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Effect of Gamma Irradiation on the Microflora of Commercial Ready-To-Use (RTU) Salads during Cold Storage

Jang-Ho Kim, Ju-Woon Lee, You-Seok Lee, Sang-Hee Oh and Myung-Woo Byun†

Radiation Food Science and Biotechnology Team, Korea Atomic Energy Research Institute, Daejeon 305-353, Korea

Abstract

Since ready-to-use (RTU) products are not fully cooked, the shelf-life of the product is comparably short and the products are easily spoiled when contaminated with food-borne pathogens. Low-dose gamma irradiation of 0.5, 1, or 2 kGy effectively reduced the total aerobic bacterial counts in 2 Korean manufactured RTU products by 1.63 to 2.95 log CFU/g during cold storage. Irradiation at 2 kGy reduced the psychrotrophic bacterial counts in most of the samples to below the limit of detection (<2 log CFU/g). Irradiation at 0.5 kGy completely eliminated *Escherichia coli* from the commercial RTU samples.

Key words: low-dose irradiation, RTU salads, total plate counts, psychrotrophic counts

INTRODUCTION

Due to consumer demand for fresh and healthy food, interest in minimally processed fruits and vegetables has increased. The production of shredded ready-to-use (RTU) or ready-to-eat (RTE) vegetables has grown especially rapidly. A variety of salad vegetables such as carrots, celery, and others are used in minimally processed products (1). RTU or RTE produces have several advantages over whole vegetables, and they are considered to be more nutritious, convenient, and efficient in meal preparation (2)

The shelf-life of commercial RTU salads is shorter than that of the unprocessed raw salad vegetables. Raw vegetables are highly vulnerable to spoilage microorganisms, including pathogens, since shredded vegetables provide a higher nutrient availability. It is difficult for the minimal processing methods for vegetables such as washing, cutting, dipping in sanitizer, and packaging to adequately reduce microbial contamination. Several researchers have reported that *Listeria monocytogenes* was found in coleslaw and a salad mix (3-5). It was reported that outbreaks of *Escherichia coli* O157:H7 were related to lettuce and vegetable salad consumption (6). Other pathogenic microorganisms including *Salmonella* sp., *Campylobacter* sp., and *Yersinia enterocolitica* have also been found in fresh-cut fruits and vegetables (7-10).

Gamma irradiation is one of the most effective antimicrobial treatments for many foods including fresh produce. Beuchat (11) stated that ionizing radiation could be an extremely effective tool for reducing the populations of pathogenic microorganisms and parasites in raw fruits and vegetables. Several researches have reported that a low dose irradiation can improve the microbial quality of various fresh-cut vegetables such as green onion leaves (12), diced celery (13), shredded iceberg lettuce (14), diced tomato (15), coriander leaves (16), cut lettuce (17), and shredded carrot (18).

The present study investigated the effect of gamma irradiation on microflora, including the psychrotrophs of 2 different commercial RTU salads produced in Korea and prepared from different ingredients.

MATERIALS AND METHODS

Salads sample preparation

Two different commercial salad products were purchased at a local grocery store in Korea. Salad G (identified by the initial of a brand name) consisted of lettuce, cabbage, red cabbage, carrot, red dandelion, Chinese kale, and chicory. Salads F consisted of oak-leaf lettuce, chicory, broccoli, lettuce, and red cabbage. Each salad sample was aseptically shredded further and mixed to obtain sample homogeneity. The salad sample was then transferred to sterile stomacher bags and the bags were sealed.

Gamma irradiation

Samples in the bags were irradiated at a dose rate of 7 kGy per hour of gamma ray to obtain 0.5, 1.0, 2.0 kGy by using a Co-60 gamma ray irradiating facility (IR-

†Corresponding author. E-mail: mwbyun@kaeri.re.kr
Phone: +82-42-868-8060. Fax: +82-42-868-8043

79, Nordion International Ltd., Ontario, Canada, 100 kCi). The irradiation dose was validated by using 5-mm diameter alanine dosimeters (Bruker Instrument, Rheinstten, Germany). Free radicals were measured using an EMS 104 EPR Analyzer (Bruker Instrument). The actual doses were within ±2% of the target doses.

