ALAN ARCHIVOS LATINOAMERICANOS DE NUTRICIÓN
Publicación Oficial de la Sociedad Latinoamericana de Nutrición
Venezuela, 8 de Enero de 2013

Trabajos de Investigación

Effect of gamma irradiation on the microbiological quality of minimally processed vegetables

L. López V[1]., S. Avendaño V[2]., J. Romero R[3]., S. Garrido[4], J. Espinoza[5], and M. Vargas[6]

1 Associated Professor, Food Microbiology. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Casilla 233, Santiago de Chile.
E-mail: llopez@uchile.cl

2 Assistant Professor, Food Microbiology. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Casilla 233, Santiago de Chile.

3 Adjunct Professor, Industrial Microbiology. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Casilla 233, Santiago de Chile.

4 Assistant Student, Food Microbiology. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. Casilla 233, Santiago de Chile.

5,6 Comisión Chilena de Energía Nuclear, Santiago, Chile

Compact title:
Effect of irradiation on vegetables

SUMMARY
Effect of gamma irradiation on the microbiological quality of minimally processed vegetables
The initial microflora of minimally processed celery and cabbage packaged under a modified atmosphere was determined. The samples came from the same producer and were sold in a supermarket chain of Santiago, Chile. Although neither *E. coli* nor *Salmonella spp*. were detected, initial total plate and Enterobacteriaceae counts were high (≥ 10^5 cfu/g), not meeting in most cases the specifications of the Chilean legislation. The D10 value for two strains of *E. coli* (ATCC 8739 and a wild type) inoculated as indicator microorganisms was determined. After irradiating with 5 D10 doses (1kGy), the variation of the microbial population and sensory quality during a 7 days storage period at 5ºC was studied. In irradiated celery, a reduction of 4.7 and 3.8 logs for total plate and *Enterobacteriaceae* counts respectively was observed. There was a decrease of 3.8 and 3.6 logs in cabbage for total plate and *Enterobacteriaceae* counts respectively. In both irradiated and non-irradiated vegetables, neither *E. coli* nor *Salmonella spp.* were detected. An increase of 1.6 - 1.7 logs in both microbiological parameters in non-irradiated samples was observed during storage. In irradiated products, only celery showed an increase of 1.2 log in total plate count. Both *Enterobacteriaceae* count in the two vegetables and Total plate count in cabbage presented essentially no variation in time. No significant differences ($p \geq 0.05$) were detected in sensory total quality between the control and the irradiated vegetable and between days of storage.

Keywords: gamma irradiation; minimally processed vegetables; *Escherichia coli*, cabbage, celery.

RESUMEN
Efecto de la radiación gamma en la calidad microbiológica de vegetales minimamente procesados
Se determinó la microflora inicial de apio y repollo minimamente procesado, envasado en atmósfera controlada, proveniente de un proveedor y disponible en una cadena de supermercados de la ciudad de Santiago de Chile. Aunque no se detectó *E. coli* ni presencia de *Salmonella spp*, los recuentos de aerobios mesófilos y de enterobacterias fueron elevados (≥ 10^5 ufc/g), sobrepasando en la mayoría de los casos las especificaciones de la legislación chilena. Se determinó el valor D10 para dos cepas de *E. coli* (ATCC 8739 y una cepa silvestre aislada de vegetales) inoculadas como microorganismos indicadores. Se estudió la variación de la población microbiana y la calidad sensorial, después de irradiar con dosis de 5 D10 (1 kGy), durante un período de almacenamiento de 7 días a 5ºC. En apio irradiado se observó una reducción de 4,7 y 3,8 ciclos logarítmicos en el recuento de aerobios mesófilos y de enterobacterias, respectivamente. En repollo la reducción fue de 3,8 y 3,6 logaritmos para recuento de aerobios mesófilos y enterobacterias, respectivamente. En ambos vegetales irradiados y no irradiados, no se detectó *E. coli* ni presencia de *Salmonella spp*. Durante el almacenamiento se observó un aumento de 1,6 - 1,7 logaritmos en ambos parámetros microbiológicos en las muestras no irradiadas. En los productos irradiados solo en apio se observó un aumento de 1,2 logaritmos en el recuento de aerobios mesófilos. En repollo el recuento de aerobios mesófilos se mantuvo sin variación en el tiempo, lo mismo se observó para el recuento de enterobacterias en ambos vegetales. No se detectaron diferencias significativas ($p \geq 0,05$) en la calidad sensorial total, entre el control no irradiado y los productos irradiados, ni entre los días de almacenamiento.

