

Effects of Low-dose Gamma Irradiation on the Shelf Life and Quality Characteristics of Cut Romaine Lettuce Packaged under Modified Atmosphere

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ABSTRACT: Cut romaine lettuce, packaged under modified atmosphere, was subjected to 0.15 and 0.35 kGy gamma irradiation. Irradiation at 0.35 kGy decreased aerobic plate counts by 1.5 logs and yeast and mold counts by 1 log; these differences were maintained through the 22-d storage. Irradiation at 0.15 kGy caused smaller reductions in microbial counts. A decrease in headspace CO₂ was observed in the 0.35-kGy-treated lettuce, although CO₂ concentrations increased in all samples with storage. O₂ concentration was not affected by irradiation. Ten percent loss in firmness was observed at 0.35 kGy, while other sensory attributes such as color, generation of off-flavor, and appearance of visual defects were not affected.

Key Words: romaine lettuce, irradiation, microbial counts, texture, color, sensory

Introduction

ROMAINE LETTUCE (*LATUCA SATIVA* VAR. *LONGIFOLIA*) IS A POPULAR salad crop in the United States. The increasing demand for ready-to-eat foods has prompted the sale of pre-cut romaine lettuce, often marketed as Caesar salad kits in retail markets and in larger quantities for food service institutions. Since the processor does all the preparation, such as trimming, cutting, and washing, the product saves labor and time for the purchasing institutions. It also reduces shipping costs since waste material is removed prior to shipment (Bolin and others 1977). However, cutting lettuce decreases its shelf life significantly compared to whole head lettuce. Elevated respiration and transpiration rates due to wounded and senescing leaf tissue, water loss, and decay caused by microorganisms contribute to the physiological processes that hasten spoilage (King and Bolin 1989). Since the lettuce is not subjected to any lethal processing, microbial counts are frequently in the range of 10⁴ to 10⁷ CFU/g (King and others 1991).

Washing with chlorinated water, refrigerated storage, and modified atmosphere packaging (MAP) are the strategies utilized to prolong shelf life and inhibit growth of microorganisms in minimally processed (MP) vegetables (Wiley 1994). Low temperature storage is highly effective in decreasing enzyme activity and microbial proliferation. Thus, it is probably the most important factor in preservation of cut lettuce (Bolin and others 1977). Still, refrigeration is not a good defense against pathogenic psychrotrophs such as *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica*, which have the potential to cause foodborne illness (Sumner and Peters 1997). Typically, MP vegetables are cleaned with water and/or chemical sanitizers to reduce surface microbial counts. Chlorine is a popular sanitizer, although its effectiveness is limited by its inability to reach into tissue crevices and its rapid inactivation from contact with organic matter in the wash water (Beuchat 1992). Modified atmosphere packaging has been used to preserve the sensory quality and to limit microbial growth. However, the hazard from certain microorganisms, such as *Listeria monocytogenes* and *Aeromonas hydrophila*, may be increased (Berrang and others 1989a, 1989b). In MAP, the most common way to modify the atmosphere is to increase CO₂ levels and decrease O₂ levels. Microbial growth can be controlled within limits. However, the

major goal of MAP is to decrease the rate of respiration and retard ripening, hence providing higher quality for a longer period of time.

Irradiation is a well-established process with clearly documented safety and efficacy. Its efficacy stems from the fact its activity is not limited to the surface, it can penetrate the product and eliminate microorganisms that are present in crevices and creases (significant for vegetables like lettuce). Langerak and Damen (1978) extended the shelf life of prepacked soup-greens stored at 10°C by treatment with 1 kGy. The shelf life of the nonirradiated greens was 1 d; irradiation at 1 kGy reduced the counts of microorganisms (total viable counts, *Enterobacteriaceae*, and yeasts) to the extent that shelf life was improved to 6 d. Howard and others (1995) examined the effect of irradiation at 1 kGy on the microbiological, sensory, and chemical quality of *pico de gallo*. They found that color, flavor, texture, odor, and sensory attributes were not affected by radiation treatment. However, aerobic mesophilic, heterofermentative, and total lactic microflora were substantially decreased. Hagenmaier and Baker (1997) reported that irradiation (at a mean dosage of 0.19 kGy) of commercially prepared fresh-cut iceberg lettuce resulted in a product that after 8 d had a microbial population of 2.9 × 10² CFU/g and yeast population of 60 CFU/g, compared with 2.2 × 10⁵ and 1.4 × 10³ CFU/g, respectively, for the nonirradiated control. They determined that a dosage of 0.15 to 0.5 kGy in combination with chlorination can significantly reduce microbial levels.

