JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

TATIANA P. NUNES, CECÍLIA G. MARTINS, JORGE H. BEHRENS, KÁTIA L. O. SOUZA, MARIA INÉS GENOVESE, MARIA TERESA DESTRO, AND MARIZA LANDGRAF*

Departamento de Alimentos e Nutrição Experimental, FCF, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 14, 05508-900 São Paulo, SP, Brazil

This work studied the radiation resistance of *Listeria monocytogenes* and *Salmonella* species and the effect of irradiation on leaf flavonoid content and sensory acceptability of minimally processed arugula. Immersion in ozone-treated water reduced the analyzed microorganisms by 1 log. *L. monocytogenes* and *Salmonella* were not isolated from samples. Samples of this vegetable were inoculated with a cocktail of *Salmonella* spp. and *L. monocytogenes* and exposed to γ irradiation. D_{10} values for *Salmonella* ranged from 0.16 to 0.19 kGy and for *L. monocytogenes* from 0.37 to 0.48 kGy. Kaempferol glycoside levels were 4 and ca. 3 times higher in samples exposed to 1 and 2 kGy, respectively, than in control samples. An increase in quercetin glycoside was also observed mainly in samples exposed to 1 kGy. In sensory evaluation, arugula had good acceptability, even after exposure to 2 and 4 kGy. These results indicate that irradiation has potential as a practical processing step to improve the safety of arugula.

KEYWORDS: ***Listeria monocytogenes*; *Salmonella* spp.; radiation; flavonoid; sensory acceptability; *Eruca sativa* Mill.**

INTRODUCTION

The international market of fruits and vegetables experienced many alterations in recent years because of the changes in consumption habits, which increased the demand for minimally processed vegetables (*1*). Moreover, the industry, researchers, and consumers have demonstrated great interest in the antioxidant capacity of flavonoids and other phytochemical compounds present in vegetables that can have a protective effect against cancer and cardiovascular diseases (*2, 3*).

Research, however, has shown that minimal processing is not enough to guarantee the safety of these foods. The majority of these products do not require any additional treatment and are consumed raw, thus becoming a potential safety issue in public health. These foods have been shown to be a source of food-poisoning outbreaks, resulting from contamination with pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* (*4–8*). Minimally processed vegetables are subjected to steps such as peeling, slicing, or shredding that could be means for cross-contamination. Garg et al. (*9*) verified that the population of mesophilic bacteria in sliced vegetables increased from 10^3-10^4 to 10^5-10^6 CFU/g due to the increase in the availability of nutrients and the surface area for these microorganisms.

Because minimal processing by itself does not guarantee the safety of these foods, ionizing radiation is one process to address this problem; it can effectively eliminate human pathogens, improving the safety and quality of minimally processed vegetables and extending the shelf life of these products (*10*). Moreover, it is the only treatment that can penetrate inside the leaves of vegetables. However, despite regulatory approval and control in the use of irradiation, barriers related to costs and consumer acceptance still prevent the commercialization of irradiated produce (*11*).

Several studies with minimal processing of the most consumed vegetables in North America are reported in the literature. However, products of great acceptance and economic importance in other countries, such as arugula, watercress, and others are not so well studied. Therefore, the aims of the present research were to determine the D_{10} value (which is the dose in kilograys for a 1 $\log_{10}$ reduction in the population) of a cocktail

* Corresponding author (telephone +55-11-30912191; fax +55-11-38154410; e-mail landgraf@usp.br).

10.1021/jf072873j CCC: $40.75
Published on Web 02/01/2008

of *Salmonella* and *L. monocytogenes* strains and the flavonoid content and the sensory quality of irradiated, minimally processed arugula.

