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Screening for Antioxidative Activity in Soybean Local Cultivars in Korea

Ill Min Chung*†, Joung Kuk Ahn*, Hee Youn Chi* and Jin Ohk Lee*

**Department of Crop Science, Konkuk Univ., Seoul, South Korea, 143-701*

ABSTRACT: Sixty local soybean cultivars were evaluated on the antioxidative activity by superoxide dismutase (SOD), 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), and chemiluminescence using the FI-CL system. Soybean were collected throughout the country, and were grown over two years (1997 and 1998) for measuring antioxidative activity in soybean seeds. There were differences in antioxidative activity depen- ding on the method of measurement and variation of the crop year. Soybeans from Kwangyang-shi-1 (76.78%) in 1997 and Kangjin-gun-3 (79.14%) in 1998 showed the highest SOD activity, whereas those from Hwasoon-gun (80.43%) in 1997 and Kangjin-gun-2 (49.82%) in 1998 exhibited the highest DPPH activity. Soybeans from Chongup-gun-2 (75.77%) in 1997 and from Yochon-shi-5 (69.17%) in 1998 exhibited the highest TBA activity, and those from Jinahn-gun (48.99%) in 1997 and Kohung-gun (49.73%) in 1998 exhibited the highest activity using the chemiluminescence method. These results suggest that it may be possible to develop soybean varieties with higher antioxidative activity.

Keywords **:** soybean, antioxidative activity, SOD, DPPH, TBA, FI-CL

Soybean (*Glycine max* (L.) Merrill) is one of the most important upland food crops in Korea. In 1997, more than 1.7 million tons was consumed, although only 160,000 tons was produced locally, amounting to a self-supply rate of less than 10% and imports of 1.6 million tons (Kim *et al.*, 1998). Domestically produced soybeans should be compared separately from imported soybeans, based on quality and diversification. The health benefits of food made from the soybean have been known for a long time and are widely recognized throughout the world (Holt, 1997; Kyoko, 1998). Nowadays, demand for this so-called "health food" is increasing in many countries. The soybean provides potentially beneficial effects for several of the most common disorders that afflict human beings, including cancer (Holt, 1997).

One of the primary effects of soybean consumption is the prevention of the active oxygen radical. Superoxide dismutases (SOD, EC 1.15.1.1), known as Cu/ZnSOD, MnSOD and FeSOD, are a group of enzymes that catalyse the dismutation of the superoxide radical (O2-) in respiring cells (Fridovich, 1975), and have been identified as an essential component in an organic defence mechanism on oxidant stress resulting from the deleterious effects of reduced oxygen species (Bowler *et al.*, 1992). Recently, many phenolic compounds, including flavonoids, phenylpropanoids and phenolic acids, contribute oxygen free radical scavenging in 1,1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) assay (Osawa *et al.*, 1992; Rice-Evans *et al.*, 1996). The occurrence and intensity of the chemiluminescence (CL) was closely related to the radical reaction and radical scavenging activity of the compounds present in the reaction mixture. A radical scavenger was shown to exhibit a very weak light emission in the presence of acetaldehyde and active oxygen (Yoshiki *et al.*, 1995). Free radical scavenging properties of flavonoids have permitted the characterization of major phenolic components of naturally occurring phytochemicals as antioxidative (Rice-Evans *et al.*, 1996).

The main purpose of this study was to screen potential antioxidative soybean varieties by estimating antioxidative activity of collected traditional soybean varieties in Korea through SOD, DPPH, TBA, and FI-CL. Results of this study may provide basic information for breeding soybean varieties with higher antioxidative activity.

MATERIALS AND METHODS

Sample preparations

Sixty varieties of soybean (*Glycine max* (L.) Merrill), 27 of which were collected from Chollanamdo, 14 from Chollabukdo, and 19 from Chungchongnamdo, were cultivated and harvested in an experimental field at the College of Agriculture and Life Science, Konkuk University, in 1997 and 1998. The harvested samples were kept in a cool chamber at below -35°C until used for this study.