Palabras claves: irradiación gamma; vegetales minimamente procesados; *Escherichia coli*, repollo, apio.

INTRODUCTION

Microbiological quality is an important factor to consider in the quality concept of a food product. Normally, fruits and vegetables carry a non-pathogenic epiphytic microflora. However, there are certain factors contributing to the microbiological contamination of these products with pathogens. Contamination may arise as a consequence of treating soil with organic fertilizers such as manure and sewage sludge and from irrigation water. Additionally, the application of technologies such as cutting, slicing, skinning and shredding remove the natural protective barriers of the intact plant, likely providing a suitable medium for the growth of contaminating microorganisms. Some of these microorganisms are capable of growing on whole, minimally processed or cut fruits and vegetables under routine handling and storage conditions. Outbreaks of human diseases associated with the consumption of raw vegetables and fruits often occur in developing countries and have become more frequent in developed countries over the past decade (1, 2).

For fresh-cut vegetables that are eaten raw, none of the c[illegible]ied on to

substantially reduce the number of contaminating microorganisms occurring during some of the steps of the food chain, such as harvest, transportation, storage, distribution and handling before consumption. Washing with antimicrobial compounds, while important, often brings about only a relatively small reduction. Eliminating the risks is difficult. Managing them is based on identifying and controlling those factors that are important in preventing contamination or limiting growth of pathogenic microorganisms between farm and plate (1, 2, 3).

Nowadays, the food market offers a great variety of minimally processed vegetables. Nevertheless, the elaboration process does not always reduce the presence of spoilage and/or pathogenic microorganisms to an adequate level. In Chile, the label in these products specifies that they are ready-to-eat, not needing a previous treatment such as washing, disinfecting, or cooking.

According to several studies, the application of ionizing irradiation is a promising technology that may be used to control spoilage and pathogenic microorganisms in order to increase shelf life, improve the safety of ready-to-eat fruits and vegetables and as a substitute for the use of decontaminating chemicals (4, 5, 6, 7).

Considering that, in order to accept a chemical product as a sanitizing agent, it is necessary that the compound kill 99.999% of a certain microorganism population (8), when using gamma radiation a 5 log reduction would be desired, that means irradiation with a 5 D10 dose.

The objective of this study was to evaluate the effect of irradiation on the microbiological and sensory quality, and changes occurring during the storage under refrigerated conditions of minimally processed celery (Apium graveolens var dulce), and cabbage (Brassica oleraceae *var capitata*). An irradiation dose corresponding to 5 D10 for two *E. coli* strains inoculated as indicator microorganism in the vegetables was used.

MATERIALS AND METHODS

Materials

Samples from minimally processed celery and cabbage were obtained from a supermarket in Santiago, Chile. Celery was presented in slices and cabbage was finely shredded. Both vegetables came from the same producer, harvested from a single source. The vegetables were sanitized using sodium hypochlorite and packaged in polyethylene bags under nitrogen atmosphere. The date of purchase was the same day the products were delivered to the market. Shelf life data printed on the package was 8 days, when stored at 5ºC.

Methods

Microbiological quality of the products

In order to obtain the indigenous micro flora of the selected products, a previous study was carried out. Twelve samples of each product, purchased 4 at a time at different dates, were analyzed, determining Total plate count (TPC), *Enterobacteriaceae* count (Ent), Most Probable Number of *E. coli* (MPN) and detection of Salmonella spp. in 25 g (Sal). TPC, MPN and Sal were carried out according to Bacteriological Analytical Manual (9) and Ent according to ISO 7402, (10).

Dosimetry study

According to ASTM D 2954-71 (11), dosimetry was conducted for each vegetable in an experimental irradiator BPCDI Nº3 (Brookhaven Portable Cesium Development Irradiator) provided with a Cs137 source. The source's activity was 53,430 Ci (1.98 x 10^{15} Bq) and the dose rate was 0.44 Gy /sec. Ferrous-sulfate cupric-sulfate dosimeters were prepared to measure the absorbed doses.

