Low-Dose Irradiation of Cut Iceberg Lettuce in Modified Atmosphere Packaging

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Irradiation at a mean dosage of 0.19 kGy of commercially prepared fresh-cut lettuce resulted in a product that had, 8 days after irradiation, microbial population of 290 cfu/g and yeast population of 60 cfu/g, compared with values of 220 000 and 1 400 cfu/g, respectively, for the nonirradiated control. Irradiation also caused moderate changes in respiration rate and headspace gas concentrations. It appears feasible to combine chlorination with irradiation at 0.15−0.5 kGy to produce fresh-cut, chopped lettuce with reduced microbial population.

Keywords: *Lettuce; irradiation; minimally processed*

INTRODUCTION

Brackett (1994) found that fresh produce has high levels of microorganisms, typically 10^4-10^6 colony forming units (cfu)/g. The levels found on minimally processed ("fresh-cut") vegetables in 15 studies summarized by Nguyen-the and Carlin (1994) were 10^4-10^8 cfu/g. King *et al.* (1991) reported values of 10^4-10^7 for lettuce. Diets with such high microbial populations are of particular concern for at-risk individuals, especially from concern for *Listeria monocytogenes* and *Pseudomonas aeruginosa*, with the result that salads are sometimes excluded from the diets of some medical patients (Remington and Schimpff, 1981; Bendig and Strangeways, 1989). Such at-risk individuals are sometimes referred to as the YOPI segment of the population: the young, old, pregnant, and immunocompromised (Baird-Parker, 1994).

In present-day commercial processes for preparing fresh-cut lettuce, chlorine is used to control the microbial population in the wash water. King *et al.* (1991) reported the microbial populations found on fresh-cut iceberg lettuce prepared with the use of chlorine water. However, according to Nguyen-the and Carlin (1994) chlorine cannot be relied on to eliminate pathogenic microorganisms such as *L. monocytogenes*.

It has been suggested that microorganisms in fresh-cut vegetables be controlled with hurdle technology, for example, the addition of ionizing irradiation to the present chlorine-based commercial process (Wiley, 1994; Thayer, 1995). Irradiation doses up to 1 kGy for fresh produce are permitted in the United States (FDA, 1995a). Because product located on the interior of an irradiated batch receives lower dosage, the 1 kGy limit means that some of the produce would receive a dosage of no more than about 0.5 kGy (Diehl, 1995). Such a low dosage would not by itself seem sufficient to assure low microbial populations, and therefore, in this study the uses of chlorine and irradiation are combined.

There is, nevertheless, a disadvantage associated with reduction of microorganism populations, this being that lettuce stored in low-oxygen packaging no longer has the protection afforded by a "high level of competing organisms", which is recognized in the Food Code as a measure of protection against growth of *Clostriduim botulinum* (FDA, 1995b). Nevertheless, low-dose irradiation has been used to reduce the microbial populations of endive and to increase shelf life (Langerak, 1978).

The present work is aimed at providing information to help determine what role irradiation, in combination with chlorine and modified-atmosphere packaging, may have in improving the quality of fresh-cut lettuce.

MATERIALS AND METHODS

The iceberg lettuce used in this study was *Lactuca sativa* var. Raleigh harvested in South Bay, FL, and *L. sativa* var. Patriot from California (Table 1). In all cases the lettuce was processed into a fresh-cut product at a large, state-of-the-art processing plant. The process consisted of removing the core and outer leaves, cutting, washing with chlorinated water, centrifuging to remove excess water, and packaging. The chlorinated water used in the process had 0.8−2.0 ppm of free chlorine, and the pH was maintained at 6.8−7.2. The lettuce pieces, classified by the industry as a $^3/_8$ in. cut, had a mean piece weight of 0.4 g. The lettuce was slightly compressed as it was packed into bags of either 1 or 5 lb net weight, and these were sealed. The sealed bags were packed into 20 lb net weight cartons (Table 1) and stored at 2 ± 2 °C. The lettuce was treated with ^{60}Co γ irradiation at Food Technology Service, Inc. (Mulberry, FL) within 2 days after packaging. Irradiation dosage was measured with 0.1−3.3 kGy dosimeters (Harwell Laboratory, Oxfordshire, U.K.). The storage times reported herein are reckoned from the time of irradiation.

