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Antioxidant Effects of *Cheonggukjang* Containing *Phellinus linteus* Extract

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Abstract This study was carried out to examine the antioxidant effects of *cheonggukjang* combined with *Phellinus linteus* extract. The electron-donating activity (EDA) of *cheonggukjang* containing 0.3% *P. linteus* extract (0.3% CPLE) was higher than that of *cheonggukjang* only. EDA of the ethanol extract from *cheonggukjang* was higher than that of the water extract. The water and the ethanol extracts showed strong antioxidant activity with regard to peroxide value. However, the ethanol extract showed a higher peroxide value than the water extract. The nitrite scavenging activity of the ethanol extract was greater than that of the water extract, corresponding to the EDA and peroxide values for each extract. Therefore, the antioxidant effects were enhanced by adding 0.3% of extract from *P. linteus* in manufacturing *cheonggukjang*. It is suggested that *P. linteus* extract could be put into practice as an effective antioxidant agent.

Keywords: DPPH radical, peroxide value, nitrite scavenging ability

Introduction

As a result of exposure to multiple types of environment stress, the human body will form reactive oxygen species (ROS): superoxide radical, hydrogen peroxide, and hydroxyl radical (1). These ROS cause damage to biological macromolecules such as DNA, lipids, and proteins, and therefore significantly affect the function of biological tissues. The defense systems against ROS include the endogenous antioxidants, superoxide dismutase (SOD), catalase, and glutathione peroxidase as well as exogenous (dietary) antioxidants such as tocopherol, ascorbic acid, and polyphenols (2). However, these systems are not always sufficient to inhibit oxidation, making it necessary to obtain additional antioxidants from foods.

Korean fermented soybean foods, namely *doenjang*, *cheonggukjang*, soy sauce, and *kochujang*, comprise a significant proportion of the Korean diet. Many recent studies have reported on the functionalities of such foods (2-4). *Cheonggukjang* is known to contain various physiological properties including fibrinolytic activity (5), immunoreactivity (6,7), along with antihypertensive and cholesterol-lowering effects (8). However, few studies have addressed the improvement of *cheonggukjang* antioxidant activity.

It is well known that some non-toxic mushrooms contain biological compounds having medicinal effects (9,10). *Phellinus linteus* is a medicinal mushroom and has been used for the treatment of inflammatory diseases and cancer in Oriental medicine (11,12). In addition, *P. linteus* extract has been reported to have antioxidant activities (10).

In order to improve the functionality of *cheonggukjang*, *P. linteus* extract was used as an additive to *cheonggukjang* in this study. We examined the scavenging effect of this combination on DPPH radicals, peroxide value, and nitrite.

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Materials and Methods

Soybeans and *Phellinus linteus* extract Soybeans were obtained from the Yeoju Experimental Farm of Konkuk University (Yeoju, Korea). *P. linteus* extract (PLE) was provided by the Cell Activation Research Institute (Seoul, Korea) and dissolved in 15 mL of distilled water.

Bacterial strains *Bacillus* sp. with high protease activity was screened from *cheonggukjang* purchased in a local market, and identified tentatively as *Bacillus subtilis* SK510 (13).

Starter culture Starter culture medium containing 0.8 g of nutrient broth, 1 g of skim milk and 100 mL of distilled water was sterilized for 15 min at 121°C, and used to cultivate *B. subtilis* SK510 for 48 hr at 37°C.

***Cheonggukjang* preparation** Washed soybeans (1 kg) were soaked in water for 18 hr, and steamed at 121°C for 50 min. When the steamed soybeans were cooled to 50°C, they were divided into 4 containers. Each group of soybeans (500 g) was inoculated with a 2%(v/w) suspension of *B. subtilis* SK510 and fermented at 37°C for 48 hr. Salt (3%) and PLE (0, 0.1, 0.3, and 0.5%) were then added to *cheonggukjang* and the mixture was aged at 20°C for 15 days.

Preparation of extract Ten g of *cheonggukjang* was shaken in 100 mL of distilled water at 37°C for 24 hr, and centrifuged at 7,000×g for 15 min. The supernatant was then filtered (Whatman No. 2) and used as water extract. For making ethanol extract, the residue from above was shaken with 100 mL of ethanol at 37°C for 24 hr, and centrifuged at 7,000×g for 15 min. The supernatant was filtered (Whatman No. 2) and used as ethanol extract.

