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Effect of Red Pepper Seed on *Kimchi* Antioxidant Activity During Fermentation

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Abstract In this study, the antioxidant activities of red pepper seed *kimchi* extracts were examined. The extracts were evaluated by various antioxidant assays that included determinations of total phenolic and flavonoid contents, DPPH radical scavenging, superoxide anion radical scavenging, nitric oxide scavenging, superoxide dismutase (SOD) activity, metal chelating activity, and reducing power. All the *kimchi* extracts showed strong antioxidant activities by the tested methods. The 7% red pepper seed *kimchi* that was fermented for 6 days possessed the highest activity according to the performed tests. Yet, the highest scavenging activity was exhibited against nitrite, by extracts from *kimchi* for 0 day of fermentation rather than 6 days. All the activities for the *kimchi* fermented for 0 day with the addition of 7% red pepper seed increased markedly with an increase in concentration. With the exception of metal chelating and SOD activities, for the antioxidant properties assayed, the red pepper seed *kimchi* extracts were found to be highly effective.

Keywords: antioxidant activity, red pepper seed *kimchi*

Introduction

Kimchi is a traditional Korean fermented vegetable food that is consumed every day as the main side dish of the Korean diet. Fermented *kimchi* generally contains high levels of lactic acid bacteria (10^{7-9} CFU/mL), organic acids, and other nutrients such as vitamins, minerals, dietary fiber, as well as functional components of the ingredients from fermentation (1). Many benefits of *kimchi* have been reported, including antimutation and anticancer effects (1), hypolipidemic effects (2), antioxidant and antiaging effects (3), and the activation of detoxifying components (4). *Kimchi* is widely known as a nutritious and health promoting food (5,6).

The antioxidant effects of *kimchi* have been defined by *in vitro*, *in vivo*, and in clinical studies. *Kimchi* can inhibited linoleic autooxidation (7) and low density lipoprotein (LDL) oxidation (8), and scavenged free radicals (9-11). Many studies have suggested that the antioxidative characteristic of *kimchi* is one of the mechanisms for its anti-mutagenecity, anti-atherogenecity (12), and anti-aging qualities (9-11). The antioxidative compounds in *kimchi*, by acting as hydrogen donors, may eliminate free radicals in the body. *Kimchi* was shown to inhibit Cu^{2+} induced LDL oxidation. Also, the dichloromethane fraction of *kimchi* showed the highest antioxidant effect against LDL oxidation by inhibiting thiobarbituric acid (TBARS) production, and had a prolonged lag-phase duration by 2-fold compared to the control (13). The liver homogenates of the experimental group containing the dichloromethane fraction of the *kimchi* inhibited LDL oxidation in the presence of Cu^{2+} by 46% (14). The low enzyme activities observed in the *kimchi* solvent fractions added to the diet groups might be due to the fact that the rate of lipid oxidation decreased less in the control. The antioxidative effects of cabbage, red pepper powder, and garlic were evaluated in rabbits fed 1% cholesterol for 3 months. Plasma TBARS and peroxide value (POV) levels were reduced in both the red pepper powder and garlic fed rabbits ($p<0.05$) compared to the control. Plasma vitamin E concentrations increased in the rabbits that were fed red pepper powder and garlic. For the hepatic antioxidative enzyme activities, catalase activity was significantly increased in the red pepper powder- and garlic-fed rabbits compared to the control. Therefore, *kimchi* ingredients such as red pepper powder and garlic play important roles in its antioxidative effects (8). In Korea, red pepper has been a valuable ingredient of *kimchi*, contributing its characteristic sensory qualities of red color and a piquant taste (15).

