Safety improvement and quality retention of gamma irradiated spinach leaves.

G. Finten[1*]; J. I. Garrido[2]; M.C. Cova[2]; P. Narvaiz[2]; R. J. Jagus[1]; M. V. Agüero[1]

[1]: Universidad de Buenos Aires (UBA). Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Tecnologías y Ciencias de la Ingeniería (INTECIN). Facultad de Ingeniería. Departamento de Ingeniería Química. Laboratorio de Microbiología Industrial: Tecnología de alimentos, Av. Int. Guiraldes 2620, (C1428EGA), Ciudad Autónoma de Buenos Aires, Argentina.
[2]: Comisión Nacional de Energía Atómica-Sección Irradiación de Alimentos-Gerencia de Aplicaciones y Tecnología de Radiaciones-Gerencia de Área Aplicaciones de la Tecnología Nuclear-(B1802AYA) Centro Atómico Ezeiza, provincia de Buenos Aires, Argentina.
***Corresponding author**
Finten, Gabriel
Laboratorio de Microbiología Industrial: Tecnología de alimentos
Departamento de Ingeniería Química
Facultad de Ingeniería, Universidad de Buenos Aires
Av. Int. Güiraldes 2620. Postal Code: C1428EGA. Buenos Aires
ARGENTINA
Tel: (+54) (011) 4576-3240
fintengabriel@hotmail.com

Abstract

The purpose of this work was to evaluate γ-irradiation effects on native microbiota, some phytochemicals and sensory attributes of fresh spinach (*Spinacia oleracea* L.) throughout 15 days of storage (6 ± 1 °C). The proposed treatment (1.5 kGy) effectively reduced native microbiota extending shelf life under refrigeration to 14 days, and the evaluated phytochemicals (polyphenols, antioxidant capacity, chlorophyll, carotenoids) were preserved with the exception of ascorbic acid, which was reduced by more than 80% after treatment. Sensory acceptability, evaluated by a consumer panel, of samples irradiated either at 1.5 or 3.0 kGy was good till the last analysis date (day 14). The target dose had a great performance, presenting scores in some attributes significantly higher than those of control samples (overall liking and appearance on day 2). Overall, the performance of gamma rays was acceptable and significantly increased product acceptability; therefore it represents a feasible alternative for spinach disinfection.

Practical Applications

Gamma irradiation has attracted attention as a potential non-thermal decontamination strategy to ensure the safety of fresh fruits and vegetables. Several studies have demonstrated the high efficiency of this technology applied on different vegetables to control typical pathogens, but vegetables tolerance to radiation can vary, so process validation is required in each case. Hopefully the results presented herein could encourage large scale adoption of this technology by the fresh produce industry.

Keywords: Non thermal technology; Leafy vegetables; *Spinacia oleracea* L.; Preservation.

1. Introduction

In the last years consumers are more prone to request high quality on food and are willing to pay a premium price for it (Parlato et al. 2014). Minimally processed vegetables (MPV) are demanded due to their wholesomeness and fast preparation (Sagong et al. 2011). Stress caused by handling, washing, cutting, packaging and storage under a low O_2 atmosphere due to respiration, in the so-called “modified atmosphere packaging” (MAP) affects their physiology and biochemistry, which impairs quality and shelf-life (Watada et al. 1996). These products are good substrates for the growth of microorganisms, either spoiling or pathogenic, during production, transport, processing, marketing and storage; consequently, the number of foodborne outbreaks associated with their consumption is increasing worldwide (Olaimat and Holley 2012). In 2006 one of the greatest foodborne outbreaks in USA took place due to an E. coli O157:H7 contamination in fresh spinach, which led to 206 reported illness cases (Hanning et al. 2009; Lynch et al. 2009). The latter should concern food scientists and governments not only because they affect people´s health, with fatal consequences in some cases, but also because of the tremendous economic losses inflicted to the health care systems (Roberts 2014). The food industry is also economically damaged due to recalls and distrust.

Pre-harvest strategies, such as the application of Good Agricultural Practices (GAPs), together with proper disinfection procedures are critical steps to ensure MPV safety (Goodburn and Wallace 2013). Unfortunately, current production and processing practices cannot be relied upon to ensure pathogen-free fresh produce (Doyle and Erickson 2008). The widespread disinfection with sodium hypochlorite solution only achieves microbial reductions between 1 and 2 log cycles, which is not enough for providing safe products (Warning and Datta 2013). Despite its popularity and convenience, the effects of chlorine on pathogens inoculated onto produce are considered inconsistent (Neal et al. 2012). Additionally, its use is criticized due to the possible formation of carcinogenic compounds (Gómez-López et al. 2014). Consequently, there is a growing interest in the development of alternative disinfection technologies to overcome this drawback (Ölmez and Kretzschmar 2009; Serna Villagomez et al. 2010).

Among disinfection technologies, γ-irradiation has attracted attention as a potential non-thermal decontamination strategy to ensure the safety of fresh fruits and vegetables (Farkas et al. 1997; Horak et al. 2006). Furthermore, USA regulations have allowed the use of ionizing radiation for pathogen control and shelf life extension of fresh iceberg lettuce and spinach up to 4.0 kGy (Food and Drug Administration, 2008). Several studies have demonstrated the high efficiency of γ-irradiation applied on different MPV to control typical pathogens associated with these products (Fan et al. 2012; Fan and Sokorai 2011a, 2008; Niemira 2007, 2003; Niemira et al. 2005; Niemira et al. 2003; among others), even on microorganisms internalized into vegetal tissues, which is a very particular capacity as compared with other preservation technologies (Doyle and Erickson 2008; Gomes et al. 2009; Niemira 2007). However, the effectiveness of this treatment is highly dependent on the microorganism under consideration, processing and storing conditions, packaging material, and food matrix. Hence, vegetables tolerance to radiation can vary and process validation is required in each case (Fan et al. 2008; Gupta et al. 2012; Niemira 2008).

Considering that fresh spinach leaves have been highly demanded by consumers which doubled their production in the last ten years, reaching roughly 23 Mt p.a. (FAOSTAT, 2015), and that recent cases of foodborne outbreaks were associated with spinach consumption, the purpose of this work was to evaluate γ-irradiation effects on native microbiota, some phytochemicals and sensory attributes of fresh spinach (*Spinacia oleracea* L.) throughout refrigerated storage.

2. Materials and methods

2.1 Raw material and sample preparation

Spinach leaves (*Spinacia oleracea* L.) were directly obtained from greenhouses in Buenos Aires province, Argentina. The leaves were harvested during November and transported under refrigeration to the laboratory within the first hour after harvest. Plants were cut to discard roots and the lower portions of stems. Only whole leaves, uniform in size and color, without visually evident defects were selected, then dipped in tap water 1:20 (w:v) for 5 min, centrifuged for 30 s in a sterile domestic salad spinner, and packed in 20 g units (experimental unit) using polyolefin PD960 (Cryovac®, Duncan, USA).

