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Effects of Gamma Irradiation on the Fermentative Microorganisms and Lactate Dehydrogenase Activity in Kimchi at Different Fermentation Stages

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Abstract

Gamma irradiation treatment was performed at the early and mid-fermentation stages of Kimchi preparation. Changes in fermentative microorganisms and lactate dehydrogenase activity during the fermentation periods were investigated to determine proper irradiation point for extending the shelf life of Kimchi. Initial levels of acid producing bacteria and yeast in Kimchi were 10^4 CFU g^{-1} and 10^1 CFU g^{-1}, and reached up to 10^9 CFU g^{-1} after 15 days and 10^7 CFU g^{-1} after fermentation for 30 days at 10°C, respectively. The radiation resistance of acid producing bacteria in the earlier stage (D_{10} value was 0.87 kGy) was higher than at the mid-fermentation stage (after 10 days at 10°C, D_{10} value was 0.69 kGy). Microbial growth and lactate dehydrogenase activity were inhibited significantly by gamma irradiation at the early fermentation stage of Kimchi and acidification was effectively delayed during the subsequent storage period. Although the growth of fermentative microorganisms was inhibited by gamma irradiation at the mid-fermentation stage of Kimchi, lactate dehydrogenase activity was maintained and acidification continued during the storage period.

Key words: Kimchi, lactate dehydrogenase, gamma irradiation, fermentation

INTRODUCTION

Kimchi is a Korean traditional fermented vegetable food and can be classified as pickles fermented with lactobacilli (1). The raw materials used for preparing Kimchi include vegetables (oriental cabbage or radish, onion, green onion, carrot, cucumber, leaf mustard, etc.), spices (hot pepper, garlic, ginger, sesame, pine nuts, etc.) and non-vegetable ingredients (fermented anchovy, fermented shrimp, boiled starch, sugar, oyster etc.) (2). Various acid producing bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus* etc.) produce lactate dehydrogenase, and lactic acid and other organic acids which accumulate during Kimchi fermentation (3). Some yeasts (*Hansenula*, *Pichia*, *Debaryomyces* etc.) are responsible for specific flavor or deterioration from the mid-fermentation stage onward (2, 4,5). After the Kimchi reaches a well-ripened stage, these microbiological and enzymatic activities continue. Finally, deterioration of Kimchi results in a sour taste, bitter taste, off-odor, and softening (2,6). Therefore, inactivation of fermentative microorganisms is essential for preserving and extending the shelf life of Kimchi. However, most existing technologies are incongruent or still have little effect (2). Recently, significant effects of gamma irradiation on the regulation microbial growth in fermented vegetables (7), fermented seafood (8), fermented soybean paste (9,10) and soy sauce (11) have been reported. It is known that irradiation is effective in controlling fermentative microorganisms but not enzyme activity. Kim et al. (12) reported that the aging of fermented soybean products continued after gamma irradiation because of residual hydrolytic enzyme activity. These results indicate that not only microbial control but also enzymatic control should be considered in irradiation treatment of fermented food. Therefore, gamma irradiation treatment was performed at different fermentation stages of Kimchi and changes in fermentative microflora and lactate dehydrogenase (LDH) activity were investigated to determine an optimal irradiation time for controlling aging and extending the shelf life of Kimchi.

MATERIALS AND METHODS

Sample preparation

Kimchi was prepared by the method of Lee et al. (13). The oriental cabbage, cut into half, was dipped in a 15%

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salt solution for 4 hrs. At that time, the salinity of the brined cabbage was 3.03±0.05%. Each piece of cabbage was washed with tap water for 1 min, drained for 1 hr and then mixed with spices and additives. The recipe of spices and additives was; sliced radish 5 g, red pepper powder 3 g, garlic 1 g, green onion 2.5 g, fermented anchovy sauce 2 g and ginger 0.5 g per 100 g of the brined cabbage. Each piece of blended cabbage (about 600 g) was packed anaerobically in polyethylene vinyl bags.

The Kimchi was irradiated at early fermentation stage (just after packaging) and mid fermentation stage (after 10 days, fermented at 10°C). Gamma irradiation was performed in a cobalt-60 gamma irradiator (point source, AECL, IR-79, Nordion, Canada) at doses of 2.5, 5 and 10 kGy. The source strength was approximately 100 kCi with a dose rate of 70 Gy min^{-1} at 15±0.5°C; the actual doses were within ±2% of the target dose. The absorbed dose was monitored with both free-radical and ceric/cerous dosimeters (14). A non-irradiated Kimchi was also prepared as a control. All samples were stored at 10°C, generally known as optimal temperature for fermentation (2) and cold chain system for distribution of Kimchi, and analyzed at 5~10 day intervals for 30 days.