Determination of D_{10} value for *E. coli*

Preparation of the inocula

Two strains of *E. coli* were used, an ATCC 8739 and a wild type, previously isolated by the authors from vegetables purchased at the local market. The cultures were grown at 35ºC for 24 h 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.

Inoculation

To inoculate the samples, 100 g of each pre-cut vegetable, disinfected by irradiating with a 25 kGy dose, were introduced in a sterile flask containing 1 liter of the diluted culture suspension and shaken for 5 min. The liquid was withdrawn through a sterile plastic bag with holes. Aseptically, 5 samples of 10 g of each vegetable were weighed, packaged in sterilized bags and sealed.

Irradiation

Samples of each vegetable were irradiated with 0.5 kGy, 1.0 kGy, 1.5 kGy and 2.0 kGy. Non-irradiated samples were kept as control. After the irradiation, the 10 g samples were homogenized with 90 ml of 0.1% peptone water (pH 7) in a Stomacher (Lab Blender Stomacher 400. Type BA 7021. Seward, London) for 1 min at normal speed (200 paddles/min). Considering that the study was performed with pure cultures of both strains, *E. coli* count was carried out plating 1 ml decimal aliquots into Petri dishes and pouring Tryptic Soy Agar (Oxoid). Plates were incubated at 35ºC for 24 - 48 h before counting. D_{10} value was calculated using the mean of 3 replicates of the experience for each vegetable.

Effect of a 5 D_{10} dose and changes occurring during the refrigerated storage of non-inoculated vegetables

Microbiological study

Aseptically, 25 g for the Sal determination and 10 g for TPC, Ent and MPN controls from each vegetable were weighed, packaged in sterilized bags and sealed. All non-inoculated samples were irradiated with the 5 D_{10} dose. Non-irradiated products were kept as control. The products were assessed after the irradiation (T0) and during storage at 5ºC for up to 7 days, controlling at 2, 5 and 7 days. The experience was replicated 4 times for each vegetable. Means and standard errors were calculated from a commercial spreadsheet (Microsoft Excel). Data were analyzed using ANOVA and Duncan Test (5% level) using Statgraphics 5.1.

Sensory evaluation

The total sensory quality, that is, the evaluation of texture, visual, odor and flavor attributes of the irradiated and non-irradiated samples, were evaluated by using the Scoring method with a scale from 1 (I dislike it extremely) to 9 (I like it extremely)

panelists. The training of the panelists was developed by presenting them with vegetal samples having different degrees of sensory damage, in order to obtain experience and the ability to objectively differentiate amongst the 9 points of the used scale.

The evaluation of the refrigerated stored samples was carried out on days 1, 4 and 7 after irradiating with a 5 D10 dose. Samples were presented to the panelists in a tray containing two dishes with 20 g of the vegetable, one corresponding to the irradiated sample and the other to the non-irradiated control. Dishes were randomly codified.

Two replicates at different dates were carried out. Data was analyzed using ANOVA and Duncan Test (5% level) using Statgraphics 5.1.

RESULTS AND DISCUSSION

Microbiological quality of the original products

Results of the microbiological analysis of celery and cabbage are presented in Figure 1 and Figure 2 respectively.

Figure 1
Microbiological analysis of celery samples
(▮) Total plate count, (≅) *Enterobacteriaceae count*

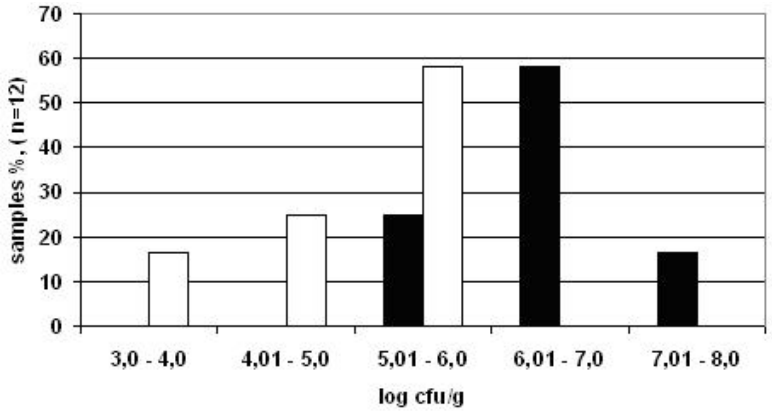
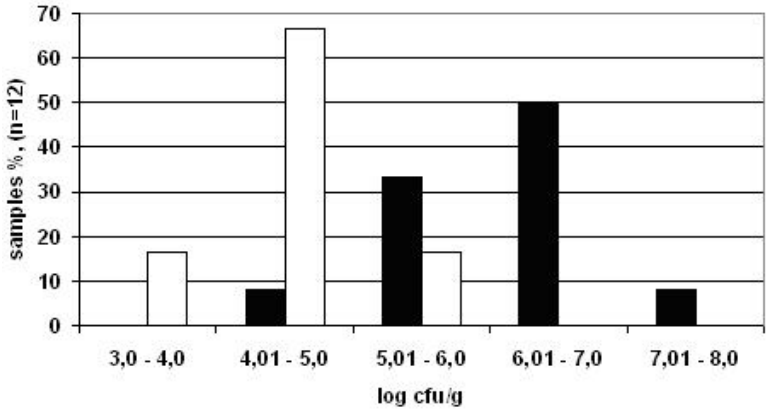


