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ScienceDirect
Food Chemistry 106 (2008) 1128–1137
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant properties of water extracts from *Monascus* fermented soybeans

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Received 2 March 2007; received in revised form 6 June 2007; accepted 17 July 2007

Abstract

Solid-state bioprocessing of soybean by *Monascus* is a biotechnological strategy to produce *Monascus*-fermented soybeans (MFS) with more beneficial components. The objective of this study was to evaluate the antioxidant properties of cold and hot water extracts from MFS as compared to uninoculated soybeans. With regard to the EC_{50} values of antioxidant activities, the effectiveness of cold water extracts was in a descending order: soybeans > MFS-31527 > MFS-31499 whereas that of the hot water extracts: soybeans ~ MFS-31499 > MFS31527. Cold water extracts showed higher reducing power and lower scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals than hot water extracts. For both extracts, MFS and soybeans showed comparable effective chelating abilities on ferrous ions. Total phenols were the major naturally occurring antioxidant components found. For both extracts, soybeans were more effective in antioxidant activity and scavenging ability on DPPH radicals whereas MFS-31499 and MFS-31527 were more effective in reducing power and scavenging ability on hydroxyl radicals. Based on the results obtained, MFS-31499, MFS-31527 and soybeans were relatively effective in the antioxidant properties assayed and might be potential antioxidants for application in food products.

Keywords: *Monascus*; Soybeans; Antioxidant activity; Reducing power; Scavenging ability; Chelating ability; Antioxidant components

1. Introduction

Fungus *Monascus* has been used as a traditional fermented food and its metabolic products have also been utilised as a food pigment or biological agents in oriental countries for centuries. Most of the strains isolated from traditional oriental foods are characterised as the genus *Monascus*, which is categorised into four species: *M. pilosus, M. purpureus, M. rubber* and *M. froridanus* (Blanc et al., 1994; Juzlová, Martinková, & Kren, 1996). Monacolin K, commercially known as lovastatin, mevinolin, cholestin and mevacor, is one of the secondary metabolites from the *Monascus* species and it has been demonstrated as a specific inhibitor of 3-hydroxy-3-methylglurtaryl-coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis (Alberts et al., 1980; Endo, 1980). Monacolin K may be used to maintain a normal blood lipid level by decreasing cholesterol synthesis (Bach, 1986; Cicero, Brancaleoni, Laghi, Donati, & Mino, 2005; Endo, 1979; Endo & Hasumi, 1997; Heber, Lembertas, Lu, Bowerman, & Go, 2001; Kennedy et al., 1999; Li et al., 1998; Wang et al., 1997).

Soybeans and soy products are rich in isoflavones and are very common foods in oriental countries, as a meat substitute. Many studies have shown that daily intakes of soy foods may help humans to prevent certain cancers (Alekel, Hasler, Juma, Drum, & Kukreja, 1998; Anthony, Clarkson, Hughes, Morgan, & Burke, 1996; Messina, Persky, Setchell, & Barnes, 1994), lower plasma cholesterol (Crouse et al., 1999; Franke, Custer, Cerna, & Narala, 1995; Gardner, Newell, Cherin, & Haskell, 2001; Ho et al., 2000; Potter et al., 1993; Wangen, Duncan, Xu, & Kurzer, 2001), lower blood pressure (Jenkins et al., 2002;

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doi:10.1016/j.foodchem.2007.07.047

Washburn, Burke, Morgan, & Anthony, 1999) and reduce the risk of coronary heart diseases (Lucas, Khalil, Daggy, & Arjmandi, 2001). Isoflavones found in soybeans, such as daidzein, genistein, daidzin and genistin, are believed to possess antioxidant and anticarcinogenic activities and inhibit melanoma cell growth (Barnes, 1995; Fritz, Seppanen, Kurzer, & Csallany, 2003; Lee et al., 2005; Liu, Chang, & Wiesenborn, 2005; Russo, Cardile, Lombardo, Vanella, & Acquaviva, 2006).

Solid-state bioprocessing (SSB) of an edible plant matrix by dietary fungi is a biotechnological strategy that may produce beneficial bioactive phytochemicals during fermentation (McCue & Shetty, 2005a). Recently, many reports on SSB of a soybean matrix by a dietary fungus exhibit that *Rhizopus oligosporus* or *Lentinula edodes* could liberate substantial amounts of free phenolic antioxidants (McCue, Horri, & Shetty, 2003; McCue, Horii, & Shetty, 2004; McCue & Shetty, 2003) and *Bacillus subtilis* and a culture mixture consisting of *Acetobacter* sp., *Lactobacillus* sp., *Saccharomyces* sp. and *Streptomyces* sp. give rise to better antioxidant capabilities (Iwai, Nakaya, Kawasaki, & Matsue, 2002; Yang, Mau, Ko, & Huang, 2000; Yokota, Hattori, Ohishi, Hasegawa, & Watanabe, 1996).

Both *Monascus* species and soybean contain functional components but in combination, SSB of a soybean matrix by *Monascus* species is a new area of investigation. The objective of this study was to evaluate the antioxidant properties of cold and hot water extracts from *Monascus*-fermented soybeans as compared to uninoculated soybean products. Antioxidant properties were assayed in termed of the inhibition of peroxidation by the conjugated diene method, reducing power, scavenging abilities on free radicals and chelating ability on ferrous ions. The contents of potential antioxidant components in cold and hot water extracts were also determined.

2. Materials and methods

2.1. Chemicals

Acetonitrile, ethanol, *n*-hexane, methanol and toluene were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Ascorbic acid, butylated hydroxyanisole (BHA), β-carotene, citric acid, 2,6-dichloroindophenol, 5,5-dimethyl pyrroline-*N*-oxide (DMPO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), ferrous ammonium sulfate, ferrozine, Folin-Ciocalteu's reagent, gallic acid, linoleic acid, potassium ferricyanide and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Ferrous chloride and hydrogen peroxide were obtained from Merck Co. (Darmstadt, Germany). Ferric chloride, potassium hydroxide, sodium carbonate, sodium phosphate, sodium sulfate and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Metaphosphoric acid was obtained from Union Chemical Works (Hsinchu, Taiwan). Pyrogallol was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). Malt extract agar, malt extract broth, potato dextrose agar and potato dextrose broth were obtained from Difco Laboratories (Sparks, MD).

