Food Sci. Biotechnol. Vol. 16, No. 3, pp. 451 ~ 456 (2007)

Food Science and Biotechnology

Comparison of Antioxidant Potentials in Methanolic Extracts from Soybean and Rice Fermented with *Monascus* sp.

Young-Hee Pyo*

Traditional Food Research Division, Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Korea

Abstract The potential antioxidant activities of methanolic extracts from soybean and rice fermented with *Monascus* sp. were investigated. *M. pilosus* IFO 480 and *M. anka* IFO 478 were screened as a suitable strain to promote the antioxidant activities in soybean- and rice- fermentation. The methanol extracts from soybean and rice after fermenting for 20 days at 30 °C resulted in a significant increase in the antioxidant capacities expressed as radical (ABTS and DPPH) scavenging assay and peroxidation inhibition (%) by thiocyanate method and increased ($p<0.01$) by a 2.6 to 3.1-fold compared with those of the unfermented products. The average antioxidant potentials of *Monascus*-fermented soybean extracts (MFSE) were significantly ($p<0.01$) stronger than *Monascus*-fermented rice extracts (MFRE). A linear correlations between free radical scavenging activity of MFSE and the total phenolics content (r=0.84) and total flavonoids content (r=0.81) were observed. These results indicated that MFSE exhibited stronger ($p<0.01$) antioxidant activity and contained significantly higher levels ($p<0.05$) of phenolics than MFRE.

Keywords: *Monascus* sp., fermentation, antioxidant activity, total phenolics content, total flavonoids content

Introduction

Free radicals and other reactive oxygen species are considered to be important causative factors in the development of cancer and cardiovascular diseases (1, 2). They are generated in living organisms as by-products through many metabolic pathways (1). Normally, free radicals are neutralized by enzymatic activity or by natural antioxidants. Thus, the generation of free radicals poses no problem as long as there remains a balance between oxygen radical production and eradication (1, 2). This relationship has led to interest in evaluating the antioxidant capacities of many dietary supplements.

The fungus *Monascus* (Monascaceae) possesses functional components effective in upholding human health. For examples, red yeast rice (red *koji*), obtained from the culture of *Monascus* during rice fermentation has been traditionally used as a medicine to improve digestion and blood circulation in East Asia (3-5). Recently, *Monascus* has been gaining more interests from food and fermentation industries as it has been proven to produce various bioactive metabolites such as natural statin (known as lovastatin, mevinolin, monacolin K, mevacor; a cholesterol synthetase inhibitors) and γ-aminobutyric acid and dimerumic acid, which show hypocholesterolemic (5, 6) and anti-hypertensive (7, 8) and antioxidant effects (9, 10) in humans. Many claimed beneficial effects of *Monascus*-fermented products may be associated to its antioxidant activity. Previous research has demonstrated that antioxidant activity of fermented soyfoods was remarkably stronger than unfermented steamed soybeans (11-13). Previous research has demonstrated that antioxidant activity of fermented soyfoods was remarkably stronger than unfermented steamed soybeans (11, 12). These antioxidant substances can be derived from the solid or semi-solid culture of fungi such as *Aspergillus* sp. and *Monascus* sp. (10, 11). Up to now, a lot of work has been done on *Monascus*-fermented rice research and production (4, 9, 10). However, the antioxidant properties of *Monascus*-fermented soybean are not available. In most recent, it was supposed that the potentially significant antioxidant proferties of *Monascus*-fermented soybean are associated with its content of bioactive mevinolins and isoflavone aglycones (14). Monascal products were usually prepared statically at incubation temperature for up to 10-20 days but the roles of fermentation time and raw material source as a solid substrate were not seriously considered. It is therefore necessary to elucidate the effect of different substrate on antioxidant capacity in solid state fermentation using *Monascus* sp.

The objectives of the present study were to compare the antioxidant potential between the soybean and the rice fermented with *Monascus* sp. The effect of *Monascus* strains on antioxidant potential during the fermentation of soybean and rice was evaluated, and investigated whether their antioxidant activity may be related to the phenolics such as total phenolics contents (TPC) and total flavonoids contents (TFC). Antioxidant properties were assayed in terms of the inhibition of linoleic acid peroxidation by the thiocyanate method (15) and scavenging ability on DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals (16), and ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] radicals (17).