Figure 2
Microbiological analysis of cabbage samples
(▮) Total plate count, (≅) *Enterobacteriaceae count*



Although the presence of no *E. coli* or Salmonella spp. was detected, and considering that these are minimally processed vegetables, the observed initial contamination for TPC and Ent is high. Total plate counts over 6.0 log cfu/g were observed in 75% and 58% of the celery and cabbage samples respectively. Prakash et al. (12) reported aerobic counts of 6.57 (log cfu/g) in diced celery, Farkas et al. (13) found levels of 6.0 (log cfu/g) for total plate counts in pre-cut carrots. The Enterobacteriaceae counts were higher than 5.0 log cfu/g in 58% and 17% of celery and cabbage respectively. Farkas et al. (13) reported counts of this bacterial group in fresh carrots, which agrees with the results mentioned in the present study. It is necessary to consider that in the cited literature, findings were obtained from fresh vegetables, that is, neither with a previous treatment nor minimally processed products like the ones used in this work.

The label on the products used for the present study states: "open and eat, no washing or disinfecting needed". This means that the consumption of these vegetables could be a potential risk for sensitive people such as the young, the old, the pregnant and the immunocompromised consumers (2). The observed microbial levels were detected in samples purchased close to the packing day. These levels are likely to increase after 8 days of refrigerated storage, recommended as expiration date.

Considering these minimally processed vegetables as ready-to-eat products, the microbiological limits specified by the Chilean legislation (14) are n=5, c=1, m= 4.7 log cfu/g and M=5.7 log cfu/g for TPC and n=5, c=1, m=3.0 log cfu/g and M=4.0 log cfu/g for Ent. Based on the M value for TPC, only 2 celery samples (17%) and 4 cabbage samples (33%) meet the recommended limits. Regarding the Enterobacteriaceae count, 2 samples (17%) of each product are within the suggested li his kind of

microbiological parameters for ready-to-eat minimally processed vegetables were found.

Dosimetry study
Dosimetry measurements indicated that a good homogeneity in the mean absorbed dose was obtained, that is, the uniformity rate of the irradiation process. Values of 1.11 and 1.16 for celery and cabbage respectively were calculated.

Determination of D_{10} value for *E. coli*
The D_{10} values obtained for both strains (ATCC and wild) in celery and cabbage are shown in Table 1.

Table 1
D10 values (kGy) for E. coli inoculated in minimally processed celery and cabbage

Vegetable	ATCC strain	Wild strain
Celery	0.18 ± 0.01[1]	0.22 ± 0.03
Cabbage	0.22 ± 0.03	0.23 ± 0.01

[1] mean from 3 trials ± standard deviation. No statistically significant differences were found ($p \geq 0.05$)

E. coli was chosen as an indicator microorganism in order to know the behavior, when irradiating, of the natural microflora in this kind of vegetable products. The study of the D_{10} value for two different strains of the microorganism (a wild and an ATCC strain) was carried out in order to determine the 5 D_{10} dose used for the irradiation of the samples. In both vegetables, the D_{10} values calculated for the ATCC strain are lightly lower than the one obtained for the wild strain. For cabbage, the values obtained for both strains were lightly higher than the one calculated for celery. However, no statistically significant differences between both strains and vegetables were found ($p \geq 0.05$).