Several studies have shown that irradiation can cause changes in pectic and cellulosic substances in plant tissues resulting in softening (Howard and Buescher 1989; Kertesz and others 1964; McArdle and Nehemias 1956; Somogyi and Romani 1964; Yu and others 1996) and discoloration of soft tissues (Bramlage and Lipton 1965). Damage to cellular membranes can lead to loss of turgor (Faust and others 1967), exacerbating the loss of firmness. However, little information is available specifically for cut-romaine lettuce. We hypothesize that low levels of irradiation (< 0.5 kGy) can achieve significant reductions in microbial counts of romaine lettuce with negligible loss of firmness. Thus, the objectives of this study were to determine the effect of low-dose irradiation on the microbial counts and sensory attributes, such as texture, color, and flavor of commercially packaged cut-romaine lettuce.

Results and Discussion

Microbiology

The change in populations of aerobic microorganisms on cut lettuce stored for 22 d at 4°C are shown in Fig. 1. In both trials, the initial load of the control sample was between 10^5 and 10^6 . At the "best-if-used-by" date (14 d after packaging), the control samples had counts of 3.5×10^7 and exceeded 10^8 by day 18. The effect of 0.35 kGy of irradiation was to decrease the microbial load by about 1.5 logs; this difference was maintained throughout the study. The 0.15 kGy treated sample displayed a less dramatic effect. Although similar results were obtained in both trials, data from the 2 trials were not combined due to the differences in dose levels.

Yeast and mold populations are shown in Fig. 2. Yeast colonies dominated this population and counts in the control samples started at 5×10^3 CFU/g and rose almost 3 logs during the 22 d study. Even after extended incubations, molds were infrequently observed at any timepoint. The effect of irradiation on this population was similar to that observed for the total aerobic population. The 0.35-kGy dose initially decreased this population by slightly more than 1 log. Although the population did increase during storage, the counts remained lower than the control at each time point. As might be predicted, the 0.15 kGy did not affect the population dramatically. Less than a 1-log reduction was initially observed, but this small difference

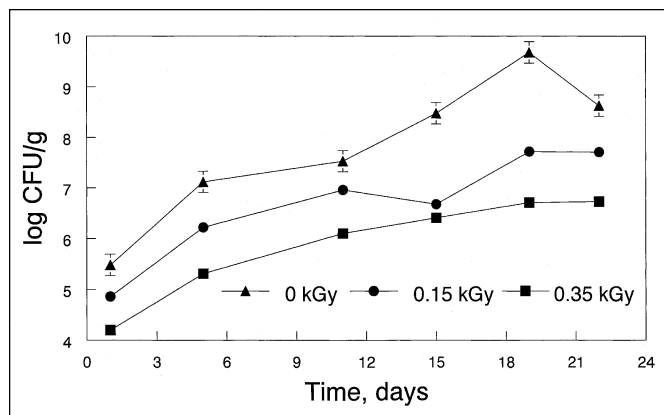


Fig. 1—Change in mean aerobic plate counts for cut romaine lettuce following irradiation at 0.15 and 0.35 kGy and storage at 4°C. Error bars, calculated from pooled variances across time points, indicate standard error of the mean.