The packaging film for trials 1 and 2 was a 44 μm thick polyolefin laminate with rated O_2 and CO_2 permeabilities of 3800 and 13000 mL m^{-2} day^{-1} atm^{-1} at 23 °C (Cryovac Division, Duncan, SC). The film for trial 3 was 51 μm thick extruded polyethylene (Para Packaging, Sunrise, FL). Temperature-abuse samples were stored for the indicated time in a room maintained at 22 °C, 65% relative humidity, either in the original sealed bags or transferred to sterile polyethylene bags of 65 μm thickness, and the open ends were folded and clamped to retard dehydration.

Bags were submerged in water to detect leaks and withdraw headspace gas samples. In case of a leak, the sample was discarded and not included in the study. The headspace gas samples were withdrawn with a syringe through a septum previously applied to the bag. The syringe was twice filled and flushed with the headspace gas to purge it of residual oxygen. The septum consisted of a 3 mm thick layer of clear Permatex RTV silicone (Loctite Corp., Cleveland, OH), adhesive aluminum foil, and tape (Scotch Patch and Repair tape, 3M, St. Paul, MN). Within 10 min after withdrawal of the

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Table 1. Irradiation Doses and Packaging

trial no.	irradiation dose[a] (kGy) min−max	irradiation dose[a] (kGy) min−max	net wt (lb)	vol[b] (cm^3)	area[c] (cm^2)	film thickness[d] (μm)	O_2 permeance[e] (mL m^{-2} day^{-1} atm^{-1})
1		0.68−0.94	5	8400	2660	44 ± 0.8	3600 ± 60
2	0.15−0.20	0.42−0.55					
3	0.16−0.22	0.41−0.50	1	1790	1390	52 ± 0.4	1900 ± 60

[a] Each trial consisted of control (not irradiated) and one or two different levels of irradiation, with the minimum kGy reading recorded at the center of the sample and the maximum at the periphery. [b] Volume of the filled package, mean for three bags. [c] Area of the film that comprised the package, mean for three packages. [d] Packaging film thickness, mean for four bags. [e] Packaging film permeance measured at 23 °C, mean of six measurements.

headspace sample, its O_2 and CO_2 concentrations were measured with a Hewlett-Packard 3890 gas chromatograph fitted with a CTR-1 column (6 ft long, $^1/_4$ and $^1/_8$ in. diameter, outer and inner columns, respectively; Alltech, Deerfield, IL). Samples were applied with a loop injector (100 mL loop). Column flow rate was 70 mL/min at 40 °C column temperature, and the thermal conductivity detector was at 120 °C.

Respiration rate at 3 ± 2 °C was determined in flow-through cells. The lettuce (200 g) was removed from the bags and placed in 1 L jars (four pooled samples per treatment), through which air was passed at 35 mL/min. After 24 h of equilibrium, the CO_2 concentration of the outlet (generally about 0.1%) was determined with a Hewlett-Packard 3890 gas chromatograph with a GSQ column (30 m × 0.53 mm i.d.; J&W Scientific, Folsom, CA). Samples were applied with a loop injector (250 μL). Column flow rate was 10 mL/min, column and thermal conductivity detector were at 40 and 120 °C, respectively. In developing the method, we measured the CO_2 concentrations after 1, 2, or 3 days, and it was determined that equilibrium had been reached after 24 h, which was sufficient time for CO_2 absorbed in the lettuce to have diffused out of the system.

Ethylene content of headspace gas was determined with the gas chromatograph using the GSQ column (at 55 °C) and flame ionization detector (at 250 °C). Injection was with split mode (20:1 split ratio), 250 °C injector temperature. Two bags per treatment were analyzed in duplicate.

Ethanol content was determined with the gas chromatograph, a FFAP column (Hewlett-Packard, Avondale, PA), and flame ionization detector. Column flow was 4 mL/min. Column temperature was 55 °C for injection and then increased at 3 °C/min. Two pooled samples per treatment were analyzed in duplicate. Each sample was blended with an equal weight of water with 1000 ppm of 1-propanol as an internal standard.

