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Inhibitory Effects of *Kimchi* Extracts on the Growth and DNA Synthesis of Human Cancer Cells

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Abstract

Effect of solvent extracts and juice supernatants from *kimchis* on the growth of various human cancer cells was studied, comparing with the actions on normal cells. Inhibitory effect of *kimchi* extracts on [^{3}H] thymidine incorporation in cancer cells was also investigated. The methanol extract, hexane extract and methanol soluble fraction (MSF) of 3-week fermented *kimchi* did not have growth inhibitory effect on Ac2F rat normal liver cells at the concentrations of 0.5~2%. However, marked decrease in the growth of AGS human gastric cancer cells was shown by the treatment of those extracts. The juice from the *kimchi* samples also suppressed the growth of K-562 human leukemia cells and MG-63 human osteosarcoma cells. Especially, the juice of 3-week fermented *kimchi* exhibited the strong growth inhibitory effect in MG-63 human osteosarcoma cells. At the photomicrographs, growth inhibition and morphological change of the cells treated with *kimchi* juice were observed. And the solvent extracts of 3-week fermented *kimchi* supressed the growth of cancer cells more than the extracts or juices from fresh and 6-week fermented *kimchi*. When AGS human gastric cancer cells were treated with the extracts of 3-week fermented *kimchi*, [^{3}H] thymidine incorporation in the cells also decreased. These results showed that *kimchi* extracts and juices had growth inhibitory effects on human osteosarcoma, leukemia and gastric cancer cells, but had no toxicity to the normal cells. We suggest that *kimchi* might have anticancer effect in part due to inhibition of the growth and DNA synthesis of cancer cells.

Key words: *kimchi*, human cancer cells, DNA synthesis

INTRODUCTION

Kimchi, a Korean traditional fermented food, is prepared with various vegetables such as Chinese cabbage, radish, spices, and other seasonings. The fermentation of *kimchi* is carried out by the microoganism, mainly the lactic acid bacteria, naturally present in the raw vegetable substances. It has been reported about 187 varieties of *kimchi* exist, depending on the ingredients and preparation methods used, where biochemical, microbiological, and nutritional characteristics are different (1,2).

Nutritionally, *kimchi* is an important source of vitamins, minerals, dietary fiber, and other nutrients. The vitamin B groups and ascorbic acid are already present in the raw materials and may be synthesized during the fermentation (3,4). It also contains high levels of organic acid and lactic acid bacteria (5).

Kimchi contains large amount of ascorbic acid (6,7), carotenoids (8), flavonoids (9) which are known to suppress the formation of carcinogenic or mutagenic compounds, and to inhibit mutagenicity induced by several carcinogens. The yellow-green vegetables, the major source of *kimchi*, have dietary fiber which can have an positive effect on the prevention of colon cancer. Park et al. (10,11) reported that around 30 vegetables that are found in Korean diets had antimutagenic activities on aflatoxin B_1, 4-nitroquinoline-1-oxide, and inhibitory activity to the growth of AZ-521 human gastric cancer cells and antitumor effect to mice. Son et al. (12,13) reported that, whether the lactic acid bacteria isolated from *kimchi* were viable or nonviable, the antimutagenicities were still active and the antimutagenic activity of the cell wall fraction was stronger than that of the cytosol fraction. The extracts from red pepper (14) and garlic (15) used as *kimchi* ingredient are also believed to have the antimutagenic and anticarcinogenic effects. Recently, several health functions of *kimchi* were reported, such as the antimutagenicity and the anticancer effects (16,17). But many studies have still reported on the fermentations and preservation of *kimchi*. A few studies reported on the protective effects and their mechanisms of *kimchi* aganist cancer.

To better understand the anticancer activity and mechanism of *kimchi*, we studied the effects of solvent extracts and juices from *kimchis* on the growth of various human cancer cells, comparing with the action on normal cells. Inhibitory effects of *kimchi* extracts on [^{3}H] thymidine incorporation in cancer cells were also investigated.

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MATERIALS AND METHODS

Preparation of *kimchi*

Chinese cabbage grown in Kimhae, was used as major raw ingredient for *kimchi*. Garlic, ginger and red pepper powder were purchased from a local market. Chinese cabbage was divided into 8 pieces, brined in 10% salt solution for 10 hours, and rinsed with fresh water. Drained chinese cabbage was cut into 4 to 5 cm in size. The ingredients and their proportions for *kimchi* are shown in Table 1. The final weight percentage of salt in *kimchi* was adjusted to 3%. The unfermented fresh *kimchi* (fresh *kimchi*) and the *kimchi* fermented for 3 and 6 weeks at 5°C (3 and 6 weeks fermented *kimchis*) were used as test samples.

