Food Sci. Biotechnol. Vol. 17, No. 4, pp. 751 ~ 754 (2008)

Food Science and Biotechnology

Combination Pretreatment of Calcium and Vitamin C to Enhance the Firmness of *Kimchi* Sterilized with High-dose Gamma Irradiation

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Abstract Texture analysis, sensory evaluation, and scanning electronic microscopic (SEM) observation were conducted to evaluate the effects of different calcium salts (calcium lactate, calcium acetate, and calcium chloride) and vitamin C on the textural properties of *kimchi*, gamma-irradiated at 25 kGy. Increase of the hardness and sensory score were observed in the *kimchi* pretreated with calcium salt or vitamin C as compared with the untreated and irradiated *kimchi*. And the hardness and sensory quality of the co-pretreated sample with 0.01% of calcium lactate and 0.3% of vitamin C were the highest after 30 days at 35°C, which indicates that the co-pretreatment is effective in preventing a decrease of the texture and sensory qualities of *kimchi* by gamma irradiation. Also, this result was supported by the SEM observation.

Keywords: *kimchi*, gamma irradiation, calcium, vitamin C, firmness

Introduction

Kimchi is a traditional Korean salted and fermented vegetable. After it reaches a well-ripened stage, its microbiological and enzymatic activities continue and result in a quality deterioration due to a sour and bitter taste, off-odor, and softening (1-3). Therefore, an inactivation of fermentative microorganisms is essential for the preservation and extension of the shelf-life of *kimchi*. In previous studies (4), a combined treatment of mild heating at 60°C and irradiation at 25 kGy was conducted to enhance the storage stability of *kimchi* at an ambient temperature. However, the textural and sensory qualities of the *kimchi* were decreased by a high energy radiation. Therefore, more studies are needed to improve the quality of *kimchi*.

Calcium salts have been used to improve the textural property of vegetables in several studies. Calcium can be added to brine to retard the softening of fermented vegetable products, such as pickles, by binding with pectin. Calcium combines with cell wall pectin in a vegetable and inhibits a demethylation of the pectin, which delays the softening of *kimchi* (5,6). But there is no report about whether a calcium treatment reduces the extent of damage to a *kimchi* texture due to high-dose irradiation.

Meanwhile, Chen and Ahn (7) suggested that certain antioxidants could interrupt a free radical chain reaction by scavenging free radicals and reduce an oxidation, off-odor formation, and softening by irradiation. As their suggest, natural antioxidants like vitamin C, vitamin E, and so on could be used to reduce the softening of *kimchi* texture by irradiation. But a study about the effect of vitamin C on *kimchi* texture treated with high-dose irradiation has not been reported.

Therefore, this study was conducted to evaluate the effects of a pretreatment of 3 calcium salts (calcium lactate, CL; calcium acetate, CA; and calcium chloride, CC) and vitamin C (Vit C) on the textural properties of *kimchi* sterilized with gamma irradiation at 25 kGy.

Materials and Methods

Preparation of the samples and gamma irradiation *Kimchi* samples were supplied from Food Research Institute, CJ Food Co., Seoul, Korea. *Kimchi* was fermented for about 2 weeks at 4°C to obtain an optimal sensory quality with a range of about pH 4.5 and 0.3% of acidity. CL, CA, CC, and Vit C (98% < purity, food grade) were prepared from Duksan Pharmaceutical Co., Ahnsan, Korea. To evaluate the effect of calcium salts and vitamin C on the quality of *kimchi*, fermented *kimchi* was cut into about 5 cm in length. Calcium salts (0.01-0.03%, w/w) and vitamin C (0.1-0.7%, w/w) were added individually into the *kimchi*. All the samples were packed in aluminium-laminated low-density polyethylene bags (Al-LDPE, Sunkyung Co., Seoul, Korea), and gamma-irradiated at 25 kGy in a cobalt-60 gamma irradiator (AECL, IR-79; MDS Nordion International Co., Ottawa, Canada). The source strength was 490 kCi with a dose rate of 70 Gy/min at 15±0.5°C and the actual doses were within 2% of the target dose. The absorbed dose was monitored with both free-radical and ceric/cerous dosimeters. And the samples were served to

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Received October 13, 2007; Revised December 18, 2007; Accepted December 24, 2007

10 panel members for an evaluation of the sensory quality of *kimchi*. After gamma irradiation, *kimchi* samples were stored at 35°C for 30 days for an evaluation of the textural and sensorial properties during storage.

Sensory evaluation Sensory acceptance of each sample was tested. The panel was composed of 10 trained panelists. Three descriptors were employed to grade the quality in terms of the texture, taste, and overall acceptance. Each member independently evaluated the *kimchi* samples for their texture, taste, overall acceptance using a 7-point scale ranging from 1 (very bad) to 7 (very good).