MATERIALS AND METHODS

Substrate. Arugula (*Eruca sativa* Mill.) samples were kindly acquired from local industry. The time elapsed between the harvest of the samples, their processing, and the beginning of this research did not exceed 24 h. All chemicals and solvents were of reagent or HPLC grade. The standards of luteolin, apigenin, kaempferol, quercetin, catechin, and epicatechin were obtained from Sigma Chemical Co. (St. Louis, MO); the standards of cyanidin, pelargonidin, delphinidin, malvidin, and peonidin were from Extrasynthese (Genay, France); naringenin, hesperetin, and the chalcones chalconaringenin and phloridzin were from Plantech (Reading, U.K.); cyanidin 3-glycoside and naringin were from Apin Chemicals (Abingdon, U.K.); and rutin was from Merck (Darmstadt, Germany).

Microorganisms. Three strains of *Salmonella* (*S.* Infantis were kindly provided by Instituto Adolfo Lutz; *S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 14028) and four strains of *L. monocytogenes* (one isolated from spinach; one from ground meat, one ATCC 7644, and one ATCC 19115) were used. The strains were maintained at −70 °C in tryptic soy broth (TSB; Oxoid, Basingstoke, U.K.) with 20% glycerol.

Vegetable Sanitation. The damaged leaves were discarded. Arugula leaves and stalks were rinsed thoroughly in cold water and sanitized by immersion in ozone-treated water (0.08 ppm) for 5 min. Then, the leaves and stalks were centrifuged to remove excess water and packed (ref *12*, with modifications).

Preparation of the Inoculum. Each strain of *Salmonella* was inoculated into TSB (Oxoid) and, for *L. monocytogenes* TSB was added with 0.6% of yeast extract (YE; Oxoid) (TSBYE) and incubated for 18–20 h at 37 °C. A loopful was transferred to 100 mL of TSB (Oxoid) for *Salmonella* and TSBYE for *L. monocytogenes* 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 to make a stock culture. The cultures were centrifuged (centrifuge Hettich, Tuttlingen, Germany) at 700*g* for 30 min, and the pellets were resuspended in 45 mL of 0.85% (w/v) NaCl solution (LabSynth, Diadema, Brazil).

Inoculation of Vegetables for Determination of Radiation D_{10} Values (*12*). The stock culture of bacteria (90 mL) was mixed with 6 L of cold (2–5 °C) distilled water to have ca.10^7–10^8 CFU/mL. The minimally processed arugula was immersed into this suspension for 15 min. Then, the arugula was spun in a sanitized salad spinner-type centrifuge to remove excess surface water. The arugula showed a contamination of ca.10^6 CFU/g. The vegetables were divided into 25 g portions and packed into polyethylene bags.

Irradiation Process. The arugula samples spiked with *L. monocytogenes* were exposed to doses of 0.5, 1.0, 1.5, 2.0, and 2.5 kGy, and the samples spiked with *Salmonella* spp. received absorbed doses of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 kGy. Each study included three samples per dose and was repeated three times. All samples were irradiated using a γ radiation ^{60}Co source (JS 7500 MDS Nordion, Kanata, Canada). The dosimetric system used was the Harwell red perspex (U.K.) dosimeter. The accuracy is 75% and the precision 72%. The slopes of the individual survivor curves were calculated with linear regression using a computer graphic program (Microsoft Excel 97 SR-2, Redmond, WA). The D_{10} value was calculated by taking the negative reciprocal of the survivor curve slope.

Enumeration of *L. monocytogenes* after Irradiation. Irradiated and non-irradiated samples were maintained under refrigeration (3–8 °C) until the beginning of microbiological tests. Each portion of 25 g was homogenized with 225 mL of 0.85% NaCl solution (LabSynth) using a Stomacher 400 (Seward, London, U.K.) during 30 s and serially diluted with 0.85% NaCl solution (LabSynth). One milliliter of each dilution was pour plated using TSAYE (ref *13*, with modifications). After solidification, an overlay of Oxford agar (Oxoid) was added to determine the population of surviving bacteria. Two pour plates per dilution were incubated at 37 °C for 24 h. Each analysis was performed three times.

Salmonella enumeration was carried out in an analogous way to the quantification of *L. monocytogenes*, and the Oxford agar (Oxoid) was replaced by MLCB agar (Oxoid) (*13*).

