The D_{10} calculated to *Escherichia coli* 0157:H7 on artificially contaminated vegetable leaves, turnip and melon were 0.11 up to 0.16 kGy. The D_{10} range of 0.16 up to 0.29 kGy was calculated for the non-pathogenic surrogate bacterium (*Listeria innocua)* in the artificially contaminated vegetables.

INTRODUCTION

Fresh fruit and vegetables are an essential part of the diet of people all around the world. Consumer's demand for high quality, fresh, nutritive and easy to prepare foods has lead to the production of minimally processed foods. The increasing demand of this kind of products as well as the trade globalisation has raised the foodborne outbreaks.

Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields or orchards, or during harvesting, postharvest handling, processing and distribution. The microbes capable of causing human diseases include bacteria, viruses and parasites that may be present in water used for irrigation or in soil where products grow. Otherwise, contamination of produce by pathogens should be expected in countries where animal wastes are used as fertilizer. Root crops and low-growing leaf and stalk crops are heavily contaminated if sewage effluent or contaminated irrigation water is used as fertilizer. Raw materials used in the preparation of minimally processed food can be contaminated with pathogens like *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella* and *Cryptosporidium* oocysts [1, 2, 3]. Extensive outbreaks of salmonellosis attributable to contaminated tomatoes, bean sprouts, celery, lettuce, cabbage, endive and watercress have occurred. *Salmonellae* also have been found in a wide range of "organic grow" products including beans, peas, sunflower seeds and alfalfa [2, 4, 5].

Raw vegetables, especially lettuce, have been identified as a common cause of traveller's diarrhoea [5, 6]. Lettuce was the source of an outbreak of *E. coli* O157:H7 involving more than 100 people in Montana during 1995. Fresh parsley has been implicated in several outbreaks in USA and Canada

caused by *Shigella sonnei* and *Escherichia coli* 0157:H7 [7]. Lettuce and green onions have been involved in some outbreaks of shigellosis [8, 9].

Listeria monocytogenes can persist for long periods and is widely distributed in soil and on plant vegetation, hence raw vegetables are potential vehicles for human listeriosis. Outbreaks of human listeriosis have been epidemiologically linked to the consumption of fresh cabbage and lettuce. High populations of *Listeria monocytogenes* can develop on the modified atmosphere package of lettuce before this vegetable is judged inedible on the basic of sensory qualities [5, 6].

Other microorganisms as *Aeromonas* sp, *B. cereus*, *Staphyloccocus* spp., *V. cholerae* and *Y. enterocolitica* were isolated from fresh fruits and vegetables (e.g.: parsley, lettuce, grated carrots, tomatoes).

Industrial processes to prepare fresh-cut products usually use chlorine in the washing water to control microbial growth. The use of disinfectants, including sodium hypochlorite, could have a low inactivation effect on some potential pathogens as shown on *L. monocytogenes* by Zang and Farber [10].

Ionising radiation as a method of food preservation, without causing heating, is an old concept [11] but its use is not spread so far. However, as a consequence of trade globalisation and the necessity of giving a prompt answer to the consumer's demand for fresh and natural products with an overly extended shelf-life, the use of irradiation is a real good tool to be used. Irradiation together with other process technologies, such as disinfection and packaging in controlled atmosphere, in a perspective of hurdle technologies is highly promising.

Food irradiation is a physical treatment in which food is exposed to ionising radiation. Irradiation has been evaluated as a treatment for disinfestations and shelf-life extension of several fruits and vegetables [12, 13, 14]. Few data are available on the effect of irradiation on minimally processed food. Doses (1 up to 6 kGy) are effective in reducing spoilage and pathogenic microorganisms [15].

The main objective of this study was to analyse the effect of gamma radiation on the quality and safety of ready to eat vegetables, namely coriander, lettuce, mint, parsley, turnip, watercress, melon and watermelon. Therefore studies of physical–chemical, sensorial properties and inactivation of microorganisms after several doses were realised. Moreover irradiated and non-irradiated produce shelf life was compared.

2. MATERIAL AND METHODS

2.1. Raw material and sample preparation

Coriander (*Coriandrum sativum* L.), lettuce (*Lactuca sativa* L. cv. Frisada), mint (*Mentha spicata* L.), parsley [*Petroselinum crispum* Mill. (A.W.Hill)], turnip (*Brassica campestris* L.), melon (*Cucumis melo* L. cv. Piel de Sapo) and watermelon [*Citrullus lanatus* (thunb.) Mansfeld] were obtained from a central warehouse. Watercress (*Nastrium officinale* L.) was also obtained from a central warehouse, but already processed with the procedure described below for vegetable leaves.

Vegetable leaves were cleaned, washed in chlorinated tap water, hand shredded and then dipped in a cold solution containing 125 ppm NaClO.

Turnips were cleaned, washed in chlorinated tap water, hand-peeled, washed, dipped in a cold solution containing 125 ppm NaClO and then cut by means of Robot - Coupe processor.

The process for watermelon and melon were: cleaning, washing in chlorinated tap water, hand-peeling and then cutting in cubes.

2.2. Packaging

The vegetables were packed in polymeric film bags (Cryovac-PE65S) using the passive mode and sealed with a Boss Vacuum packaging machine GM 2002 (W. Germany). The melon and watermelon were packed in a Nutrip-PS (polystyrene) tray and then in polymeric film bags.

2.3. Irradiation

The irradiation was carried out in the cobalt-60 plant at the Sacavém campus of the Nuclear and Technological Institute (ITN), with a planar irradiator with the activity approximately of $1.1x10^{16}$ Bq (296 kCi) (1988/11/24) and upgraded up to $1.1x10^{16}$ Bq (295 kCi) (2003/11/24). Samples were irradiated at the dose rate range of 0.7 kGy h^{-1} up to 4 kGy h^{-1}.

Eighteen packages of each product were irradiated at a range of planned doses from 0.5 up to 1 kGy in two sets of three for three irradiation batches (3 batches x 2 sets x 3 packages = 18 packages) in the same local. Lettuce and turnip were irradiated from 0.5 up to 3 kGy. In each set was placed three dosimeters, Gamachrome YR for 0.5 kGy and Amber Perspex for higher doses. Dosimeters were placed as following: two in the extremity of the set and the third on the opposite side, in the middle. The coriander, lettuce, mint, parsley, watercress, turnip and melon inoculated with *Listeria innocua* (ATCC 33090) and *Escherichia coli* O157:H7 (ATCC 35150) were irradiated at 0.1, 0.2, 0.3, 0.4 and 0.5 kGy (planned doses).

Dose uniformity was approximately 1.1.

A non-irradiated sample was processed and analysed in parallel.

The overall process for all items did not take more than 24 hours after harvest, including irradiation.

2.4. Storage

In order to study the shelf life, the vegetables and fruits were stored at 4ºC in a refrigerated stove (Zanotti, Vizuete, S.L) until one of the quality factors was degraded to an unacceptable level.

Several attributes were measured during the storage of products, in order to monitor quality evolution: physiological, physical, microbiological and sensorial parameters. Evaluation was carried out after different days in conformity with product.

All analyses were carried out on three packages for each sampling day.

2.5. Microbiological analysis

Detection of pathogenic bacteria was carried out based on ISO standards:

Salmonella spp.:	(ISO 6579) [16]
Listeria monocytogenes:	(ISO 11290-1) [17]
Escherichia coli O157:H7:	(ISO 16654) [18]
Shigella spp.:	(ISO 21567) [19]
Yersinia enterocolitica:	(ISO 10273) [20]

Total aerobic mesophilic and psycrotrophic, total coliform and *Enterobacteriaceae* counts were also carried out. Ten grams of product were homogenised with 90 mL of maximum recovery diluent (MRD - Oxoid CM 733) in a Stomacher for two minutes. After serial 10 fold dilutions aliquots were pour plated at TGYE (Oxoid CM 325) and incubated at 30ºC during three days for aerobic mesophilic determination and at 7ºC during 14 days for psychrotrophic determination. For total coliforms counts the aliquots were pour plated at violet red bile agar (VRBA - Oxoid CM107) and incubated at 37ºC

for 24-48 h. For *Enterobacteriaceae* counts the aliquots were pour plated at violet red bile glucose agar (VRBGA - Oxoid CM 0485) at 37ºC for 24-48 h.

Microbial counts were expressed by the mean of log CFU/g (Colony Forming Units/g).

2.6. Artificial contamination of vegetables with bacteria reference strains

Based on Niemira et al. [21] protocol, before inoculation *Listeria innocua* ATCC 33090 and *Escherichia coli* O157:H7 ATCC 35150 were activated in tryptic soy broth (TSB) from the stock cultures (temperature: -20ºC) for 16 h at 37ºC. After growth, microorganisms were streaked onto tryptic soy agar (TSA) and incubated at 37ºC for 48 h. *Listeria innocua* and *Escherichia coli* colonies were incubated into TSB at 37ºC for 16 h. A control of this culture (starting inoculum) was made on TSA to ensure the population concentration (ca. 10^9 CFU/mL).

For inoculation of vegetables, aliquots of 200 mL of starting inoculum were mixed with 1800 mL of sterile MRD to make the working inoculum.

The vegetables, before use in the experiments, were sanitized using a solution of 300 ppm sodium hypopchlorite at room temperature. The vegetable material was submerged and gently agitated for 3 min. The vegetables were thoroughly rinsed under running distilled water, and spun in a sterile salad spinner-type centrifuge to remove excess surface water.

Working inoculum (1000 mL) was added to sanitized vegetables. The material was spun twice to remove excess inoculum from the surface of the pieces. Vegetables samples (45 g) were placed in No. 400 stomacher bags. The samples were refrigerated (4ºC) until irradiation.

After irradiation, the samples were refrigerated until the microbiological sampling. Sterile MRD (180 mL) was added to the stomacher bags with irradiated and non-irradiated vegetables and agitated for 60 seconds. A 1 mL sample was withdrawn for serial dilution with sterile MRD. The samples were diluted, pour plated and streaked onto TSA and incubated at 37ºC for 24h.

2.7. Physiological, physical and chemical analysis

At each sampling day, the atmosphere composition in the packages was determined using a food pack gas analyser (PBI Dansensor A/S CheckMate 9900 – Denmark). Texture, pH, ºBrix, exudate and colour (L*, H* and C*) were evaluated for parsley, watercress and watermelon;

For vegetables, the textural attributes profile analyses were carried out using a texturometer TA-Hdi (Stable Micro System, UK) with a computer junction to a “texture expert” analyser. Four analyses per sample were performed with a Kramer Shear Cell 10 blade at 20ºC, with a vertical drop of 5 mm s^{-1}.

The texture of watermelon and melon was measured using TA-XDi Analyser with a 50 N load cell, crosshead at 1.5mm.sec^{-1}, and 6mm plunger and penetration distance of 10mm.

The pH was measured with a potentiometer Crison- Micro pH 2002 (Crison Instruments SA, Barcelona, Spain) with a glass electrode.

Soluble solids content (ºBrix) was performed with a hand refractometer ATAGO (Atago Co, Ltd, Tokyo, Japan) on the juice extract.

Exudate of vegetables was measured by a method modified from Carlin et al. [22]. The exudate of watermelon and melon was determined through the liquid released from the pieces of fruit in the tray.

In colour analysis, samples and controls were observed during storage for the development of brown, grey or black discoloration. This procedure was performed using a Minolta Chroma Meter CR 200b (Minolta Corp. Tokyo, Japan), a reflectance instrument with a diameter of 8 mm, standardised with a

white tile (L* = 97.5; a* = 0.4; b* = 1,9). Analysis of L*, a*, b* values during storage, Hue angle (H* = arc tang (b*/a*) and (Chroma, $C^* = (a^{*2}+b^{*2})^{1/2}$).

2.8. Sensorial analysis

A test panel made up of eight trained people evaluated the sensory quality of samples, initially and during storage. For vegetables, the general overall visual quality, aroma and flavour were scored using a scale of 1 up to 9 (1= dislike extremely and 9= like extremely); a score of 5 was considered the limit of acceptance. For fruits the general overall visual quality, aroma and flavour were scored using a scale of 1 up to 5 (1= dislike extremely and 5= like extremely); a score of 3 was considered the limit of acceptance.

2.9. Statistical analysis

Data analyses were performed using the Statistical Analysis System (SAS) software, (SAS, 1999). The effect of radiation dose and storage time, as well as the interaction between two factors were evaluated. The General Linear Models Procedure (F-test on type III sum of squares) was used for analysis of variance, with main effect means separated by Duncan's test. Significance was defined at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Microbiological analysis

3.1.1. Total aerobic mesophilic and psychrotrophic, coliforms and Enterobacteriaceae counts

Total aerobic mesophilic and psychrotrophic, coliforms and *Enterobacteriaceae* counts were determined in non-irradiated and irradiated vegetables and fruits packages, along storage time. Figures 1 and 2 present the effect of irradiation and the evolution in mesophilic and psychrotrophic counts.

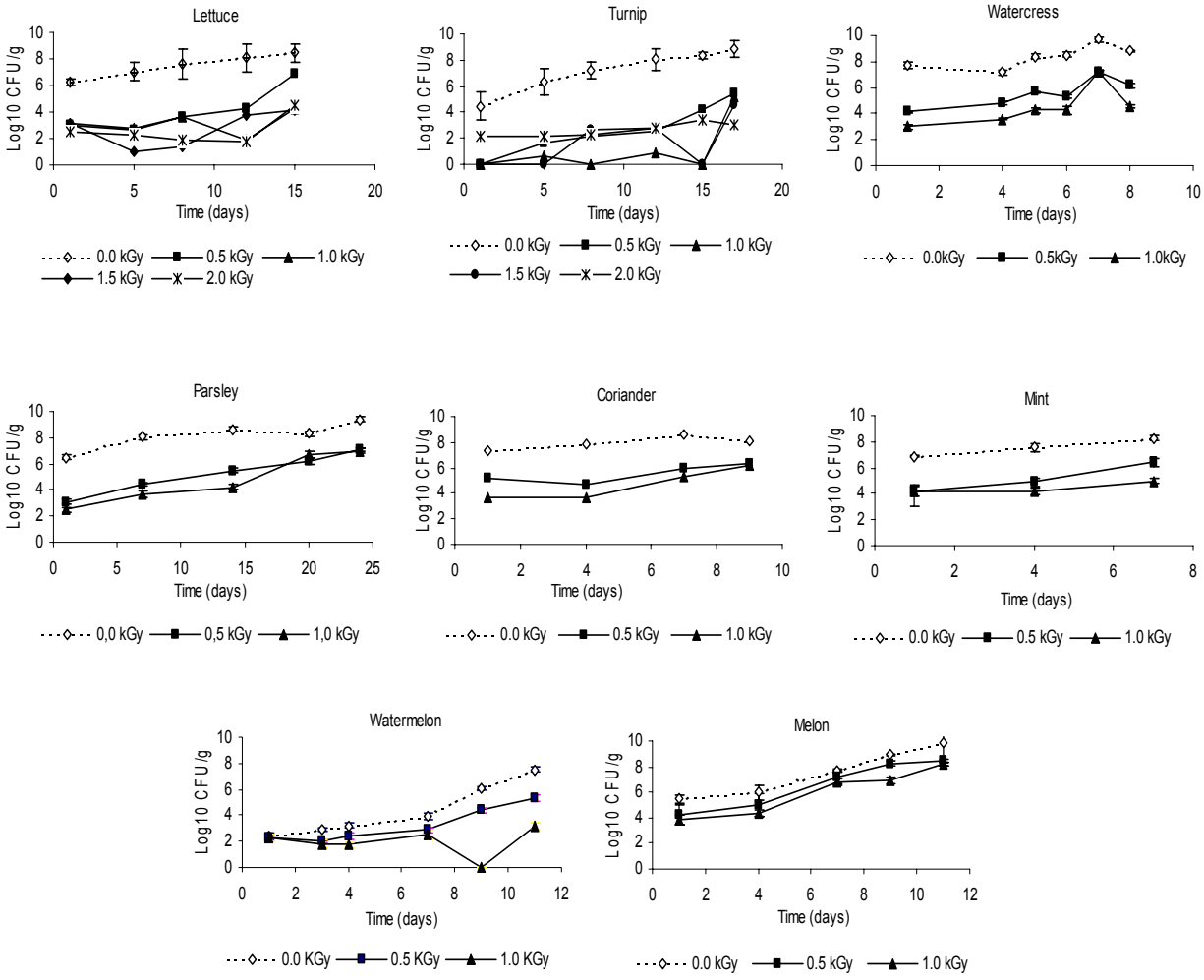

FIG. 1. Total aerobic mesophilic counts in lettuce, turnip, watercress, parsley, coriander, mint, watermelon and melon, submitted to several irradiation doses along storage time.

The irradiation treatment with 0.5 to 2 kGy in lettuce caused a reduction of 3.13 to 4 log in mesophilic and psycrotrophic counts, respectively. In relation to turnip there was a 4.2 log reduction.

These results agreed with Hagenmaier et al.and Prakash et al., who informed that low-dose irradiation (0.15-0.5 kGy) resulted in a reduction of 1.5-5 log of mesophilic counts on iceberg and romaine lettuce [23, 24]. Zhang et al. also informed that the total bacterial counts on fresh-cut lettuce irradiated with 1 kGy were reduced by the order of 2.35 log [25]. On the other hand, Lafortune et al. [26] informed that uncoated mini carrots irradiated at 0.5 and 1 kGy under air, presented a reduction of 3.5 and 4 log CFU/g, respectively.

The irradiation treatment in parsley caused a reduction of 3-4 log cycles in mesophilic and psycrotrophic counts. In relation to watercress there was a 4.69 log reduction.

A dose of 0.5 kGy resulted in reductions of 2.23 and 2.68 log of mesophilic bacterial counts in coriander and mint, respectively. With 1 kGy there was a 3.67 and 2.70 log reduction.

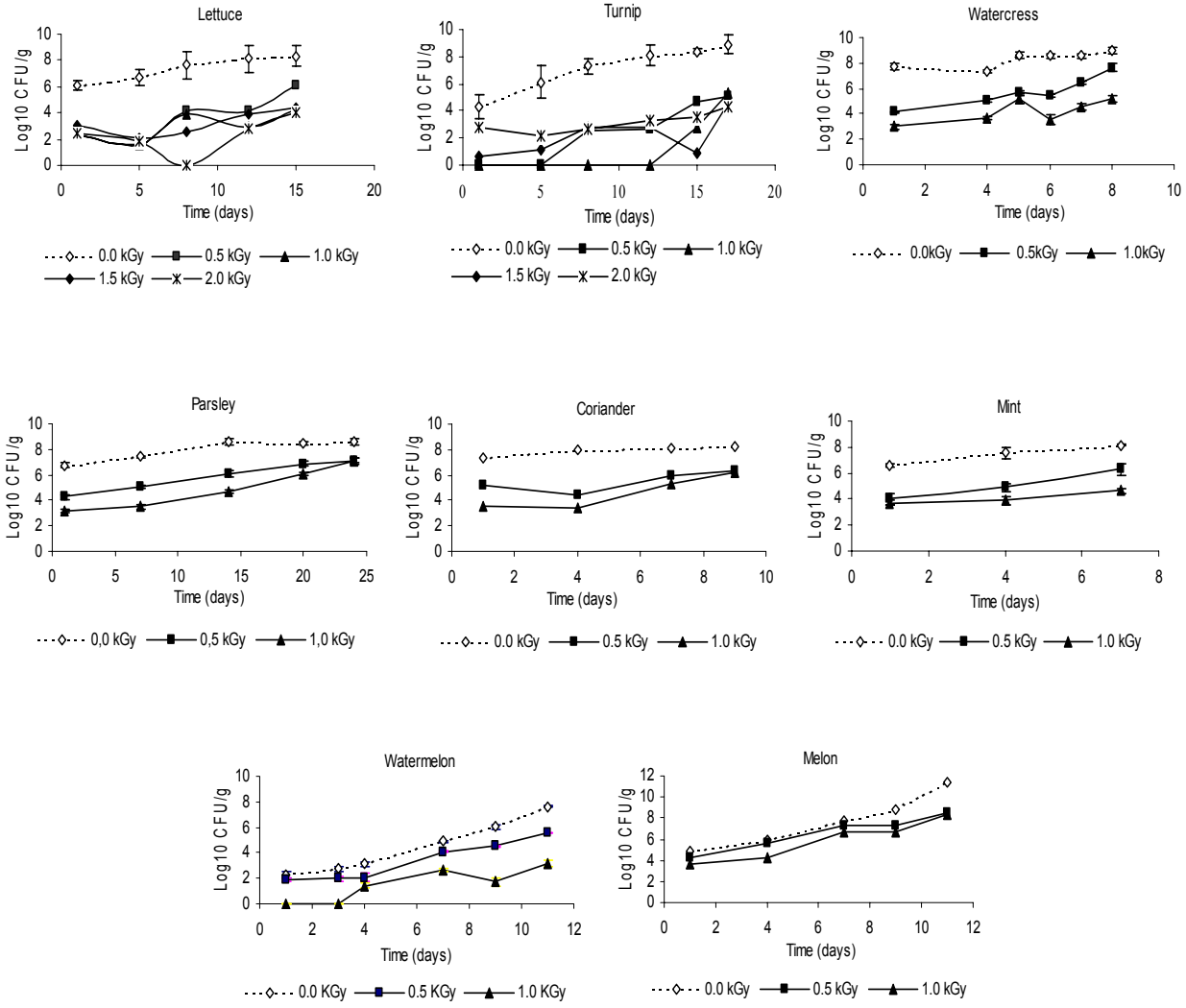

FIG. 2. Total aerobic psychrotrophic counts in lettuce, turnip, watercress, parsley, mint, melon and watermelon, submitted to several irradiation doses along storage time.

In relation to coliforms and *Enterobacteriaceae*, a dose of 0.5 kGy in coriander resulted in reductions of 1.52 and 1.72 log, respectively. In mint, the reduction was 3.57 and 3.66 log. Fan et. al. [27] have reported that doses up to 2 kGy did not significantly influence overall visual quality, decay, colour, texture, nutritional values, aroma or amount of the volatile compounds of fresh coriander and reduced microbial loads with 2.5 log.

The impact of radiation in mesophilic and psycrotrophic counts of watermelon and melon was very small.

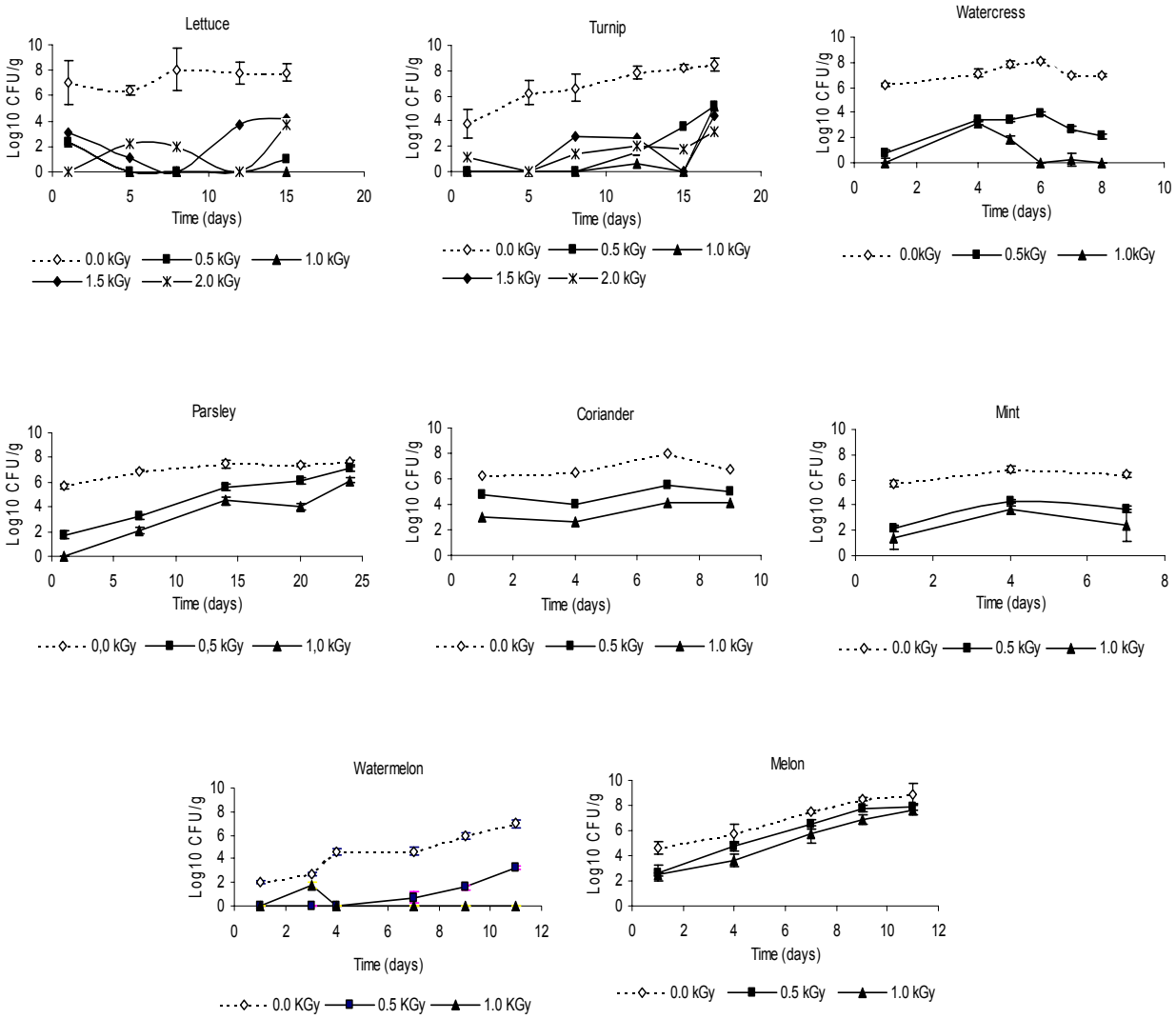

FIG. 3. Coliforms counts in lettuce, turnip, watercress, parsley, coriander, mint, watermelon and melon, submitted to several irradiation doses along storage time.

Radiation caused a reduction in coliforms and *Enterobacteriaceae* of 5 to7 log in lettuce and 4 log in turnip (Figs 3 and 4). During the trial period this difference remained constant.

Radiation caused a reduction in coliforms and *Enterobacteriaceae* of 5.69 log in parsley and 6.2 log in watercress. During the trial period (24 days to parsley and eight days to watercress) this difference remained constant.

The counts of *Enterobacteriaceae* and coliforms were reduced *circa* 2 log. During the trial period this difference remained constant.

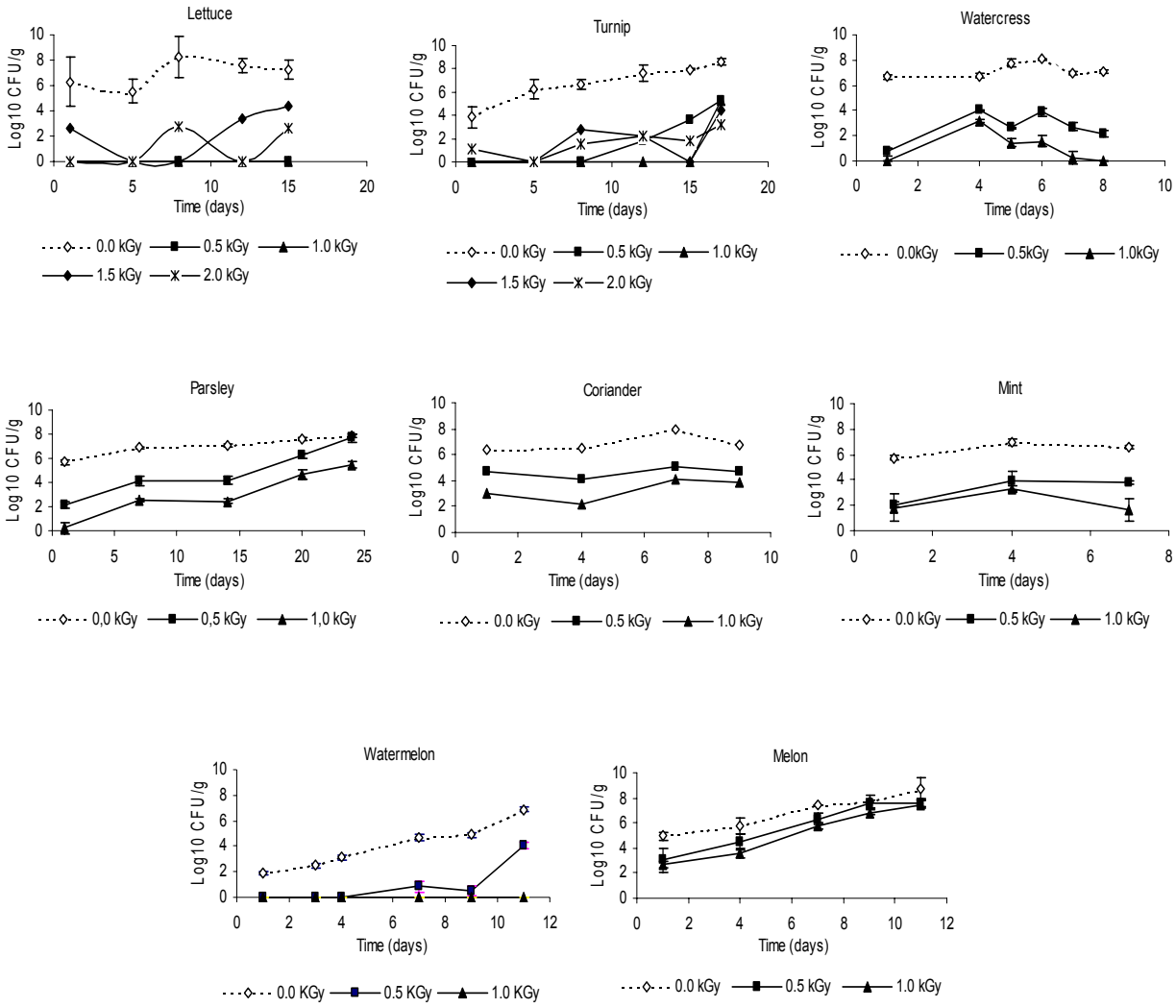

FIG. 4. Enterobacteriaceae counts in lettuce, turnip, watercress, parsley, coriander, mint, watermelon and melon, submitted to several irradiation doses along storage time.

In our study the results obtained in different vegetables are in accordance with early reports about reduction of different microorganisms, which is followed by regrowth of microorganisms on irradiated vegetables during storage [28, 29].

3.1.2. Pathogenic bacteria

Pathogenic bacteria *(Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Shigella* spp., *Yersinia enterocolitica*) were searched in non-irradiated samples of lettuce (n=4), organic turnip (n=5), parsley (n=5) and watercress (n=5). The pathogenic bacteria mentioned above were not detected in any of the samples.

3.1.3. Artificial contamination of vegetables with bacteria reference strains

Determination of *Listeria innocua* (ATCC33090) and *Escherichia coli* O157:H7 ATCC 35150 radiation sensitivity was performed on artificial contaminated vegetable leaves, turnip and melon. Irradiation effectively reduced the population of *Listeria innocua* and *Escherichia coli* O157:H7, as are presented in Figures 5 and 6.

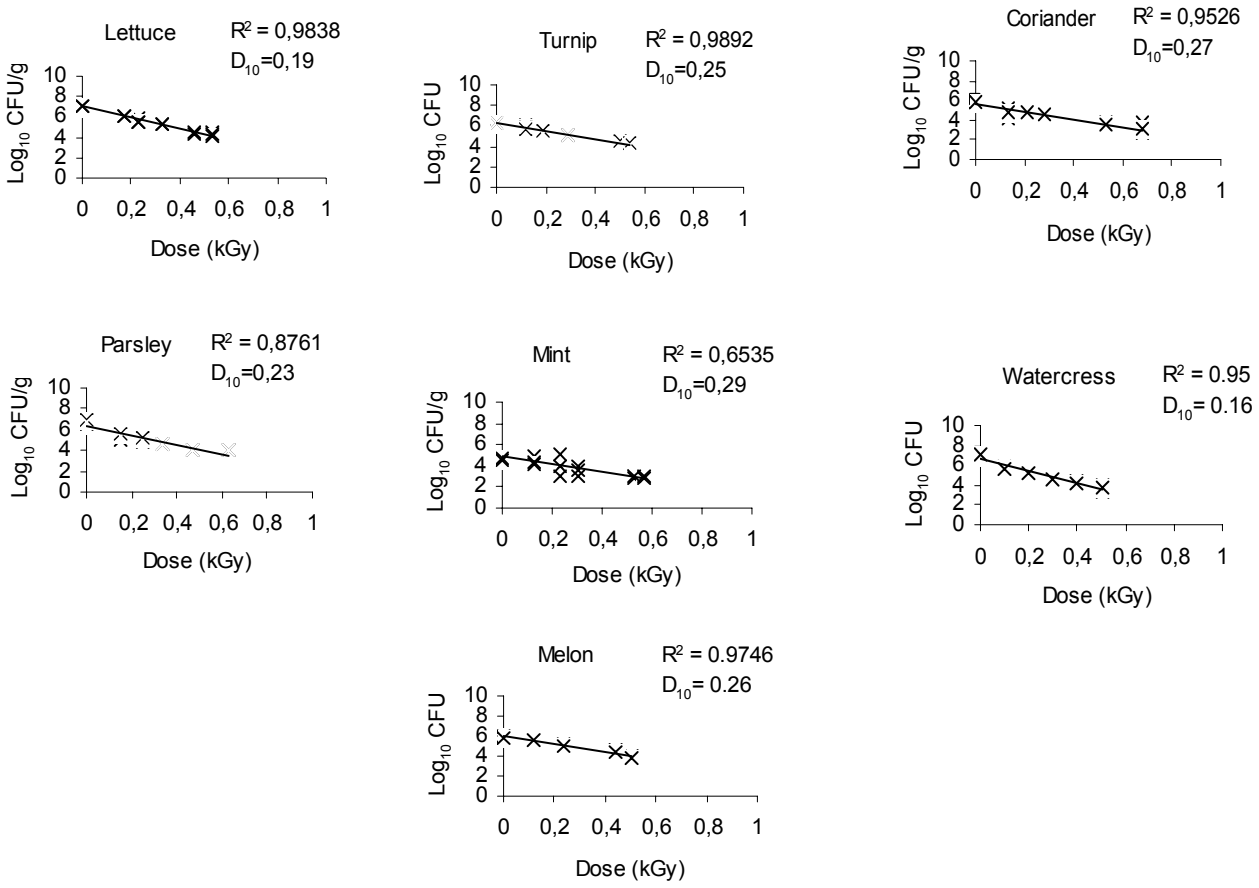

FIG. 5. Radiation sensitivity of Listeria innocua ATCC 33090 on lettuce, turnip, watercress, parsley, mint, coriander and melon surface

Listeria innocua in watercress (0.16 kGy), lettuce (0.19 kGy) and parsley (0.23 kGy) was more sensitive to radiation than in turnip (0.25 kGy), coriander (0.27 kGy), mint (0.29 kGy) and melon (0.26 kGy). The D_{10}-values obtained are in agreement with the D_{10}-value for *L. monocytogenes* and *L. inoccua* reported on endive and other food substrates [30, 31, 32].