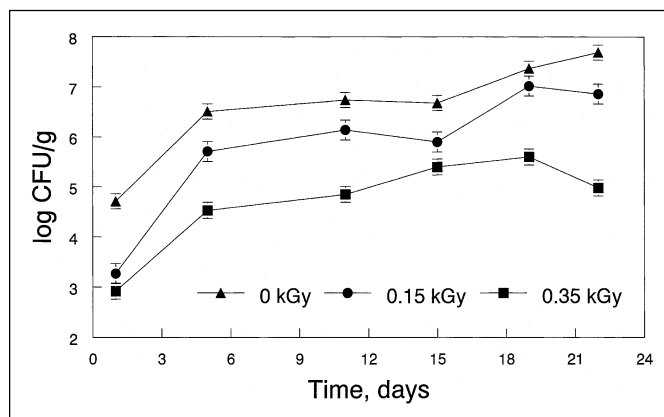


Fig. 2—Change in mean yeast and mold counts for cut romaine lettuce following irradiation at 0.15 and 0.35 kGy and storage at 4°C. Error bars, calculated from pooled variances across time points, indicate standard error of the mean.

was also maintained over time.

Hagenmaier and Baker (1997) found that refrigerated cut iceberg lettuce that had been irradiated at 0.2 kGy after a chlorine wash and MAP had only 3.2×10^2 CFU/g 8 d after irradiation. At the same time, the control had a count of 1.99×10^5 CFU/g. Thus, the authors observe almost a 3-log difference in the aerobic microbial load of irradiated in contrast to control sample compared to the 1.5-log difference observed in this study at a higher dose level. Although the reasons for the difference are unclear, several factors could contribute, such as type of lettuce and headspace gas concentrations.

The microbial counts of the nonirradiated romaine lettuce are within the range reported for ready-to-eat salads in other studies. Hagenmaier and Baker (1998) obtained values ranging from 2×10^5 to 7.6×10^8 CFU/g on the day of purchase. On the expiration date (generally 14 d after packing), the values ranged from 1.4×10^7 to 6.5×10^8 CFU/g. Garcia-Gimeno and Zurera-Cosano (1997) observed initial psychrotrophic counts of 1.07×10^5 CFU/g in mixed vegetable packaged salad, which rose to 1.48×10^7 in 8.5 d. In a survey of commercially available Caesar salad kits, Lopez-Galvez and others (1997) found average initial counts of 7.9×10^5 CFU/g with counts of 1.3×10^8 CFU/g after 20 d. Although there is no legislation in the United States for microbial counts of bagged salad, French legislation specifies a maximum of 5×10^5 CFU/g at production and 5×10^7 at the use-by date (Nguyen-the and Carlin 1994). Using those criteria, the irradiated romaine lettuce was acceptable for the length of the study (22 d), while the control exceeded 5×10^7 CFU/g between days 14 and 18.

Headspace Gases

In all samples, CO_2 levels gradually increased from initial concentrations of 11% to 25% on day 21 (Fig. 3), and O_2 levels decreased from 0.3% initially to 0.21% on day 21 (Fig. 4). After the 1st testing period, the 0.35 kGy samples had significantly lower CO_2 levels compared to the control and the 0.15 kGy samples for the remainder of the study. Hagenmaier and Baker (1997) reported that the respiration rate for irradiated cut iceberg lettuce was 33% higher than the control 1 d after treatment, the same after 8 d, and slightly lower after 13 d. Although the respiration rate was not measured, the lower CO_2 levels for the 0.35 kGy lettuce after day 1 indicates a lower respiration rate relative to the control and 0.15 kGy samples. For the most part, O_2 levels were similar for all samples, except on days 1 and 8, in which the 0.35 kGy samples were significantly different. The spike in oxygen values on day 8 accompanied by a large standard error

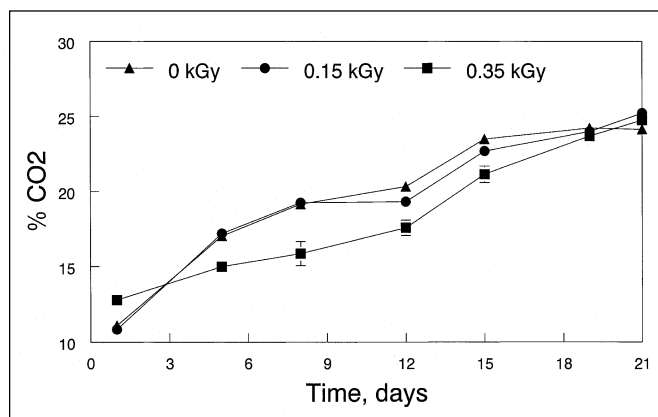


Fig. 3—Headspace CO_2 concentration in packages of cut romaine lettuce after irradiation at 0.15 and 0.35 kGy. Error bars indicate standard error of the mean.

most likely reflects the presence of an atypical bag in that sample set.