2.2 Irradiation treatments

They were carried out at the semi-industrial ^{60}Co facility (Ezeiza Atomic Centre-National Atomic Energy Commission, Buenos Aires province, Argentina), with an activity of roughly 600 kCi. Spinach bags were arranged in styrofoam refrigerated boxes during transportation to the irradiation facility and treatment. Samples were irradiated at 0 (control), 1.5 and 3.0 kGy while being maintained at 4 ± 1 °C. The minimum employed radiation dose was chosen considering D_{10} value of *Listeria innocua* ATCC 33090, employed as surrogate of *Listeria monocytogenes* in a previous experience, which turned out to be 0.25 kGy (Rubinstein et al. 2013). Listeria as a gender is considered one of the most radioresistant non-spore-forming bacteria (Niemira et al. 2003). The maximum employed radiation dose was tested in order to consider possible dose dispersions at a potential industrial scale treatment (Horak et al. 2006). Measurements of absorbed radiation doses were performed using silver dichromate dosimeters (ISO, 2003). After treatment, samples were brought to the laboratory and stored under refrigerated conditions (6 ± 1 °C) for 15 days.

2.3 Microbiological analysis

Mesophilic bacteria (MB), psychrotrophic bacteria (PB) and yeasts and molds (Y&M) counts were determined as the average of four independent replicates according to ICMSF (1983). A criterion was adopted in order to determine the microbiological acceptability of spinach leaves. For mesophilic bacteria, a maximum count of 7.7 log CFU.g^{-1}, taken from French regulations, was established as an acceptable level in ready-to-eat vegetables (Corbo et al. 2006; Corbo et al. 2004); for yeasts and molds, Fleet (1992) suggested a maximum level of 5 log CFU.g^{-1}.

2.4 Phytochemicals analysis

2.4.1 Ascorbic acid

Ascorbic acid content was determined according to AOAC 967.21(AOAC International, 1995) by titration with 2,6-dichloroindophenol; results were expressed as mg of ascorbic acid.100 g^{-1} fresh tissue (FT). Values reported are the mean of four independent determinations.

2.4.2 Antioxidant capacity and total phenolic content

The extraction was carried out following the methodology proposed by Viacava et al. (2015). Antioxidant capacity was evaluated by the DPPH radical scavenging assay according to Viacava et al. (2015), results were expressed as mg ascorbic acid.100 g^{-1} FT. Total phenolic content was determined by the Folin-Ciocalteu method (Singleton et al. 1999), being results expressed as mg gallic acid.100 g^{-1} FT. Measurements were done in triplicate.

2.4.3 Determination of chlorophylls and carotenoids

AOAC methodology 942.04 (AOAC International, 1995) was employed to obtain chlorophyll and carotenoids extracts. Chlorophyll, expressed as mg.100 g^{-1} FT, and carotenoid, expressed as mg.100 g^{-1} FT, were determined according to the equations proposed by AOAC International (1995) and Scott (2001), respectively. Measurements were done in triplicate.

2.5 Sensory evaluation

Sensory analysis were carried out by a consumer panel composed of 30 – 35 members aged between 20 and 60, with basic to no knowledge about food irradiation, who evaluated appearance, odor, taste, texture and overall liking of control and irradiated samples along storage using a 9-point hedonic scale (ASTM, 1996). Samples consisted of spinach leaves (3-4), randomly taken from a pool of 10 – 12 experimental units, which were placed into codified polypropylene disposable trays covered with PVC-film. Samples were analyzed at days 2 and 6, while at day 14 only irradiated samples were tested.

2.6 Statistical analysis

Data were analyzed using R 3.2.2 version (R Core Team, 2015). For all experiments, General Linear Model procedure was used for analysis of variance (ANOVA). For all cases, differences between levels of factors under analysis were assessed by Dunnett's test (adjusted p-values by Bonferroni's methodology). The fulfillment of the ANOVA requirements, the normal distribution of the data and the homoscedasticity, were tested by means of the Shapiro-Wilk's and the Bartlett's tests, respectively. Additionally, linear models were checked by analyzing distribution of residuals and residuals vs. fitted values. Two-sample t-test and Welch two-sample t-test, the latter when variances were not equal by means of the F test, were performed when required. The significance level for all the statistical procedures was 5%.

3. Results and discussion

3.1 Effect of irradiation treatments on native microbiota

Figure 1 shows the evolution of native microbiota: mesophilic bacteria (MB), psychrotrophic bacteria (PB), and yeasts and molds (Y&M) in control and irradiated samples during refrigerated storage. For MB and PB populations, the statistical analysis demonstrated significant main effects of the treatment and time factors, and their interactions. However, for Y&M only the main effects of factors were significant. Gamma irradiation at 1.5 kGy achieved initial reductions of 4.1, equal or greater than 4.1 (below detection limit), and 1.4 log CFU.g^{-1} with respect to control samples on day 1 for MB, PB and Y&M; meanwhile at 3.0 kGy reductions of 4.7, equal or greater than 4.1 and equal or greater than 1.9 log CFU.g^{-1} were detected for MB, PB and Y&M, respectively. These results clearly demonstrate that the proposed irradiation achieved greater initial bactericidal and fungicidal power than those typically registered with sodium hypochlorite disinfection. In fact, in a previous work with the same raw material (Finten et al. 2015), this traditional disinfection treatment (200 ppm, pH 6.5) gave reductions of 1.9, 1.9, and 1.2 log CFU.g^{-1} for MB, PB, and Y&M, respectively. Furthermore, it has been demonstrated that the efficacy of γ-irradiation is not limited to the surface (Niemira 2007); it can reduce microorganisms that are protected in crevices and creases (Prakash et al. 2000a).

Control samples presented a fast increase in MB counts since the first storage days, exceeding the established criterion by day 8 (**Fig. 1A**). On the contrary, MB counts of 1.5 and 3.0 kGy samples maintained the low values achieved after irradiation for several days and remained below the acceptability criterion throughout storage time. Differences in MB counts between control and irradiated samples were significant for the whole period. Even though microbial counts in irradiated samples were below the established criteria by day 15, further sampling was cancelled due to unacceptable visual quality noticed on the following days. A similar behavior was reported by Gupta et al. (2012), Prakash et al. (2000a), and Prakash et al. (2000b) who studied the effect of irradiation on native microbiota of other vegetables. Slightly different results were found by Kamat et al. (2003): they reported initial reductions in total bacteria of roughly 2, 4 and 5 log cycles, respectively for coriander leaves irradiated at 1, 2 and 3 kGy.

Regarding PB, changes in their counts during storage were similar to those registered for MB (**Fig. 1B**). Initially, PB counts in irradiated samples were reduced under the detection limit and remained stable during the first 2 and 3 days, respectively for samples subjected to 1.5 and 3.0 kGy. Since day 3, PB growth in irradiated samples was reassumed but their counts were still significantly lower than in control samples, between 3 and 4 log cycles for 1.5 and 3.0 kGy samples, respectively. Even though psychrotrophic microorganisms are expected to be the dominant microbiota under refrigerated storage (Ragaert et al. 2007), they are not regularly assessed by other researchers and there is no information available for comparison with other studies.