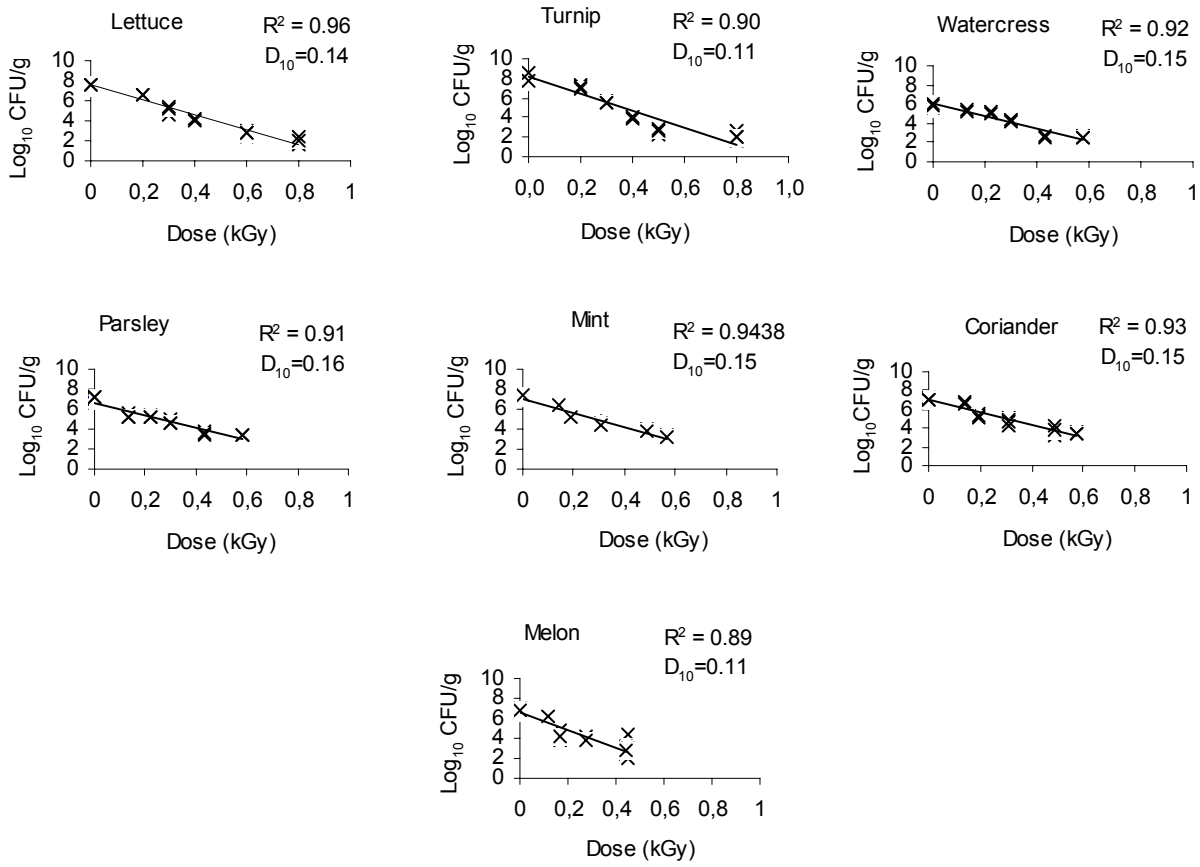

FIG. 6. Radiation sensitivity of E. coli O157:H7 ATCC 35150 on lettuce, turnip, watercress, parsley, mint, coriander and melon surface.

E. coli O157:H7 on all products showed a similar sensitivity, with D_{10}-values between 0.11 to 0.16 kGy. Comparable values, ranging from 0.108 to 0.122 kGy, were reported for *E. coli* on iceberg lettuce [33]. Irradiation at 0.55 kGy and 1.05 plus chlorination at 200 µg/mL produced a 5.4 and 7 log reduction in *E. coli* O157:H7 on lettuce and coriander respectively, while maintaining product quality [34, 35].

Prakash et al. [28] observed a 5-log reduction of *L. monocytogenes* and *E. coli* on the surface of diced celery irradiated with 1 kGy, with safeguarding the quality characteristics and shelf life.

3.2. Physiological, physical and chemical analysis

3.2.1. Atmosphere composition

Oxygen (O_2) and carbon dioxide (CO_2) percentage (%) were analysed in non-irradiated and irradiated samples.

The lettuce samples irradiated at 0.5 and 1 kGy showed the same behavior than non-irradiated samples. Lettuce samples irradiated at 1.5 - 3 kGy showed a decrease in O_2 levels (from 20% to 6%) and an increase in CO_2 levels (from 0% to 7%) (1.5 kGy) and 10% (2 kGy) on the first day.

The changes of O_2 and CO_2 levels in the headspace of irradiated lettuce samples were faster than those of non-irradiated samples. Packages with irradiated samples had higher CO_2 and lower O_2 levels during the storage period [23, 36, 37]; different authors assumed that the changes in the O_2 and CO_2 concentrations within the packages are a result of a higher respiration. The results seem to indicate that respiration would be stimulated by irradiation. On the other hand, Han et al. [38] informed of higher O_2 and lower CO_2 levels in the headspace of romaine lettuce hearts packages, this could be due to the use of lettuce hearts instead of cut leaves used. Niemira et al. [29] also found a CO_2 increase from less than 1% at the start of endive study to 18-23% at the final sampling time and the levels of O_2 declined from initial measurements of 18-19% to final levels of 1-6%.

Irradiated and non-irradiated turnip packages did not present any difference of atmosphere conditions measurements until 12 days of storage. On the first day the concentration of O_2 decreased from 20% to 0.16% and the concentration of CO_2 increased from 0 to 12% and were relatively stable during 17 days storage.

The percentage of O_2 inside of parsley packages decreased from 20% to 14% along the time of storage for the overall samples (irradiated or not). However a slight decrease of O_2 concentration (%) was observed for the irradiated samples *versus* the non-irradiated samples. The concentration rate of CO_2 for non-irradiated and irradiated parsley, showed a slight increase from 0% to 1.8% during the 25 days storage time.

In watercress, a decrease in O_2 concentration was observed, and an increase in CO_2 concentration for the irradiated samples *versus* the non-irradiated samples. During the storage there was a diminution of O_2 concentration from 20 to 1% in all packages. The CO_2 concentration was not affected by storage time. The percentage of O_2 inside watercress packages decreased in a higher rate than in lettuce (irradiated at 0.5 and 1 kGy), parsley and coriander, which points out a faster degradation on watercress.

In the first day the O_2 percentage of the irradiated mint samples atmosphere (15%) was less than in the non-irradiated samples (18%). At the end of shelf-life (9^{th} day) the O_2 concentration was the same in irradiated and non-irradiated samples. CO_2 concentrations in the package atmosphere among all samples were quite similar.

Along storage time the concentration of O_2 of the coriander package atmosphere decreased (20 to 15%), but no variation of the O_2 and CO_2 percentage between irradiated and non-irradiated samples was observed.

The irradiation dose treatments yielded watermelon samples with significantly lower O_2 levels in the headspace (1.3%) than non-irradiated (3%). The percentage of O_2 inside watermelon packages, decreased during the first four days of storage for the overall samples (irradiated or not) to values near zero. The inverse behaviour happened with CO_2 concentration, whose values (5%) remained constant till the end of 11 days storage.

For the melon samples, measurements were done on the 1^{st}, 4^{th}, 7^{th}, 9^{th} and 11^{th} day, however the readings obtained on the 4^{th} and 7^{th} day were rejected due to technical anomalies of the equipment used. Even so, variations in O_2 concentrations in the package atmosphere between the samples irradiated at different doses (0, 0.5 and 1 kGy) were not observed. The CO_2 percentage increased between the 9^{th} and the 11^{th} in all samples.

There were no variations in O_2 and CO_2 concentrations in irradiated melon package atmosphere. The CO_2 percentage increased between the 9^{th} and the 11^{th} day in all samples.

3.2.2. Texture

The energy of irradiation could affect the firmness of products. Textures measurements of non-irradiated and irradiated products were performed and analysed along the storage life.

Irradiated lettuce samples (0.5 and 1 kGy) showed a significant decrease in texture (56 N/g), when comparing with non-irradiated samples (61 N/g). The storage time did not affect the texture until the last storage day (15^{th}). Fan and Sokorai [36] found that firmness levels for samples irradiated at 1 and 2 kGy were statistically similar to those for non-irradiated samples, although texture of romaine lettuce hearts decreased by 15% at 1 kGy [38].

Irradiated turnip at 0.5 kGy presented more firmness (54 N/g) than the irradiated ones at upper doses and non-irradiated turnip (46 N/g). The storage did not affect the texture of the samples.

Texture results point out that the irradiation at 0.5 and 1 kGy did not affect the firmness of mint and coriander. In mint the firmness values increased at the end of storage (7^{th} day), this fact could be explained by the lost of moisture. In coriander the firmness value increased at 4^{th} day; until the end of storage this values remained constant. However, Fan et al. [27] found a firmness loss after irradiation at 1 kGy, but during storage the loss of firmness was less in irradiated samples than in non-irradiated.

Irradiated watercress samples (0.5 and 1 kGy) showed a significant increase in texture (94 N/g), when comparing with non-irradiated samples (85 N/g). The storage did not affect the texture of the samples.

Irradiated parsley at 1 kGy showed a significant decrease in texture (176 N/g) when compared with non-irradiated and irradiated at 0.5 kGy (187 N/g). However, the storage did not affect the texture of parsley.

Texture results point out that irradiation at 0.5 and 1 kGy did not affect the firmness of melon and watermelon.

3.2.3. Soluble solids content (°Brix) and pH

No effect of irradiation was observed, in terms of °Brix and pH, on the analysed samples of coriander, lettuce, mint, parsley, watercress and turnip as well as melon and watermelon.

No significant effect of irradiation was observed in the extention of the shelf-life of the produce studied in terms of pH and °Brix during storage time. (Data are not shown.)

3.2.4. Exudate

Exudate was measured in order to determinate cellular sap release.

Irradiated and non-irradiated samples of lettuce and turnip presented no differences until five days of storage. After the fifth day the increase of exudates in non-irradiated samples could be explained by microorganism's growth.

The exudate of irradiated and non-irradiated parsley, watercress, coriander, mint, melon and watermelon samples presented no relevant differences.

3.2.5. Colour (L, H° and C*)*

Surface colour analyses were evaluated by means of lightness (L*), chroma (C*) and Hue angle (H*) parameters.

Results point out for no detectable differences of turnip surface colour after irradiation and along storage time.

L* values have increased on lettuce during storage. A decrease of chroma values obtained from irradiated and non-irradiated lettuce were observed during the time of storage, no considerable changes were noticed on Hue values. Irradiation had no effect on L*, chroma and Hue values of lettuce.

Fan and Sokorai [36] did not find consistent difference in Hunter L, a, or b values among treatments (0 up to 4 kGy), but this data could be due to the heterogeneity of lettuce leaf tissues.

Results point out no detectable differences of parsley surface colour after irradiation. However, L* values increased and the Hue values decreased during storage. Irradiation had no effect on L*, chroma and Hue values of parsley.

Irradiation had no effect, in the first four days, in the colour parameters of mint. After 7 days of shelf life the mint irradiated with 1 kGy presented a decrease on Hue and L* values, these changes indicate that mint lost greenness and became darker.

L* and Hue values decreased after irradiation (1st day) on watermelon, nevertheless these differences decreased with storage time.

Irradiation had no effect on watercress, coriander and melon colour. Fan et al. [27] also found that a dose up to 3 kGy did not affect hue values of coriander.

No differences in colour values (E) were found between the irradiated at 1 kGy and non-irradiated diced celery [28].

3.3. Sensorial analysis

A test panel made up of eight trained people evaluated the sensorial quality of samples, initially and during storage. The general overall visual quality and aroma were scored using a scale of 1 to 9 (1= dislike extremely and 9= like extremely). A score of 5 is considered the limit of acceptance. For fruits the general overall visual quality, aroma and flavour were scored using a scale of 1 up to 5 (1= dislike extremely and 5= like extremely); a score of 3 was considered the limit of acceptance.

Sensorial results point out for an extended shelf life (up to 12 days) of lettuce irradiated at 0.5 kGy and 1 kGy compared with irradiated at 2 kGy and non-irradiated (8 days). Lettuce sensorial results are non conclusive for 1.5 kGy. Lettuce, right after irradiation at 2.5 kGy and 3 kGy, showed surface oxidation. Fan et al. [36] showed that after 14 days of storage, the overall visual quality of samples irradiated at 1 and 2 kGy was significantly better than that of control samples. Surface browning was reduced by irradiation at 1 and 2 kGy.

Sensorial results of irradiated and non-irradiated turnip also point out an extended shelf life for irradiated turnip at 0.5 kGy and 1 kGy (up to 15 days) compared with non-irradiated (12 days); turnip irradiated at 1.5 kGy and 2 kGy showed equivalent shelf life as non-irradiate turnip (12-13 days). Turnip showed shorter shelf life after irradiation at 2.5 kGy and 3 kGy (11 days).

Parsley irradiated at 0.5 and 1 kGy showed similar sensorial characteristics as non-irradiated after 24 days (end of trial).

Sensorial results point out for a small extended shelf life (up to seven days) of watercress irradiated at 0.5 kGy compared with non-irradiated watercress and irradiated at 1 kGy (six days).

The shelf life of coriander irradiated at 0.5 kGy was nine days in opposition to the samples irradiated at 1 kGy and non-irradiated (seven days).

The non-irradiated and irradiated (0.5 kGy) mint exhibited a better shelf life (7 days) than the ones irradiated at 1 kGy (4 days).

Sensorial results of irradiated and non-irradiated watermelon also point out an extended shelf life for irradiated watermelon at 0.5 kGy (up to nine days) compared with non-irradiated (four days). The watermelon irradiated at 1 kGy showed a shorter shelf life (three days).

Irradiated melon at 0.5 kGy and 1 kGy showed similar shelf-life (nine days) to non-irradiated ones.

Papaya irradiated at 0.75 kGy was considered optimum for retaining fruit firmness, with only slight control of storage decay [39, 40].

4. CONCLUSIONS

1. Irradiation of lettuce caused a microbial reduction of 3-5 log in the population of the bacteria determined. Irradiated (0.5 and 1 kGy) and non-irradiated lettuce packages did not present differences in atmosphere conditions measurements; however, after irradiation at higher doses (1.5 to 3 kGy) there was a depletion of O_2 and an increase of CO_2 concentration. Lettuce texture showed a slight decrease after irradiation at 0.5 and 1 kGy comparing with non-irradiated samples (0 kGy). For colour, overall results point out no detectable differences after irradiation of lettuce comparing with non-irradiated. Sensorial results showed that lettuce shelf life was longer after irradiation at 0.5 kGy and 1 kGy (plus 4 days).

2. In turnip, the radiation caused a microbial reduction of 4-5 log in the population of the bacteria determined. There were no differences in the atmosphere conditions measurements until 12 days, between irradiated and non-irradiated packages. Irradiated turnip at 0.5 kGy presented more firmness than the irradiated at upper doses and the non-irradiated ones. There was no detectable difference in turnip surface colour after irradiation, along storage time. Sensorial results showed that turnip shelf life was three days longer after irradiation (0.5 kGy and 1 kGy).

3. The irradiation of parsley caused a microbial reduction of 3-5 log in the population of the bacteria determined. The percentage of O_2 inside of parsley packages decreased 6% along the time of storage for the overall samples (irradiated or not) and a slight decrease of O_2 concentration (%) was observed for the irradiated samples *versus* the non-irradiated samples. Colour and texture results point out for no detectable differences after irradiation. Sensorial results of irradiated (0.5-1 kGy) and non-irradiated parsley showed no alterations until 24 days.

4. In watercress, the radiation caused a microbial reduction of 4-6 log in the population of the bacteria determined. It was observed a higher decrease in O_2 concentration for the irradiated samples comparing with non-irradiated samples. During the storage there was a diminution of O_2 concentration from 19% in all packages. Irradiated watercress showed a significant increase in texture when comparing with the non-irradiated. Irradiated and non-irradiated watercress did not present differences in colour results. Sensorial results showed that shelf life of watercress irradiated at 0.5 kGy was only one day longer than non-irradiated and irradiated at 1 kGy.

5. The irradiation of mint caused a microbial reduction of 2-3 log in the population of the bacteria determined. The irradiation caused a decrease in the O_2 concentration. Mint irradiated at 1 kGy lost greenness and became darker after seven days. Texture results point out for no detectable differences after irradiation. Sensorial results showed that shelf life of mint non-irradiated and irradiated at 0.5 kGy were three days longer than in those irradiated at 1 kGy.

6. The irradiation of coriander caused a microbial reduction of 1.5-3.7 log in the population of the bacteria determined. Irradiated and non-irradiated coriander did not present differences in atmosphere conditions measurements. Colour and texture results point out for no detectable differences after irradiation. Sensorial results showed that shelf life of coriander irradiated at 0.5 kGy was two days longer than in those non-irradiated and irradiated at 1 kGy.

7. The irradiation of watermelon caused a microbial reduction of 1-2 log in the population of the bacteria determined. The irradiation yielded watermelon samples with lower O_2 levels in the headspace than in non-irradiated. The colour was a slightly darker after irradiation (1st day), nevertheless this difference lessened along storage time. Texture results point out for no detectable differences after irradiation. Sensorial results showed that shelf life of watermelon irradiated at 0.5 kGy were four days longer than in those non-irradiated and irradiated at 1 kGy.

8. In melon, the radiation caused a microbial reduction of 1-2 log in the population of the bacteria determined. Irradiated and non-irradiated melon did not present differences in atmosphere conditions measurements. Colour and texture results point out no detectable differences after irradiation, along storage time. Irradiated melon at 0.5 kGy and 1 kGy showed similar shelf life (nine days) to non-irradiated ones.

9. *E. coli* O157:H7 on all products showed a similar sensitivity, with D-values between 0.11 to 0.16 kGy. *Listeria innocua* in watercress (0.16 kGy), lettuce (0.19 kGy) and parsley (0.21 kGy) was more sensitive to radiation than in turnip (0.25 kGy), melon (0.26 kGy), coriander (0.27 kGy) and mint (0.29 kGy).

The amount of radiation necessary to kill 10^5 *E. coli* was 0.55 to 0.8 kGy and for *Listeria innocua* vary between 0.8 and 1.45 kGy. Since such populations are considerably greater than those occasionally found in these vegetables, the application of 0.5-1 kGy would result in high inactivation of these pathogens.

Taking into account all of the parameters studied, the optimum doses to be applied would be 1 kGy for lettuce, turnip, parsley, watercress, watermelon and melon, and only 0.5 kGy for mint and coriander.

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REFERENCES

[1] GOMBAS, D.E, CHEN, Y., CLAVERO, R.S., SCOTT, V.N., Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. **66** (003) 559-569.

[2] HORBY, P.W, O'BRIEN, S.J, ADAK, G.K, GRAHAM, C., HAWAKER, J.I, HUNTER, P., LANE, C., LAWSON, A.J, MICTCHELL, R.T, REACHER, M.H, THRELFALL, E.J., WARD, L.R., A national outbreak of multi-resistent *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. Epidemiology and Infection (2003) 169-178.

[3] MONGE, R., CHINCHILLA, M., Presence of *Cryptosporium Oocysts* in fresh vegetables. J. Food Prot. **59** (1996) 202-203.

[4] ICMSF, Micro-organisms in foods 6. Microbial Ecology of Food Commodities, Cap. 5 Vegetables and vegetables products. Blackie Academic & Professional, London (1998).

[5] BEUCHAT, L.R., Pathogenic microorganisms associated with fresh produce. J. Food Prot. **59** (1996) 204-16.

[6] WACHTEL, M.R., CHARKOWSKI, A.O., Cross-contamination of lettuce with *Escherichia coli* O157:H7. J. Food Prot. **65** (2002) 465-470.

[7] NAIMI, T.S., WICKLUND, J.H., OLSEN, S.J., KRAUSE, G., WELLS, J.G., BARTKUS, J.M., BOXRUD, D.J., SULLIVAN, S., KASSENBORG, H., BESSER, J.M., MINTZ, E.D., OSTERHOLM, M.T., HEDBERG, C.W., Concurrent outbreaks of *Shigella sonnei* and enterotoxigenic *Escherichia coli* infections associated with parsley: implications for surveillance and control of foodborne illness. J. Food Prot. Apr. **66** (2003) 535-41.

[8] HAMMOND, R.M., BODAGER, D., WARD, K.V., ROWAN, A., Case studies in foodborne illness in Florida from fresh produce. HortScience **36** (2001) 22-25.

[9] MARTIN, D.L., GUSTAFSON T.L., PELESI, J.W., SUAREZ, L., PIERCE, G.V., Contamination produce – a commom source for two outbreaks of *Shigella gastroenteritis*. Am. J. Epidemiol. **124** (1986) 299-305.

[10] ZHANG, S., FARBER, J.M., The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiology **13** (1996) 311-321.
[11] SCHWARTZ, B., Effect of X-rays on trichinae, *J. Agriculture Research* 20 (1921) 845.
[12] YU, L., REITMEIER, C.A, GLEASON, M.L., NONNECKE, G.R. OLSON, D.G., GLADON, R.J., Quality of electron beam irradiated strawberries. J. Food Sci. **60** (1995) 1084-1087.
[13] BHUSHAN, B., THOMAS, P., Quality of apples following gamma irradiation and cold storage. Intern. J. Food Sci. Nutr. **49** (1998) 485-492.
[14] LALAGUMA, F., Response of 'Galia' muskmelons to irradiation as a quarantine treatment. HortScience, 33 (1998) 118-120.
[15] AKAMINE, E.K., MOY, J.H., Delay in post-harvest ripening and senescence of fruits In E.S. Josephson, M.S. Peterson (eds) - Preservation of Food by Ionizing Radiation, Vol. III, CRC Press, Boca Raton, FL. (1983) 129-158.
[16] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO 6579: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. (2002).
[17] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO 11290-1: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method (1996).
[18] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO 16654 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Escherichia coli* O157. (2001).
[19] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION ISO, 21567 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella* spp. (2004).
[20] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO 10273: Microbiology - General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica* (1994).
[21] NIEMIRA, B.A., SOMMERS, C.H., FAN. X., Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157:H7. J. Food Prot**. 65** (2002) 1388-1393.
[22] CARLIN, F., NGUYEN, T.C., CUDENNEC, P., REICH, M., Microbiological spoilage of fresh ready-to-use shredded carrots. Sci Alim. **9** (1989) 371-386.
[23] HAGENMAIER, R.D., BAKER, R.A., Low-dose irradiation of cut iceberg lettuce in modified atmosphere packaging. J. Agric. Food Chem. **45** (1997) 2864-2868.
[24] PRAKASH, A., GUNER, A.R., CAPORASO, F., FOLEY, D.M., Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food Sci. **65** (2000) 549-553.
[25] ZHANG, L., LU, Z., LU, F., BIE, X., Effect of γ irradiation on quality-maintaining of fresh-cut lettuce. Food Control **17** (2006) 225-228.
[26] LAFORTUNE, R., CAILLET, S., LACROIX, M., Combined effects of coating, modified atmosphere packaging, and gamma irradiation on quality maintenance of ready-to-use carrots (*Daucus carota*). J. Food Prot. **68** (2005) 353-359
[27] FAN, X., NIEMIRA, B.A., SOKORAI, K.J.B., Sensorial, nutritional and microbiological quality of fresh coriander leaves as influenced by ionizing radiation and storage. Food Research International **36** (2003) 713-719.
[28] PRAKASH, A., INTHAJAK, P., HUIBREGTSE, H., CAPORASO, F., FOLEY, D.M., Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Sci. **65** (2000) 1070-1075.
[29] NIEMIRA, B.A., FAN, X., SOKORAY, K.J.B., Irradiation and modified atmosphere packaging of endive influences survival and regrowth of *Listeria monocytogenes* and sensory qualities. Radiat. Phys. Chem. **72** (2005) 41-48.
[30] MONK, J.D., BEUCHAT, L.R. AND DOYLE, M.P., Irradiation inactivation of food-borne microorganisms. J. Food Prot **58** (1995) 197–208.
[31] NIEMIRA, B.A., FAN, X., SOKORAI, K.J.B., SOMMERS, C.H., Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*). J. Food Prot**. 66** (2003) 993-998.
[32] NIEMIRA, B.A., Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on four lettuce types. J. Food Sci. **68** (2003) 2784-2787.

[33] GOULARTE, L., MARTINS, C.G., MORALES-AIZPURÚA, I.C., DESTRO, M.T., FRANCO,B.D.G.M., VIZEU, D.M., HUTZLER, B.W., LANDGRAF, M., Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.). Radiat. Phys. Chem. **71** (2004) 155-159.
[34] FOLEY, D.M., DUFOUR, A., RODRIGUEZ, L., CAPORASO, F., PRAKASH, A., Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation Radiat. Phys. Chem. 63 (2002) 391-396.
[35] FOLEY, D., EUPER, M., CAPORASO, F., PRAKASH, A., Irradiation and chlorination effectively reduces *Escherichia coli* O157:H7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. J. Food Prot. **67** (2004) 2092-2098
[36] FAN, X., SOKORAI, K.J.B., Sensorial and chemical quality of gamma-irradiated fresh-cut iceberg lettuce in modified atmosphere packages. J. Food Prot. **65** (2002) 1760-1765.
[37] FAN, X., TOIVONEN, P.M.A., RAJKOWSKI, K.T., SOKORAI, K.J.B. Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. J. Agric. Food Chem. **51** (2003) 1231-1236.
[38] HAN, J., GOMES-FEITOSA, C.L., CASTELL-PEREZ, E., MOREIRA, R.G., SILVA, P.F., Quality of packaged romaine lettuce hearts exposed to low-dose electron bean irradiation. Lebensm.-Wiss. U.-Techhnol. **37** (2004) 705-715.
[39] PAULL, R.E., Response of tropical horticultural commodities to insect disinfestation treatments. HortScience **29** (1994) 988-996.
[40] PAULL, R.E., Ripening behavior of papaya (Carica papaya L.) exposed to gamma irradiation. Postharvest Biol. Technol. **7** (1999) 359-370.

USE OF IRRADIATION TO IMPROVE THE SAFETY AND QUALITY OF MINIMALLY PROCESSED FRUITS AND VEGETABLES

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Abstract

Minimally processed (MP) vegetables are prepared to meet the demands of providing ready- to-eat or ready-to-use products. In MP vegetables, contamination of foodborne pathogenic bacteria and their multiplication during storage is a serious health concern. Fresh carrots are the components of various Turkish salads such as season salad or Mediterranean salad. The reduction of the microbial population during processing and the D_{10}-values for *Listeria monocytogenes* and *Escherichia coli* inoculated on shredded carrots (*Daucus carota* L.) as well as the sensory quality of the irradiated product were evaluated. Low dose irradiation (1 kGy) was effective in eliminating *Listeria monocytogenes* and *Escherichia coli* with no evidence of re-occurrence and adverse effect on sensory attributes during storage. Mixed salad samples - Radicchio and Butterhead lettuce, Red lettuce (*L. sativa)*, Green lettuce (*Lactuca. sativa*) - were gamma irradiated at room temperature to determine the radiation sensitivity of the inoculated strains of *Listeria monocytogenes* ATCC 7644 and *Salmonella* Enteritidis ATCC 13076 and the effect of the recommended radiation dose on the ascorbic acid content and sensory attributes. The radiation doses necessary to reduce the bacterial population by 90% (D_{10}-values) for *Listeria monocytogenes* and *Salmonella* Enteritidis were determined as 0.26 kGy and 0.19 kGy, respectively. Sensory attributes such as odor and flavour were not affected at 1.5 kGy irradiation. Irradiaton process was effective in the reduction of total viable cell, yeast and molds, coliform and *E.coli* counts as well as extension of the shelf-life. *Salmonella* spp. was eliminated on the soybean sprout samples with irradiation at 1.0 kGy dose. Consequently, irradiation at 1.0-1.5 kGy dose was effective in activating the foodborne pathogens such as *Listeria monocytogenes, Salmonella* spp. and *E. coli* 0157:H7.

1. INTRODUCTION

Consumer demand for minimally processed fresh produce has been increasing due to premium product quality, convenience and fresh-like character [1]. Also, a large number of minimally processed (MP) products are commercially available in supermarkets and in food service facilities. Increase in consumption has resulted in increased frequency of outbreaks of illness associated with raw fruits and vegetables [2].

Turkey produces a variety of good quality fruits and vegetables. But their post-harvest and handling process losses (approximately 40% according to latest data) are major problems. Consumption of fresh salad vegetables and fruits has increased in quantity and variety lately in Turkey.

Contamination by foodborne pathogenic bacteria and their multiplication during storage is of serious health concern, because most of the MP vegetables are consumed without major processing [3].

In recent years, because of health interest and diet trends, leafy vegetables and salads are gaining increasing importance in the human diet. For weight conscious persons also, due to their high vitamins and fiber contents, salads are very beneficial [4]. Fresh pre-cut products and other minimally processed foods of plant origin are often consumed as such, without cooking or after undergoing a microbial inactivation process. Occurrence of some of the pathogenic organisms in raw vegetables and their implication in causing illness resulting in diarrhea/dysentery or serious diseases like yersiniosis and listeriosis have been documented in recent years [5]. MP vegetables in particular are capable of supporting microbial pathogens because they have a pH>4.6 and water activity >0.85 [6]. The high moisture and numerous cut surfaces of precut vegetables provide excellent conditions for the growth of microorganisms [7].

Commercial processes for preparing fresh-cut carrots usually use chlorine in the wash water to control the microbiological population. However, chlorine cannot be relied on to eliminate pathogenic microorganisms such as *L. monocytogenes* [2]. Irradiation has been shown to be effective in reducing the microbial population of shredded carrots.

Listeria monocytogenes and *Salmonella* Enteritidis are foodborne pathogens that are responsible for numerous foodborne illness outbreaks and product recalls. These bacteria can contaminate a variety of food products. As *L. monocytogenes* is capable of growth at refrigeration temperatures, salad vegetables that are generally chlorinated but not blanched for use in salads, even those held chilled or refrigerated, may therefore pose risk. Survival and subsequent growth of *Salmonella* and other pathogens on produce between the time of minimal processing and consumption may result in increased risk of illness [8].

Sprouts of mung bean, alfalfa, rice, wheat and soy which formed part of traditional oriental foods, have now gained popularity in many parts of the world. Although consumption of seed sprouts has a 'healthy' image, seed sprouts have been demonstrated to have been the vehicle for transmission in a number of foodborne outbreaks of infection [9]. Sprouts are produced under warm and humid conditions, which promote growth of pathogenic microorganisms [10]. It was informed that there have been several outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infections associated with commercial sprouts [11]. These outbreaks of infection have included salmonellosis and *Escherichia coli* O157 infection, and implicated seed sprouts included alfalfa, clover, cress, mung bean, radish and soy.

Irradiation is a well-established process with clearly documented safety and efficacy systems from the fact that its activity is not limited to the surface, it can penetrate into the product and eliminate microorganisms that are present in crevices and creases. Irradiation has been proven to inhibit microbial growth, and extend the shelf life of minimally processed fruits and vegetables [12].

Irradiation has gained attention as an effective tool for assuring food safety [13]. Irradiation is an effective control measure for eliminating pathogenic bacteria and parasites from the surface of fruits and vegetables [14, 15, 16, 17].

2. MATERIALS AND METHODS

2.1. Shredded carrots

2.1.1. Microbiological quality of carrots

Shredded carrots were prepared by combining 25 g of samples with 225 ml of diluents solution. The dilution liquid used was saline with 0.1% peptone and 0.85% NaCl. Samples were homogenized and serial dilutions were made. Then, the microbial counts were estimated by surface spreading. Counts of total bacteria were determined by dilution plate method using plate count agar (PCA, Merck) and counts of total yeast and mold were done by using potato dextrose agar (PDA, Merck). Total coliform bacteria were determined by using lauryl-sulfate tryptose broth supplemented with MUG (LST+ MUG, Merck) and MPN numbers were calculated from the MPN Table [18]. Thirty carrot samples were collected from different local markets.

2.1.2. Determination of D_{10}- values of Escherichia coli and Listeria monocytogenes

2.1.2.1. Strains and preparation of inocula

Reference cultures of *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 25922 were maintained in 50% glycerol at -36°C. Frozen samples of these bacteria were cultured in tryptic soy broth (TSB, Merck) for 16 h at 37°C. The cell density of the main inocula was determined by serial dilutions with sterile

0.1% buffered peptone water and plating on tryptic soy agar (TSA, Merck). The cell densities were typically 10^9 CFU/ml and 10^8 CFU/ml for *E. coli* and *L. monocytogenes*, respectively.

2.1.2.2. Preparation of samples

Fresh carrots (*Daucus carota* L.) purchased from a local market were used in these studies. The carrots were brought to the laboratory in polyethylene (PE) bags. After washing manually under running tap water, the excessive water was drained out. The samples were sorted for bruised and damaged carrots. Only healthy, firm and fresh looking samples were selected. The carrots were peeled before cutting with stainless steel peelers. The peeled carrots were manually cut into 2 mm thick slices with stainless steel knives. The cut samples were also packaged in polyethylene bags. All the samples were kept at 5ºC (overnight) before irradiation.

Shredded-carrot samples were divided into 10 portions (25 g each) in sterile polyethylene bags. Four samples from each of two groups were inoculated with *E. coli* (10^7 MPN/ml final concentration) and *L. monocytogenes* (approximately 10^6 CFU/ml final concentration) while the remaining samples were used as control (1 ml for 10 g sample). After inoculation, each shredded-carrot sample was individually hand massaged under sterile conditions for 1 min to evenly distribute the inocula on the samples prior to irradiation. The irradiated samples were then stored at 5°C for 10 days. Microbiological analyses were performed after 0, 5 and 10 days of storage.

2.1.2.3. Enumeration of Escherichia coli and Listeria monocytogenes after irradiation

Irradiated and non-irradiated samples were maintained under refrigeration (5ºC). Each portion of 25 g was homogenized with 225 ml of 0.1% peptone water using a stomacher during 2 min and serially diluted with 0.1% peptone water. 0.1 milliliter of each dilution was inoculated into three lauryl sulphate tryptose broth + MUG tubes to determine the population of surviving *E. coli*. All inoculated tubes were incubated at 37 ºC for 24-48 h. After the incubation period, three of the eight consecutive dilutions were used for MPN enumeration. Each analysis was performed two times.

For MPN enumeration, 0.1 milliliters of each serial dilution was inoculated into three *Listeria* enrichment broth tubes (LEB, Merck). The inoculated tubes were incubated at 30ºC for 24 h. After incubation, tubes in which growth was observed were streaked on supplemented Palcam agar (Merck). Plates were incubated at 37ºC for 24-48 h. Only colonies that were typical of *L. monocytogenes* were counted. The microbial counts were estimated as MPN/g by using the MPN table.

2.1.2.4. Irradiation process

Inoculated shredded carrot samples were treated with 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 kGy. All radiation treatments were carried out at 12ºC using Cobalt-60 Gamma-cell irradiator (Issledovatelj) with a dose rate 2.67 kGy/h. The irradiation dose received was measured by using Gammachrome YR dosimeters (Chromwell, UK). The change in absorbance was measured spectrophotometrically.

2.1.3. Microbiological, chemical and sensorial analysis during storage

To examine the microbial load and to perform the sensorial analysis of shredded carrots, the samples were divided into two groups. One group was irradiated at 1 kGy and the other group was not irradiated. Both of them were stored at 8ºC for 10 days in the refrigerator. The microbiological and chemical analyses were done for every five-day storage period.

All radiation treatments were carried out at 12ºC using Cobalt-60 Gamma-cell irradiator (Issledovatelj) with a dose rate 2.50 kGy/h.

2.1.3.1. Microbiological analysis during storage

Non-irradiated and irradiated shredded carrots were prepared by combining 25 g of samples with 225 ml of diluent solution. The dilution solution used was saline water with 0.1% peptone and 0.85% NaCl. Samples were homogenized and serial dilutions were made. Total bacterial counts were determined by plate method using plate count agar. The same procedure was used for total yeast and mold counts with plating on potato dextrose agar. *E. coli* was determined by using lauryl- sulfate tryptose broth supplemented with MUG. Gas positive tubes were examined for fluorescence by UV lamp. The microbial counts were estimated as MPN/g by using the MPN table.

To evaluate the presence/absence of *L. monocytogenes*, 25 g of irradiated and non-irradiated samples were placed aseptically into 225 ml of Listeria enrichment broth (LEB, Merck) and incubated at 30^{o}C for 48 hours and then streaked on Palcam agar. Inoculated plates were incubated at 37^{o}C and examined after 24 hours and if necessary, after another 24 hours in order to check for the possible presence of *L. monocytogenes* colonies.

In order to determine the presence/absence of *E. coli* in the treated and untreated samples, 25 g of the samples were placed aseptically into 225 ml of LST broth and incubated at 37ºC for 24 hours and then streaked on violet red bile agar + MUG (VRB+ MUG, Merck). Streak plates were incubated at 37ºC and examined after 16-18 hours. The colonies were examined for fluorescence by UV lamp (Merck).