DPPH radical scavenging Scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assessed using the method of Blois with modification (14). Extract to be tested (0.6 mL) was added to 2.4 mL of 4×10^{-4} M DPPH solution (dissolved in absolute ethyl alcohol), and vortexed for 10 sec. After 10 min, the absorbance at 526 nm was measured with a spectrophotometer. The control contained distilled water instead of extract. Electron-donating ability (EDA) was calculated as a percentage of DPPH decoloration using the following formula:

EDA (%)=(1−absorbance of sample/absorbance of control)×100

Measurement of peroxide value For making a linoleic acid-ethanol solution, 1 g of linoleic acid was dissolved in 20 mL of ethanol, and 25 mL of 0.2 M phosphate buffer (pH 7.0) was added. Five mL of extract was then added and the solution was incubated at 45°C for 9 days in an incubator. Subsequently, the solution was taken once in 2 days, and it was shaken with 25 mL of chloroform in a separatory funnel. Thereafter, 25 mL of glacial acetic acid and 1 mL of saturated potassium iodine (KI) solution were added to the flask, which was kept in the dark for 5 min. The solution was mixed with 50 mL of distilled water, and titrated with 0.01 N sodium thiosulfate solution ($Na_2S_2O_3$). The peroxide value was calculated with the following formula (15):

Peroxide value (meq/kg)=$(V_1-V_0)\times F\times0.01/S\times1000$

V_1 is the titration value of 0.01 N $Na_2S_2O_3$, V_0 is the titration value of blank test, F is factor of 0.01 N $Na_2S_2O_3$, S is the mass of linoleic acid (g), and 0.01 is the equivalent amount of peroxide in 1 mL of 0.01 N $Na_2S_2O_3$.

Nitrite scavenging activity Nitrate scavenging activity was measured according to the method described by Gray *et al.* (16). One mL of extract was added to 2 mL of 1 mM $NaNO_2$, and the test tube was filled to 10 mL with 0.1 N HCl (pH 1.2) or 0.2 M citric acid buffer solution (pH 3.0, 6.0). The solution was then incubated at 35°C for 1 hr. After 1 hr, 1 mL of the reaction was removed and added to 5 mL of 2% acetic acid and 0.4 mL of Griess Reagent (equal volumes of 1% sulfanylic acid and 1% naphtylamine in 30% acetic acid). The mixture was incubated for 15 min at room temperature and the absorbance was measured at 520 nm with a spectrophotometer. The nitrite scavenging effect was determined on the basis of the following formula:

Nitrite scavenging activity (%) = [1−(absorbance of $NaNO_2$ solution − absorbance of samples/absorbance of $NaNO_2$ solution)]×100

Statistical analysis Each experiment was repeated 3 times, and the results were expressed as the means±standard deviation for 3 samples. Data analysis were performed using SPSS12.0 (Statistical Package for Social Sciences, SPSS Inc., Chigo, IL, USA).

Results and Discussion

Scavenging effects of *cheonggukjang* on DPPH radicals The changes in electron-donating ability (EDA) of the water and ethanol extracts of *cheonggukjang* during aging are shown in Table 1 and 2. The EDA of the water and ethanol extracts increased gradually during the aging period, and reached the highest EDA values by the 9th day of aging. The water and ethanol extracts of *cheonggukjang* containing 0.3% *P. linteus* extract (0.3% CPLE) had higher EDA values than those of the control or 0.1% CPLE.

Song *et al.* (17) reported that *P. linteus* was shown to directly scavenge the stable DPPH radical over a concentration range of 10 μL/mL (30.01±2.72% inhibition) to 300 μL/mL (85.5±4.2% inhibition). Kim (1) also reported that *P. linteus* is a natural antioxidant with a high scavenging effect on DPPH radicals.

However, 0.5% CPLE exhibited a lower EDA than

Table 1. Electron donating activity (EDA)[1] by water extract from *cheonggukjang* with aging time (Unit: %)

Treatment[2]	Aging time (day)				
	1	3	5	7	9
Control	0	4.35±0.18	6.24±0.77	11.87±0.38	12.31±0.17
0.10%	0	6.70±0.29	7.45±0.49	11.84±0.07	12.21±0.64
0.30%	0	8.34±0.01	11.69±0.65	12.98±0.83	13.87±0.22
0.50%	0	6.20±0.50	10.78±0.75	12.01±0.43	12.92±0.38

[1]EDA (%)=(1−absorbance of sample/absorbance of control)×100.
[2]0.1, 0.3, and 0.5% mean the *cheonggukjang* with 0.1, 0.3 and 0.5% of *P. linteus* extract, respectively.