For determination of microbial populations a 100 g sample was mixed with an equal weight of buffered water and agitated for 90 s in a paddle blender (the Masticator, IUL, S.A., Barcelona, Spain). Three appropriate dilutions of the liquid were pipetted from the blended samples to determine the colony forming units per gram of lettuce. Plate count agar (Difco Laboratories, Detroit, MI), incubated at 35 °C for 2 days, was used to determine total mesophilic microorganisms. Potato dextrose agar (Difco), incubated at 25 °C for 3 days, was used to determine yeasts and molds. For trials 1 and 2, each sample consisted of two samples from each of two 5 lb bags. For trial 3, each sample is from a separate 1 lb bag. The colonies grown on five Petri dishes of plate-count agar (average of 500 colonies per treatment) were visually separated into morphological type and five of each type Gram-stained.

Oxygen permeability of the packaging material was determined at 30 °C, 70% relative humidity, with an Ox-Tran 100 (Modern Controls, Inc., Minneapolis, MN) that had been calibrated with Polyester Film No. 1470 from the National Bureau of Standards and also an industry standard with permeance of 2300 mL/(m^2 day).

Texture was determined with the Instron Model 1011 (Canton, MA), using a Kramer shear cell containing 35 g of sample. A 5000 N transducer was used, set at a load range of 2000 N. Crosshead speed was 50 mm/min. Six pooled samples were taken for each treatment.

Separate samples from five bags were taken for analysis of headspace gases and microorganisms. Statistical calculations were performed with Statistix software (Analytical Software, Tallahassee, FL). The error bars in figures show the pooled standard error, except where this is smaller than the symbol.

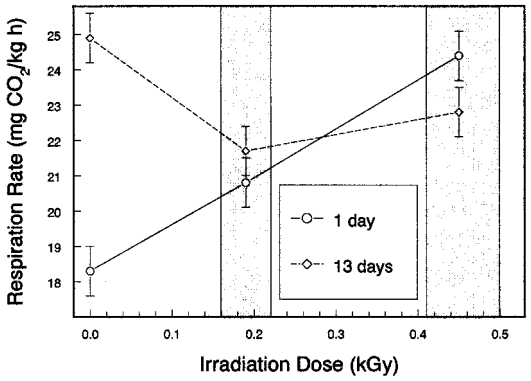

Figure 1. Respiration rate of lettuce in air at 3 °C after storage in sealed bags at 2 °C (trial 3). The width of the shaded band represents the minimum−maximum range of irradiation dosage. The error bars show the standard error, except where this is smaller than the symbol.

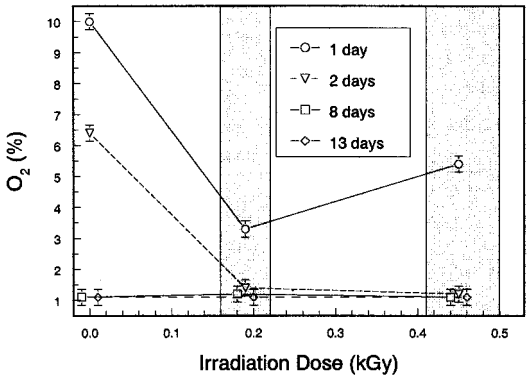

Figure 2. O_2 content of package headspace after different storage times at 2 °C in sealed bags (trial 3).

RESULTS AND DISCUSSION

Respiration Rate and Headspace Gases. The respiration rate for irradiated lettuce (Table 1) was about 33% higher than that for the control samples 1 day after irradiation, virtually the same after 8 days at 2 °C, and slightly lower after 13 days at 2 °C. In an earlier trial, the respiration was 36% higher 2 days after the lettuce had been irradiated at a dosage of 0.81 kGy.