Enzyme extraction for measurement of SOD activity

Crude extracts were prepared by homogenizing 2 g of

†Corresponding author: (Phone) +82-02-450-3730 (E-mail) imcim@konkuk.ac.kr <Received October 28, 2000>

ground soybean seed with the seed coat in 5 mL of a buffer solution (pH 7.0, 100 mM phosphate, 10 mM ascorbate, 5 mM EDTA) and 0.2 g of insoluble poly vinyl polypyrrolidone (PVP). The homogenate was then centrifuged at 15,000 rpm for 10 min. The supernatants were desalted on a Sephadex G-25 column, pre-equilibrated with a buffer (pH 7.0, 100 mM phosphate, 10 mM ascorbate, 0.2 mM EDTA), and used as a testing solution (Chung *et al.*, 1995).

Test of SOD activity

SOD activity of collected soybean varieties was measured using the indirect spectrophotometric method of Beyer and Fridovich (1987). Test tubes containing reaction solution were illuminated for 7 min in an aluminium foil lined box containing two 20-W Sylvania Groux fluorescent lamps at 25°C. After exactly 7 min, the absorbance at 560 nm of the blank solution and reaction solution was measured with a spectrophotometer (UV, Hitachi Ltd., Tokyo, Japan). The SOD activity was expressed as the inhibition percentage of the Nitro Blue Tetrazolium (NBT) reduction method (Asada *et al.*, 1974) and referred to as the inhibition rate.

Inhibition (%) = [(A–B)/A] × 100

A : absorbance of blank

B : absorbance of samples

Sample preparation for the measurement of DPPH and TBA activity

The 5 g ground soybean seed samples, including seed coat, were extracted with 100 mL of 80% methanol, stirred for 24 h at room temperature, and filtered through Whatman No. 4 filter paper. The filtrate was dried in a rotary vacuum evaporator at below 40°C, then freeze-dried. The obtained crude samples were used to measure antioxidative activity and were kept in a cool chamber at below -35°C until extracted.

Measurement of the DPPH value

Free radical scavenging activity using the DPPH method was measured according to the method of Yoshida et al. (1989): 2.5 mL of 0.35 mM DPPH and 0.25 mL of 1% sample solution were homogenized and allowed to react for 10 min at room temperature, and then the optical density was measured at 517 nm. The activities were calculated against the control, which used ethanol. Activity is indicated as inhibition rate.

Inhibition (%) = [(A-B)/A] × 100

A : absorbance of blank

B : absorbance of samples

Preparation of substrate solution for the measurement of TBA activity

Linoleic acid was added to a 30 mM solution (100 mM phosphate buffer and ethanol 4 : 1), which was used as the substrate solution. Phosphate buffer (19.2 mL, 100 mM) and 0.8 mL of each sample solution were added to an Erlenmeyer flask containing 20 mL of substrate solution, and mixed with a shaker at 100 rpm at 40°C for 24 h. This solution was used as the optimal reaction solution.

Measurement of TBA value

After preparation of the reaction solution, 2.0 mL of reaction solution, 1.0 mL of 35% trichloroacetic acid (TCA) and 2.0 mL of 0.75% TBA were put into a test tube and mixed with a vortex for 30 s. The mixture was allowed to react at 95°C in a water bath for 40 min, and, after cooling to room temperature, 1.0 mL of acetic acid and 2.0 mL of chloroform was added to the test tube. After agitation, the above solution was centrifuged at 3000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and used as TBA values. The inhibitory activity was calculated against the TBA value of the control (Mitsuda *et al.*, 1966; Sidwell *et al.*, 1954) and referred to as the inhibition rate.