Table 2. Electron donating activity (EDA)[1] by ethanol extract from *cheonggukjang* with aging time (Unit: %)

Treatment[2]	Aging time (day)				
	1	3	5	7	9
Control	15.14±0.38	16.63±0.77	17.95±0.56	20.88±0.28	20.98±0.38
0.10%	17.44±0.09	18.31±0.60	19.71±0.31	20.83±0.30	21.08±0.65
0.30%	17.18±0.28	18.42±0.39	19.74±0.33	21.13±0.32	22.13±0.33
0.50%	15.02±0.49	17.98±0.05	19.25±0.43	21.35±0.28	21.57±0.07

[1]EDA (%)=(1−absorbance of sample/absorbance of control)×100.
[2]0.1, 0.3, and 0.5% mean the *cheonggukjang* with 0.1, 0.3 and 0.5% of *P. linteus* extract, respectively.

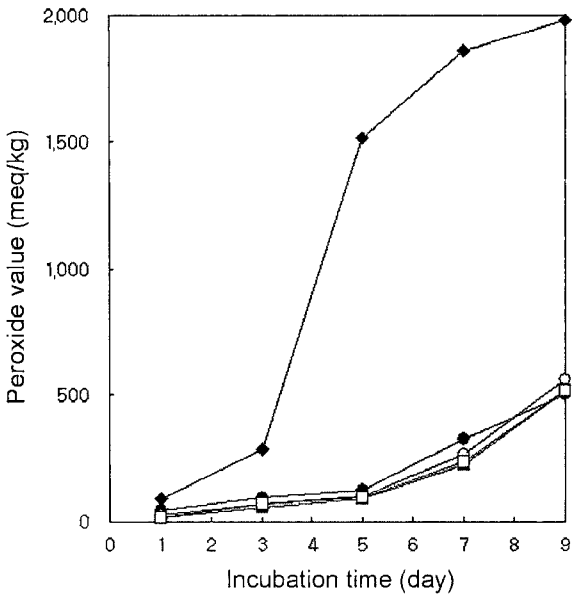

Fig. 1. Changes in peroxide value of linoleic acid by the addition of water extract from *cheonggukjang*. ◆, Non-extracts; ●, *cheonggukjang* only (control); ○, *cheonggukjang* with 0.1% *P. linteus* extract; ■, *cheonggukjang* with 0.3% *P. linteus* extract; □, *cheonggukjang* with 0.5% *P. linteus* extract.

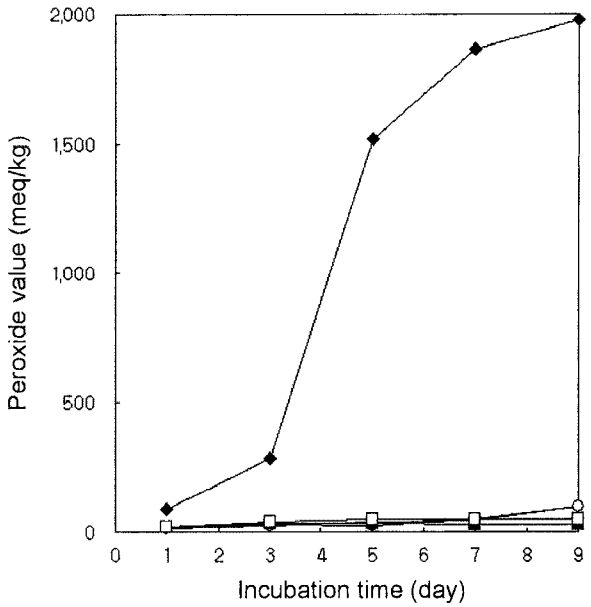

Fig. 2. Changes in peroxide value of linoleic acid by the addition of ethanol extract from *cheonggukjang*. ◆, Non-extracts; ●, *cheonggukjang* only (control); ○, *cheonggukjang* with 0.1% *P. linteus* extract; ■, *cheonggukjang* with 0.3% *P. linteus* extract; □, *cheonggukjang* with 0.5% *P. linteus* extract.