Microbiological evaluation

A piece of Kimchi was homogenized using a lab blender (Hanil, FM 680T, Seoul, Korea) for 60 sec, and then filtered (No. 2, Whatmann, Kent, England). Each filtrate (1 mL) was diluted and poured in triplicate on a MRS agar (Difco Lab, Detroit. MI, USA), containing 3% sodium chloride and 0.002% bromophenol blue, and incubated at 30°C for 48 hr, and then yellow halo forming colonies were counted as acid producing bacteria. Each filtrate (1 mL) was diluted and poured in triplicate on a potato dextrose agar (PDA) (Difco Lab, Detroit. MI, USA), containing 3% sodium chloride, 0.25% lactic acid, and 0.01% chloramphenicol, and incubated at 30°C for 48 hr, and then colony formation unit (CFU) per mL was counted as yeast.

Calculation of radiation sensitivity

Viable microbes of irradiated and non-irradiated samples on the MRS and PDA agar plates were counted and radiation sensitivities were calculated as D_{10} value. The D_{10} value is an expression of the radiation dose needed to reduce the number of microorganisms by 10 fold and was calculated with the equation derived from survival plots (15).

pH and acidity

Homogenized, filtered (No. 2, Whatmann, Kent, England) and 10-fold diluted samples were used to determine the change of pH and acidity. The pH was determined with a pH meter (Orion 520A, Boston, MA, USA) and acidity was expressed as content of lactic acid (weight %) by measuring the titration volume of 0.1 N NaOH to adjust pH at 7.3.

Lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was determined by using a LDH assay kit (Sigma Co.). A piece of Kimchi was homogenized using a lab blender (Hanil, FM 680T, Seoul, Korea) for 60 sec, and then filtered (No. 2, Whatmann, Kent, England). 10 mL of filtrate was collected and sonicated (Sonicator W 700, Ultrasonics Inc., UK) at 4°C for a total period of 5 min. The cellular debris and solid materials were removed by centrifugation at 10,000×g at 4°C. The supernatant was used as the cell free crude extract. The reaction mixture contained 93 μM of β-NADH, 22.7 mM Na-pyruvate, 0.1 M phosphate buffer (pH 7.5), and 0.05 mL of cell-free crude enzyme in a final volume of 3.0 mL. The substrate was replaced by water in a blank mixture. The mixture was incubated at 25°C in a cuvette with a 1 cm light path. The reaction was started by the addition of Na-pyruvate and was measured by changes in absorbance at 340 nm with a spectrophotometer equipped with a thermostatically controlled cuvette holder and recorder. The absorbance was monitored at 30 second-intervals for 3 min and LDH activity (units/mL) was calculated by the equation, provided with the kit;

$$\text{LDH activity} = \Delta A \text{ (absorbance) per min} \times 20{,}000 \times \text{TCF (1 at 25°C)}$$

Relative percentage of LDH activity was compared with maximum LDH level (100%) of the control.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effect of irradiation dose on the microbiological and LDH changes by SAS (SAS Institute, Cary, NC USA) software (16). Student-Newman-Keul's multiple range test was used to compare differences among means at $p<0.05$ (17).

RESULTS AND DISCUSSION

Gamma irradiation on early fermentation stage of Kimchi

Viable cell counts of the acid producing bacteria were 10^4 CFU g^{-1} at the early fermentation stage and reached up to 10^9 CFU g^{-1} after 15 days at 10°C in the non-irradiated sample (Fig. 1). The D_{10} value of acid producing bacteria was 0.87 kGy (data not shown). The growth rates of acid producing bacteria in the irradiated samples were lagged with 2~7 decimal reductions until the late-stage of fermentation. Viable cell counts of the