Inhibition (%) = [(A–B)/A] × 100

A : absorbance of blank

B : absorbance of samples

Sample preparation for the measurement of chemiluminescence using the FI-CL system

The 5 g ground soybean seeds samples, including seed coat, were extracted with 100 mL of 80% methanol containing 0.01% EDTA, stirred for 24 h at room temperature, and filtered through a Whatman No. 4 filter paper. The EDTA was added as a chelating agent, to prevent the inactivation of active compounds by metals, and the temperature was for the protection from thermal decomposition of active compounds. The filtrate was dried in a rotary vacuum evaporator at below 40°C and concentrated filtrates were adjusted to 0.1% of the solid concentration with 50% methanol to allow the measurement of radical scavenging using the FI-CL method.

Measurement of CL

Analysis of CL was performed using a modification of the

method of Choi *et al.* (2000). For the measurement of CL, cytochrome C and luminol were purchased from Sigma Chemical (St. Louis, Mo, USA) and hydrogen peroxide was obtained from Hayashi Pure Chemical Industry (Osaka, Japan). CL was measured with the filter-equipped photon counting type spectrophotometer (CLD-110, Tohoku Electronic Industry) connected to a pump (Gilson Model 303) and a sample injection valve (Rheodyne Model 7125). The dispersed light at the grating was simultaneously detected on the photocathode with the image sensor in the range of 300 nm to 650 nm. Photons were counted in the range of 300 nm 650 nm and computed as a total spectral intensity. The mobile phase was 50 mM phosphate buffer (pH 7.4) containing 50% methanol (for solvent-sample), cytochrome C (10 mg/L) and luminol (2 mg/L), and the flow rate was maintained at 1.0 mL/min. For measuring the abilities of radical scavengers, the mixture of 0.06% H_2O_2 (5 μL) and scavenger solution was injected. The reduced CL intensity of the mixture, compared with the CL intensity of 0.06% H_2O_2 (5 μL), enables the quantitative analysis of radical scavenging activity. The radical scavenging activity percentage was calculated using the following equation:

Radical scavenging activity (%) = [(A–B)/A] × 100

A : CL intensity generated from the injection of H_2O_2 only

B : CL intensity generated from the injection of H_2O_2+scavenger

Statistical analysis

Analysis of variance for all data was accomplished using the general linear model procedure of the SAS program, and all of the experiments incorporated three replications with a completely randomized design. The pooled mean values were separated on the basis of LSD at the 0.05 probability (SAS Institute, 1985).

Table 1. The SOD activity on 60 soybean varieties in 1997 and 1998.

Local cultivars	Inhibition (%)			Local cultivars	Inhibition (%)		
	1997	1998			1997	1998	
Damyang-gun	22.96	21.54	ns	Chongup-gun-2	20.34	5.85	ns
Hampyoung-gun	12.18	67.75	*	Iksan-shi-1	29.71	59.24	ns
Hwasoon-gun	42.34	0.00	*	Iksan-shi-2	60.56	14.35	*
Kangjin-gun-1	65.03	37.72	*	Imshil-gun	38.71	67.04	*
Kangjin-gun-2	53.11	14.50	*	Jinahn-gun	49.97	68.57	ns
Kangjin-gun-3	36.31	79.14	*	Kunshan-shi-1	0.00	15.77	*
Kohung-gun	70.57	66.60	ns	Kunshan-shi-2	58.80	77.81	*
Kurye-gun-1	63.85	47.17	*	Namwon-shi-1	9.23	67.68	*
Kurye-gun-2	56.49	35.11	*	Namwon-shi-2	0.00	48.82	*
Kwangyang-shi-1	76.78	33.25	ns	Ckku-gun	71.48	65.06	ns
Kwangyang-shi-2	70.98	65.57	ns	Soonchang-gun	25.67	55.14	*
Kwangyang-shi-3	34.46	79.15	*	Asan-gun	68.71	27.17	*
Mokpo-shi	71.13	65.85	ns	Boryoung-shi-1	51.35	32.62	ns
Sunchon-shi-1	12.82	28.20	ns	Boryoung-shi-2	68.16	0.00	*
Sunchon-shi-2	61.36	54.41	ns	Chonan-shi	20.47	19.56	ns
Yochon-shi-1	50.07	59.17	ns	Kongju-shi-1	45.35	43.55	ns
Yochon-shi-2	26.48	53.32	ns	Kongju-shi-2	50.56	39.11	ns
Yochon-shi-3	18.22	59.62	*	Kumsan-gun	48.90	72.76	*
Yochon-shi-4	7.16	63.30	*	Nonsan-shi	71.98	0.00	*
Yochon-shi-5	5.33	55.22	ns	Onyang-shi-1	63.95	71.17	ns
Yochon-shi-6	68.25	69.28	ns	Onyang-shi-2	22.07	52.56	*
Yochon-shi-7	64.91	65.82	ns	Puyeo-gun-1	45.14	58.15	ns
Yosu-shi-1	66.96	61.49	ns	Puyeo-gun-2	56.70	72.87	*
Yosu-shi-2	73.13	75.54	ns	Seochun-gun-1	14.27	0.00	ns
Yosu-shi-3	58.60	11.93	*	Seochun-gun-2	6.02	60.95	*
Youngam-gun	12.14	11.91	ns	Seosan-shi-1	18.21	52.13	*
Youngkawang-gun	0.00	64.81	*	Seosan-shi-2	39.53	62.30	*
Changsu-gun-1	66.31	0.00	*	Taeahn-gun	50.55	69.14	ns
Changsu-gun-2	26.43	34.24	ns	Yesan-gun-1	65.80	7.96	*
Chongup-gun-1	73.06	27.48	*	Yesan-gun-1	13.79	18.92	ns
				CV(%)	23.41	28.92	
				LSD(0.05)	15.89	20.36	