Red pepper (*Capsicum annuum* L.) is a vegetable known for its rich antioxidant content. Fresh red peppers are especially high in ascorbic acid, with 116 mg per 100 g. Their attractive red color is due to various carotenoid pigments that include β-carotene with pro-vitamin A activity, and oxygenated carotenoids such as capsanthin, capsorubin, and cryptocapsin, which are distinct to this genus and are shown to be effective free radical scavengers (16). Red peppers also contain high levels of natural phenolics and flavonoids like quarcetin, luteolin, and capsaicinoids (17). Capsicum cultivars have been identified as vegetables with potentially high antioxidant activities (18). Research in our laboratory on the biological effects of red pepper has focused on their influence in lipid metabolism, their action in digestive acceleration, the beneficial influence of their hypocholesterolemic effect on cholesterol gallstone disease and diabetic nephropathy, and their antioxidant influence on inflammatory disease (19). Red pepper's lipolytic effect in fat cells has been observed, and lipolytic activity increases with the degree of piquant taste, implicating that capsaicin is the compound responsible for this activity. In addition, red pepper has

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shown a higher antioxidant activity than other *kimichi* seasonings. However, limited information is available on the antioxidant content and activity of red pepper seed *kimchi*. In this study, we used *kimchi* containing added red pepper seed to examine the antioxidant effects of the red pepper seed *kimchi*, as well as its antioxidant content over the fermentation period.

Materials and Methods

Chemicals Potassium ferricyanide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA), trizma base, 2 N Folin-Ciocalteu reagent, and naphthyl-ethylenediamine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ferrous chloride and ethylenediamine tetracetic acid (EDTA) were purchased from Junsei Chemical (Tokyo, Japan). Pyrogallo and sulphanilamidein were purchased from Yakuri Pure Chemical (Tokyo, Japan). All other chemicals and solvents used were of commercial analytical grade.

Materials Chinese cabbage, garlic, scallion, ginger, onion, fermented anchovy juice, and salt were purchased on the day of *kimchi* preparation at a local market in Seoul, Korea and stored in a refrigerator at 5°C. The red pepper seed was purchased from Banhemaru Co. (Umsung, Korea). The Chinese cabbage was cut into 4 pieces and steeped in 10% salt water for 12 hr and then rinsed 3 times with water. The standardized recipe for *kimchi* was 3.0 g of onion, 1.0 g of garlic, 1.0 g of scallion, 1.0 g of fermented anchovy juice, 1.0 g of glutinous rice flour, 0.5 g of ginger, 0.2 g of sugar, and 0.3 g of salt in proportion to 100 g of salted Chinese cabbage. The final salt concentration was adjusted up to 2.5%. The red pepper seed *kimchi* was made by the above (control *kimchi*) recipe. The additions of red pepper seed in proportion to 100 g of salted Chinese cabbage were 1, 3, 5, and 7%. The prepared *kimchi* (0 day) was put into jars and fermented for 0 and 6 days at 14°C.

Plant materials and preparation of extracts The freeze-dried red pepper seed *kimchi* samples (100 g) were extracted by stirring with 500 mL of 70% ethanol at 80°C for 3 hr 2 times, and then filtered through Whatman No. 2 filter paper. The solvent of the combined extracts was evaporated under a reduced pressure using a rotary vacuum-evaporator at 50°C, and the remaining water was removed by lyophilization. The obtained freeze-dried extract was used directly to determine the total phenolic and total flavonoid contents, as well as to assess antioxidant capacity through various chemical assays.

The amounts of phenolic compounds and flavonoids The total phenolic contents of the red pepper seed *kimchi* extracts were determined using Folin-Ciocalteu reagent (20). The red pepper seed *kimchi* extracts (200 μL) were mixed with 400 μL of 2 N Folin-Ciocalteu reagent and 0.8 mL of 10% sodium carbonate. The mixtures were shaken thoroughly and allowed to stand for 1 hr. Next, the absorbance at 750 nm was determined. The phenolic contents were determined using a standard curve obtained from various concentrations of gallic acid (GAE).

The total flavonoid contents of the red pepper seed *kimchi* extracts were determined using the David deformed method (21). The red pepper seed *kimchi* extracts (1.0 mL) were mixed with 10 mL of diethylene glycol and 0.1 mL of 1 N NaOH. The mixtures were shaken thoroughly and allowed to stand for 1 hr at 37°C. Then the absorbance at 420 nm was determined. The flavonoid contents were acquired using a standard curve obtained from various concentrations of rutin (RE).