Materials and Methods

Chemicals DPPH, ammonium thiocyanate, ferrous chloride, trolox, potassium persulfate, catechin(+), linoleic acid, and ABTS were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Sodium phosphate monobasic, sodium phosphate dibasic, hydrogen peroxide, $NaNO_2$, and $AlCl_3 \cdot 6H_2O$ were from the Wako Pure Chemical Co.

*Corresponding author: Tel: 82-31-780-9305; Fax: 82-31-709-9876
E-mail: rosapyo@hotmail.com
Received January 23, 2007; accepted April 11, 2007

(Osaka, Japan). Folin-Ciocalteu's reagent was from Merk Chemical Co. (Darmstadt, Germany). All other reagents were of the highest grade available unless otherwise indicated.

***Monascus*-fermented soybean extract (MFSE) and rice extract (MFRE)** *M. anka* IFO 478, *M. anka* IFO 873, *M. pilosus* IFO 480, *M. pilosus* IFO 520, *M. purpurea* IFO 482, *M. rubber* IFO 492, and *M. kaoliang* ATCC 592, which were previously determined to be the good strains for mevinolin production without citrinin, a toxic fungal secondary metabolite (18, 19), were obtained from Korea Food Research Institutes (KFRI, Seongnam, Korea). The fungi were inoculated onto potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) and incubated at 30 °C for 7 days. After cultivation, colonies of spores that appeared on the plates were transferred (about 1 cm^2) and inoculated into 100 mL of nutrient broth (3.4 rice powder, 1.1 peptone, 2.6 glycine, 12.9 glucose; %w/v, and initial pH 6.0) and incubated at 30°C for 4 days with shaking at 150 rpm. Whole soybeans (Pyungchang, Korea, 2004) and polished rice (Icheon, Korea, 2004) were washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans (moisture content, 55-60%) and rice (moisture content, 45-50%) were weighed to 100 g in Erlenmeyer flask with a baffle and autoclaved (121°C) for 30 min. After cooling, the substrates were inoculated with 10 mL (10%, v/w) of nutrient broth (pH 6.0) and incubated at 30°C for 30 days (19). At 5 days intervals, samples were collected asceptically from each flask and lyophilized. Each sample (20 g) was ground and extracted in 200 mL methanol for 24 hr, three times. The combined extract was then rotary evaporated at 40°C to dryness. The yields (g/100 g) were in a descending order of MFSE (17.71 g) > MFRE (10.45 g) > unfermented soybean extract (4.92 g) > unfermented rice extract (2.45 g). Each extract was used directly for analyses of antioxidant activities.

Inhibition of linoleic acid peroxidation assay The inhibition of linoleic acid peroxidation was measured by the method of Yen *et al.* (15). Linoleic acid emulsion was prepared by adding equal amount of linoleic acid and Tween 20, followed by serially dilution to a final concentration of 0.02 M with sodium phosphate buffer (0.2 M, pH 7.0) and the mixture was homogenized. Aliquots of each sample were mixed with 2.5 mL linoleic acid emulsion and 2 mL sodium phosphate buffer and the mixture was incubated at 37°C for 24 hr. A 4 mL of ethanol solution (75%), 0.1 mL ammonium thiocyanate and 0.08 mL ferrous chloride (0.02 M in 5.0% HCl) were added to 0.08 mL reaction mixture. After 3 min, the absorbance of the mixture at 500 nm was measured by a spectrophotometer (U-2000; Hitachi, Tokyo, Japan). The inhibition ratio was calculated as the follows: Inhibition ratio (%) = $[1 - (A_s - A_b)/(A_c - A_b)] \times 100$, where A_s, A_b, and A_c represented absorbances measured for sample, blank, and control, respectively.

DPPH radical scavenging assay The ability of the extracts to scavenge the DPPH radical was assessed spectrophotometrically (16). A 20 μL aliquot of each extract in methanol (1-500 mg/mL) was mixed in a test tube with 1.0 mL methanol containing 0.1 mM DPPH, which is a stable free radical and has a typical absorbance at 517 nm. The decrease in absorbance at 517 nm was measured at 0, 10, and then every 10 min until the reaction reached a plateau. The scavenging capacity of MFSE and MFRE was calculated by the following equation: Scavenging activity (%) = $[1- (As - A_b) / (Ac- A_b)] \times 100$, where As, A_b, and Ac represented absorbances measured for sample, blank, and control, respectively. Trolox in methanol (10-1,000 μM) was also analyzed for comparison.