ns, *: Nonsignificanty or Significant at 0.05, respectively

RESULTS AND DISCUSSION

Measurement of SOD activity

The SOD enzyme activity of 60 soybean varieties is presented in Table 1. The soybean variety and region they were collected from were different in regards to SOD activity in 1997 and 1998. The higher activity during the two years was for the soybeans from Kohung-gun, Kwangyang-shi-2, Mok- po-shi, Yochon-shi-6, Yosu-shi-2, and Okku-gun. In 1997 (CV = 23.41%), soybeans from Kwangyang-shi-1 (76.78%), Yosu-shi-2 (73.13%), Nonsan-shi (71.98%), and Okku-gun (71.48%) showed the higher SOD activity when compared with soybeans from the other regions. In 1998 (CV = 28.92%), soybeans from Kangjin-gun-3 (79.14%), Kwangyang-shi-3 (79.15%), Kunshan-shi-2 (77.81%), and Yosushi-2 (75.54%) showed the highest SOD activity when compared with soybeans from the other regions. The SOD activity varied according to the year of cultivation and soybean variety. This study showed that antioxidative activity present in soybean varieties varies, and it is suggested that genetic variations in regards to inhibitory effects exist among soybean varieties. These results are supported by the high CV value. Therefore, we believe that it would be possible to effectively breed soybean varieties with high SOD activity.

Oxidative stress, which is an important phenomenon in many biological systems, arises from the deleterious reactions of oxygen. It is an unfortunate consequence of life for any aerobic organism. These reactions are mediated by reduced oxygen species such as superoxide radicals and hydrogen peroxide. SOD have been identified as an essential component in an organism's defence mecha-

Table 2. The antioxidant activity using the DPPH method on extractd soybeans in 1997 and 998.