Scavenging activity on DPPH radicals Each extract (1.0 mg/mL) in ethanol (4 mL) was mixed with 1 mL of ethanolic solution containing DPPH radicals, resulting in a final concentration of 0.15 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm against a blank (22).

Superoxide anion radical (O_2^-) scavenging activity The superoxide anion scavenging activity of the samples (red pepper seed *kimchi* extracts) was measured via the method of Robak and Gryglewski (23). Aliquots of 1 mL each of the following solutions (prepared in 0.1 M phosphate buffer at pH 7.4) were added to 1 mL (1.0 mg/mL) of the red pepper *kimchi* extracts: 150 μM NBT, 60 μM PMS, and 468 μM NADH, respectively. Scavenging activity on the O_2^- was expressed as: (1−absorbance at 560 nm in the presence of sample / absorbance at 560 nm in the absence of sample)×100.

Metal chelating activity Metal chelating activity was determined according to the method of Dinis *et al.* (24). Each extract (1.0 mg/mL) in water (1 mL) was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank.

Nitric oxide (NO) scavenging activity NO was generated from sodium nitroprusside and measured by the Greiss reaction according to the method described by Kato *et al.* (25). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO (16), which interacts with oxygen to produce nitrite ions that can be estimated by using Greiss reagent. Scavengers of NO compete with oxygen, leading to reduced production of NO. Using Griess reagent, the red pepper seed *kimchi* extracts (1.0 mg/mL) were used to determine nitrite scavenging activity at different conditions (pH 1.2) by measuring the absorbance at 520 nm. The sodium nitrite (1 mM) was mixed with different concentrations of various red pepper seed *kimchi* extracts dissolved in the suitable solvent systems, incubated at 37°C for 1 hr, and then reacted with Greiss reagent (1% sulphanilamidein in 30% acetic acid, and 0.1% naphthylethylenediamine dihydrochloride in 30% acetic acid). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and the subsequent coupling with naphthylethylenediamine was read at 520 nm, and referred to the absorbance of potassium nitrite standard solutions that were similarly treated with Griess reagent. Thus, the nitrite scavenging activity (%) was calculated with the following equation:

Nitrite scavenging activity (%)=[1−(absorbance of 1 mM $NaNO_2$ added sample after standing for 1 hr−absorbance of control) / absorbance of 1 mM $NaNO_2$]×100

Reducing power The reducing power was determined according to the method of Oyaizu (26). Each extract (1.0 mg/mL) in water (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After 2.5 mL of 100 mg/mL trichloroacetic acid were added, the mixture was centrifuged at 1,017×g for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 1 mg/mL ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

Superoxide dismutase (SOD) activity SOD activity was determined according to the method of Kim *et al.* (27). Each extract (1 mg/mL) in water (0.2 mL) was mixed with 3 mL of 50 mM Tris-HCl buffer [50 mM Tris (hydroxymethyl) amino-methane+10 mM EDTA, pH 8.5] and 0.2 mL of 7.2 mM pyrogallo, and the mixture was incubated 25°C for 10 min. The reaction was stopped by the addition of 1 mL of 1 N HCl. The absorbance of the mixture was determined at 420 nm against a blank.

Statistical analysis The results of the treatments were expressed as means±standard deviations (SD). The data were analyzed by one-way analyses of variance (ANOVA) with SPSS (Statistical Analysis Program, version 12.0) to determine the effects of the red pepper seed additions and fermentation periods. Significant differences between the treatment means were determined using Duncan's multiple range test ($p<0.05$).