As expected according to scientific knowledge, yeasts and molds were the least sensitive of the tested microbial populations to γ-irradiation *(*Josephson and Peterson 1983), being their growth slope during storage time smaller than those of MB and PB (**Fig. 1C**). At the 8th storage day, control samples reached the adopted acceptability limit while irradiated samples had Y&M counts between 1 and 2 log cycles lower, according to dose. Irradiated samples reached the criterion towards the end of the storage period (15 days), without significant differences with control sample. Neal et al. (2010), who irradiated spinach leaves with electron beam up to a maximum dose of 1.4 kGy, and Kamat et al. (2003) found similar microbial growth patterns.

A strict maintenance of low temperatures, less than 5 ºC, is recommended in order to control the potential growth of *Clostridium botulinum* on fresh-cut vegetables packaged in a modified atmosphere (Austin et al. 1998). This is doubtfully achievable in practice throughout the commercialization period, particularly in developing countries. So the packaging material of irradiated fresh spinach should have enough oxygen permeability to avoid this risk, considering that as shelf life is extended by the irradiation treatment, carbon dioxide concentration due to leaves respiration increases into the package favoring anaerobic bacterial growth. Besides, irradiation at these low doses causes a microbiota selection, eliminating more radio-sensitive bacteria than *C. botulinum*. Attempts to extend shelf life by inhibiting the normal spoilage microorganisms should be balanced against the possibility of favoring *C. botulinum* growth and toxin production (Lilly et al. 1996). Nevertheless, *C. botulinum* is not likely to be a risk in the product proposed in the present research; mainly due to the high oxygen permeability of the packaging material and to the lower respiration rate that fresh whole leaves have as compared to fresh-cut leaves, which in this case resulted in oxygen levels into the package of 16% at the 15th refrigerated storage day (data not shown).

3.2 Effect of irradiation treatments on phytochemicals

As the nutritive value of spinach leaves has been reported not to be impaired by ionizing radiation treatments, particularly as far as folic acid is concerned (Müller and Diehl 1996), phytochemicals were chosen to be analyzed in the present work. They are non-nutritive plant substances that have health-promoting effects. Consumption of natural, fresh plant produce rich in phytochemicals has been reported to overcome some of the degenerative diseases that affect humans. However, improper processing, handling, and long-term storage of produce might result in minimal availability of these health-promoting compounds (Alothman et al. 2009). **Figure 2** shows the effect of γ-irradiation on ascorbic acid (A), total polyphenols (B), and antioxidant capacity (C) of spinach samples along storage.

Fan and Sokorai (2011b) reported that spinach was more tolerant to ionizing radiation than other leafy vegetables such as lettuce (Zhang et al. 2006), probably due to its higher ascorbic acid content, and so, higher antioxidant capacity. Ascorbic acid is the primary water soluble antioxidant in plant tissues, interacting with reactive species produced by water radiolysis, thus protecting cell constituents from oxidative damage (Cocetta et al. 2014). Ascorbic acid in the present study was significantly affected by factors time and treatment, their contents in irradiated samples were reduced since the first days of storage (**Fig. 2A**). Control samples presented higher contents but they were reduced by more than half on day 13. Similarly, reported losses in ascorbic acid at the 14th storage day were 33, 73, 84, 90 and 94% for 0, 1, 2, 3 and 4 kGy fresh-cut spinach leaves, respectively (Fan and Sokorai 2011b). Similar reductions were also found in baby spinach leaves treated with electron beam radiation up to 1 kGy (Gomes et al. 2008). Furthermore, Lester et al. (2010) who treated baby spinach leaves at doses up to 2 kGy, found that ascorbic acid was significantly reduced at 2 kGy, and affected at lower doses, depending on cultivar. On the other hand, ascorbic acid in carrot and kale juice was stable when irradiated at 3 kGy and stored (10 ºC) for 3 days (Song et al. 2006). However, in spite of their high ascorbic acid content**,** generally spinach leaves are not considered a source of this vitamin by populations with a varied dietary intake in which citric fruits are consumed**.**

Phenolics are a large class of natural phytochemicals that can be found in many edible plant products exhibiting powerful antioxidant activities. In such a capacity they are able to scavenge reactive oxygen species (ROS) generated endogenously and by chemical carcinogens. It has been reported that irradiation treatments can generate free radicals, thus leading to an induction of stress responses in plant foods, which in turn may lead to an increase in the antioxidant synthesis (Oms-Oliu et al. 2012). Consistently with this, Song et al. (2006) observed that total phenolic content of carrot and kale juices substantially increased by applying an irradiation treatment.

Irradiation can influence phytochemicals content and also the capacity of a specific plant to produce them. Under certain favorable conditions such as exposure to radiation sources, wounding, storage at low temperatures, and/or exposure to extreme temperatures, the concentration of plant phytochemicals might be enhanced (An et al. 2004; Antonio et al. 2011). Usually low and medium radiation doses have insignificant effects on antioxidants, depending on their radiosensitivity and the effect of irradiation itself on other food constituents that might be responsible for the production and/or the accumulation of phytochemicals in the plant (Alothman et al. 2009).

Polyphenols content did not show a clear pattern but values among samples were very alike in each analysis date (**Fig. 2B**). Similar results were found by Fan (2005), on lettuce and endive irradiated up to 2 kGy; Arjeh et al. (2015), who treated sour cherry juice with γ-radiation doses up to 6.0 kGy; Banerjee et al. (2015), irradiating shredded cabbage at doses up to 2.0 kGy; and Brandstetter et al. (2009) studying the effect of 10 kGy of gamma irradiation on sage, thyme and marjoram. Ahn et al. (2005), found slightly increased antioxidant activity and phenolic contents on minimally processed Chinese cabbage irradiated at 0.5 kGy, though lower polyphenols values over 1 kGy. An et al. (2004), irradiated green tea leaves at 40 kGy and found that antioxidant capacity was preserved, and anti-microbial activity was enhanced.

Regarding antioxidant capacity, it was significantly affected by storage time. Lower values were found on irradiated samples at days 2 and 7, though in both cases, without significant differences (**Fig. 2C**). The latter could be partially explained by the loss of antioxidant activity due to the reduction observed in ascorbic acid content (**Fig. 2A**).

In **Figure 3** chlorophyll and carotenoids contents are shown; neither treatment nor storage time had significant effects. However, in irradiated samples their contents were barely reduced over time. Gomes et al. (2008) found resembling chlorophyll and carotenoids results when using electron beam irradiation up to 1 kGy. Lester et al. (2010), with doses of γ-rays up to 2 kGy, reported slightly lower values in carotenoids content at the end of storage. Furthermore, Nunes et al. (2013) did not find significant changes when irradiating arugula up to 2 kGy. Chlorophylls and carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system susceptible to oxidation, isomerization and other chemical changes during processing and storage (Oms-Oliu et al.2012). However, in the present study they could have been protected by the radical scavenging capacity of ascorbic acid.