Local cultivars	Inhibition (%)			Local cultivars	Inhibition (%)		
	1997	1998			1997	1998	
Damyang-gun	67.85	40.69	*	Chongup-gun-2	69.24	26.35	*
Hampyoung-gun	32.22	17.48	*	Iksan-shi-1	11.11	12.49	ns
Hwasoon-gun	80.43	44.98	*	Iksan-shi-2	23.97	18.16	*
Kangjin-gun-1	49.17	33.42	*	Imshil-gun	13.68	12.51	ns
Kangjin-gun-2	53.06	49.82	ns	Jinahn-gun	39.10	37.90	ns
Kangjin-gun-3	14.78	22.19	ns	Kunshan-shi-1	45.45	16.12	*
Kohung-gun	78.39	49.05	*	Kunshan-shi-2	64.01	27.61	*
Kurye-gun-1	46.19	39.89	ns	Namwon-shi-1	57.44	18.61	*
Kurye-gun-2	11.04	39.04	*	Namwon-shi-2	14.42	12.14	ns
Kwangyang-shi-1	55.53	13.36	*	Okku-gun	14.10	10.55	ns
Kwangyang-shi-2	54.51	14.91	*	Soonchang-gun	12.92	12.21	ns
Kwangyang-shi-3	42.67	18.46	*	Asan-gun	34.40	39.69	*
Mokpo-shi	31.11	42.23	*	Boryoung-shi-1	51.77	27.01	*
Sunchon-shi-1	39.94	30.51	ns	Boryoung-shi-2	28.39	32.69	ns
Sunchon-shi-2	59.69	27.77	*	Chonan-shi	14.02	15.01	ns
Yochon-shi-1	50.70	13.34	*	Kongju-shi-1	13.42	15.86	*
Yochon-shi-2	55.32	14.56	*	Kongju-shi-2	17.95	22.52	ns
Yochon-shi-3	52.97	14.28	*	Kumsan-gun	35.84	27.87	*
Yochon-shi-4	48.85	16.22	*	Nonsan-shi	24.06	37.44	*
Yochon-shi-5	17.65	41.98	*	Onyang-shi-1	7.79	13.50	ns
Yochon-shi-6	27.24	36.07	*	Onyang-shi-2	19.62	18.66	ns
Yochon-shi-7	40.35	29.89	*	Puyeo-gun-1	33.21	28.89	ns
Yosu-shi-1	54.59	19.23	*	Puyeo-gun-2	25.83	19.36	*
Yosu-shi-2	41.55	17.27	*	Seochun-gun-1	73.32	23.96	*
Yosu-shi-3	65.76	12.99	*	Seochun-gun-2	26.48	38.44	*
Youngam-gun	29.44	38.42	ns	Seosan-shi-1	31.56	34.11	ns
Youngkawang-gun	50.25	38.22	ns	Seosan-shi-2	27.58	24.92	ns
Changsu-gun-1	12.09	15.38	ns	Taeahn-gun	20.16	15.28	ns
Changsu-gun-2	74.26	27.25	*	Yesan-gun-1	25.53	14.95	*
Chongup-gun-1	15.55	14.99	ns	Yesan-gun-1	21.81	14.79	*
				CV (%)	21.12	11.50	
				LSD (0.05)	12.81	4.66	

ns, * : Nonsignificant of Significant at 0.05, respectively

Table 3. The antioxidant activity using the TBA method on extracted soybeans in 1997 and 1998.