Although no literature data for D_{10} values were found regarding minimally processed vegetables inoculated with this microorganism, a comparison between 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 (15) and Adams and Moss (16) respectively, although no further details on food matrices and bacterial strain are given. Doyle (17) presents values of 0.23 - 0.35 kGy when inoculating in fresh foods of animal origin. Studies with a O157:H7 strain showed results of 0.27 kGy, 0.24 - 0.27 kGy and 0.24 kGy in poultry, fresh foods and meat respectively (5, 17, 18). ICMSF (19) reports different values for ground poultry and ground meat that range between 0.241 and 0.39 kGy, using wild and O157:H7 strains. The radiation source, gaseous phase and temperature are detailed. According to this literature data, the results found in this study could be considered within the range of reported D_{10} for *E. coli.*

Effect of the 5 D_{10} dose
According to the obtained D_{10} values, the 5 D_{10} irradiation dose that should be used for the experience would be 0.90 kGy and 1.10 kGy for the ATCC and wild strains in celery respectively, and 1.10 kGy and 1.15 kGy for the ATCC and wild strain in cabbage respectively. Considering the application of this irradiation treatment at industrial level, and the fact there were no statistically significant differences in the calculated D_{10} values between both strains and the vegetables, a dose of 1.0 kGy was used for the irradiation of all samples.

Irradiation effect on microbiological counts
Figures 3 and 4 present the results of the irradiation effect and the variation of microbiological counts (log cfu/g) of the products during storage at 5ºC.

Figure 3
Effect of radiation and changes in TPC and Ent counts in celery during refrigerated storage.
(•) TPC and (▮) Ent Control (non-irradiated),
(O) TPC 1.0 kGy, (≅) Ent 1.0 kGy.
Error bars indicate standard deviation

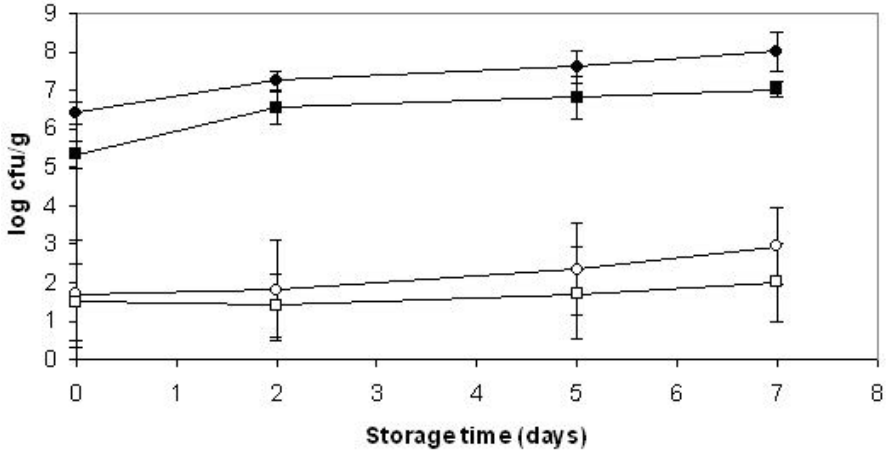
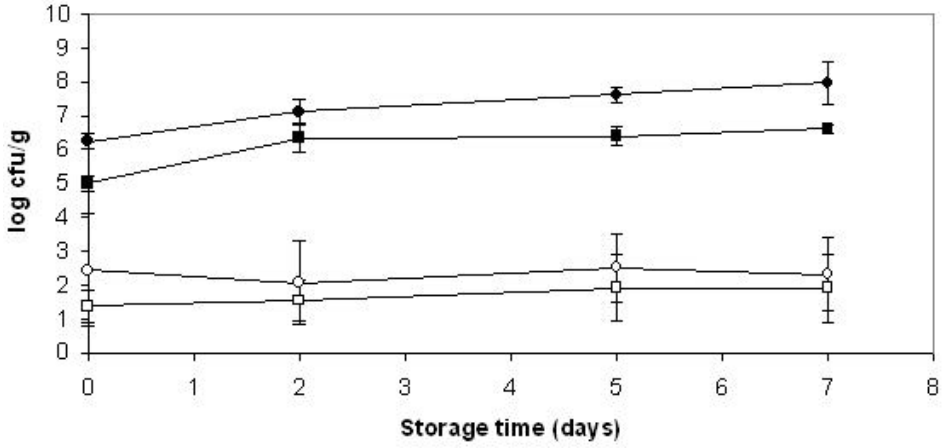