3.3Sensory analysis

Figure 4 shows results corresponding to the sensory evaluation of control and irradiated spinach leaves by the consumer panel along storage time**.** On day 2 aspect and overall liking were significantly improved by the irradiation treatments and it could be associated with the slight increase in chlorophyll and carotenoids contents averages on day 2 (**Fig. 3**) possibly due to an increased bioavailability after irradiation treatments. On the other hand, odor, taste and texture were evaluated as alike in every sample. On day 6 there were no significant differences in any of the evaluated attributes; nevertheless, 3.0 kGy samples were afforded the lowest scores of the three but still above 5: neither like nor dislike.

At the end of storage, day 14, control samples could not be analyzed due to their high microbial load (**Fig. 1**). Samples irradiated at 1.5 were significantly better graded in every attribute, with the exception of odor, than those irradiated at 3.0 kGy, being all of them above score 5. Some panelists noticed differences in odor, texture and aspect according to radiation dose; “loss of turgor and sogginess” was sometimes mentioned after tasting 3.0 kGy samples. Texture softening has been associated with changes in pectic substances, hydrolysis of polygalactouronides and cellulose; as a consequence, turgor loss due to cellular damage (Prakash et al. 2000a).

It is clear that the higher the radiation dose the greater its negative impact on sensory quality. However, this study indicates that spinach leaves irradiated and stored for 14 days can tolerate at least up to 3 kGy of γ-radiation even though the minimum target dose was smaller (1.5 kGy). Lower scores in every attribute evaluated at the end of the storage period were probably due to leaves senescence as it was previously observed by Piagentini et al. (2002). As a consequence of senescence, water loss is induced resulting in spinach wilting and shriveling which associated with microbial growth leads to product spoilage (Neal et al. 2010).

Many authors have reported sensory quality data of irradiated leafy vegetables: Prakash et al. (2000a) (lettuce), Kamat et al. (2003) (coriander), Zhang et al. (2006) (lettuce), Fan and Sokorai (2008) (lettuce, cilantro, parsley, red cabbage, and spinach among other vegetables), Gomes et al. (2008) (spinach), Nunes et al. (2008) (arugula), Neal et al. (2010) (spinach), Fan and Sokorai (2011a) (lettuce), Fan and Sokorai (2011b) (spinach), and Fan et al. (2012) (lettuce and spinach), employing different methodologies, radiation doses, packaging, atmosphere and storage conditions which led to some different results. Nevertheless, those authors who evaluated spinach leaves agreed that irradiation up to 1 kGy did not impair sensory quality (Fan et al. 2008). Contrary to the present results, Neal et al. (2010) found that spinach treated at 1.4 kGy showed significantly lower hardness scores as assessed by a trained panel. Fan et al. (2012) did not find significant differences, as compared with control, on overall appearance and sogginess of refrigerated spinach leaves even when irradiated at 3 and 4 kGy, and stored for 14 days; but they reported a significantly lower purchase intention.

Low-dose gamma irradiation as a disinfection treatment for spinach leaves at low doses resulted in acceptable sensory quality even after 14 days of refrigerated storage. The target dose, 1.5 kGy, had a great performance presenting scores in some attributes higher than those in control samples. Undesirable effects mainly on texture arose at day 14 in samples treated with 3.0 kGy, but this drawback could be tackled employing a different product loading configuration which allows lowering dose uniformity ratio (Dmax/Dmin) (ISO, 2011). Combination of ionizing radiation with other preservation technologies as hurdles should be studied in order to minimize doses and so optimize spinach leaves tolerance (Gomes et al. 2011; Goularte et al. 2004; Gupta et al. 2012; Moosekian et al. 2014; Neal et al. 2008).

4. Conclusion

Gamma irradiation at 1.5 kGy could constitute a feasible alternative for the disinfection of fresh spinach leaves. In the present work the adequacy of the proposed treatment was proved as native microbiota was effectively reduced which extended shelf life under refrigeration to 14 days, the evaluated phytochemicals were preserved with the exception of ascorbic acid, and the sensory acceptability was good. Product quality was still good even at the maximum absorbed dose of 3.0 kGy, which fulfils the requisite Dmax/Dmin ≤ 2 established in some national regulations such as the Argentine. Hopefully these results could encourage large scale adoption of this technology by the fresh produce industry.

Acknowledgements

This research was supported by Universidad de Buenos Aires (UBACyT 20020130100176BA and 2002012030008BA), Agencia Nacional de Promoción Científica y Tecnológica (PICT 2013/0636), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the International Atomic Energy Agency (IAEA): Coordinated Research Project (2010-2015): "The development of irradiated foods for immuno-compromised patients and other potential target groups"). RC-15744.

Authors gratefully acknowledge the collaboration of INTA (BANOR-12715012), technician Maritza Guerrero Martínez, sensory panel members, staffs of the High Dose Dosimetry Section and Semi-industrial Irradiation Facility, Ezeiza Atomic Centre.

References

Ahn, H. J., Kim, J. H., Kim, J. K., Kim, D. H., Yook, H. S., & Byun, M. W. (2005). Combined effects of irradiation and modified atmosphere packaging on minimally processed Chinese cabbage (*Brassica rapa* L.). *Food Chemistry*, *89*, 589-597. http://dx.doi.org/10.1016/j.foodchem.2004.03.029

Alothman, M., Bhat, R., & Karim, A. A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends in Food Science & Technology*, *20*, 201-212. http://dx.doi.org/10.1016/j.tifs.2009.02.003

An, B. J., Kwak, J. H., Son, J. H., Park, J. M., Lee, J. Y., Jo, C., & Byun, M. W. (2004). Biological and anti-microbial activity of irradiated green tea polyphenols. *Food Chemistry*, *88*, 549-555. http://dx.doi.org/10.1016/j.foodchem.2004.01.070

Antonio, A. L., Fernandes, A., Barreira, J. C. M., Bento, A., Botelho, M. L., & Ferreira, I. C., (2011). Influence of gamma irradiation in the antioxidant potential of chestnuts (*Castanea sativa* Mill.) fruits and skins. *Food and Chemical Toxicology*, *49*, 1918-1923. http://dx.doi.org/10.1016/j.fct.2011.02.016

AOAC (Association of Official Analytical Chemists) International (1995). *Official Method of Analysis (16th ed.)*. Arlington, VA: AOAC International.

Arjeh, E., Barzegar, M., & Sahari, M. A. (2015). Effects of gamma irradiation on physicochemical properties, antioxidant and microbial activities of sour cherry juice. *Radiation Physics and Chemistry*, *114*, 18-24. http://dx.doi.org/10.1016/j.radphyschem.2015.05.017

ASTM (1996). *Sensory testing method: 2nd Edition.* Manual 26. Philadelphia: American Society for Testing Materials (ASTM).

Austin, J. W., Dodds, K. L., Blanchfield, B., & Farber, J. M. (1998). Growth and toxin production by *Clostridium botulinum* on inoculated fresh-cut packaged vegetables. *Journal of Food Protection*, *61*, 324-328. Available at: http://www.ingentaconnect.com/content/iafp/jfp/1998/00000061/00000003/art00012#expand/collapse [Accessed 24 May 2016].