Results and Discussion

The amounts of phenolic compounds and flavonoids The extracts were also analyzed for total polyphenol content (Table 1). The order of polyphenol content in the respective *kimchi* sample extracts was as follows; 0 day 0%, 0 day 1%, 0 day 3%, 0 day 5%, 0 day 7%=6 days 0%, 6 days 1%, 6 days 3%, 6 days 5%, and 6 days 7% as gallic acid equivalents. The total phenolics and gallic acid equivalents of the fermented *kimchi* extracts of the raw samples were significantly higher than those of the nonfermented *kimchi* extracts. Peppers contain moderate to high levels of phytochemicals that can contribute to antioxidant activity, such as phenolic acids, which are important components that may reduce the risk of degenerative diseases (17,28). Although increased phenolic compound levels with maturation have been described in pepper fruits (29), other authors have found that it depends on the pepper cultivar (30). For example, Gnayfeed *et al.* (31) found differences between cultivars with respect to capsaicinoid development during maturation, and losses during maturation by some pepper cultivars were related to

Table 1. Comparative total phenolic and flavonoid contents of red pepper seed *kimchi* extracts[1)]

Sample	GAE (mg/g)	RE (mg/g)
0 day 0 %	16.10±0.13[a]	4.55±0.01
0 day 1%	16.96±0.27[b]	4.55±0.01
0 day 3%	17.74±0.26[c]	5.26±0.18
0 day 5%	18.41±0.18[d]	5.71±0.06
0 day 7%	18.99±0.05[e]	6.31±0.12
6 days 0%	18.99±0.45[e]	5.27±0.06
6 days 1%	20.86±0.14[f]	5.51±0.05
6 days 3%	21.50±0.21[g]	7.73±0.15
6 days 5%	22.36±0.32[h]	8.50±0.33
6 days 7%	23.52±0.45[i]	11.37±0.72

[1)]The total phenolic and flavonoid contents are expressed as gallic acid equivalents (GAE) and rutin equivalents (RE), mg/g dry weights of the samples, respectively; the values are means±SD; [a-i]values with different letters differ significantly ($p<0.001$).

peroxidase activity. The results of the DPPH radical assay and Folin-Denis test indicate a strong association between antioxidant activity and phenolic compound content, suggesting that phenolic compounds are probably responsible for the antioxidant activity of red pepper seed. Many previous studies conducted with vegetables and fruit have also found a positive correlation between total phenolic compounds and antioxidant activity, indicating that high total phenol contents increase antioxidant activity (32,33). The total phenol content of *kimchi* increased fermented stage. Uda *et al.* (34) reported that the amount of phenol compounds increases with fermentation time. This is similar to that reported by Woo and Jeong (35), in which the total amount of phenol compounds was high in germinated brown rice *kimchi* after 9-12 days of fermentation compared with the amount measured immediately after its preparation. This increase was due to the presence of phenolic acids such as σ-coumaric and ferulic acids and their interactions with microbes that produced ethyl or vinyl derivatives phenol with antioxidative activity.

Flavonoids are one of the most diverse and widespread groups of natural compounds. Flavones, isoflavones, flavonones, anthocyanins, and catechins are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging and strong antioxidant capacities (36). Most flavonoids possess strong antioxidant properties that follow chain breaking mechanisms (37). Likewise, the total flavonoid contents of the red pepper seed *kimchi* extracts were determined by the David deformed method and are presented as ruin equivalents (RE) in Table 1. Here, the nonfermented *kimchi* contained less flavonoids (RE value 4.55±0.01-6.31±0.12 mg/g) compared to the fermented *kimchi* (RE value 5.27±0.06-11.37±0.72 mg/g).

Scavenging activity on DPPH radicals DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (38). It has been found that compounds such as cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (*p*-nitrophenol diamine, *p*-aminophenol), reduce and bleach DPPH by their hydrogen donating abilities

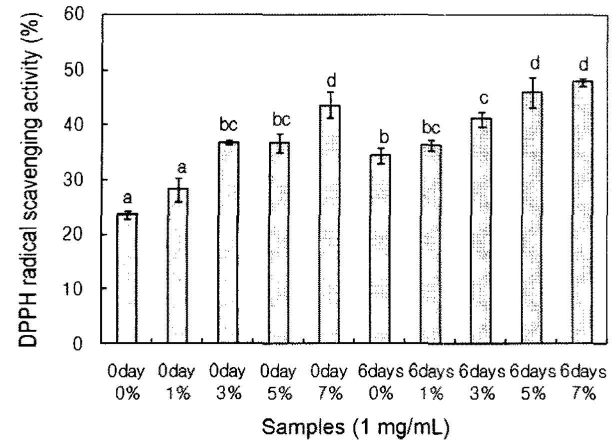

Fig. 1. Free radical DPPH scavenging activities of red pepper seed *kimchi* extracts. Each value represents means±SD (n=3). [a-d]Values with different letters among columns differ significantly (p<0.001).