2.1.3.2. Chemical analysis during storage

Moisture loss was examinated at 105°C in Genlab Wab Osr oven (Genlab Ltd, England) until constant weight was achieved.

The pH of the irradiated and non-irradiated sliced-carrot samples were measured with pH-meter Mettler Toledo MP 220 (Mettler Ltd, Switzerland).

Juiciness was calculated by the ratio of the juice weight to the carrot weight. The juice extract was obtained using Arzum Prokit (Arzum Ltd., Turkey).

Soluble solid content of the samples were performed with refractometer Atago (Atago Co. Ltd., Japan).

Colour analysis was observed using Minolta Chroma Meter CR 310 (Minolta Corp., Japan). The Hunter La*b* values were used for color analysis.

2.1.3.3. Sensory analysis during storage

The sensory qualities of the irradiated and non-irradiated shredded carrots were evaluated at the beginning and the end of storage period with twelve people. A panel of twelve untrained judges evaluated the samples for color, odor, texture and general acceptability using a 5 point-scale where 1 indicates extremely dislike and five extremely like. The samples were coded by using standard random numbers. The sensory evaluations were conducted at room temperature under normal laboratory light conditions and the panelists were free to judge any sample.

2.1.4. Statistical analysis

Presence of any statistically significant difference between the average values was evaluated by using two-factor factorial experiment. Comparisons of the differences between the average values were evaluated by the Duncan test.

2.2. Mixed salad

2.2.1. Determination of microbiological quality and shelf life of commercially packaged mixed salad

Mixed salad consisted of Radicchio, Butterhead lettuce, red lettuce and green lettuce. In order to screen the native microflora of this product, 10 raw samples from the same plant were analysed for total viable cell count (TVC) (ISO 4833:2003) [19], total yeast and molds, total coliforms and *E. coli* with MUG-LST [15, 7] and determination of *Salmonella* spp., *Listeria* spp., and *E. coli* O157:H7 in 25 g. *Salmonella* and *Listeria* analyses were carried out according to ISO 6579:2002 [20], and 11290-1:1996 [21], respectively, and *E. coli* O157:H7 analysed according to BAM [22].

Commercially packaged samples were stored for 0 to 10 days (0, 1, 3, 5, 7 and 10) at 5°C until analysis was carried out. The samples were brought to room temperature and opened aseptically prior to analysis. Microbiological and sensory analyses were carried out simultaneously. During storage period analyses of total viable cell, total yeast and molds, total coliforms and *E. coli*, determination of *Salmonella* spp., *Listeria* spp., and *E. coli* O157:H7 were carried out in same manner as described above.

2.2.2. Inoculation of the mixed salad for determination of radiation D-values

2.2.2.1. Strains and preparation of inocula

Reference cultures of *L. monocytogenes* ATCC 7644 and S. Enteritidis ATCC 13076 were maintained in 50% glycerol at -36°C. Frozen samples of these bacteria were cultured in tryptic soy broth (TSB, Merck) for 16 h at 37°C with agitation and streaked onto tryptic soy agar (TSA, Merck). They were incubated at 37°C for 24 h to form single colonies. These colonies were used to inoculate fresh TSB for each experiment, grown for 16 h at 37°C with agitation. The cell density of the main inoculum was determined by serial dilution with sterile 0.1% buffered peptone water and plating on TSA. The cell density was typically 10^9 CFU/ml for each bacterium. For inoculation of leaf pieces, aliquots of 100 ml of starting inoculums were mixed with 900 ml of sterile buffered peptone water to make the working inoculum.

2.2.2.2. Inoculation of mixed salad samples

Before using in the experiments, mixed salad sample was sanitized using a solution of 300 ppm sodium hypochloride at room temperature. Mixed salad sample was submerged and gently agitated for 3 min in this disinfectant solution. The sample was thoroughly rinsed with sterile distilled water, and spun in a sterile salad spinner-type centrifuge to remove excess surface water [23]. The microflora of sanitized mixed salad sample was measured by surface washing with 0.1 % buffered peptone water, serial dilution plating on tryptic soy agar and incubation at 37°C for 24 h. Mixed salad samples were inoculated separately with these pathogens. Sanitized mixed salad samples (10 g) were transferred to a sterile beakerglass, inoculated in a biological laminar flow cabinet, and 1000 ml of the working inoculum was added. The sample was agitated gently for 2 min. to completely submerge each piece, and then, transferred to a sterile salad spinner-type centrifuge (Ucel, Istanbul Turkey). The sample was spun twice to remove excess inoculum from the surface of the leaf pieces. Samples (10 g) were placed in stomacher bags. The samples were refrigerated (5°C) until irradiation, typically 60 min.

2.2.2.3. Irradiation process

Inoculated mixed salad samples were treated with 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 kGy. All radiation treatments were carried out at 12ºC using Cobalt-60 Gamma-cell irradiator (Issledovatelj) with a dose rate 1.88 kGy/h.

2.2.3. Enumeration of Salmonella Enteritidis and Listeria monocytogenes after irradiation

Irradiated and non-irradiated samples were maintained under refrigeration (5ºC). Each portion of 10 g was homogenized with 90 ml of 0.1% buffered peptone water (Merck) using a Stomacher during

2 min and serially diluted with 0.1% buffered peptone water (Merck). 0.1 milliliter of each dilution was spread onto XLT_4 (Merck) to determine the population of surviving bacteria. Two spread plates per dilution were incubated at 37ºC for 24-48 h. After the incubation period, approximately five colonies from each plate were tested with Latex agglutination test (Oxoid). Each analysis was performed two times.

For direct plating, 0.1 milliliters of serial dilutions were plated in duplicate onto supplemented Palcam agar (Merck). Plates were incubated at 37ºC for 24-48 h. Only colonies that were typical of *L. monocytogenes* were counted. Five random colonies per sample were verified as *L. monocytogenes* through biochemical tests (Xylose, Rhamnose, Mannitol and Hemolysis).

2.2.4. Determination of ascorbic acid and sensory evaluation

Ascorbic acid concentrations of irradiated and untreated samples were determined by HPLC (Schimadzu Corporation, VP series, Kyoto-Japan), equipped with Schimadzu pump, UV-VIS detector and Schimadzu software in two replicates.

A panel of seven untrained judges evaluated the samples for color, odor, texture and overall acceptability using a 5 point scale where 1 indicates extreme dislike and 5 extreme like. The samples were coded using standard random numbers. The sensory evaluations were conducted at room temperature under normal laboratory light conditions and the panelists were free to judge any sample. Data were analysed with ANOVA and Duncan Test.

2.3. Soybean sprouts

2.3.1. Microbiological analysis on the commercially packaged soybean sprouts

The commercially packaged soybean sprout samples were stored for 10 days at 5^0C until analysis. These samples were examined for total viable count, total count of yeast and molds, total coliform and *Escherichia coli* counts at different storage periods (0, 1 ,3, 5, 7 and 10 days). The experiment was performed two times.

Twelve commercially packaged soybean sprout samples were obtained from local markets and examined for pathogens *(L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7), microbiological quality (total viable count, total yeast and molds, total coliform and *E. coli* counts).

2.3.2. Determination of the D_{10} -values of the selected pathogenes

2.3.2.1. Sanitizing of the soybean sprouts samples

Before using in the experiments, the soybean sprout material was sanitized using a solution of 300 ppm sodium hypochloride at room temperature. The soybean sprout material was submerged and gently agitated for 3 minutes in the disinfectant solution. The soybean sprouts were thoroughly rinsed with sterile distilled water and spun in a sterilized salad spinner-type centrifuge (Ucel, Istanbul, Turkey) to remove excess surface water. The remaining microflora was determined in a 10 gram portion of the sanitized sample that was homogenized in a 90 ml 0.1% buffered peptone water (BPW, Merck 1.07228) followed by serial dilution and plating on tryptic soy agar (TSA, Merck 1.0558), and incubation at 37°C for 24-48 hours.

2.3.2.2. Main inoculum

Reference cultures of *Listeria monocytogenes* RSKK 472,1/26, *Salmonella* Enteritidis ATCC 13076 and *E. coli* O157:H7 RSKK No:232 were maintained in 50% glycerol at –36°C. Frozen samples of the bacteria were cultured in tryptic soy broth (TSB, Merck 1.05459) for 16 hours at 37°C and streaked onto TSA. Plates were incubated at 37°C for 24 hours and single colonies were used to inoculate fresh TSB and incubated at 37°C for 16 hours for each experiment. The cell number of the main inoculum

was determined by serial dilution with sterile 0.1% BPW and plating on TSA. The cell density was typically 10^9 CFU/ml for each bacteria. The initial inoculum was used directly to inoculate soybean sprout. For inoculation of soybean sprout pieces, aliquots of 100 ml of starting inoculum were mixed with 900 ml of sterile BPW to make the working inoculum. All the experiments were carried out according to Niemira et al. [23].

2.3.2.3. Inoculation of the soybean sprouts samples

The soybean sprout samples were inoculated separately with these pathogens. Sanitized soybean sprouts (100 g) were aseptically transferred to a sterile beaker containing 1000 ml of the working inoculum on the sprouts. The material was agitated gently for 2 minutes to completely submerge each piece, and then transferred to a sterilized salad spinner-type centrifuge. The material was spun twice to remove excess inoculum from the surface of the soybean sprout pieces. Samples (10 g) were placed in stomacher bags. In these experiments, 10 g samples instead of 45 g were used due to the limitation of Gamma-cell dimension. The samples were refrigerated (5°C) until irradiation, typically for 60 minutes. All these experiments were carried out according to Niemira et al. [23].

2.3.3. Examination of the effect of the irradiation on survival of pathogenic microorganisms on soybean sprouts

2.3.3.1. Radiation treatment of the soybean sprouts

Inoculated soybean sprout samples were irradiated at different doses which were 0.00 (control), 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50 kGy.

2.3.3.2. Sampling

After irradiation, the samples were refrigerated until microbiological analysis; typically for 60-90 minutes; the samples and sterile 0.1% BPW (90 ml) were added to the stomacher bag and homogenized for two minutes. Serial dilutions were made in sterile 0.1 % BPW.

2.3.3.2. Determination of survival of pathogens

Salmonella Enteritidis: Homogenized samples were diluted and plated onto XLT4 Agar (Merck 1.13919) supplemented with XLT4 Selective Supplement (Merck 1.08981). Inoculated plates were incubated at 37°C for 48 hours and typical colonies were counted. These counts were transfered into log colony forming units (CFU) per gram of soybean sprout.

Listeria monocytogenes: Homogenized samples were diluted and plated onto Palcam listeria selective agar (Merck 1.11755) supplemented with Palcam listeria selective supplement (Merck 1.12122). Inoculated plates were incubated at 37°C for 48 hours and typical colonies were counted. These counts were transfered into log colony forming units (log CFU) per gram of soybean sprout.

E.coli O157:H7: Homogenized samples were diluted and plated onto sorbitol Mac Conkey agar (Merck 1.09207) supplemented with CT Supplement (Merck 1.09202). Inoculated plates were incubated at 37°C for 48 hours and typical colonies were counted. These counts were transfered into log colony forming units (CFU) per gram of soybean sprout.

2.3.4. Effect of irradiation and storage at 5°C on commercially packaged soybean sprouts

Commercial soybean sprout packages were irradiated in gamma-cell at different doses (0.0, 1.0, 1.5, 2.0 and 2.5 kGy). After irradiation, the commercially packaged samples were stored for 0 to 13 days at 5°C until analysis were carried out (day 0, 1, 3, 5, 7, 10 and 13). At each sampling day, microbiological, sensory and nutritional analyses were carried out simultaneously.

2.3.4.1. Microbiological, nutritional and sensory evaluation of the effect of the irradiation on commercially packaged soybean sprouts during storage period

a. Microbiological analysis

During storage period, microbiological analysis were done for total viable count, total mold and yeast, total coliform and *Escherichia coli* counts, and presence or absence of *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 at different storage days (day 0, 1, 3, 5, 7, 10 and 13). The experiment was performed two times.

Total microbial counts were estimated by surface spreading on plate count agar (Merck 1.05463). The counts were determined as log CFU/g fresh weight.

Homogenized samples were diluted serially and then surface spreaded on yeast extract glucose chloramphenicol agar FIL-IDF (MERCK 1.16000). Plates were incubated at 30°C for five days. The total yeast and mold counts were determined as log CFU/g fresh weight.

For total coliform and E. coli counts homogenized samples were serially diluted and then dilutions were inoculated into triplate tubes containing lauryl-sulphate tryptose broth containing MUG (LST-MUG) (Merck 1.12588) and incubated at 37°C for 24-48 hours. Growth and gas-positive tubes were examined for fluorescence by UV lamp (Merck, Germany). Of all dilutions, three were used for MPN calculation.

b. Determination of Ascorbic Acid

The soybean sprout samples were irradiated with different doses (0.0, 1.0, 1.5, 2.0 and 2.5 kGy). Then HPLC analysis was performed with a model HP-1100 HPLC system (Hewlett Packard, Waldbron, FRG) for vitamin C content. Sprouts were crushed in a porcelain mortar. 1 g of the crushed material was weighed accurately. These 1 g samples were extracted five times with 10 ml of 2% metaphosphoric acid and filtered. Then the filtered solution was completed to 50 ml with the mobile phase that was used in the HPLC analysis. These extracts were used as stock solutions. 5 ml of these stock solutions were filtered through Cartridge column (Seppak C_{18}, Waters), completed to 10 ml with the mobile phase and 5 µl of each was injected to the HPLC column for the analysis.

The samples of soy bean sprouts that contain ascorbic acid were prepared and analysed with the HPLC method.

c. Sensory evaluation

A test panel with seven panelists was performed sensory evaluation during storage (at 0, and after 1^{st}, 3^{rd} and 5^{th} days). The organoleptic properties including appearance, odor, taste, texture and general acceptibility of irradiated and non-irradiated commercially packaged soybean sprout samples were evaluated.

d. Statistical Analyis

Presence of any statistically significant difference between the mean values was evaluated by using two factorial analyses. Comparison of the differences between the mean values was done by using Duncan test.

e. Evaluation of the presence of Listeria monocytogenes, Salmonella spp and E. coli O157:H7

The soybean sprout samples were analysed according to ISO 11290-1:1996 [21]. Half Fraser broth (Merck 1.10398) was used for primary enrichment. Incubation was performed at 30°C for 24 hours. Secondary enrichment was carried out in Fraser broth (35-37°C for 24 hours). Oxford agar (Merck

1.07006) and Palcam agar (Merck 1.11755) were used for plating out and identification. Incubation was performed at 35-37°C for 24-48 hours.

ISO 6579 was applied for the detection of *Salmonella* spp [20]. Buffered peptone water (Merck 1.07228) was used for pre-enrichment. Then Rappaport-Vassiliadis (RVS) broth (Merck 1.07700) and Muller-Kaufmann tetrathionate (MKTT) broth (Merck 1.10863), as selective enrichment liquid media were used. RVS broth was inocubated at 41.5 ± 1°C for 24 ± 3 hours and MKTT broth at 37°C ± 1°C for 24 ± 3 hours. Two selective solid media, XLT4 (Merck 1.13919) and Rambach agar (Merck 1.07500) were used for plating out and identification of *Salmonella* spp. colonies.

E. coli O157:H7 was analysed according to FDA method [22]. Enrichment of *E. coli* O157:H7 was performed in tryptic soy broth supplemented with novobiocin (Merck 1.09205), incubated at 35-37°C for 18-24 hours. After incubation, suspensions from enrichment broth were streaked on sorbitol-MacConkey agar (Merck 1.09207) followed by incubation at 35-37°C for 24 hours.

3. RESULTS AND DISCUSSION

3.1. Shredded carrot

3.1.1. Microbiological quality of raw carrots

Thirty carrot samples were collected from different markets. Local market survey of fresh carrots showed large variation in the bacterial load (3.78-6.68 log CFU/g), molds and yeasts (4.40-6.86 log CFU/g) and coliform bacteria (0.32-4.04 log MPN/g) (data are not shown). Like other MP (fresh-cut) vegetables, shredded carrots may have high levels of microorganisms [6]. In commercial shredded carrots, mesophilic counts of about 10^6 to 10^7 have been reported [2, 24].

Initial total bacterial counts were determined as approximately 10^5 CFU/g in shredded carrots after preparation of the sample. Irradiation at all doses was effective in reducing total bacterial counts in the shredded carrot samples. Dose of 1.0 kGy suppressed the population of total bacteria to undetectable levels. The regression coefficient was determined as r = -0.99.

Fig. 1 shows the inactivation of *L. monocytogenes* inoculated on shredded carrots and irradiated up to 2.5 kGy. D_{10}-value of *L. monocytogenes* in shredded carrots was calculated as 0.29 kGy and it should be noted that the regression coefficient for the irradiation treatment was calculated as r =-0.96. Niemiera et al. [25] reported that the population of *L. monocytogenes* on inoculated endive was suppressed by 0.42 kGy, a dose calibrated to achieve a 99% reduction.

The radiation dose necessary to reduce the bacterial population by 90% (D_{10}-values) for *L. monocytogenes* differed significantly among vegetables at each irradiation temperature. D_{10} increased significantly with decreasing temperature for all vegetables, with each vegetable showing a different response pattern. At an irradiation temperature of -5 ^{o}C, D_{10} ranged from 0.505 kGy for broccoli to 0.613 kGy for corn. At -20 ^{o}C, D_{10} ranged from 0.767 kGy for lima beans to 0.916 kGy for peas [26].

Inactivation of *E. coli* inoculated on shredded carrots and irradiated up to 2.5 kGy is shown in Fig. 2. D_{10}- value of *E. coli* was calculated as 0.29 kGy and it should be noted that the regression coefficient for the irradiation treatment was calculated as r = -0.99.

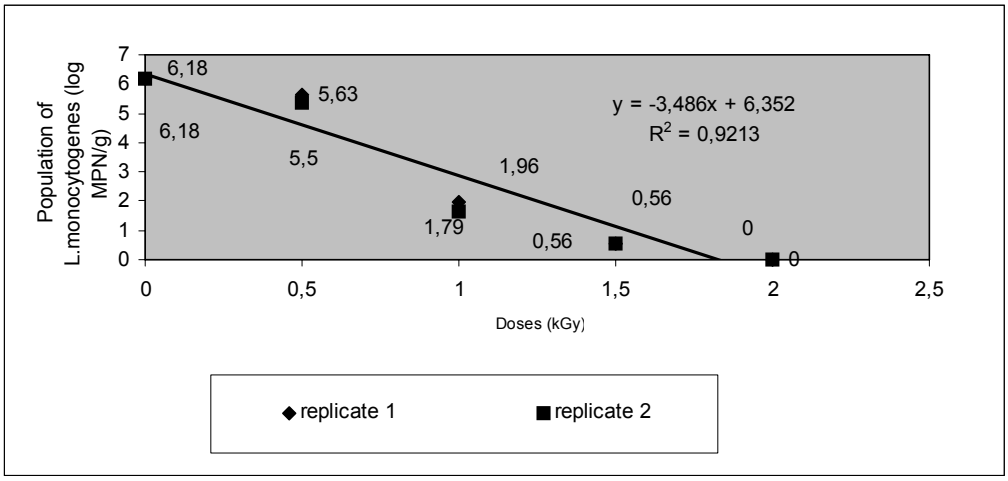

FIG. 1. Radiation sensitivity of L. monocytogenes inoculated on shredded carrots.

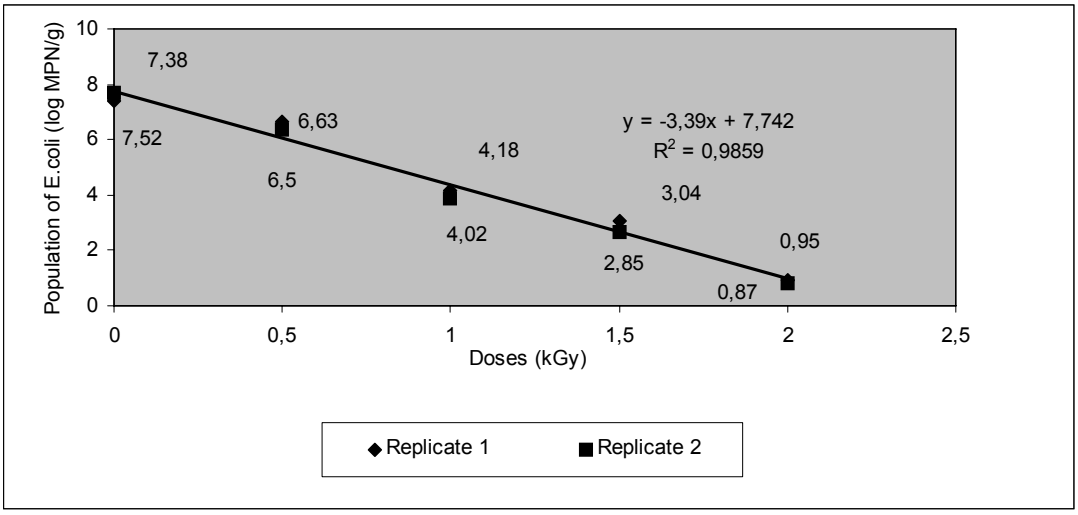

FIG. 2. Effect of irradiation on E. coli inoculated on shredded carrots.

The dose of 1.0 kGy resulted in the 3 log-cycle reduction of *E. coli* and *L. monocytogenes* counts. Thus, doses > 1.0 kGy would be adequate to reduce the 5 log of these pathogens in the minimally processed carrots. Irradiation has been shown to be effective in reducing the microbial population of shredded carrots.

3.1.3. Microbiological analysis during storage

Microbiological evaluation of foods is of primary importance due to the presence of harmful human pathogens, and their inactivation is essential to ensure the hygienic quality of food material. The initial bacterial load was 5.72 log CFU/g in control samples and reached to 7.77 log CFU/g and 8.27 log CFU/g after 5 and 10 days of storage, respectively (Table 1). A dose of 1 kGy reduced the microbial load to 3.84 log CFU/g, which were 5.22 log CFU/g and 5.23 log CFU/g after 5 and 10 days of storage, respectively. These results are in agreement with earlier observations on carrots [24, 28], lettuce [9], cilantro leaves [29] and other vegetables [15, 30].

TABLE 1. EFFECT OF IRRADIATION ON THE TOTAL BACTERIA AND YEAST AND MOLD COUNTS OF SHREDDED CARROTS DURING STORAGE

	0 kGy			1 kGy		
Storage time (day)	0	5	10	0	5	10
Total bacteria count (log CFU/g)	5.72	7.77	8.27	3.84	5.22	5.23
Yeast and mold count (log CFU/g)	5.76	8.15	8.00	3.70	4.43	5.21

No *L. monocytogenes* or *E. coli* were detected in any sample whether irradiated or non-irradiated just after irradiation or during the entire storage period of 10 days in minimally processed carrots. Lacroix and Lafortune [31] determined that the application of 0.6 kGy under MAP was able to assure complete inhibition of *E. coli* in grated carrots. However, a dose of 0.9 kGy was necessary to achieve the same effect when the treatment was done under air.

The fungal count of control sample was 5.76 log CFU/g initially and increased to 8.15 log CFU/g and 8.00 log CFU/g after 5 and 10 days of storage, respectively (Table 1). The fungal count of irradiated sample was 3.70 log CFU/g initially and increased to 5.21 log CFU/g after storage period.

3.1.4. Chemical and sensory analysis during storage

The pH values ranged from 6.33 to 5.38 during the storage period in non-irradiated sliced carrots. For the irradiated sample, the pH increased from 6.15 to 6.64. While the soluble solid content (^{0}Brix) varied from 7.10 to 5,84 in non-irradiated samples, in irradiated samples from 6.93 to 6.27 through the storage (data are not shown).

At the beginning of the storage period, the Huntercolor L, a* and b* values in non-irradiated and irradiated samples were found as follows: 63.103; 26.513; 41.500 and 63.167; 25.087; 38.867, respectively. At the end of the storage, the Hunter color L, a* and b* values in non-irradiated and irradiated samples were found as follows: 62.197; 21.673; 33.260 and 65.530; 20.590; 33.583, respectively. Juiciness (w/w, %) changed from 48.61 to 45.41 in non-irradiated samples and from 46.31 to 48.90 in irradiated samples. The moisture loss (w/w, %) varied from 9.01 to 7.94 in non-irradiated samples while from 9.05 to 8.10 in irradiated samples during the storage (data are not shown).

Irradiation and storage showed no significant effect on soluble solid content, Hunter color L value, juiciness and moisture loss of the sliced carrot samples ($p > 0.05$).

For pH, the interaction between irradiation and day was significant. Although no significant difference was observed between the pH values at the first and fifth day in non-irradiated samples ($p > 0.05$), the pH value at the end of the storage differed from the first and fifth day ($p \leq 0.05$). For the irradiated samples, the difference in pH between the first and last day and between the fifth and last day was found to be significant ($p \leq 0.05$). Through the storage period, no significant difference was observed between the non-irradiated and irradiated samples in pH values, but for the last day the difference was significant ($p \leq 0.05$).

While changes in a* and b* values through the storage period was observed as significant, irradiation had no effect on these values ($p > 0.05$). The a* and b* values at the first day were significantly different from the fifth and last day. The a* and b* values at the fifth and last day were similiar.

Dose of 1.0 kGy did not cause softening of shredded carrots and sensory attributes were not adversely affected. Farkas et al. [30] reported that 1 kGy dose of gamma radiation was sufficient to reduce the

bacterial load, improve the microbiological shelf life and extend the sensorial quality of precut peppers and carrots. However, doses up to 2 kGy did not lessen the sensory quality of carrots [24, 32].

TABLE 2. INTERACTION BETWEEN IRRADIATION DOSE AND STORAGE PERIOD FOR ODOR, TASTE AND GENERAL ACCEPTABILITY IN SHREDDED CARROT SAMPLES

Irradiation Dose	Days	Sensory Analysis		
		Odor	Taste	General Acceptability
0 kGy	0	$5,583^{Aa}$	$6,000^{Aa}$	$6,028^{Aa}$
	10	$3,556^{Ab}$	$3,444^{Ab}$	$3,693^{Ab}$
1 kGy	0	$5,167^{Aa}$	$5,972^{Aa}$	$6,028^{Aa}$
	10	$5,389^{Ba}$	$5,278^{Ba}$	$5,833^{Ba}$

a;b: means within columns for each carrot (irradiated or non-irradiated) in days followed by the same letter are not significantly different at $p > 0.05$; A;B: means within columns for each day (irradiated or non-irradiated) not followed by the same letter are significantly different at $p \leq 0.05$.

The results of the sensory analysis is given in Table 2. While significant differences in odor, taste and general acceptability of the non-irradiated samples of shredded coconut between the first and last day was observed ($p \leq 0.05$), no significant difference was observed for the irradiated samples ($p > 0.05$). Chaudry et al. [33] determined that minimally processed carrots may be treated with 2 kGy dose to keep the appearance and flavor quality acceptable with extension of shelf life up to 14 days at refrigerated temperature.

3.2. Mixed salad

3.2.1. Microbiological analysis of raw mixed salads

Analysed raw mixed salad presented high level of total viable cell and total yeast and molds counts. *Listeria* spp. was detected in all samples. In only one sample *E. coli* O157 and *Salmonella* spp. were detected. *E. coli* O157 was non H7.

Results of total viable cell (TVC) and total yeast and molds (TYM) count are given in Table 3.

TABLE 3. TVC AND TYM IN MIXED SALAD

	TVC log CFU/g	TYM log CFU/g
A1	7.32	6.97
A2	7.15	6.90
A3	7.11	7.11
A4	7.26	6.94
A5	7.70	6.95
A6	7.20	7.08
A7	7.18	7.20
A8	7.20	7.15
A9	7.18	7.18
A10	7.23	7.54

3.2.2. Result of microbiological analysis of commercially packaged mixed salad during storage at 5ºC

Since shelf life is normally five days at 5^{o}C, this trial was carried out for ten days for the determination of extra shelf life.

TVC and TYM counts for the determination the shelf life of commercial packages before irradiation are given in Table 4.

TABLE 4. TVC AND TOTAL YEAST AND MOLDS OF MIXED SALAD IN COMMERCIAL PACKAGES DURING THE STORAGE

Day	Total viable count log CFU/g		Total yeast and mold log CFU/g	
	Replicate I	Replicate II	Replicate I	Replicate II
0	7.28	6.94	6,.	6.64
1	6.86	6.67	6.66	6.28
3	7.11	7.11	6.83	7.04
5	7.41	8.18	6.93	8.00
7	7.60	8.00	7.81	7.99
10	7.61	7.76	7.6	7.72

Results showed that processed mixed salad in the commercial package sold in Turkey have high level total viable count and total yeast and mold. These results point out that the processing plant has a sanitizing problem related to the processing of mixed salad. In addition, levels of TVC and TYM increased during refrigerated storage.

TABLE 5. COLIFORM AND E. COLI COUNTS OF COMMERCIAL PACKAGED MIXED SALAD DURING STORAGE AT 5ºC BEFORE IRRADIATION TREATMENT

Day	Coliform count MPN/g		E. coli count MPN/g	
	Replicate I	Replicate II	Replicate I	Replicate II
0	4.38	3.38	1.63	1.63
1	3.38	3.04	0.95	<0.48
3	3.32	3.63	0.60	0.60
5	3.38	3.97	<0.48	0.60
7	3.38	2.66	<0.48	<0.48
10	3.04	3.66	0.60	<0.48

Table 5 shows that coliform count of replicate I was higher than that of replicate II at the beginning of storage, and in replicate I samples, coliform count decreased during storage. *E. coli* counts of commercial mixed salad samples decreased during storage.

Minimally processed, chilled vegetables are frequent carriers of *Pseudomonas*, *Enterobacteriaceae*, and lactic acid bacteria as natural microbiota. The high moisture and numerous cut surfaces of precut vegetables provide excellent conditions for the growth of microorganisms [14].

Mixed salad exhibited large variations in microbiological quality; indicating possible contamination during growth, harvesting and post-harvest handling, transportation and storage conditions, controlled by various factors. Initial total bacterial counts were determined as approximately 10^7 CFU/g in mixed salad samples. The use of sodium hypochloride as a sanitizer reduced the total bacterial count by approximately 3 log (pre-sanitation 7.11 log CFU/g and post sanitation 4.18 log CFU/g). Goularte et al. [34] reported that the use of sodium hypochloride as a sanitizer reduced the population of Enterobacteriaceae by 2 log and washing thoroughly with water reduced those microorganisms by 1 log. The effectiveness of treatment with water containing up to 200μg /ml chlorine in reducing numbers of naturally occurring microorganisms and pathogenic bacteria is minimal; often not exceeding 2 logs on lettuce [35].

3.2.3. Determination of D_{10}-values of the pathogens

Figs. 3 and 4 show the inactivation of *S.* Enteritidis and *L. monocytogenes* inoculated in minimally processed mixed salad irradiated up to 2.5 kGy. The D_{10}-values for *L. monocytogenes* (0.26 kGy) were higher than those for *S.* Enteritidis (0.19 kGy). Goularte et al. [34] determined that the D-values of *Salmonella* spp. were 0.16, 0.22 and 0.23 kGy on lettuce. Farkas [36] obtained D-values of 0.46 and 0.59 kGy for *Salmonella* using cauliflower and cooked potato, respectively. In general, the D-values determined by several authors varied from 0.10 to 1.29 kGy for *Salmonella* spp. [36, 37].

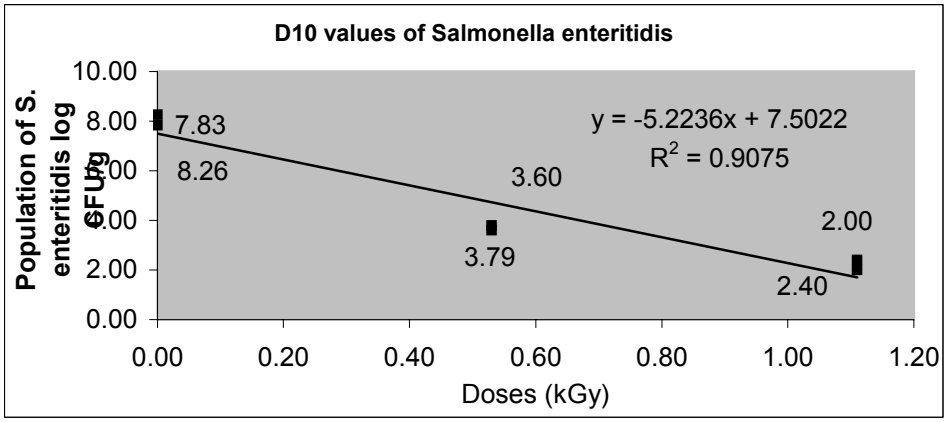

FIG. 3. Radiation sensitivity of S. Enteritidis inoculated on mixed salad.

The dose of 1 kGy resulted in five-log cycles and four-log cycles of reduction of *S.* Enteritidis and *L. monocytogenes*, respectively. Thus, approximately 1.5 kGy would be adequate to eliminate the load of these pathogens naturally present in mixed salad.

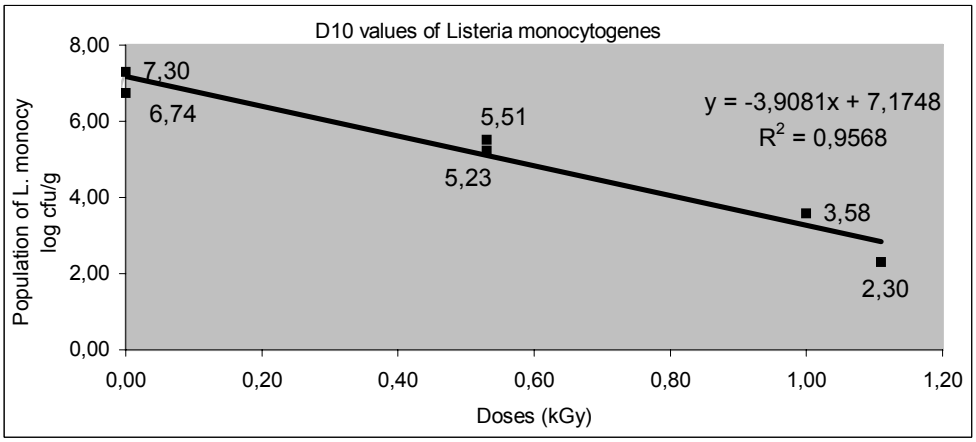

FIG. 4. Radiation sensitivity of Listeria monocytogenes.

3.2.4. Storage after irradiation treatment

Effect of irradiation treatment on TYM and TVC and counts of mixed salad during storage period is shown in Tables 6 and 7.

TABLE 6. TYM COUNTS OF COMMERCIALLY PACKAGED MIXED SALAD DURING STORAGE AT 5^0C AFTER IRRADIATION TREATMENT (log CFU/g)

Replicate	Day	Irradiation doses				
		0	1.0	1.5	2.0	2.5
1	0	7.15	<2	<2	<2	<2
	1	7.08	<2	<2	<2	<2
	3	7.2	<2	<2	<2	<2
	5	6.89	<2	<2	<2	<2
	7	7.66	3.86	3.18	<2	<2
2	0	6.85	<2	<2	<2	<2
	1	6.66	<2	<2	<2	<2
	3	6.8	<2	<2	<2	<2
	5	7.69	<2	<2	<2	<2
	7	7.26	3.86	3.18	<2	<2

Table 6 shows that initial TYM counts of non-irradiated samples of mixed salad were very high, about 7 log CFU/g, and slight increase was observed during storage. TYM counts of all irradiated samples were below the detection limit (2 log CFU/g) until the seventh day of refrigerated storage. In samples irradiated at 1 and 1.5 kGy, surviving yeasts and molds were able to regrow at the end of storage period.

The initial total viable cell counts of packaged mixed salad samples were also very high (Table 7). Irradiation with 1.0 kGy was able to reduce TVC below detection limit. Surviving bacteria were able to regrow after 5 days of storage in samples irradiated with 1.0 and 1.5 kGy, while irradiation with 2.0 and 2.5 kGy resulted in total bacterial counts below detection limit during 13 days of storage.