Banerjee, A., Suprasanna, P., Variyar, P. S., & Sharma, A. (2015). Gamma irradiation inhibits wound induced browning in shredded cabbage. *Food Chemistry*, *173*, 38-44. http://dx.doi.org/10.1016/j.foodchem.2014.09.166

Brandstetter, S., Berthold, C., Isnardy, B., Solar, S., & Elmadfa, I. (2009). Impact of gamma-irradiation on the antioxidative properties of sage, thyme, and oregano. *Food and Chemical Toxicology*, *47*, 2230-2235. http://dx.doi.org/10.1016/j.fct.2009.06.009

Cocetta, G., Baldassarre, V., Spinardi, A., & Ferrante, A. (2014). Effect of cutting on ascorbic acid oxidation and recycling in fresh-cut baby spinach (*Spinacia oleracea* L.) leaves. *Postharvest Biology and Technology*, *88*, 8-16. http://dx.doi.org/10.1016/j.postharvbio.2013.09.001

Corbo, M. R., Altieri, C., D'amato, D., Campaniello, D., Del Nobile, M. A., & Sinigaglia, M. (2004). Effect of temperature on shelf life and microbial population of lightly processed cactus pear fruit. *Postharvest Biology and Technology, 31,* 93-104. http://dx.doi.org/10.1016/S0925-5214(03)00133-9

Corbo, M. R., Del Nobile, M. A., & Sinigaglia, M. (2006). A novel approach for calculating shelf life of minimally processed vegetables. *International Journal of Food Microbiology, 106,* 69-73. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.05.012

Doyle, M. P., & Erickson, M. C. (2008). Summer meeting 2007–the problems with fresh produce: an overview. *Journal of Applied Microbiology, 105,* 317-330. http://dx.doi.org/10.1111/j.1365-2672.2008.03746.x

Fan, X. (2005). Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. *Journal of the Science of Food and Agriculture, 85*(6), 995-1000. http://dx.doi.org/10.1002/jsfa.2057

Fan, X., & Sokorai, K. J. (2008). Retention of Quality and Nutritional Value of 13 Fresh-Cut Vegetables Treated with Low-Dose Radiation. *Journal of Food Science, 73*, S367-S372. http://dx.doi.org/10.1111/j.1750-3841.2008.00871.x

Fan, X., Niemira, B. A., & Prakash, A. (2008). Irradiation of fresh fruits and vegetables. *Food Technology, 62,* 36-43. Available at: https://www.ift.org/food-technology/access-denied.aspx?ReturnUrl=%2fFood-Technology%2fPast-Issues%2f2008%2fMarch%2fFeatures%2fIrradiation-of-Fresh-Fruits-and-Vegetables.aspx [Accessed 24 May 2016].

Fan, X., & Sokorai, K. J. (2011) (a). Effects of gamma irradiation, modified atmosphere packaging, and delay of irradiation on quality of fresh-cut iceberg lettuce. *Horticultural Science**,** 46*, 273-277. Available at: http://hortsci.ashspublications.org/content/46/2/273.full.pdf+html [Accessed 24 May 2016].

Fan, X., & Sokorai, K. J. (2011) (b). Changes in Quality, Liking, and Purchase Intent of Irradiated Fresh-Cut Spinach during Storage. *Journal of Food Science, 76*, S363-S368. http://dx.doi.org/10.1111/j.1750-3841.2011.02207.x

Fan, X., Guan, W., & Sokorai, K. J. (2012). Quality of fresh-cut Iceberg lettuce and spinach irradiated at doses up to 4kGy. *Radiation Physics and Chemistry, 81*, 1071-1075. http://dx.doi.org/10.1016/j.radphyschem.2011.11.022

FAOSTAT, FAO. (2015). Statistics of the Food and Agriculture Organization of the United Nations [electronic document]. URL http://faostat3.fao.org/browse/Q/QC/E *[last access: October 20th, 2016]*.

Farkas, J., Saray, T., Mohacsi, C. F., Horti, K., & Andrassy, E. (1997). Effects of low dose gamma radiation on shelf life and microbiological safety of pre-cut/prepared vegetables. *Advances in Food Science, 19*, 111-119.

Finten, G., Finten, F., Agüero, M. V., & Jagus, R. J. (2015). Efectividad del ácido cítrico como reemplazante del hipoclorito de sodio en el lavado de hojas de espinaca (*Spinacia oleracea* L.). In Aguirre, A., González, A., and Strumia, M. (Eds.), *Investigaciones y avances en conservación y almacenamiento de los alimentos : trabajos completos del V CICYTAC 2014*. Córdoba: Ministerio de Industria, Comercio, Minería y Desarrollo Científico Tecnológico . Secretaría de Ciencia y Tecnología, 2015. Available at: http://cicytac.cba.gov.ar/TrabajosCompletos/Conservacion%20y%20almacenamiento.pdf [Accessed 3 Feb. 2016].

Fleet, G. (1992). Spoilage yeasts. *Critical Reviews in Biotechnology, 12,* 1-44. http://dx.doi.org/10.3109/07388559209069186

Food and Drug Administration, HHS. (2008). Irradiation in the production, processing and handling of food. Final rule. *Federal Register, 73*(164), 49593. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18958941 [Accessed 24 May 2016].

Gomes, C., Moreira, R., Castell-Perez, M., Kim, J., Da Silva, P., & Castillo, A. (2008). E-beam irradiation of bagged, ready-to-eat spinach leaves (*Spinacea oleracea*): an engineering approach. *Journal of Food Science, 73,* E95-E102. http://dx.doi.org/10.1111/j.1750-3841.2007.00629.x

Gomes, C., Da Silva, P., Moreira, R. G., Castell-Perez, E., Ellis, E. A., & Pendleton, M. (2009). Understanding E. coli internalization in lettuce leaves for optimization of irradiation treatment. *International Journal of Food Microbiology, 135,* 238-247. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.08.026

Gomes, C., Moreira, R. G., & Castell-Perez, E. (2011). Microencapsulated antimicrobial compounds as a means to enhance electron beam irradiation treatment for inactivation of pathogens on fresh spinach leaves. *Journal of Food Science, 76,* 479-488. http://dx.doi.org/10.1111/j.1750-3841.2011.02264.x

Gómez-López, V. M., Lannoo, A. S., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157: H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control, 42*, 132-138. http://dx.doi.org/10.1016/j.foodcont.2014.01.034

Goodburn, C., & Wallace, C. A. (2013). The microbiological efficacy of decontamination methodologies for fresh produce: A review. *Food Control, 32*, 418-427. http://dx.doi.org/10.1016/j.foodcont.2012.12.012

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. (2004). Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.). *Radiation Physics and Chemistry, 71*, 157-161. http://dx.doi.org/10.1016/j.radphyschem.2004.03.076

Gupta, S., Chatterjee, S., Vaishnav, J., Kumar, V., Variyar, P. S., & Sharma, A. (2012). Hurdle technology for shelf stable minimally processed French beans (*Phaseolus vulgaris*): A response surface methodology approach. *LWT-Food Science and Technology, 48*, 182-189. http://dx.doi.org/10.1016/j.lwt.2012.03.010

Hanning, I. B., Nutt, J. D., & Ricke, S. C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease, 6*, 635-648. http://dx.doi.org/10.1089/fpd.2008.0232

Horak, C., Pietranera, M. A., Malvicini, M., Narvaiz, P., Gonzalez, M., & Kairiyama, E. (2006). Improvement of hygienic quality of fresh, pre-cut, ready-to-eat vegetables using gamma irradiation. In *Use of Irradiation to Ensure the Hygienic Quality of Fresh, Pre-Cut Fruits and Vegetables and Other Minimally Processed Food of Plant Origin* (pp. 23-40). TECDOC 1530. Vienna: International Atomic Energy Agency (IAEA). Available at: http://www-pub.iaea.org/MTCD/Publications/PDF/te_1530_web.pdf#page=30 [Accessed 24 May 2016].