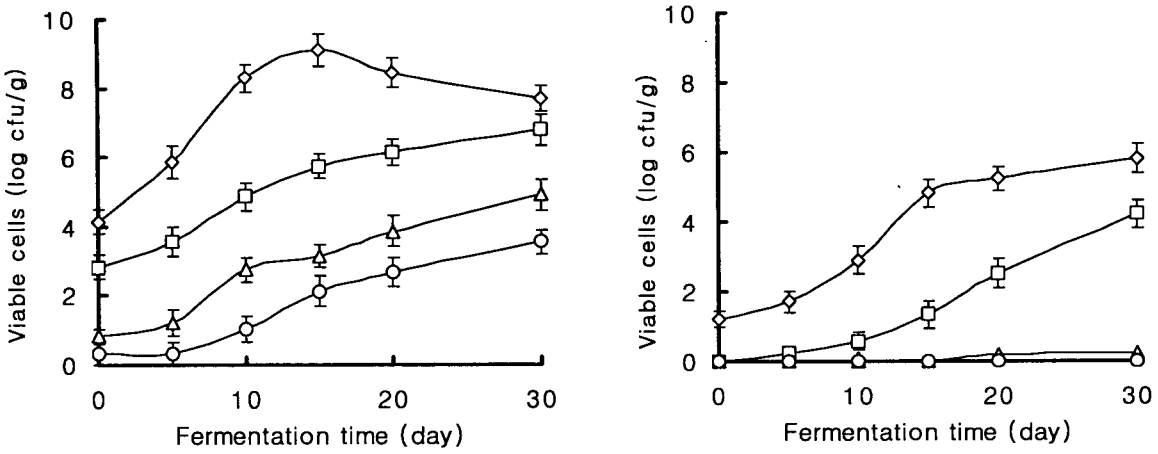

Fig. 1. Growth of acid producing bacteria (left) and yeast (right) in Kimchi after gamma irradiation at the early fermentation stage (D+0 day). Symbols are ◇, non-irradiated control; □, 2.5 kGy; △, 5 kGy; ○, 10 kGy irradiated Kimchi.

yeast were 10^1 CFU g^{-1} at the early fermentation stage and reached up to 10^5 CFU g^{-1} after 30 days in the non-irradiated sample (Fig. 1). The growth rate of yeast in the irradiated sample with a dose of 2.5 kGy lagged behind non-irradiated samples by about 10~15 days. Yeast groups were nearly eliminated and were not detected in 5 and 10 kGy irradiated samples during the fermentation period. Therefore, it is expected that gamma irradiation at the early fermentation stage of Kimchi might effectively delay acidification by controlling the growth of acid producing bacteria, and prevent a bitter taste, off-odor and softening by controlling putrefactive yeast growth.

Irradiation dose-dependently reduced the pH and lactic acid concentration of the fermenting kimchi (Fig. 2). The initial pH was 5.60 at the early fermentation stage, gradually lowered and reached 3.42 after 30 days in the non-irradiated sample (Fig. 2). The pH of 2.5, 5 and 10 kGy-irradiated samples maintained higher levels than that of the control and reached 3.66, 4.22 and 4.46 after 30 days, respectively. Lactic acid concentration was 0.17 % at the early fermentation stage, and gradually increased to 1.29 % after 30 days in the non-irradiated control (Fig. 2). Concentrations of lactic acid in the 2.5, 5 and 10 kGy-irradiated samples remained lower than that of the non-irradiated sample, and reached 1.02, 0.90 and 0.75% after 30 days, respectively. These results confirm that gamma irradiation at the early fermentation stage of Kimchi effectively reduces acidification.

The initial LDH activity was 4.57% of maximum at the early fermentation stage, reached the maximum activity (100%) after 15 days, and gradually decreased until the late stage in the non-irradiated control (Fig. 3). The LDH activities were lagged and significantly lowered by irradiation dose dependently, and decreased to 87.5, 71.3 and 54.2% of maximum after 30 days in 2.5, 5 and 10 kGy-irradiated samples, respectively.

Thus, it can be seen that gamma irradiation at the early fermentation stage of Kimchi fermentation inactivates and lags the growth of fermentative microbes, lower the LDH activity, and delays the acidification effectively.

