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Antioxidant Activity of Fermented Soybean Extract

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Free radicals are considered to be important causative factors in the development of cancer and cardiovascular diseases. This relationship has led to interest in evaluating the antioxidant capacities of many dietary supplements. Fermented soybean extract is produced by symbiotic fermentation of organic soybean with 20 types of *Lactobacillus* and yeast. In vitro and in vivo models are used in this study to evaluate the antioxidant effect of fermented soybean extract. Several in vitro models are used to detect the antioxidant capacity of the fermented soybean extract, which is compared to vitamin C and Trolox. The results demonstrate that the fermented soybean extract has strong antioxidant activity against unsaturated fatty acid peroxidation compared to vitamin C and Trolox. By the means of the test system developed by Y. Toshiki et al., it is shown that the fermented soybean extract can function both as an antioxidant and as a free radical acceptor that can convert free radicals into harmless substances through an energy-decreasing procedure. An in vivo study examines the effects of fermented soybean extract on the activity of antioxidant enzymes. The activities of the antioxidant enzymes (AOE) including total superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) are measured in liver, kidney, and brain from male Sprague−Dawley rats. The activities of CAT, SOD, and GPX are increased in the liver. However, the SOD activity is decreased in the kidney. SOD and GPX activities are decreased in the brain. These results lead to the conclusion that fermented soybean extract not only has antioxidant activity but also has an effect on the activity of antioxidant enzymes in liver.

KEYWORDS: Fermented soybean extract; antioxidant; antioxidant enzymes; free radicals; symbiotic fermentation; superoxide dismutase; catalase; glutathione peroxidase

INTRODUCTION

Free radicals are atoms or molecules with an unpaired electron in the outer orbit. They are the byproducts of many normal reactions within the body that include energy generation, breakdown of lipids and proteins, the catecholamine response, and the inflammatory process (*1*). This electron imbalance makes them highly reactive to be able to oxidize lipids, proteins, DNA, and carbohydrates. This eventually causes disruption of cell membranes, leading to the release of the cell contents and death. The unpaired electron may also take an electron from another molecule, join with another molecule, or completely disengage itself and reattach itself to another molecule, thus producing more free radicals. 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.

If the antioxidant defenses are not completely efficient, increased free radical formation in the body leads to increased damage. The term "oxidative stress" is often used to refer to this effect. If mild oxidative stress occurs, tissues often respond by making extra antioxidant defenses. However, severe oxidative stress can cause cell injury and death (*2*). Endogenous oxidative damage to proteins, lipids, and DNA is thought to be an important etiologic factor in aging and the development of chronic diseases including carcinogenesis (*3*), the formation of foam cells in atherosclerotic plaques (*4*), and the development of vasogenic and cytotoxic edema after head injury (*5*). Additionally, oxidative stress is associated with the progression of many diseases including cancer, stroke, heart disease, cataracts, macular degeneration, depression, and the rate of aging (*6*−*9*).

In the present study, the potential antioxidant effect of a commercial fermented soybean extract (FSE) was investigated.

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FSE consists of a mixture of soybean extracts and the secondary metabolites of lactobacillus and yeasts. Unlike other traditional fermented soy foods including tempeh, miso, soy sauces, natto, and fermented tofu, FSE was fermented by a coculture system of lactobacillus and yeast. Moreover, FSE has been used as a traditional remedy to prevent cancer and cardiovascular disease progression, as an adjuvant drug for the reduction of chemotherapy side effects, for the prevention of nosocomial infection, etc. After taking FSE, many cancer patients experience positive responses including immune function enhancement, appetite improvement, and fatigue reduction during chemotherapy or radiotherapy (*10*). Apart from these effects, FSE can function as a nutritional supplement for infants, pregnant women, and individuals after major surgery. On the basis of these effects, this study was constructed to scientifically evaluate the potential antioxidant effects of this product against free radicals. Because some oxidative damage is created through normal body functions, our own body cells produce specific enzymes designed to reduce oxidative damage. Two of these important enzymes produced by the body are superoxide dismutase (SOD) and catalase (CAT). These enzymes are our front line of defense against oxidative damage. Besides the antioxidant nutrients (vitamins A, C, and E) found in foods, antioxidant enzymes (SOD and CAT) are equally as important in protecting the body from oxidative damage. Hence, the study was further designed to understand whether FSE could elevate the activation of antioxidant enzymes to increase antioxidant effect in vivo.