TABLE 7. TVC COUNTS OF COMMERCIALLY PACKAGED MIXED SALAD DURING STORAGE AT 5OC AFTER IRRADIATION TREATMENT (log CFU/g)

Replicate	Day	Irradiation doses				
		0	1.0	1.5	2.0	2.5
1	0	7.36	<2	<2	<2	<2
	1	7.08	<2	<2	<2	<2
	3	7.18	<2	<2	<2	<2
	5	7.15	3.76	3.49	<2	<2
	7	8	3.32	3.18	<2	<2
	10	7.08	4.15	4.08	<2	<2
	13	7.9	4.74	4.18	<2	<2
2	0	6.86	<2	<2	<2	<2
	1	6.7	<2	<2	<2	<2
	3	6.99	<2	<2	<2	<2
	5	7.04	<2	<2	<2	<2
	7	7.81	3.62	<2	<2	<2
	10	8.26	4.26	<2	<2	<2
	13	7.28	5.84	4.23	<2	<2

Coliform and total psychrophilic counts for the determination the shelf life of commercial packages after irradiation are given in Tables 8 and 9.

TABLE 8. COLIFORM COUNTS OF COMMERCIALLY PACKAGED MIXED SALAD DURING STORAGE AT 5^{O}C AFTER IRRADIATION TREATMENT (log MPN/g)

Replication	Day	Irradiation doses				
		0	1.0	1.5	2.0	2.5
1	0	5.04	<0.48	<0.48	<0.48	<0.48
	1	4.18	<0.48	<0.48	<0.48	<0.48
	3	3.04	<0.48	<0.48	<0.48	<0.48
	5	2.66	<0.48	<0.48	<0.48	<0.48
	7	2.66	<0.48	<0.48	<0.48	<0.48
	10	2.32	<0.48	<0.48	<0.48	<0.48
	13	2.18	<0.48	<0.48	<0.48	<0.48
2	0	5.04	<0.48	<0.48	<0.48	<0.48
	1	3.63	<0.48	<0.48	<0.48	<0.48
	3	2.66	<0.48	<0.48	<0.48	<0.48
	5	2.32	<0.48	<0.48	<0.48	<0.48
	7	2.66	<0.48	<0.48	<0.48	<0.48
	10	2.38	<0.48	<0.48	<0.48	<0.48
	13	2.18	<0.48	<0.48	<0.48	<0.48

In non–irradiated samples of mixed salad coliforms were present in high numbers (5.04 log MPN/g), and counts decreased during 13 days of storage. Coliform bacteria were not detected in any of the irradiated samples.

TABLE 9. TOTAL PSYCROPHILIC COUNTS OF COMMERCIALLY PACKAGED MIXED SALAD DURING STORAGE AT 5^{O}C AFTER IRRADIATION TREATMENT (log CFU/g)

Replicate	Day	Irradiation doses				
		0	1.0	1.5	2.0	2.5
1	0	6.15	<2	<2	<2	<2
	1	6.04	<2	<2	<2	<2
	3	6.18	<2	<2	<2	<2
	5	6.2	4.04	3.04	<2	<2
2	0	4.4	<2	<2	<2	<2
	1	4.11	<2	<2	<2	<2
	3	6.93	<2	<2	<2	<2
	5	6.41	4.04	3.92	<2	<2

Table 9 shows that psychrophilic bacteria were reduced by low dose irradiation below the detection limit. Growth of psychrophilic bacteria was observed in control samples after three days of storage and after five days of storage in samples irradiated with 1.0 and 1.5 kGy.

Contamination of psychrotrophic pathogens and their multiplication during storage is of major concern to food technologists [3]. Except *Listeria* presence/absence test, *Listeria* spp. was counted on Palcam listeria selective agar in the non-irradiated samples. Initial counts of *Listeria* were about 5 log CFU/g. Although 1 kGy irradiation treatment reduced the number of *Listeria* by four logs, *Listeria* spp was detected in 1.0 and 1.5 kGy irradiated samples during the storage period. Determination of D_{10}-value for *Listeria monocytogenes* confirmed that 1.5 kGy irradiation is not enough to eliminate this pathogen in the mixed salad when present in high numbers. *Listeria* spp. is a big problem in Turkey. Combined process (combination of irradiation and other suitable technology) and HACCP must be implemented to improve safety of the MP vegetables in Turkey.

3.2.4.1. Determination of ascorbic acid

Initial ascorbic acid concentration of non-irradiated samples was determined as 19.58 ppm. When irradiated with 1 kGy, it was determined as 10.46 ppm. Radiation dose of 1 kGy caused 47% decrease in ascorbic acid content. Abdellaoui et al. [38] reported that the doses adequate for quarantine purposes showed no significant loss in the Vitamin C content of several citrus fruit. Moshonas and Shaw [7] demonstrated that 1 kGy of gamma irradiation had no effect on Vitamin C content of grapefruit. It is possible that Vitamin C may not be the primary defense mechanism of fruit against the oxidative stress induced by gamma irradiation in early harvest. Yanez et al. [39] stated that a previous study reported that irradiation doses of more than 0.25 – 2.5 kGy in grapefruit showed a decreasing trend in Vitamin C content [40].

Kilcast [41] reported that irradiation can cause a partial conversion of ascorbic acid to dehydroascorbic acid which could account for the losses of ascorbic acid observed. It is also possible that some degradation of the vitamin could have occurred through its attack by free radicals [42].

3.2.4.2. Sensory Analysis

1.5 kGy dose of irradiation did not cause softening of the mixed salad and sensory attributes were not adversely affected (Table 10). Our results are in agreement with those of Prakash et al. [12], who noted no effect of radiation doses on the sensory attributes of lettuce and those of Fan et al. [29], who reported no significant effect on the visual quality, decay, color, texture and reduction in bioload of cilantro leaves irradiated at 2 kGy dose. They further stated that foodborne pathogens often observed on cilantro leaves may be inactivated by 2 kGy dose.

According to Niemira et al. [27], irradiation doses up to 1.0 kGy had no significant effect on color of endive leaf material either taken from the leaf edge or the leaf midrib.

TABLE 10. SENSORY PROPERTIES (ODOUR & FLAVOUR) OF MINIMALLY PROCESSED MIXED SALAD AT DIFFERENT IRRADIATION DOSES

	Treatments (kGy)				
	0	1.0	1.5	2.0	2.5
Odor	2.190 A	2.080 AB	2.030 AB	1.930 BC	1.850 C
Flavor	2.170 A	2.080 AB	1.970 BC	1.900 BC	1.850 C

All readings are the means of 14 observations. Figures with the same letters are not statistically different at 95 % level

The results showed that there were not large differences between controls and irradiated (with 1.0 and 1.5 kGy) mixed salad samples in odor and flavour.

3.3. Soybean Sprouts

3.3.1. Microbiological analysis of commercial packaged soybean sprouts

Microbiological analysis for total viable count (TVC), total yeast and molds (TYM), total coliform and *E. coli* were performed on the sprout samples in commercial packages to examine the shelf life profile during storage. The results are given in the following Tables and Figures.

TABLE 11. VC AND TYM OF COMMERCIAL SOYBEAN SPROUTS PACKAGES DURING STORAGE AT 5°C

Day	Total Viable Cell (log CFU/g)*	Total Yeast and Mold (log CFU/g)**
0	8.16^{c}	6.63
1	8.28^{bc}	6.18
3	8.36^{bc}	6.43
5	8.40^{a}	6.62
7	8.61^{ab}	6.65
10	8.74^{abc}	6.64

*means within column for each day (TVC) not followed by same letter are significantly different ($p \leq 0.05$)
** In all samples only yeast colonies were observed.

As seen in Table 11, initial total viable count of the soybean sprouts was approximately 8.2 log CFU/g. During storage up to 10 days, there was slight increase in bacterial counts. Counts by two replicates at the end of storage increased significantly by approximately 0.5 log CFU/g.

The change in total viable cell count was significant by statistical analysis. Total viable cell count changed by storage period ($p=0.043$; $p\leq0.05$).

The level of yeast and mold contamination initially was 6.63 log CFU/g (Table 11). Total yeast and mold counts did not change by the storage period ($p=0.078$, $p\leq0.05$) statistically.

Total coliform counts were very high (average 6.78 log MPN/g) and the level of total colifom did not change significantly ($p=0.979$, $p\leq0.05$) during storage period (Table 12).

Initial *E.coli* count was 3.62 log MPN/g and the counts did not change significantly during storage period ($p=0.212$, $p\leq0.05$).

TABLE 12. TOTAL COLIFORM AND TOTAL E.COLI COUNT OF COMMERCIAL SOYBEAN SPROUTS PACKAGES

Day	Total Coliform Count (log MPN/g)	Total *E.coli* Count (log MPN/g)
0	6.78	3.62
1	6.90	3.63
3	6.79	3.60
5	6.73	3.68
7	6.76	3.72
10	6.72	3.78

Commercially packaged soybean sprout samples were obtained from local markets and examined for microbiological quality and pathogenic microorganisms. Total viable count (TVC), total yeast and molds (TYM), total coliform and *E. coli,* and pathogens (*Salmonella* spp*., L. monocytogenes* and *E. coli* O157H:7) were performed on twelve commercially packaged samples. The results are given in Table 13.

TABLE 13. RESULT OF TVC, TYM AND TOTAL COLIFORM AND E.COLI COUNT OF COMMERCIAL PACKAGES OF SOYBEAN SPROUTS

Sample	Total viable cell	Total yeast and molds	Total Coliform count	Total *E.coli* count
	(log CFU/g)	(log CFU/g)	(MPN/g)	(MPN/g)
1	8.15	7.05	7.97	4.38
2	8.17	6.83	7.18	4.38
3	8.10	6.52	8.08	4.63
4	7.90	6.69	7.30	5.45
5	7.78	6.18	6.87	3.63
6	7.54	6.28	6.36	4.38
7	7.63	6.18	6.63	3.38
8	7.89	6.53	6.63	4.63
9	7.68	6.47	6.97	3.97
10	7.97	6.70	7.17	4.32
11	8.12	6.51	6.87	5.17
12	8.03	6.24	7.30	3.63

Analysis showed that soybean sprout samples had high level of total viable cell and total yeast and molds counts. Also, total coliform and *E. coli* counts were very high. The average values of counts for the 12 samples were calculated to be 7.91 log CFU/g, 6.51 log CFU/g, 7.11 log MPN/g and 4.33 log MPN/g for total viable cell, yeast and molds, coliform and *E. coli*, respectively.

L. monoctogenes and *E. coli* O157 H:7 were not detected in any of the samples, but *Salmonella* spp. was detected in 9 samples out of twelve samples.

These results showed that, in Turkey, commercially packaged soybean sprouts have high levels of microbial contamination. These results pointed out the sanitation problem during processing of soybean sprout. However, after refrigerated storage for ten days, increase in microorganism counts was not high.

3.3.2. Determination of D_{10}-values of the pathogens

D_{10}-values for *L. monocytogenes*, *S.* Enteritidis and *E. coli* O157:H7 in soybean sprout samples were determined. Radiation sensitivity of these pathogenes are shown in Figures 5, 6, and 7.

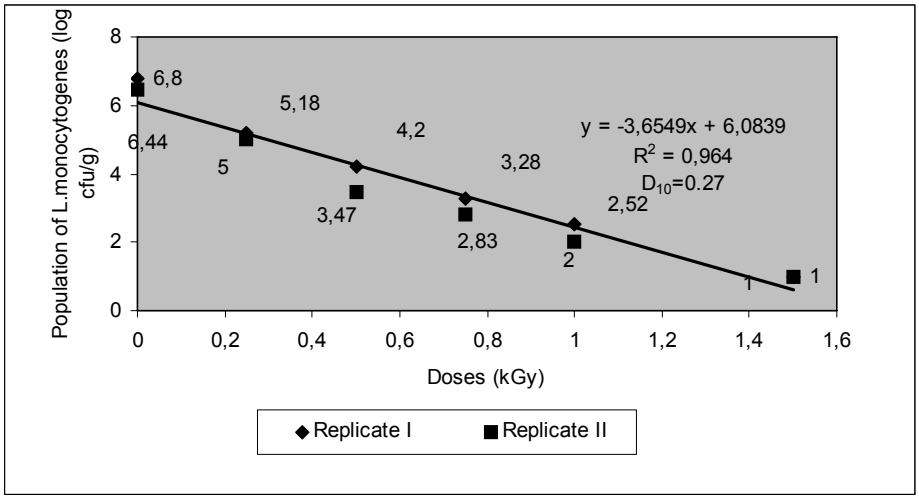

FIG 5. Radiation sensitivity of L. monocytogenes inoculated on soy sprouts.

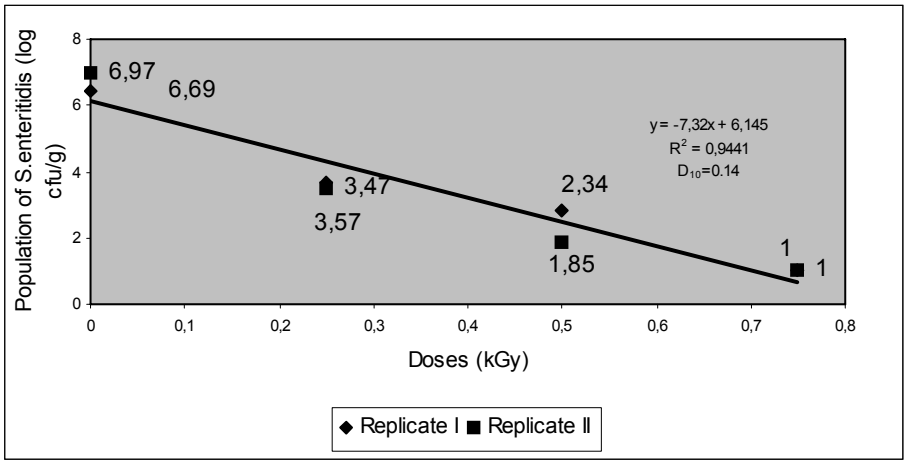

FIG. 6. Radiation sensitivity of s. Enteritidis inoculated on soy sprouts.

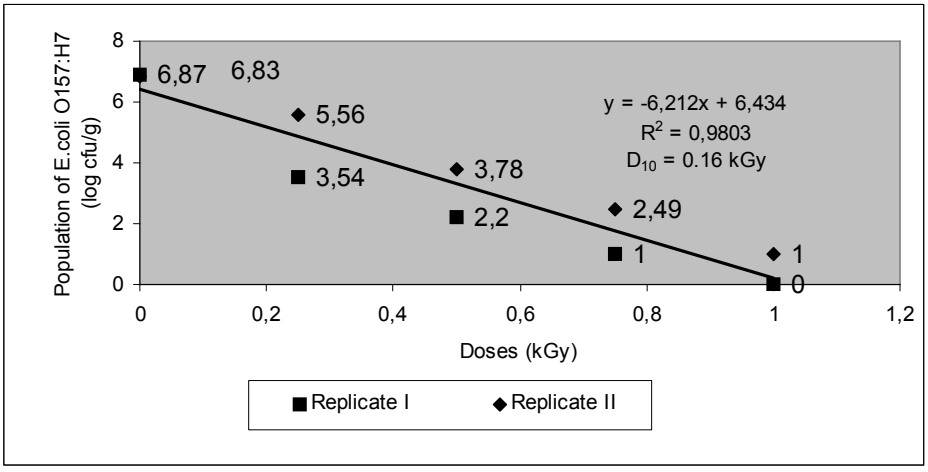

FIG 7. Radiation sensitivity of E. coli O157H:7 inoculated on soy sprouts

D_{10} -values were determined for *L. monocytogenes*, S. enteritidis and *E. coli* O157:H7 as 0.27, 0.14 and 0.16 kGy, respectively. Compared to that of the other two pathogens *L. monocytogenes* was found to be the most resistant to irradiation process.

3.3.3. Microbiological analysis of commercial packaged soybean sprouts after irradiation during storage period

Counts for the total viable counts, yeast and molds, coliform and *E. coli* counts were determined on the commercially packaged soybean sprouts after irradiation (0.0, 1.0, 1.5, 2.0 and 2.5 kGy) during storage period (0, 1, 3, 5, 7, 10, 13 days). Also, detection of pathogenic microorganisms (*Salmonella* spp., *E.coli* O157H:7 and *L.monocytogenes*) were performed on the soybean sprouts. The results are shown in Figures 8-11.

Total viable cell, total yeast and mold counts, and coliform and *E. coli* counts were below the detection limit (<10 log CFU/g and <0.30 log MPN/g) for the soybean sprout samples that were irradiated at 2.0 and 2.5 kGy doses. Also *E. coli* cells were below the detection limit (<0.30 log MPN/g) when irradition dose of 1.0 kGy was used.

Low dose irradiation treatment (1.0 and 1.5 kGy) in soybean sprout samples caused a reduction in the total viable cell counts (Figure 8). Growth of total cell counts was observed in irradiated samples during storage, but at the end of 13 days storage the TVC of irradiated samples was 3-4 log-cycles less than that of the control sample.

In the statistical analysis for total viable cell count, the interaction between irradiation doses and storage period was significant ($p=0.001$, $p \leq 0.05$) as shown in Table 14. In non-irradiated soybean samples no significant growth in TVC was observed until 10 days of storage ($p>0.05$). TVC of irradiated samples (1.0 and 1.5 kGy) were significantly lower than in non-irradiated samples, and significant growth was observed in 1.0 kGy samples after three days and in 1.5 kGy samples after five days of storage.

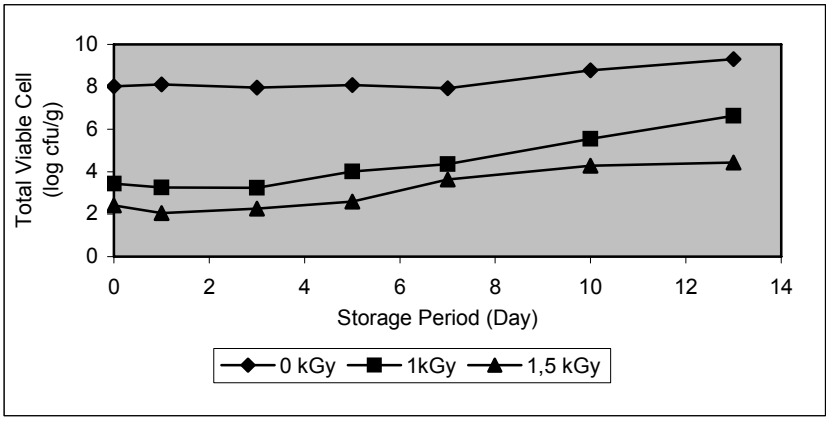

FIG. 8. TVC counts of commercially packaged soybean sprouts during storage at 5^0C after irradiation treatment.

TABLE 14. TVC IN COMMERCIALLY PACKAGED SOYBEAN SPROUT SAMPLES DURING STORAGE AT 5^0C AFTER IRRADIATION TREATMENT

Doses	Storage Period (Day)						
(kGy)	0	1	3	5	7	10	13
0	8.02^{Ba}	8.11^{Ba}	7.965^{Ba}	8.09^{Ba}	7.94^{Ba}	8.78^{Aa}	9.31^{Aa}
1	3.44^{DEb}	3.26^{Eb}	3.25^{Eb}	4.01^{Cdb}	4.58^{Cb}	5.55^{Ab}	6.63^{Bb}
1.5	2.41^{Cc}	2.05^{Cc}	2.26^{Cc}	2.59^{Cc}	3.63^{Bc}	4.28^{Ac}	4.43^{Ac}

Means within rows for each day not followed by the same letter (upper case) are significantly different at ($p \leq 0.05$).
Means within columns for each irradition doses followed by the same letter (lower case) are not significantly different at ($p>0.05$)

The effect of irradiation on the reduction of yeast and mold counts of soybean sprout samples is given in Figure 9. The reduction in initial counts was approximately 4 log CFU/g.

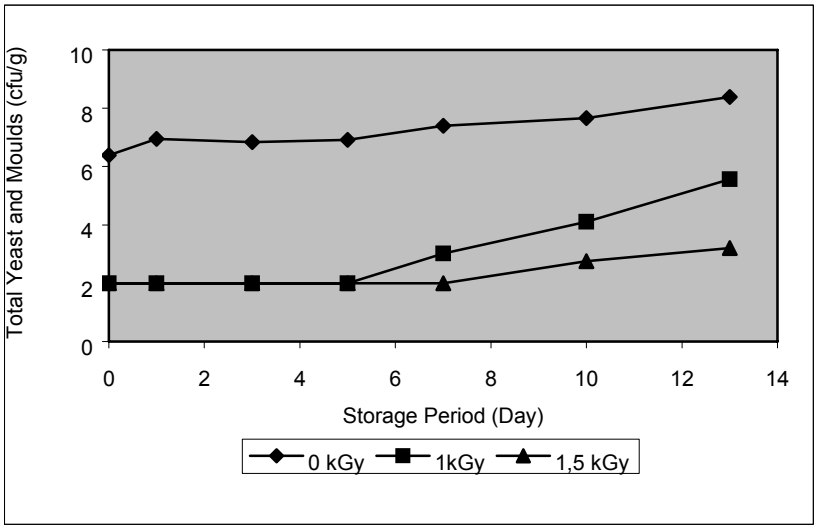

FIG. 9. TYM counts of commercially packaged soybean sprouts during storage at 5^0C after irradiation treatment.

The interaction between doses and days by Duncan's test is given in Table 15. Statistically significant ($p=0.00$, $p \leq 0.05$) growth of yeast and mold counts was observed in 1.0 kGy irradiated soybean sprout samples after five days, the increase was approximately 3.50 log cfu/g at the end of storage. Similar trend for yeast and molds counts was observed when irradiation dose of 1.5 kGy was applied. At this dose, counts did not show differences until 7^{th} day, and increased by approximately 1.0 log cfu/g to the 13^{th} day.

TABLE 15. TMY IN COMMERCIALLY PACKAGED SOYBEAN SPROUT SAMPLES DURING STORAGE AT 5^0C AFTER IRRADIATION TREATMENT

Doses	Storage Period (Day)						
(kGy)	0	1	3	5	7	10	13
0	6.39 ^{Da}	6.95 ^{Ca}	6.84 ^{Ca}	6.92 ^{Ca}	7.40 ^{Ba}	7.66 ^{Ba}	8.39 ^{Aa}
1	2.00 ^{Db}	2.00 ^{Db}	2.00 ^{Db}	2.00 ^{Db}	3.02 ^{Cb}	4.10 ^{Bb}	5.57 ^{Ab}
1.5	2.00^{Cb}	2.00^{Cb}	2.00^{Cb}	2.00^{Cb}	2.00 ^{Cc}	2.76 ^{Bc}	3.21 ^{Ac}

Means within rows for each day not followed by the same letter (upper case) are significantly different at ($p \leq 0.05$).
Means within columns for each irradition doses followed by the same letter (lower case) are not significantly different at ($p>0.05$)

Figure 10 shows the coliform counts after irradiation during storage period. The statistical evaluation of total coliform counts is given in Table 16. No significant difference was observed between storage days, but significant differences was found for irradiation doses ($p=0.00$; $p\leq0.05$). Irradiation at 1.0 and 1.5 kGy doses decreased total coliform counts approximately by 5.3 and 6.0 log MPN/g, respectively. Total coliform counts, both for irradiated and non-irradiated samples, did not change during storage period ($p>0.05$).

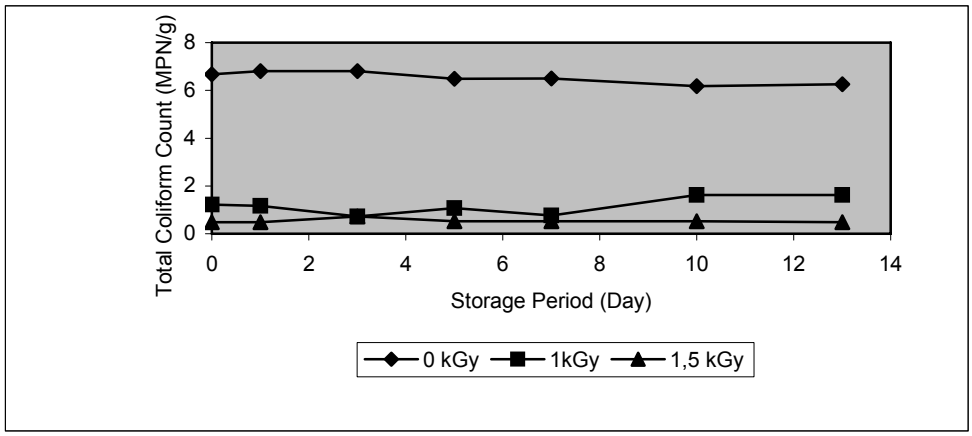

FIG. 10. Total coliforms counts of commercially packaged soybean sprouts during storage at 5^0C after irradiation treatment.

TABLE 16. CHANGE IN COLIFORM COUNTS IN COMMERCIALLY PACKAGED SOYBEAN SPROUT SAMPLES AFTER IRRADIATION TREATMENT

	Irradiation Doses (kGy)		
	0.0	1.0	1.5
coliform (log MPN/g)	6.527 [a]	1.173 [b]	0.5314 [c]

Means within raws for each not followed by the same letter are significantly different at ($p \leq 0.05$)

Because *E.coli* counts were below detection limit in all the samples irradiated above 1.0 kGy doses, results for the non-irradiated samples (control) are given Figure 10. According to statistical analysis, total *E. coli* count did not change during storage period (p=0.924, p>0.05).

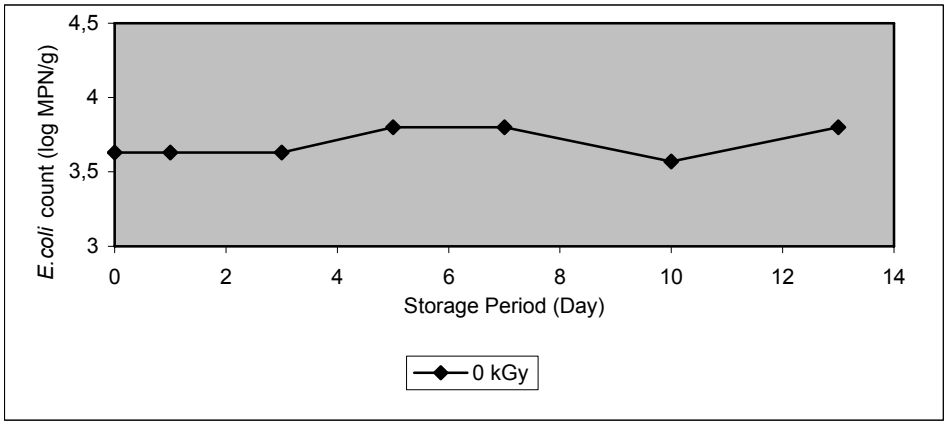

FIG. 11. Total E. coli counts of control samples of commercially packaged soybean sprouts at 5^0C storage period.

In addition, the samples were tested for pathogenic microorganisms (*Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7) besides the microbiological examination of soybean sprouts after irradiation during storage period. Among the pathogens tested, while *L. monocytogenes* and *E. coli* O157:H7 were not detected on the samples at the first day and during storage period, *Salmonella* spp. was detected in non-irradiated samples and in samples irradiated at 1.0 kGy during storage period. *Salmonella* spp. was not observed in 1.5, 2.0 and 2.5 kGy irradiated samples. *Salmonella* analysis during storage period shows that 1.0 kGy irradiation was not enough to eliminate this pathogen in soybean sprout samples.

3.3.4. Determination of ascorbic acid on commercial packaged soybean sprouts

Vitamin C content of non-irradiated (control group) soybean sprout samples were found to be 8.81 ppm. When irradiation dose of 1.0 kGy was used, vitamin C was found to be 2.68 ppm. Vitamin C was reduced approximately to 1/3 with 1.0 kGy dose.

3.3.5. Sensory Analysis

The organoleptic quality of irradiated and control soybean sprout samples were examined in the experiment during storage period (0, 1st, 3 rd and 5th days).

Irradiation doses up to 2.5 kGy and storage period had no effect on the appearance, odor, taste, texture, and general acceptibility of the soybean sprout samples. Statistical evaluation of the sensory analysis showed that there was no interaction between dose and days, and the organoleptic quality of soybean sprouts did not change by the storage days and irradiation doses ($p>0.05$). The panelists were pleased with the organoleptic properties of all the samples at each day.

4. CONCLUSIONS

4.1. Shredded carrots

It can be concluded from these studies that a dose of 1.0 kGy is sufficient to reduce the non-pathogenic and the pathogenic bioload of minimally processed carrots without affecting the sensorial quality. D_{10}-values for *L. monocytogenes* and *E. coli* were determined as 0.29 kGy and 0.29 kGy, respectively. At 1.0 kGy irradiation, approximately 4-log reduction in *E. coli* and *L. monocytogenes* counts and lack of adverse effects on sensory attributes indicate that low dose irradiation can improve food safety of shredded carrots for sale or food service. In addition, *Listeria* and *E. coli* were not recovered from any sample during the storage periods, even after selective enrichment, for product treated with 1 kGy. Treatment with 1 kGy was effective to reduce the pathogens in the shredded carrots.

While irradiation had an effect only on pH, the other chemical properties, soluble solid content, HunterLa*b* values, juiciness and moisture loss were not affected by irradiation. HunterLa*b* values a* and b* changed during the storage period.

Irradiation had no effect on the appearance and texture of sliced-carrot samples. Panelists observed that storage affected the sensory quality of the samples. Panelists preferred the irradiated to the non-irradiated sliced-carrot samples regarding the odor, taste and general acceptability.

4.2. Mixed salad

It can be concluded from these studies that a dose of 1.5 kGy is sufficient to maintain the sensorial quality and the reduction of pathogenic bioload of minimally processed mixed salad. The five-log reduction in *S.* Enteritidis counts and 4 log reduction in *L. monocytogenes* counts and lack of adverse effects on sensory attributes indicate that low dose irradiation can improve food safety of mixed salad for sale or food service.

4.3. Soybean sprouts

Total viable cell, yeast and molds, coliform and *E. coli* counts changed by storage period. D_{10}-values were determined for *L. monocytogenes*, *S.* Enteritidis and *E.coli* O157:H7 as 0.27, 0.14 and 0.16 kGy, respectively. Compared to the other two pathogens, *L. monocytogenes* was found to be the most resistant to irradiation process. Irradiaton process was effective in the reduction of total viable cell, yeast and molds, coliform and *E.coli* counts. Also, shelf-life of the food was increased. *Salmonella* spp. was eliminated on the soybean sprout samples with irradiation at 1.0 kGy dose. 1.0 kGy dose decreased Vitamin C content approximately by 1/3. Irradiation had no effect on the organoleptic properties of soybean sprout samples.

In conclusion, the radiation process can be used to increase the shelf-life and inhibit microbial growth of shredded carrots, mixed salad and soybean sprouts. D_{10}-values of each concerned vegetables were observed differently. 1.0 kGy irradiation dose was effective to reduce pathogens in the shredded carrots and soybean sprouts however 1.5 kGy was sufficient in the mixed salad. The sensory quality of the fresh vegetables was not affected by applied irradiation doses.

REFERENCES

[1] OHLSSON, T., Minimal processing of the future - an overview, Trends in Food Sci. Technol. **5** (11) (1994) 341-344.

[2] NGUYEN-THE, C., CARLIN, F., The microbiology of minimally processed fresh fruits and vegetables, Crit. Rev. Food Sci. Nutr. **43** (4) (1994) 371-401.

[3] BHARATHI, M.N., RAMESH, M.N., VARADARAJ, M.C., Predicting the behavioral pattern of *Escherichia coli* in minimally processed vegetables, Food Cont. **12** (2001) 275-284.

[4] KAMAT, A., K., PINGULKAR, K., BHUSHAN, B., GHOLAP, A., THOMAS, P., Potential application of low dose gamma irradiation to improve the microbiological safety of fresh coriander leaves, Food Control **14** (2003) 529-537.

[5] BEUCHAT, L.R., *Listeria monocytogenes*: incidence in vegetables, Food Control **7** (1996) 23-228.

[6] BRACKETT, R.E., Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In: Wiley, R. C. (Ed.), Minimally Processed Refrigerated Fruits and Vegetables, Chapman & Hall, New York (1994) 269-312.

[7] MOSHONAS, M.G., SHAW, P.E., Effects of low dose gamma irradiation on grape-fruit products, J. Agric. Food Chem. **32** (1984) 1098–1101.

[8] WEISSINGER, W.R., CHANTARAPANONT, W., BEUCHAT L.R., Survival and growth of *Salmonella* baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as sanitizer, Int. J. Food Micro. **62** (2000) 123-131.

[9] TAORMİNA, P.J., BEUCHAT, L.R., SLUTSKER, L., Infections associated with eating seed sprouts: an international concern, Emerging Infect. Dis**. 5** (5) (1999) 626-634.

[10] ROSAS, C.J., ESCARTİN, E.F., Incidence and germicide sensitivity of *Salmonella typhi* and *Vibrio cholerae* O1 in alfalfa sprouts, J. Food Saf. **19** (1999) 137-146.

[11] National Advisory Committee on Microbiological Criteria for Foods NACMCF (NACMF), Microbiological safety evaluations and recommendations on sprouted seeds, Int. J. Food Microbiol. **52** (1999) 123-153.

[12] PRAKASH, A., GUNER, A.R., CAPORASO, F., FOLEY, D.M., Effects of low dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere, J. Food Sci. **65** (2000) 549-553.

[13] THAYER, D.W., RAJKOWSKI, K.T., Developments of irradiation of fresh fruits and vegetables, Food Technol. **53** (11) (1999) 62-65.

[14] KADER, A.S., Potential applications of ionizing radiation in postharvest handling of fresh fruits and vegetables, Food Technol. **40** (6) (1986) 117-121.

[15] LANGERAK, D.I., DAMEN, G.A.A., Influence of irradiation on keeping quality of prepacked soup greens stored at 10^0C. In: Food Preservation by Irradiation, International Atomic Energy Agency, Vienna (1978) 275-282.

[16] FARKAS, J., Irradiation of minimally processed foods. In: Molins, R. (Ed), Food Irradiation Principles and Applications, John Wiley & Sons, New York

[17] World Health Organization (WHO), Surface decontamination of fruits and vegetables eaten raw: a review. In: WHO Unpublished Document WHO/FSF/FOS/98.2 (1998).

[18] FENG, P., Rapid methods for detecting food borne pathogens. In: Bacteriological Analytical Manual, AOAC International, Gaithersburg, MD, (1998) App. 1.01– App. 1.16.

[19] International Organization for Standardization (ISO) EN ISO 4833:2003, Microbiology of food and animal feeding stuffs. Colony-count technique at 30 degrees C.

[20] International Organization for Standardization (ISO) EN ISO 6579:2002, Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp.

[21] International Organization for Standardization (ISO) EN ISO 11290-1:1996, Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1: Detection method.

[22] U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, Bacteriological Analytical Manual (BAM) Online, Chapter 4A Diarrheagenic Escherichia coli, www.cfsan.fda.gov.