Microbiological Evaluation before and after Minimal Processing. According to analysis recommended by ANVISA, RDC12 (*14*), fecal coliforms (*15*), and *Salmonella* spp. (*16*) were done. Besides these, enumeration of mesophilic (*17*), psycotrophic (*18*), lactic acid bacteria (*19*), *Pseudomonas* spp. (*20*), and the presence/absence of *L. monocytogenes* (*21*) were also performed.

Flavonoid Extraction. Flavonoid extraction was performed according to the method in ref *22* and that of Arabbi et al. (*23*) with slight modifications, as follows. Samples were thoroughly homogenized by powdering in liquid nitrogen. Duplicate samples of the fresh powder (20 g) were extracted three times in methanol/water (100 mL the first time, 50 mL the next two times) with a final solvent composition of 70:30, including the water of samples, at speed 5 for 1 min (Brinkmann homogenizer, Polytron-Kinematica GmbH, Kriens-Luzern, Sweden), in an ice bath. The homogenate containing the polyphenolics was filtered under reduced pressure through filter paper (Whatman no. 06). The extracts obtained were concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120, Buchi, Flawil, Sweden) at 40 °C and made up to 25 mL with water for subsequent application to solid-phase extraction (SPE) columns.

Solid-Phase Extraction. Aliquots (10 mL) of the extracts obtained above were passed through polyamide SC 6 (Macherey-Nagel GmbH and Co., Duren, Germany) columns (1 g/6 mL) previously conditioned with 20 mL of methanol and 60 mL of water. The columns were washed with water (20 mL) and eluted with 50 mL of methanol followed by 50 mL of 99.5:0.5 methanol/ammonia. Each eluate was evaporated to dryness under reduced pressure at 40 °C, redissolved in methanol or methanol/acetic acid 95:5 (1 mL), and filtered through a 0.22 μm poly(tetrafluoroethylene) (PTFE) filter (Millipore Ltd., Bedford, MA) prior to HPLC analysis.

Analytical HPLC. Identification and quantification of flavonoids was achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with an autosampler and a quaternary pump coupled to a diode array detector. The column used was a Prodigy 5 μ ODS 3 reversed-phase silica (250 mm × 4.6 mm i.d., Phenomenex Ltd.), and elution solvents A and B were water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1) and acetonitrile, respectively. The solvent gradient was in the proportion of 17% B for 2 min, increasing to 25% B after 5 min, to 35% B after a further 8 min, and to 50% B after 5 min. Eluates were monitored at 270, 370, and 525 nm. Samples were injected in duplicate, and flavonoids were quantified using the respective external standards. Flavonoid standard solutions were prepared by dissolving in HPLC grade methanol and stored at −20 °C between analyses. Calibration was performed by injecting the standard three times at five different concentrations. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. Chromatography was used when necessary. Quantification of quercetin glycosides was performed using quercetin as the standard, except for rutin, for which the rutin standard was used. Results are expressed as milligrams of aglycon per 100 g of fresh weight (FW), as mean [standard deviation (SD)].

Sensory Evaluation of Minimally Processed Irradiated Arugula. Three packages of minimally processed arugula, of approximately 2 kg each, were acquired from local industry. These samples were minimally processed and exposed to radiation doses of 2 and 4 kGy.

For the acceptability evaluation, panelists were asked to rate each sample for overall liking on a hybrid 10 cm hedonic scale (0 = extremely dislike; 10 = extremely like) (*24*). This panel (aged 20–55 years) was composed of 50 nontrained members of the faculty, staff, and students of the University of São Paulo (USP). They evaluated the appearance, flavor, aroma, and texture of the products. Arugula was maintained under refrigeration (ca. 7 °C) and served (10 g) in white plastic dishes coded with three-digit random numbers. The samples were evaluated under white light in individual cabins, in the Sensory Food Analysis Laboratory, Faculty of Pharmaceutical Sciences, USP, in São Paulo, SP. Data were submitted to a two-way ANOVA (SAS Institute, Inc., Cary, NC) followed by Tukey's means comparison test ($p \leq 0.05$).