MATERIALS AND METHODS

Reagents. Iron(III) chloride and 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) were obtained from Wako (Osaka, Japan). Acetate anhydride, ammonium thiocyanate, deoxyribose, bicinchoninic acid (BCA), biotinylate alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), catalase, copper sulfate, cumene hydroperoxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), glutathione reductase, glycine, glutathione (GSH), horseradish peroxidase (HRPO), hydrogen chloride (HCl), linoleic acid, 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), NADH, NADPH, nitroblue tetrazolium (NBT), phenol red, phenazine methosulfate (PMS), potassium cyanide (KCN), potassium dihydrogen phosphate, potassium phosphate, pyrogallo, sodium azide, streptavidin, sucrose, tris-base, Triton X-100, and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Ascorbic acid and iron(II) chloride tetrahydrate were bought from Merck & Co., Inc. (Darmstadt, Germany). Trolox (soluble vitamin E) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

FSE. FSE, a concentrated solution of fermented soybean extract, was provided by the Microbio Co., Ltd. The microorganisms used in the fermenting process included *Lactobacillus paracasei*, *Lactobacillus burglarious*, and *Saccharomyces cerivisiae*, which are often found in the human intestinal tract or in some traditional fermented products. The final products with a weight of 1.136 g/mL were subjected to a sterilization process to ensure that they did not carry any foodborne pathogens. The moisture, crude protein, crude fat, crude fiber, carbohydrate, and ash contents in the FSE were 71.49, 5.45, 0.16, 0.15, 17.6, and 5.15%, respectively.

NBT Method. The measurement of reduction ability was according to the method of Robak et al. (*11*). Test samples of different concentrations were mixed with 0.15 mM NADH and 0.74 mM NBT in sequence and then added promptly to 0.05 mM PMS to obtain a reduced form of NBT. The amount of NBT in the reduction state was determined by absorbance at a wavelength of 560 nm. A reduction in absorbance is therefore proportional to a sample's ability to eliminate superoxide anion oxygen free radicals.

H_2O_2 Reduction Method. This assay is described by Keisari et al. (*12*) and is based on the HRPO-mediated oxidation of phenol red by H_2O_2, which results in the formation of a compound absorbing at 610 nm. Phenol red (0.07 mg/mL) was mixed with the samples at different concentrations; then 10 mM H_2O_2 was added, and the mixture was left to stand for 5 min. HRPO (0.1 unit) was added and mixed followed by a wait of 5 min. Finally, 0.01 N NaOH was added.

DPPH Reduction Method. The ability to eliminate free radicals was measured according to the method of Das et al. (*13*). DPPH is a free radical with unpaired electrons. Samples at different concentrations were mixed with 0.18 mM DPPH and then allowed to stand for 5 min. Finally, the absorbance at a wavelength of 517 nm was determined.

Total Reactive Antioxidant Potential (TRAP) Reduction Method. The method of Francesco et al. was used (*14*). Glycine buffer (975 mM) at pH 8.6, 0.1 mM luminol (LH_2), and 6.66 mM ABAP were mixed in a dish in order. The sample was then placed on a magnetic stirrer in the chamber of an ultraweak chemiluminescence analyzer (CLD-110, Tohoku, Japan) for 10 s at 25 °C. Samples at different concentrations were then added, and chemiluminescence was determined for 20 s.

Conjugated Diene Hydroperoxide Production Method. The method for determining the production of conjugated diene hydroperoxide by oxidation of linoleic acid was presented by Liegeois et al. (*15*). Samples at different concentrations were mixed with a 9 mM linoleic acid emulsion and heated to 37 °C. Samples (0.05 mL) were removed at 0 and 6 h and mixed with 0.95 mL of 80% methanol, and the absorbance at a wavelength of 234 nm was read.