Determinations of pH, acidity and the level of reducing sugar

The blended *kimchi* samples were filtered by cheese cloths. The pH of filterate was measured with pH meter. The acidity was determined by the method of AOAC (18). 0.1% phenolphthalein was dropped to the filtrate and titrated with 0.1 N NaOH, and then the lactic acid content was calculated and expressed as the acidity (%). To analyze the content of reducing sugar, the filtrate was diluted with distilled water. The Fehling solution was added to test sample and the mixture was heated. After cooling, the amount of reducing sugar was determined by the method of Schoorl (19), using titration with 0.1 N $Na_2S_2O_3$ standard solution.

Solvent extraction of *kimchi*

Kimchi samples (fresh *kimchi*, 3 and 6 weeks fermented *kimchis*) were freeze-dried and minced by a blender. The minced *kimchi* samples (25 g) were extracted with methanol (500 ml), three times, by shaking for 8 hours and then taken as methanol extract. Another minced *kimchi* samples (25 g) were extracted with hexane (500 ml) by the same method as methnol extraction. After taking hexane extract, 500 ml of methanol was added to the residues and shaken for 16 hours, followed by reflux for 90 minutes at 70~80°C water bath. After filtration, methanol soluble fraction (MSF) was taken. The *kimchi* extracts were dried by rotary vaccum evaporator (Buchi 011 & 461, Switzerland) and then dissolved in dimethyl sulfoxide (DMSO).

Juice supernatant of *kimchi*

Kimchi was minced by a blender and centrifuged at 10,000 rpm for 10 minutes. The supernatant was filtrated through 0.45 μm (pore size) membrane and then used for test.

Table 1. Compositions (%) of ingredients when the *kimchi* was prepared

Ingredient	Composition
Chinese cabbage	100% (3000 g)
Red pepper powder	2% (60 g)
Crushed garlic	2% (60 g)
Crushed ginger	0.5% (15 g)
Final salt concentration	3.0%

Chemicals

Dulbecco's modified eagle's medium (DMEM), fetal calf serum (FCS), 0.05% trypsin-0.02 EDTA, penicillin-streptomycin were obtained from Gibco Chemical Co. (Grand Island, NY, USA). Phosphated buffered saline (PBS, pH7.2) and 24 wells microplates were purchased from Sigma Chemical Co. (St. Louis MO, USA) and Costar Co. (Cambridge, MCA, USA), respectively. All chemicals used in the present experiment were sterilized through millipore membrane filtration or autoclaved.

Inhibitory effects on the growth of cancer cells

Normal cell

Ac2F cells, rat normal liver cells were obtained from Japanese Cell Line Collection (Tokyo, Japan). The medium used for the cells was DMEM supplemented with 20% fetal calf serum (FCS) and 100 unit/ml of penicillin-streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C. A medium change was made on the 2nd day after seeding. The cells were transferred every 8 days, using PBS and 0.05% trysin-0.02% EDTA, and new flasks were seeded with 5×10^4 cells in 5ml of medium each.

Cancer cells

MG-63 (human osteosarcoma cells), K-562 (human leukemia cells) and AGS (human gastric cancer cells) cells were obtained from Korean Collection for Type Culture (Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FCS and 100 unit/ml of penicillin-streptomycin at a humidified atmosphere of 5% CO_2 at 37°C. A medium change was made on the 2nd day after seeding. The cells were transferred every 6~7 days, using PBS and 0.05% trypsin-0.02 EDTA. After 10 times of passage, new cancer cells were taken from liquid nitrogen tank and cultured again.

Growth inhibition assay

Ac2F, MG-63, K-562 and AGS cells were plated at a concentration of 4×10^4 cells per well of 24 well microplate. The cells were allowed to adhere in each well for overnight at 37°C in a 5% CO_2. The medium was changed with DMEM supplemented with 1% (10 μl per ml) of *kimchi* samples and 20% (for normal cell) or 10% (for cancer cells) FCS. A medium change was made on the 2nd day after seeding and the medium was changed every two days. Following 6 days, the cells were washed with PBS, seperated with 0.05% trysin-0.02 EDTA and counted with hemocytometer. Morphological appearances of the cells were also observed with optical microscope.