Local cultivars	Inhibition (%)			Local cultivars	Inhibition (%)		
	1997	1998			997	1998	
Damyang-gun	7.91	46.28	ns	Chongup-gun-2	75.77	50.66	ns
Hampyoung-gun	49.17	35.44	*	Iksan-shi-1	41.94	47.08	ns
Hwasoon-gun	72.25	45.82	*	Iksan-shi-2	44.29	53.35	ns
Kangjin-gun-1	48.44	62.60	ns	Imshil-gun	43.17	29.88	ns
Kangjin-gun-2	57.06	59.94	ns	Jinahn-gun	63.06	57.23	ns
Kangjin-gun-3	35.64	41.03	ns	Kunshan-shi-1	74.45	40.70	*
Kohung-gun	49.33	47.34	ns	Kunshan-shi-2	65.06	50.10	ns
Kurye-gun-1	49.92	66.41	*	Namwon-shi-1	65.98	33.4 6	*
Kurye-gun-2	47.62	63.30	ns	Namwon-shi-2	32.83	37.15	ns
Kwangyang-shi-1	64.17	62.30	ns	Okku-gun	45.92	29.23	ns
Kwangyang-shi-2	62.38	46.44	*	Soonchang-gun	45.12	57.40	*
Kwangyang-shi-3	26.72	24.96	ns	Asan-gun	62.98	53.85	ns
Mokpo-shi	43.14	65.65	*	Boryoung-shi-1	64.30	45.46	*
Sunchon-shi-1	56.15	48.52	ns	Boryoung-shi-2	46.33	28.90	*
Sunchon-shi-2	39.19	47.35	ns	Chonan-shi	42.73	28.22	*
Yochon-shi-1	66.04	59.96	ns	Kongju-shi-1	38.25	25.79	*
Yochon-shi-2	45.49	62.98	ns	Kongju-shi-2	51.23	46.20	ns
Yochon-shi-3	61.66	57.03	ns	Kumsan-gun	47.33	47.16	ns
Yochon-shi-4	61.04	62.70	ns	Nonsan-shi	33.67	40.23	ns
Yochon-shi-5	50.46	69.17	*	Onyang-shi-1	47.27	19.98	ns
Yochon-shi-6	53.61	40.13	ns	Onyang-shi-2	47.25	18.87	*
Yochon-shi-7	49.80	52.85	ns	Puyeo-gun-1	44.67	47.71	ns
Yosu-shi-1	56.03	61.05	ns	Puyeo-gun-2	37.41	46.52	ns
Yosu-shi-2	25.38	57.45	*	Seochun-gun-1	74.68	42.22	*
Yosu-shi-3	58.93	61.64	ns	Seochun-gun-2	52.99	64.15	*
Youngam-gun	59.47	41.11	ns	Seosan-shi-1	58.41	51.11	ns
Youngkawang-gun	48.72	60.34	ns	Seosan-shi-2	53.70	41.01	ns
Changsu-gun-1	48.49	50.49	ns	Taeahn-gun	60.37	45.08	ns
Changsu-gun-2	63.93	68.63	ns	Yesan-gun-1	53.01	52.44	ns
Chongup-gun-1	49.62	50.89	ns	Yesan-gun-1	53.76	52.06	ns
				CV (%)	21.81	21.19	
				LSD (0.05)	18.37	16.58	

ns, * : Nonsignificant or Significant at 0.05, respectively

nism (Bowler *et al.*, 1992). As higher plants have been known to contain three classes of SOD enzymes (Cu/ZnSOD, MnSOD, and FeSOD) (Fridovich, 1975), the identification of SOD isozymes on crude soybean extracts, which show high SOD activity, needs to be investigated in more detail.

Measurement of DPPH, TBA and FI-CL

Although there are many arguments about the mechanism of antioxidation reaction, the reactive oxygen theory is regarded as a typical mechanism (Mitsuda *et al.*, 1966; Yoshida *et al.*, 1989). There are various methods to measure antioxidative activity; DPPH, TBA and the CL method for H_2O_2 scavenging activity are most often used. Results of these methods are presented in Tables 2, 3 and 4.

The DPPH activity in 1997 was higher than in 1998. Soybeans from Hwasoon-gun (80.43%) and Kohung-gun (78.39%) showed higher activities in 1997 (CV = 21.12%), although in 1998 (CV = 11.50%) soybeans from Kangjin-gun-2 (49.82%) and Kohung-gun (49.05%) showed higher activities.

In regards to the TBA method, soybeans from Chongup-gun-2 (75.77%), Seochun-gun-1 (74.68%), and Kunshan-shi-1 (74.45%) exhibited higher activities in 1997 (CV = 21.81%), although in 1998 (CV = 21.19%), soybeans from Yochon-shi-5 (69.17%) and Chansu-gun-2 (68.63%) exhibited higher activities.