ABTS radical scavenging assay This assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical in 6 min (17). This method was not only a rapid and reliable test of total antioxidant capacity but also an advantageous assay applicable to both hydrophilic and lipophilic antioxidants (17). The blue green ABTS was produced through the reaction between 7 mM ABTS and 2.45 mM potassium persulfate in water. This solution was stored in the dark for 12-16 hr before use. The concentrated ABTS solution was diluted with phosphate buffered saline (PBS), pH 7.4 to a final absorbance of 0.70±0.02 at 734 nm at 37°C (i.e., an ABTS concentration of approximately 47 μM). After the addition of 990 μL of ABTS solution to 10 μL of each sample (1-500 mg/mL) or trolox (10-1,000 μM) standards in ethanol or PBS, the absorbance was measured exactly 6 min after the initial mixing. This was compared to a blank where 10 μL of the solvent was added to 990 μL of the ABTS solution. The equation used for calculating scavenging capacity of MFSE and MFRE was the same as that for DPPH radical scavenging assay.

Determination of total phenolics content The total phenolic content (TPC) was determined by a Folin-Ciocalteu assay (20) using gallic acid (GA) as the standard. Each sample (200 μL), distilled water (3 mL), 1.0 mL of Folin-Ciocalteu's reagents solution, and 0.8 mL sodium carbonate (7.5%) were mixed in a tube and incubated for 10 min at room temperature. A 20 mL of distilled water was added. The mixture was allowed to stand for 1 hr at room temperature. The absorbance was measured at 765 nm against distilled water as a blank. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/g sample) through the calibration curve of gallic acid.

Determination of total flavonoids content Total flavonoid content (TFC) was determined by a colorimetric method (21). Briefly, 0.25 mL of the extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, and followed by adding 75 mL of a 5% $NaNO_2$ solution. After 6 min, 150 mL of a 10 % $AlCl_3 \cdot 6H_2O$ solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1 M NaOH. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm. The results were expressed as mg catechin equivalents (CAE) per g sample using the calibration curve of (+)-catechin.

Statistical analysis Results are presented as mean value ± standard deviation (SD). Statistical comparisons were made by ANOVA procedure followed by a Duncan's multiple range tests, $p<0.01$ and $p<0.05$ were considered significantly different.

Results and Discussion

Effect of *Monascus* strains on antioxidant potentials of MFSE and MFRE The antioxidant activity of methanol extract from soy and rice fermented with 7 strains of *Monascus* for 15 days at 30°C have been evaluated respectively (Table 1). Significant difference ($p<0.01$) in antioxidant capacities were found between the fermented products and unfermented products of soybean and rice. Among the seven strains showing antioxidant activity, *M. pilosus* IFO 480 and *M. anka* IFO 478 exhibited marked antioxidant activities in MFSE and MFRE. MFRE (83.8%) fermented with *M. anka* IFO 478 exhibited stronger antioxidant effect for radical scavenging than MFSE (75.5%) fermented with *M. pilosus* IFO 480. The results corresponded to the studies of Aniya *et al.* (9). They suggested that the antioxidant component of *M. anka* is the dimerumic acid which has the potential antioxidant and protective against CCl_4-induced liver injury. *M. pilosus* IFO 480, however, was the only strain that exhibited a markedly antioxidant activity on lipid peroxidation in both MFSE (86.9%) and MFRE (66.8%). This result might be because the methanol extracts from soy and rice fermented with *M. pilosus* IFO 480 contain more antioxidative substances than the other extracts. As seen in Table 1, the ability of MFSE to inhibit the peroxidation of linoleic acid with inhibition rate in the range 58.9-86.9%, varied with the strains. There were no significant differences ($p>0.01$) in antioxidant activity of MFSE and MFRE between *M. pilosus* IFO 480 and *M. anka* IFO 478; both strains scavenged average 76.9% of the radicals and inhibited 75.7% of the peroxidation of linoleic acid. MFRE showed better antioxidant properties (56.8-83.8%) in elimination of DPPH and ABTS radicals than in inhibition (48.6-67.8%) of linoleic acid peroxidation. The results indicated that MFRE might react as free radical scavengers, and contribute hydrogen from hydroxyl groups themselves; thereby forming stable free radicals which do not initiate or propagate further oxidation of lipids (22, 23). Among the various samples of fermented rice tested, the extract from rice fermented with *M. anka* IFO 478 exhibited the highest inhibition rate (83.8%) on DPPH and ABTS radicals. The greatest antioxidant activity among MFSE and MFRE was observed in *M. pilosus* IFO 480, which exhibited an 86.9 % inhibitory effect on linoleic acid peroxidation. Therefore, *M. anka* IFO 478 and *M. pilosus* IFO 480 might be selected as good strain for antioxidants production in *Monascus*-fermentation using rice and soybean. *M. anka* IFO 478 and *M. pilosus* IFO 480 were used for further evaluation of antioxidant potentials of MFRE and MFSE respectively.