[^{3}H] thymidine incorporation assay

Cancer cells were plated at a concentration of 4×10^4 cells/well in 24 well microplate and cultured for overnight at 37°C under 5% CO_2. The medium was changed with DMEM supplemented with 10% FCS and 1% (10 μl per ml) of *kimchi* samples, and cultured for 48 hours at 37°C. The cells were refeeded by the medium with [^{3}H] labelled thymidine (3 μCi per ml) and cultured again. After 2 times of washing with

PBS, the cells were added by 5% of TCA (cold) and then left for 1 hour at 4°C. TCA was discarded and, for the seperation of cells, 1% of SDS was added. Those were maintained at 55°C for 1 hour. The cells were transferred to scintillation vial, washed with 125 μl of distilled water and 3.5 ml of scintillation cocktail was added. After vortexing, the radioactivity was determined by Berkman LS 250 scintillation counter.

Statistical analysis

Data were presented in means±SD after one-way ANOVA analysis. Significant differences of treatment from the control were determined by using the Student's *t* test.

RESULTS AND DISCUSSION

pH, acidity and reducing sugar level of *kimchis*

The pH of *kimchi* decreased during fermentation, as shown in Table 2. The pH of fresh (unfermented) *kimchi* was 5.7 but those of 3 and 6 week-fermented *kimchis* were 4.3 and 3.9, respectively.

Fermentation increased the acidity of *kimchi* which was calculated as the amount of lactic acid (Table 2). The acidity of fresh *kimchi* was 0.33%. After fermentation of 3 and 6 weeks, the acidities of the *kimchis* were 0.59% and 0.77%, respectively. The reducing sugar level of fresh *kimchi* was 2.37, but those of 3 and 6 weeks fermented *kimchis* were 1.07 g% and 0.64 g%, respectively (Table 2). It was known that the sugars presented in baechu *kimchi* were glucose, mannose, fructose, galactose, and arabinose (20,21). And, during fermentation, sugar compounds dissolved slowly and moved to the liquid from tissue (20,21). As the fermentation was proceeded, while the amount of reducing sugar and pH decreased, the acidity increased. It has been reported that an organic acids and a related decrease in pH are typical phenomena in *kimchi* fermentation (5).

Effect of *kimchi* extracts on normal cell

Before the treatment of cancer cells, the extract of 3 week fermented *kimchi* were administered to the culture system of Ac2F cells, rat normal liver cell, for 6 days. As shown in Table 3, the growth of Ac2F cells did not decrease by methanol extract and hexane extract after 6 days. Methanol soluble fraction (MSF) did not also exhibit inhibitory effect on the growth of Ac2F cells. These results suggested that the extracts of properly ripened *kimchi* did not have the growth inhibitory effect on the normal cell.

Table 2. Changes in level of reducing sugar, acidity, and pH during fermentation of *kimchi* at 5°C

	Fresh *kimchi*	3 weeks fermented *kimchi*	6 weeks fermented *kimchi*
Reducing sugar (g%)	2.37±0.04[1)]	1.07±0.02	0.64±0.02
Acidity (%)	0.33±0.01	0.59±0.01	0.77±0.03
pH	5.7	4.3	3.9

[1)]Data are means±SD.

Table 3. Growth inhibitory effect of various concentration of methanol extract, hexane extract and methanol soluble fraction (MSF) from 3 week fermented *kimchi* in Ac2F normal liver cells after 6 days of incubation at 37°C

Concetration (%)	Number cells ($\times 10^4$/ml)
Control	35.8±4.9
Methanol ext.	
0.5%	36.7±4.7
1.0%	35.5±1.7
2.0%	30.8±2.3
Hexane ext.	
0.5%	33.2±2.0
1.0%	35.3±3.0
2.0%	37.3±2.9
MSF	
0.5%	32.2±2.5
1.0%	36.2±1.9
2.0%	36.8±3.6

[1)]Data are means±SD.

Growth inhibitory effects of solvent extracts and juice supernatants of *kimchis* in cancer cells

Fig. 1 shows the effect of extracts of fresh *kimchi* on the growth of AGS cells, human gastric cancer cell, after 6 days of incubation. Methanol extract, hexane extract and MSF from fresh *kimchi* significantly inhibited the growth of AGS cells at the concentrations of 1~2% compared to control ($p<0.05$). By the treatment with 2% methanol extract, the growth of AGS cells was reduced by 31%. Hexane extract (1%) and MSF (2%) also inhibited the growth of AGS cells by 24% and 34%, respectively. But when 0.5% of each extracts from fresh *kimchi* was added to the medium, growth of AGS cells was not inhibited after 6 days.