2.2. Materials and preparation of extracts

Soybean (*Glycine max* Merrill) was purchased at a local market in Taichung City, Taiwan. *Monascus purpureus* Went (BCRC 31499) and *Monascus pilosus* K. Sato ex D. Hawksworth & Pitt (BCRC 31527) were obtained from the Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu City, Taiwan. The fungi of *M. purpureus* and *M. pilosus* were inoculated onto malt extract agar and potato dextrose agar, respectively, and both incubated at 25 °C for 72 h. After a pure culture was obtained, the *M. purpureus* and *M. pilosus* mycelium was re-inoculated into malt extract broth and potato dextrose broth, respectively and both mycelia were incubated at 25 °C for 7 days as the inoculum. Each inoculum was then homogenised in a Warring blender and inoculated into autoclaved soybeans at a rate of 5 ml/100 g. The corresponding products, *M. purpureus* BCRC 31499 fermented soybeans (MFS-31499) were produced after the colonisation of fungal mycelia for 6 days at 30 °C, whereas *M. pilosus* BCRC 31527 fermented soybeans (MFS-31527) were produced after the colonisation of fungal mycelia for 7 days at 25 °C. Two *Monascus* fermented soybean products as well as uninoculated soybeans, which were also autoclaved and used as controls, were air-dried in an oven at 50 °C.

For each product, three dried samples (~100 g each) were randomly selected and prepared for analyses. A coarse powder (8 opening/cm) was obtained using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany). For the cold water extract, a subsample (10 g) was extracted by shaking with 100 ml of deioinised water, at 25 °C at 20*g* for 24 h and filtering through Whatman No. 1 filter paper. For the hot water extract, a subsample (10 g) was extracted by boiling with 100 ml of deioinised water at 100 °C for 10 min and filtering. Both residues were then extracted with two additional 100 ml portions of deioinised water as described above. The combined cold water and hot water extracts were then freeze dried. The dried extract was used directly for analyses of antioxidant components or redissolved in deionised water to a concentration of 50 mg/ml and were then diluted to 0.01, 0.1, 1, 5, 10, 20 mg/ml for further uses.

2.3. Antioxidant activity

Antioxidant activity was determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). Each extract (0.01–20 mg/ml, 100 μl), in deioinised water, was mixed with 2 ml of 10 mmol/l linoleic acid emulsion (pH 6.6) in test-tubes and placed in darkness at 37 °C to accelerate oxidation. After incubation for 0 h or 15 h,

0.1 ml of each tube was mixed 7 ml of 800 ml/l methanol in deionised water and the absorbance of the mixture was measured at 234 nm, against a blank in a Hitachi U–2001 spectrophotometer (Tokyo, Japan). Antioxidant activity was calculated as follows: antioxidant activity (%) = [(ΔA_{234} of control – ΔA_{234} of sample)/ΔA_{234} of control] × 100. A value of 100% indicates the strongest antioxidant activity. An EC_{50} value (mg extract/ml) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHA and α-tocopherol were used as positive controls and diluted to 0.01, 0.1, 1, 5, 10, 20 mg/ml for further uses.

2.4. Reducing power

Reducing power was determined according to the method of Oyaizu (1986). Each extract (0.01–20 mg/ml, 2.5 ml), in deionised water, was mixed with 2.5 ml of 200 mmol/l 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 200*g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 1 mg/ml ferric chloride, and the absorbance was measured at 700 nm, against a blank. A higher absorbance indicates a higher reducing power. An EC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid, BHA and α-tocopherol were used as positive controls and diluted to 0.01, 0.1, 1, 5, 10, 20 mg/ml for further uses.

2.5. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Each extract (0.01–20 mg/ml, 4 ml), in deionised water, was mixed with 1 ml of a methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mmol/l 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 (Shimada, Fujikawa, Yahara, & Nakamura, 1992). An EC_{50} value (mg extract/ml) is the effective concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid, BHA and α-tocopherol were used as positive controls and diluted to 0.01, 0.1, 1, 5, 10, 20 mg/ml for further uses.

2.6. Scavenging ability on hydroxyl radicals

Hydroxyl radicals reacted with the nitrone spin trap DMPO and the resultant DMPO–OH adducts were detected by an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 8 min after mixing 200 μl of each extract (5–20 mg/ml) in deionised water with 200 μl of 10 mmol/l H_2O_2, 200 μl of 10 mmol/l ferrous ammonium sulfate and 200 μl of 10 mmol/l DMPO using a Bruker EMX-10 EPR spectrometer (Bruker Biospin, Rheinstetten, Germany) at the following settings: 0.3480-T magnetic field, 1.0×10^{-4} T modulation amplitude, 0.5 s time constant and 200 s scan period (Shi, Dalal, & Jain, 1991). An EC_{50} value (mg extract/ml) is the effective concentration at which hydroxyl radicals were scavenged by 50%. BHA was used as a positive control.

2.7. Chelating ability on ferrous ions

Chelating ability was determined according to the method of Dinis, Madeira, and Almeida (1994). Each extract (0.01–20 mg/ml, 1 ml) in deionised water, was mixed with 3.7 ml methanol and 0.1 ml 2 mmol/l ferrous chloride. The reaction was initiated by 0.2 ml 5 mmol/l ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. An EC_{50} value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50%. EDTA was used as a positive control and diluted to 0.01, 0.1, 1, 5, 10, 20 mg/ml for further uses.

2.8. Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each dried extract (100 mg) was extracted with 10 ml 10 mg/ml metaphosphoric acid for 45 min at room temperature and filtered. The filtrate (1 ml) was mixed with 9 ml 2,6-dichloroindophenol and the absorbance was measured within 15 s at 515 nm against a blank. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid.

β-Carotene was extracted and analysed as described by Rundhaug, Pung, Read, and Bertram (1988). Each dried extract (100 mg) was extracted with 1 ml ethanol, 2 ml *n*-hexane containing BHA (25 μg/ml) and 1 ml deionised water, at 20*g* for 45 min, at room temperature and then centrifuged at 400*g* for 10 min. After the removal of the *n*-hexane layer by N_2 gas, the volume was adjusted to 1 ml using *n*-hexane and filtered through a syringe-driven filter unit (13 mm, Millipore, Billerica, MA) using a 0.45-μm PVDF non-sterile filter paper. Immediately after filtration, the filtrate was injected onto a high performance liquid chromatograph (HPLC).

The HPLC system consisted of a Shimadzu LC-10AT VP pump (Tokyo, Japan), a Shimadzu FCV-10AL VP controller, a Rheodyne 7725i injector, a 20-μl-sample loop, a Hitachi D–2500 chromato-integrator, a Shimadzu SPD-10 A VP UV–Vis detector and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck). The mobile phase was 75 ml methanol/25 ml toluene at a flow rate of 1.5 ml/min and UV detection was at 450 nm. The β-Carotene content was calculated on the basis of the calibration curve of authentic β-carotene.