Hamza and others (1996) observed that CO₂ levels up to 10% and O₂ levels decreased to 1% reduced browning and improved the visual quality of minimally processed romaine lettuce. However, increasing CO₂ levels to 15% enhanced brown lesions and decreasing O₂ levels to 0.5% intensified browning and development of anaerobiosis. Naturally developed modified atmosphere (after vacuum sealing) in cut lettuce packages with CO₂ levels less than 20% and O₂ levels of 1% to 4% induced little discoloration or fermentation (McDonald and Risse 1990). In this study, the suboptimal CO₂ levels (>20%) and O₂ levels (<0.5%) probably accelerated the discoloration and formation of off-odors.

Texture

The firmness of all samples decreased with time, and the rate of change was similar for all treatments (Fig. 5). Over the course of 21 d, lettuce firmness decreased by 40%. Change in texture of lettuce is usually associated with loss of moisture, which decreases turgidity in the cells. It becomes apparent as lettuce leaves become limp and lose their crispness. According to Ryall and Lipton (1972), more than 5% water loss can cause texture breakdown in lettuce. In addition, high CO₂ levels can enhance tissue softening (Hamza and others 1996).

The 0.35-kGy irradiated lettuce was significantly less firm (approximately 10%) relative to the control throughout the study except day 18. Yet the sensory panelists detected differ-

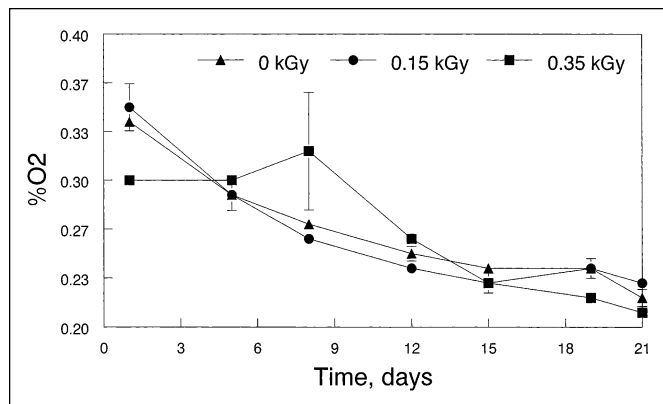


Fig. 4—Headspace O₂ concentration in packages of cut romaine lettuce after irradiation at 0.15 and 0.35 kGy. Error bars indicate standard error of the mean.

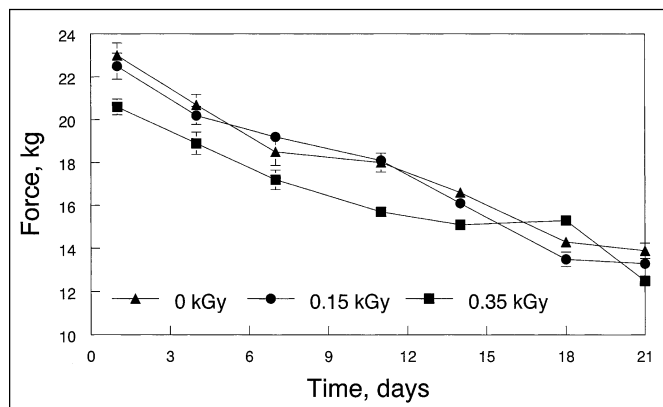


Fig. 5—Change in texture of cut romaine lettuce as measured by Kramer Shear Force following irradiation and storage at 4°C. Error bars indicate standard error of the mean.

Table 1—Change in sensory attributes (color, texture, and off-flavor) of cut-romaine lettuce measured by a trained sensory panel using unstructured, anchored 15 cm scales. Data shown are the means from 6 panelists ± standard deviation.