Effect of fermentation time on antioxidant potentials of MFSE and MFRE As shown in Table 2, both MFSE and MFRE at 50 mg/mL showed significant scavenging effects on the DPPH and ABTS radical and inhibition effects on the linoleic acid peroxidation ($p<0.05$). MFSE and MFRE after 15 days of fermentation exhibited a strengthened potential, followed by a slow increase throughout the fermentation. The increased antioxidant activity of *Monascus*-fermented products observed in the present study is consistent with previous reports (10, 12). Soybean extract showed strong inhibitory activity towards hydrogen peroxide than rice extract. The extracts (50 mg/mL) from soybean after 20 days fermentation showed a particularly high antioxidant activity (91.7% radical scavenging effect and 93.2% inhibition effects of linoleic acid peroxidation) and increased by a 2.7 to 3.1-fold compared with those of the unfermented products. These findings implied that enhanced antioxidant ability of fermented products was derived from the substrate and the mycelia during *Monascus*-fermentation. Recent study demonstrated that the antioxidant capacity of monascal soybean is associated with the content of mevinolins and isoflavone aglycones, which were derived from the

Table 1. Effect of *Monascus* strain on antioxidant potentials of methanol extracts (50 mg/mL) from soybean and rice fermented with *Monascus* sp. at 30°C for 15 days[1)]

	Radical scavenging (%)[2)]		Inhibition of lipid peroxidation (%)	
Strains	Soybean	Rice	Soybean	Rice
Control[3)]	30.8±0.6^a	35.2±0.3^a	42.3±0.4^a	30.4±0.5^a
anka IFO 478	70.1±1.1^b	83.8±1.4^b	81.4±2.0^b	67.8±1.5^b
anka IFO 873	60.1±1.2^c	70.2±1.1^c	65.2±0.8^c	56.3±1.6^c
pilosus IFO 480	75.5±1.4^b	78.1±1.1^b	86.9±2.6^b	66.8±1.2^b
pilosus IFO 520	54.7±0.6^c	56.8±0.7^d	78.5±0.7^b	49.4±0.6^c
purpurea IFO 482	60.8±1.2^c	62.4±0.8^d	58.9±0.7^c	48.6±0.6^c
ruber IFO 492	57.7±1.1^c	65.9±0.7^d	64.2±0.6^c	59.4±1.6^b
kaling ATCC 592	68.2±1.5^b	60.6±1.4^d	70.3±1.7^c	50.2±0.9^c

[1)]Each value is mean±SD (n=3); Different letters (vertical comparison) indicate significant difference ($p<0.01$).
[2)]Radical scavenging activity (%DPPH + %ABTS / 2).
[3)]Unfermented products.