As shown in Fig. 2, 2% of methanol extract from 3 weeks fermented *kimchi* suppressed the growth of AGS cells by 63%. With the addition of 1% of hexane extract and 2% of MSF

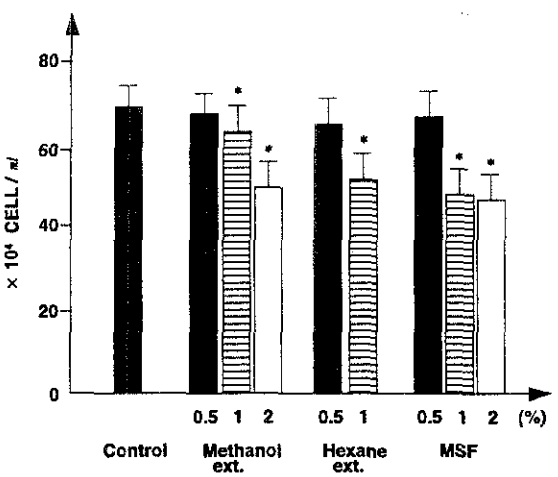

Fig. 1. Growth inhibitory effect of various concentrations of methanol extract, hexane extract and methanol soluble fraction (MSF) of fresh *kimchi* in AGS human gastric cancer cell after 6 days of incubation at 37°C. *Significantly different at the $p<0.05$ level by student t-test.

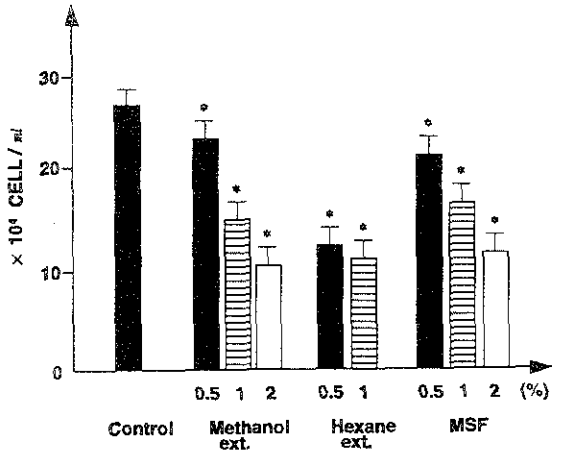

Fig. 2. Growth inhibitory effect of various concentrations of methanol extract, hexane extract and methanol soluble fraction (MSF) of 3 week fermented *kimchi* in AGS human gastric cancer cell after 6 days of incubation at 37°C. *Significantly different at the $p<0.05$ level by student t-test.

to the culture system, the growth of AGS cells was inhibited by 61% and 59%. In previous study (22), the extracts of 3 weeks fermented *kimchi* had inhibitory activity for His to His^+ reverse-mutations induced by AFB_1 in *Salmonella typhimurium* TA98. In *Drosophila* wing hair spot test, mutation clone frequency induced by AFB_1 was considerably inhibited by the *kimchi* extracts (22). The results indicated that 3 week fermented *kimchi*, the properly ripened *kimchi*, might have much antimutagenic and anticarcinogenic compounds compared with fresh *kimchi*.

Inhibitory effects of extracts from 6-week fermented *kimchi* on the growth of AGS cells were weaker than those of extracts from 3 weeks fermented *kimchi*, but stronger than those from fresh *kimchi* (Fig. 3). The treatment of 2% of methanol extract and 1% of hexane extract suppressed AGS cells by 45% and 58%, respectively. MSF also inhibited the growth of AGS cells by 56%, at a concentration of 2%. Among extracts of 6 weeks fermented *kimchi*, effect of hexane extract was the strongest.

When AGS cells were treated with *kimchi* juices for 6 days, 1% of juices inhibited the growth more than 2% of those (Fig. 4). The trends of inhibitory effects of juices from fresh *kimchi* were similars with those of 3 and 6 weeks fermented *kimchis*.