Figure 4
Effect of radiation and changes in TPC and Ent counts in cabbage during refrigerated storage.
(•) TPC and (▮) Ent Control (non-irradiated),

(O) TPC 1.0 kGy, (≅) Ent 1.0 kGy.
Error bars indicate standard deviation



Initial total plate and Enterobacteriaceae counts are similar to those obtained in the preliminary study. No E. coli or Salmonella spp. during the studied period were detected. After irradiation, a 4.7 and a 3.8 log reduction in TPC and Ent respectively was observed in celery. Prakash et al. (12) 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 irradiating carrots cubes with the same dose was achieved by Farkas et al (13), in TPC and Ent respectively.

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 on the 8th storage day; 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 (13, 12, 20). At day 7, *Enterobacteriaceae* counts increased in 1.7 and 1.6 logs in celery and cabbage respectively. Values informed by Farkas (13) in sliced bell peppers showed an increase of nearly 3 logs. Statistically significant differences ($p \leq 0.05$) were found between day 0 and days 2, 5 and 7 for both microbiological parameters. Comparing these results may give a trend of the behavior of the microbial population, but the kind of vegetable studied, the treatment or preparation of the samples, the type of the indigenous microflora and storage time must be considered.

During the storage of the irradiated samples, the increase of the surviving flora was lower than the obtained in the non-irradiated products. In general, TPC and Ent counts remained essentially stable during storage of both products. The higher count at day 7 was observed in celery, where TPC was 1.2 log higher than the result obtained at the initial day. Prakash et al. (12) reported variation in TPC of 1.9 log after 8 days at 5°C, in diced celery irradiated with 1 kGy. 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 obtained from the irradiated samples presented a great variety between the replicate samples, which may be due to a non homogeneous microflora of the initial products. This could depend on a non-standardized process or treatment during the elaboration of the minimally processed products and/or different culture conditions of the vegetable. It is possible to point out that when irradiating with a 1 kGy dose, an important reduction of the initial microflora can be achieved. Moreover, these levels of contamination could be maintained during storage within the microbiological limits recommended by the Chilean legislation, which is not achieved with the non-irradiated samples.

Sensory evaluation

Table 2 presents a comparison of the results for total sensory quality, between the non-irradiated samples and the irradiated vegetables during the refrigerated storage. No significant differences ($p \geq 0.05$) were observed between the non-irradiated and the irradiated samples, and between days of refrigerated storage. According to the obtained results, the panelists were unable to distinguish significant changes in the total quality of each vegetable. At the end of the storage period, the score obtained for irradiated celery was 5.3 ("I like it a little") and 6.8 ("I like it more or less") for cabbage. In both cases, the acceptability at day 7 was better for the irradiated samples when comparing with the scores obtained for the not irradiated vegetables. The score considered as a limit for acceptability was 3 (I dislike it a little). Clark (6) reported that irradiation of whole fruits and vegetables was not successful, because many products have a low tolerance for radiation and develop skin damage and internal browning; however, fresh cut products do not exhibit these reactions. The vegetables used in the present study can be considered as fresh cut products, agreeing with the statement cited above.

Table 2
Total sensory quality of celery and cabbage (non-irradiated and irradiated) during the refrigerated storage using scoring method with a 1 to 9 points scale[1]

Days	Celery		Cabbage	
	Non irradiated	1.0 kGy	Non irradiated	1.0 kGy
1	6.2 ± 0.90[2]	6.7 ± 0.90	7.0 ± 0.21	6.7 ± 0.21
4	6.0 ± 0.48	5.7 ± 0.48	6.5 ± 0.57	5.2 ± 0.57
7	4.5 ± 0.49	5.3 ± 0.49	5.7 ± 0.59	6.8 ± 0.59

[1] 1 = I dislike it extremely, 9 = I like it extremely

[2] mean from 2 trials and scores obtained from 6 panelists ± standard deviation. No statistically significant differences were found ($p \geq 0.05$)

Venezuela, 8 de Enero de 2013

As a conclusion to the present study, it is necessary to point out the high levels of total plate and *Enterobacteriaceae* counts detected in these samples of minimally processed celery and cabbage from the Chilean market. Irradiation with a 1 kGy doses reduced this contamination to acceptable levels without changing the acceptability of the samples during the recommended shelf life. The remaining microflora in the irradiated products does not show a significant increase during the refrigerated storage recommended by the producer. Irradiation of this kind of ready-to-eat products is a feasible alternative treatment to be considered in order to improve the microbiological quality without affecting the sensory attributes and to extend the shelf life of the products.

