

Gamma-Irradiation Provides Microbiological Protection While Maintaining Sensory Quality Change of Fresh Kale Juice During Storage

– Research Note –

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Abstract

The effect of gamma-irradiation on microbiological growth in kale juice during storage was studied. Fresh kale juice was prepared and irradiated at 0, 1, 3, 5, 7, 10, and 15 kGy. D values for total bacteria, yeast and mold, *Salmonella*, *E. coli*, and *Pseudomonas* were 3.6, 4.0, 3.2, 1.4, and 1.6 kGy, respectively. *E. coli* and *Pseudomonas* were eliminated completely at 5 and 7 kGy, respectively. Gamma-irradiation also reduced total viable bacteria during storage. Therefore, these results indicate gamma-irradiation can prevent microbial spoilage of fresh kale juice by inactivating pathogenic microorganisms.

Key words: gamma-irradiation, kale juice, microbial spoilage

INTRODUCTION

Fresh kale juice is a traditional drink product for health promotion in Korea, and is known to have anticarcinogenic and antimutagenic properties (1,2). Kale contains flavonoids such as quercetin and kaempferol, which are known to have anticarcinogenic properties (2,3). Fresh kale juice has great potential as functional food, provided its microbial safety is assured during marketing. However, un-pasteurized fruit and vegetable juices have been implicated in food-borne diseases (4). Raw juices are highly perishable and difficult to store in a fresh state, and have a very short shelf-life. Vegetable juices, in particular, have a relatively high pH making them susceptible to bacterial growth (5). Gamma-irradiation is an alternative treatment to thermal processing since it is known to be an effective method for controlling microbial levels in ready-to-eat vegetables. Irradiation has been approved by the Food and Drug Administration for use on fruits and vegetables at low doses, below 10 kGy (6). There have been reports on the effectiveness of gamma-irradiation for extending the post-harvest life of fresh fruits and vegetables (7). Howard et al. (8) reported decreased microbial levels in irradiated *pico de gallo* at 1 kGy. Chervin and Boisseau (9) found that a dose of 2 kGy inhibited the growth of aerobic and lactic acid-producing microorganisms in shredded carrots.

This study examined the effect of gamma-irradiation on microbiological changes in fresh kale juice during storage, and suggest the appropriate processing treatment in marketing of fresh kale juice.

MATERIALS AND METHODS

Sample preparation

Fresh kale was purchased from a local market in Daejeon, Korea and homogenized in a blender at 4°C. The kale juice was then aseptically transferred into a sterile eppendorf tube.

Sample irradiation

Kale juice samples were irradiated at room temperature using a ⁶⁰Co gamma irradiator Type IR-79 (MDS Nordion International Inc., Ontario, Canada) at 1, 3, 5, 7, 10, and 15 kGy. Samples (1 mL) were then sealed in sterile test tubes and stored at 4°C.

Microbiological analysis

Following irradiation, kale juice samples were diluted with peptone water for microbial count. Serial dilutions were performed in triplicate on each selective agar plate. Total viable bacterial counts were obtained by plating the appropriately ten-fold diluted samples onto plate count agar (Difco Co., Detroit, MI, USA). Samples were evenly spread on the surface of the plates with a sterile glass rod. Diluted samples were plated on potato dextrose agar for yeast and mold counts (Difco Co., Detroit, MI, USA). Both plates were incubated at 30°C for 48 hr. Selective media for *E. coli*, *Salmonella*, and *Pseudomonas* were EC Medium, tryptic soy broth agar, and *Pseudomonas* agar F (Difco, USA), respectively. All plates were incubated for 48 hr and colony forming units (CFU) were counted. Incubation temperatures for *Pseudomonas*, *salmonella*, and *E. coli* were 20°C, 30°C, and 35°C, respectively. The effect of

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refrigerated storage at 4°C, was investigated by determining changes in residual total bacteria count, yeast and mold, *Pseudomonas*, *Salmonella*, and *E. coli* counts after storage. All the experiments were carried out in duplicate and each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU/g.

Determination of D values

To obtain the D values (radiation doses needed to decrease a microbial population by 90%), the slopes of the individual survivor curves were determined by linear regression. D values were then calculated by taking the negative reciprocal of the slope of the survival curve.

RESULTS AND DISCUSSION

Irradiation inactivation study

Inactivation kinetics appeared to be of the first order, although evidence of tailing was observed. Irradiation inactivation curves were constructed for various irradiation doses, up to 15 kGy, and the slopes of the individual survival curves were determined by linear regression (Fig. 1). The numbers of total bacteria, yeast and mold, *Salmonella*, and *Pseudomonas* of fresh kale juice were around 10^5 CFU/g, while the number of *E. coli* was around 10^4 CFU/g, depending on the method of preparation of the fresh kale juice samples. Gamma-irradiation effectively reduced microbial populations in kale juice. *E. coli* and

Pseudomonas were eliminated by doses of 5 kGy and 7 kGy, respectively. By contrast, total bacteria, yeast and mold, and *Salmonella* required 20 kGy to be completely eliminated from the fresh kale juice prepared under the experimental conditions of this study.