Lipid Peroxidation Method. Lipid peroxidation was determined indirectly by measuring malondialdehye (MDA) formation, which was described by Janero et al. (*16*). At low pH and elevated temperature, MDA readily participates in a nucleophilic addition reaction with TBA, and this generates a red, fluorescent MDA/TBA 1:2 complex. The ability to easily detect MDA (as the free aldehyde or its TBA derivative) has led to the routine use of MDA determination and, particularly, the "TBA test" for the detection and quantification of lipid peroxidation in a wide array of sample types. Rat microsome fraction was used as source of lipid, and NADPH was used to produce enzymatic lipid peroxidation. After the samples had been mixed at various concentrations with the microsome fraction and NADPH, the absorbance at a wavelength of 535 nm was used to estimate the MDA concentration and thus the extent of lipid peroxidation.

Chemiluminescence Measurement for the Presence of Active Oxygen. The photon emission (chemiluminescence, CL) of FSE in the presence of active oxygen species was detected according to the method of Yoshiki et al. (*17*). The CL intensity [P] in the presence of active oxygen species [X], catalytic species [Y] and receptors [Z] is predicted by $[P] = k[X][Y][Z]$. This formula determines the antioxidant ability of test compound to eliminate free radicals from the intensity of chemiluminescence (P). Hydrogen peroxide (H_2O_2) and acetaldehyde (MeCHO) were used as X and Z, respectively. Y is the sample with antioxidant activity to be measured. The photon constant, *k*, is calculated for the characteristics of the interactions of the active oxygen species, catalytic species, and receptor molecules.

The following mixture, 0.6 mL of 250 mM H_2O_2 and 0.6 mL of 379 mM MeCHO (dissolved in 50% methanol), was placed in a small dish. The dish was transferred to the chamber of an ultraweak chemiluminescence detector and allowed to stabilize for 200 s at 37 °C. Next, 0.3 mL of FSE at different concentrations was added as the antioxidant, followed by the determination of the intensity of chemiluminescence for 200 s.

Animal Preparation. Male Sprague−Dawley rats (156−168 g) were provided by the animal center of Tzu Chi University. FSE was diluted to 2% and administrated orally at a dose of 10 mL/kg/day for 10 days. After all rats were sacrificed, the activities of antioxidant enzymes including CAT, SOD, and glutathione peroxidase (GPX) in the liver, kidney, and brain were tested.

Antioxidant Enzyme Activity Measurement. Organ samples (0.4 g) were mixed with 4 mL of solution (0.25 M sucrose and 0.5 mM EDTA) in a homogenizor at 10000 rpm for 80 s. Protein was quantitated with BCA protein assay, and samples were diluted to the concentration of 5 mg/mL. The measurements of antioxidant enzymes were according to the method of Lai et al. (*18*). The activity of total SOD was measured by inhibition of pyrogallol autoxidization. Manganese superoxide

Table 1. Antioxidant Activities of FSE, Trolox, and Vitamin C

	samples		
method	FSE IC_{50}[a], mean ($n = 3$)	Trolox IC_{50}, mean ($n = 3$)	vitamin C IC_{50}, mean ($n = 3$)
NBT reduction	65.3 μg/mL	27.3 μg/mL	19.3 μg/mL
H_2O_2 reduction	2.84 mg/mL	10.1 μg/mL	1.32 μg/mL
DPPH reduction	6.91 mg/mL	4.59 μg/mL	4.45 μg/mL
TRAP reduction	13.5 μg/mL	4.7 ng/mL	4.7 ng/mL
conjugated diene	3.11 mg/mL	3.75 μg/mL	NA
lipid peroxidation	3.39 mg/mL	2.28 μg/mL	NA

[a] IC_{50} is the inhibitory concentration of the test samples that inhibits 50% substrate.

dismutase (Mn SOD) activity was measured by the addition of 30 mM KCN in the mixture to inhibit copper zinc superoxide dismutase (Cu/Zn SOD) activity. The activity was determined from the absorbance at a wavelength of 420 nm at 25 °C for 3 min. One unit was taken as the activity that inhibited the reaction by 50%.