Tocopherols were extracted and analysed according to the method of Carpenter (1979). Each dried extract (100 mg) was suspended in 6 ml pyrogallol (60 mg/ml in

ethanol) and 4 ml of 600 mg/ml aqueous potassium hydroxide solution and the resulting mixture was saponified at 70 °C for 20 min. Deionised water (15 ml) was added and the mixture was extracted with 15 ml of *n*-hexane. The organic layer was washed with deionised water to neutral, dried over anhydrous sodium sulfate, and rotary evaporated to dryness. The residue was redissolved in 5 ml of *n*-hexane and filtered prior to HPLC injection in the same manner as in the β-carotene assay.

The HPLC system was the same as for the β-carotene assay. The mobile phase was 85 ml acetonitrile/15 ml methanol at a flow rate of 1.5 ml/min and UV detection was at 295 nm. The content of each tocopherol was calculated on the basis of the calibration curve of each authentic tocopherol.

Total phenols were determined according to the method of Taga, Miller, and Pratt (1984). Each dried extract (100 mg) was dissolved in a solution of 5 ml of 3 mg/ml in 60 ml methanol/40 ml deionised water and the resulting mixture (100 μl) was added to 2 ml of 20 mg/ml aqueous sodium carbonate solution. After 2 min, 100 μl of 500 mg/ml Folin-Ciocalteu's reagent were added to the mixture. After 30 min of standing, absorbance was measured at 750 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid.

2.9. Statistical analysis

For each of the cold and hot water extractions, three samples were prepared for assays of every antioxidant attribute and component. The experimental data was subjected to an analysis of variance for a completely random design to determine the Fisher protected least significant difference at the level of 0.05. For the correlations, the CORR procedure (SAS Institute Inc., Cary, NC, 1988) was used to determine the Pearson's correlation coefficient (r) between the content of each antioxidant component and the EC_{50} value of each antioxidant attribute.

3. Results and discussion

3.1. Extraction yield

Following the extraction with cold or hot water, the yields of MFS-31499 were higher than those of soybeans and in turn higher than those of MFS-31527 (Table 1). The yields of the cold and hot water extracts were similar for MFS-31499 or soybeans. However, for MFS-31527, hot water extracted more than cold water. Usually, *Monascus* colonisation degrades the substrate soybean into small water soluble substances for energy to grow. Therefore, the higher yields of MFS-31499 were expected whereas the lower yields of MFS-31527 might be due to the rapid decomposition of small water substances which occurred immediately after those were degraded from the substrate.

Table 1
Extraction yield of cold and hot water extracts from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans

	Extraction yield[a] (g/100 g)		
	MFS-31499	MFS-31527	Soybeans
Cold water	a39.6 ± 0.45A[b]	b25.7 ± 1.78C	a34.6 ± 3.14B
Hot water	a39.0 ± 0.92A	a30.2 ± 0.63C	a35.1 ± 1.26B

[a] Extracted from air-dried materials. Each value is expressed as mean ± standard error ($n = 3$).
[b] Means with different capital letters within a row are significantly different ($P < 0.05$). Means with different small letters within a column are significantly different ($P < 0.05$).

3.2. Antioxidant activity

Using the conjugated diene method, antioxidant activities of the cold and hot extracts from MFS-31499, MFS-31527 and soybeans increased with increased concentrations (Fig. 1). For the cold extracts, soybeans exhibited higher antioxidant activity than MFS-31499 and MFS-31527. At 5 mg/ml, cold water extracts from MFS-31499 and MFS-31527 exhibited moderate antioxidant activities of 61.4% and 70.5%, respectively whereas that from

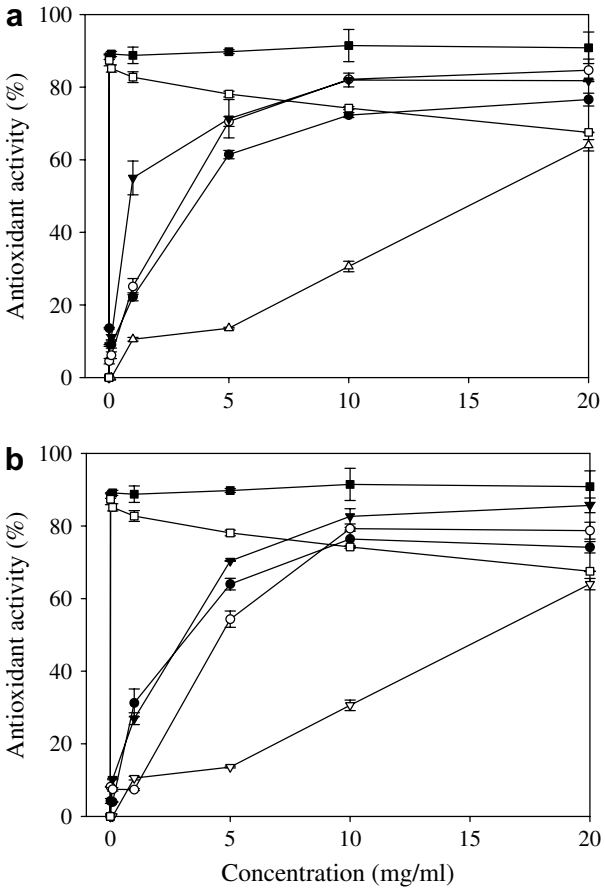

Fig. 1. Antioxidant activity of cold (a) and hot water extracts (b) from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans. Each value is expressed as mean ± SE ($n = 3$). (●) MFS-31499, (○) MFS-31527, (▼) Soybeans, (▽) ascorbic acid, (■) BHA, (□) α-tocopherol.

soybeans showed antioxidant activity of 55.0% at 1 mg/ml. For the hot extracts, antioxidant activities of soybeans were comparable to those of MFS-31499 and MFS-31527. At 5 mg/ml, hot water extracts from MFS-31499, MFS-31527 and soybeans exhibited moderate antioxidant activities of 64.0%, 54.3% and 70.4%, respectively. Consistently for three samples, cold and hot water extracts showed similar antioxidant activities. It seems hot water might not cause the thermal destruction of certain antioxidant components. However, antioxidant activities were 88.4% and 87.4% at 0.01 mg/ml for BHA and α-tocopherol, respectively and 64.0% at 20 mg/ml for ascorbic acid.

The antioxidant activity assayed was the ability to inhibit the peroxidation of linoleic acid. The antioxidant activity of both extracts might be due to the reduction of hydroperoxide, inactivation of free radicals or complexing with metal ions or combination thereof. This good antioxidant activity of cold and hot water extracts from MFS-31499, MFS-31527 and soybeans might be attributed to the presence of phytochemicals, such as isoflavones (Wang & Wixon, 1999). Taylor and Richardson (1980) found that cysteine exhibited better protection of a linoleate emulsion than BHA, butylated hydroxytoluene (BHT) and α-tocopherol against the oxidation by haemoglobin. Furthermore, it seems that MFS-31499, MFS-31527 and soybeans might contain some amount of cysteine, which is in soluble soy protein and thereby, probably contributing to the better antioxidant activities of cold and hot water extracts.