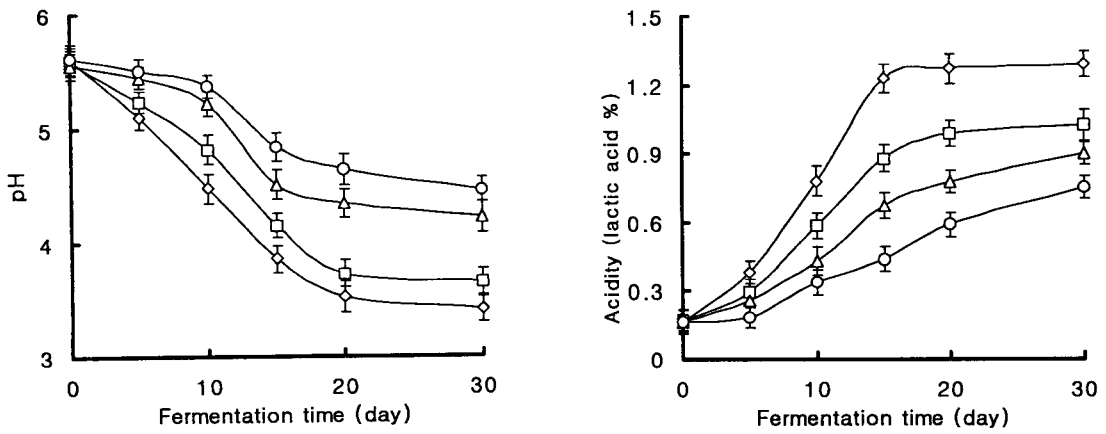

Fig. 2. Changes in pH and lactic acid concentration in Kimchi after gamma irradiation on the early fermentation stage (D+0 day). Symbols are ◇, non-irradiated control; □, 2.5 kGy; △, 5 kGy; ○, 10 kGy irradiated Kimchi.

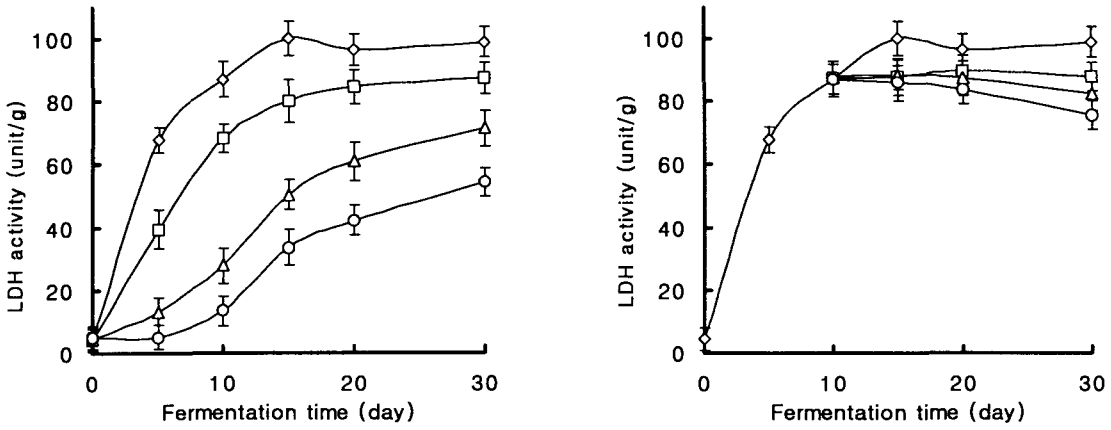

Fig. 3. Changes in lactate dehydrogenase activity in Kimchi after gamma irradiation at the early fermentation stage (D+0 day, left) and mid fermentation stage (D+10 day, right). Symbols are ◇, non-irradiated control; □, 2.5 kGy; △, 5 kGy; ○, 10 kGy irradiated Kimchi.

Gamma irradiation on mid-stage of Kimchi fermentation

Viable cell counts of the acid producing bacterial group were 10^8 CFU g^{-1} at the mid fermentation stage. Gamma irradiation inactivated acid producing bacteria in a dose-dependent manner with a D_{10} value of 0.63 kGy (data not shown). This result was similar to previous studies which reported that radiation sensitivity of the microorganism was higher in log phase than lag or the stationary phase (11). The viable cell counts of acid producing bacteria in the irradiated samples were maintained with 2~7 decimal reductions until the late stage of fermentation (Fig. 4). Yeast counts were 10^3 CFU g^{-1} at the mid fermentation stage, but were eliminated by >5 kGy of gamma irradiation (Fig. 4).

The pH was 4.48 at the mid-fermentation stage and reached 3.42 after 30 days in the control (Fig. 5), and 3.51 in the 2.5 kGy irradiated sample. The pH of 5 and 10 kGy-irradiated samples were higher levels than those of control and 2.5 kGy-irradiated samples, and reached 3.69 and 3.87 after 30 days, respectively. The content of lactic acid was 0.78% at the mid fermentation stage, and gradually increased and reached 1.29% after 30 days in the non-irradiated control (Fig. 5). From the mid- to late fermentation period, lactic acid contents in the 2.5, 5 and 10 kGy-irradiated samples remained slightly lower compared to the control, and reached 1.27, 1.18 and 1.14% after 30 days, respectively. However, these values were higher than those of the early fermentation stage irradiated samples (Fig. 2). The LDH activity at the mid-fermentation stage was 87%, and gradually increased and reached its maximum activity (100%) after 15 days (Fig. 3). The increase in LDH activity was nearly prevented by >5 kGy of gamma irradiation, but residual activities were maintained. These results were similar to previous studies of protease in Korean fermented soybean food (12). Therefore, it was concluded that gamma irradiation at the mid-stage of Kimchi fermentation effectively inac-