Color Day	Control	0.15 kGy	0.35 kGy
1	11.5 ± 0.48	11.60 ± 0.47	11.60 ± 0.37
4	11.57 ± 0.54	10.82 ± 0.92	10.68 ± 1.09
7	11.3 ± 1.65	10.63 ± 1.68	10.81 ± 1.52
11	11.56 ± 0.39	10.96 ± 0.82	10.22 ± 1.12
14	11.15 ± 0.45	10.37 ± 1.01	11.00 ± 0.18
18	10.16 ± 1.84	10.64 ± 0.45	10.20 ± 0.70
21	11.22 ± 0.76	9.06 ± 2.23	9.98 ± 0.52

Texture Day	Control	0.15 kGy	0.35 kGy
1	8.2 ± 1.05	7.78 ± 0.58	8.68 ± 1.38
4	7.41 ± 0.31	7.65 ± 0.65	7.32 ± 0.59
7	7.0 ± 1.26	7.43 ± 1.30	6.88 ± 1.16
11	6.70 ± 0.61	6.86 ± 0.61	6.82 ± 0.37
14	6.30 ± 0.69 ^a	5.58 ± 1.14 ^a	4.68 ± 1.36 ^b
18	3.66 ± 1.55 ^b	5.30 ± 1.48 ^a	5.85 ± 1.01 ^a
21	5.33 ± 1.55	5.33 ± 1.74	5.21 ± 1.51

Off-flavor Day	Control	0.15 kGy	0.35 kGy
1	0.52 ± 0.66	0.88 ± 0.76	0.58 ± 0.79
4	0.55 ± 0.32	0.62 ± 0.22	0.65 ± 0.33
7	1.3 ± 1.10	1.0 ± 0.67	1.41 ± 1.01
11	0.93 ± 1.05	3.88 ± 3.26	3.83 ± 2.40
14	2.70 ± 1.19	3.70 ± 0.29	5.65 ± 1.40
18	11.20 ± 1.11 ^a	8.54 ± 2.28 ^b	7.68 ± 1.06 ^b

^{a,b}Different letters within a row are significantly different (P < 0.05).

ences among samples only on days 14 and 18. Irradiation at 0.15 kGy did not cause a significant change in lettuce firmness except on days 18 and 21. Textural changes induced by irradiation have been associated with changes in pectic substances as well as other cell components, such as cellulose, hemicellulose, and pectin enzymes (Yu and others 1996). Hydrolysis of polygalactouronides and cellulose into low molecular weight fractions (Maxie and Abdel-Kader 1966) and loss of turgor due to damage to cellular membranes (Faust and others 1967) contribute to softening.

Color

There was no difference in the color of the lettuce samples due to irradiation (data not shown). The change in total color with time was mostly due to an increase in “a” values, indicating a loss of green, with small increases in “b” values (increasing yellow) as well as increased darkening (decreasing “L”). Chlorophyll breakdown would increase “a” values (Bolin and Huxsoll 1991), as would an increase in red discoloration due to phenolic oxidation (King and Bolin 1989). Phenolic oxidation and bacterial spoilage can cause darkening of the leaves over time (King and Bolin 1989).

Sensory evaluation

As storage time increased, panelists gave lower scores for color (less green) and texture (less firm) and higher scores for off-flavor for all samples (Table 1). The data indicate that the panelists were unable to distinguish between the samples in terms of color. This result is in agreement with the instrumental evaluation of color, in which there were no differences due to treatment. Although, instrumental texture analysis showed the 0.35-kGy sample to be significantly less firm than the other samples throughout the study, the panelists perceived differences in texture only on days 18 and 21. The 0.35 kGy sample was perceived to be less firm on day 18. However, on day 21, the control

was considerably less firm. Until day 11, off-flavor was ranked low by the panelists but increased rapidly thereafter. Peiser and others (1997) observed a rapid increase in acetaldehyde and ethanol concentration between days 10 and 16 in bags of ready-to-eat salad. According to Wiley (1994), at low oxygen concentrations, anaerobic metabolism is responsible for the production of CO₂, ethanol, aldehydes, and other chemical compounds that produce off-flavor, off-odors, and discoloration. Lopez-Galvez and others (1997) compared the quality changes in several commercially available packaged salads, including Caesar salad. They report that development of off-odor starts at day 10 and is a common problem in commercial salad products.