3.3. Reducing power

The reducing powers of cold and hot water extracts from three samples increased in two patterns with increased concentrations, i.e., a moderate increase for MFS-31499 and MFS-31527 and a slow increase for soybeans (Fig. 2). For both water extracts, the reducing powers of MFS-31499 and MFS31527 were higher than that of soybeans. For cold water extracts, the reducing powers of MFS-31499 and MFS-31527 were both 0.54 at 5 mg/ml whereas that of soybeans was 0.55 at 10 mg/ml. For hot water extracts, the reducing powers of MFS-31499 and MFS-31527 were 0.48 and 0.31 at 5 mg/ml, respectively, whereas that of soybeans was 0.37 at 10 mg/ml. Apparently, cold water extracts showed higher reducing powers than hot water extracts. It seems that heating might cause the thermal destruction of certain reducing components. However, reducing powers were 1.25 and 1.10 for ascorbic acid and α-tocopherol at 1.0 mg/ml, respectively, and 1.15 for BHA at 0.1 mg/ml.

Furthermore, reductones such as ascorbic acid can react directly with peroxides and also with certain precursors and thereby, prevent the formation of peroxide (Shimada et al., 1992). The reducing power of cold and hot water extracts might be due to their hydrogen-donating ability, as described by Shimada et al. (1992). Furthermore, Yang et al. (2000) reported that fermented soybean broth by *Acetobacter* sp., *Lactobacillus* sp., *Saccharomyces* sp. and

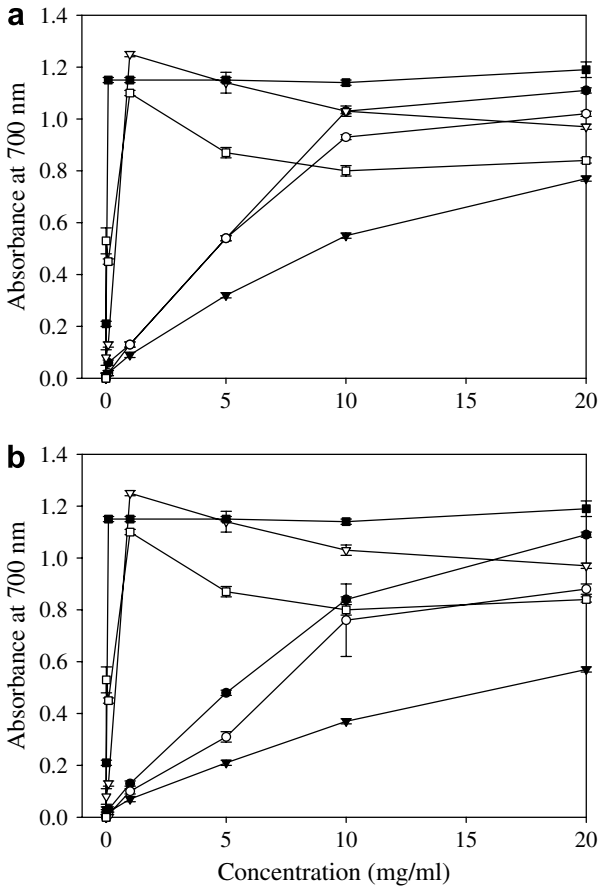

Fig. 2. Reducing power of cold (a) and hot water extracts (b) from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans. Each value is expressed as mean ± SE ($n = 3$). (●) MFS-31499, (○) MFS-31527, (▼) Soybeans, (▽) ascorbic acid, (■) BHA, (□) α-tocopherol.

Streptomyces sp. exhibited higher reducing powers than soybean broth. Therefore, MFS-31499 and MFS-31527 might contain reductones formed during fermentation, which could react with free radicals to stabilise and terminate radical chain reactions.

3.4. Scavenging ability on 1,1-diphenyl–2-picrylhydrazyl radicals

For both cold and hot water extracts, soybeans showed higher scavenging abilities on DPPH radicals than MFS-31499 and MFS-31527 (Fig. 3). At 5 mg/ml, scavenging abilities of the cold water extracts from MFS-31499, MFS-31527 and soybeans on DPPH radicals were 36.5%, 56.4% and 89.1%, respectively. For hot water extracts, MFS-31499, MFS-31527 and soybeans exhibited scavenging abilities of 76.8%, 92.2% and 94.7% on DPPH radicals at 5 mg/ml, respectively. Obviously, hot water extracts showed better scavenging DPPH abilities than cold water extracts. Contrary to the reducing power, heating might impart certain components with better scavenging ability on DPPH radicals. However, the scavenging abilities of BHA and α-tocopherol were 93.1% and 93.3% at 0.1

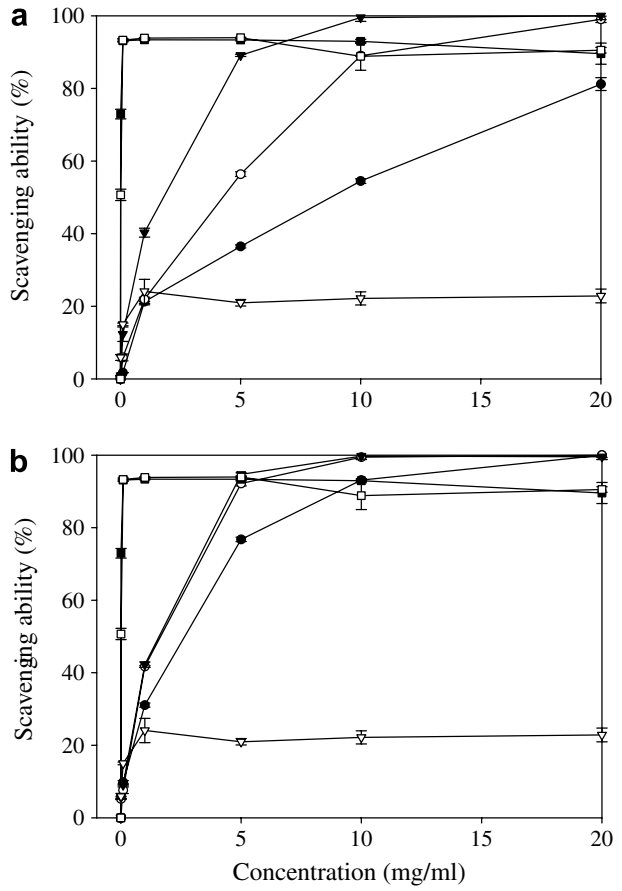

Fig. 3. Scavenging ability of cold (a) and hot water extracts (b) from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans on 1,1-diphenyl–2-picrylhydrazyl radicals. Each value is expressed as mean ± SE ($n = 3$). (●) MFS-31499, (○) MFS-31527, (▼) Soybeans, (▽) ascorbic acid, (■) BHA, (□) α-tocopherol.

mg/ml, respectively and that of ascorbic acid was 24.1% at 1 mg/ml.