Overall attractiveness

A trained panelist evaluated the overall appearance of the salad samples for attractiveness based on the method of Kader et al. (19). Scores from 1~9 were used to describe the overall appearance quality, where 9 – excellent, 7 – good, 5 – fair, 3 – poor, and 1 – extremely poor. Browning, russet spotting, rotting, and cutting damage were examined to evaluate the appearance quality of the salad samples during 7 days of cold storage.

Microbiological analysis

After irradiation, samples were placed in cold storage (7°C). On days 0, 3, 5, and 7 a portion of each sample was removed and blended with 0.1% (w/v) peptone solution (Difco Laboratories, Detroit, MI) using a stomacher (BagMixer® 400, Interscience Inc., St. Nom, France) at a high speed for 2 min. After serial dilutions, 0.1 mL aliquots were plated onto plate count agar (Difco Laboratories) for the total aerobic and psychrotrophic bacterial counts, and onto eosin methylene blue agar (Difco Laboratories) for counting *E. coli* using a standard spread plate method. Duplicate plates for each sample were incubated at 30°C for 48 hrs for the total bacterial counts, at 5°C for 7 days for the psychrotrophic counts, and at 37°C for 48 hrs for *E. coli*. For the detection of *E. coli* or *E. coli* O157:H7, both samples were pre-enriched in a half strength trypticase soy broth (Difco Laboratories) at 37°C for 6 hrs, enriched in EC broth at 45°C for 15 hrs, and streaked onto Fluorocult® *E. coli* media (Merck, Darmstad, Germany) at 37°C for 24 hrs.

Statistical analyses

Microbial count data was analyzed using an analysis of variance (ANOVA) and General Linear Models Procedure of SAS (20). Fisher's Least Significant Difference (LSD) test was used to separate the means at a significance of $p<0.05$.

RESULTS AND DISCUSSION

Total aerobic bacterial counts of the irradiated salad samples during cold storage

In the non-irradiated salad samples, the initial total aerobic bacterial counts of salads F and salads G were 5.86 log CFU/g and 7.29 log CFU/g, respectively (Fig. 1). These initial microbial populations were relatively lower than the results found by Marchetti et al. (21); they reported 10^8 CFU/g of psychrotrophic bacteria, approximately 10^5 CFU/g of lactic acid bacteria, and 10^4 CFU/g of yeasts. In general, the initial microbial population of the various raw vegetables exceeds a level of 10^6 CFU/g (22) and varies according to salad composition, initial contents of raw matter, type of packaging film, atmospheres, and initial gas concentrations (23). In the non-irradiated samples, the total aerobic bacterial counts increased in proportion to the storage time (Fig. 1), and the overall appearance qualities on storage day 7 were poor when compared to the irradiated samples (Table 1). In the irradiated samples, however, the initial (immediately following irradiation in irradiated samples) total aerobic bacterial counts were significantly lower than those of the non-irradiated samples by 1.63 to 2.95 log CFU/g as the irradiation dose increased (Fig. 1). As the storage time proceeded, the 1 and 2 kGy-irradiated samples showed no significant difference in the total aerobic bacterial count (Fig. 1) or overall appearance quality (Table 1). This result is consistent with the results of other microbiological studies on minimally processed