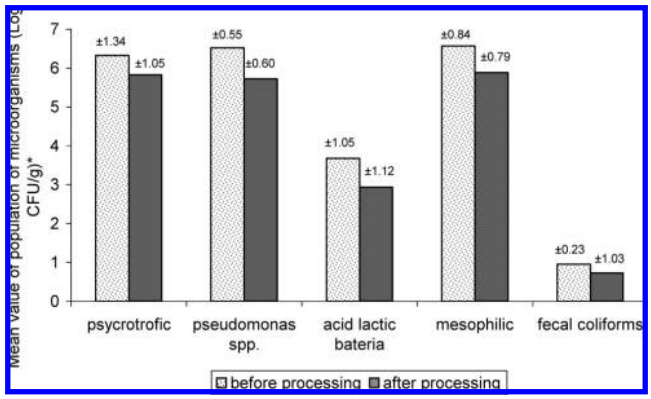

Figure 1. Microbiological evaluation of arugula before and after minimal processing (* ± SD).

RESULTS AND DISCUSSION

Figure 1 shows the effect of minimal processing in the reduction of the natural microflora present in these vegetables. It is possible to observe a reduction by <1 $\log_{10}$ for the analyzed group of bacteria after washing in ozone-treated water (0.08 ppm).

The reduction obtained was lower than the values reported by Martins et al. (*25*) and Goularte et al. (*26*), although these researchers used sodium hypochlorite solution (200 ppm) as a sanitizer. On the other hand, Tsuhako (*27*), who also used hypochlorite (in lettuce) verified that the minimum processing provided a reduction superior to that found in our research, with reductions by 2.68 $\log_{10}$ CFU/g for acid lactic bacteria, 3.35 $\log_{10}$ CFU/g for *Pseudomonas*, and 2.93 $\log_{10}$ CFU/g for psycrotrophics.

Koseki and Isobe (*28*) evaluated the effect of the ozone concentration (3, 5, and 10 ppm) on the microbiological growth of minimally processed lettuce and verified that the bacterial count was reduced in response to the increase of the ozone concentration. However, the reduction observed by these authors was 1.4 $\log_{10}$ CFU/g at concentrations >5 ppm of ozone.

E. coli, when detected, was reduced by ca. 1 log CFU/g, after processing, achieving undetected levels.

Salmonella and *L. monocytogenes* were not found in the analyzed arugula samples, agreeing with Tsuhako (*27*), who did not find any of these pathogenic bacteria in samples of minimally processed lettuce. However, such a fact was observed by Martins et al. (*29*), who detected *L. monocytogenes* in spinach sample and *Salmonella* in four minimally processed vegetable samples (watercress, lettuce, chicory, and prepacked salad with lettuce, carrot, and radicchio).

These results show that the sole treatment with chlorine or ozone solutions was not sufficient to eliminate the bacteria from minimally processed vegetables. Therefore, the combination with irradiation could improve the quality of this produce.

The radiation resistances of *Salmonella* and *L. monocytogenes* are the most studied because of their importance in public health, besides being considered the most resistant microorganisms among non-spore-forming pathogens to the radiation process.

Figures 2 and **3** show the inactivation of *L. monocytogenes* and *Salmonella* inoculated on minimally processed arugula and irradiated up to 2.5 kGy. The D_{10} values for *L. monocytogenes* (0.48, 0.42, and 0.37 kGy) were higher than those for *Salmonella* (0.19, 0.19, and 0.16 kGy) when inoculated on arugula.

Niemira (*30*) reported that the radiation sensitivity of a microorganism is influenced by the different types or cultivars of the same vegetable. Four types of lettuce (Boston, iceberg, green leaf, and red lettuce) had presented different D_{10} values for *Salmonella*: 0.24, 0.25, 0.31, and 0.23 kGy, respectively.