The same conclusions might be arrived at from the values for composition of headspace gases (Figures 2 and 3). Headspace O_2 was markedly lower and CO_2 markedly higher than control 1−2 days after irradiation (Figures 2 and 3). However, 8 or 14 days after irradiation, headspace gases were virtually the same for control and irradiated samples (Figure 2). The lettuce bags were sealed without gas flushing with modified atmosphere gases, and therefore the composition of the headspace gases would have been 21% O_2 and approximately 0.04% CO_2 at the time of packaging. If we

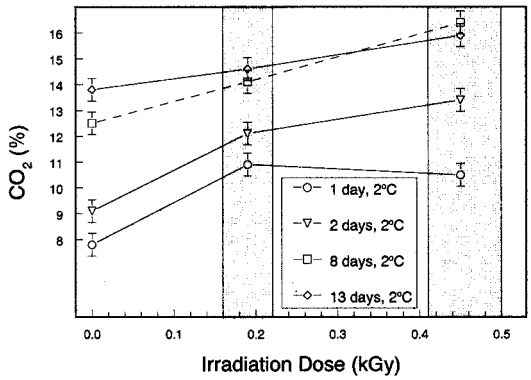

Figure 3. CO_2 content of package headspace after different storage times at 2 °C in sealed bags (trial 3).

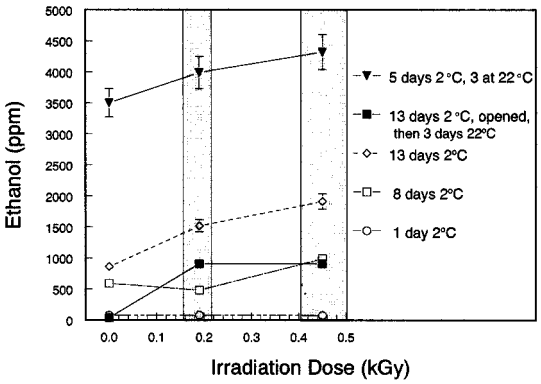

Figure 4. Ethanol content of cut lettuce after various storage conditions (trial 3).

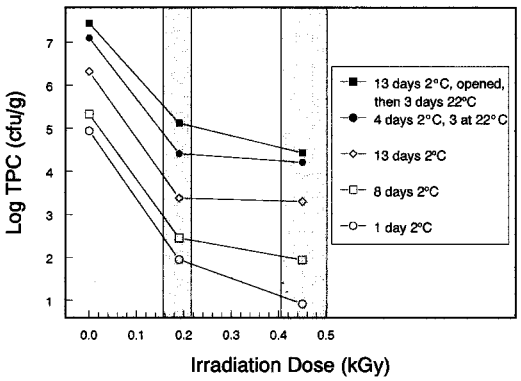

Figure 5. Microbial populations (TPC) of lettuce stored under various conditions.

make the normal assumption that subsequent lowering of O_2 concentration and increase in CO_2 is caused by respiration, the faster initial changes in these gases for irradiated samples indicate temporarily higher respiration rates for the irradiated samples. However, the fact that the relatively low O_2 and high CO_2 of the irradiated samples did not persist (Figures 2 and 3) suggests the high respiration rate was only temporary. Further, headspace CO_2 was virtually the same for irradiated and control, and all samples stored at 2 °C had <20% CO_2, which is the value considered to be the maximum tolerated (McDonald *et al.*, 1990), all of which suggest that the packaging film used was equally suitable for both irradiated and control.

The headspace gas of lettuce stored for 4 days at 2 °C and then for 5 days at 22 °C had about 26% CO_2 and 1.0% O_2 at irradiation levels of 0, 0.19, and 0.45 kGy (data not shown). These CO_2 contents were markedly higher, but the O_2 levels were virtually the same as the headspace gases of lettuce stored at 2 °C. Similar results were observed for all three experiments. In general, the headspace O_2 did not fall below 1% at 22 °C or below 1.1% at 2 °C. These oxygen concentrations are not high enough to prevent production of *C. botulinum* toxin (Nguyen-the and Carlin, 1994).

The amount of headspace O_2 at the time of irradiation (Figure 2) is of some importance because the effectiveness of irradiation in killing microorganisms has been reported to be less at low oxygen levels (Epp *et al.*, 1968).

Ethanol. Ethanol content increased with storage time for all samples in sealed bags, whether or not irradiated. Ethanol tended to be higher for samples that had been irradiated, although the increase due to irradiation was much smaller than that caused by storage at higher temperature. The ethanol content decreased after the bags were opened, presumably because of oxidation (Figure 4).