Several factors can influence the resistance of microorganisms to inactivation by gamma-irradiation (10). One of the factors is the chemical composition and physical state of the medium during irradiation. Initial microbial load of the sample is also important. Refrigeration can be an adequate barrier to the growth of pathogenic bacteria in some foods, especially foods such as fruit juices which are usually acidic (11). For apple juice, 1.8 kGy was sufficient to eliminate *E. coli* O157:H7, depending on the pH and the amount of suspended solids (1). The pH of fresh kale juice was 6.1, which is not low enough to inhibit microbial growth. Therefore, irradiation may be useful, since the inactivation of microorganisms in foods is dependent on the pH of the sample. D values were determined by linear regression. D values for total bacteria, yeast and mold, *Salmonella*, *E. coli* and *Pseudomonas* were 3.6, 4.0, 3.2, 1.4, and 1.6 kGy, respectively. These results indicate that *E. coli* and *Pseudomonas* were more sensitive to gamma-irradiation than were yeast and mold, total bacteria, and *Salmonella*. These results also suggest that 6D processing is more than sufficient for microbial safety of fresh kale juice.

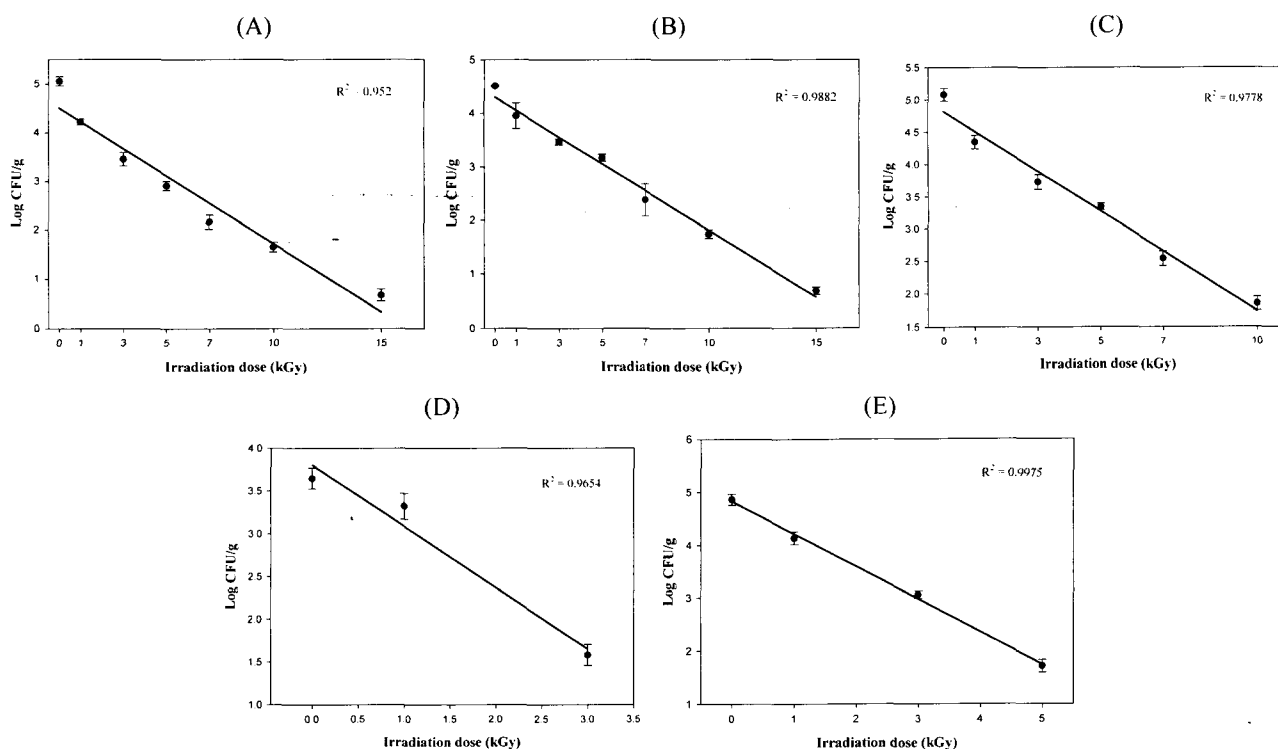


Fig. 1. Changes in populations of (A) total bacteria, (B) yeast and mold, (C) *Salmonella*, (D) *E. coli*, (E) *Pseudomonas* in fresh kale juice by gamma-irradiation at various radiation doses. Bars represent standard error.