0.3% CPLE. It is likely that the extra PLE in 0.5% CPLE had a negative effect on the EDA of *cheonggukjang*. It is mainly due to the growth inhibition effect of mushrooms on Gram-positive bacteria (18).

The control also showed a comparatively high EDA, which would be contributed by many kinds of antioxidants present in *cheonggukjang*, such as isoflavones, phenolic acids, tocopherol, amino acid, peptides, aromatic amines, phospholipids, and saponins (19-22). The EDA value was depended on the type of *cheonggukjang* extract. In all *cheonggukjang* samples, the ethanol extract showed a higher EDA than the water extract during the aging period. The ethanol and water extracts showed a range of EDA values from 15.02-22.13 and 0-13.87%, respectively. Choi (3) reported that ethanol extract had a higher EDA than water extract from fermented soybeans made with *Bacillus* sp. FB52. On the contrary, Kim (23) reported that water extract had a higher EDA than ethanol extract from *doenjang* made with molds. Therefore, the 0.3% addition of extract from *P. linteus* increased the EDA value of *cheonggukjang*.

Peroxide value in linoleic acid Changes in the peroxide value by water and ethanol extracts of *cheonggukjang* aged for various periods of time are shown in Fig. 1 and 2. We chose *cheonggukjang* aged for 3 days as for a measurement of peroxide value. Linoleic acid solution was incubated at 45°C for 9 days, and the peroxide value was measured on alternate days (1, 3, 5, 7, and 9 days). On the last day of incubation, the peroxide value increased to 1,978 meq/kg for the extract free sample, however the ethanol and water extract treated samples showed peroxide values of 16-100 and 15-560 meq/kg, respectively. However, there were small differences in the peroxide values of the CPLE water extracts throughout the incubation period. The CPLE ethanol extracts exhibited similar changes in peroxide values. It is thought that the strong antioxidant activities of both PLE and *cheonggukjang* are due to the many kinds of antioxidants each contains (1,10,21). The peroxide value also depends on the type of *cheonggukjang* extract. In all cases, the ethanol extract of *cheonggukjang* had a lower peroxide value than the corresponding water extract during the aging period. On the contrary, Choi (3) reported that the ethanol extract made from fermented soybean made by *Bacillus* sp. FB52 had a higher peroxide value than the water extract, and Kim (23) reported that the water extract had a higher peroxide value than the ethanol extract from *doenjang* made with molds. It is estimated that ethanol extract of PL has higher antioxidant activity than water extract.

Nitrite scavenging ability Changes in the nitrite scavenging ability of the water and ethanol extracts of *cheonggukjang* are shown in Fig. 3 and 4. *Cheonggukjang* ripened for 3 days was sampled for the measurement of nitrite scavenging ability. The nitrite scavenging ability of the water extract ranged from 29.37-31.34 and 9.61-11.53% at pH 1.2 and 3.0, respectively. The nitrite scavenging ability of the ethanol extract ranged from 39.79-40.85 and 34.54-35.95% at pH 1.2 and 3.0, respectively. Overall, the nitrite scavenging abilities of the water and ethanol extracts at the same pH conditions showed a similar pattern. The nitrite scavenging ability of the ethanol extract, however, was greater than the water extract. This is in accordance with the EDA and peroxide values for each extract. Lee and Han (24) reported that the nitrite scavenging ability of *Ulmus devidiana* ethanol extract was higher than that of water extract. Lee *et al.* (25) has also reported that the 80% ethanol extract of olive leaf fractions showed 72.8% nitrite