Soybeans from Jinahn-gun (48.99%), Seochun-gun-1 (48.76%), and Hwasoon-gun (48.60%) showed high activities in 1997 with the CL measurement using the FI-CL system (CV = 1.61%). However, in 1998, soybeans from Kohung-gun (49.73%), Kangjin-gun-2 (49.15%), and Kurye-gun-2 (48.97%) showed the higher activities (CV = 2.54%). There were differences between the DPPH, TBA, and CL

Table 4. The antioxidative activity by flow injection chemilumiescence method on extracted soybean in 1997 and 1998.

Local cultivars	Inhibition (%)			Local cultivars	Inhibition(%)		
	1997	1998			1997	1998	
Damyang-gun	48.11	48.72	*	Chongup-gun-2	47.35	47.24	ns
Hampyoung-gun	47.53	46.15	*	Iksan-shi-1	41.19	41.16	ns
Hwasoon-gun	48.60	48.89	*	Iksan-shi-2	42.85	42.96	ns
Kangjin-gun-1	44.01	48.13	*	Imshil-gun	40.10	40.85	ns
Kangjin-gun-2	45.67	49.15	*	Jinahn-gun	48.99	47.84	*
Kangjin-gun-3	41.57	41.39	ns	Kunshan-shi-1	45.06	40.40	*
Kohung-gun	47.27	49.73	*	Kunshan-shi-2	46.95	46.90	ns
Kurye-gun-1	44.56	48.48	*	Namwon-shi-1	45.20	42.84	*
Kurye-gun-2	44.09	48.97	*	Namwon-shi-2	41.04	40.97	ns
Kwangyang-shi-1	44.03	43.02	ns	Okku-gun	40.46	39.96	ns
Kwangyang-shi-2	43.03	43.59	ns	Soonchang-gun	38.53	40.14	ns
Kwangyang-shi-3	40.78	41.82	ns	Asan-gun	48.13	48.52	*
Mokpo-shi	46.57	48.34	*	Boryoung-shi-1	44.42	47.42	*
Sunchon-shi-1	45.74	47.30	ns	Boryoung-shi-2	47.54	48.59	*
Sunchon-shi-2	45.20	47.23	*	Chonan-shi	40.01	40.56	ns
Yochon-shi-1	42.53	42.62	ns	Kongju-shi-1	42.14	41.80	ns
Yochon-shi-2	42.53	44.30	ns	Kongju-shi-2	40.39	41.30	ns
Yochon-shi-3	42.85	43.83	ns	Kumsan-gun	47.57	47.27	ns
Yochon-shi-4	42.73	43.93	*	Nonsan-shi	46.82	48.75	*
Yochon-shi-5	46.05	48.92	*	Onyang-shi-1	39.52	40.73	ns
Yochon-shi-6	47.56	48.91	*	Onyang-shi-2	41.77	42.73	*
Yochon-shi-7	47.87	48.33	ns	Puyeo-gun-1	48.32	46.10	*
Yosu-shi-1	42.36	44.77	*	Puyeo-gun-2	44.80	44.43	ns
Yosu-shi-2	39.99	41.56	ns	Seochun-gun-1	48.76	46.86	*
Yosu-shi-3	47.87	41.78	*	Seochun-gun-2	47.30	48.68	*
Youngam-gun	47.94	48.78	*	Seosan-shi-1	47.63	47.96	ns
Youngkawang-gun	45.22	48.44	*	Seosan-shi-2	45.87	46.00	ns
Changsu-gun-1	41.28	40.74	ns	Taeahn-gun	45.17	43.85	ns
Changsu-gun-2	47.99	47.74	ns	Yesan-gun-1	46.55	43.86	*
Chongup-gun-1	40.30	41.22	ns	Yesan-gun-1	44.23	43.55	ns
				CV (%)	1.61	2.54	
				LSD (0.05)	1.56	1.85	

ns, * : Nonsignificant of Significant at 0.05, respectively

methods in the measurement of antioxidative activity. These results were similar to those of Chung *et al.* (1998), who noted different antioxidative activity between the DPPH and TBA methods. It was considered that the extracts used in this study were not pure, but rather crude extracts.

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