Table 4 shows the effects of juice supernatants of *kimchis* on the growth of K-562 human leukemia cells and MG-63 human osteosarcoma cells after 4 days of incubation. At a concentration of 10 μl/ml of juice supernatant, growth inhibitory effects of 3 weeks fermented *kimchi* were stronger than those of fresh and 6 weeks fermented *kimchi*, and the effect of fresh *kimchi* was the weakest in K-562 human leukemia cells. But growth inhibitory effects of fresh and 3 weeks fermented *kimchi* were 84% and 81%, respectively, by the addition of 20 ul/ml. On the growth of MG-63 cells, juice supernatnants of 3-week fermented *kimchi* had stronger inhibitory effect than those of other *kimchis* at a concentration of 20 μl/ml. However, growth inhibitory effect of juice supernatnants from *kimchis* in MG-63 cells were weaker than those in

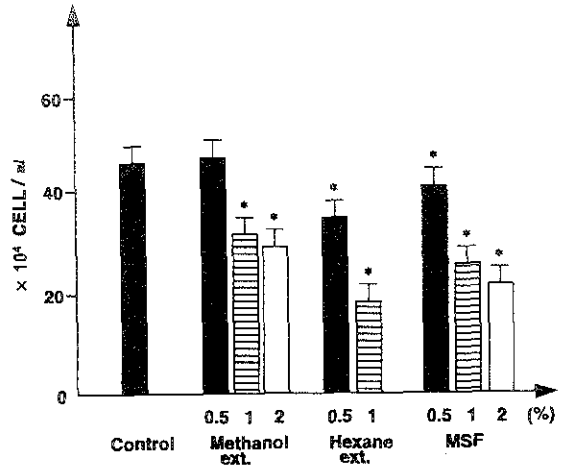

Fig. 3. Growth inhibitory effect of various concentrations of methanol extract, hexane extract and methanol soluble fraction (MSF) of 6 week fermented *kimchi* in AGS human gastric cancer cell after 6 days of incubation at 37°C. *Significantly different at the $p<0.05$ level by student t-test.

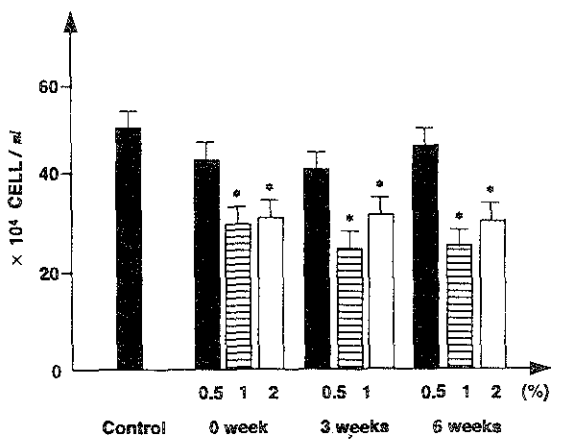

Fig. 4. Growth inhibitory effect of various concentrations of juice supernatants from *kimchis* fermented for different time (week) at 5°C in AGS human gastric cancer cells after 6 days of incubation at 37°C. *Significantly different at the $p<0.05$ level by student t-test.

Table 4. Growth inhibitory effect of juice supernatants of *kimchis* in K-562 human leukemin cells and MG-63 human osteosarcoma cells after 4 days of incubation at 37°C

Sample juice supernatant	Human leukemia K-562 cells		Human osteosarcoma MG-63 cells
	10 μl/ml	20 μl/ml	20 μl/ml
Control	104± 5[1)]	83±11	68±10
Fresh *kimchi*	83± 4 (20)[3)]*	13± 2 (84)*	57± 7 (13)*
kimchi (3wk)[2)]	29±12 (72)*	20± 4 (81)*	23± 3 (66)*
kimchi (6wk)	56±10 (46)*	51± 2 (51)*	30± 4 (56)*

[1)]Data are means±SD.
[2)]*Kimchi* was fermented at 5°C for 3 or 6 weeks
[3)]Inhibition rate
*Significantly different at the $p<0.05$ level by student t-test.

K-562 cells.

Fig. 5 shows morphological appearence of MG-63 cells treated with 20 μl/ml of juice supernatant from 3 weeks fermented *kimchi* for 2 days at 37°C. At the photomicrographs, growth inhibition and morphological change of the cells were observed.