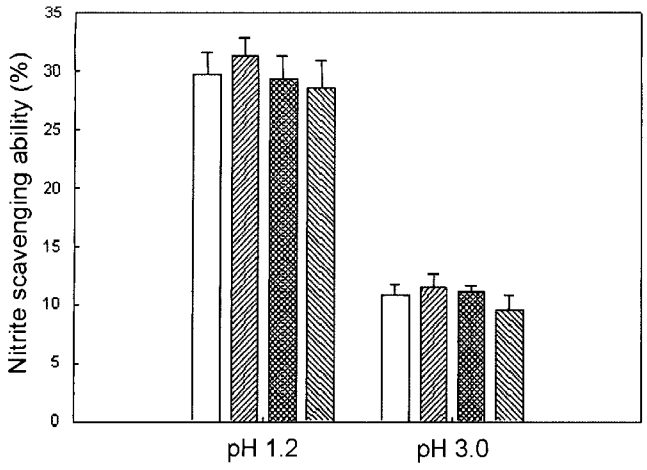

Fig. 3. Nitrite scavenging activity of water extract from *cheonggukjang* with *Phellinus linteus* extract fermented by *Bacillus subtilis* SK510 at 20°C. □, *Cheonggukjang* only (control); ▨, *cheonggukjang* with 0.1% *P. linteus* extract; ▩, *cheonggukjang* with 0.3% *P. linteus* extract; ▧, *cheonggukjang* with 0.5% *P. linteus* extract. Means±SD based on 3 samples. Data analysis was performed using SPSS 12.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA).

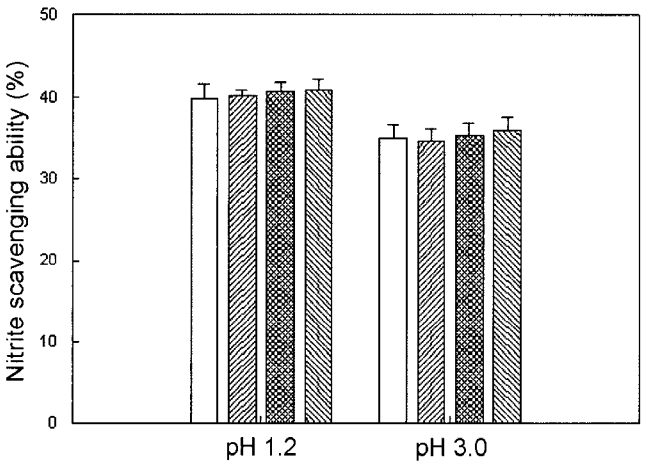

Fig. 4. Nitrite scavenging activity of ethanol extract from *cheonggukjang* with *Phellinus linteus* extract fermented by *Bacillus subtilis* SK510 at 20°C. □, *Cheonggukjang* only (control); ▨, *cheonggukjang* with 0.1% *P. linteus* extract; ▩, *cheonggukjang* with 0.3% *P. linteus* extract; ▧, *cheonggukjang* with 0.5% *P. linteus* extract.

scavenging activity, while the water fraction showed only 47.8% nitrite scavenging activity. However, opposite results are seen for food substances such as green tea (26) and pine needle extracts (26,27). Choi (3) has also reported the opposite result with the water extract of fermented soybeans showing a higher nitrite scavenging ability than the ethanol extract due to the reduced sugar, organic acid, and fatty acid contents following soybean fermentation.

Both *cheonggukjang* extracts showed higher nitrite scavenging activity at pH 1.2 than pH 3.0 since low pH is associated with high nitrite scavenging activity (28). Lee and Han (24) and Hong *et al.* (27) also reported that all extracts showed a higher nitrite scavenging activity at pH 1.2 than at pH 3.0. In this study, addition of 0.3% of extract from *P. linteus* increased the antioxidant effects in *cheonggukjang*. Hence, it is suggested that *P. linteus* extract will be put into practice as an effective antioxidant agent.