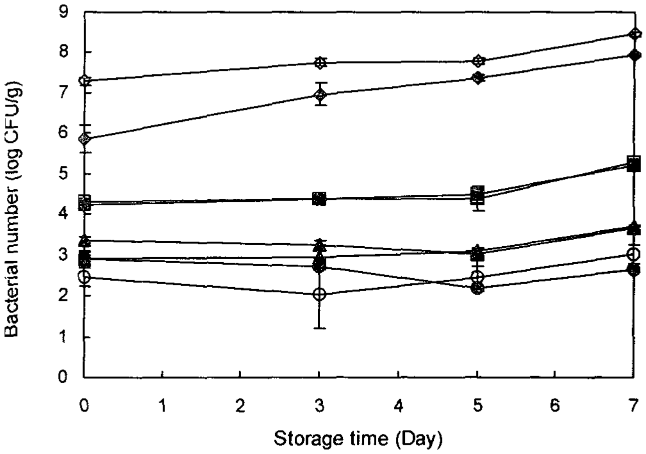

Fig. 1. Effect of gamma irradiation on the total plate counts of commercial RTU salad sample F and G during cold storage. -◆-, non-irradiated salad F; -■-, 0.5 kGy-irradiated salad F; -▲-, 1 kGy-irradiated salad F; -●-, 2 kGy-irradiated salad F; -◇-, non-irradiated salad G; -□-, 0.5 kGy-irradiated salad G; -△-, 1 kGy-irradiated salad G; -○-, 2 kGy-irradiated salad G.

Table 1. Overall attractiveness of irradiated commercial RTU salads stored at 7°C

Storage time (day)	0 kGy	0.5 kGy	1 kGy	2 kGy
0	9	9	9	9
3	8	9	9	9
5	8	9	9	9
7	6	8	9	9

Scoring system: 9 - excellent, free from defects; 7 - good, minor defects but not objectionable; 5 - fair, slightly to moderately objectionable defects; 3 - poor, excessive defects; 1 - extremely poor, not usable.

vegetables using gamma-irradiation. Foley et al. (14) reported that irradiation of lettuce at 0.55 kGy effectively reduced standard plate counts and yeast and mold counts and did not cause softening or sensory defects. Kamat et al. (16) found that irradiation at 1 kGy was sufficient for bacterial decontamination and elimination of potential pathogens and to maintain the quality of the coriander leaves up to 14 days during cold storages. According to our results, the total aerobic bacterial counts of the irradiated samples were significantly lower than those of the non-irradiated samples through the entire cold storage period (Fig. 1). These results indicate that irradiation at low doses, upto 2 kGy, is very effective at controlling bacterial contamination without any adverse effect on the overall quality of the commercial vegetable salads.

Psychrotrophic bacterial counts during cold storage

Psychrotrophic (cold tolerant) bacterial load in minimally processed vegetable salads is an important factor which affects the quality of the products since commercial vegetable salads are mostly kept and sold at a cold temperature. For this reason, the psychrotrophic bacterial growth was monitored during 7 days of cold storage at 7°C (Fig. 2). In the non-irradiated salad samples, the psychrotrophic bacterial counts in salads F and salads G on day 0 of storage were 5.31 log CFU/g and 5.69 log CFU/g, respectively (Fig. 2). These initial psychrotrophic bacterial counts of the salad samples were similar to the results found by Garg et al. (24) and Garcia-Gimeno & Zurera-Cosano (23), but lower than that found by Marchetti et al. (21) who reported 10^8

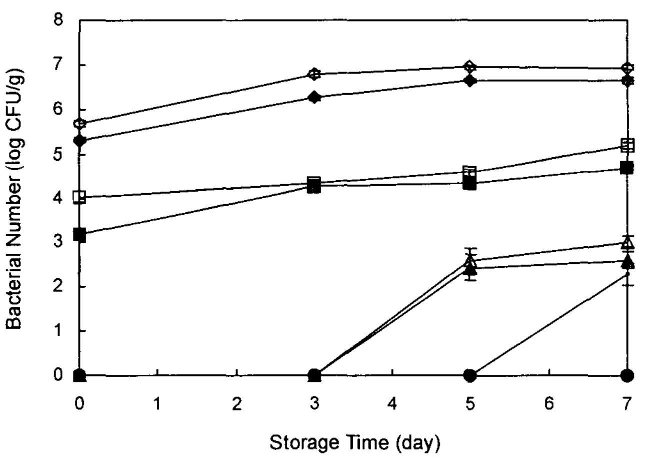

Fig. 2. Effect of gamma irradiation on the psychrotrophic bacterial counts of commercial RTU salad samples F and G during cold storage.
-◆-, non-irradiated salad F; -■-, 0.5 kGy-irradiated salad F; -▲-, 1 kGy-irradiated salad F; -●-, 2 kGy-irradiated salad F; -◇-, non-irradiated salad G; -□-, 0.5 kGy-irradiated salad G; -△-, 1 kGy-irradiated salad G; -○-, 2 kGy-irradiated salad G.