Table 2. Effect of fermentation time on antioxidant potentials of MFSE and MFRE[1)]

Time (day)	Radical scavenging (%)[2)]		Inhibition of lipid peroxidation (%)	
	Soybean	Rice	Soybean	Rice
0	34.2±0.6[a]	35.2±0.3[a]	30.1±0.2[a]	21.4±0.7[a]
5	40.8±1.3[b]	42.3±1.6[b]	40.6±1.1[b]	18.6±0.9[a]
10	64.6±1.2[c]	65.5±1.2[c]	60.2±1.6[c]	28.6±0.9[b]
15	80.8±1.6[d]	90.1±1.7[d]	78.6±1.7[d]	58.6±1.4[c]
20	91.7±1.4[e]	88.7±1.1[d]	93.2±1.1[e]	55.8±1.6[c]
30	90.9±0.9[e]	87.8±0.8[d]	91.4±0.9[e]	60.2±1.2[c]

[1)]The concentration of each sample is 50 mg/mL; Each value is mean± SD (n=3); Different letters (vertical comparison) indicate significant difference (p<0.05).
[2)]Radical scavenging activity (%DPPH + %ABTS / 2).

soybean during *Monascus*-fermentation (14, 24). Meanwhile, MFRE reacted with the free radical more efficiently than hydrogen peroxides. For examples, MFRE showed the inhibitory ratio against linoleic acid peroxidation by 60.2%. However, after 15 days of fermentation, MFRE exhibited 90.1% inhibition on the radicals of DPPH and ABTS, which was significantly 2.6 times higher than that of control (p<0.01). Moreover, the EC_{50} values (mg sample/mL) of DPPH of MFRE (38.2 mg) was lower than that of MFSE (42.7 mg) (Table 3). EC_{50} value is the effective concentration at which the free radicals were scavenged by 50% and obtained by interpolation from linear regression analysis. MFSE established better antioxidant properties in scavenging of ABTS radical (EC_{50} 35.3 mg/mL) than in elimination of DPPH radical (EC_{50} 42.7 mg/mL). The scavenging effect on DPPH and ABTS radicals of MFSE had greater antioxidant activity (average EC_{50} 39.0 mg/mL) than MFRE (average EC_{50} 59.6 mg/mL). The effect of dose-dependent on antioxidant potential of MFSE and MFRE are compared with Trolox and shown in Table 4. MFSE had significant scavenging effects (p<0.05) on the DPPH and ABTS radical and the effects increased with increasing concentration in the range 20-200 mg/mL. Compared with those of Trolox (90.6% radical scavenging, 91.2% inhibition of peroxidation at 1 mM), both radical scavenging (95.5%) and peroxidation inhibitory activities (92.1%) of MFSE were higher at 200 mg/mL. There were no significant difference (p>0.05) in the scavenging effects between 100 and 200 mg/mL of MFRE (Table 4). Rice showed stronger radical scavenging activities for DPPH radicals than soybean, while soybean reacted with hydrogen peroxide more efficiently than rice. It has been demonstrated that many polyphenolics have antioxidant capacities (25, 26). These compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (25-27). Thus, the influence of total phenolics and total flavonoids on the observed antioxidant properties in MFSE and MFRE was investigated.

Table 3. EC_{50} values of MFSE and MFRE in antioxidant properties

Test	EC_{50} value[1)] (mg sample/mL)	
	Soybean	Rice
Scavenging effect on DPPH·	42.7±0.6[a2)]	38.2±0.2[a]
Scavenging effect on ABTS·	35.3±0.3[b]	80.9±0.7[c]

[1)]EC_{50} value; The effective concentration at which the DPPH and ABTS radicals were scavenged by 50%; EC_{50} value was obtained by interpolation from linear regression analysis; Each value is mean±SD (n=3).
[2)]Different letters (horizontal comparison) indicate significant difference (p<0.01).

Relationship between antioxidant activity and total phenolics and total flavonoids contents The phenolic compounds are wide spread plant secondary metabolites and contribute to the overall antioxidant activities of plant foods (27, 28). In order to examine the potential role of the phenolic compounds on antioxidant activity of MFRE and MFSE, total phenolics and total flavonoids contents were analyzed and results expressed in mg GAE and mg CAE per g samples respectively are presented in Table 5. TPC ranged from 4.6 mg in control to 7.5 mg in fermented soybean. Also, TPC of rice ranged from 1.4 mg in control to 3.8 mg in fermented products. *Monascus*-fermented products resulted in a significant increase (p<0.05) in TPC with the increasing fermentation time until 20 days. Significant differences (p<0.05) in TPC were found between MFSE and MFRE. The highest concentration (7.5 mg) of TPC was obtained from fermented soybean. The average TPC value (6.1 mg) of soybean during the fermentation was 2.2 times higher than that of the fermented rice (2.8 mg).