References

1. Kim TH. Antioxidant and free radical-scavenging properties of *Phellinus baumi* extracts. MS thesis. Gyeongsang National University, Yeosu, Korea (2002)
2. Chung KS, Kim JY, Hong SW, Lee BK. Isolated of bacteria producing a B-cell-specific biological response modifier found in Korean fermented soybean paste. J. Microbiol. Biotechn.16: 126-135 (2006)
3. Choi JY. Antioxidative properties and nitrosation inhibition effect of fermented soybean. MS thesis. Konkuk University, Seoul, Korea (2002)
4. Kim YS, Park YS, Lim MH. Antimicrobial activity of *Prunus mume* and *Schizandra chinenis* H-20 extract and their effect on quality of functional *kochujang*. Korean J. Food. Sci. Technol. 35: 893-897 (2003)
5. Paik HD, Lee SK, Heo S, Kim Y, Lee HH, Kwon TJ. Purification and characterization of the fibrinolytic enzyme produced by *Bacillus subtilis* KCK-7 from *cheonggukjang*. J. Microbiol. Biotechn. 14: 829-835 (2004)
6. Park HJ. Effect of fermented soybean paste *cheonggukjang* on the immunoreactivity in ovariectomized mice. MS thesis, SookMyung Women's University, Seoul, Korea (2006)
7. Lee CH, Yang EI, Song GS, Chai OH, Kim YS. Effects of *cheonggukjang* on immune response and gastrointestinal functions in rats. Food Sci. Biotechnol. 15: 19-23 (2006)
8. Yoo JY. Present status of industries and research activities of Korean fermented soybean products. Microorgan. Ind. 23: 13-30 (1998)
9. Lee JS, Baik HS, Park SS. Purification and characterization of two novel fibrinolytic proteases from mushroom, *Fomitella fraxinea*. J. Microbiol. Biotechn. 16: 264-271 (2006)
10. Lee JW, Bang KW. Biological activity of *Phellinus* spp. Food Ind. Nutr. 6: 25-33 (2001)
11. Lee JH, Lee SJ, Choi YH, Chung KT, Jeong YK, Choi BT. Effect of mycelial culture of *Phellinus linteus* on ethanol-induced gastric ulcer in rats. Phytother. Res. 20: 396-402 (2006)
12. Chung HY, Kim TW. Isolation and characterization of a water-soluble polysaccharide from the mycelia of solid cultured *Phellinus linteus*. Food Sci. Biotechnol. 14: 783-787 (2005)
13. Jiang CK. Quality characteristics, antioxidant, and antitumor effects of *cheonggukjang* with *Phellinus linteus* extract. MS thesis, Konkuk University, Seoul, Korea (2007)
14. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 181: 1199-1202 (1958)
15. Naohiko Y. Antioxidative activities of *miso* and soybean sauce on linoleic acid. Nippon Shokuhin Kogyo Gakk. 26: 20-25 (1979)
16. Gray JI, Dugan JR. Inhibition of N-nitrosamine formation in model food systems. J. Food Sci. 40: 981-984 (1975)
17. Song YS, Kim SH, Sa JH, Jin CB, Lim CJ, Park EH. Anti-angiogenic, antioxidant, and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. J. Ethnopharmacol. 88: 113-116 (2003).
18. Park SS, Lee KD, Min TJ. Study on the screening and development of antibiotics in the mushrooms. Korean J. Mycol. 23:28-36 (1995)
19. Kim SH, Yang JL, Song YS. Physiological functions of *cheonggukjang*. Food Ind. Nutr. 4: 40-46 (1999)
20. Kim MH, Im SS, Yoo YB, Kim GE, Lee JH. Antioxidative materials in domestic *meju* and *doenjang*. J. Korean Soc. Food Nutr. 23: 792-798 (1994)
21. Jang CH, Lim JK, Kim JH, Park CS, Kwon DY, Kim YS, Shin DH, Kim JS. Change of isoflavone content during manufacturing of *cheonggukjang*, a traditional Korean fermented soyfood. Food Sci. Biotechnol. 15: 643-645 (2006)
22. Yang SO, Chang PS, Lee JH. Isoflavone distribution and beta-glucosidase activity in *cheonggukjang*, a traditional Korean whole soybean-fermented food. Food Sci. Biotechnol. 15: 96-101 (2006)
23. Kim JH. Quality properties of soybean pastes made with molds

isolated from traditional *meju*. MS thesis. Konkuk University, Seoul, Korea (2004)
24. Lee YJ, Han JP. Antioxidative activities and nitrite scavenging abilities of extracts from *Ulmus davidiana*. J. Korean Soc. Food Sci. Nutr. 29: 893-899 (2000)
25. Lee OH, Lee HB, Son JY. Antimicrobial activities and nitrite-scavenging ability of olive leaf fractions. J. Food Cook. Sci. 20: 204-210 (2004)
26. Cho YS, Sung SK, Kim SM, Lee IG, Lee SH, Kim SG. Antioxidative and nitrite scavenging ability of pine needle and green tea extracts. Korean J. Food Sci. Anim. Resour. 22: 13-19 (2002)
27. Hong TG, Lee YR, Yim MH, Hyun CN. Physiological functionality and nitrite scavenging ability of fermentation extracts from pine needles. Korean J. Food Preserv. 11: 94-99 (2004)
28. Park YB, Lee TG, Kim OK, Do JR, Yeo SG, Park YH, Kim SB. Characteristics of nitrite scavenger derived from seeds of *Cassiatora*. L. Korean J. Food Sci. Technol. 27: 124-128 (1995)