ISO. (2003). Practice for use of a dichromate dosimetry system. In *Standards on dosimetry for radiation processing.* ISO/ASTM 51401:2003. Geneva: ISO/ASTM International.

ISO. (2011). *Food irradiation - Requirements for the development, validation and routine control of the ionizing radiation process used for the treatment of food.* ISO 14470:2011. Geneva: ISO International.

ICMSF. (1983). Métodos recomendados para el análisis microbiológico en alimentos. In *Microorganismos de los alimentos I. Técnicas de análisis microbiológicos* (2nd ed., pp. 105-280). Zaragoza: Acribia S.A.

Josephson, E. S., & Peterson, M. S. (1983). *Preservation of food by ionizing radiation.* Boca Raton, FL: CRC Press.

Kamat, A., Pingulkar, K., Bhushan, B., Gholap, A., & Thomas, P. (2003). Potential application of low dose gamma irradiation to improve the microbiological safety of fresh coriander leaves. *Food Control, 14*, 529-537. http://dx.doi.org/10.1016/S0956-7135(02)00116-0

Lester, G. E., Hallman, G. J., & Pérez, J. A. (2010). γ-Irradiation dose: effects on baby-leaf spinach ascorbic acid, carotenoids, folate, α-tocopherol, and phylloquinone concentrations. *Journal of Agricultural and Food Chemistry*, *58*, 4901-4906. http://dx.doi.org/10.1021/jf100146m

Lilly, T., Solomon, H. M., & Rhodehamel, E. J. (1996). Incidence of *Clostridium botulinum* in vegetables packaged under vacuum or modified atmosphere. *Journal of Food Protection*, *59*(1), 59-61. Available at: http://www.ingentaconnect.com/content/iafp/jfp/1996/00000059/00000001/art00012 [Accessed 24 May 2016].

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, *137*, 307-315. http://dx.doi.org/10.1017/S0950268808001969

Moosekian, S. R., Jeong, S., & Ryser, E. T. (2014). Inactivation of sanitizer-injured *Escherichia coli* O157: H7 on baby spinach using X-ray irradiation. *Food Control*, *36*, 243-247. http://dx.doi.org/10.1016/j.foodcont.2013.08.024

Müller, H., & Diehl, J. F. (1996). Effect of ionizing radiation on folates in food. *LWT-Food Science and Technology*, *29*, 187-190. http://dx.doi.org/10.1006/fstl.1996.0026

Neal, J. A., Cabrera-Diaz, E., Márquez-Gonzaález, M., Maxim, J. E., & Castillo, A. (2008). Reduction of *Escherichia coli* O157: H7 and *Salmonella* on baby spinach, using electron beam radiation. *Journal of Food Protection*, *71*, 2415-2420. Available at: http://www.ingentaconnect.com/content/iafp/jfp/2008/00000071/00000012/art00006 [Accessed 24 May 2016].

Neal, J. A., Booren, B., Cisneros-Zevallos, L., Miller, R. K., Lucia, L. M., Maxim, J. E., & Castillo, A. (2010). Shelf life and sensory characteristics of baby spinach subjected to electron beam irradiation. *Journal of Food Science*, *75*, S319-S326. http://dx.doi.org/10.1111/j.1750-3841.2010.01664.x

Neal, J. A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L. M., O'Bryan, C. A., Crandall, P. G., Ricke, S. C., & Castillo, A. (2012). Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157: H7 on spinach (*Spinacia oleracea*) leaves. *Food Research International*, *45*, 1123-1128. http://dx.doi.org/10.1016/j.foodres.2011.04.011

Niemira, B. A. (2003). Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on 4 lettuce types. *Journal of Food Science*, *68*, 2784-2787. http://dx.doi.org/10.1111/j.1365-2621.2003.tb05805.x

Niemira, B. A., Fan, X., Sokorai, K. J., & Sommers, C. H. (2003). Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*). *Journal of Food Protection*, *66*, 993-998. Available at: http://www.ingentaconnect.com/content/iafp/jfp/2003/00000066/00000006/art00012 [Accessed 24 May 2016].

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

Niemira, B. A. (2007). Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate *Escherichia coli* O157:H7 internalized in leaves of Romaine lettuce and baby spinach. *Journal of Food Protection*, *70*, 2526-2532. Available at: http://www.ingentaconnect.com/content/iafp/jfp/2007/00000070/00000011/art00010 [Accessed 24 May 2016].

Niemira, B. A. (2008). Irradiation compared with chlorination for elimination of *Escherichia coli* O157:H7 internalized in lettuce leaves: influence of lettuce variety. *Journal of Food Science*, *73*, M208-M213. http://dx.doi.org/10.1111/j.1750-3841.2008.00746.x

Nunes, T. P., Martins, C. G., Behrens, J. H., Souza, K. L., Genovese, M. I., Destro, M. T., & Landgraf, M. (2008). Radioresistance of *Salmonella* species and *Listeria monocytogenes* on minimally processed Arugula (*Eruca sativa* Mill.): Effect of irradiation on flavonoid content and acceptability of irradiated produce. *Journal of Agricultural and Food Chemistry*, *56*, 1264-1268. http://dx.doi.org/10.1021/jf072873j

Nunes, T. P., Martins, C. G., Faria, A. F., Bíscola, V., de Oliveira Souza, K. L., Mercadante, A. Z., Cordenunsi, B. R., & Landgraf, M. (2013). Changes in total ascorbic acid and carotenoids in minimally processed irradiated Arugula (*Eruca sativa* Mill.) stored under refrigeration. *Radiation Physics and Chemistry.*, *90*, 125-130. http://dx.doi.org/10.1016/j.radphyschem.2013.03.044

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, *32*, 1-19. http://dx.doi.org/10.1016/j.fm.2012.04.016

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food Science and Technology*, *42*, 686-693. http://dx.doi.org/10.1016/j.lwt.2008.08.001

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Elez-Martínez, P., & Martín-Belloso, O. (2012). Stability of health-related compounds in plant foods through the application of non thermal processes. *Trends in Food Science and Technology*, *23*, 111-123. http://dx.doi.org/10.1016/j.tifs.2011.10.004