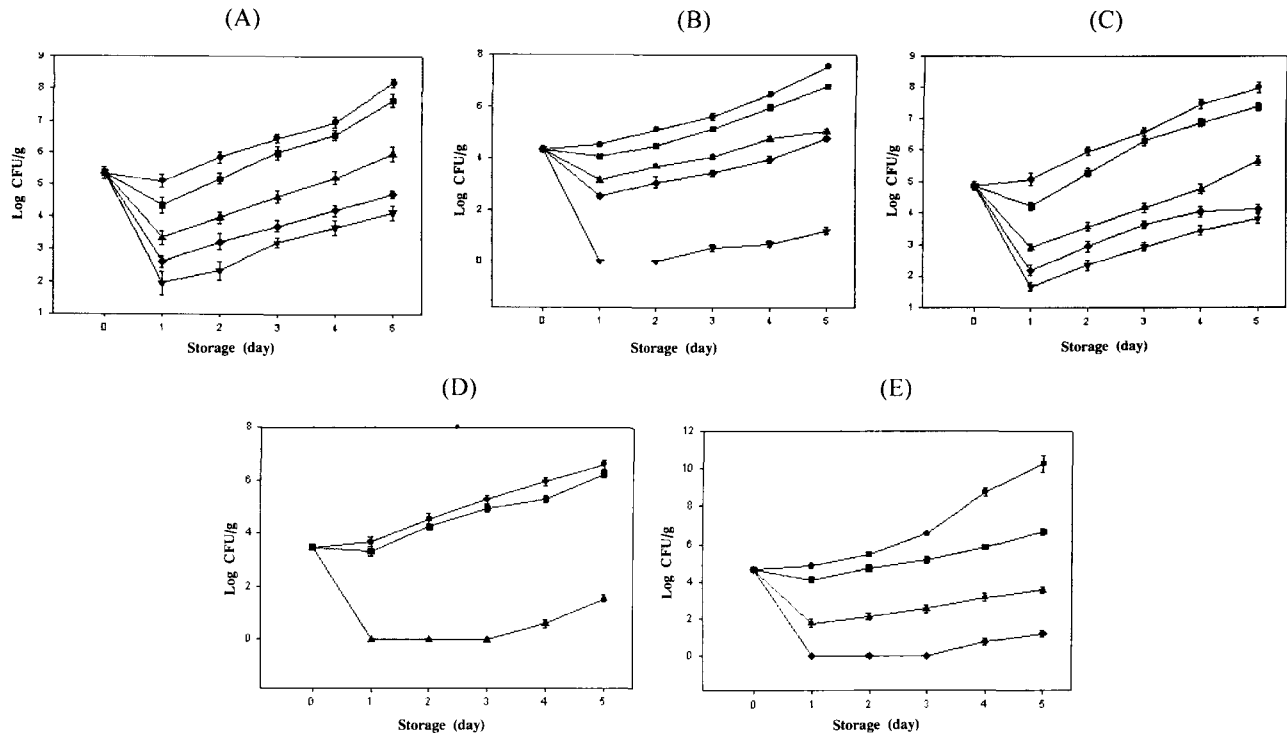


Fig. 2. Changes in populations of (A) total bacteria, (B) yeast and mold, (C) *Salmonella*, (D) *E. coli*, (E) *Pseudomonas* during storage of fresh kale juice irradiated at various radiation doses. Bars represent standard error.

—●— 0 kGy, —■— 1 kGy, —▲— 5 kGy, —◆— 7 kGy, —▼— 10 kGy

Microbiological analysis during storage

Gamma-irradiation effectively reduced total viable bacterial populations in fresh kale juice during storage without significantly affecting sensory qualities (Fig. 2 A). The levels of total bacteria were similar in irradiated and non-irradiated samples after day 1, but irradiated samples maintained lower levels during further storage. The difference between the irradiated and non-irradiated samples on day 1 varied by 5 log cycles. Treatment with a dose 15 kGy eliminated the total viable bacteria, while a dose of 5 kGy reduced initial counts by 2 log cycles.

Yeasts and molds, and *Salmonella*, also exhibited significant differences between the non-irradiated and the irradiated samples (Fig. 2 B and C). Like total bacteria, irradiation with 15 kGy completely eliminated yeast, mold, and *Salmonella*. Irradiation reduced the initial levels of yeast and mold by 0.5 to 3 log cycles on day 1, while the number of *Salmonella* decreased by 1 to 3.5 log cycles. Non-irradiated samples had rapid proliferations of yeast, mold, and *Salmonella*, exceeding 10^8 CFU/g after 5 days.

E. coli, was eliminated by irradiation with a 5 kGy dose (Fig. 2 D). At day 1, *E. coli* grew at similar rate in irradiated and non-irradiated samples, but irradiated samples maintained lower counts by 2 log cycles during the entire storage period. *Pseudomonas*, was eliminated by irradiation with a 7 kGy dose (Fig. 2 E). At day 1, the difference between the irradiated and non-irradiated samples was

varied by 3 log cycles; *Pseudomonas* in non-irradiated samples grew by up to 10 log cycles after 5 days. These results confirm that *E. coli* and *Pseudomonas* were the most irradiation-sensitive.

In conclusion, gamma-irradiation significantly decreased the number of microorganisms while simultaneously maintaining the quality of fresh kale juice. These results provide insight into the irradiation doses needed to ensure microbial safety for fresh kale juice during marketing.

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