Catalase activity was measured as the rate of disappearance of H_2O_2 at 240 nm for 1 min. Samples were treated with a sonicator and then were centrifuged at 500*g* and 4 °C for 10 min. The upper layer of supernatant was removed, and 1% anhydrous alcohol and 1% Triton X-100 were added to stabilize CAT activity and to separate CAT from cell membrane. The reaction was incubated for 1 h and stored at −20 °C in a freezer. Frozen samples (100 mL) were reacted with a solution of 2.8 mL of 50 mM potassium phosphate buffer (pH 7.0) and 0.1 mL of 10 mM H_2O_2. Catalase activity was expressed in units per milligram of protein.

The structure of GPX was constructed with four selenium-contained protein subunits, which had the ability to decompose hydrogen peroxide (H_2O_2) and other organic hydroperoxide (ROOH). The protein subunits without selenium would decompose only the ROOH compound. Both reactions have to use glutathione to complete the reaction. Cumene hydro peroxide or H_2O_2 was used as substrate of glutathione. Consumption of NADPH was used to determine the activities of total GPX and selenium-contained GPX. One unit of GPX activity was defined as the production of 1 mmol of GSSG at 25 °C and pH 7.0 per minute.

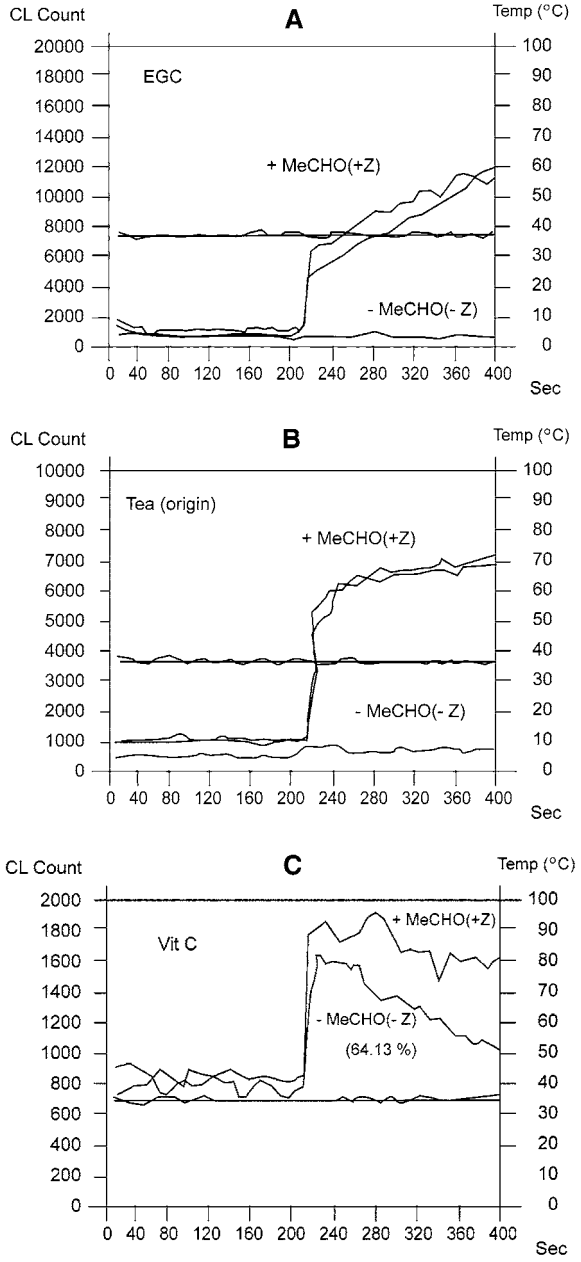
Statistical Methods. All data were presented as mean values ± standard errors. The unpaired Student's *t* test was used to determine the significant difference. Data would show statistical difference when *P* value is below 0.05.