Parlato, A., Giacomarra, M., Galati, A., & Crescimanno, M. (2014). ISO 14470: 2011 and EU legislative background on food irradiation technology: The Italian attitude. *Trends in Food Science and Technology*, *38*, 60-74. http://dx.doi.org/10.1016/j.tifs.2014.04.001

Piagentini, A. M., Güemes, D. R., & Pirovani, M. E. (2002). Sensory characteristics of fresh-cut spinach preserved by combined factors methodology. *Journal of Food Science*, *67*, 1544-1549. http://dx.doi.org/10.1111/j.1365-2621.2002.tb10319.x

Prakash, A., Guner, A., Caporaso, F., & Foley, D. (2000a). Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. *Journal of Food Science*, *65*, 549-553. http://dx.doi.org/10.1111/j.1365-2621.2000.tb16046.x

Prakash, A., Inthajak, P., Huibregtse, H., Caporaso, F., & Foley, D. (2000b). Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. *Journal of Food Science*, *65*, 1070-1075. http://dx.doi.org/10.1111/j.1365-2621.2000.tb09420.x

R Core Team (2015). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing. Vienna: R Core Team. Available at: https://www.R-project.org/ [Accessed 24 May 2016].

Ragaert, P., Devlieghere, F., & Debevere, J. (2007). Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. *Postharvest Biology and Technology*, *44*, 185-194. http://dx.doi.org/10.1016/j.postharvbio.2007.01.001

Roberts, P. B. (2014). Food irradiation is safe: Half a century of studies. *Radiation Physics and Chemistry*, *105*, 78-82. http://dx.doi.org/10.1016/j.radphyschem.2014.05.016

Rubinstein, A. J., Cova, M. C., Narvaiz, P., Jagus, R. J., & Agüero, M. V. (2013). Radiorresistencia de *Listeria innocua* en espinaca lavada. In *Libro de actas del XIV Congreso Argentino de Ciencia y Tecnología de los Alimentos (CYTAL).* Rosario: Asociación Argentina de Tecnólogos Alimentarios (AATA).

Sagong, H. -G., Lee, S. -Y., Chang, P. -S., Heu, S., Ryu, S., Choi, Y. -J., & Kang, D. -H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157: H7, *Salmonella* typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology*, *145*, 287-292. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.010

Scott, K. (2001). Detection and measurement of carotenoids by UV/VIS spectrophotometry. In R. Wrolstad (Ed.), *Current protocols in food analytical chemistry*. New York: Wiley.

Serna Villagomez, N., Neri Herrera, E. A., Orozco, L., Wild-Padua, G., & Iturriaga, M. H. (2010). Effect of storage time on the efficacy of chemical and irradiation treatments to inactivate Salmonella on cilantro (*Coriandrum sativum* L.). *Journal of Food Safety*, *30*, 584-598. http://dx.doi.org/10.1111/j.1745-4565.2010.00227.x

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, (299C), 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

Song, H. P., Kim, D. H., Jo, C., Lee, C. H., Kim, K. S., & Byun, M. W. (2006). Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. *Food Microbiology*, *23*, 372-378. http://dx.doi.org/10.1016/j.fm.2005.05.010

Viacava, G. E., Roura, S. I., & Agüero, M. V. (2015). Antioxidant activity of Butterhead lettuce: factors significantly affecting antioxidant extraction and quantification. *Journal of Food Measurement and Characterization*, *9*, 206-214. http://dx.doi.org/10.1007/s11694-015-9225-8

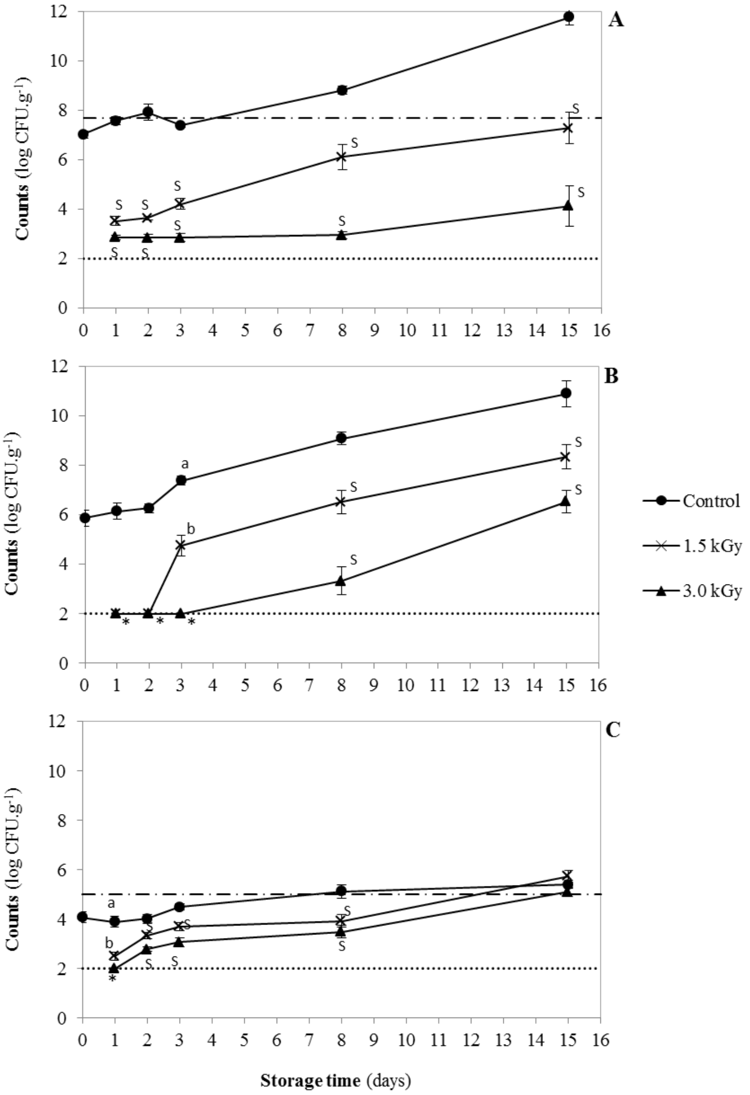
Warning, A., & Datta, A. K. (2013). Interdisciplinary engineering approaches to study how pathogenic bacteria interact with fresh produce. *Journal of Food Engineering*, *14*, 426-448. http://dx.doi.org/10.1016/j.jfoodeng.2012.09.004

Watada, A. E., Ko, N. P., & Minott, D. A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*, *9*, 115-125. http://dx.doi.org/10.1016/S0925-5214(96)00041-5

Zhang, L., Lu, Z., & Wang, H. (2006). Effect of gamma irradiation on microbial growth and sensory quality of fresh-cut lettuce. *International Journal of Food Microbiology*, *106*, 348-351. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.07.005

FIGURE 1. Evolution of native microbiota in control and irradiated spinach samples during refrigerated storage.