The total flavonoids contents were analyzed and results expressed in mg CAE per g samples are presented in Table 5. TFC of soybean and rice ranged 1.8-3.3 and 0.45-0.68 mg, respectively. Significant differences (p<0.05) in TFC were found between soybean and rice. The highest concentration (3.3 mg) of TFC was obtained from MFSE after 20 days of fermentation. A linear correlation between free radical scavenging activity and polyphenolic concentration has been reported in an extensive range of vegetables, fruits, and beverages (28-30). As you shown in

Table 4. Effect of dose-dependent on antioxidant potentials of MFSE and MFRE[1)]

mg/mL	Radical scavenging (%)[2)]		Inhibition of lipid peroxidation (%)	
	Soybean	Rice	Soybean	Rice
20	31.6±0.3[a]	25.5±0.2[a]	34.2±0.3[a]	10.8±0.6[a]
50	58.5±1.1[b]	65.4±0.6[b]	70.8±0.6[b]	30.9±1.1[b]
100	80.3±1.6[c]	85.9±1.1[c]	86.6±0.8[c]	45.8±2.2[c]
200	95.5±0.9[d]	93.1±1.2[c]	92.1±1.6[c]	70.5±1.4[d]
Trolox[3)]	90.6±0.5		91.2±0.8	

[1)]Each value is mean±SD (n=3); Different letters (vertical comparison) indicate significant difference (p<0.05).
[2)]Radical scavenging activity (%DPPH + %ABTS / 2).
[3)]The concentration of trolox is 1 mM.

Table 5. Determination of total phenol contents (TPC) and total flavonoids contents (TFC) of MFSE and MFRE[1)]

	TPC (mg GAE/g)		TFC (mg CAE/g)	
Time (day)	Soybean	Rice	Soybean	Rice
0	4.6±0.1[a]	1.4±0.1[a]	2.1±0.1[a]	0.45±0.06[a]
5	4.8±0.2[a]	1.6±0.3[a]	1.8±0.2[a]	0.46±0.04[a]
10	5.6±0. 3[b]	2.6±0.1[b]	2.6±0.1[b]	0.52±0.05[b]
15	6.8±0. 1[c]	3.6±0.2[c]	2.9±0.3[b]	0.68±0.02[c]
20	7.5±0.4[d]	3.8±0.1[d]	3.3±0.2[c]	0.66±0.02[c]
30	7.3±0.1[b]	3.7±0.2[d]	3.1±0.2[c]	0.64±0.03[c]

[1)]Each value is mean±SD (n=3); Different letters (vertical comparison) indicate significant difference ($p<0.05$).