Measurement of the hardness The hardness of each sample was analyzed by a penetrating test (8) by using a texture analyzer system (TA-XT2i; Stable Micro Systems, Godalming, UK). Square-type samples (3×3 cm) were prepared by cutting the stump of *kimchi* at 5 cm distances from the bottom. Operational condition of the texture analyzer was as follows; probe (P2, 5 mm cylinder probe), travel distance (65%), pretest speed (5.0 mm/sec), test speed (1.0 mm/sec), and post test speed (5.0 mm/sec). The hardness was expressed in g (maximum penetration force).

Scanning electron microscope (SEM) *Kimchi* tissue (1×1 cm) from around the midrib was cut and freeze dried for 24 hr. The dried *kimchi* was then coated with gold by using Carbon Coater (108-CA; Jeol, Tokyo, Japan). Microscopic observation of the epidermis was performed by using a 30 kV, 10 μÅ SEM (JSM-6335F; Jeol).

Statistical analysis Analysis was carried out in triplicate, and the results were expressed as means±standard deviations. One-way analysis of a variance (ANOVA) was used to determine the effects of different calcium salts and vitamin C on the textural properties of gamma-irradiated *kimchi* using the SPSS software (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare the differences among the means at $p<0.05$ (13).

Results and Discussion

Determination of the optimal addition level of calcium salt and vitamin C The effects of 3 kinds of calcium salts on the sensory quality of *kimchi* are shown in Table 1. The result showed that *kimchi* added with 0.01% of calcium salt had significantly higher sensory acceptance scores than the other samples regardless of the kinds of calcium salts ($p<0.05$). As the concentration of the calcium salt was increased above 0.02%, the bitter taste was strengthened in all the samples. Although beneficial for an improvement of the textural properties, the use of calcium salts might impart a bitterness or flavor differences in food products (9,10). Meanwhile, the sensory acceptance of *kimchi* added with above 0.4% of vitamin C was significantly reduced ($p<0.05$). Therefore, their optimal addition levels were tentatively determined to add 0.01% of calcium salt and 0.3% of vitamin C to *kimchi*, respectively. And these concentrations were used in a subsequent experiment to evaluate their effect on the textural property of the gamma-irradiated *kimchi*.

Effect of the pretreatment of calcium salts and vitamin C on the hardness of irradiated *kimchi* The changes of the hardness of the gamma-irradiated *kimchi* added with different calcium salts (0.01%, w/w) and vitamin C (0.3%, w/w) during storage at 35°C are shown in Table 2. After the gamma irradiation, the hardness of the *kimchi* was reduced by almost 50% from 1,953 to 976 g. Hardness of each sample added with CL, CC, and Vit C before gamma irradiation was significantly higher than the gamma-irradiated sample at 25 kGy ($p<0.05$). Hardness of the CL treatment sample (1,326 g) was the highest among the different calcium salts and similar to vitamin C (1,359 g). And the co-treatment of CL and Vit C (1,558 g) was

Table 1. Effect of the concentration of calcium salts and vitamin C on the sensory properties of *kimchi*

Sample[1)]	Concentration (%)	Sensory scores			
		Flavor	Texture	Taste	Overall acceptance
CON	0	6.7±0.4$^{a2)}$	6.6±0.3^{a}	6.9±0.3^{a}	6.9±0.7^{a}
CL	0.01	6.7±0.7^{a}	6.7±0.5^{a}	6.8±0.6^{a}	6.8±0.5^{a}
	0.02	6.8±0.5^{a}	6.5±0.5^{a}	5.2±0.4^{b}	5.1±0.4^{b}
	0.03	6.9±0.8^{a}	6.8±0.6^{a}	4.8±0.2^{b}	4.7±0.2^{b}
CA	0.01	6.7±0.8^{a}	6.6±0.5^{a}	6.5±0.5^{a}	6.5±0.3^{a}
	0.02	6.6±0.3^{a}	6.8±0.7^{a}	4.9±0.2^{b}	4.8±0.2^{b}
	0.03	6.8±0.6^{a}	6.7±0.3^{a}	4.4±0.4^{b}	4.3±0.3^{b}
CC	0.01	6.6±0.6^{a}	6.6±0.4^{a}	6.2±0.5^{a}	6.3±0.6^{a}
	0.02	6.8±0.3^{a}	6.7±0.5^{a}	4.5±0.3^{b}	4.4±0.3^{b}
	0.03	6.7±0.7^{a}	6.6±0.6^{a}	4.1±0.4^{b}	4.1±0.4^{b}
Vit C	0.1	6.8±0.5^{a}	6.7±0.6^{a}	6.8±0.4^{a}	6.8±0.6^{a}
	0.3	6.7±0.6^{a}	6.5±0.4^{a}	6.9±0.5^{a}	6.8±0.5^{a}
	0.5	6.6±0.3^{a}	6.6±0.7^{a}	5.8±0.4^{b}	6.0±0.5^{ab}
	0.7	6.6±0.2^{a}	6.8±0.5^{a}	4.9±0.3^{c}	5.1±0.4^{b}

[1)]CON, *kimchi* non-added with calcium salt and vitamin C; CL, calcium lactate; CA, calcium acetate; CC, calcium chloride; Vit C, vitamin C.
[2)a-c]Values with different letters within a column differ significantly ($p<0.05$).