It was noted that leaf surface browning (LSB), leaf edge browning (LEB), and pinking were the main defects that contributed to the decrease in visual quality. Russett spotting was minimal. Therefore, in the 2nd trial, 2 to 3 panelists evaluated visual quality based on a scoring system reported by Lopez-Galvez and others (1996). Although the number of panelists does not allow for statistical analysis of the data, these observations may have practical value. The occurrence of LSB and LEB were similar in all samples, with a rapid increase in occurrence after day 12. Pinking, a quality loss in lettuce caused by the action of polyphenol oxidase enzyme, was fairly limited; however,

it was noted that lettuce, in bags which had been opened for a few days, rapidly developed pinking. High CO₂ levels have been shown to inhibit the production of phenolic compounds (Mateos and others 1993), thus protecting against pinking. Overall visual quality (OVQ) was constant until day 5, after which there was a gradual decline. There was no consistent effect of irradiation treatment on any of the defects for the duration of the study. On day 22, the OVQ was the same for all samples. The low levels of irradiation as used in this study did not exaggerate the visual defects.

Conclusion

LOW-DOSE IRRADIATION INCREASES THE MICROBIOLOGICAL shelf life of MAP fresh cut romaine lettuce. 0.35 kGy irradiation causes softening of the lettuce although the change in texture was not apparent to a trained sensory panel. Other sensory attributes, such as visual quality and off flavor development, are not adversely affected. The decrease in microbial counts could offer protection against pathogens of human significance, although this assumption needs to be verified by inoculation studies. In addition, studies are needed to determine the optimum gas composition and irradiation levels to optimize microbiological and sensory shelf life.

Materials and Methods

Sample preparation

10-oz (280-g) bags of cut romaine lettuce were obtained from a local fresh produce packinghouse and transported to the laboratory in coolers. The lettuce had been trimmed, cored, cut, washed in chlorinated water (80 to 100 ppm NaOCl), spun-dried, and packaged in laminated polyethylene bags with oxygen permeability of 1550 to 2015 mL m⁻² d⁻¹ atm⁻¹. During packaging, the bags were flushed with nitrogen gas and then sealed to give an initial concentration of approximately 1.5% O₂ and 4% CO₂.

Irradiation treatment

Lettuce packages (contained in coolers) were exposed to gamma radiation from a Co⁶⁰ source at Sterigenics Inc., a contract irradiation facility (Tustin, Calif., U.S.A.). The samples were placed in coolers containing ice packs at a precise location from the source to receive irradiation at a dose rate of 1 kGy/hr. The dose received was confirmed by gamma chrome dosimeters (Harwell Dosimeters Ltd., Oxfordshire, U.K.) placed in the front and back of the coolers. Midway through the process, the coolers were rotated 180° to ensure that a uniform dose was delivered. The measured levels in the 1st trial were 0.15 kGy front and back, and 0.34 and 0.36 kGy front and back, respectively. For the 2nd trial, the dose levels achieved were 0.09 kGy both front and back and 0.26 and 0.28 kGy front and back. The temperature of the lettuce prior to irradiation was measured at 5 °C and after treatment was measured at 7°C.

The lettuce was stored in a refrigerator at 4°C for the length of the study. The 3 sets of samples (0, 0.15, and 0.35 kGy) were subjected to the following analyses twice every week for 3 wk. The study was done twice.

Headspace gas analysis

Both headspace oxygen and carbon dioxide were analyzed using a calibrated Pac Check™ 650 (Mocon, Minneapolis, Minn., U.S.A.) in automatic mode. The syringe needle was in-

serted through a 1-cm² square of adhesive rubber applied to the package to prevent air leaking from or into the package. The volume extracted by the syringe was 8 ml. Four replicates were made for each bag. The manufacturer's reported accuracy for this machine is ± 0.05% from 0.1% to 9.99% O₂ and ± 2% from 1% to 100% CO₂.