The high scavenging ability of cold and hot water extracts from MFS-31499, MFS-31527 and soybeans might be attributed to the presence of isoflavones (Lee et al., 2004; Lee et al., 2005). In addition, McCue and Shetty (2005b) reported that bioprocessing of soymilk by Kefir cultures containing *Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetylactis, Lactobacillus plantarum, Lactobacillus casei, Saccharomyces fragilis and Leuconostoc crenoris* could increase phenolic contents and thus enrich the scavenging abilities on DPPH radicals. It seems that MFS-31499 and MFS-31527 might contain phenolic components, formed during fermentation, which contribute to this free radical scavenging ability.

Herraiz, Galisteo, and Chamorro (2003) found out that an essential amino acid, L-tryptophan, could react with phenolic aldehydes in food to form phenolic tetra hydro-β-carboline alkaloids that scavenged 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) effectively. Therefore, the presence of L-tryptophan in cold and hot water extracts from MFS-31499, MFS-31527 and soybeans might most likely account for the scavenging ability on DPPH radicals.

3.5. Scavenging ability on hydroxyl radicals

For cold water extracts, scavenging abilities on hydroxyl radicals were in the range of 22.8–33.2% at 20 mg/ml (Table 2). For hot water extracts, at 20 mg/ml, scavenging abilities on hydroxyl radicals were in the descending order: MFS-31499 > MFS-31527 > soybean. For both extracts, MFS-31499 and MFS-31527 exhibited better scavenging abilities than soybean. It seems that *Monascus* growth on soybean might produce some components effective in scavenging hydroxyl radicals. However, at 20 mg/ml, the scavenging ability of BHA was only 18.3%.

Iwai et al. (2002) reported that low-molecular-weight viscous substances (Mw < 100,000) from Natto exhibited higher scavenging abilities on hydroxyl radicals than high-molecular-weight viscous substances (Mw > 100,000) and water extracts from Natto. In addition, fermented soybean broth by *Acetobacter* sp., *Lactobacillus* sp., *Saccharomyces* sp. and *Streptomyces* sp., showed better scavenging abilities on hydroxyl radicals than soybean broth (Yang et al., 2000). It reveals that MFS-31499 and MFS-31527 might possess more hydroxyl radical scavenging components which are formed during fermentation.

Halliwell, Gutteridge, and Aruoma (1987) indicated that mannitol, histidine and adenosine monophosphate (AMP) showed scavenging abilities on hydroxyl radicals. Both water extracts might contain these components, which were responsible for the low scavenging ability on hydroxyl radicals.

3.6. Chelating abilities on ferrous ions

At 5 mg/ml, the chelating abilities of cold water extracts from MFS-31499, MFS-31527 and soybeans on ferrous ions were 42.6%, 63.3% and 58.6%, respectively (Fig. 4). For hot water extracts, at 5 mg/ml, MFS-31499, MFS-31527 and soybeans chelated 74.2%, 46.7% and 51.7% of ferrous ions,

Table 2
Scavenging ability of cold and hot water extracts from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans on hydroxyl radicals

Amount (mg/ml)	Scavenging ability[a] (%)		
	MFS-31499	MFS-31527	Soybeans
Cold water			
5.0	–[b]	–	–
10.0	–	5.15 ± 0.32	–
20.0	26.4 ± 2.51B	33.2 ± 0.73A	22.8 ± 0.68C
Hot water			
5.0	–	–	–
10.0	15.0 ± 0.27A	5.32 ± 0.64C	6.20 ± 0.17B
20.0	35.9 ± 0.43A	19.5 ± 0.33B	8.37 ± 0.36C

[a] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).
[b] No effect.

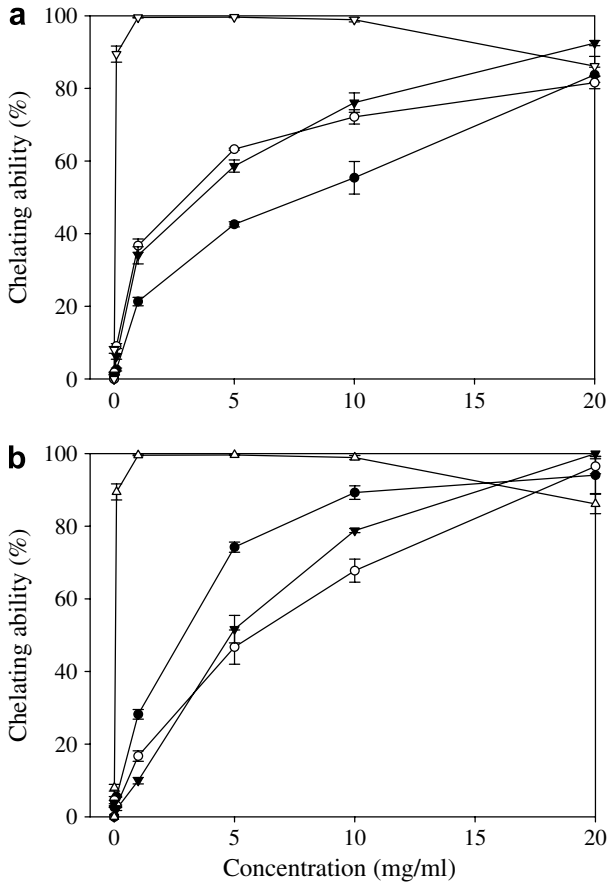

Fig. 4. Chelating ability of cold (a) and hot water extracts (b) from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans on ferrous ions. Each value is expressed as mean ± SE ($n = 3$). (●) MFS-31499, (○) MFS-31527, (▼) Soybeans, (▽) EDTA.

respectively. For both extracts, *Monascus* fermented soybeans and soybeans showed comparable effective chelating abilities on ferrous ions. However, EDTA was an excellent chelating agent for ferrous ions and its chelating ability was 89.4% at 0.1 mg/ml.

Since ferrous ions were the most effective pro-oxidants in the food system, (Yamaguchi, Tatsumi, Asano, Kato, & Ueno, 1988), and are commonly found in foods, the high ferrous ion chelating abilities of the cold and hot water extracts from MFS-31499, MFS-31527 and soybeans would be beneficial.

3.7. EC_{50} values in antioxidant properties

The antioxidant properties assayed herein were summarised in Table 3 and the results were normalised and expressed as EC_{50} values (mg water extracts per ml) for comparison. Effectiveness of antioxidant properties inversely correlated with their EC_{50} values. With regard to the EC_{50} values of antioxidant activities by the conjugated diene method, the effectiveness of cold water extracts was in a descending order: soybeans > MFS-31527 > MFS-31499 whereas that of hot water extracts was in a descending order: soybeans ~ MFS-31499 > MFS31527. For both extracts, effectiveness in their reducing powers was in a descending order: MFS-31499–MFS-31527 > soybeans. Cold water extracts were more effective than hot water extracts as evidenced by lower EC_{50} values for the three samples.