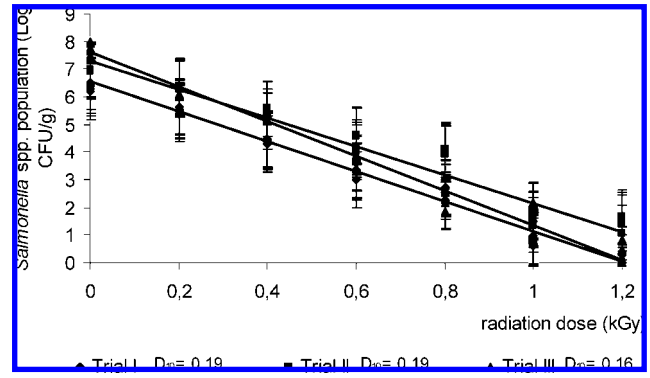

Figure 2. Population of *Salmonella* spp. in arugula exposed to γ radiation.

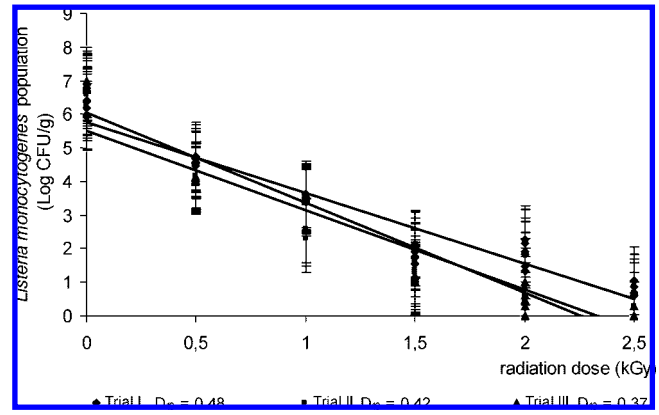

Figure 3. Population of *L. monocytogenes* in arugula exposed to γ radiation.

Goularte et al. (*26*) obtained D_{10} values ranging from 0.16 to 0.23 kGy for *Salmonella* spp. in minimally processed lettuce. These results are similar to the values found in this research, but somewhat lower than those found by Martins et al. (*31*), who found D_{10} values ranging from 0.33 to 0.42 kGy in minimally processed watercress.

Figure 2 shows D_{10} values for *Salmonella*. These values (0.16–0.19 kGy) are lower than that observed by Dhokane et al. (*32*), who found *Salmonella* D_{10} values of 0.312 and 0.345 kGy after inoculation on carrot and cucumber, respectively.

The D_{10} values for *L. monocytogenes* established herein (0.37–0.48 kGy) were higher than those reported by Bari et al. (*33*), Martins et al. (*25*), Niemira (*30*), Niemira et al. (*34*), and Tsuhako (*27*). In those studies, the D_{10} values for *L. monocytogenes* ranged from 0.19 to 0.25 kGy in several vegetables analyzed.

Although a clear mechanism has not yet been established for the variation in sensitivity of *Salmonella* and *L. monocytogenes* observed in the present study and that in the literature, there are a number of complicating factors which may play a role. First and foremost is the nature of the suspending material, arugula, which has not been previously evaluated in irradiation studies. The specific impact and influence of the suspending substrate on radiation sensitivity of inoculated bacteria is poorly understood (*30*). Other potentially significant factors may be the microorganism itself, the water activity, the temperature during the radiation process, the food composition, and the presence of oxygen. Also, the presence of compounds that can protect or not the microorganisms by the action of radiolytic products may play an important role.

Table 1. Flavonoid Content (Kaempferol and Quercetin) in Arugula Samples Exposed to 1 and 2 kGy[a]

	content (mg/100 g of sample in FW[b]) at a radiation dose of		
flavonoid	0 kGy	1 kGy	2 kGy
kaempferol	20.2 ± 0.2	88 ± 2	55.2 ± 0.8
quercetin	8.0 ± 0.1	15.1 ± 0.2	8.00 ± 0.07

[a] Values are mean ± SD. [b] FW, fresh weight;

Table 2. Mean Score of Sensory Acceptability of Minimally Processed Arugula (*Eruca sativa* Mill.) Exposed to Different Doses of γ Radiation[a]

	irradiation dose		
	0 kGy	2 kGy	4 kGy
mean score of sensory acceptability	7.1a	6.3ab	6.0b

[a] Means followed by the same letters are not significantly different at 95% confidence levels.