Microbial analysis

Total aerobic and yeast and mold colony forming units (CFU) were determined by standard spread plate methodology. Twenty-five-gram samples of lettuce was mixed with 225 mL sterile Butterfield's phosphate buffer in a sterile bag, then agitated for 1 min in a paddle blender (Masticator, IUL, Barcelona, Spain). Duplicate packages of lettuce were tested for each dosage. Samples were then placed in an ice chest until all samples were ready for the dilution step. One mL of the supernatant was then serially diluted in 9 mL Butterfield's phosphate buffer. Selected dilutions (0.1 mL) were plated in duplicate on plate count agar (PCA) (Difco, Detroit, Mich., U.S.A.) or potato dextrose agar (PDA) (Difco) containing 25 µg/mL chloramphenicol (Sigma, St. Louis, Mo., U.S.A.). On day 1, each dilution was plated. After determining the CFU for each sample at this time point, 3 dilutions were plated for each time point thereafter. PCA plates were incubated at 30 °C for 48 h, and PDA plates were plated at 30°C for 72 h. Plates with CFUs between 25 and 300 were utilized to calculate the CFU/g. The CFUs reported reflect the average of 4 to 6 plates.

Color analysis

The Hunter Lab D25-PC2 model colorimeter (Hunter Lab, Reston, Va., U.S.A.) was used to assess lettuce color. Following calibration with black and white standard tiles, samples of about 10 g each were placed into a standard glass container and read at opponent color scales: L (100 = bright, 0 = dark), a (positive = red, negative = green), and b(positive = yellow, negative = blue). Triplicate measurements were used for each determination.

Total Color Difference (ΔE) was calculated by using the

following formula:

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2},$$

where L_0 , a_0 , and b_0 represent the readings on the 1st day.

Texture analysis

Texture was determined using a Kramer Shear Press with 5 blades (TA-91) attached to a Stable Micro System Texture Analyzer (Model TA-XT2, Texture Technology Corp. Scarsdale, N.Y., U.S.A., and Stable Microsystems, Godalming, Surrey, U.K.). Ten-g samples (with ribs removed) were placed into the square metal container and then a 5 flat-plate plunger was forced through the lettuce. The probe was set at 50 mm from the bottom of the 5 flat-plate plunger and moved downward at 1.5 mm/s. The maximum force, kg, was recorded automatically by the Texture Expert software program, v. 1.6 (Texture Technology Corp, Scarsdale, N.Y., U.S.A.). Five measurements were performed for each sample.

Sensory evaluation

Analytical sensory testing was conducted at Chapman University's Sensory Evaluation Laboratory in individual booths in a controlled environment evaluation room following American Society for Testing and Materials (ASTM) guidelines (ASTM

1981; ASTM 1996). Panelist selection was based on interest, performance on preliminary screening tests, and availability. The selected panelists were all students from the Food Science Department at Chapman University and were trained in 5 60-minute sessions to identify and evaluate the intensity of key lettuce sensory characteristics (color, texture, and off flavor). During training, intensity scaling methods were explained, score sheets were developed, and samples covering the type and range of changes expected during the study were evaluated. Color, texture, and off-flavor were rated from none to intense on unstructured, anchored scales (15-cm horizontal lines) (Poste and others 1991). The same 6 trained panelists evaluated the lettuce samples throughout the investigation. Lettuce samples were given to panelists in cups coded with 3-digit random numbers (Poste and others 1991). Samples were evaluated routinely for up to 24 d of storage.

Statistical analysis

Analysis of variance using the Tukey-Kramer test of multiple comparisons of the means ($p \leq 0.05$) was used to determine the presence of significant differences (ASTM 1996) among the samples over time. The sensory and instrumental measurements for color and texture were correlated. Statistical analysis was performed using JMP®, program v. 3.5 (SAS Institute, Cary, N.C., U.S.A.).

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- MS 19990915 received 9/9/99; revised 12/22/99; accepted 3/30/00.

We wish to thank Thomas Tape for his technical assistance, Ready Pac Corp. for providing the lettuce, and Sterigenics International Inc. for gamma irradiation of the samples. This work was supported by a grant from the Irvine Health Foundation.

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