With regard to the scavenging ability on DPPH radicals, both extracts were effective in a descending order: soybeans > MFS-31527 > MFS-31499. Hot water extracts were more effective than cold water extracts as evidenced by lower EC_{50} values for the three samples. With regard to the scavenging ability on hydroxyl radicals, cold water extracts of the three samples were effective in the descending order: MFS-31527 > MFS-31499 > soybeans whereas hot water extracts were effective in the descending order: MFS-31499–MFS-31527 > soybeans. Cold water extracts were more effective than hot water extracts. With regard

Table 3
EC_{50} values of cold and hot water extracts from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans

	EC_{50} value[a] (mg extract/ml)		
	MFS-31499	MFS-31527	Soybeans
Cold water			
Antioxidant activity	a3.83 ± 0.09A[b]	b3.19 ± 0.12B	b0.90 ± 0.07C
Reducing power	b4.61 ± 0.08B	b4.61 ± 0.08B	b8.91 ± 0.18A
Scavenging ability on DPPH radicals	a8.76 ± 0.13A	a4.26 ± 0.06B	a1.79 ± 0.07C
Scavenging ability on hydroxyl radicals	a29.1 ± 1.49B[c]	b26.0 ± 0.28C[c]	b32.0 ± 0.54A[c]
Chelating ability on ferrous ions	a8.14 ± 1.02A	b3.00 ± 0.13B	b3.59 ± 0.26B
Hot water			
Antioxidant activity	b3.28 ± 0.26B[b]	a4.64 ± 0.14A	a3.12 ± 0.06B
Reducing power	a5.28 ± 0.11C	a7.26 ± 0.70B	a16.5 ± 0.41A
Scavenging ability on DPPH radicals	b2.66 ± 0.04A	b1.66 ± 0.02B	b1.59 ± 0.04C
Scavenging ability on hydroxyl radicals	a26.7 ± 0.21B[c]	a41.6 ± 0.20B[c]	a213 ± 15.2A[c]
Chelating ability on ferrous ions	b2.89 ± 0.10B	a5.73 ± 0.87A	a4.86 ± 0.29A

[a] EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) or hydroxyl (OH) radicals were scavenged by 50%; and ferrous or cupric ions were chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.

[b] Each value is expressed as mean ± SE ($n = 3$). Means with different capital letters within a row are significantly different ($P < 0.05$). Means with different small letters within a column at a specific antioxidant attribute are significantly different ($P < 0.05$).

[c] Obtained by extrapolation from linear regression analysis.

to chelating ability on ferrous ions, cold water extracts were effective in a descending order: soybeans ~ MFS-31527 > MFS-31499. On the contrary, hot water extracts were effective in the descending order: MFS-31499 > soybeans ~ MFS-31527.

Apart from scavenging abilities on hydroxyl radicals, most EC_{50} values were less than 10 mg/ml, indicating that both extracts from the three samples effective in these antioxidant properties. Among the antioxidant properties assayed, cold extracts from the three samples were more effective in reducing power and scavenging ability on hydroxyl radicals whereas hot water extracts from three samples was more effective in scavenging ability on DPPH radicals. For both extracts, soybeans were more effective in antioxidant activity and scavenging ability on DPPH radicals whereas MFS-31499 and MFS-31527 were more effective in reducing power and scavenging ability on hydroxyl radicals. Overall, MFS-31499 and MFS-31527 were comparable to soybeans in most antioxidant properties.

When the extraction yields were taken into consideration, the EC_{50} values for antioxidant activity (mg dried sample per ml) of the cold water extracts were 9.67, 12.4 and 2.60 mg/ml whereas those of the hot water extracts were 8.42, 15.4 and 8.90 mg/ml for MFS-31499, MFS-31527 and soybeans, respectively. The EC_{50} values for reducing power of the cold water extracts were 11.6, 17.9 and 25.7 mg/ml whereas those of the hot water extracts were 13.6, 24.1 and 47.1 mg/ml for MFS-31499, MFS-31527 and soybeans, respectively. The EC_{50} values for scavenging ability on DPPH radicals of the cold water extracts were 22.1, 16.6 and 5.17 mg/ml whereas those of the hot water extracts were 6.83, 5.50 and 4.53 mg/ml for MFS-31499, MFS-31527 and soybeans, respectively.

The EC_{50} values for scavenging ability on hydroxyl radicals of the cold water extracts were 73.4, 101 and 92.3 mg/ml whereas those of the hot water extracts were 68.6, 138 and 607 mg/ml for MFS-31499, MFS-31527 and soybeans, respectively. The EC_{50} for chelating ability on ferrous ions of the cold water extracts were 20.6, 11.7 and 10.4 mg/ml whereas those of the hot water extracts were 7.42, 19.0 and 13.9 mg/ml for MFS-31499, MFS-31527 and soybeans, respectively. Most EC_{50} values of the cold water and hot water extracts were below 30 mg/ml on the basis of dry sample except for that of hot water extracts from soybeans for reducing power, those of the both water extracts for scavenging abilities on hydroxyl radicals. It seems that these two extracts were effective for the antioxidant properties assayed.

Although BHA, ascorbic acid and/or α-tocopherol were good for antioxidant activity, reducing power and scavenging ability on DPPH radicals and EDTA was a good chelator for ferrous ions, they were additives and used or present in mg levels in foods. Both water extracts of MFS-31499, MFS-31527 and soybeans could be used in g or hundreds of g levels as a soymilk or food processing products such as tofu. Therefore, fermented soybeans (MFS-31499 and MFS-31527) in human diets might supply a new alternative for health protection to help humans reduce oxidative damage daily.

3.8. Antioxidant components

Naturally occurring antioxidant components, including ascorbic acid, tocopherols and total phenols, were found in the cold and hot water extracts from MFS-31499, MFS-31527 and soybeans (Table 4). However, β-carotenes were not detected in both water extracts due to their fat-soluble nature. Contents of ascorbic acid were low in both extracts of MFS-31527. Total tocopherol contents were in the range from 0.04 to 0.11 mg/g. Total phenols were the major naturally occurring antioxidant components found and the contents in cold water extracts were higher than those in hot water extracts. The contents of total antioxidant components assayed were in the descending order: MFS-31527 (10.81 mg/g) > MFS-31499 (10.14 mg/g) > soybeans (7.93 mg/g) for the cold water extracts and MFS-31499 (8.50 mg/g) > MFS-31527 (6.05 mg/g) > soybeans (5.82 mg/g) for the hot water extracts. The higher amount of total phenols in cold water extracts might explain their increased effectiveness in reducing power and scavenging ability on hydroxyl radicals. The results of higher total phenol contents were consistent with the findings in McCue and Shetty (2005b).