Table 2. Change of the hardness of the irradiated *kimchi* added with different calcium salts (0.01%) and vitamin C (0.3%) during storage at 35°C (g, maximum penetration force)

Sample[1)]	Storage period (day)	
	0	30
CON	1,953±94$^{aA2)}$	361±32^{bE}
IR	976±46^{aD}	683±32^{bD}
CL-IR	1,326±59^{aC}	814±31^{bB}
CA-IR	1,085±41^{aCD}	636±27^{bC}
CC-IR	1,265±53^{aB}	773±36^{bB}
Vit C-IR	1,359±41^{aC}	512±37^{bD}
CL-Vit C-IR	1,558±52^{aB}	931±48^{bA}

[1)]CON, non-treated and non-irradiated *kimchi*; IR, gamma-irradiated *kimchi* at 25 kGy; CL-IR, gamma-irradiated *kimchi* at 25 kGy after treatment of 0.01% of calcium lactate; CA, calcium acetate; CC, calcium chloride; Vit C, vitamin C.
[2)]$^{a-b,A-D}$Values with different letters within a row and column, respectively, differ significantly ($p<0.05$).

considered as a pretreatment method to efficiently reduce the extent of damage to the *kimchi* texture due to irradiation. During storage at 35°C for 30 days, the hardness of the control sample was drastically reduced from 1,953 to 361 g. But the hardness of the co-treatment of CL and Vit C (931 g) was the highest in all the samples during storage. In general, the fermentation of *kimchi* is progressed with a decrease of the pH and a decline of the hardness by the action of fermentation microorganisms (11-13). Even though irradiation technology can inactivate microorganisms, a high ionizing energy can also weaken the cellulose structure of *kimchi* (8,14). As reported in several researches, addition of calcium could retard a decrease of the hardness of *kimchi* during storage. Generally, the crispiness of *kimchi* is closely related to the cell wall polysaccharides, such as pectin (15). Calcium dissolved from a calcium salt combines with pectin and increases the firmness of the tissue (16,17), thus the crispiness of the *kimchi* is improved. It was found that a calcium dip increased the firmness of sliced apples, because the calcium prevented an irradiation-induced softening in thin apple slices (18). Magee *et al.* (19) also found that dipping sliced Roma tomato with 1% of calcium chloride solution or 2% of calcium lactate solution enhanced the firmness and decreased the water-soluble pectin. Fan *et al.* (20) reported that fruit slices were softened during irradiation and storage, but this decrease in the firmness during storage was reduced by a calcium treatment.

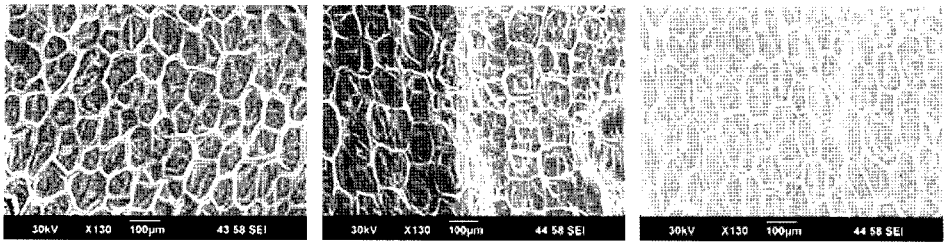
Meanwhile, antioxidants are regarded as compounds that are able to delay, retard, or prevent oxidation processes. They can interfere with an oxidation by reacting with free radicals, chelating metals and also by acting as oxygen scavenger, a triplet form as well as a singlet form and by transferring hydrogen atoms to a free radical structure (20). The result from the texture analysis indicated that the co-pretreatment of CL and Vit C was considered as an efficient method to retard the softening of the *kimchi* gamma-irradiated at 25 kGy.