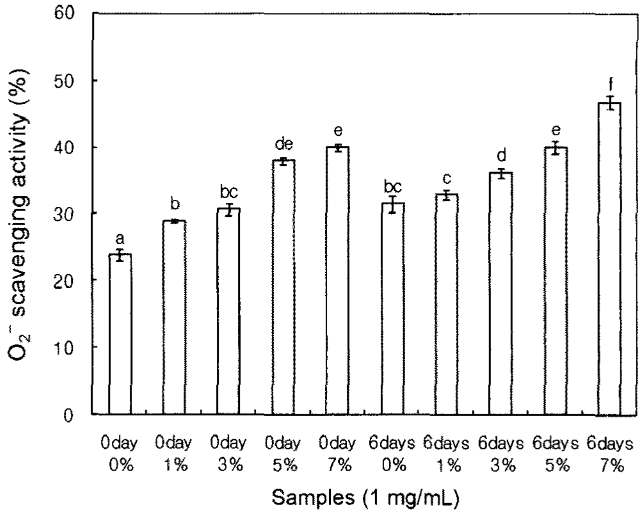

Fig. 2. Superoxide anion radical (O_2^-) scavenging activities of red pepper seed *kimchi* extracts by the non-enzymatic phenazine methosulfate-nicotin amide adenine dimucleotide (PMS-NADH) method. Each value represents means±SD (n=3). [a-f]Values with different letters among columns differ significantly (p<0.001).

(39,40). Previous studies have also shown linear relationships between total phenolic or anthocyanin content and antioxidant capacity in some berry crops (41).

The DPPH radical scavenging activities of the red pepper *kimchi* extracts are shown in Fig. 1. Antioxidant activities, as measured by the DPPH radical, ranged from 23-43% in the nonfermented *kimchi*, and from 34-47% in the fermented *kimchi*. These variations may have been caused by differences either in potency, or in the concentration of reducing substances (mainly phenolics). Red peppers contain numerous phenolic compounds, and it's possible not all genotypes contain similar profiles or relative proportions of compounds within the profile. Differences among these profiles may subsequently result in complex changes in antioxidant activity or other bioactivities (42). Various researchers have used different assay systems to determine the antioxidant activity of Capsicum (29,30). Therefore, red pepper seed may increase the antioxidant activity of *kimchi* in addition to the fermentation time. This is similar to that reported by Woo and Jeong (35) in which DPPH radical scavenging activity increased significantly with increasing concentrations of germinated brown rice and fermentation time. Generally, many studies have reported that red pepper was effective in scavenging free radicals, and this activity is also contained in red pepper seed.

Superoxide anion radical (O_2^-) scavenging activity Despite its involvement in many pathological processes, superoxide by itself is not as reactive as the well known hydroxyl radicals. Yet, it can give rise to the more toxic hydroxyl radicals, damaging biomacromolecules directly and indirectly (43). Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Some of the electrons escaping from the chain reactions of mitochondria directly react with oxygen and form superoxide anion. This anion plays an important role in the formation of other reactive oxygen species in living systems, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen (44). Superoxide radicals, as such, have been implicated in playing crucial roles in ischaemia-reperfusion injury (45).