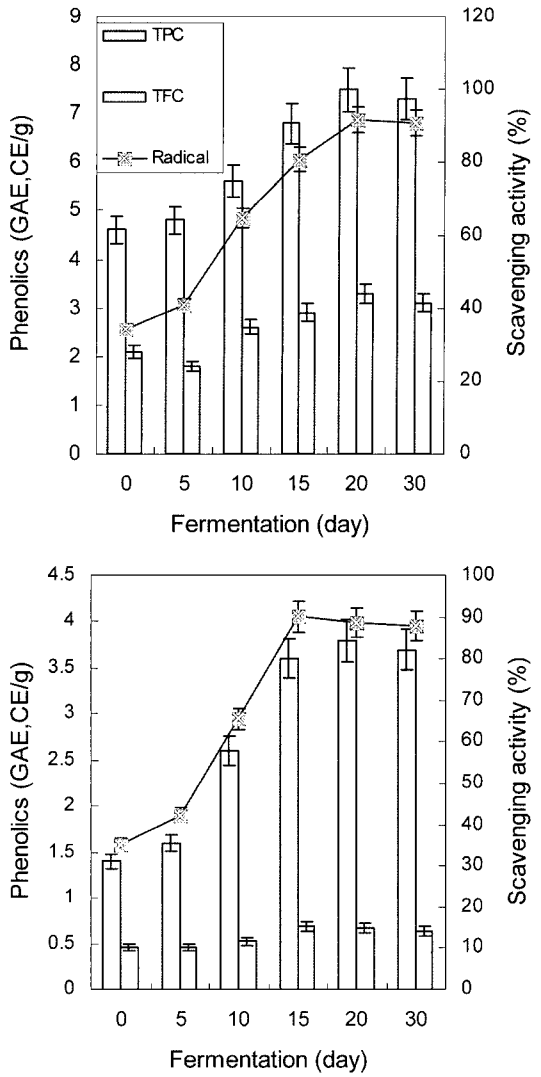

Fig. 1. The changes of total phenolics contents (TPC, mg GAE/g sample) and total flavonoids contents (TFC, mg CE/g sample) and in percentage of radical scavenging activity (% DPPH + %ABTS/2) of MFSE (top) and MFRE (bottom). Each value is mean±SD (n=3).

Fig. 1, the overall relationship between radical scavenging activity and total phenolic and total flavonoids contents for MFSE and MFRE was a positive linear correlation (TPC/TFC: r=0.79/0.77). In the case of MFSE, there was a good linear correlation between the scavenging effects for DPPH and ABTS radicals and the content of total phenols (r=0.84) and total flavonoids (r=0.81). The results indicated that the free radical scavenging effect of MFSE might be mostly related to its content of phenolics. For examples, MFSE, that was shown the highest antioxidant activity (91.7%) after 20 days of fermentation had the highest contents of total phenolics (7.5 mg) and total flavonoids (3.3 mg) respectively. Generally, compounds in soybean with antioxidant properties include flavonoids (isoflavones), tri-terpenoids, carotenoids, tocopherols, and saponins (30). Among them, the major active components of soybean are isoflavones, including daidzein and genistein which are antioxidants (31, 32). In the previous study, it was reported that liberation of aglycones isoflavone, genistein, and daidzein through the catalytic action of β-glucosidase during lactic acid-fermentation may account for the increased antioxidant capacity found with the fermented soybean (33). In view of these results, it can be concluded that the high contents of phenolic compounds are responsible for the greater antioxidant activity of MFSE compared to the MFRE, with its much lower phenolic content.

In summary, the antioxidant capacities of MFSE and MFRE were evaluated with three different *in vitro* testing systems. The total free radical scavenging (DPPH and ABTS radicals) and lipid peroxidation inhibitory activities of both soybean and rice enriched by using *Monascus*-fermentation. MFRE was highly effective in scavenging DPPH radicals, while MFSE not only has the radical scavenging ability but also has the ability to inhibit peroxidation of linoleic acid. The antioxidant potentials of MFSE were significantly ($p<0.01$) stronger than those of MFRE. A positive and significant correlation existed between antioxidant properties and total phenolics and total flavonoids content of MFSE, revealing that phenolic compounds were the dominant antioxidant components in the soybean fermented with *Monascus pilosus* IFO 480. Thus, *Monascus*-fermented soybean may be used as natural and potent dietary antioxidative additives or supplements due to its effective activities on free radical scavenging and peroxidation inhibition.

Acknowledgments

This work was supported by a Korea Research Foundation Grant funded by the Korean Government (MOEHRD, no. R03-2004-000-10025-0).

References

1. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74: 139-162 (1994)
2. Belch JJF, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. Brit. Heart J. 65: 245-248 (1991)
3. Juzlova P, Martinkova L, Kren V. Secondary metabolites of the fungus *Monascus*: a review. J. Ind. Microbiol. 16: 163-170 (1996)
4. Ma J, Li Y, Ye Q, Li J, Hua Y, Ju D, Zhang D, Cooper R, Chang M. Constituents of red yeast rice, a traditional Chinese food and