CFU/g of psychrotrophic bacteria in salad samples. Garg et al. (24) reported that the initial psychrotrophic bacterial counts were 1.8×10^5 CFU/g in cabbage, 2.7×10^5 CFU/g in lettuce, and 9.5×10^4 CFU/g in carrot salad. Garcia-Gimeno & Zurera-Cosano (23) reported that the initial psychrotrophic bacterial count in salads started at 1.07×10^5 CFU/g and increased to 1.48×10^5 CFU/g after 9 days of cold storage at 4°C. On the other hand, Abdul-Raouf et al. (25) reported that the psychrotrophic bacterial counts starting at 7.76×10^4 CFU/g in a modified-atmosphere reached 10^9 CFU/g after 14 days. Manvell and Ackland (26) found that the psychrotrophic bacterial count increased by 5 log after 8 days. In this experiment, the psychrotrophic bacterial counts in the non-irradiated salad samples F and G increased by 1.34 and 1.25 log CFU/g, respectively after 7 days of cold storage. In the irradiated samples, even the lowest irradiation dose reduced the psychrotrophic bacterial counts to below the initial psychrotrophic bacterial counts of the non-irradiated salad samples after 7 days of cold storage (Fig. 2). These results indicate that an irradiation dose of 1 kGy effectively inhibits the growth of psychrotrophic microorganisms and maintains the attractiveness of salads during cold storage, and an irradiation dose of 2 kGy reduced the psychrotrophic bacterial counts in most of the samples to below the limit of detection ($<$2 log CFU/g) (Fig. 2). These results indicate that a low dose irradiation of fresh-cut produce is an effective treatment for eliminating the potential risk caused by pathogenic psychrotrophic bacteria such as *L. monocytogenes*.

Detection of *E. coli* in the salad samples during cold storage

E. coli counts in the non-irradiated samples were below the limit of detection ($<$2 log CFU/g). However, *E. coli* was detected in the non-irradiated samples during the cold storage after enrichment with trypticase soy broth and a selective media, but was not detected in the irradiated samples (Table 2). Several studies have reported that strains of *E. coli* have been implicated in human food-borne illness due to the consumption of con-

Table 2. Detection of *Escherichia coli* in irradiated commercial RTU salads stored at 7°C

Storage time (day)	0 kGy	0.5 kGy	1 kGy	2 kGy
0	+[1)]	-[2)]	-	-
3	+	-	-	-
5	+	-	-	-
7	+	-	-	-

[1)] +: *E. coli* detected.
[2)] -: *E. coli* not detected.

taminated commercial raw vegetable salads (6,27). *E. coli* grows on raw salad vegetables that have been contaminated due to improper processing and storage conditions. In this study, the lowest irradiation dose, 0.5 kGy, eliminated *E. coli* from the salad samples during cold storage. These results are consistent with other studies: Foley et al. (14) also reported similar results that *E. coli* counts were effectively decreased throughout storage in 0.55 kGy-irradiated lettuce, and remained undetectable through day 7. Prakash et al. (13) found that an irradiation at 0.5 kGy eliminated detectable *E. coli* from celery after the initial storage. In general, fresh-cut vegetables need to be irradiated at a low-dose (>2 kGy) since a high-dose irradiation may introduce organoleptic defects such as textural change. Thus, it is concluded that a low-dose irradiation is an efficient technology to improve the quality of RTU produce such as fresh-cut vegetables during cold storage.

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