The superoxide anion scavenging activities of the red pepper seed *kimchi* extracts are shown in Fig. 2. All of the extracts had scavenging activities on the superoxide radical at the 1 mg/mL concentration. The superoxide scavenging activities of the red pepper seed *kimchi* extracts increased markedly with the elevations of concentration and fermentation time. The scavenging activity of the red pepper seed *kimchi* showed that its inhibitory potential follows the order; 0 day 0%<0 day 1%<0 day 3%<6 days 0%<6 days 1%<6 days 3%<0 day 5%<0 day 7%<6 days 5%<6 days 7%. The O_2^- scavenging activity ranged from 23-40% in the nonfermented *kimchi*, and from 31-47% in the fermented *kimchi*. Especially, the O_2^- scavenging activity increased with increasing concentrations of red pepper seed and fermentation time. This suggested that differences in antioxidative activity are related to the products produced during fermentation. This is similar to that reported by Woo and Jeong (35) in which O_2^- scavenging activity was low in the control group (9-20%), but increased with the germinated brown rice concentration to the range of 27 to 71%. Hong *et al.* (46) reported that it was high (56-65%) in a fermented pine needle extract. Overall, the potential scavenging abilities of the phenolic substances may be due to the active hydrogen donor ability of hydroxyl substitution (47). Similarly, Hagerman *et al.* (48) suggested that high molecular weights, and the proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging by tannins than their specific functional groups.

Metal chelating activity Transition metals such as ions can stimulate lipid peroxidation by generating hydroxyl radicals through Fenton reactions, as well as accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore, driving the chain reaction of lipid peroxidation. The extracts' chelating activities for ferrous ion were assayed by the inhibition of the formation of red-colored ferrozine, and the ferrous

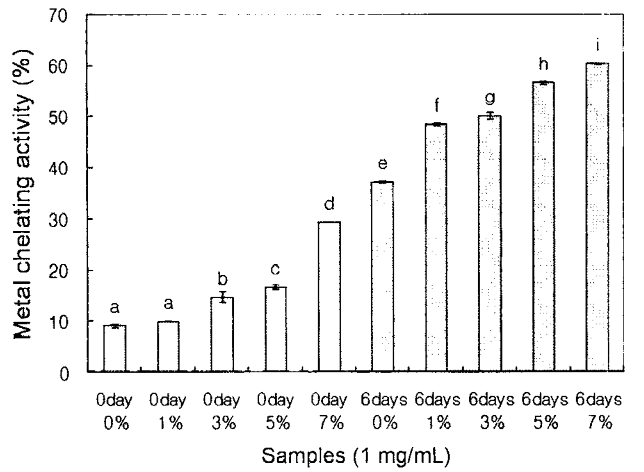

Fig. 3. Ferrous ion chelating activities of red pepper seed *kimchi* extracts. Each value represents means±SD (n=3). [a-i]Values with different letters among columns differ significantly (p<0.001).

complex (49). Significant differences in chelating activity were observed among the varieties. Metal chelating activity ranged from 9-30% in the nonfermented *kimchi*, and from 37-60% in the fermented *kimchi*. The metal chelating activity increased with increasing concentrations of red pepper seed and fermentation time. The antioxidant effect of lactic acid bacteria has been reported only recently (50). Lin and Yen (51) reported that lactic acid bacteria was high elimination of Fe^{2+}, Cu^{2} . Red pepper seed has strong ferrous ion chelating activity. It was reported that chelating agents, which form σ-bonds with metals, are effective as secondary antioxidants because they reduced the redox potential, thereby stabilizing the oxidized form of metal ions (52). The data obtained from Fig. 3 reveals that the extracts demonstrate an effective capacity for iron binding, suggesting that red pepper's action as an antioxidant may be related to its iron-binding capacity.

NO scavenging activity NO reactive nitrogen species are formed during reactions with oxygen or with superoxide, and NO_2, N_2O_4, N_3O_4, NO_3 , and NO_2^- are very reactive. These compounds are responsible for altering the structures and functional behaviors of many cellular components (52).