[23] NIEMIRA, B.A., SOMMERS, C.H., FAN, X., Suspending lettuce type influences recoverability and radiation sensitivity of *E. coli* O157:H7, J. Food Prot. **65** (2002) 1388-1393.
[24] CHERVIN, C.L., BOISSEAU, P., Quality maintenance of ready-to-eat shredded carrots by gamma irradiation, J. Food Sci. **59** (1994) 359-361.
[25] NIEMIRA, B.A., FAN, X., SOKORAI, K.J.B., SOMMERS, C.H., Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *L. innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*), J.Food Prot**. 66** (2003) 993-998.
[26] PRAKASH, A., INTHAJAK, P., HUIBREGTSE, H., CAPORASO, F., FOLEY, D.M., Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery, J. Food Sci. **65** (6) (2000) 1070-1075.
[27] HAGENMAIER, R.D., BAKER, R.A., Microbiological population of shredded carrots in modified atmosphere packaging as related to irradiation treatment, J. Food Sci. **63** (1998) 156-164.
[28] FAN, X., NIEMIRA, B.A., SKORAI, K.J.B., Sensorial, nutritional and microbiological quality of fresh cilantro leaves as influenced by ionizing radiation and storage, Food Res. Int. **36** (2003) 649-760.
[29] FARKAS, J., SARAY, T., MOHACSI-FARKAS, C., HORTI, K., ANDRASSY, E., Effect of low dose gamma radiation on shelf life and microbiological safety of pre-cut/prepared vegetables, Adv.Food Sci. **19** (3/4) (1997) 111-119.
[30] LACROIX, M., LAFORTUNE, R., Combined effects of gamma irradiation and modified atmosphere packaging on bacterial resistance in grated carrots (*Daucus carota*), Radiation Physics and Chemistry **71** (2004) 77–80.
[31] SCANDELLA, D., FOURES, M., Effect de I' ionissation sur le maintien de la qualite des legumes de quatrieme gamme. Ind. Agric. Alim. **104**: (1987) 911-916.
[32] CHAUDRY, M.A., BIBI, N., KHAN, M., KHAN, M., BADSHAH, A., QURESHI, M.J., Irradiation treatment of minimally processed carrots for ensuring microbiological safety, Radiation Physics and Chemistry **71** (2004) 169-173.
[33] GOULARTE, L., MARTINS, C.G., MORALES-AIZPURU, I.C., DESTRO, M.T., FRANCO, B.D.G.M., VIZEU, D.M., HUTZLER, B.W., LANDGRAF, M., Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.), Radiation Physics and Chemistry **71** (2004) 155–159.
[34] ADAMS, M.R., HARTLEY, A.D., COX, I.J., Factors affecting the efficacy of washing procedures used in the production of prepared salads, Food Microbiol. 6 (1989) 69-77.
[35] FARKAS, J., Irradiation as a method for decontaminating food: a review, Int. J. Food Microbiol. **44** (1998) 189-204.
[36] RADOMYSKI, T., MURANO, E.A., OLSON, D.G., MURANO, P.S., Elimination of pathogens of significance in food by low-dose irradiation: a review, J. Food Prot. **57** (1994) 73–86.
[37] ABDELLAOUI, S., LACROIX, M., JOBIN, M., BOUBEKRI, C., GAGNON, M., Effect of gamma irradiation combined with hot water treatment on phytochemical properties, Vitamin C content and organoleptic quality of clementines, Sci. Aliment. **15** (1995) 217-235.
[38] YANEZ, G.M., ARTEGA, G.A., MIRANDA, J.F., PARDO, A., SAMPERE, E., CASTILLO, E., SERRANO, G., Stability of Vitamin C content in grapefruit treated with gamma irradiation, Agroquim. Technol. Aliment. **30 (**1990) 409-415.
[39] PATIL, B.S., VANAMALA, J., HALLMAN, G., Irradiation and storage influence on bioactive components and quality of early and late season 'Rio Red' grapefruit *(Citrus paradisi* Macf.), Postharvest Biology and Technology **34** (2004) 53–64
[40] KILCAST, D., Effect of irradiation on vitamins, Food Chem. **49** (1994) 157-164.
[41] DUODU, K.G., MINNAAR, A., TAYLOR, J.R.N., Effect of cooking and irradiation on the labile vitamins and antinutrient content of a traditional African sorghum porridge and spinach relish, Food Chemistry. **66** (1999) 21-27.

EFFECT OF GAMMA RADIATION ON THE MICROBIOLOGICAL QUALITY OF SEEDS AND SEED SPROUTS

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Abstract

Irradiation (2 kGy) treatment alone or in combination with a decontamination wash regime using calcium hypochlorite, eugenol (oil of clove) or oregano oil did not significantly affect the quality of alfalfa seeds during sprouting. Irradiation (2 kGy) and oregano oil (0.1%) did result in significantly lower total counts during storage of the sprouts for 5 days at 5°C. However, the microbial counts in all cases were > log 8 by day 5 of storage and all the samples appeared spoiled. *Pantoea* spp. was the dominant bacterium present in all samples. These results suggest that even if the initial microbial quality of the seeds is good (< 1 log/g) sufficient microorganisms are present to grow rapidly during the sprouting process, resulting in sprouts with high total counts.

1. INTRODUCTION

One of the first reported outbreaks of human illness associated with seed sprouts was in 1973 [1] with *Bacillus cereus* being the causative agent. Since then there have been many more outbreaks associated with a range of pathogens including *Salmonella* spp. and *Escherichia coli* O157:H7 [2]. It is well established that contaminated seeds are a major source of the problem. The sprouting process, with conditions of high moisture, high nutrients and a temperature of 20-25°C, provides excellent conditions for the rapid multiplication of microorganisms. Even if seeds with a very low initial contamination are used, the resultant sprouts may contain high numbers of pathogens.

A logical approach to control this problem would be to treat the seeds and so eliminate any pathogens before the sprouting process begins. Ideally, any intervention strategy should deliver a 5-log reduction in numbers of *Salmonella* spp. and enterohemorrhagic *E. coli* O157 [3].

There have been numerous studies to determine effective treatment strategies for seed decontamination. A variety of chemical treatments have been evaluated. Many were found to be effective in reducing the initial level of contamination but, to date, none have been successful in delivering a 5-log reduction of both *Salmonella* and *E. coli* O157:H7. For example, free chlorine (2,040 ppm) reduced the population of *S.* Stanley inoculated on to alfalfa seeds from 10^2 colony forming units (CFU)/g to less than 1 CFU/g [4]. In study 2,000 ppm chlorine, 6% H_2O_2 or 80% ethanol reduced *Salmonella* populations on alfalfa seeds by more than 3 logs but did not eliminate the pathogen [5]. Treatments with 20,000 ppm chlorine and a liquid prototype wash product (Fit) reduced the numbers of *Salmonella* and *E. coli* O157:H7 in alfalfa seeds by log 2.5 and log 5.4 respectively [6]. However, neither treatment eliminated completely these pathogens as evidenced by their detection on enrichment of treated seeds. Application of heat to kill pathogens on alfalfa seeds has also been investigated. Treatment of the seeds at 60°C for five minutes was effective in giving approximately a 2 log reduction in numbers of *S.* Stanley but higher temperatures or longer times significantly decreased germination of the seeds (4). The use of gamma radiation to reduce the numbers of *Salmonella* spp. and strains of *E. coli* O157:H7 in seed sprouts has been investigated by Rajkowski and Thayer [7]. Radiation D_{10} values for various *Salmonella* isolates varied between 0.46 and 0.54 kGy and between 0.30 and 0.34 kGy for *E. coli* O157:H7 isolates. This would suggest that a radiation dose of 2.7 kGy would be required to achieve the target of a 5-log reduction in the pathogens. It is not known what effect this treatment would have on the sensory quality of the sprouts. Also, there is little published information on the effect of irradiation on the microbiological quality and sprouting efficiency of seeds. Another approach to the problem of pathogen contamination could be the irradiation of the seed

prior to sprouting. In addition, other decontamination washes have been proposed, including the use of natural essential oils.

The key objectives of this investigation were:

1. Determine radiation D-values of relevant microorganisms inoculated on to a variety of seeds used for sprouting.
2. Assess the effect of irradiation on the sprouting efficiency of the seeds.
3. Investigate the effect of irradiation and decontamination washes (calcium hypochlorite and eugenol (clove oil) on the microbiological quality of the seed.
4. Investigate the combined effect of oregano oil and irradiation on the microbial quality of seeds and seed sprouts.

2. MATERIALS AND METHODS

2.1. Radiation D-values of *E. coli* and *Listeria innocua* in alfalfa, radish, broccoli and clover seeds

2.1.1. Preparation of inoculum

Stationary phase cultures of *Listeria innocua* (MP2418) isolated from a poultry plant and *Escherichia coli* (NCTC11288), both designated as hazard group 1 organisms, were prepared as described by Patterson [8].

2.1.2. Irradiation treatments

All radiation treatments were carried out at 12^{o}C using a cobalt-60 Gammabeam 650 irradiator (Nordion International, Canada) with a dose rate of 2.0 kGy/h. The irradiation dose received was measured using Gammachrome YR dosimeters (AEA Technology, UK). The change in absorbance was measured spectrophotometrically as per manufacturer's instructions.

2.1.3. Preparation and inoculation of seeds

Seeds (10g aliquots) were irradiated to 6 kGy to reduce the background microflora. Seeds were inoculated with one of the cultures as described by Patterson [8] and plated on to trytptose soya agar containing 3% yeast extract (TSAYE) within two hours of the irradiation treatment. The plates were incubated at 37^{o}C for 24 hr, before being counted. The D_{10} values for each organism in each seed type was calculated as described previously [8].

2.2. Effect of irradiation on sprouting efficiency of alfalfa, radish, broccoli and clover seeds

On two separate occasions the seeds were irradiated as described above to 3 or 6 kGy or left unirradiated as controls. The seeds (100-200) were placed on moist sterile filter paper in a sterile jar and incubated at 20^{o}C for up to five days. The number of germinated seeds was counted for each treatment. The experiment was repeated once. The sprouting efficiency was calculated as the percentage of seeds that germinated.

2.3. Effect of irradiation and decontamination washes (calcium hypochlorite and eugenol) on the microbiological quality of alfalfa seeds

Alfalfa seeds were subjected to 4 decontamination treatments: (i) non-irradiated seeds washed 5 times x 5 min in sterile water (controls samples), (ii) seeds irradiated to 2 kGy as described above and soaked in 25,000 ppm calcium hypochlorite for 10 minutes followed by a neutralisation wash using 0.1% sodium thiosulphate. This was followed by rinsing in water. The neutralisation and rinsing process was repeated four times and the residual chlorine levels monitored, (iii) seeds irradiated to 2 kGy and soaked in 600 mg/l eugenol for 10 min and then rinsed 5 x 5 min in water. The rinsing

process was repeated 4 times, (iv) seeds irradiated to 2 kGy and then washed in 25,000 ppm calcium hypochlorite for 10 min, then neutralised in 0.1% sodium thiosulphate as before. This was followed by soaking in eugenol and washing in water as before.

Aliquots (10g) of seeds were placed in sterile jars, covered with muslin and incubated at 20^{o}C for four days. Each day the sprouting seeds were rinsed in 100 ml sterile water to simulate commercial sprouting conditions. Serial dilutions were prepared from the rinse water and samples were plated out daily on to TSAYE. The plates were incubated for 24 hr at 37^{o}C to obtain a total viable aerobic (TVC) count. The experiment was repeated twice. In the final experimental run, isolates were selected at random, using a Harrison disc, from the TSAYE plates obtained after four days. Pure cultures were obtained for each isolate and they were presumptively identified to genus level using the Schewan scheme. A minimum of 20 colonies were obtained for each treatment. Representative identifications were confirmed using API strips (Biomeriux Ltd.).

2.4. Effect of irradiation (2 kGy) and oregano oil wash on the microbiological quality of alfalfa seeds and sprouts during storage at 5^{o}C

2.4.1. Treatment of alfalfa seeds

Alfalfa seeds (10g aliquots) were irradiated to 2 kGy as before or left unirradiated. The seeds were subjected to four different wash regimes: (i) control seeds were not irradiated but soaked in distilled water for 10 min followed by 4 x 5 min rinses in water; (ii) irradiated (2 kGy) seeds soaked in distilled water for 10 min followed by 4 x 5 min rinses in water; (iii) irradiated (2 kGy) seeds soaked in 0.1% oregano oil solution for 10 min followed by 4 x 5 min rinses in water and (iv) non-irradiated seeds soaked in 0.1% oregano oil solution for 10 min followed by 4 x 5 min rinses in water. The seeds were placed in sterile jars, covered with muslin and incubated at 20^{o}C for four days to allow sprouting to take place. Each day the sprouting seeds were rinsed in 100 ml sterile water to simulate commercial sprouting conditions. Serial dilutions were prepared from the rinse water and TSAYE was used to obtain a TVC as described previously.

After four days the sprouted seeds were removed from the jars and aseptically transferred into sterile tubes, which were sealed and stored at 5^{o}C for a further five days to simulate the holding conditions of sprouted seeds. Each day three samples of sprouted seeds from each treatment were plated on to TSAYE. The plates were stored at 37^{o}C for 24 hr. The whole experiment was repeated once.

3. RESULTS AND DISCUSSION

3.1. Radiation D-values of *E. coli* and *Listeria innocua* in alfalfa, radish, broccoli and clover seeds

The radiation D_{10} values for *E. coli* and *L. innocua* were 0,35 kGy and 0.45 kGy, respectively. There was no significant difference between the seed type. **(data not shown)**

3.2. Effect of irradiation on sprouting efficiency of alfalfa radish, broccoli and clover seeds

Results are given in Table 1. Increasing the irradiation dose decreased the sprouting efficiency significantly at 6 kGy for the alfalfa seeds (Fig. 1). For the other seeds, a dose of 3 kGy significantly decreased the sprouting efficiency. It was decided that a 2 kGy treatment would be used for all future work. This would be sufficient to potentially give a 5.7 log reduction in *E. coli* and 4.4 log reduction in *L. innocua*.

TABLE 1. EFFECT OF IRRADIATION ON SPROUTING EFFICIENCY OF SEEDS

	Sprouted seeds (%)		
Seed type	**Control (0 kGy)**	**3kGy**	**6 kGy**
Alfalfa	83	78	66
Broccoli	89	80	31
Clover	90	77	34
Radish	95	70	22

3.3. Effect of irradiation and decontamination washes (calcium hypochlorite and eugenol) on the microbiological quality of alfalfa seeds

Results of the four decontamination treatments are given in Table 2. Initially the contamination levels were very low. However, within one day of the sprouting process, counts had risen significantly in all treatments. The decontamination washed containing calcium hypochlorite gave significantly lower counts than the controls or eugenol alone at this stage. By the end of the sprouting process (day 4), there was no significant difference between the treatments. In all cases the TVC was greater than log 9. These results agree with other observations, in that even when the initial count is below detectable levels, sufficient microorganisms survive the decontamination procedures and can grow to reach very high numbers in a short period of time.

The results of the microflora analysis is given in Table 3. The different treatments did not have a significant effect on the microflora of the sprouted seeds. *Pantoea* spp. dominated the microflora in all samples.

TABLE 2. EFFECT OF IRRADIATION (2 kGy) AND DECONTAMINATION WASHES ON THE MICROBIOLOGICAL QUALITY OF ALFALFA SEEDS DURING SPROUTING AT 20^{O}C

Treatment	**Log_{10} TVC during sprouting process***				
	0	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Control (no irradiation, water wash)	<1[a]	7.07[a**]	8.73[a]	9.46[bc]	9.31[a]
Irradiation and wash in calcium hypochlorite	<1[a]	5.99[b]	8.81[a]	9.32[ab]	9.23[a]
Irradiation and wash in Eugenol	2.59[b]	6.48[c]	8.83[a]	9.56[c]	9.26[a]
Irradiation and wash in calcium hypochlorite and Eugenol	<1[a]	6.22[b]	9.42[b]	9.15[a]	9.22[a]

* means of 3 replicates.
**values in the same column not bearing a common superscript are significantly different (p=0.05). Least significant difference (LSD) = 0.234

TABLE 3. MICROFLORA OF ALFALFA SEEDS AFTER 4 DAYS SPROUTING AT 20^{O}C

	% isolates			
	Control (no irradiation, water wash)	Irradiation and wash in calcium hypochlorite	Irradiation and wash in Eugenol	Irradiation and wash in calcium hypochlorite and Eugenol
Pantoea spp.	90	90	85	85
Acinetobacter spp.	10	0	0	15
Pseudomonas spp	0	5	0	0
Staphylococcus spp.	0	5	5	0
Bacillus spp.	0	0	10	0

3.4. Effect of irradiation (2 kGy) and oregano oil wash on the microbiological quality of alfalfa seeds and sprouts during storage at 5^{o}C

The TVC results of the effect of the four different decontamination procedures on the microbial quality of the seeds during germination are given in Table 4. Overall, the combined effect of radiation and oil treatment gave the lowest counts during germination. However, as before, the TVC were very high by the end of the sprouting process, irrespective of decontamination treatment. The effects of the treatments on the subsequent microbiological quality of the alfalfa sprouts during storage at 5^{o}C are given in Table 5. The combined irradiation and oil treatments resulted in sprouts with the lowest TVC at the end of the storage period. However, the numbers were still very high and there was a spoilage odour observed in all samples.

TABLE 4. EFFECT OF IRRADIATION (2 kGy) AND OREGANO OIL WASH ON THE MICROBIOLOGICAL QUALITY OF ALFALFA SEEDS DURING SPROUTING AT 20^{O}C

	Log_{10} TVC during sprouting process*			
	Day 1	**Day 2**	**Day 3**	**Day 4**
Control (no irradiation, water wash)	3.3^{a**}	8.42^{a}	9.73^{a}	9.99^{a}
Radiation, water wash	2.3^{b}	7.93^{b}	9.49^{a}	9.69^{ab}
No irradiation, oregano wash	2.05^{b}	7.3^{c}	9.09^{b}	9.62^{bc}
Irradiation and oregano wash	1.52^{c}	6.76^{d}	8.64^{c}	9.32^{c}

*means of 3 replicates.
** values in the same column not bearing a common superscript are significantly different (p=0.05). Least significant difference (LSD) = 0.32

TABLE 5. EFFECT OF IRRADIATION (2 kGy) AND OREGANO OIL WASH ON THE MICROBIOLOGICAL QUALITY OF ALFALFA SPROUTS DURING STORAGE AT 5^{O}C

	Log_{10} TVC during storage of sprouted seeds*		
	Day 1	**Day 4**	**Day 5**
Control (no irradiation, water wash)	9.9^{a**}	9.29^{a}	8.91^{a}
Radiation, water wash	9.58^{b}	9.02^{b}	8.67^{b}
No irradiation, oregano wash	9.52^{b}	9.16^{ab}	8.75^{b}
Irradiation and oregano wash	9.36^{b}	8.75^{c}	8.45^{c}

*means of 3 replicates.
** values in the same column not bearing a common superscript are significantly different (p=0.05). Least significant difference (LSD) = 0.22

4. CONCLUSIONS

1. Irradiation (2 kGy) of alfalfa seeds reduced the initial microbial loead to below the level of detection (< 10 cells). However, surviving microorganisms did grow rapidly during the sprouting process, irrespective of the decontamination treatment.
2. Irradiation (2 kGy) treatment alone or in combination with a decontamination wash regime (using calcium hypochlorite, eugenol (oil of clove) or oregano oil) did not significantly affect the quality of alfalfa seeds during sprouting.
3. Irradiation (2 kGy) and oregano oil (0.1%) did result in significantly lower total counts during storage of the sprouts for five days at 5^{o}C. However, the microbial counts in all cases were > log 8 by day 5 of storage and all the samples appeared spoiled. *Pantoea* spp. was the dominant bacterium present in all samples.

4. These results suggest that even if the initial microbial quality of the seeds is good (< 1 log/g) sufficient microorganisms are present to grow rapidly during the sprouting process, resulting in sprouts with high total counts.

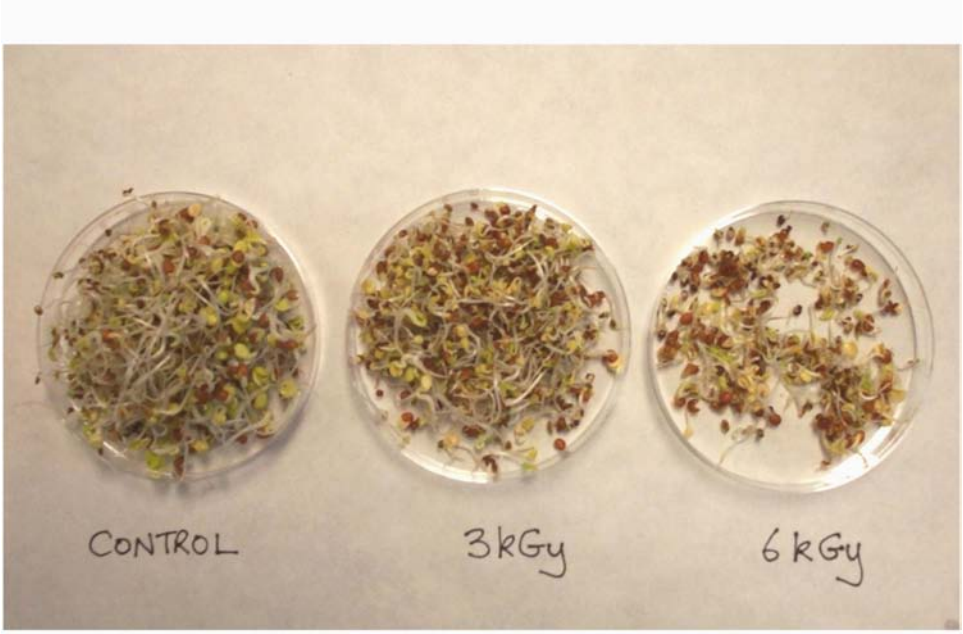

FIG. 1 Effect of irradiation on sprouting efficiency of alfalfa seeds.

REFERENCES

[1] PORTNOY, B.L., GOEPFERT, J.M. HARMON, S.M., An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. Am. J. Epidemiol. **103** (1976) 589-594.

[2] TAORMINA, P.J., BEUCHAT, L.R., SLUTSKER, L., Infections associated with eating seed sprouts: An international concern. Emerging Infect. Diseases **5** (1999) 626-634.

[3] ANON, Microbiological safety evaluations and recommendations on sprouted seeds. Int. J. Food Microbiol. **52** (1999) 123-153.

[4] JACQUETTE, C.B., BEUCHAT, L.R., MAHON, B.E., Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl. Environ. Microbiol. **62** (1996) 2212-2215.

[5] BEUCHAT, L.R., Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. J. Food Microbiol. **34** (1997) 204-216.

[6] BEUCHAT, L.R., WARD, T.E., PETTIGREW, C.A. Comparison of chlorine and a prototypes produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* 0157:H7 on alfalfa seeds. J. Food Prot. **64** (2001) 1520158.

[7] RAJKOWSKI, K.T., THAYER, D.W. Reduction of *Salmonella* spp. and strains of *Escherichia coli* 0157:H7 by gamma radiation of inoculated sprouts. J. Food Prot. **63** (2000) 871-875.

[8] PATTERSON. M.F. Sensitivity of *Campylobacter* spp. to irradiation in poultry meat. Let. Appl. Microbiol **20** (1995) 338-340.

ELECTRON BEAM INACTIVATION OF ENTERIC VIRUSES ON CANTALOUPE SURFACES

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Abstract

Minimally processed vegetables have been implicated in the transmission of food borne viral infections. Studies using Poliovirus Type 1 and bacteriophage MS2 were performed to determine whether electron beam irradiation (10 MeV) could be used to inactivate these viruses on cantaloupe *Cucumis melo reticulatus* surfaces. Cantaloupe rinds measuring 5 x 5 cm^2 were used as the test material. The D_{10} values of Poliovirus Type 1 and MS2 bacteriophage were 4.76 and 4.54 kGy respectively. These results suggest that E-beam irradiation can be used to destroy enteric viruses on cantaloupe surfaces. However, further studies are still needed to determine the actual dose that should be employed if E-beam irradiation is used as a final step of a comprehensive HACCP process to reduce viral contamination in minimally processed vegetables.

1. INTRODUCTION

The Centre for Disease Control and Prevention (CDC) has recognized that among the food-borne disease outbreaks in the USA between 1993 and 1997, over half of the cases of "unknown etiology" exhibited characteristics of viral illnesses [1]. In 2000, viral agents accounted for 28% of all documented food borne illness cases, whereas bacterial agents accounted for 25% of all food-borne disease outbreaks [2]. Mead et al. [1] have suggested that enteric viruses may actually account for as much as 67% of all food-borne disease related gastroenteritis in the United States. The large number of enteric virus infections can be attributed in part to their low infectious doses (100 PFU) and their stability in the environment [3, 4, 5]. Kurdziel et al. [6] have reported that poliovirus can survive for extended periods of time on the surfaces of soft fruit and salad vegetables at storage temperatures used within households.

Though the incidence of food-borne illnesses associated with fresh produce is relatively low, the number of outbreaks associated with fresh produce has recently doubled in numbers [7]. We have recently reported that carrots, cilantro and parsley can harbor fecal indicator viruses [8, 9]. Cantaloupes are also of particular concern with a number of recent gastro enteritis linked to contaminated cantaloupes. Cantaloupes, melons and other produce can get contaminated either by contaminated irrigation water supplies, during processing and packaging due to contaminated process water, equipment and by personnel [10, 11].

Though there have been a number of published reports suggesting that ionizing irradiation can be used to inactivate bacterial and fungal pathogens, reports on the use of ionizing irradiation for inactivating enteric viruses are relatively limited. Historically, it has been assumed that ionizing irradiation is ineffective against viruses. However, there have been some recent published reports suggesting that ionizing irradiation can be effective against viruses [12]. Studies in our laboratory have also shown that enteric viruses suspended in waste water effluents can be inactivated using electron beam irradiation [13].

Given the ability of enteric viruses to be transmitted via cantaloupes, the objective of this study was to identify the D_{10} values of model enteric viruses namely MS2 bacteriophage and attenuated poliovirus on cantaloupe surfaces using electron beam irradiation. The MS2 bacteriophage is a bacteriophage that is commonly isolated from the GI tracts of poultry and routinely used as a surrogate for fecal viruses. Attenuated poliovirus Type 1(vaccine strain) is also routinely used as a model enteric virus to study the efficacy of inactivating agents.

2. MATERIALS AND METHODS

2.1. Cantaloupes Samples

Studies were conducted using a 5 x 5 cm^2 piece of cantaloupe rind (with a thickness of ~4 mm and average weight of ~17 grams) as the test material. Preliminary experiments were performed to identify this particular sample shape as ideal for the experimental objectives. The use of cantaloupe rinds facilitated the efficient recovery of the test viruses as well as the ability to control the inoculation area. Previous studies evaluating the ecology of pathogens on cantaloupe surfaces used rinds as the test surface [14].

2.2. Virus Suspensions and Inoculation

The titer in the virus suspension was 1.04 x 10^{12} PFU/mL (bacteriophage) and 4.85 x 10^{7} PFU/mL (poliovirus Type 1). Ten fold serial dilutions using phosphate buffer (pH 6.0) were prepared for enumeration purposes. The bacteriophage titer was assayed using trypticase soy agar plates amended with ampicillin-streptomycin and the host bacteria E.coli Famp (, 2001a). For the poliovirus, the serial dilutions were prepared in Minimum Essential Media (MEM) and the assays were conducted in tissue culture wells containing confluent BGMK cells. The cantaloupe samples were prepared, in triplicate, by inoculating the surface of with 0.1mL of a virus (bacteriophage and poliovirus) stock suspension. The inoculant was allowed to completely absorb into only the surface of the rind prior to exposing them to E-beam.

3.3. Electron Beam Irradiation

The cantaloupes rinds were placed in separate sterile Zip-Lock™ bags and heat sealed. These bags were then placed in another heat sealed bag prior to irradiation. (This procedure was followed as per the standard operating procedures for the E-beam facility when introducing human pathogens). The samples were subjected to electron beam (E-beam) irradiation doses (on a horizontal profile in a tray on a conveyor belt at approximately 3°C using the 10MeV linear accelerators at the Electron Beam Food Research Facility (EBRF) on the Texas A&M University campus. Preliminary dose-mapping studies were performed using L-α–alanine dosimeter tablets (Gamma-Service Produktbestrahlung GmbH, GERMANY) and measured using EPR (Bruker, E-scan, Bruker BioSpin, Corp., USA). The target doses for the bacteriophage inoculated samples were 2.5 kGy, 7.5 kGy and 10 kGy while the target doses for the poliovirus Type I inoculated samples were 2.5 kGy, 5.0 kGy and 10 kGy of irradiation. The actual measured doses and the intended target doses are shown in Table 1. All samples were maintained cold (on blue-ice) prior to irradiation and prior to enumeration.

Table 1. MEASURED AND TARGET ELECTRON BEAM DOSES

Target Virus	Target Dose (kGy)	Measured Dose (kGy)
MS2 bacteriophage	2.5	2.88
	7.5	7.89
	10.0	10.59
Poliovirus Type 1	2.5	2.63
	5.0	5.61
	10.0	10.16

2.4. Recovery of Viruses from Cantaloupes and Enumeration

Using sterile techniques, the rinds were removed from the heat-sealed bags and placed into another sterile bag containing 10 mL phosphate buffer (pH 6.0) and allowed to rotate on a shaking platform for five minutes. Aliquots were thereafter removed from these bags for enumeration. The MS2 samples were enumerated using USEPA- standardized protocols for enumerating bacteriophages [15] and the poliovirus type I samples were enumerated using BGMK cells followed by crystal violet staining for plaque enumeration [16, 17].

2.5. Data Analysis

The PFU/mL data was transformed into log10 PFU/mL and plotted as a function of the measure dose. The D_{10} values (the dose required to reduce the viral populations by 1 log cycle) were calculated by using the linear regression analysis (Figures 1 and 2).

3. RESULTS AND DISCUSSION

The inactivation of MS2 bacteriophage and Poliovirus Type I when exposed to E-beam irradiation are shown in Figures 1 and 2.

The D_{10} value for MS2 bacteriophage was 4.54 kGy compared to 4.76 kGy for Poliovirus Type 1 strain. It is evident that the D_{10} values for these enteric viruses are significantly higher than that of enteric bacteria [18]. No sensory evaluations were conducted as part of these studies since the primary focus was on identifying the E-beam dose that can achieve virus inactivation. There was a difference in the D_{10} value of these viruses on cantaloupes as compared to when these were suspended in water. In distilled water and secondarily treated waste effluents, MS2 virus exhibited D_{10} values of 1.83 kGy and 2.04 kGy respectively while poliovirus Type I virus exhibited D_{10} values of 1.83 kGy and 2.84 kGy respectively [13]. The difference in the inactivation kinetics between water samples and cantaloupes rinds could be attributed to "scavenger" molecules such as sugars on the cantaloupes rinds that could have attenuated the OH free radicals.

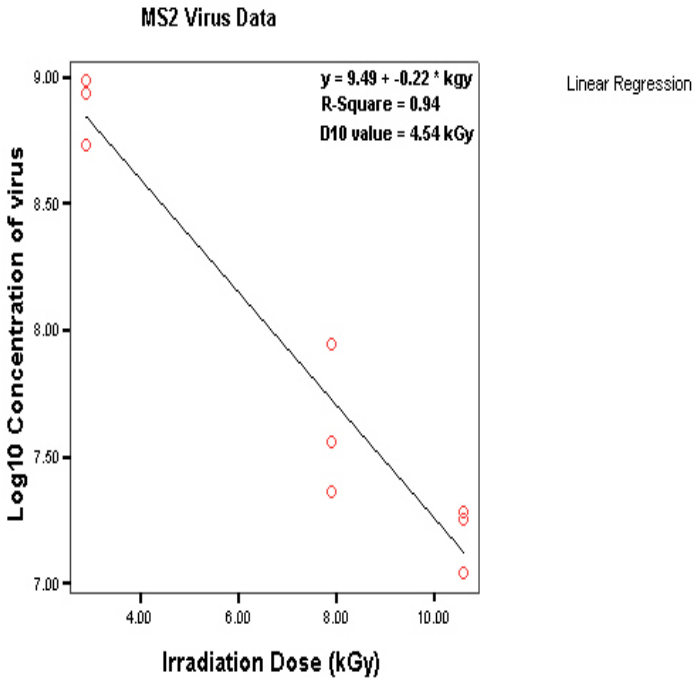

FIG. 1. Inactivation of MS2 bacteriophage on cantaloupe surfaces.

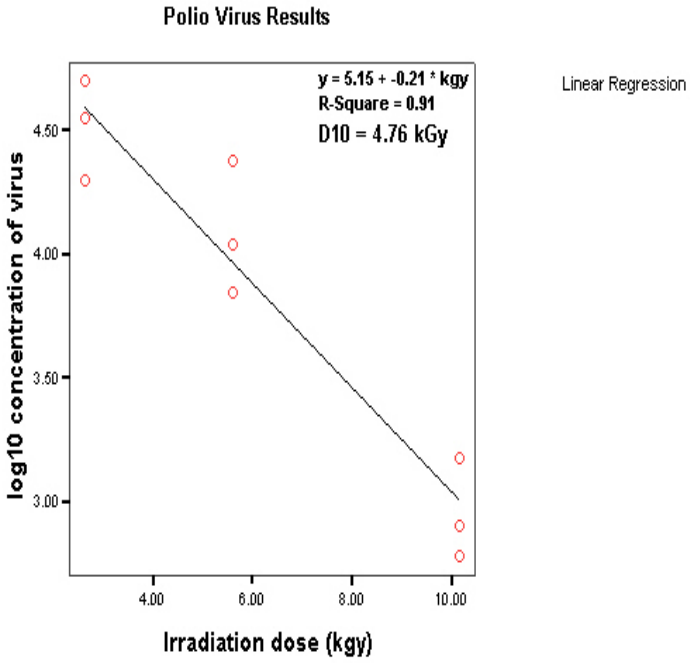

FIG. 2. Inactivation of Poliovirus Type I on cantaloupe surfaces.

It may be argued that based on this data, achieving a 6-log reduction of enteric viruses on cantaloupes may require excessively high E-beam doses. However, it must be emphasized that if ionizing irradiation such as E-beam is used as an integral step of a comprehensive HACCP plan, a 6-log reduction of target organisms in not necessarily needed. Under such scenarios, the anticipated loads of enteric viruses would be at very low levels and thus achieving even a 1-log reduction of the viruses may be a significant food safety accomplishment [19]. Nevertheless, studies are still needed to identify whether novel packaging methods such as modified atmosphere packaging in conjunction with E-beam can further enhance the inactivation efficiency of enteric viruses [20].

REFERENCES

[1] MEAD, P. S., SLUTSKER, L., DIETZ, V., MCCAIG, L.F., BRESEE, J.S., SHAPIRO, C., GRIFFIN, P.M., TAUXE, R.V., Food-related illness and death in the United States, Emerg. Infect. Dis. **5** (1999) 607-25.

[2] CENTRAL FOR DISEASE CONTROL AND PREVENTION (CDC) 2000. U.S. Foodborne disease outbreaks. Foodborne Outbreak Response and Surveillance Unit. http://www.cdc.gov/foodborneoutbreaks/ Accessed December 20, 2004.

[3] ALLWOOD, P. B., MALIK, Y.S. HEDBERG, C.W., GOYAL, S.M., Effect of temperature and sanitizers on the survival of feline calicivirus, *Escherichia coli*, and F-specific coliphage MS2 on leafy salad vegetables, J. Food Prot. **67** (2004) 451-6.

[4] ALLWOOD, P. B., MALIK, Y.S., HEDBERG, C.W., GOYAL, S.M. Survival of F-specific RNA coliphage, feline calicivirus, and *Escherichia coli* in water: a comparative study, Appl. Environ. Microbiol. **69** (2003) 5707-5710.

[5] TAKU, A., GULATI, B.R., ALLWOOD, P.B., PALAZZI, K., HEDBERG, C.W., GOYAL, S.M., Concentration and detection of caliciviruses from food contact surfaces, J. Food Prot. **65** (2002) 999-1004.

[6] KURDZIEL, A.S., WILKINSON, N., LANGTON, S., COOK, N. Survival of poliovirus on soft fruit and salad vegetables, J. Food Prot. **64** (2001) 706-709.