Effect of the pretreatment of calcium salts and vitamin C on the sensory properties of irradiated *kimchi* The changes of the sensory properties of gamma-irradiated *kimchi* added with different calcium salts and vitamin C during storage at 35°C are shown in Table 3. The result was similar to that of the texture analysis. Gamma irradiation deteriorated the sensory quality of the *kimchi* in respect to texture, taste, and overall acceptance. But it appeared that an individual addition of the calcium salts and vitamin C inhibit a softening of the *kimchi* due to a radiation treatment. Among the calcium salts, CL was more effective in inhibiting the deterioration of the sensory quality of *kimchi* by irradiation. During storage at 35°C for 30 days, the sensory acceptance of the control sample was drastically reduced ($p<0.05$) because pH and acidity of untreated *kimchi* (CON) sample are considerably changed after 30 days (data not shown). But the texture, taste, and overall acceptance of the gamma-irradiated *kimchi* at 25 kGy after a treatment of 0.01% of CL and 0.3% of Vit C (CL-Vit C-IR) were the highest in all the samples after 30 days at 35°C. Powerful radicals generated from an ionization of water by radiation, randomly react with the molecules in *kimchi*, and the glycosidic bonds of carbohydrate are cleaved by the reaction of the radicals with ester bond and then the texture of the vegetable is tenderized (21,22). The result from the sensory evaluation reconfirmed that the co-pretreatment of CL and VC could be used to retard or inhibit the deterioration of textural quality occurred by high-dose irradiation.

Table 3. Change of the sensory properties of the irradiated *kimchi* added with different calcium salts (0.01%) and vitamin C (0.3%) during storage at 35°C

Sample[1)]	Texture		Taste		Overall acceptance	
	0 day	30 day	0 day	30 day	0 day	30 day
CON	6.9±0.7$^{aA2)}$	5.2±0.5^{bA}	6.7±0.5^{aA}	2.3±0.2^{bC}	6.7±0.7^{aA}	2.1±0.2^{bC}
IR	4.3±0.3^{aC}	3.1±0.4^{bC}	4.3±0.3^{aC}	4.4±0.5^{bB}	4.6±0.5^{aB}	4.5±0.3^{bB}
CL-IR	6.1±0.5^{aA}	4.8±0.3^{bAB}	6.2±0.5^{aA}	5.8±0.5^{bA}	6.1±0.5^{aA}	5.9±0.4^{bA}
CA-IR	5.2±0.4^{aB}	3.7±0.3^{bBC}	5.5±0.6^{aB}	5.1±0.4^{bAB}	5.6±0.6^{aAB}	5.3±0.5^{bAB}
CC-IR	5.7±0.6^{aAB}	4.4±0.4^{bB}	5.7±0.4^{aAB}	5.3±0.5^{bA}	5.9±0.5^{aA}	5.4±0.6^{bA}
Vit C-IR	5.8±0.4^{aA}	4.1±0.5^{bBC}	5.2±0.4^{aB}	5.4±0.2^{bA}	6.2±0.4^{aA}	5.4±0.5^{bA}
CL-Vit C-IR	6.2±0.5^{aA}	5.7±0.6^{aA}	6.3±0.3^{aA}	6.0±0.4^{aA}	6.5±0.5^{aA}	6.2±0.5^{bA}

[1)]CON, non-treated and non-irradiated *kimchi*; IR, gamma-irradiated *kimchi* at 25 kGy; CL-IR, gamma-irradiated *kimchi* at 25 kGy after treatment of 0.01% of calcium lactate; CA, calcium acetate; CC, calcium chloride; Vit C, vitamin C.
[2)]$^{a-b,A-D}$Values with different letters within a row and column, respectively, differ significantly ($p<0.05$).



CON IR CL-Vit C-IR

Fig. 1. Scanning electron microscope photographs (130×) of the epidermis tissue of the *kimchi*. CON, *kimchi* non-treated with calcium salt, vitamin C, and gamma irradiation; IR, *kimchi* only gamma irradiated at 25 kGy; CL-Vit C-IR, *kimchi* added with calcium lactate (0.01%), vitamin C (0.3%), and gamma-irradiated at 25 kGy.

Microscopic photograph of the *kimchi* epidermis tissue

Figure 1 shows the microscopic photographs on the epidermis tissue of CON, gamma-irradiated *kimchi* at 25 kGy (IR), and CL-Vit C-IR. The microstructure of CON was arranged well, and the cell walls are the thickest in those of the other samples. But gamma irradiation at 25 kGy induced to a collapse of the microstructure and destroyed the cell walls in the epidermis tissue of *kimchi*. The cell walls and microstructure of CL-Vit C-IR sample relatively thick and well arranged compared to the IR sample. Nayak *et al.* (23) reported on a progressive breaking up of a cell wall structure of potato under a light microscope with an increase in the irradiation dose up to 12 kGy, which resulted in softening of the irradiated potato samples, as evident by the hardness data. Therefore, the microscopic observation of the *kimchi* samples indicates that the co-pretreatment of CL and Vit C was an efficient method for enhancing the firmness of the *kimchi* sterilized with high-dose irradiation.

Acknowledgment

This research was supported by the Top Brand Project Research of Korea Science and Engineering Foundation; Ministry of Science and Technology; Korean government through its National Nuclear Technology Program; and the Daegu University Research Fund, 2006.

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