A: mesophilic bacteria; B: psychrotrophic bacteria; C: yeasts and molds. Continuous dotted lines indicate the detection limit of the microbiological method (2 log CFU.g^{-1}) and the semi-continuous dotted line (upper region) indicate the maximum acceptable level set for mesophilic bacteria and yeasts and molds. Data are presented as the means ± standard errors expressed as vertical bars (n= 4). Letter "S" at each day indicates significant differences with control (Dunnett's test, p<0.05). Letters "a" and "b" indicate significant differences assessed by a two-sample t test (p<0.05).



---

* *Microbial counts were lower than 2 log CFU.g^{-1}.*

FIGURE 2. Evolution of ascorbic acid (A), total polyphenols content (B), and antioxidant capacity (C) during refrigerated storage for control, and irradiated spinach samples.

Data are presented as the means ± standard errors expressed as vertical segments (n=4 for A, and n=3 for B and C). Letter “S” at each day indicates significant differences with control (Dunnett’s test, p<0.05).

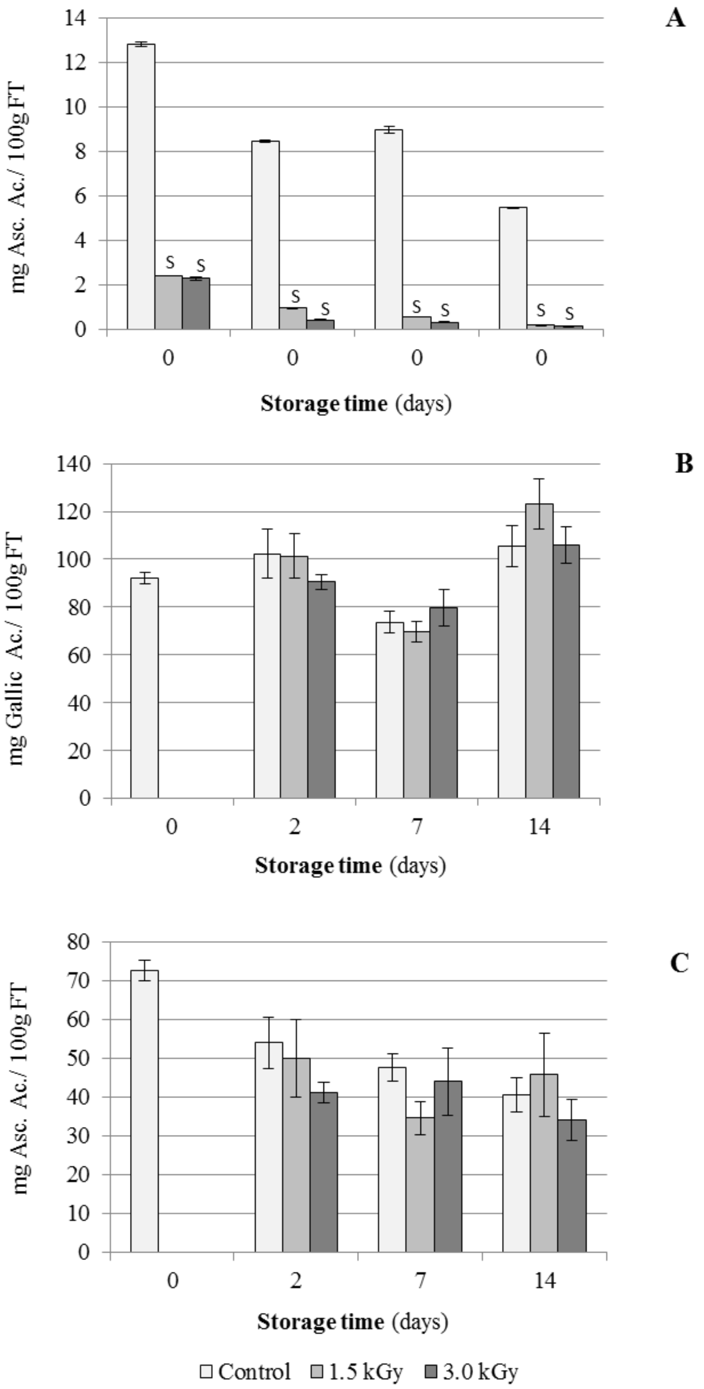


FIGURE 3. Evolution of total chlorophyll content (A), and carotenoids content (B) during refrigerated storage for control, and irradiated spinach samples.

Data are presented as the means ± standard errors expressed as vertical segments (n=3). No significant differences between control and irradiated samples were found (Dunnett's test, p<0.05).

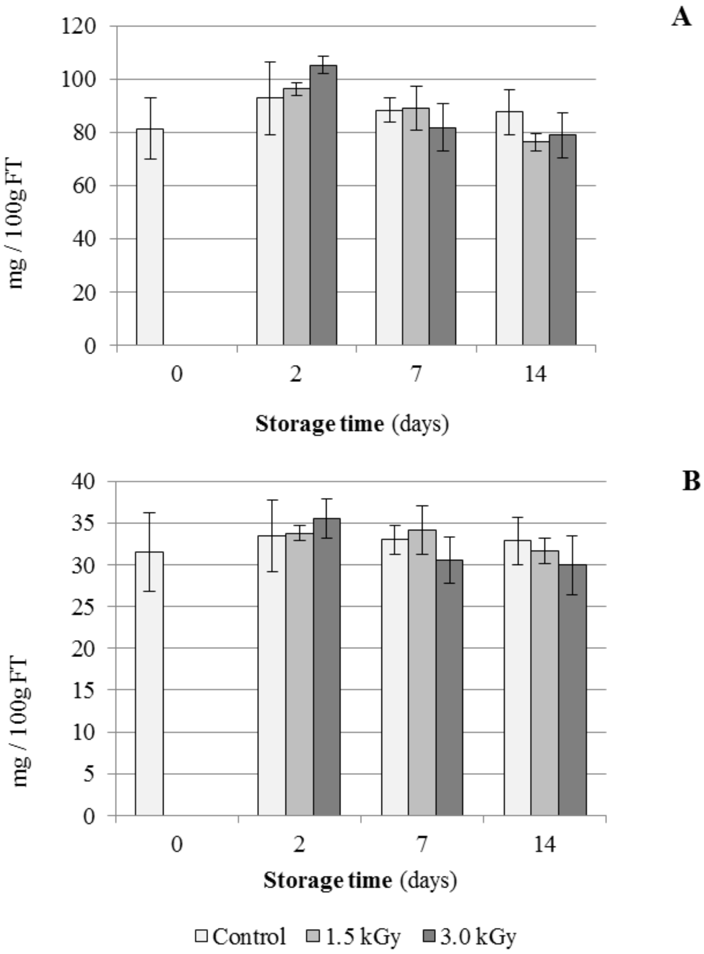


FIGURE 4. Sensory analysis carried out on control and irradiated spinach samples during refrigerated storage on days 2 (A), 6 (B), and 14 (C).

Data are presented as the means ± standard errors expressed as vertical segments (n= 26-33). Letter “S” at each day indicates significant differences with control (Dunnett’s test, $p<0.05$). Letters “a” and “b” indicate significant differences assessed by Welch two-sample t test ($p<0.05$).