[7] BEAN, N. H., GOULDING, J.S., DANIELS, M.T., ANGULO, F.J., Surveillance for foodborne disease outbreaks - United States, 1988-1992, J. Food Prot. **60** (1997)1265-1286.
[8] ENDLEY, S., JOHNSON, E., PILLAI, S.D., A simple method to screen cilantro and parsley for fecal indicator viruses, J. Food Prot. **66** (2003) 1506-1509.
[9] ENDLEY, S., LU, L., VEGA, E., HUME, M.E., PILLAI, S.D. Male-specific coliphages as an additional fecal contamination indicator for screening fresh carrots, J. Food Prot. **66** (2003) 88-93.
[10] DUFFY, E.A., LUCIA, L.M., KELLS, J.M., CASTILLO, A., PILLAI, S.D., ACUFF, G.R., Concentrations of *Escherichia coli* and genetic diversity and antibiotic resistance profiling of *Salmonella* isolated from irrigation water, packing shed equipment, and fresh produce in Texas. J. Food Prot. **68** (2005) 70-70.
[11] GAGLIARDI, J.V., MILLNER, P.D., LESTER, G., INGRAM, D., On-farm and postharvest processing sources of bacterial contamination to melon rinds, J Food Prot. **66** (2003) 82-87.
[12] DE RODA HUSMAN A.M., BIJKERK, P., LODDER, W., VAN DEN BERG, H., PRIBIL, W., CABAJ, A., GEHRINGER, P., SOMMER, R., DUIZER E. Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation, Appl Environ Microbiol. **70** (2004) 5089-93.
[13] PILLAI, S.D., ESPINOSA , I.Y., E-beam inactivation of RNA and DNA containing viruses. Abstract. Annual Meeting of the Amer. Soc. Microbiol. Washington, DC (2003).
[14] RICHARDS, G.M., BEUCHAT, L.R., Metabiotic associations of molds and Salmonella Poona on intact and wounded cantaloupe rind, Int. J Food Microbiol. **97** (2005) 327-39.
[15] US Environmental Protection Agency (USEPA), Method 1602. Male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. http://www.epa.gov/nerlcwww/1602ap01.pdf. USEPA. Office of Water. Washington DC (2001).
[16] US Environmental Protection Agency (USEPA), USEPA Manual of Methods for Virology. http://www.epa.gov/nerlcwww/about.htm. USEPA. Microbiology. Washington D.C. (2001).
[17] VEGA, E., SMITH, J., GARLAND, J., MATOS, A., PILLAI, S.D. Variability of virus attachment to lettuce, J. Food Prot **68** (2005) 2112-2117.
[18] SMITH, S., PILLAI, S.D., Irradiation and Food Safety, Scientific Status Summary. Food Technology **58** (2004): 48-55.
[19] MENA, K.D., PILLAI, S.D. Developing Quantitative Risk-based Microbial Standards for Irrigation Water, J. Water and Health (*in review*).
[20] PILLAI, SD., Food Irradiation, In: Preharvest and PostHarvest Food Safety, RC BIER, SD. PILLAI, TD PHILLIPS & RL ZIPRIN (eds), IFT Press, Blackwell Publishing, 375-387 (2004).

RESPONSE OF *LISTERIA MONOCYTOGENES*-INOCULATED LEAFY SALAD VEGETABLES FOLLOWING IRRADIATION AND REFRIGERATED STORAGE UNDER MODIFIED ATMOSPHERE

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Section 1.

Radiation sensitivity and quality of fresh cut vegetables exposed to gamma radiation

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Abstract

This section summarizes research on quality of irradiated vegetables. Thirteen fresh-cut (minimally processed) vegetables (broccoli (*Brassica oleracea italica)*, cilantro (*Coriandrum sativum)*, red cabbage (*Brassica oleracea capitata)*, endive (*Cichorium endivia)*, parsley (*Petroselinum crispum)*, green and red leaf lettuce (*L. sativa)*, Iceberg (*Lactuca sativa* var. *capitata*) and Romaine lettuce (*Lactuca sativa)*, spinach (*Spinacia oleracea)*, carrots (*Daucus carota)*, green onions, *(Allium cepa)* and celery (*Apium graveolens dulce*) plus alfalfa sprouts (*Medicago sativa)* were gamma irradiated at doses up to 3 kGy at 0.5 kGy intervals. The samples were then stored in air at 4°C for 14 days. At the beginning and end of 14 days, electrolyte leakage, visual quality, and sogginess were assessed. Results showed that electrolyte leakage of vegetables increased linearly with increasing radiation doses when measured immediately after irradiation. Radiation dose threshold, defined as the dose at which electrolyte leakage was significantly ($P<0.05$) increased, varied among vegetables, ranging from 2.44 kGy for broccoli to 0.60 kGy for carrots. Irradiation had no effect on visual quality on the day of irradiation. After 14 days of storage, the responses of individual vegetables to ionizing radiation varied greatly. Irradiation, especially at lower doses, improved visual quality, and reduced sogginess and electrolyte leakage of many vegetables. It appears that most tested vegetables can tolerate 0.6 kGy radiation based on appearance and electrolyte leakage measurements. The implication and limitation of the results are discussed.

1. INTRODUCTION

Consumption of fresh-cut (minimally processed) fruits and vegetables in the USA has increased in recent years, presumably due to demand for convenience and freshness. The fresh-cut industry, which is relatively new, having started in the early 1980's, has become a multi-billion enterprise and continues to grow rapidly [1]. However, there is a concern for the microbiological safety of fresh-cut fruits and vegetables. Although the percentage of fresh-cut vegetables contaminated with food-borne human pathogens is very low, several outbreaks of food-borne illness have been found to be associated with consumption of fresh and fresh-cut vegetables [2-4]. Various sanitizing agents used in commercial processing lines, such as chlorine, have a limited effect on microbial populations [5].

Ionizing irradiation is a non-thermal technology that eliminates food-borne pathogens and extends shelf-life of various foods, including fresh vegetables. Many earlier studies focused on whole fruits and vegetables, dealing with effects of ionizing radiation on quality and shelf-life of whole produce. For example, Kader [6] summarized the relative tolerance of fresh fruits and vegetables to ionizing radiation and grouped them into three categories (high, moderate, and low). All vegetables fell into the low tolerance category. Fresh-cut vegetables are prepared from the raw products by cutting, shredding, trimming, washing, and drying. The resulting products are still fresh and have a short shelf-life (10-14 days). There are a limited number of publications on ionizing irradiation of fresh-cut vegetables. Earlier research focused on selected commodities (i.e. lettuce). For example, Prakash and others [7] irradiated fresh-cut romaine lettuce up to 0.35 kGy and found no difference in sensory attributes except for a 10% loss in firmness. Foley and others [8] found that 0.55 kGy radiation in combination with chlorination treatment produced a 5.4 log reduction in *E. coli O157:H7* and no adverse effect on

sensory quality. Fan and Sokorai [9] found that iceberg lettuce packaged in modified atmosphere film bags can tolerate up to 1.0 kGy radiation.

The objectives of this study were to investigate the impact of irradiation on visual quality and membrane damage of a wide range of fresh-cut vegetables, and to estimate radiation sensitivity using electrolyte leakage.

2. MATERIALS AND METHODS

2.1. Sample preparation

Fourteen vegetables were used including Romaine, Iceberg, red and green leaf lettuce, alfalfa sprouts, cilantro, parsley, green onions, carrots, broccoli, endive, red cabbage, spinach, and celery. These vegetables were used because of economic importance or an association with contamination of food-borne pathogens and outbreaks of illnesses. The fresh-cut vegetables were either prepared at the laboratory from intact produce or purchased pre-cut from local supermarkets. Lettuce, spinach and endive were cut with sharp stainless knives into 3-cm squares. Cilantro and parsley leaves were cut into ~2 cm long pieces. Broccoli was prepared using only florets without major stalks. Green onions and celery were cut into 1-cm long pieces. The cut pieces were then rinsed with deionizing water, drained and spin-dried using hand operated kitchen spinners (Wilton Industries Inc., Woodridge, IL). Alfalfa sprouts, shredded red cabbage and shredded carrots were purchased and used without further preparation. All samples were placed into zipper bags which had been perforated with four holes (0.6 cm in diameter). There were four bags of samples for each treatment (dose), and each bag was treated as a replicate. Each bag contained 15 g of sample. The samples were then irradiated at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy at 4°C. The samples were equilibrated to 4°C prior to irradiation. After irradiation, samples were stored at 4°C for 14 days before quality assessment.

2.2. Irradiation and dosimetry

The samples were irradiated using a self-contained cesium-137 gamma radiation source (Lockheed Georgia Company, Marietta, GA) with an average dose rate of 0.096 kGy/min. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field, by irradiating them within a polypropylene container (4-mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during the entire study. During irradiation, temperature (4 ± 2°C) in the radiation chamber was controlled by flushing the gas phase above liquid nitrogen into the upper portion of chamber. Routine dosimetry was performed using 5-mm-diameter alanine pellets (Bruker, Inc. Billarna, MA., USA). The pellets were placed into 1.2 ml cryogenic vials (Nalgene, Rochester, NY., U.S.A.), and the cryogenic vials were taped to the bags prior to irradiation. Alanine pellets were read using a Bruker EMS 104 EPR analyser and compared with a standard curve. Actual dose was typically within 5% of the targeted doses.

2.3. Electrolyte leakage

Five grams of each sample were incubated for 1 hr at 23°C in 100-ml glass bottles containing 50-70 ml deionized water [10]. During incubation, samples were agitated using a shaker (model M49125, Barnstead International, Dubuque, Iowa, USA.) at a speed of 100 rpm. Electrical conductivity of the bathing solution was measured using a CON 100 conductivity meter (Oakton Instruments, Singapore). The samples were then autoclaved (121°C) for 25 min, and total conductivity of bathing solution was measured after cooling. Electrolyte leakage was expressed as percent total conductivity.

2.4. Visual quality assessment

Each sample was visually rated according to the scoring system developed by Kader et al. [11]. For overall quality, the scale was 1-9 with 9 as excellent and 1 as unusable. The sogginess was rated as 1 (none) to 5 (severe) [12]. Visual quality of samples in the bags was rated by three judges under fluorescent laboratory light.

2.5. Statistical analysis

The experiments were repeated twice for cilantro, red-leaf lettuce, green leaf lettuce, endive, parsley, and green onions, and the results were pooled. There were four replicates per experiment/treatment. Data were subjected to statistical analysis using SAS ver. 6.12 (SAS Institute, Raleigh, NC, U.S.A.). The least significant difference was analysed using general linear models (GLM) procedure.

3. RESULTS AND DISCUSSION

Thirteen fresh cut vegetables plus alfalfa sprout were irradiated with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 4°C. Electrolyte leakage was measured on the same day of irradiation.

Electrolyte leakage increased linearly with increasing radiation dose for all vegetables (Table 1). The R^2 values for most vegetables were above 0.90 while the R^2 for broccoli was the lowest (0.56), suggesting that electrolyte leakage may be used for assessing radiation sensitivity of fresh-cut vegetables. To evaluate the radiation sensitivity of fresh-cut vegetables, a novel parameter, dose threshold was introduced. Dose threshold was defined as radiation dose at which electrolyte leakage was significantly ($P<0.05$) increased, and was calculated by dividing $LSD_{0.05}$ values with the rate of increase in electrolyte leakage as a function of dose (LSD/rate). The rate of increase in electrolyte leakage and dose threshold was negatively correlated ($P=0.03$). There was a large variation in dose thresholds ranging from 2.44 kGy for broccoli to 0.60 kGy for carrots. All vegetables had radiation thresholds of at least 0.6 kGy. The dose thresholds for most of the fresh-cut vegetables were between 1 and 2 kGy. There was no significant difference in dose threshold among most vegetables due to large standard deviations. Broccoli, endive and red cabbage, however, had significantly ($P<0.05$) higher dose threshold than cilantro, green onions and carrots when evaluated using LSD or Duncan's analysis. Fan and Sokorai [13] defined radiation thresholds as doses that increased electrolyte leakage by 50% (EL_{50}) over the non-irradiated controls. The radiation thresholds using EL_{50} varied among vegetables from 0.3 to 2.0 kGy. Red cabbage, broccoli and endive had the highest radiation resistance while celery, carrot and green onion were the most sensitive to radiation. Even though the dose thresholds calculated using the EL_{50} were slightly lower than those in the present study, both calculations suggest that broccoli, red cabbage and endive were more resistance to radiation while carrots and green onions were more susceptible to irradiation**.** The endogenous antioxidant capacity and total phenolic content of the vegetables also varied significantly among the tested vegetables [13]. The antioxidants, however, did not correlate well the radiation thresholds.

TABLE 1. ELECTROLYTE LEAKAGE (%) OF VEGETABLES AS A FUNCTION OF RADIATION DOSE

Vegetables	0	0.5	1.0	1.5	2.0	2.5	3.0	LSD[a]	R^2	Dose Threshold (kGy)[b]
Broccoli	0.8	0.8	0.8	0.7	0.8	1.3	1.3	0.3	0.56	2.4
Endive	1.4	1.7	2.0	2.1	2.5	2.9	3.1	0.6	0.98	1.8
Red cabbage	1.4	1.4	1.5	2.1	1.9	2.1	3.1	0.4	0.80	1.6
Parsley	1.7	1.8	2.5	3.2	3.0	3.3	3.9	1.0	0.92	1.3
Green leaf lettuce	1.8	2.2	2.5	2.5	2.5	4.0	4.1	1.0	0.84	1.3
Romaine lettuce	1.7	3.0	3.9	4.6	4.4	5.0	5.5	1.4	0.90	1.3
Iceberg lettuce	1.5	2.3	2.5	2.7	3.0	3.9	5.1	1.3	0.91	1.2
Spinach	3.6	4.7	4.5	5.3	5.9	6.3	6.8	1.5	0.97	1.2
Alfalfa sprouts	5.4	5.7	6.1	6.7	7.9	7.5	8.9	1.4	0.93	1.2
Red leaf lettuce	3.5	4.0	4.4	4.9	5.5	8.2	9.8	1.6	0.87	1.1
Celery	2.6	4.6	5.0	7.0	10.8	11.9	11.6	2.6	0.93	1.0
Green onion	3.0	4.9	5.1	4.9	7.8	12.5	11.5	2.4	0.85	0.8
Cilantro	0.9	1.5	1.8	2.0	2.9	3.3	3.3	0.8	0.96	0.8
Carrots	3.3	3.8	4.3	5.2	5.8	7.2	9.1	0.6	0.94	0.6

[a] The least significant difference at $P<0.05$ level.
[b] Dose thresholds were defined as the dose at which electrolyte leakage was significantly ($P<0.05$) increased.

Thirteen fresh-cut vegetables plus alfalfa sprouts were exposed to 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 4°C. After 14 days storage at 4°C, visual quality was rated using a scale of 9-1 with 9 as excellent and 1 as unusable.

The visual quality of the samples was not significantly affected by irradiation at any dose when assessed on the day of irradiation .The visual quality scores of irradiated endive, green onions, celery and spinach were generally higher than non-irradiated ones. Green leaf lettuce irradiated at all doses except 3.0 kGy had better visual quality than non-irradiated controls (Table 2). Visual quality of broccoli, red cabbage, parsley, cilantro, carrots, red leaf lettuce and alfalfa sprouts were not affected by irradiation at any dose. In contrast, visual quality of Iceberg and Romaine lettuce deteriorated with higher radiation doses, particularly at doses above 1.0 kGy. The reduced visual quality in Iceberg and Romaine lettuce was mostly due to increased tissue browning (data not shown). The results on visual quality indicated that all fresh-cut vegetables can tolerate gamma radiation at doses up to 1.0 kGy.

TABLE 2. VISUAL QUALITY OF FRESH-CUT VEGETABLES AS A FUNCTION OF RADIATION DOSE

Vegetables	0	0.5	1	1.5	2.0	2.5	3.0	LSD[a]
Broccoli	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0.5
Endive	5.8	6.3	6.5	6.2	6.6	6.2	6.7	0.5
Red cabbage	8.4	7.9	8.2	8.5	7.9	7.5	8.5	0.5
Parsley	6.2	7.0	7.6	7.8	7.8	7.5	7.3	0.9
Green leaf lettuce	5.4	6.7	7.1	6.6	6.6	6.2	5.2	0.7
Romaine lettuce	6.8	6.9	6.0	5.6	5.0	4.5	4.4	1.1
Iceberg lettuce	6.8	6.2	6.2	5.3	4.3	3.5	4.0	0.7
Spinach	5.0	5.5	6.9	7.4	7.4	7.4	7.0	1.3
Alfalfa sprouts	7.8	7.9	8.0	8.0	8.0	8.0	7.9	0.2
Red leaf lettuce	4.0	5.1	4.3	4.4	4.5	3.7	3.4	0.7
Celery	3.9	4.7	4.9	6.2	5.9	5.6	5.7	0.8
Green onion	3.7	4.8	5.3	5.4	5.5	5.6	5.3	0.5
Cilantro	5.5	6.4	6.2	6.3	6.3	5.8	5.5	1.2
Carrots	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0.5

[a] The least significant difference at $P<0.05$ level.

Thirteen fresh-cut vegetables plus alfalfa sprouts were irradiated with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 4°C. After 14 days storage at 3°C, sogginess was scored using a scale of 1-5 with 1 as none and 5 as severe.

Sogginess was the symptom of soaked appearance, and may be related to leakage of fluids from tissue and/or loss of tissue integrity [12]. The sogginess scores of broccoli, red cabbage, endive, Iceberg lettuce, and carrots were low, and generally not affected by irradiation at any dose (Table 3). For many vegetables (parsley, green leaf lettuce, cilantro, spinach, red leaf lettuce, green onions), the non-irradiated samples had the highest sogginess scores, and samples irradiated at 1.0 or 1.5 kGy had the lowest scores. As dose increased from 1.0 or 1.5 to 3.0 kGy, sogginess generally increased. Irradiation at low doses had little effect on sogginess of Romaine lettuce and celery, however, at high doses (particularly at 3.0 kGy), sogginess scores increased. Overall, sogginess of irradiated samples was less severe or similar to non-irradiated controls.

TABLE 3. SOGGINESS OF FRESH-CUT VEGETABLES AS A FUNCTION OF RADIATION DOSE

Vegetables	0	0.5	1.0	1.5	2.0	2.5	3.0	$LSD_{0.05}$[a]
Broccoli	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
Endive	1.8	1.7	1.5	1.7	1.6	1.7	1.4	0.3
Red cabbage	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
Parsley	1.9	1.3	1.2	1.2	1.5	1.6	1.7	0.4
Green leaf lettuce	2.3	1.7	1.3	1.4	1.3	1.3	2.1	0.5
Romaine lettuce	1.0	1.0	1.0	1.0	1.3	1.7	2.0	0.3
Iceberg lettuce	1.0	1.0	1.0	1.0	1.0	1.0	1.0	----
Spinach	2.2	1.9	1.4	1.3	1.0	1.0	1.3	0.3
Alfalfa sprouts	1.1	1.1	1.0	1.0	1.0	1.0	1.2	0.2
Red leaf lettuce	3.0	2.2	2.6	2.5	2.4	2.8	3.0	0.5
Celery	1.0	1.0	1.0	1.0	1.0	1.1	1.5	0.2
Green onion	4.3	2.7	2.2	2.2	2.3	2.3	2.6	0.4
Cilantro	2.1	1.6	1.5	1.3	1.8	1.5	1.6	0.3
Carrots	1.0	1.0	1.0	1.8	1.0	1.0	1.0	0.2

[a] The least significant difference at $P<0.05$ level.

Thirteen fresh-cut vegetables plus alfalfa sprouts were irradiated with 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 4°C. Electrolyte leakage was measured after 14 days of storage at 4°C.

After 14 days of storage, electrolyte leakage of non-irradiated samples varied greatly among the vegetables, reflecting differences in structure, tissue, and/or concentration of ionic (both inorganic and organic ions) concentration among the vegetables (Table 4). Electrolyte leakage of broccoli, red cabbage and spinach was not affected by irradiation at any dose. For carrots, Iceberg and Romaine lettuce, electrolyte leakage increased with radiation dose. However, for most of the fresh-cut vegetables (endive, parsley, green leaf lettuce, cilantro, red leaf lettuce, green onions, and celery) and alfalfa sprouts, non-irradiated samples had higher electrolyte leakage than those irradiated at 0.5 or 1.0 kGy. But as doses further increased, electrolyte leakage also increased. For these vegetables, either the non-irradiated sample or those irradiated at 3.0 kGy had the highest electrolyte leakage. Overall, samples that received 0.5 or 1.0 kGy radiation had less or similar electrolyte leakage than non-irradiated samples 14 days after irradiation.

TABLE 4. ELECTROLYTE LEAKAGE (%) OF FRESH-CUT VEGETABLES AS A FUNCTION OF RADIATION DOSE.

Vegetables	0	0.5	1	1.5	2.0	2.5	3.0	LSD[a]
Broccoli	0.5	0.4	0.4	0.3	0.4	0.4	0.3	0.2
Red cabbage	0.7	0.9	1.2	0.9	1.0	1.4	0.9	0.3
Endive	16.9	4.7	2.8	8.0	6.5	7.6	8.1	6.4
Parsley	12.2	4.0	4.0	3.5	3.0	4.0	4.0	4.1
Green leaf lettuce	5.8	2.8	2.3	6.2	1.6	5.2	10.0	6.0
Romaine lettuce	1.0	1.5	2.3	3.3	3.7	6.3	5.1	1.2
Iceberg lettuce	1.3	2.4	1.7	2.7	3.7	4.5	5.8	2.5
Spinach	14.3	16.9	15.9	12.7	11.7	10.8	13.7	5.2
Alfalfa sprouts	11.3	8.5	11.3	10.5	10.2	13.6	18.3	5.5
Red leaf lettuce	8.5	3.6	2.2	3.6	3.9	4.4	5.5	3.3
Celery	6.3	3.5	3.9	7.4	7.4	7.7	11.9	6.5
Green onion	33.6	8.4	10.8	14.7	15.1	15.3	20.8	7.9
Cilantro	9.4	3.4	5.6	6.7	6.5	8.6	8.0	4.3
Carrots	1.9	2.3	2.4	2.6	2.8	3.4	3.6	0.4

[a] The least significant difference at $P<0.05$ level.

There was some correlation between sogginess and electrolyte leakage after 14 days of storage. For example, many non-irradiated samples had the most severe sogginess as well as the highest electrolyte leakage. Also both the lowest scores of sogginess and electrolyte leakage were observed in some samples that received doses of 0.5 or 1.0 kGy.

Measurement of electrolyte leakage in plant tissues has been used for estimating membrane permeability and integrity in relation to environmental stresses, growth and development, and microbial attack. Irradiation is known to increase electrolyte leakage in a number of plant tissues [14, 15]. Besides irradiation, there are many other factors that may also increase membrane deterioration and electrolyte leakage. For example, as vegetables senesce, cells and tissues lose their integrity and function, resulting in cellular electrolyte leakage. Another common factor is the decay caused by microbial attack. Proliferation of microorganisms can cause damage to the cell wall, membrane and tissue, and increases cellular leakage [16]. Our results showed that after 14 days of storage, the non-irradiated control samples and those irradiated at higher doses (2.5 and 3 kGy) often had the highest electrolyte leakage. The high electrolyte leakage in control samples is likely related to senescence and/or decay. It is known that irradiation delays senescence, inactivates microorganisms and reduces decay. However, high doses of radiation can also cause membrane damage and increase electrolyte leakage as observed in samples irradiated at 2.5 or 3.0 kGy.

Our results showed irradiation induced increases in electrolyte leakage in all vegetables when measured immediately after irradiation. Therefore it is possible to use electrolyte leakage measurement to assess membrane damage and radiation sensitivity of various vegetables.

The radiation threshold values calculated from changes in electrolyte leakage were not significantly correlated to post-storage visual quality changes of irradiated vegetables. Visual quality assessed after 14 days of post-irradiation storage at 4°C suggested that most of the vegetables can tolerate at least 1 kGy radiation. Of course, other quality attributes in addition to visual quality, can limit the

acceptability of irradiated vegetables. Many factors other than membrane permeability can influence quality attributes. Quality losses (such as browning) can be a result of chemical changes not necessarily related to membrane permeability. Irradiation increases phenolic content in vegetables [8, 17, 18], which may in turn influence appearance, flavor and nutritive values. Irradiation can inactivate spoilage microorganisms present in vegetables, resulting in extended shelf life of vegetables. The low scores of visual quality in some of control samples may partially be due to decay caused by spoilage microorganisms. Furthermore, post-irradiation conditions such as maturity of produce, storage temperature and headspace atmosphere, can influence shelf-life and quality parameters of vegetables. The radiation sensitivity measured using electrolyte leakage should be used only as indicators of potential post-storage quality.

It should be pointed out that there are several limitations of this study in terms of implications for commercial use of ionizing radiation on the fresh-cut vegetables. First, the samples used in the study were purchased from local supermarkets. Therefore, the ages of the materials were unknown. Maturity and age of the raw materials may affect the response of samples to irradiation. Secondly, many commercially available fresh-cut vegetables are packaged in modified atmosphere film bags which maintain lower O_2 and higher CO_2 levels. Under modified atmosphere packaging, the browning and senescence of samples may be reduced. Consequently, visual quality may be maintained. Third, this study only investigated visual quality and membrane damage. Many other quality parameters, such as texture, nutritional, and flavor changes may also affect overall quality and acceptance of irradiated samples. Also, the visual quality was judged by only three people using a scaling system. Although the system is routinely used by many researchers, a large non-trained panel may be used to better evaluate consumer acceptance of the irradiated produce. Nevertheless, our results using both electrolyte leakage measurements and visual quality suggest most of the vegetables tested can tolerate 1.0 kGy radiation. The results were similar to our earlier observations on iceberg lettuce, green onions and alfalfa sprouts [9, 19-21] with an exception for cilantro. An earlier study [10] suggested that cilantro leaves could tolerate 2 kGy radiation while the calculated dose threshold basing on electrolyte leakage was only 0.8 kGy. Langerak [22] showed that radiation at 1 kGy resulted in reductions of bacterial populations while doubling the shelf life of cut endive. Farkas and others [23] showed that 1 kGy radiation reduced loads of spoilage bacteria, improved microbiological shelf life and extended sensorial keeping quality of pre-cut peppers and carrots.

Our results indicate that many vegetables are tolerant to low dose irradiation. There was no deterioration of visual quality or membrane damage for all samples that received doses of 0.5 or 1.0 kGy compared to non-irradiated controls after 14 days post-irradiation storage at 4 °C. Further research is needed to study the effect of irradiation on other quality attributes under modified atmosphere packaging and/or under abusive temperatures.

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Section 2.

Studies of post-irradiation survival of *Listeria monocytogenes* and *L. Innocua* on endive, regrowth under modified atmosphere packaging and product sensory qualities

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Abstract

This section summarizes research on the efficacy of ionizing radiation to eliminate the pathogen *Listeria monocytogenes* (ATCC 49594) or the non-pathogenic surrogate *Listeria innocua* (ATCC 51742) from endive (*Cichorium endiva*), the potential for combining irradiation with modified atmosphere packaging (MAP) to prevent re-growth of the pathogen during storage, and the effects of treatment on product sensory qualities. It was determined that the radiation sensitivity of the pathogen and the surrogate were similar, validating the use of the surrogate for studies of irradiation of salad vegetables. During refrigerated storage (in air) following irradiation, the population of *L. monocytogenes* on inoculated endive was briefly suppressed by 0.42 kGy, a dose calibrated to achieve a 99% (2 log) reduction. However, the pathogen regrew after 5 days until it exceeded the bacterial levels on the control after 19 days in storage. Treatment with 0.84 kGy, equivalent to 99.99% (4 log) reduction, suppressed *L. monocytogenes* throughout the course of refrigerated storage. Doses up to 1.0 kGy had no significant effect on color of endive leaf material, either taken from the leaf edge or the leaf midrib. The texture of leaf edge material was unaffected by doses up to 1.0 kGy, while the maximum dose tolerated by leaf midrib material was 0.8 kGy. In combination studies, cut pieces of endive were inoculated with *L. monocytogenes*, packaged in gas-impermeable bags in air, 5/5/90 or 10/10/80 percent CO_2, O_2 and N_2 (AAir-0@, A5/5" and A10/10" respectively) and irradiated to 0.0 (control), 0.3 or 0.6 kGy. During refrigerated storage *L. monocytogenes* and background microflora regrew during storage on Air-0 samples, but not on 5/5 or 10/10 samples. In each of the three atmospheres, O_2 declined and CO_2 increased, irrespective of radiation dose. Irradiated leaf material in Air-0 tended to retain color attributes during storage better than non-irradiated; color retention was more variable under 5/5 and 10/10 packaging. After 8 days, maximum shear force relative to the initial level was significantly reduced in 5/5 at all radiation doses, was not significantly changed in Air-0, and was dose-dependent in 10/10. By 14 days, the texture of all samples had degraded significantly. These results indicate that irradiation and MAP can be combined to prevent the regrowth of *L. monocytogenes* during post-irradiation refrigerated storage, thereby improving product safety.

1. INTRODUCTION

Fresh produce has been associated with numerous outbreaks of food-borne illness in North America in recent years [2]. *L. monocytogenes* is a food-borne bacterium responsible for numerous food-borne illness outbreaks and product recalls [24, 25]. This pathogen can be found as a contaminant in a variety of food products, including vegetables [2, 26]. The mortality frequency for listeriosis patients is approximately 20%; *L. monocytogenes* is subject to zero-tolerance regulation in ready-to-eat (RTE) products in the United States [27, 28]. In environments where pathogens may not be used, the non-pathogenic bacterium *L. innocua* is used commonly in decontamination studies as a surrogate for *L. monocytogenes* [29]. Surrogate organisms can yield important information about how their associated pathogen might respond to a given antimicrobial treatment. However, *L. innocua* has been shown to differ in from *L. monocytogenes* with regard to attachment behavior [30]; this has implications for intervention studies which address the formation and/or removal of biofilms in which *L. monocytogenes* may be a participant. Thus, a given non-pathogenic surrogate may be a good model with regard to one parameter (e.g. sensitivity to an antimicrobial agent or process) but a poor model with regard to another (e.g. attachment). Different species within a given bacterial genus can have significantly different sensitivity to ionizing radiation, even when treated in the same product, under the same conditions [31].

Modified atmosphere packaging (MAP) is used commercially to suppress the growth of spoilage organisms and extend the shelf life of vegetable and meat products [32]. MAP may be passive, in which packages are sealed in air, or active, in which a defined mixture of gases are used to flush the package, typically with reduced O_2 and increased CO_2, with the balance composed of N_2. For vegetables packaged under either system, there is no single ideal or standard gas mixture. The mixture

of gases within the package changes over time in response to the respiration of the produce, the gas permeability of the packaging material, and the specific vegetable under consideration [33].

The potential for combining MAP with low-dose irradiation has been explored in a variety of foods. However, the extent to which headspace gas composition influences the regrowth of irradiated *L. monocytogenes* on vegetables is poorly understood, particularly with regard to the bacteriostatic effects of elevated CO_2 levels on spoilage and pathogenic bacteria [32]. Understanding the behavior of vegetable-associated pathogens after irradiation treatment will help to determine the applicability of irradiation to these products as an antimicrobial intervention.

The objectives of this study were to determine 1) the radiation sensitivity of a pathogen, *L. monocytogenes*, and a commonly used surrogate, *L. innocua*, when inoculated onto endive (*Cichorium endiva*), a leafy salad vegetable, 2) the survival and potential for regrowth of *L. monocytogenes* on irradiated endive stored in air and in various MAP conditions, and 3) the effect of efficacious doses of radiation on the texture and color of endive leaves.

2. MATERIALS AND METHODS

2.1. Products

Whole heads of endive were purchased from local markets the day of each experiment. The outer leaves were discarded. For the purposes of microbiological testing, cut leaf pieces were prepared from the entire head. The basal portion of the head was removed, approximately 5 cm from the end. The leaves were sliced as a group into pieces weighing approximately 0.5 g. Since fresh produce typically carries a significant native microbial load [9], before use in the experiments, the leaf material was surface-sanitized with a 300 ppm sodium hypochlorite solution according to the method of Niemira et al. [27]. Briefly, the leaf pieces were gently agitated in the room-temperature sanitizing solution for 3 min., thoroughly rinsed in distilled water, and spun in a sterile salad spinner-type centrifuge to remove excess surface water (Oxo International, New York, N.Y.). This design of salad spinner incorporates a container base which captures all of the water removed from the leaf surface, and prevents the formation of aerosolized droplets. The microflora of sanitized leaf material was measured using a surface wash with Butterfield's phosphate buffer (BPB Applied Research Institute, Newtown, CT), serial dilution, pour plating with tryptic soy agar (TSA, Difco, Detroit, MI) and incubation at 37EC for 24 h. The post-sanitization population was found to be less than 20 cfu/g leaf tissue.

Homogenized leaf tissue was used as a model solution to determine the effect of internal leaf chemistries on the radiation sensitivity of internalized bacterial [27]. Homogenized leaf suspensions were prepared using sanitized cut leaf tissue. A sample of 45 g of leaf material was placed into a sterile Oster-style blender jar (Thomas Scientific, Swedesboro, NJ) with 180 ml of sterile BPB. This was blended at high speed using a laboratory-grade Osterizer-style blender (Thomas Scientific, Swedesboro, NJ) for 5 s to completely homogenize the leaf material. The homogenate was poured through 4 layers of sterile cheesecloth into a sterile beaker.

2.2. Microorganisms

A representative strain of *L. monocytogenes* (ATCC 49594, American Type Culture Collection, Manasass, VA) and a non-pathogenic strain of *L. innocua* (ATCC 51742) was maintained on 50% glycerol at -70°C. A frozen culture was regrown to late log phase in tryptic soy broth (TSB, Difco) for 16 h at 37°C with agitation and streaked onto Palcam agar (Difco). This was incubated at 37°C for 48 h to form single colonies. These colonies were used to inoculate fresh TSB for each experiment, grown for 16 h at 37°C with agitation. The cell density of the starting inoculum was determined by serial dilution with sterile BPB and pour plating with TSA, 37°C for 48 h. The cell density was typically 10^9 cfu/ml.

To determine the amount of radiation necessary to eliminate 90% (1 log) of the population (D_{10}), the homogenized leaf suspensions (99 ml) were first inoculated with 1 ml of either *L. monocytogenes* or

L. innocua culture. Aliquots (5 ml) of the inoculated suspensions were dispensed into sterile glass tubes. One tube was used per culture per dose. The experiment was performed three times.

Cut leaf pieces were inoculated separately according to the method of Niemira et al. [27]. Because of the potential for aerosolization of the inoculum associated with this method, all material preparation involving the microorganisms was conducted in a biological airflow hood under strict adherence to worker safety guidelines. Sanitized leaf pieces were transferred to a sterile glass inoculation dish (22 cm H 33 cm H 5 cm) and 1000 ml of the working inoculum of either *L. monocytogenes* or *L. innocua* was added. The material was agitated gently for 120 s to completely submerge each piece, and then transferred to a sterile salad spinner-type centrifuge. The material was spun twice to remove excess inoculum from the surface of the leaf pieces.

In the case of material to be evaluated in combination with MAP, two strains of *L. monocytogenes* (ATCC 49594 and ATCC 43256) were used to make a cocktail inoculum. The cultures were maintained, regrown and cultured as described above, with each culture treated separately until they were combined in a 1:1 ratio to make the starting inoculum. The cell density of the starting inoculum was determined by serial dilution with sterile BPB and pour plating with TSA, as described above. The cell density was typically 10^9 cfu/ml. The starting inoculum was diluted 1:10 with sterile BPB to make a working inoculum.

2.3. Packaging for storage studies

In separate studies to determine the survival and recovery of *L. monocytogenes* on irradiated endive during refrigerated storage, cut leaf pieces were inoculated with *L. monocytogenes* and bagged. In all cases, samples (45 g) of each lettuce type were placed in No. 400 stomacher bags (Tekmar, Inc., Cincinnati, OH). The samples were refrigerated (4°C) until irradiation, typically 30-60 min. For samples to be evaluated under air storage, the bags were loosely secured so as to provide for gas exchange with the air. Multiple bagged samples were prepared for a storage study of 19 days duration. Each experiment was performed three times.

For material to be stored under MAP, the samples were bagged as follows. In order to ensure that the material would experience a relatively high-CO_2, low-O_2 environment, samples (45 g) were placed in a laminated foil/plastic barrier-type bag (MIL-B-131, Bell Fibre Products, Columbus, GA). Bagged samples were placed in a self-contained gas packer/sealer (model #A300/16 MC, Multivac Inc., Kansas City, MO). The bags were flushed with air (passive MAP, AAir-0"), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 (A5/5") or 10% CO_2, 10%O_2, 80% N_2 ("10/10") (Scott Specialty Gases, Plumsteadville, PA). In separate experiments for sensory analyses, non-inoculated samples of endive leaves were bagged in the three atmospheres as described. The ratio headspace gas to leaf pieces was approximately 10:1 (v/v). The samples were refrigerated (4°C) until irradiation, typically 30-60 min. Each experiment was performed three times.

2.4. Irradiation

The samples were irradiated using the Lockheed-Georgia cesium-137 self-contained gamma radiation source described in Section 1, with a dose rate of 0.098 kGy/min. Calibration and dosimetry were as previously described.