RESULTS

Antioxidant Potential of FSE. Six different methods were used to evaluate the antioxidant potential of FSEE; vitamin C and Trolox (water-soluble vitamin E) as control samples were also detected. As the results show in **Table 1**, FSE was able to eliminate superoxide anions, H_2O_2, DPPH free radicals and trap free radicals; the average IC_{50} values were 65.3 μg/mL, 2.84 mg/mL, 6.91 mg/mL, and 13.5 μg/mL, respectively, which were higher than those of vitamin C and Trolox. Regarding the antioxidant effect of FSE on unsaturated fatty acid, its IC_{50} value was 3.11 mg/mL, in comparison to that of Trolox at 3.75 μg/mL. Additionally, FSE had an anti-lipid peroxidation effect with an IC_{50} of 3.39 mg/mL compared to that of Trolox at 2.28 μg/mL. In the reactions of linoleic acid and lipid oxidation, there were no significant antioxidant activities on vitamin C.

Elimination of Free Radical Energy through Energy Degradation. Under the method developed by Yoshiki et al., the chemiluminescence intensities of epigallocatechin (EGC), a polyphenol, tea, and vitamin C were measured. EGC gave intense chemiluminescence. If no MeCHO was added, there was no chemiluminescence (**Scheme 1A**). Using tea as antioxidant without added MeCHO, it was found that the chemiluminescence intensity was only 5.79% (**Scheme 1B**) of that with MeCHO. However, when vitamin C was used as the test sample without added MeCHO, the chemiluminescence intensity was 64.13% (**Scheme 1C**) of that with MeCHO, showing that the chemiluminescence mechanism of vitamin C was different from that of the others.

Scheme 1. Antioxidant Effects of Tea and Vitamin C[a]



[a] At a temperature of 37 °C, chemiluminescence, in CL counts, was measured in the presence of H_2O_2 as a peroxide without the presence of acetaldehyde (Z) as a radical receptor and with an addition of various antioxidants, that is, EGC, tea, vitamin C, and FSE, at 200 s. (**A**) shows the chemiluminescence determined with or without acetaldehyde when EGC was added as the antioxidant at 200 s; (**B**) shows that when tea was added as the antioxidant at 200 s, its chemiluminescence emitted in the presence of acetaldehyde (+Z) is 5.79% of that in the absence of acetaldehyde; (**C**) shows that when vitamin C was used as the antioxidant, the chemiluminescence intensity in the absence of acetaldehyde was 64.13% of that detected in the presence of acetaldehyde.

The data of the chemiluminescence spectrum of FSE are summarized in **Table 2**. The chemiluminescence intensity of FSE was associated with the concentration, and the presence of MeCHO did not affect the chemiluminescence signal. The ratio of *P*(−MeCHO)/*P*(+MeCHO) remained at 94% when FSE was diluted 100-fold (11.38 μg/mL). These data show FSE has

Table 2. Anti-Free Radical Activity of FSE with/without MeCHO

FSE diluted with 50% MeOH	P[a] (+MeCHO)	P (–MeCHO)	P (–MeCHO)/ P (+MeCHO) (%)
1:1	378937	167235	44.13
1:10	135701	86366	63.64
1:100	19210	18060	94.01
1:500	7615	6865	90.15

[a] Total photon counts/200 s = anti-free radical activity.

Table 3. SOD Activity in Liver, Kidney, and Brain after Rats Were Treated with Distilled Water or 2% FSE (10 mL/kg/Day) for 10 Days[a]

parameter	liver	kidney	brain
SOD			
control group	8.6 ± 0.4	2.3 ± 0.2	1.74 ± 0.22
tested group	10.3 ± 0.4*	1.6 ± 0.2*	0.27 ± 0.03**
Cu/Zn SOD			
control group	8.2 ± 0.4	1.9 ± 0.3	1.52 ± 0.17
tested group	9.9 ± 0.4	1.3 ± 0.3	0.21 ± 0.03
Mn SOD			
control group	0.4 ± 0.1	0.4 ± 0.1	0.22 ± 0.06
tested group	0.4 ± 0.1	0.2 ± 0.1	0.06 ± 0.01

[a] Data are presented as mean [±standard errors (tested group, $n = 5$; control group, $n = 4$]. SOD activity is presented as units per mg of protein. *, $P < 0.05$; **, $P < 0.01$.