Microorganisms. Compared to the control, fewer microorganisms were found in irradiated samples (Figures 5 and 6; Table 2). Generally, the higher the dosage, the lower the microbial population, although increasing the dose above 0.2 kGy had decreasing benefit. The same was true of the yeast population. Microorganism populations at all storage conditions increased markedly with storage time and temperature, although no attempt was made to quantify the growth rates (Figure 6).

Irradiation at 0.2 kGy combined with a chlorine wash resulted in fresh-cut lettuce that had, at its 10-day expiration date (8 days after irradiation) a mean microbial population of only 290 cfu/g and a yeast population of 60 cfu/g, compared to values of 220 000 and 1 400 cfu/g, respectively, for nonirradiated control (Table 2). Somewhat longer storage life than 10 days is claimed by other producers, and therefore the microbial populations observed after 13 days are also of some importance. Lettuce irradiated at this same dose had, 13 days after irradiation, a mean microbial population of 2 400 cfu/g and yeast population of 180 cfu/g (Table 2). Considerably lower populations were achieved with dosage of 0.41−0.50 kGy (Table 2). Nevertheless, when considering the benefits of irradiation, we would be prudent to consider only the microbial reductions achieved at the lower dosage of 0.16−0.22 kGy because dose (D) uniformity ratios (D_{max}/D_{min}) of 2−3 are considered acceptable in commercial irradiation situations (Diehl, 1995). Thus, even if a maximum dosage of 0.5 kGy is received by some bags of lettuce, others could receive a dosage of as little as one-third of that amount.

The microflora grown on plate-count agar consisted of about 96% bacteria with about 4% yeasts (Table 2). Of the bacteria, 96−97% were Gram-negative rods (Table 2). Similar proportions of Gram-negative bacteria were found on iceberg lettuce by King *et al.* (1991) and on other fresh-cut produce by Garg *et al.* (1990). The colonies grown from irradiated lettuce were morphologically similar to those from control, and no changes were apparent at different storage time, although Mossel *et al.* (1995) proposed that because Gram-negative rods are more sensitive to irradiation, other forms of bacteria are expected to predominate after irradiation.

Texture. Lettuce irradiated at 0.81 kGy (trial 1) required a mean force of 1236 N to force it through the shear cell, compared to 1311 N for control, measured 2−10 days after irradiation (19 trials, SE = 14 N, data not shown). This difference in texture was readily apparent visually; the irradiated lettuce tended to settle

Table 2. Microbiological Populations of Lettuce Stored at 2 °C for 8 and 13 Days after Irradiation[a]

dose (kGy)	time (days)	microbial population[b] (log[cfu/g]) mean	microbial population[b] (log[cfu/g]) range	yeast population[c] (log[cfu/g]) mean	yeast population[c] (log[cfu/g]) range	coliforms (MPN)	colony identification[d] Gram-negative rods (%)	colony identification[d] yeasts (%)
0.00	8	5.3	5.2−5.5	3.1	3.0−3.2	0.9	95	2
0.00	13	6.3	6.2−6.4	4.9	4.8−4.9	0.7	92	5
0.16−0.22	8	2.5	2.4−2.5	1.8	1.6−1.8	0.1	86	11
0.16−0.22	13	3.4	3.3−3.5	2.3	2.2−2.3	<0.02	92	5
0.41−0.50	8	2.0	1.8−2.2	1.2	1.0−1.4	<0.02	88	9
0.41−0.50	13	3.3	3.2−3.5	1.8	1.7−1.9	<0.02	93	3

[a] Results for five bags per treatment. [b] SE was 0.05. [c] Yeast population from potato dextrose agar. SE was 0.04. [d] From examination of colonies on plate-count agar. The remaining 3−4% consisted of Gram-negative cocci, Gram-positive rods, and cocci.