For determination of D_{10} values, the inoculated samples of homogenized leaf tissue were treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8 or 1.0 kGy. Inoculated cut leaf pieces were treated with 0.0 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.75 or 1.0 kGy. For the storage study, varying doses were applied, based on the intent of the study. For pieces stored under air, the inoculated cut leaf pieces were treated with 0.0 (control), 0.42 or 0.84 kGy. These doses were selected to achieve 0, 2 or 4 $\log_{10}$ reductions (approximately), based on the D_{10} values obtained for *L. monocytogenes*. For MAP studies, the bagged samples were given doses of 0.0 (control), 0.3 or 0.6 kGy. The samples for the three replications of each study were irradiated concurrently.

2.5. Microbiological sampling

After irradiation, the samples were returned to refrigerated storage (4EC) until microbiological sampling, typically 30-60 min. For determination of D_{10} values, aliquots (1 ml) of irradiated leaf homogenates were serially diluted with sterile BPB. Pour plating with TSA was used to determine the surviving bacterial population. Three pour plates per dilution were incubated for 24 h at 37°C and counted with an automatic plate counter. In the case of irradiated cut leaf pieces, sterile BPB (180 ml) was added to the stomacher bag, and agitated for 60 s. A 1 ml sample was withdrawn for serial dilution with sterile BPB. The samples were diluted, pour plated with TSA and incubated as described.

The bacterial reduction data were normalized against the control and plotted as the $\log_{10}$ reduction using the nominal doses. The slopes of the individual survivor curves were calculated with linear regression using a computer graphics program (SigmaPlot 5.0, SPSS Inc., Chicago, IL). The D_{10} value for *L. monocytogenes* and *L. innocua* on each leaf preparation was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp. Ottawa, Ontario, Canada). The significance of differences between the regression lines was determined using analysis of covariance (Excel, Microsoft Corp., Redmond, WA).

For the storage studies, the irradiated leaf pieces were stored, either in air or under MAP at 4°C until sampling. Samples were collected immediately after irradiation (day 0), and on days 2, 5, 14 and 19. For all material under MAP, additional samples were taken at day 1 and at day 9. The samples were surface washed with BPB, serially diluted with BPB and pour plated with TSA as described. Simultaneous samples were plated on Palcam agar and on TSA to distinguish regrowth of *L. monocytogenes* among regrowth of other aerobes. For key sampling times, analysis of variance (ANOVA) was used to evaluate the difference among the dose levels (SigmaStat, SPSS, Chicago, IL).

2.6. Sensory properties

2.6.1. Immediately post-irradiation

Cut leaf pieces of endive were prepared from heads of endive purchased fresh from local markets. The outer leaves and the base of the heads were removed as described above. The proximal portions of the leaves, consisting primarily of leaf midrib tissue, were separated *en masse* from the distal portions of the leaves, consisting primarily of leaf edge tissue. These were separately cut into pieces, sanitized, rinsed and spun dry as described above. Five samples of each type of leaf tissue were bagged for irradiation, 10 g per sample. The samples were treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8 or 1.0 kGy as described, and held at 2°C until sampling, typically 90-120 min. The study was performed three times, with samples irradiated concurrently. Data was pooled and analysed with ANOVA as described.

2.6.2. Post-irradiation and MAP storage

MAP samples given 0.0 (control), 0.3 or 0.6 kGy were evaluated for color and texture after 1, 8 and 14 days in refrigerated storage. The samples consisted of one bag per dose/time/atmosphere combination. The experiment was performed three times.

2.6.3. Color

Color values were taken with a Hunter Lab Miniscan XE meter (Hunter Laboratory, Inc., Reston, VA) to determine the brightness (L-value), greenness/redness (a-value) and blueness/yellowness (b-value) of the material. The meter was calibrated using white and black standard tiles. Illuminant D65, 10^{o} standard observer, and a 2.5 cm port/viewing area were used.

2.6.4. Texture

The maximum shear strength of the leaf sections was measured with a TA.XT2i texture analyser running the TextureExpert v.1.22 software package (Texture Technologies, Scarsdale, NY) using a TA-91 Kramer Shear Press with 5 blades.

2.7. Headspace gas sampling of MAP samples

At each of the sampling times (1, 2, 5, 9, 14 and 19 days), the sample bags were inspected for leaks. A 0.5ml aliquot of the headspace gas in the sample bag was taken using a syringe and a fine gauge needle, pierced through the bag material. The sampling hole was resealed with electrical tape. The gas samples were analysed with a Gow-Mac series 580 gas chromatograph (Gow-Mac Instrument, Bridgewater, NJ), equipped with a 183 cm CTR I column (Alltech Associates, Inc., Deerfield, IL) and a thermal conductivity detector. The injector, oven and detector temperatures were held at ambient (23-25°C). The carrier gas was helium with a flow rate of 120 ml/min. CO_2 and O_2 levels were calculated in comparison to a standard (Alltech Associated, Deerfield, IL). After the headspace gas sampling was completed, the bagged samples were set aside for microbiological sampling.

3. RESULTS

3.1. D_{10} values

Irradiation effectively reduced the population of *L. monocytogenes* and *L. innocua* in leaf homogenates and on cut leaf pieces. The D_{10} values obtained did not differ significantly ($P<0.05$) between *L. monocytogenes* and *L. innocua* on either leaf homogenates or leaf pieces. However, the D_{10} value for *L. innocua* was significantly ($P<0.05$) lower on leaf homogenates vs. leaf pieces, while the D_{10} value for *L. monocytogenes* was not sensitive to leaf preparation method (Table 1).

3.2. Storage and regrowth

3.2.1. Air storage

Irradiation with either 0.42 or 0.84 kGy reduced the initial population of *L. monocytogenes* on endive pieces by 2.6 or 4.0 $\log_{10}$ factors relative to the untreated control, close to levels of reduction predicted by the calculated D_{10} value (Fig. 1A, Palcam agar). The *L. monocytogenes* population was slightly reduced on the untreated controls after two days of refrigerated storage, but remained stable thereafter through the 19 days of the study. Following 0.42 kGy, the population similarly declined slightly until five days, then rebounded and increased at the 14 day period. At the final sampling time, the population of *L. monocytogenes* was slightly (0.4 $\log_{10}$ units) greater than on the control, a statistically significant difference ($P<0.05$). Following 0.84 kGy, the levels of *L. monocytogenes* increased slightly during storage, although the population remained significantly lower than the control throughout the course of the study.

The total aerobic plate counts (TAPC) obtained after incubation at 37°C were generally 0.5 to 1.0 $\log_{10}$ units greater than counts on Palcam agar at the comparable dose-time combinations (Fig. 1B, TSA), indicating that *L. monocytogenes* was the dominant member of the microbial community. The behavior of the TAPC in response to irradiation was generally similar to that of the *L. monocytogenes* population. TAPC increased on samples treated with 0.42 kGy until, at day 14 and 19, they were not significantly different from the controls. TAPC was reduced following 0.84 kGy, but by days 14 and 19, the counts were significantly higher than immediately after treatment (Fig. 1B).

3.2.2. MAP storage

The recoverable counts following inoculation (day 0) were approximately 6.5 $\log_{10}$ cfu/g leaf material. After two days in storage, the non-irradiated Air-0 samples showed significantly ($P<0.05$) increased counts of *L. monocytogenes* (Fig. 2A) and total microflora (Fig. 3A), rising from the relatively high

initial level of associated bacteria immediately following inoculation, 6.2-6.8 $\log_{10}$ cfu/g and remaining consistent for the remainder of the study. In contrast, on non-irradiated 5/5 and 10/10 samples the populations of *L. monocytogenes* (Fig. 2B, Fig. 2C, respectively) and total microflora (Fig. 3B, Fig. 3C, respectively) tended not to increase during storage, and were not significantly ($P<0.05$) different than the initial levels. On non-irradiated samples, the populations observed during storage tended to be more variable on 5/5 than in either Air-0 or 10/10. Also in contrast to the regrowth observed in Air-0 samples, the *L. monocytogenes* and total microbial populations on the irradiated 5/5 and 10/10 samples at the final sampling times (day 19) were either significantly ($P<0.05$) lower than or not significantly different from the initial levels.

Irradiation reduced the levels of *L. monocytogenes* and total microflora in each of the three atmospheres examined. A dose of 0.6 kGy resulted in significant ($P<0.05$) reductions of 3.09 (Fig. 2A), 2.41 (Fig. 2B) and 2.53 (Fig. 2C) $\log_{10}$ cfu per gram of *L. monocytogenes* in Air-0, 5/5 and 10/10, respectively. The same dose significantly ($P<0.05$) reduced the total microflora by 2.34 (Fig. 3A), 2.20 (Fig. 3B) and 2.26 (Fig. 3C) $\log_{10}$ cfu per gram in Air-0, 5/5 and 10/10, respectively. On irradiated Air-0 samples (Fig. 2A), at both 0.3 kGy and 0.6 kGy, *L. monocytogenes* and the total microflora regrew from the initial reductions until the population was equal to or significantly greater ($P<0.05$) than the non-irradiated samples by the last sampling time (19 days).

In contrast to the results obtained in the Air-0 packages, *L. monocytogenes* did not regrow following irradiation on endive packaged in 5/5 (Fig. 2B) or 10/10 (Fig. 2C). Recoverable counts from the latter sampling dates under both gas regimes were either significantly ($P<0.05$) lower than, or not different from, the levels seen immediately post-irradiation (day 0). Total microbiological counts showed a similar pattern of persistent post-irradiation suppression under 5/5 (Fig. 3B) and 10/10 (Fig. 3C) packaging.

3.3. Sensory response

3.3.1. Color

Immediately post-irradiation, doses up to 1.0 kGy had no significant effect on the color of leaf tissue (Table 2). Material taken from the leaf edge was generally darker and greener than material taken from the leaf midrib.

Following MAP storage, all gas/dose combinations were subject to significant ($P<0.05$) loss of greenness (i.e higher “a” values) during storage (Table 3). Neither radiation dose nor the packaging atmosphere employed had a consistent effect on “a” values.

In the non-irradiated samples in each of the three atmospheres, day 14 samples were significantly darker (i.e. lower “L” values) than the day 1 samples (Table 4). In material which had received 0.3 kGy, this pattern was repeated for 10/10 samples, but the loss did not rise to the level of significance for Air-0 and 5/5 samples. Following 0.6 kGy, there was significant darkening for 5/5 and 10/10 samples at day 14, but not for Air-0 samples.

A somewhat similar pattern was observed with regard to loss of yellowness (i.e. lower “b” values) (Table 5). In the non-irradiated samples in each of the three atmospheres, day 14 samples were significantly ($P<0.05$) less yellow than the day 1 samples. Following 0.3 kGy, 5/5 samples showed significant loss of yellowness by day 14, but Air-0 and 10/10 samples did not. Following 0.6 kGy, there was a significant loss of yellowness for 5/5 and 10/10 samples by day 14, but not for Air-0. It should be noted that for each of these color assessments, the results obtained at the final sampling time, day 14, are of limited value, as the loss of texture at this time would render the product essentially unsalable.

3.3.2. Texture

Immediately post-irradiation, doses up to 1.0 kGy had no effect on texture of leaf material taken from the leaf edge, with the maximum shear force obtained at each dose being not statistically different (ANOVA, $P<0.05$) from the control (Fig. 4). Material taken from the leaf midrib was similarly insensitive to doses up to 0.8 kGy; however, at the highest dose examined, 1.0 kGy, the maximum shear force was significantly less (ANOVA, $P<0.05$) than that of the control (Fig. 4).

Following MAP storage, there were no significant ($P<0.05$) differences in texture (maximum shear force) resulting from irradiation in any atmosphere, at any given sampling date (Table 6). Similarly, there were no significant differences among the atmospheres at any given sampling date (Table 6). In Air-0 samples, there was no significant ($P<0.05$) difference between the day 1 and day 8 samples at any of the three doses. Samples packaged in 5/5 and 10/10 generally had a significant loss of texture by day 8. However, extended time in storage caused an essentially similar pattern of loss of texture for all gas/dose combinations, in that samples which were to have been evaluated at day 14 were degraded to the point that meaningful measurements of shear force could not be taken (Table 6).

3.3.3. Headspace gas in MAP samples

In each of the atmospheres used, the percentage O_2 decreased and that of CO_2 increased (Fig. 5A). For Air-0 samples, CO_2 increased from less than 1% at the start of the study to 18-23% at the final sampling time, with no significant difference among the three radiation levels. Levels of O_2 declined from initial measurements of 18-19% to final levels of 1-6%, also with no significant difference resulting from irradiation. For samples packaged in both the 5/5 (Fig. 5B) and 10/10 (Fig. 5C) atmospheres, O_2 levels dropped to below detectable levels (less than 1%) by 5 days post-packaging for all three radiation doses. Levels of CO_2 rose during storage in samples packed in both 5/5 and 10/10, but tended to be more variable in 5/5.

Elevated levels of CO_2 can have a bacteriostatic or bactericidal effect on spoilage pathogens [32]. By day 5, CO_2 levels in the Air-0 samples rose to 8-13% and O_2 levels fell to 4-8% (Fig. 5A), yet the irradiated samples were still subject to a regrowth of *L. monocytogenes* and total microflora. However, the combination of elevated CO_2 and very low O_2 levels in the 5/5 and 10/10 samples during the course of storage prevented the regrowth of bacteria on the irradiated samples in both atmospheres.

4. DISCUSSION

Ionizing radiation was effective in reducing the population viability of both *L. monocytogenes* and *L. innocua* on endive leaf preparations. In this study, the responses of the strains of *L. monocytogenes* and *L. innocua* to irradiation did not differ from each other on either cut leaf pieces or leaf homogenate. These results suggest that, on this food substrate, *L. innocua* can be an effective surrogate for *L. monocytogenes*. The radiation D_{10} value of *L. monocytogenes* was insensitive to changes in leaf tissue preparation, i.e. a liquid leaf homogenate suspension vs. surface inoculation. *L. innocua*, in contrast, was significantly more sensitive to irradiation in leaf homogenate than on the leaf surface. The leaf homogenate is intended as a simplified model for the type of chemistries which an internalized bacterial population may encounter, albeit a model with acknowledged limitations [27]. The results of this study suggest that *L. innocua* which is internalized in a leaf may be somewhat more sensitive than surface-associated contamination.

On endive leaf material stored in air, inoculated *L. monocytogenes* remained present on the non-irradiated control material at levels approximately equal to that of the initial inoculation. The regrowth of *L. monocytogenes* on endive following 0.42 kGy demonstrated herein support earlier reports of initial reduction, followed by the regrowth in storage of *L. monocytogenes* on irradiated vegetables and meats stored in aerobic packaging. In those studies, *L. monocytogenes*-inoculated celery [34], frankfurters [35] and beef bologna [36] were treated with doses resulting in initial reductions of approximately 2 $\log_{10}$ cfu per g, comparable to those obtained in the present work. The pattern of regrowth following a low radiation dose is consistent, despite the fact that the D_{10} value, i.e. the

radiation dose required to achieve a 1 $\log_{10}$ reduction, for *L. monocytogenes* is typically higher on meats than on vegetables. Low radiation doses, i.e. doses roughly equivalent to 2 D_{10} units, therefore provide a transitory reduction of *L. monocytogenes* population.

L. monocytogenes is able to conduct injury repair and grow at refrigeration temperatures. Therefore, for material stored under air, a higher initial radiation dose, equivalent to 4 or more D_{10} units is required to effect a more complete elimination, and avoid the possibility of regrowth of *L. monocytogenes* after an inadequate treatment. In contrast, under MAP, packaging with air allowed regrowth, while enhanced CO_2 gas mixes did not. These results demonstrate that the antimicrobial effects of lower radiation doses can be made to persist during refrigerated storage by altering the headspace gas composition.

Endive leaf pieces were seen to be generally insensitive to changes in color or texture immediately following antimicrobially efficacious radiation doses, i.e 0.8 kGy. Under MAP, no significant differences were evident among the various irradiation doses applied. However, all samples suffered from loss of quality during storage. As commercially packaged leafy salad vegetables have a salable shelf life of 12-14 days [37, 38], the loss of texture at the final sampling time in this study is not unexpected.

5. SUMMARY AND CONCLUSIONS

1. This study has shown that on a leafy green vegetable, *L. innocua* ATCC 51742 has a similar response to *L. monocytogenes* ATCC 49594 and may therefore be regarded as a valid surrogate model organism on this product for evaluations of radiation sensitivity.

2. Following very low radiation doses, equivalent to 2 D_{10} units, an initial decline in *L. monocytogenes* population was fully recovered by 19 days in storage.

3. Higher doses result in a more lasting suppression of *L. monocytogenes* on endive stored in air, and these doses (0.8 -1.0 kGy) have little or no significant impact on the product's sensorial properties.

4. The reduction in *L. monocytogenes* in the 5/5 and 10/10 samples following low-dose irradiation can be made to persist during storage by forcing the O_2 content of the atmosphere to very low levels. The resultant loss of texture and color resulting from anaerobic conditions highlight the equally important need for providing the leaves with an opportunity for gas exchange. The use of gas permeable plastics to control gas exchange, or the application of acidified rinses, to control color loss, are tools to be explored further in achieving this balance.

5. A challenge for future research is balancing the preservation of quality while preventing the regrowth of *L. monocytogenes* following low-dose irradiation plus MAP.

ACKNOWLEDGEMENTS

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TABLE 1. RADIATION D10 VALUES OF LISTERIA MONOCYTOGENES AND L. INNOCUA INOCULATED ONTO ENDIVE PIECES AND ENDIVE LEAF HOMOGENATE

	Endive homogenate	Cut leaf pieces	
L. monocytogenes	0.20 ∀ 0.01[a]	0.21 ∀ 0.01	NSD[b]
L. innocua	0.19 ∀ 0.01	0.22 ∀ 0.01	P<0.05
	NSD	NSD	

[a] Numbers are D_{10} values plus or minus the standard error, in kGy.
[b] For each inoculum type, and for each leaf preparation, the results of analysis of covariance are shown, either as P value or "NSD" (i.e. no significant difference).

TABLE 2. COLOR PARAMETERS OF IRRADIATED ENDIVE PIECES

	Leaf edge sections			Leaf midrib sections		
Dose (kGy)	L[a]	a[b]	b[c]	L	A	B
0.0	37.47a[d]	-9.13a	25.89a	57.35a	-5.59a	25.23a
0.2	39.05a	-8.79a	25.06a	53.57a	-6.44a	27.32a
0.4	38.22a	-8.57a	24.89a	53.39a	-6.10a	24.86a
0.6	37.70a	-9.04a	25.45a	55.14a	-5.72a	24.80a
0.8	37.13a	-8.52a	23.85a	56.91a	-5.66a	26.21a
1.0	37.51a	-8.91a	24.42a	55.02a	-6.09a	25.42a

[a] Brightness ("L"): 0 = white, 100=black.
[b] Green/Red ("a"): negative "a" values indicate greenness; positive "a" values indicate redness.
[c] Blue/Yellow ("b"): negative "b" values indicate blueness; positive "b" values indicate yellowness.
[d] For a given product, dose-temperature combinations followed by the same letter are not significantly different. P<0.05, analysis of variance, Tukey test.

TABLE 3. COLOR VALUES (GREENNESS) OF IRRADIATED ENDIVE STORED AT 4°C FOR 14 DAYS [a]

Day	Gas	0.0 kGy		0.3 kGy		0.6 kGy	
1	Air-0	-8.9∀0.16	dx	-8.6∀0.16	ex	-8.6∀0.17	Cx
8	Air-0	-7.8∀0.27	cdx	-8.3∀0.12	dex	-7.6∀0.39	Bcx
14	Air-0	-5.0∀0.40	bxy	-6.5∀0.38	abcx	-5.6∀0.67	Aby
1	5/5	-8.5∀0.14	cdx	-8.3∀0.20	cdex	-8.8∀0.12	Cx
8	5/5	-7.4∀0.27	cx	-6.9∀0.24	abcdxy	-6.2∀0.27	Aby
14	5/5	-3.9∀0.20	ax	-4.3∀0.24	ax	-3.9∀0.39	Ax
1	10/10	-8.7∀0.22	dx	-8.6∀0.13	ex	-8.6∀0.16	Cx
8	10/10	-8.2∀0.21	cdx	-7.4∀0.36	bcdex	-7.7∀0.24	Bcx
14	10/10	-5.7∀0.30	bx	-5.3∀0.21	abx	-4.8∀0.37	Ax

[a] Note: within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different (P<0.05). Within each row, means indicated by the same letter (x through z) are not significantly different (P<0.05) (∀ SE).

TABLE 4. COLOR VALUES (BRIGHTNESS) OF IRRADIATED ENDIVE STORED AT 4°C FOR 14 DAYS [a]

Day	Gas	0.0 kGy		0.3 kGy		0.6 kGy	
1	Air-0	36.1∀0.96	abx	34.5∀1.10	abx	35.9∀1.02	Abx
8	Air-0	35.0∀0.79	abcx	36.1∀0.80	abx	37.3∀1.82	Ax
14	Air-0	28.7∀1.50	dx	33.5∀1.41	aby	35.4∀0.79	Aby
1	5/5	36.3∀0.63	abx	36.0∀0.92	abx	37.6∀1.09	Ax
8	5/5	34.0∀0.97	bcx	33.2∀0.76	abx	33.4∀1.09	abcx
14	5/5	27.9∀1.11	dx	31.6∀1.44	bx	30.0∀1.45	Cx
1	10/10	38.5∀0.80	ax	37.5∀0.82	ax	37.3∀0.85	Ax
8	10/10	35.0∀0.89	abcx	34.0∀1.15	abx	36.3∀1.18	Abx
14	10/10	31.0∀0.79	cdx	31.8∀0.99	bx	31.1∀1.11	Bcx

[a] Note: within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different (P<0.05). Within each row, means indicated by the same letter (x through z) are not significantly different (P<0.05) (∀ SE).

TABLE 5. COLOR VALUES (YELLOWNESS) OF IRRADIATED ENDIVE STORED AT 4°C FOR 14 DAYS[a]

Day	Gas	0.0 kGy		0.3 kGy		0.6 kGy	
1	Air-0	23.3∀0.45	ax	22.2∀0.41	abcx	21.8∀0.51	abx
8	Air-0	22.1∀0.40	abx	22.9∀0.40	abx	22.3∀0.63	Ax
14	Air-0	20.3∀0.85	bcx	23.0∀0.74	ay	22.5∀0.39	Ay
1	5/5	22.4∀0.55	abx	22.2∀0.31	abxc	23.2∀0.60	Ax
8	5/5	22.6∀0.47	abx	22.4∀0.31	abx	22.2∀0.38	Ax
14	5/5	18.6∀0.52	cx	19.8∀0.78	cx	19.2∀0.46	Cx
1	10/10	23.5∀0.47	axy	21.9∀0.43	abcx	22.4∀0.48	Ay
8	10/10	23.4∀0.51	ax	21.9∀0.78	abcx	21.7∀0.59	abx
14	10/10	20.3∀0.56	bcx	20.5∀0.55	bcx	19.6∀0.60	bcx

[a] Note: within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different (P<0.05). Within each row, means indicated by the same letter (x through z) are not significantly different (P<0.05). (∀ SE)

TABLE 6. TEXTURE (MAXIMUM SHEAR FORCE IN G) OF IRRADIATED ENDIVE STORED AT 4°C FOR 14 DAYS[a]

Day	Gas	0.0 kGy		0.3 kGy		0.6 kGy	
1	Air-0	25357∀1170	abx	24744∀1602	abx	24997∀1622	abx
8	Air-0	20398∀1398	bcx	23208∀1667	bcx	19852∀1356	bcx
14	Air-0	N/A		N/A		N/A	
1	5/5	26741∀1349	abx	28735∀1083	ax	26055∀1252	Ax
8	5/5	19535∀2119	bcx	18732∀1306	cx	18654∀1686	Cx
14	5/5	N/A		N/A		N/A	
1	10/10	24204∀1217	abx	26322∀936	abx	28383∀952	Ax
8	10/10	17422∀1516	cx	18811∀1171	cx	22926∀1648	abcx
14	10/10	N/A		N/A		N/A	

[a] Note: within each of the three doses, means for the different samples which are followed by the same letter (a through e) are not significantly different (P<0.05). Within each row, means indicated by the same letter (x through z) are not significantly different (P<0.05).(∀ SE)
N/A: data not available due to sample degradation.

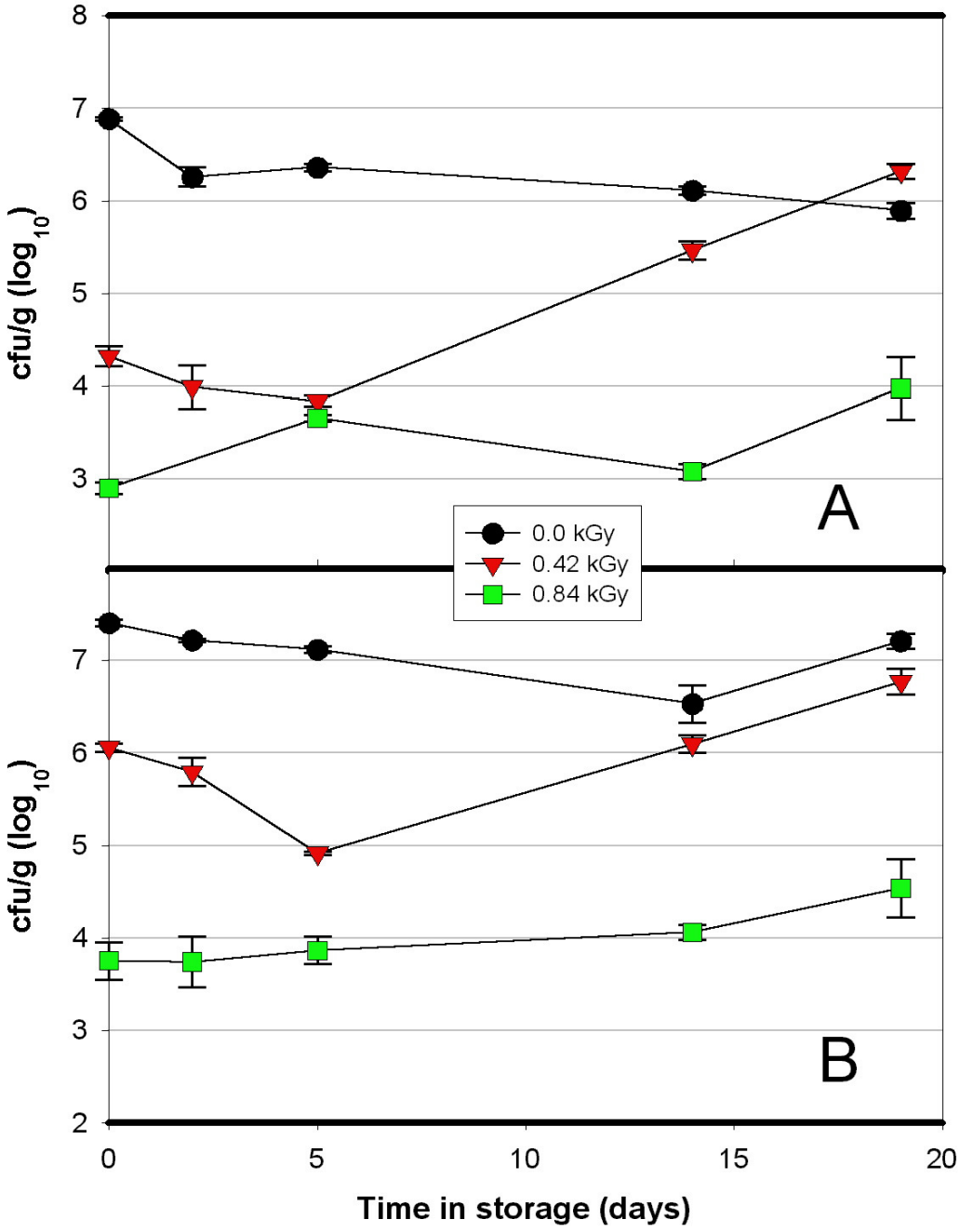

FIG 1. Survival and regrowth during refrigerated storage of L. monocytogenes on irradiated endive leaf pieces.

Graphs represent growth on Palcam agar (A) or tryptic soy agar (B). Error bars indicate standard error, n=9 per sampling time.

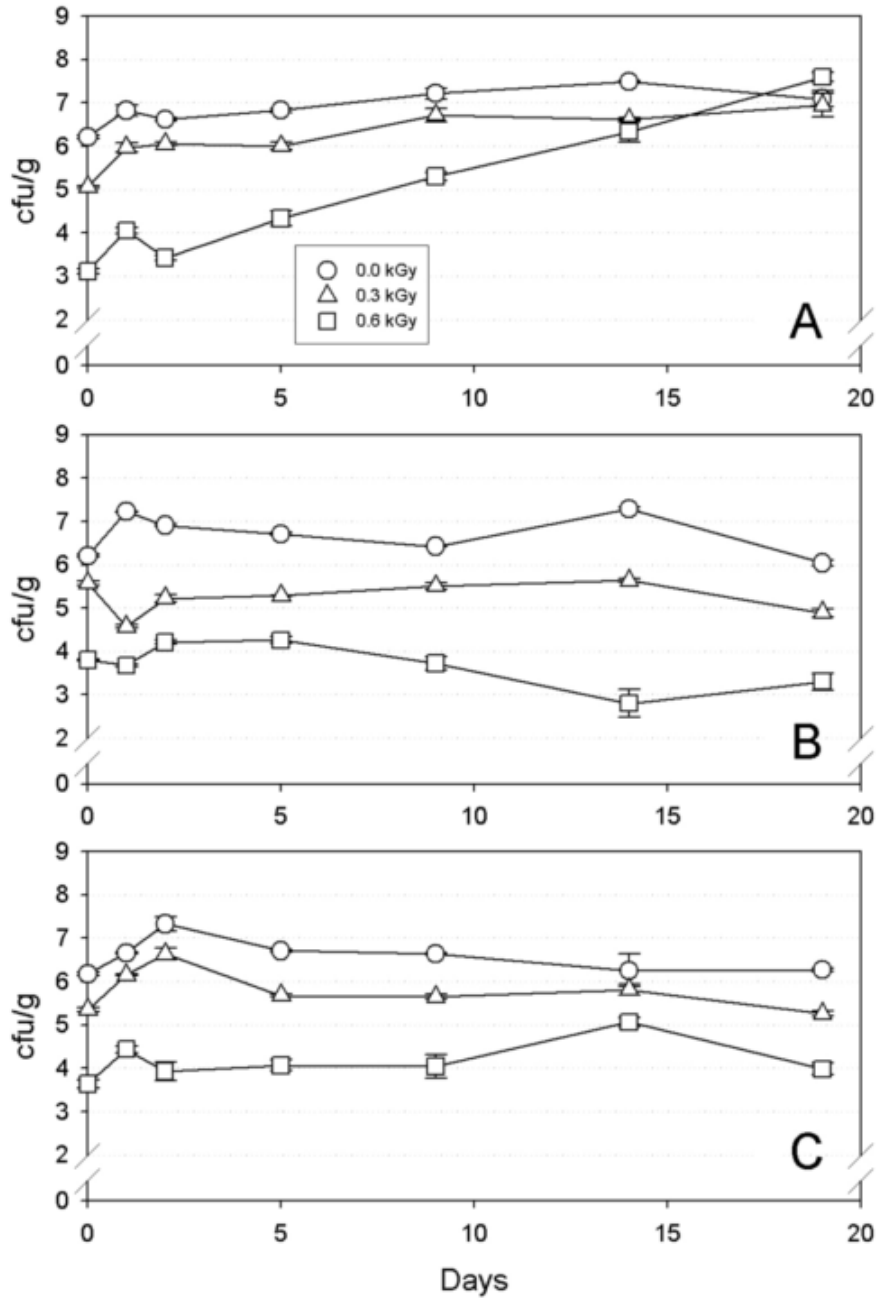

FIG 2. Populations of L. monocytogenes from packaged, irradiated endive, plated on Palcam agar.

Packaging atmospheres are air (AAir-0", graph 2A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 (A5/5", graph 2B) or 10%CO_2, 10%O_2, 80% N_2 (A10/10", graph 2C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, n=9.

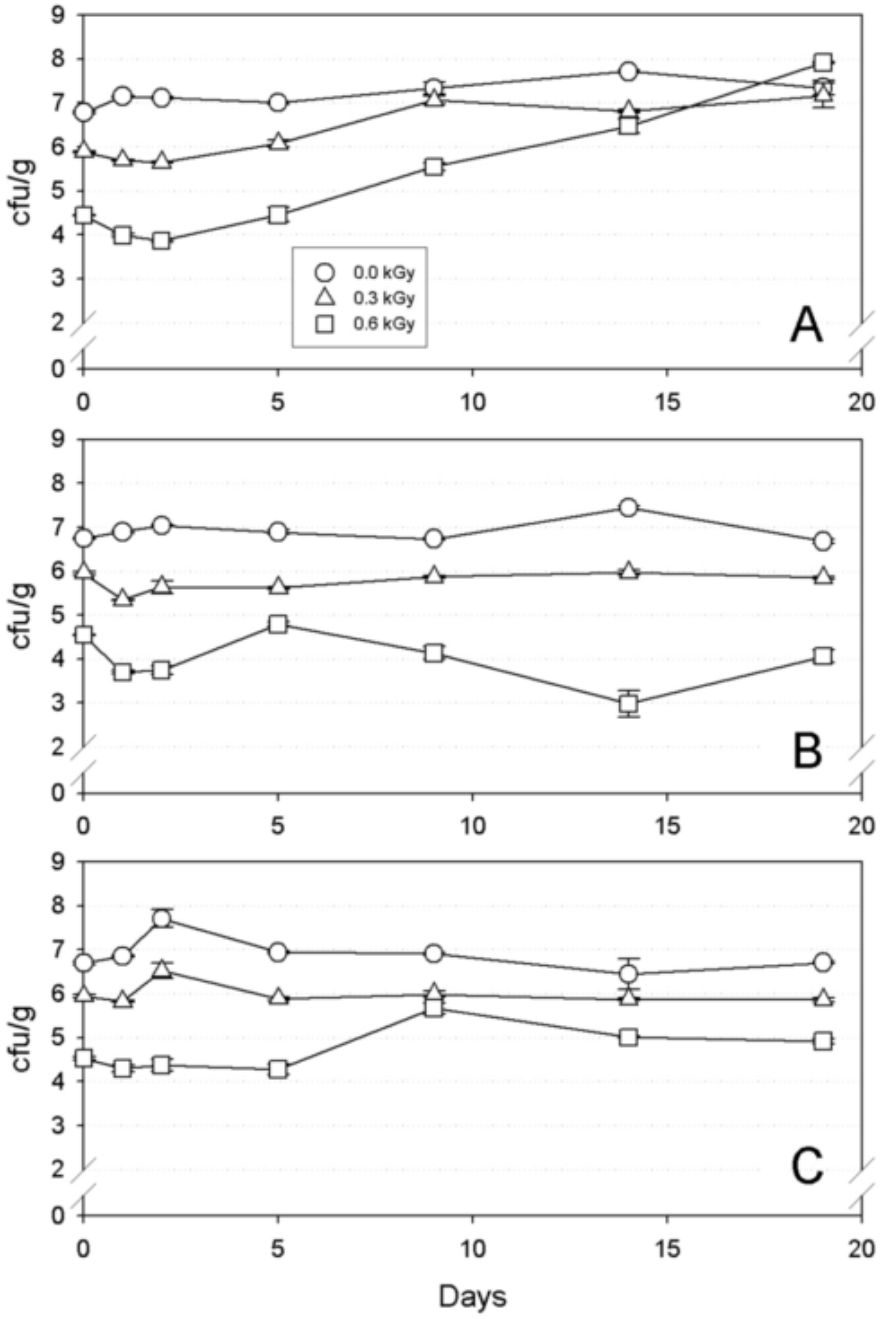

FIG 3. Total microbial populations from packaged, irradiated endive, plated on tryptic soy agar.

Packaging atmospheres are air (AAir-0", graph 3A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 (A5/5", graph 3B) or 10%CO_2, 10%O_2, 80% N_2 (A10/10", graph 3C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, n=9.