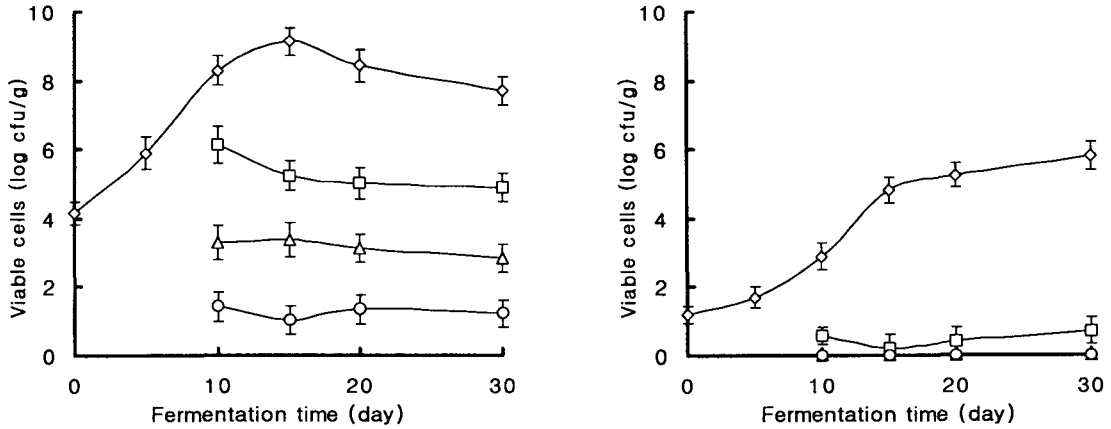

Fig. 4. Growth of acid producing bacteria (left) and yeast (right) in Kimchi after gamma irradiation at the mid fermentation stage (D+10 day). Symbols are ◇, non-irradiated control; □, 2.5 kGy; △, 5 kGy; ○, 10 kGy irradiated Kimchi.

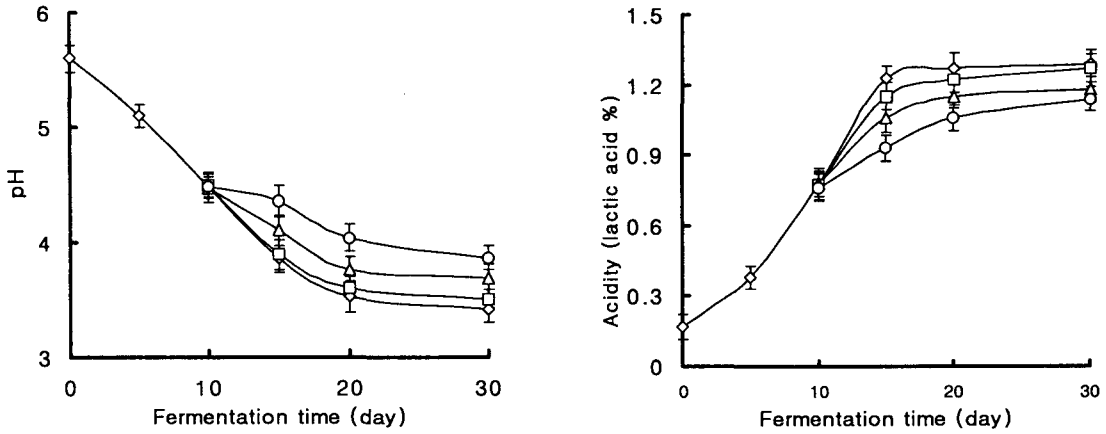

Fig. 5. Changes in pH and lactic acid concentration in Kimchi after gamma irradiation on the mid fermentation stage (D + 10 day). Symbols are ◇, non-irradiated control; □, 2.5 kGy; △, 5 kGy; ○, 10 kGy irradiated Kimchi.

tivates fermentative microbes. However, LDH activity was hardly influenced by gamma irradiation and residual LDH induced acidification continued.

In summary, we demonstrated that gamma irradiation treatment, up to 10 kGy, at the early stage of Kimchi fermentation dose dependently inactivates fermentative microbes, lowers LDH activity, and delays acidification. Although gamma irradiation at the mid-fermentation stage of Kimchi preparation inactivated fermentative microbes effectively, LDH activity remained at high residual levels, and LDH induced acidification continued. In this study, 5 kGy of gamma irradiation at the early fermentation stage was more effective in aging control than irradiation at the mid-fermentation stage.

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