From Fig. 4 we can see that the scavenging of nitric oxide by the samples was concentration dependent. One can also see that all the samples likely had NO scavenging activity. For the NO scavenging activity of the red pepper seed *kimchi*, the inhibitory potential followed the order of 6 days 0%<6 days 1%<6 days 3% <0 day 1%<0 day 3%<6 days 5%<6 days 7%<0 day 0% <0 day 5%<0 day 7%. The NO scavenging activity ranged from 61-67% in the nonfermented *kimchi*, and from 53-61% in the fermented *kimchi*. The 7% red pepper seed *kimchi* that was fermented for 6 days possessed the highest activity according to the other tests. But, the highest scavenging activity was measured against NO scavenging activity, by extracts from *kimchi* for 0 day of fermentation rather than 6 days. The NO scavenging activity increased with the increasing concentrations of red pepper seed. This finding is similar to that reported by Woo and Jeong (35). The scavenging rates were 57.94, 62.98, 61.71, and 64.43%

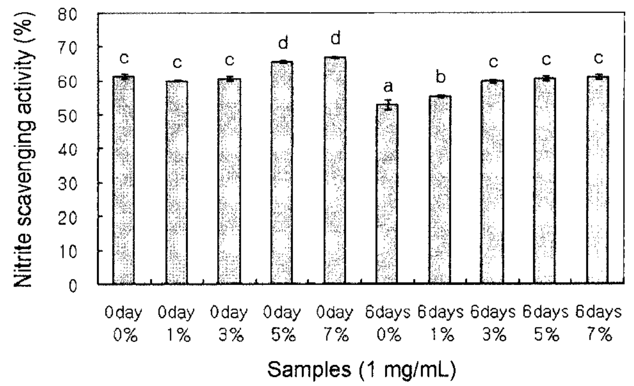

Fig. 4. Nitrite scavenging activities of red pepper seed *kimchi* extracts. Each value represents means±SD (n=3). [a-d]Values with different letters among columns differ significantly (p<0.001).

immediately after preparation in the control group and in the 1, 3, and 5%(w/w) germinated brown rice groups, which suggests that the scavenging effect increases with the germinated brown rice concentration. Nitrite scavenging rates 12 days after *kimchi* preparation were 90.15, 88.82, and 90.31% in the 1, 3, and 5%(w/w) germinated brown rice groups, respectively. On the basis of these result, red pepper seed *kimchi* extracts had higher NO scavenging activity. However, red pepper seed fermentation extracts inhibited antioxidant activity of lactic acid bacteria.

The various roles of NO in numerous disease states have generated a considerable amount of discussion over the past several years, ever since the journal *Science* named it the molecule of the year in 1992. NO is an essential bioregulatory molecule required for several physiological processes such as neural signal transmission, immune response, control vasodilatation, and blood pressure; however, the elevation of NO results in several pathological conditions, including cancer. Spices may have properties for counteracting the effects of NO formation, and in turn may be of considerable interest for preventing the negative effects of excessive NO generation *in vivo* (16).

Reducing power To examine the reducing power of the samples, Fe^{3+} to Fe^{2+} reduction was investigated. Earlier authors (53,54) observed a direct correlation between the antioxidant activity and reducing power of certain plant extracts. Reducing properties are generally associated with the presence of reductones (53), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (52).

The reducing power results for the red pepper seed *kimchi* extracts are given in Fig. 5. The extracts showed comparable reducing powers in the following order; 0 day 0%, 6 days 0%, 0 day 1%, 0 day 3%, 0 day 5%, 6 days 1%, 6 days 3%, 6 days 5%, 0 day 7%, and 6 days 7%, respectively. Like the antioxidant activity, the reducing power of the red pepper seed *kimchi* extracts increased with increasing amounts of sample and fermentation time. In our study, most of the red pepper seed *kimchi* extracts had higher reducing powers, which suggests that the reducing activity of the tested extracts contributed