medicine. J. Agr. Food Chem. 48: 5220-5225 (2000)
5. Manzoni M, Rollini M. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl. Microbiol. Biot. 58: 555-564 (2002)
6. Endo A, Hasumi K, Negishi S. Monacolins J and L new inhibitors of cholesterol biosynthesis produced by *Monascus ruber*. J. Antibiot. 38: 420-422 (1985)
7. Su YC, Wang JJ, Lin TT, Pan TM. Production of the secondary metabolites gamma-aminobutyric acid and monacolin K by *Monascus*. J. Ind. Microbiol. Biot. 30: 41-46 (2003)
8. Kuba M, Tana S, Tawata S, Tawata M, Yasuda M. Production of angiotensin I- converting enzyme inhibitory peptides from soybean protein with *Monascus purpureus* acid proteinase. Process Biochem. 40: 2191-2196 (2005)
9. Aniya Y, Ohtani I, Higa T, Miyagi C, Gibo H, Shimabukuro M, Nakanishi H, Taira J. Dimerumic acid as an antioxidant of the mold, *Monascus anka*. Free Radical Bio. Med. 28: 999-1004 (2000)
10. Yang JH, Tseng YH, Lee YL, Mau JL. Antioxidant properties of methanolic extracts from monascal rice. LWT-Food Sci. Technol. 39: 740-747 (2006)
11. Murakami H, Asakawa T, Terao J, Matsushita S. Antioxidative stability of *tempeh* and liberation of isoflavones by fermentation. Agr. Biol. Chem. Tokyo 48: 2971-2975 (1984)
12. Nam HY, Min SG, Shin HC, Kim HY, Fukushima M, Han KH, Choi KD, Lee CH. The protective effects of isoflavone extracted from soybean paste in free radical initiator treated rats. Food Sci. Biotechnol. 14: 586-592 (2005)
13. Jang MY, Cho JY, Cho JI, Moon JH, Park KH. Isolation of compounds with antioxidative activity from quickly fermented soy-based foods. Food Sci. Biotechnol. 15: 214-219 (2006)
14. Pyo YH, Lee TC. The potential antioxidant capacity and angiotensin I-converting enzyme inhibitory activity of *Monascus*-fermented soybean extracts: Evaluation of *Monascus*-fermented soybean extracts as multifunctional food additives. J. Food Sci. 72: S708-S713 (2007)
15. Yen GC, Hsieh CL. Antioxidant activity of extracts from *du-zhong* (*Eucommia ulmoids*) toward various peroxidation models *in vitro*. J. Agr. Food Chem. 46: 3952-3957 (1998)
16. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 26: 25-30 (1995)
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
18. Pyo YH. Optimum condition for production of mevinolin from the soybean fermented with *Monascus* sp. Korean J. Food Sci. Technol. 38: 256-261 (2006)
19. Pyo YH, Lee, YC. Mevinolin production by *Monascus pilosus* KFRI-1140 in solid state fermentation using soymeal. Food Sci. Biotechnol. 15: 647-649 (2006)
20. Singleton VL, Rossi JA. Colorimetry of total phenolic with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Viticult. 16: 144-158 (1965)
21. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559 (1999)
22. Dziezak JD. Antioxidants. Food Technol.-Chicago 40: 94-102 (1986)
23. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclotextrin emulsion. J. Agr. Food Chem. 40: 945-948 (1992)
24. Pyo YH. Production of a high value-added soybean containing bioactive mevinolins and isoflavones. J. Food Sci. Nutr. 12: 29-34 (2007)
25. Rice-Evans C, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio. Med. 20: 933-956 (1996)
26. Catherine A, Rice-Evans C, Nicholas JM, George P. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2: 152-159 (1997)
27. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism, and structure-activity relationship. J. Nutr. Biochem. 13: 572-584 (2002)
28. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74: 2157-2184 (2004)
29. Amarowicz R, Troszynska A, Barylko-Pikielna N, Shahidi F. Polyphenolics extracts from legume seed: Correlations between total antioxidant activity, total phenolics content, tannins content, and astringency. J. Food Lipids 278-286 (2004)
30. Lopez-Amoros ML, Hernandez T, Estrella I. Effects of germination on legume phenolic compounds and their antioxidant activity. J. Food Compos. Anal. 19: 277-283 (2006)
31. Meng QH, Lewis P, Wahala K, Adlercreutz H, Tikkanen MJ. Incorporation of esterified soybean isoflavones with antioxidant activity into low-density lipoprotein. Biochim. Biophys. Acta 1438: 369-376 (1999)
32. Mitchell JH, Gardner PT, Mcphail DB, Morrice PC, Collins AR, Duthie GG. Antioxidant efficacy of phytoestrogens in chemical and biological model system. Arch. Biochem. Biophys. 360: 142-148 (1998)
33. Pyo YH, Lee TC, Lee YC. Effects of lactic acid fermentation on enrichment of antioxidant properties and bioactive isoflavones in soybean. J. Food Sci. 70: S215-S220 (2005)