ACKNOWLEDGMENTS
This research was supported by the FAO/IAEA - Co-ordinated Research Project - CHI 11902

REFERENCES

1. Beuchat L. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Issues, Food Safety Unit, World Health Organization; 1998.
2. European Commission. Health & Consumer Protection Directorate General. Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw; 2002 URL: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html
3. Food Safety & Hygiene. A bulletin for the Australian Food Industry. Packaged minimally processed fresh-cut vegetables; 2000 URL: http://www.dfst.csiro.au/fshbull/fshbull21.htm
4. Thayer D. and Rajkowski K. Developments in irradiation of fresh fruits and vegetables. Food Tech 1999; 53(11):62-65.
5. Federighi M. and Tholozan J.L. Traitments ionisants et hautes pressions des aliments. Paris: Ed. Economica; 2001. p. 59.
6. Clark P. Extending the Shelf Life of Fruits and Vegetables. Food Techn 2002; 56(4):98-106.
7. FAO/OIEA/OPS. Consulta Técnica Conjunta sobre el uso de Irradiación como medida de intervención en Salud Pública para el control de Enfermedades Transmitidas por Alimentos, Washington, D.C. Organismo Internacional de Energía Atómica, Viena; 1992.
8. Ayres J.C., Mundt J.O and Sandine W.E. Microbiology of foods. San Francisco: W. H. Freeman and Company; 1980. p. 131.
9. Food and Drug Administration. Bacteriological Analytical Manual on line; 2001. URL: http://www.cfsan.fda.gov/~ebam/bam-toc.html
10. ISO 7402:1993 (E). Microbiology - General guidance for the enumeration of Enterobacteriaceae without resuscitation - MPN technique and colony-count technique. 1993.
11. ASTM D2954-71. Standard Test Methods for Absorbed Gamma and Electron Radiation Dose with the Ferrous Sulfate - Cupric Sulfate Dosimeter. ASTM, Philadelphia, P.A.; 1977.
12. Prakash A., Inthajak P., Huibregtse F., 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 2000a; 65(6):1070-1075.
13. Farkas J., Sáray T., Mohásci-Farkas C., Horti K. and Andrássy E. Effects of Low-dose Gamma Radiation on Shelf-life and Microbiological Safety of Pre-cut/Prepared Vegetables. Advances in Food Science 1997; 19(3/4):111-119.
14. Ministry of Health. Nuevo Reglamento Sanitario de los Alimentos. Santiago de Chile: Ediciones Publiley; 2000. p. 89.
15. Garbutt J., Essentials of Food Microbiology. London: Ed. Arnold; 1997. p. 113.
16. Adams M.R., Moss M.O. Food Microbiology. Cambridge: The Royal Society of Chemistry; 1995. p. 76.
17. Doyle M., Beuchat L. and Montville T. Microbiología de los Alimentos. Fundamentos y Fronteras. Zaragoza: Ed. Acribia; 1997. p. 533.
18. Olsson D.G. Irradiation of food. IFT Expert Panel on Food Safety and Nutrition. Food Tech 1998; 52 (1):56-62.
19. International Commission on Microbiological Specifications for Foods, ICMSF. Microbiological Specifications of Food Pathogens. London: Published by Blackie Academic & Professional; 1996. p. 135.
20. Prakash A., Guner A., Caporaso F. and 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 2000b; 65(3):549-553.

ALAN-VE ISSN 0004-0622 - Depósito Legal: pp 199602DF83
Sociedad Latinoamericana de Nutrición
Venezuela, 8 de Enero de 2013

Producción editorial en Venezuela: Capítulo Venezolano - RIF: J-30843129-0
Urbanización Santa María, primera transversal, No. 417-214, Planta Alta
Tele-Fax: (+58-212) 283.8618
E-mail info@alanrevista.org
Código Postal: 1070
Caracas - Venezuela