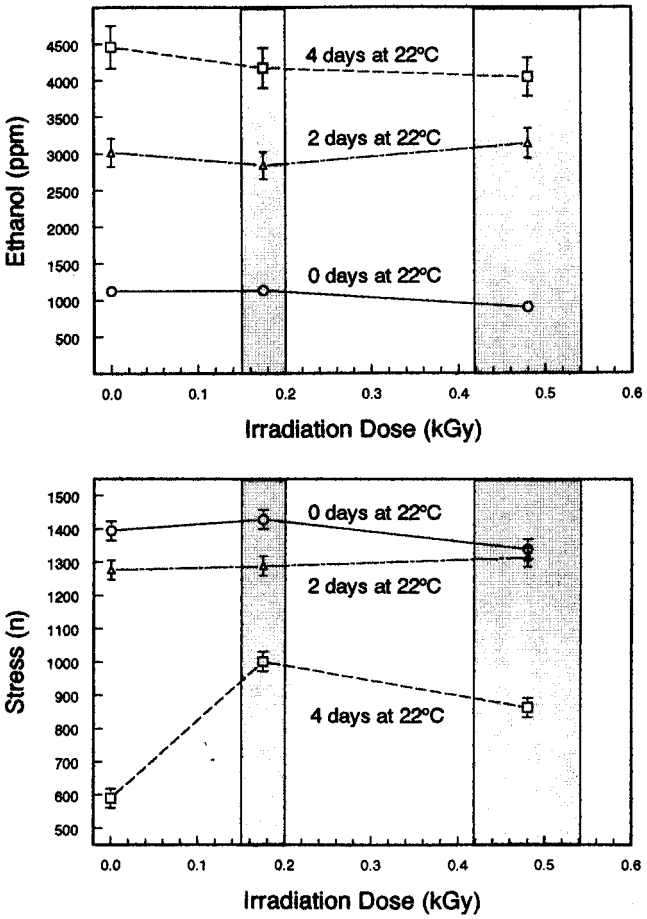

Figure 6. Ethanol (top) and stress (bottom) in Kramer cell of lettuce stored at 22 °C in sealed bags after prior storage for 5 days at 2 °C (trial 2).

in the bag. For trials 2 and 3, when the dosage was reduced to 0.2 and 0.5 kGy, the shear force was virtually the same for irradiated and control, and no difference in texture was apparent (Figure 6). After 0 and 2 days of storage at 22 °C there was no significant difference in texture at the different levels of irradiation (Tukey test, $\alpha = 0.05$).

Spoilage. Lettuce stored at 22 °C in sealed bags became inedible by its appearance after a few days at that temperature. This was evident from the putrid odor, swelling of the bag, appearance and texture of the lettuce, and the presence of free liquid that had exuded from the lettuce. After 4 days at 22 °C, the texture had decreased considerably and the ethanol content increased (Figure 6, data for trial 2). As it had for trial 3 (Figure 5), the microbial population increased rapidly for the samples stored at 22 °C. For trial 2, nonirradiated lettuce had populations of 1×10^5, 9×10^7, and 6×10^8 cfu/g after 0, 2, and 4 days at 22 °C, respectively, and lettuce irradiated at a mean dose of 0.48 kGy had populations of 1.5×10^2, 8×10^5, and 5×10^7 cfu/g after 0, 2, and 4 days at 22 °C, respectively (data not shown). By all measures used (texture, ethanol and microbial population, and general appearance) the irradiated samples did spoil at roughly the same rate as did the control samples, although the data are not adequate to define spoilage rates.

As mentioned, normal microbial spoilage affords some measure of protection against growth of *C. botulinum* (FDA, 1995b; Petran *et al.*, 1995).

Ethylene. Ethylene content of the headspace gas inside the bags was 0.6−1.8 ppm for irradiated (0.81 kGy) and control samples stored for 2−19 days at 2 °C or for up to 4 days at elevated temperature (12 or 22 °C) (data not shown in tabular form). The amount of ethylene in the headspace gas was virtually the same for irradiated and control samples and did seem high enough to be a spoilage factor.

Conclusion. The present study demonstrates that a workable dosage range for irradiating fresh-cut lettuce is 0.15−0.5 kGy. This dosage, combined with chlorination, gives a product with about 300 cfu/g microbial population and 60 cfu/g yeast count, measured at the expiration date, 10 days after packaging. Such a product would seem of value as food for those individuals most sensitive to microflora. Low-dose irradiation might also be considered for lettuce-containing salads that contain egg, poultry, meat, or other ingredients that may pose a microbiological risk.

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