In results above, the solvent extracts from 3-week fermented *kimchi* inhibited the growth of cancer cells more than the extracts or juices of fresh and 6-week fermented *kimchis*. In our previous report (23), the hexane extract and MSF of 3 weeks fermented *kimchi* suppressed carcinogen-induced cytotoxicity and transformation in C3H/10T1/2cells. Since the effects revealed from all the *kimchi* extracts and juices, it seemed that active compounds are more than one, might be water soluble and lipid soluble ones or both.

Inhibitory effects of the *kimchi* extracts on DNA synthesis in cancer cells

In above result, growth inhibitory effects of extracts from 3-week fermented *kimchi* on the growth of AGS cells were very strong. Therefore the effects of those extracts on [^{3}H] labelled thymidine incorporation in AGS cells were studied after 4 days of incubation.

As shown in Table 5, methanol extract, hexane extract and MSF of 3-week fermented *kimchi* significantly suppressed DNA incorporation of [^{3}H] thymidine in AGS cells at the concentration of 0.5~2%, compared to control ($p<0.05$). The more the concentration of extract was, the higher the inhibition rate of DNA incorporation was. These results showed that *kimchi* extracts decreased the DNA synthesis.

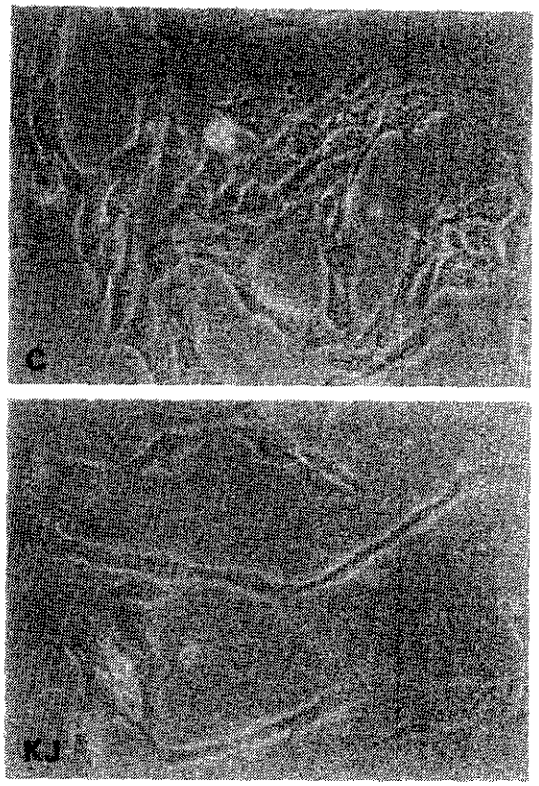

Fig. 5. Photomicrogrophs of MG-63 human osteosarcoma cells treated 20 μl/ml of juice supernatant of 3 week fermented *kimchi* for 2 days at 37°C (×200). C: control, KJ: juice supernatant treatment

Table 5. [^{3}H] Thymidine incorporation at different concentration of methanol extract, hexane extract and methanol soluble fraction (MSF) from 3 weeks fermented *kimchi* in AGS human gastric cancer cells

Concentration	[^{3}H] Thymidine incorporation /well×10^3 cpm	Inhibition
Control	13.5±0.6[1)]	
Methanol ext.		
0.5%	12.2±0.4*	10
1.0%	7.4±0.4*	46
2.0%	6.5±0.6*	52
Hexane ext.		
0.5%	5.7±0.5*	58
1.0%	4.5±0.2*	66
MSF		
0.5%	12.4±0.3*	8
1.0%	9.2±0.6*	32
2.0%	8.3±0.3*	38

[1)]Data are means±SD.
*Significantly different at the $p<0.05$ level by student t-test.

Oh et al. (24) reported that high consumption of *kimchi* subjects might prevent the colon cancer and the factors in *kimchi* inhibit the activities of β-glucuronidase and nitroreductase that mediate the conversion of precarcinogens to carcinogens involved in colon cancer. And *kimchi* contains high levels of vitamin C and carotenoids which are well known as antioxidative, antimutagenic and anticancer compounds (25,26).

The major ingredients of *kimchi* are green-yellow vegetables which are known to have antimutagenic and anticancer effects (10,11). And *kimchi* is fermented by lactic acid bacteria which have anticancer function (12,13,27,28) and can be prepared with the anticarcinogenic foods. Therefore, *kimchi* can be a district anticancer functional food.

To elucidate the anticarcinogenic function of *kimchi*, further study on *in vivo* effects and action mechanism of it is needed. More research regarding the anticancer compounds that might be produced during the fermentation will have to be continued.

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