Table 4. CAT Activity in Liver, Kidney, and Brain after Rats Were Treated with Distilled Water or 2% FSE (10 mL/kg/Day) for 10 Days[a]

CAT	liver	kidney	brain
control group	162.6 ± 7.6	31.56 ± 1.88	0.273 ± 0.06
tested group	191.8 ± 3.0*	34.62 ± 2.27	0.205 ± 0.01

[a] Data are presented as mean [±standard errors (tested group, $n = 5$; control group, $n = 4$]. CAT activity is presented as units per mg of protein. *, $P < 0.05$.

a complete capability to eliminate free radicals by way of energy degradation, not just by transfer of free radical energy from one substance to another.

Antioxidant Enzyme Activities in Liver, Kidney, and Brain. In the Sragueâ€“Dawly rat study, the SOD activities in liver, kidney, and brain were measured after the rats were fed with FSE or distilled water for 10 days. The activities of total SOD and Cu/Zn SOD were significantly increased in the liver; however, the total SOD activity was decreased in kidney. The SOD activity and Cu/Zn SOD activities were decreased in the brain as shown in **Table 3**. The result shown in **Table 4** demonstrated that the CAT activity was also significantly increased in the liver and that there were no differences in the kidney and brain. Total GPX activity was significantly increased in the liver; however, the Se-GPX activity was increased in the kidney, but non-Se-GPX was decreased. As for the brain, only the total GPX activity was significantly decreased as shown in **Table 5**. Using a Western blot test, there was no significant difference between the protein contents of CAT and Cu/Zn SOD in liver and Cu/Zn SOD in brain (data not shown).

DISCUSSION

FSE apparently acts as an antioxidant and against free radicals. This action is extensive and effective in eliminating free radicals ranging from the superoxide anion to H_2O_2 to lipid peroxide free radical, as well as having an antioxidant effect on unsaturated fatty acids and lipids. Although the antioxidant potentials of the control samples Trolox and vitamin C were stronger than that of FSE, the Trolox and vitamin C used were pure compounds. FSE contains 70% water, and the administration of FSE generally requires 100-fold dilution. This suggests that FSE has excellent and effective antioxidant potential as a dietary supplement.

Table 5. GPX Activity in Liver, Kidney, and Brain after Rats Were Treated with Distilled Water or 2% FSE (10 mL/kg/Day) for 10 Days[a]

	liver	kidney	brain
GPX			
control group	240.2 ± 44.6	162.7 ± 8.4	9.1 ± 0.1
tested group	405.9 ± 33.0*	167.3 ± 6.9	6.2 ± 0.3*
Se -GPX			
control group	178.5 ± 41.1	127.1 ± 4.2	8.3 ±1.0
tested group	280.7 ± 41.1	151.6 ± 5.6**	6.1 ± 0.5
non Se-GPX			
control group	61.7 ± 54.8	35.5 ± 6.6	0.8 ± 0.2
tested group	155.1 ± 48.4	15.7 ± 4.7*	0.2 ± 0.2

[a] Data are presented as mean [±standard errors (tested group, $n = 5$; control group, $n = 4$]. GPX activity is presented as units per g of protein. *, $P < 0.05$; **, $P < 0.01$.

According to the method of Yoshiki et al., the antioxidant can accept the electrons of free radicals and transfer them to the electron receptor; finally, the electrons return to ground state, emitting the surplus energy ($h\nu$) as chemiluminescence. This was verified using gallic acid as the antioxidant. The antioxidants of EGC and tea also gave the same results. However, the antioxidant vitamin C displayed 64.13% chemiluminescence in the absence of electron receptor. This reflects a different antioxidant mechanism for vitamin C. Thus, we propose that there are three types of antioxidants:

(1) The antioxidant is turned into a free radical after the acceptance of a free radical.

(2) The antioxidant transfers the free radical to the free radical receptor and eliminates the free radical (e.g., gallic acid).