Phenols such as BHT and gallate were known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Due to their scavenging abilities for free radicals and chelating abilities on ferrous ions, phenols might possess good antioxidant, antimutagenic and anticancer properties (Lotito & Fraga, 1998; Ahmad & Mukhtar, 1999). In addition, contents of total phenols were moderately to highly associated with reducing power ($r = 0.751$), scavenging ability on DPPH radicals ($r = -0.731$) and hydroxyl radicals ($r = 0.623$). Therefore, high contents of total phenols in the extracts might be responsible for their effec-

Table 4
Contents of ascorbic acid, tocopherols and total phenols of cold and hot water extracts from *Monascus* fermented soybeans (MFS-31499 and MFS-31527) and soybeans

Compound	Content[a] (mg/g)		
	MFS-31499	MFS-31527	Soybeans
Cold water extract			
Ascorbic acid	0.23 ± <0.01B	0.11 ± 0.01C	0.34 ± 0.02A
α-Tocopherol	0.01 ± <0.01A	0.01 ± <0.01A	0.01 ± <0.01A
γ-Tocopherol	0.02 ± <0.01C	0.04 ± <0.01B	0.05 ± <0.01A
δ-Tocopherol	0.01 ± <0.01A	0.02 ± 0.01A	0.01 ± <0.01A
Total phenols	10.1 ± 0.04B	10.8 ± 0.01 A	7.93 ± 0.04 C
Hot water extract			
Ascorbic acid	0.35 ± 0.05A	0.16 ± 0.07B	0.22 ± 0.03B
α-Tocopherol	0.01 ± <0.01B	0.05 ± 0.02A	0.01 ± <0.01B
γ-Tocopherol	0.05 ± <0.01B	0.05 ± 0.01B	0.07 ± <0.01A
δ-Tocopherol	0.01 ± <0.01A	0.01 ± <0.01A	0.01 ± <0.01A
Total phenols	8.50 ± 0.02A	6.05 ± 0.02B	5.82 ± 0.04C

[a] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($P < 0.05$).

tive antioxidant properties. However, on the basis of the results obtained, MFS-31499, MFS-31527 and soybeans were relatively effective for the antioxidant properties assayed. Accordingly, MFS might be potential antioxidants for use in food products and could be developed as a new dietary supplement and functional foods. Finally, the active antioxidant components will be further studied.

Acknowledgements

The study was supported by the National Science Council, ROC, Grant No. NSC 95-2313-B005-056-MY2.

References

Ahmad, N., & Mukhtar, H. (1999). Green tea polyphenols and cancer: Biologic mechanisms and practical implications. *Nutrition Review, 57*, 78–83.

Alberts, A. W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., et al. (1980). Mevinolin, a higher potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase. *Proceedings of the National Academy of Sciences of USA, 77*, 3957–3961.

Alekel, L., Hasler, C. M., Juma, S., Drum, B. W., & Kukreja, S. C. (1998). Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. *American Journal of Clinical Nutrition, 68*, 1358S–1363S.

Anthony, M. S., Clarkson, T. B., Hughes, C. L., Morgan, T. M., & Burke, G. L. (1996). Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal Rhesus monkeys. *Journal of Nutrition, 126*, 43–50.

Bach, T. J. (1986). Hydroxymethylglutaryl-CoA reductase, a key enzyme in phytosterol synthesis. *Lipids, 21*, 82–88.

Barnes, S. (1995). Effect of Genistein on in vitro and in vivo models of cancer. *Journal of Nutrition, 125*, 777S–783S.

Blanc, P. J., Loret, M. O., Santerre, A. L., Pareilleux, A., Prome, D., Prome, J. C., et al. (1994). Pigments of Monascus. *Journal of Food Science, 59*, 862–864.

Carpenter, A. P. (1979). Determination of tocopherols in vegetable oils. *Journal of the American Oil Chemists' Society, 56*, 668–672.

Cicero, A. F. G., Brancaleoni, M., Laghi, L., Donati, F., & Mino, M. (2005). Antihyperlipidaemic effect of a Monascus purpureus brand dietary supplement on a large sample of subjects at low risk for cardiovascular disease: A pilot study. *Complementary Therapies in Medicine, 13*, 273–278.

Crouse, J. R., III, Morgan, T., Terry, J. G., Ellis, J., Vitolins, M., & Burke, G. L. (1999). A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Archives of Internal Medicine, 159*, 2070–2076.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics, 315*, 161–169.

Endo, A., & Hasumi, K. (1997). Mevinic acids. In T. Anke (Ed.), *Fungal Biotechnology* (pp. 162–172). Chapman & Hall.

Endo, A. (1979). Monacolin K, a new hypocholesterolemic agent produced by a Monascus species. *Journal of Antibiotics, 32*(8), 852–854.

Endo, A. (1980). Monacolin K, a new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Journal of Antibiotics, 33*, 334–336.

Franke, A. A., Custer, L. J., Cerna, C. M., & Narala, K. (1995). Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Proceedings of the Society for Experimental Biology for Medicine, 208*, 18–26.

Fritz, K. L., Seppanen, C. M., Kurzer, M. S., & Csallany, S. (2003). The in vivo antioxidant activity of soybean isoflavones in human subjects. *Nutrition Research, 23*, 479–487.

Gardner, C. D., Newell, K. A., Cherin, R., & Haskell, W. L. (2001). The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. *American Journal of Clinical Nutrition, 73*, 728–735.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Heber, D., Lembertas, A., Lu, Q. Y., Bowerman, S., & Go, V. L. (2001). An analysis of nine proprietary Chinese red yeast rice dietary supplements: Implications of variability in chemical profile and contents. *Journal of Alternative and Complementary Medicine, 7*(2), 133–139.

Herraiz, T., Galisteo, J., & Chamorro, C. (2003). L-Tryptophan reacts with naturally occurring and food-occurring phenolic aldehydes: Activity as antioxidants and free radical scavengers. *Journal of Agricultural and Food Chemistry, 51*, 2168–2173.

Ho, S. C., Woo, J. L., Leung, S. S. F., Sham, A. L. K., Lam, T. H., & Janus, E. D. (2000). Intake of soy products is associated with better plasma lipid proriles in the Hong Kong Chinese population. *Journal of Nutrition, 130*, 2590–2593.

Iwai, K., Nakaya, N., Kawasaki, Y., & Matsue, H. (2002). Inhibitory effect of natto, a kind of fermented soybeans, on LDL oxidation in vitro. *Journal of Agriculture and Food Chemistry, 50*, 3592–3596.