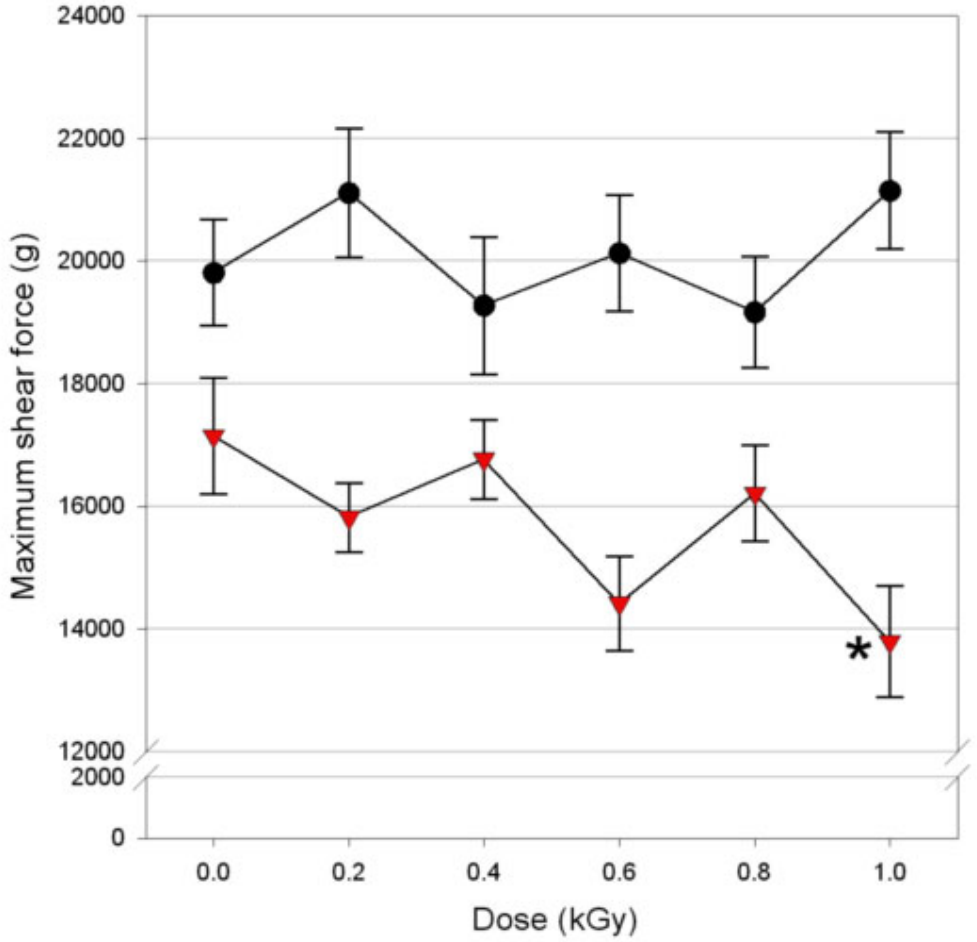

FIG 4. Texture of irradiated endive - leaf edge segments (circle) or leaf midrib segments (triangle).

For each type of leaf segment, doses denoted with an asterisk (*) are significantly (P<0.05) different from their respective control by analysis of variance, ANOVA. Error bars indicate standard error, n=15.

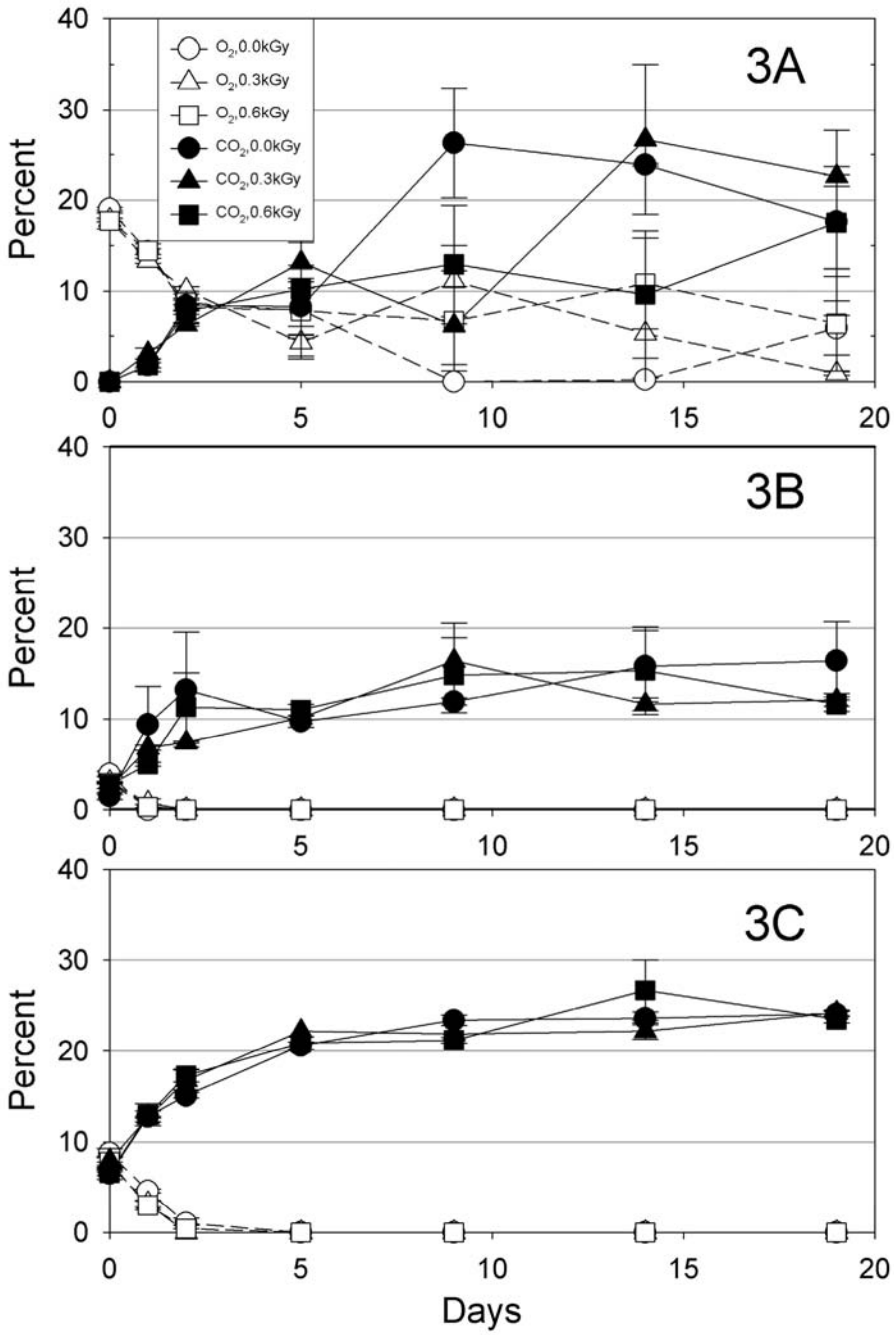

FIG 5. Concentration of O_2 (white) and CO_2 (black) in packaged, irradiated endive.

Packaging atmospheres are air (AAir-0", graph 5A), a pre-mixed atmosphere of 5% CO_2, 5% O_2, 90% N_2 (A5/5", graph 5B) or 10%CO_2, 10%O_2, 80% N_2 (A10/10", graph 5C). Irradiation doses are 0.0 (circle), 0.3 (triangle) or 0.6 kGy (square). Error bars indicate standard error, n=3.

Section 3.

Studies on the irradiation destruct values for *Shigella Sonnie* in liquids or on fresh produce, *Salmonella* and *Escherichia Coli* O157:H7 on sprouts and post irradiation survival and regrowth of indigenous microflora on vegetable sprouts and on warm or cold water-washed cut Iceberg Lettuce

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Abstract

Consumption of contaminated fresh produce with *Salmonella* spp., *Escherichia coli* O157:H7 or *Shigella sonnie* resulted in confirmed food-borne outbreaks in the United States and elsewhere. Irradiation destruct values of the produce related isolates are not known and were determined after being inoculated on fresh lettuce or sprouts using a gamma source. The resulting destruct values for *Salmonella, E. coli* O157:H7 and *Shigella sonnie* were 0.46 ± 0.02, 0.30 ± 0.02, and 0.24 ± 0.03 kGy, respectively. These values are comparable with the published values for the meat-related food-borne isolates. Ionizing irradiation was used as an intervention to reduce the indigenous microbial populations on fresh sprouts and cut lettuce washed in 5 and 47°C water. Microbiological profiles were monitored during refrigerated (4°C) storage and analysed each week for up to three weeks to determine the keeping quality. Regardless of the initial background, after irradiating to 2 kGy, a two log reduction was observed for the total aerobic and coliform counts on the sprouts or lettuce samples. During storage the bacterial counts of the irradiated samples increased but not to the level of the non-irradiated samples. The results indicate that a 2 kGy dose improved the microbial keeping quality of the fresh produce, inhibited microbial spoilage and achieved a 5 log reduction for both the *E. coli* O157:H7 and *Shigella sonnie*. The reduced bacterial counts would also provide a margin of safety by also reducing food-borne pathogen levels.

1. INRODUCTION

In the United States there have been confirmed *Shigella* related food borne outbreaks since 1995 [39, 40, 41]. As a result of the awareness that this pathogen can adulterate food [42-45], particularly fresh produce [46, 47, 48, 49, 50, 51], the U.S. Food and Drug Administration included *Shigella* identification as a contaminant in their 2000 survey of domestic and imported fresh produce. *Shigella* was isolated from domestically grown cantaloupes, cilantro, green onions and parsley [52]. The bacteria was isolated in nine out of 1003 imported products, including, cantaloupe, celery, lettuce, parsley and scallions [53].

The objectives of this study are to determine the radiation sensitivity of *Shigella sonnei,* isolated from produce related outbreaks, that were inoculated onto iceberg lettuce, radish sprouts and into liquids; the radiation sensitivity of *Salmonella* and *Escherichia coli* inoculated on radish sprouts; and the efficacy of irradiation in improving the microbial keeping quality of fresh produce.

2. METHOD AND MATERIAL

2.1. Microorganisms

All bacteria isolates were obtained from produce related outbreaks. The isolates were: *Shigella sonnei* F6129 and 10304-98; *Salmonella enteritidis* Antum F4317, Stanley H0558, Newport H1275 and Infantis F4319; and *Escherichia coli* O157:H7 C7927, Sea13B88 and F4546.

2.2. Products

Filter sterilized lettuce extract broth, sterile deionized water, and irradiation sanitized (6 kGy) lettuce pieces or vegetable sprouts were used.

2.3. Inoculation

A single 18 h culture was obtained for each isolate. The cells were harvested by centrifugation, washed and resuspended in either sterile deionized water or vegetable broth to a log 10^{8-9}.

Cells suspended in water were used for the produce inoculation. A cocktail of *Salmonella* or *E. coli* was made by combining equal volumes of the separate isolates for the produce inoculum. Each Shigella strain was cultured and used separately. The irradiated sanitized produce was inoculated by submersion 1 kg/1 liter for one min. The produce was drained in a colander to remove excess liquid inoculum, bagged, and refrigerated 24 h before the D_{10} determination.

2.4. Irradiation

The samples were irradiated using the Lockheed-Georgia cesium-137 self-contained gamma radiation source described in Section 1, with a dose rate of 0.098 kGy/min. Calibration and dosimetry were as previously described. Doses used to obtain D_{10} values were 0.0, 0.2, 0.4, 0.8, 1.0, 1.2, 1.4, 1.6, 2.0 and 2.4 kGy. Doses for the microbial keeping quality study were 0, 1, and 2 kGy.

2.5. Sampling

The liquid sample used to obtain the D_{10} value for *Shigella* were serially diluted in 0.1 % peptone water and plated on both tryptic soy broth plus agar (TSBA) and TSBA plus pyruvate (TSBA+). The initial dilution for all produce samples was made with 1 % buffered peptone water and serials diluted with 0.1 % peptone water. The microbial keeping quality counts were done on tryptic soy agar or Aerobic and E.coli/Enterobactericeae Petrifilm plates. The counts for the *Shigella* inoculated produce were done using both TSBA and TSBA+.

3. RESULTS

3.1. D_{10} value for *Shigella*

The D_{10} curves for the *Shigella* F6129 and 10304-98 isolates are shown in Fig. 1. The D_{10} values were 0.26 ± 0.03 and 0.30 ± 0.02 kGy, respectively, for all samples tested. There was no difference between the counts obtained using TSBA or TSBA+ for the irradiation D_{10} recovery. These values are comparable with those values published by Mossel for D_{10} values for *Shigella* in seafood; when the two isolates were compared for their thermal sensitivity, there was a dramatic difference: Strain F6129 was significantly more thermally sensitive than the 10304-98, whereas the radiation sensitivity was the same.

3.2. D_{10} value for *Salmonella*

The D_{10} curves for the *Salmonella* cocktail on alfalfa sprouts was 0.46 kGy (Figure 2). This value compares well with those published values determined for *Salmonella* inoculated on meat products.

3.3. D_{10} value for *E.* coli O157:H7

The D_{10} curve for the *E. coli* cocktail on alfalfa sprouts was 0.30 kGy (Figure 3). This value compares well with those published values determined for *E. coli* inoculated on meat products.

3.4. Microbial keeping quality

The 2 kGy dose reduced the initial microbial background counts (total aerobic and coliform) on alfalfa sprouts (Figure 4). For the cut lettuce that was washed before the irradiation treatment and kept at refrigeration temperature (4 ± 2 ^{o}C) the reduction in both aerobic and coliform counts was maintained for up to two weeks (Figure 5).

4. CONCLUSION

The microbial keeping quality was greatly enhanced by giving the fresh produce an irradiation treatment of 2 kGy [54]. However, when a 5 log reduction is needed to guarantee a *Salmonella* free product, a >2 kGy dose would be needed. The 2 kGy level did not cause any undesirable nutrient loss.

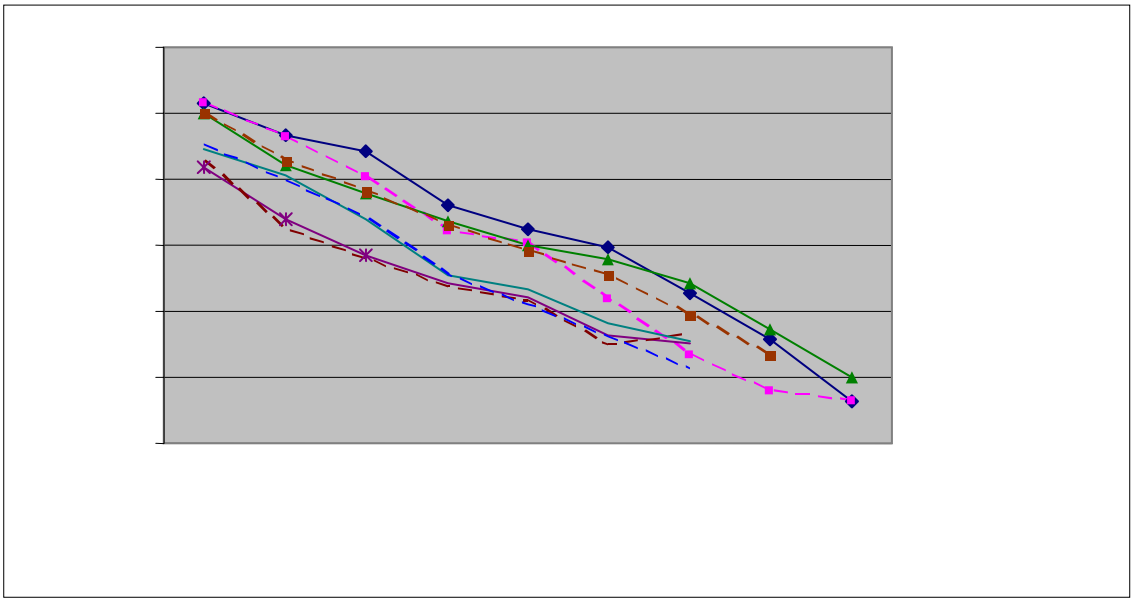


FIG 1. D_{10} Curves of Shigella sonnie F6129 in liquid and on fresh produce. The + indicates retrieved on TSBA+.

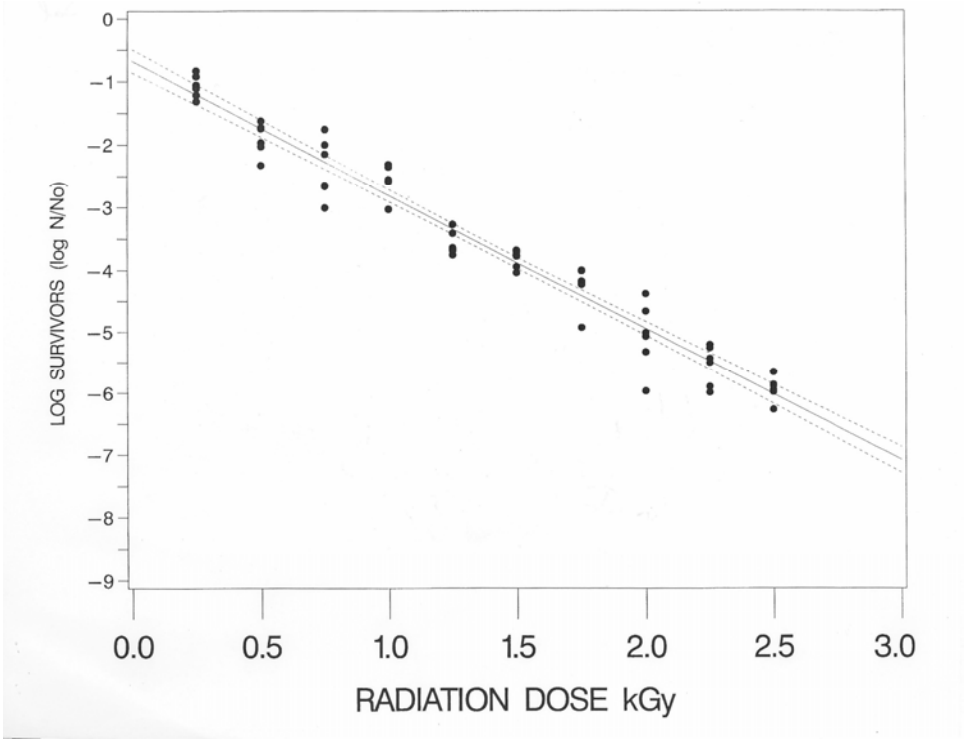


FIG 2. D_{10} curves for Escherichia coli O157:H7 cocktail on alfalfa sprouts.

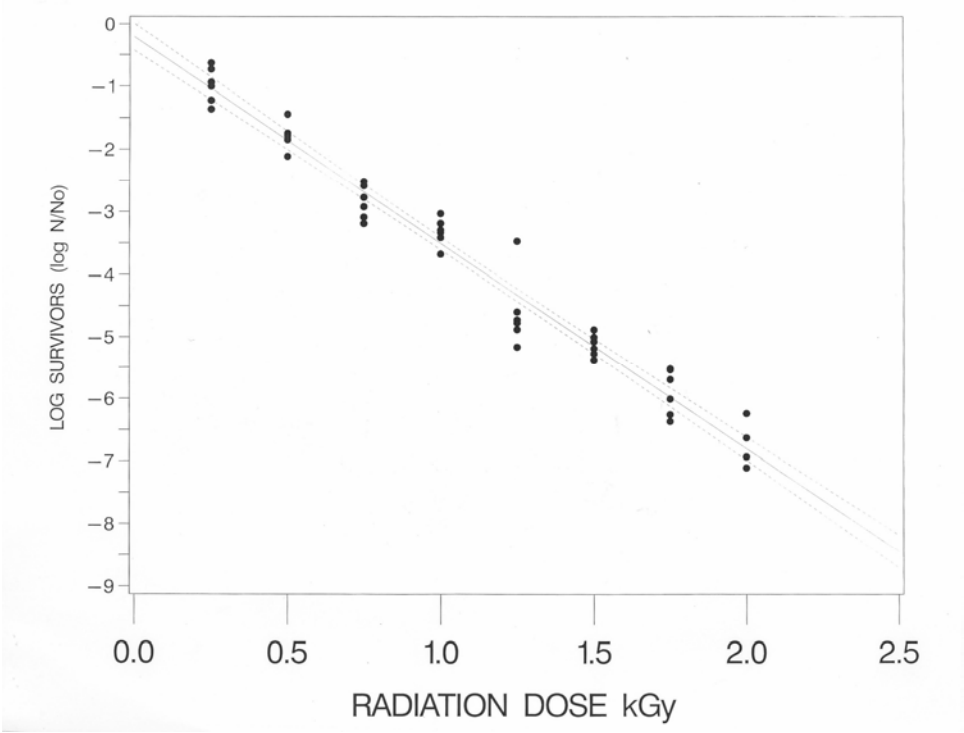


FIG 3. D_{10} curves for Salmonella cocktail on alfalfa sprouts.

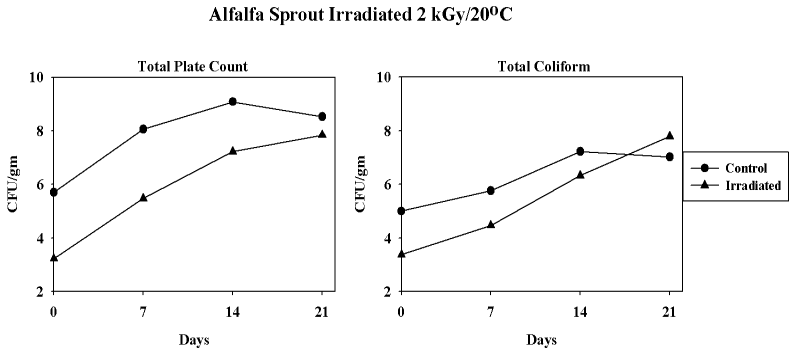
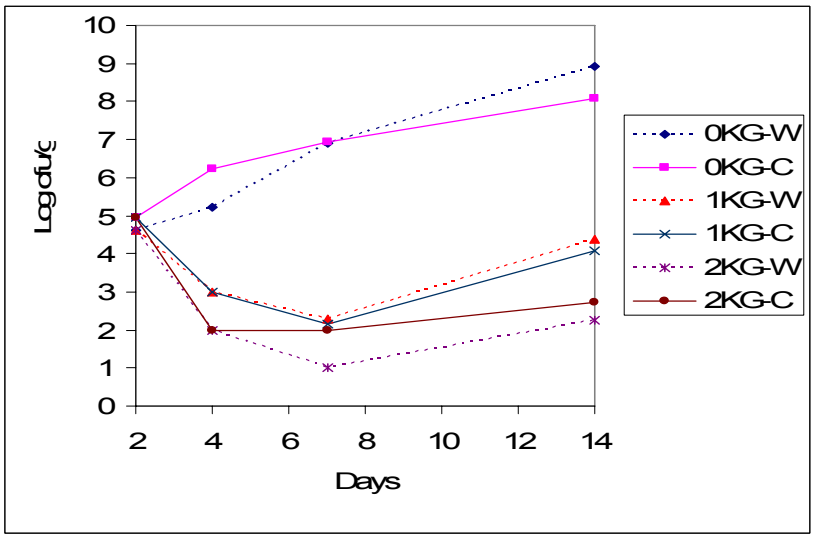


FIG 4. Microbial keeping quality of total aerobic and coliform counts of alfalfa sprouts irradiated at 2 kGy at 20° C and kept refrigerated for up to 3 wks.



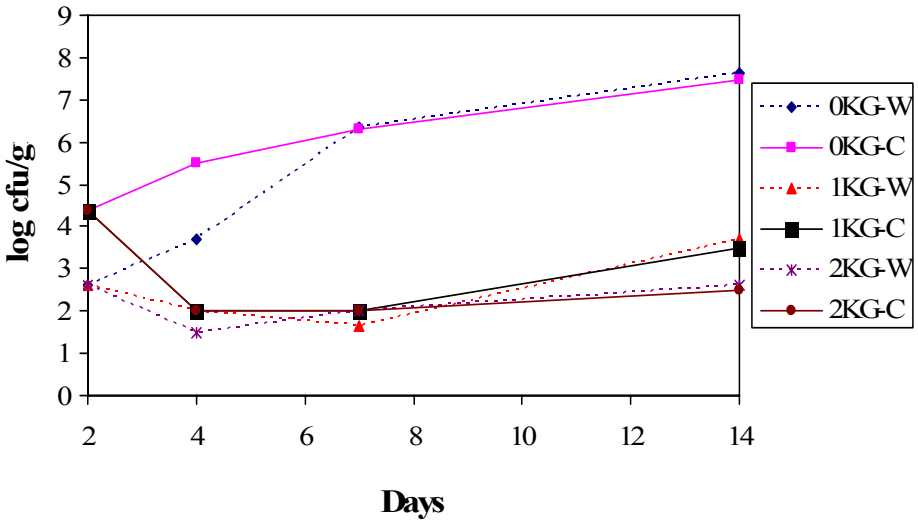


FIG 5: Microbial keeping quality of warm (W) and cold (C) washed cut iceberg lettuce irradiated at 1 and 2 kGy stored refrigerated for 2 wks.

Top figure is aerobic plate counts and bottom figure is coliform counts.

REFERENCES

[1] GLASER, L.K., THOMPSON, G.D., HANDY, C.R., Recent changes in marketing and trade practices in the U. S. Lettuce and fresh-cut vegetable industries. Agric. Info. Bulletin **767** (2001). USDA, Economic Research Service, Washington DC.

[2] BEUCHAT, L. R. *Listeria monocytogenes*: incidence in vegetables. Food Control **7** (1996) 223-228.

[3] SIVAPALASINGAM S., FRIEDMAN, C.R., COHEN, L., TAUXE, R.V., Fresh Produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. **67** (2004) 2342-2353.

[4] WACHTEL, M.R., CHARKOWSKI, A.O., Cross-contamination of lettuce with *Escherichia coli* O157:H7. J. Food Prot. **65** (2002) 465-470.

[5] ZHANG, S., FARBER, J.M., The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiol. **13** (1996) 311-321.

[6] KADER, A.A. Potential applications of ionizing radiation on postharvest handling of fresh fruits and vegetables. Food Technol. **40** (1986) 117-121.

[7] PRAKASH, A., GUNER, A.R., CAPORASO, F., FOLEY, D.M., Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food Sci. **65** (2000) 549-553.

[8] FOLEY, D.M., DUFOUR, A., RODRIGUEZ, L., CAPORASO, F., PRAKASH, A., Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. Radiation Physics Chem. **63** (2002) 391-396.

[9] FAN, X., SOKORAI, K.J.B., Sensorial and chemical quality of gamma irradiated fresh-cut iceberg lettuce in modified atmosphere packages. J. Food Prot. **65** (2002) 1760-1765.

[10] FAN, X., NIEMIRA, B.A., SOKORAI, K.J.B., Sensorial, nutritional and microbiological quality of fresh cilantro leaves as influenced by ionizing irradiation and storage. Food Research Intern. **36** (2003) 713-719.

[11] KADER, A.A., LIPTON, W.J., MORRIS, L.L., Systems for scoring quality of harvested lettuce. HortScience. **8** (1973) 408-409.

[12] FAN, X., TOIVONEN, P.M.A., RAJKOWSKI, K.T., SOKORAI, K., Warm water treatment in combination with modified atmosphere packaging reduced undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. J. Agric. Food Chem. **51** (2003) 1231-1236

[13] FAN, X., SOKORAI, K.J.B., Assessment of radiation sensitivity of fresh-cut vegetables using electrolyte leakage measurement. Postharvest Biol. Technol. **36** (2005)191-197.

[14] HAYASHI, T., TODORIKI, S., NAGAO, A., Effect of gamma irradiation on the membrane permeability and lipid composition of potato tubers. Environ. Exp. Bot. **32** (1992) 265-271

[15] LESTER, G.E., WOLFENBARGER, D.A., Composition of cobalt-60 gamma irradiation dose rates on grapefruit flavedo tissues and on Mexican fruit fly mortality. J. Food Prot. **53** (1990) 329-331.

[16] FINLAYSON, J.E., PRITCHARD, M.K., RIMMER, S.R., Electrolyte leakage and storage decay of five carrot cultivars in response to infection by *Sclerotinia sclerotiorum*. Can. J. Plant. Pathol. Rev. Can. Phytopathol. **11** (1989) 31??-316.

[17] FAN, X., Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. J. Sci. Food and Agric. **85** (2005) 995-1000

[18] QUFEDJIKH, H., MAHROUZ, M., AMOIT, M.J., LACROIX, M., Effect of γ-irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of citrus clementina Hort. Ex. Tanaka. J. Agric. Food Chem. **48** (2000) 558-565.

[19] FAN, X., THAYER, D.W., Quality of irradiated alfalfa sprouts. J. Food Prot. **64** (2001) 1574-1578.

[20] FAN, X., NIEMIRA, B.A., SOKORAI, K.J.B., Use of ionizing radiation to improve sensory and microbial quality of fresh-cut green onion leaves. J. Food Sci. **64** (2003) 1478-1481.

[21] KIM, H.J., HAO, F., STOYAN, T. A., FAN, X., Effect of sequential treatment of warm water dip and low dose gamma irradiation on the quality of fresh-cut green onions. J. Food Sci. **70** (2005) M179-185.

[22] LANGERAK, D.I., The influence of irradiation and packaging on the quality of prepacked vegetables. Ann. Nutr. Aliment. **32** (1978) 569-586.
[23] FARKAS, J., SARAY, T., MOHACSI-FARKAS, C., HORTI, C., ANDRASSY, E., Effects of low-dose gamma irradiation on shelf life and microbiological safety of precut/prepared vegetables. Adv. Food Sci. **19** (1997) 111-119.
[24] NIEMIRA, B. A. Irradiation of fresh and minimally processed fruits, vegetables and juices. pp. 279-300. *in* NOVAK, J. S., SAPERS, G. M., JUNEJA, V. K. (eds.). The Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL (2003).
[25] ANONYMOUS, Multistate outbreak of listeriosis-United States, 1998. Morbid. Mortal. Wkly. Rep. **47** (1998) 1085–1086.
[26] BARNES, R., ARCHER, P., STRACK J., AND ISTRE, G. R., Epidemiological notes and reports: Listeriosis associated with consumption of turkey franks. Morbid. Mortal. Wkly. Rep. **38** (1989) 268-269.
[27] NIEMIRA, B. A., SOMMERS, C. H. AND FAN, X., Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157:H7. J. Food Prot. **65** (2002) 1388-1393.
[28] USDA, Revised policy for controlling *Listeria monocytogenes*. Fed. Reg. 54:22345-22346. Food Safety and Inspection Service, U.S. Dept. Of Agriculture, Washington, D.C. (1989).
[29] SOMMERS, C., KOZEMPEL, M., FAN, X. AND RADEWONUK, E. R., Use of vacuum-steam-vacuum and ionizing radiation to eliminate *Listeria innocua* from ham. J. Food Prot. **65** (2002) 1981-1983.
[30] MEYLHEUC, T., GIOVANNACCI, I., BRIANDET, R. AND BELLON-FONTAINE, M. N., Comparison of the cell surface properties and growth characteristics of *Listeria monocytogenes* and *Listeria innocua*. J. Food Prot. **65** (2002) 786-793.
[31] NIEMIRA, B. A., SOMMERS, C. H. AND BOYD, G., Irradiation inactivation of four *Salmonella* species in orange juices with varying turbidity. J. Food Prot. **64** (2001) 614-617.
[32] YUAN, J.T.C. Modified atmosphere packaging for shelf-life extension. pp. 205-219. *in* NOVAK, J. S., SAPERS, G. M., JUNEJA V. K. (eds.), The Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, FL, **(**2003).
[33] AL-ATI, T. AND HOTCHKISS, J.H., Application of packaging and modified atmosphere to fresh-cut fruits and vegetables. pp. 305-338 *in* LAMIKANRA, O., Fresh-cut fruits and vegetables, science, technology and market. CRC Press, Boca Raton, FL. (2002).
[34] PRAKASH, A., INTHAJAK, P., HUIBREGTSE, H., CAPORASO, F. AND FOLEY, D.M., Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Sci. **65** (2000) 1070-1075.
[35] SOMMERS, C.H., FAN, X., Irradiation (gamma) of fine emulsion sausage that contained sodium diacetate. J. Food Prot. **66** (2003) 819-824
[36] SOMMERS, C.H., FAN, X., NIEMIRA, B.A. AND SOKORAI, K., Radiation (gamma) resistance and postirradiation growth of *Listeria monocytogenes* suspended in beef bologna containing sodium diacetate and potassium lactate. J. Food Protection. **66** (2003) 2051–2056.
[37] GARRETT, E.H., Fresh-cut produce: tracks and trends. pp. 1-10. *in* LAMIKANRA, O. (ed.) Fresh-cut fruits and vegetables, science, technology and market. CRC Press, Boca Raton, FL., (2002).
[38] CARLIN, F., NGUYEN-THE, C. AND MORRIS, C. E., Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Cichorium endivia* var. *latifolia*). J. Food Prot. **59** (1996) 698-703.
[39] FROST, J. A., MCEVOY, M. B., BENTLEY, C. A., ANDERSSON Y. AND ROWE, B., An outbreak of *Shigella* sonnei infection associated with consumption of iceberg lettuce. http://www.cdc.gov/ncidod/eid/vol1no1/frost.htm.
[40] MEAD, P. S., SLUTSKER, L., DIETZ, V., MCCAIG, L. F., BRESEE, J. S., SHAPIRO, C., GRIFFIN, P. M. AND TAUXE, R. V., Food-related illness and death in the United States. Emerging Infect. Dis. **5** (1999) 607–625.
[41] CENTER FOR DISEASE CONTROL, Outbreaks of *Shigella sonnei* infection associated with eating fresh parsley – United States and Canada, July-August 1998. MMWR **48** (1999) 285-289.

[42] LEONARDOPOULOS, J., PAPAKONSTANTINOU, A., KOURTI, H. AND PAPAVASSILIOU, J., Survival of *Shigellae* in soil. Zbl. Bakt. Hyg., I. Abt. Orig. B **171** (1980) 459-465.
[43] SLAM, M. S., HASAN, M.K. AND KHAN, S.I., Growth and survival of *Shigella flexneri* in common Bangladeshi foods under various conditions of time and temperature. Appl. Environ. Microbiol. 59 (1993) 652-654.
[44] FEHLHABER, K., Studies into properties of *Shigella* with relevance to food hygiene. Archiv. Exper. Vet. Med. **35** (1981) 955-964.
[45] FERNANDEZ ESCARTIN, E., CASTILLO AYALA A. AND LOZANO, J. SALDANA, Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. J. Food Prot. **52** (1989) 471-472.
[46] SATCHELL, F. B., STEPHENSON, P. W., ANDREWS, H., ESTELA, L. AND ALLEN G., The survival of *Shigella sonnei* in shredded cabbage. J. Food Prot. **63** (1990) 568-572.
[47] SIMANGO, C AND RUKURE, G., Survival of bacterial enteric pathogens in traditional fermented foods. J. Appl. Bacteriol. **73** (1992) 37-40.
[48] SMITH, J. L., *Shigella* as a foodborne pathogen. J. Food Prot. **50** (1987) 788-801.
[49] SMITH, J. L. AND DELL, B. J., Capability of selective media to detect heat-injured *Shigella* flexneri. J. Food Prot. **53** (1990) 141-144.
[50] WU, F. M., DOYLE, M. P., BEUCHAT, L. R., WELLS, J. G., MINTZ, E. D. AND SWAMINATHAN, B., Fate of *Shigella sonnei* on parsley and methods of disinfection. J. Food Prot. **63** (2000) 568-572.
[51] LAMPEL, K. A. AND MAURELLI, A. T., *Shigella* Species. *In* International Handbook of Foodborne Pathogens, MILIOTIS, M. D. AND BIER, J. W. (ed.), Marcel Dekker, Inc. New York, NY. , (2003) 167-180.
[52] U. S. Dept. Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition. FDA Survey of domestic fresh produce. http://www.cfsan.fda.gov/~dms/prodsu10.html. (2003).
[53] U. S. Dept. Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. FDA Survey of imported fresh produce. http://www.cfsan.fda.gov/~dms/prodsu6.html.
[54] MOSSEL, D. A. A., Irradiation: An effective mode of processing food for safety. *In:* Food Irradiation Processing, Proceedings of an International Symposium. Washington, DC STI/PUB/695, Vienna:I, (1985) 251-279.

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