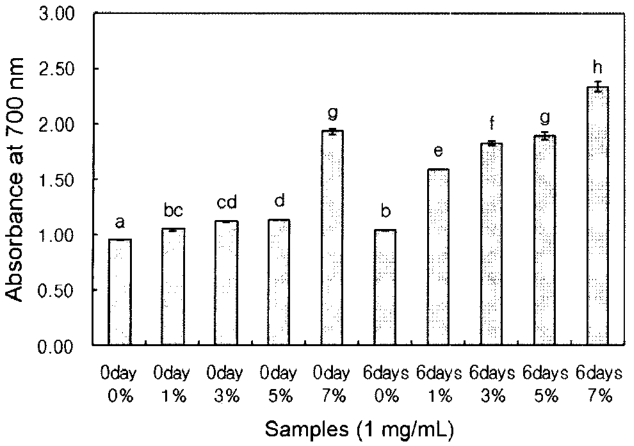

Fig. 5. Reducing power of red pepper seed *kimchi* extracts. Each value represents means±SD (n=3). [a-h]Values with different letters among columns differ significantly (p<0.001).

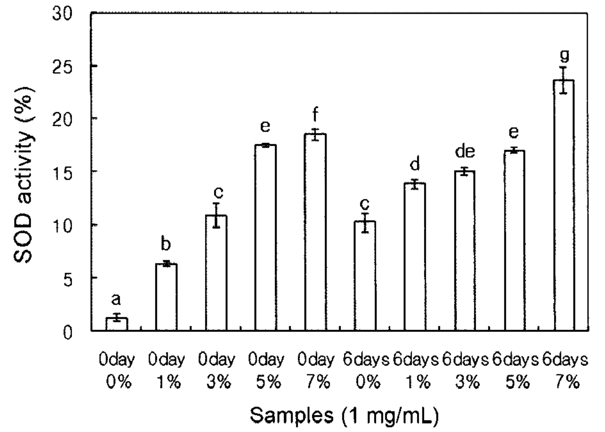

Fig. 6. Superoxide dismutase (SOD) activities of red pepper seed *kimchi* extracts. Each value represents means±SD (n=3). [a-g]Values with different letters among columns differ significantly (p<0.001).

significantly to their antioxidant effects, and there are likely reductones of some sort in red pepper seed (36). Red pepper seeds were previously shown to be a source of phenolic compounds (55), and earlier researchers have also observed similar differences in the total reducing phenolic contents of different crops (56-58).

SOD activity SOD is concerned with the elimination of O_2^- that develops from natural substances and is retained in the body, SOD activity is necessary to restrain this development as by free radical oxidation is an obstacle and enemy to the body (59). SOD activity is principally measured by the automatic oxidation reaction of pyrogallo, which reacts with superoxide and displays browning material. Animals defend against free radicals by SOD reactions, which catalyze the decomposition of superoxide anion to generate hydrogen peroxide. SOD is one of the most important factors in oxidative defense systems. From studies on aerobic organism, 4 types of SOD (Mn-SOD, Fe-SOD, Cu/Zn-SOD, Ni-SOD) have been identified. Each SOD requires the indicated metal cofactor (60).

As shown in Fig. 6, the red pepper seed *kimchi* reacted directly with, and quenched SOD to different degrees, showing increased activities at higher concentrations. The SOD activity was observed for 0 day 0%, 0 day 1%, 6 days 0%, 0 day 3%, 6 days 1%, 6 days 3%, 6 days 5%, 0 day 5%, 0 day 7%, and 6 days 7%, respectively. The SOD activity ranged from 1-19% in the nonfermented *kimchi*, and from 10-24% in the fermented *kimchi*. The SOD activity increased with the increasing concentrations of red pepper seed and fermentation time. Zitzeisberger *et al.* (61) reported that free radical oxidation can be detoxified by scavenger enzymes such as Mn-SOD of lactic acid bacteria.

In conclusion, the antioxidant activities of red pepper seed *kimchi* were evaluated in this study with various antioxidant assays. All the extracts showed strong antioxidant activity in the tested methods. Overall, the antioxidant activity increased with the increasing concentrations of red pepper seed and fermentation time. The red pepper seed *kimchi* extracts were highly effective for the antioxidant assayed, with the exceptions of metal chelating activity, and SOD activity. These results suggest that red pepper seed is healthy food having radical scavenging and antioxidant activities.

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