The presence of bioactive compounds, flavonoids, carotenoids, insoluble fibers, and ascorbic acid, adds market value and desirability, and results in increased vegetable consumption. However, the different processing technologies to which foods are submitted can increase or reduce the amounts of these components.

Table 1 shows the effect of irradiation on flavonoid content in minimally processed arugula. Irradiation caused a very significant increase in flavonoid content. Kaempferol glycoside levels were 4 and ca. 3 times higher in samples exposed to 1 and 2 kGy, respectively, than those in control samples. Quercetin glycoside content was ca. 2 times higher when 1 kGy was applied, but did not differ from the control when exposed to 2 kGy.

These results indicate that irradiation induced de novo synthesis of flavonoids, probably involving an increase in phenylalanine ammonia-lyase (PAL) activity, which catalyzes the first reaction of the biosynthesis of flavonoids.

Arabbi et al. (*23*) analyzed the flavonoid content in several vegetables most commonly consumed in Brazil and reported that the main sources of flavonoids are orange (70%), lettuce (9%), and tomatoes (2.5%). The flavonoids found in largest abundance were glycosides of quercetin.

Vanamala et al. (*35*) reported an increase in flavonoid content (naringin and narutin) in irradiated and freeze-dried samples of grapefruit when compared to control samples. Increase in the flavonoid content was also observed by Variyar et al. (*36*) with soybean exposed to 0.5 and 5 kGy. The latter dose provided the highest increase. On the other hand, irradiation can, sometimes, reduce the flavonoid content as observed by Breitfellner et al. (*37*). The authors studying the effect of γ radiation on flavonoid content in strawberry observed that 3 kGy, approved in many countries for use in this type of food, did not result in a significant reduction of these compounds, not causing, therefore, any impact on its antioxidant capacity.

Although irradiation can improve the microbiological safety of minimally processed vegetables, this process can cause sensory changes in some vegetables, depending on the applied dose. In the case of arugula, however, the mean scores of sensory acceptability (**Table 2**) indicated that both control and irradiated samples (2 and 4 kGy) were accepted. However, very high doses of radiation (4 kGy) caused a significant reduction ($p \leq 0.05$) in the acceptability of arugula when compared to the control sample. On the other hand, Martins et al. (*31*) did not find significant difference in the initial irradiated minimally processed watercress acceptability exposed to 1, 3, and 4 kGy.

Comments from the panel about the appearance of the vegetable were very contradictory. Different groups of consumers considered the appearance of the vegetable to be both of "better appearance" and "bad appearance". In relation to the flavor, aroma, and texture attributes, the irradiated samples elicited more negative comments when compared to control samples. Whereas the non-irradiated sample got a higher number of comments described as "good" flavor and "characteristic" flavor, the samples exposed to 1 kGy were described as "bitter", "strong", or "spicy". In contrast, samples irradiated with 2 kGy were also described as the sample with "more soft" flavor.

In conclusion, these results indicate that irradiation can reduce the risk of pathogenic bacteria in minimally processed vegetables and increase flavonoid content in arugula, when exposed to 1 kGy. Sensorially, it was verified that the product had good acceptability, even when exposed to 2 kGy.

ACKNOWLEDGMENT

We thank B. Niemira for revising the manuscript.