Jenkins, D. J. A., Kendall, C. W. C., Jackson, C. J. C., Connelly, P. W., Parker, T., Faulkner, D., et al. (2002). Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *American Journal of Clinical Nutrition, 76*, 365–372.

Juzlová, P., Martinková, L., & Kren, V. (1996). Secondary metabolites of the fungus Monascus: A review. *Journal of Industrial Microbiology, 16*, 163–170.

Kennedy, J., Auclair, K., Kendrew, S. G., Park, C., Vederas, J. C., & Hutchinson, R. C. (1999). Modulation of polyketode synthase activity by accessory proteins during lovastatin biosynthesis. *Science, 284*, 1368–1372.

Klein, B. P., & Perry, A. K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *Journal of Food Science, 47*, 941–945, 948.

Lee, C. H., Yang, L., Xu, J. Z., Yeung, S. Y. V., Huang, Y., & Chen, Z. Y. (2005). Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chemistry, 90*, 735–741.

Lee, J., Renita, M., Fioritto, R. J., Martin, S. K. S., Schwartz, S. J., & Vodovotz, Y. (2004). Isoflavone characterization and antioxidant activity of ohio soybeans. *Journal of Agriculture and Food Chemistry, 52*, 2647–2651.

Li, C., Zhu, Y., Wang, Y., Zhu, J. S., Chang, J., & Kritchevsky, D. (1998). Monascus purpureus fermented rice (red yeast rice): A natural food product that lowers blood cholesterol in animal models of hypercholesterolemia. *Nutrition Research, 18*, 71–78.

Lingnert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidative effect in model system. *Journal of Food Processing and Preservation, 3*, 87–103.

Liu, J., Chang, S. K. C., & Wiesenborn, D. (2005). Antioxidant properties of soybean isoflavone extract and tofu in vitro and in vivo. *Journal of Agriculture of Food Chemistry, 53*, 2333–2340.

Lotito, S. B., & Fraga, C. G. (1998). (+)-Catechin prevents human plasma oxidation. *Free Radical Biology and Medicine, 24*, 435–441.

Lucas, E. A., Khalil, D. A., Daggy, B. P., & Arjmandi, B. H. (2001). Ethanol-extracted soy protein isolate does not modulate serum cholesterol in golden Syrian hamsters: A model of postmenopausal hepercholesterolemia. *Journal of Nutrition, 131*, 211–214.

Madhavi, D. L., Singhal, R. S., & Kulkarni, P. R. (1996). Technological aspects of food antioxidants. In D. L. Madhavi, S. S. Deshpande, & D.

K. Salunkhe (Eds.), *Food antioxidants: Technological, Toxicological, and Health Perspectives* (pp. 159–265). New York: Marcel Dekker.

McCue, P., & Shetty, K. (2003). Role of carbohydrate-cleaving enzymes in phenolic antioxidant movilization from whole soybean fermented with Rhizopus oligosporus. *Food Biotechnol, 17*(1), 27–37.

McCue, P. P., & Shetty, K. (2005a). A model for the involvement of lignin degradation enzymes in phenolic antioxidant mobilization from whole soybean during solid-state bioprocessing by Lentinus edodes. *Process Biochemistry, 40*, 1143–1150.

McCue, P. P., & Shetty, K. (2005b). Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures. *Process Biochemistry, 40*, 1791–1797.

McCue, P., Horii, A., & Shetty, K. (2004). Mobilization of phenolic antioxidants from defatted soybean powders by Lentinus edodes during solid-state bioprocessing is associated with enhanced production of laccase. *Innovative Food Science and Emerging Technologies, 5*(3), 385–392.

McCue, P., Horri, A., & Shetty, K. (2003). Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by Rhizopus oligosporus: Role of carbohydrate-cleaving enzymes. *Journal of Food Biochemistry, 27*(6), 501–514.

Messina, M. J., Persky, V., Setchell, K. D., & Barnes, S. (1994). Soy intake and cancer risk; a review of the in vitro and in vivo data. *Nutrition and Cancer, 21*, 113–131.

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

Potter, S. M., Bakhit, R. M., Essex-Sorlie, D. L., Weingartner, K. E., Chapman, K. M., Nelson, R. A., et al. (1993). Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *American Journal of Clinical Nutrition, 58*, 501–506.

Rundhaug, J. E., Pung, A., Read, C. M., & Bertram, J. S. (1988). Uptake and metabolism of β-carotene and retinal by C3H/10T1/2 cells. *Carcinogenesis, 9*, 1541–1545.

Russo, A., Cardile, V., Lombardo, L., Vanella, L., & Acquaviva (2006). Genistin in habits UV light-induced plasmid DNA damage and cell growth in human melanoma cells. *Journal of Nutritional Biochemistry, 17*, 103–108.

Shi, X., Dalal, N. S., & Jain, A. C. (1991). Antioxidant behaviour of caffeine: Efficient scavenging of hydroxyl radicals. *Food Chemistry and Toxicology, 29*, 1–6.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry, 40*, 945–948.

Taga, M. S., Miller, E. E., & Pratt, D. E. (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society, 61*, 928–993.

Taylor, M. J., & Richardson, T. (1980). Antioxidant activity of cysteine and protein sulfhydryls in a linoleate emulsion oxidized by hemoglobin. *Journal of Food Science, 45*, 1223–1227, 1230.

Wang, C., & Wixon, R. (1999). Phytochemicals in soybeans: Their potential health benefits. *Inform, 10*(4), 315–321.

Wang, J., Lu, Z., Chi, J., Wang, W., Su, M., Kou, W., et al. (1997). A multi-center clinical trial of the serum lipid lowering effects of a Monascus purpureus (red yeast) rice preparation from traditional Chinese medicine. *Current Therapeutic Research, 58*, 964–978.

Wangen, K. E., Duncan, A. M., Xu, X., & Kurzer, M. S. (2001). Soy isoflavones improve plasma lipids in normocholesterolemic and mild hypercholesterolemic postmenopausal women. *American Journal of Clinical Nutrition, 73*, 231–255.

Washburn, S., Burke, G. L., Morgan, T., & Anthony, M. (1999). Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. *Menopause, 6*, 7–13.

Yamaguchi, R., Tatsumi, M., Asano, M., Kato, K., & Ueno, Y. (1988). Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agricultural and Biological Chemistry, 52*, 849–850.

Yang, J.-H., Mau, J.-L., Ko, P.-T., & Huang, L.-C. (2000). Antioxidant properties of fermented soybean broth. *Food Chemistry, 71*, 249–254.

Yokota, T., Hattori, T., Ohishi, H., Hasegawa, K., & Watanabe, K. (1996). The effect of antioxidant-containing fraction from fermented soybean food on atherosclerosis development in cholesterol-fed rabbits. *LWT – Food Science and Technology, 29*, 751–755.