LITERATURE CITED

(1) Ragaert, P.; Verbeke, W.; Devlieghere, F.; Debevere, J. Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Qual. Pref.* **2004**, *15*, 259–270.
(2) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216.
(3) Ness, A. R.; Powles, J. W. Fruit and vegetables, and cardiovascular disease: a review. *Int. J. Epidemiol.* **1997**, *26* (1), 1–13.
(4) Beuchat, L. R. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* **1996**, *59*, 204–216.
(5) De Roever, C. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* **1998**, *9*, 321–347.
(6) Center for Disease Control and Prevention. Update on Multi-State Outbreak of *E. coli* O157:H7 Infections from Fresh Spinach, September 19, 2006a. Available at http://www.cdc.gov/ecoli/2006/september/updates/091906.htm, accessed Sept 28, 2006.
(7) Center for Disease Control and Prevention. Multistate Outbreak of *E. coli* O157 Infections, November−December 2006b. Available at http://www2a.cdc.gov/HAN/ArchiveSys/ViewMsgV.asp?AlertNum=00256, accessed Jan 10, 2007.
(8) FSNET 2007 Jan. 11/07 II. FSAI product recall: florette watercress and florett spinach, watercress & rocket. Available at http://www.fsai.ie/news/press/pr_07/pr20070111.asp, accessed Jan 12, 2007.
(9) Garg, N.; Churey, J. J.; Splittstoesser, D. F. Effect of processing conditions on the microflora of fresh-cut vegetables. *J. Food Prot.* **1990**, *53*, 701–703.
(10) Labuza, T. P.; 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.* **1989**, *13*, 1–69.
(11) Ornellas, C. B. D.; Gonçalves, M. P. J.; Silva, P. R.; Martins, R. T. Atitude do consumidor frente à irradiação de alimentos. *Cienc. Tecnol. Aliment.* **2006**, *26*, 211–213.
(12) Niemira, B. A.; Sommers, C. H.; Fan, X. Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157:H7. *J. Food Prot.* **2002**, *65*, 1388–1393.
(13) Kang, D. H.; Fung, D. Y. C. Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*. *J. Food Prot.* **1999**, *62* (11), 1346–1349.
(14) Brazil Ministério da Saude Agência Nacional de Vigilância Sanitária. RDC 12, de 2 de janeiro de 2001; available at http://e-egis.anvisa.gov.br/leisref/public/showAct.php?id=144&word=, accessed April 12, 2006.
(15) Kornachi, J. L.; Johnson, J. L. *Enterobacteriaceae*, Coliforms, and *Escherichia coli* as quality and safety indicators. In *Compendium of Methods for the Microbiological Examination of*

Foods, 4th ed.; Downes, F. P., Ito, K., Eds.; American Public Health Association: Washington, DC, 2001; pp 69–82.
(16) Andrews, W. H.; Flowers, R. S.; Silliker, J.; Bailey, J. S. Salmonella. In *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed.; Downes, F. P., Ito, K., Eds.; American Public Health Association: Washington, DC, 2001; pp 357–380.
(17) Morton, D. R. Aerobic plate count. In *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed.; Downes, F. P., Ito, K., Eds.; American Public Health Association: Washington, DC, 2001; pp 63–67.
(18) Cousin, M. A.; Jay, J. M.; Vassavada, P. C. Psychrotrophic microorganisms. *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed.; Downes, F. P., Ito, K., Eds.; American Public Health Association: Washington, DC, 2001; pp 159–166.
(19) Hall, P. A.; Ledenbach, L.; Flowers, R. S. Acid-producing microorganisms. In *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed.; Downes, F. P., Ito, K., Eds.; American Public Health Association: Washington, DC, 2001; pp 201–207.
(20) Mead, G. C.; Adams, B. W. A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. *Br. Poult. Sci* **1977**, *18*, 661–667.
(21) Hitchins, A. D. Detection and enumeration of *Listeria monocytogenes* in foods (Chapter 10). In *Bacteriological Analytical Manual Online*, January 2003; available at http://vm.cfsan.fda.gov/~ebam/bam-10.htm, accessed March 21, 2005.
(22) Price, K. R.; Prosser, T.; Richetin, A. M. F.; Rhodes, M. J. C. A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. *Food Chem.* **1999**, *66*, 489–494.
(23) Arabbi, R.; Genovese, M. I.; Lajolo, F. M. Flavonoids in vegetable foods commonly consumed in Brazil and estimated by Brazilian population. *J. Agric. Food Chem.* **2004**, *52*, 1124–1131.
(24) Villanueva, N. D. M.; Petenate, A. J.; Silva, M. A. A. P. Performance of three affective methods and diagnosis of the ANOVA model. *Food Qual. Pref.* **2000**, *11*, 363–370.
(25) Martins, C. G.; Behrens, J. H.; Hutzler, B. W.; Vizeu, D. M.; Franco, B. D. G. M.; Destro, M. T.; Landgraf, M. Gamma irradiation to improve the microbiological safety of minimally processed watercress. Presented at the *19th International ICFMH Symposium Food Micro*, Portoroz, Slovenia, 2004.
(26) Goularte, L.; Martins, C. G.; Morales-Aizpurúa, 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.* **2004**, *71*, 155–159.
(27) Tsuhako, V. P. Irradiação de alface (*Lactuca sativa* L.): aspectos microbiológicos e sensoriais. Dissertação de mestrado, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, 2005; 53 pp.
(28) Koseki, S.; Isobe, S. Effect of ozonated water treatment on microbial control and on browning of Iceberg lettuce (*Lactuca sativa* L.). *J. Food Prot.* **2006**, *69*, 154–160.
(29) Martins, C. G.; Froder, H.; Souza, K. L. O.; Destro, M. T.; Franco, B. D. G. M.; Landgraf, M. Ecologia microbiana de vegetais folhosos minimamente processados. In *Congresso Brasileiro de Microbiologia*; Anais: Florianópolis, Brazil, 2003.
(30) Niemira, B. A. Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on 4 lettuce types. *J. Food Sci.* **2003**, *68*, 2784–2787.
(31) Martins, C. G.; Behrens, J. H.; Destro, M. T.; Franco, B. D. G. M.; Vizeu, D. M.; Hutzler, B.; Landgraf, M. γ Radiation in the reduction of *Salmonella* spp. inoculated on minimally processed watercress (*Nasturtium officinalis*). *Radiat. Phys. Chem.* **2004**, *71*, 87–91.
(32) Dhokane, V. S.; Hajare, S.; Shashidar, R.; Sharma, A.; Bandekar, J. R. Radiation processing to ensure safety of minimally processed carrot (*Daucus carota*) and cucumber (*Cucumis sativus*): optimization of dose for the elimination of *Salmonella typhimurium* and *Listeria monocytogenes*. *J. Food Prot.* **2006**, *69* (2), 444–448.
(33) Bari, M. L.; Nakauma, M.; Todoriki, S.; Juneja, V. K.; Isshiki, K.; Kawamoto, S. Effectiveness of irradiation treatments in inactivating *Listeria monocytogenes* on fresh vegetables at refrigeration temperature. *J. Food Prot.* **2005**, *68*, 318–323.
(34) Niemira, B. A.; Fan, X.; Sokorai, K. J. B.; Sommers, C. H. Ionizing radiation sensitivity of *L. monocytogenenes* ATCC49594 and *L. Innocua* ATCC51742 inoculated on endive (*Chicorium endive*). *J. Food Prot.* **2003**, *66*, 993–998.
(35) Vanamala, J.; Cobb, G.; Turner, N. D.; Lupton, J. R.; Yoo, K. S.; Pike, L. M.; Patil, B. S. Bioactive compounds of grapefruit (*Citrus paradisi* cv. Rio red) respond differently to postharvest irradiation, storage, and freeze drying. *J. Agric. Food Chem.* **2005**, *53*, 3980–3985.
(36) Variyar, P. S.; Limaye, A.; Sharma, A. Radiation-induced enhancement of antioxidant contents of soybean (*Glycine max* Merril). *J. Agric. Food Chem.* **2004**, *52*, 3385–3388.
(37) Breitfellner, F.; Solar, S.; Sontag, G. Effect of gamma irradiation on flavonoids in strawberries. *Eur. Food Res. Technol.* **2002**, *215*, 28–31.

Received for review September 27, 2007. Revised manuscript received December 14, 2007. Accepted December 18, 2007.

JF072873J