(3) The antioxidant acquires a free radical, transfers the molecule without electron receptor, delivers the free radical to the solvent directly, and then eliminates the free radical (e.g., FSE). This is close to an ideal antioxidant.

FSE is a natural liquid mixture, and the results show that it has antioxidant potential and may use one or two of the above mechanisms. First, FSE may act as a catalytic agent and an electron receptor to eliminate the free radical. Second, FSE may deliver the free radicals directly to the solvent and eliminate them in the same way as vitamin C.

In vivo study has demonstrated that after being treated with FSE, the body weight and organ weight of rats have not shown significant difference (data not shown). However, SOD, CAT, and GPX activities are significantly increased in the liver. SOD activity is decreased in kidney; nonetheless, CAT and GPX activities have not changed in the kidney. On the other hand, SOD and GPX activities are decreased in the brain, where there is no difference of CAT activity. These findings demonstrate that FSE has different effects on antioxidant enzymes in the liver, kidney, and brain, respectively.

The FSE consists of isoflavones, saponins, lecithin, amino acids, vitamins, etc. It provides better absorption and bioavailability than soy-based products. Because FSE absorbed by each organ of rat is different, the metabolic enzymes in organs would not be the same as well. Therefore, the antioxidant enzymes vary in each organ. The changes of SOD in liver, kidney, and brain are mainly due to the change on Cu/Zn SOD activity, but not Mn SOD. It has been known that Cu/Zn SOD is located in the cytoplasm and Mn SOD is found in the mitochondria matrix. It can be concluded that the influence caused by FSE should be in the cytoplasm, but not in the mitochondria matrix.

Cu/Zn SOD activity may be related to the ability of metal insertion. In addition, the sulfur hydrogen compounds, such as reductive glutathione and 2-mercaptopropionylglycine, can be used to elevate Cu/Zn SOD activity (*19*). Contrarily, the combination of Cu/Zn SOD and succinylation or carbamoylation would cause a decrease of enzyme activity (*20*). FSE may elevate the substances for increasing Cu/Zn SOD activity in liver. On the other hand, it may raise the substances for decreasing Cu/Zn SOD activity in kidney and brain or directly decrease Cu/Zn SOD activity.

CAT activity is increased only in the liver. It has been reported that CAT activity is increased by integration with estrogen and progestin (*21*). Isoflavones in FSE have a structure and effect to those of estrogen and progestin, which may be one of the components to increase CAT activity. Feeding genistein has been shown to increase CAT activity in liver (*22*).

The present study demonstrates that GPX, Se-GPX, and non-Se-GPX activities are significantly increased in liver. However, Se-GPX activity is the major part being decreased and only little influence is shown on non-Se-GPX activity. Both Se-GPX and non-Se-GPX are modulated by glutathione. Selenium is the main factor for regulating enzyme activity. Therefore, further study is needed to investigate FSE on the effect of glutathione and selenium metabolism.

We hypothesize that the change of enzyme activity would be due to the activation or inhibition of the enzyme itself and also affect the enzyme content. Western blot was used to confirm this hypothesis. However, the results show that there is no difference in the amount of CAT and Cu/Zn SOD enzymes in liver and Cu/Zn SOD in brain. It is observed that enzyme activity change should be related to the components or metabolites of FSE, which have the ability to affect enzyme activation or inhibition, and not due to the enzyme content.

Many dietary supplements have been developed as the result of the finding of potential antioxidant phytochemicals such as phenolics or polyphenols. Flavonoids represent the most common and widely distributed group of plant phenolics. Some research has demonstrated that many phenolics, including flavonoids and phenolic acids, have antioxidant capacities that are much stronger than those of vitamins C and E (*14*, *15*). Also, it has been suggested that soy isoflavones show a direct free radical quenching ability, with genistein and daidzein being particularly effective (*16*, *17*). FSE shows many characteristics of an ideal antioxidant. It can be concluded that FSE not only has antioxidant ability but also has the ability to activate antioxidant enzymes.

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NOTE ADDED AFTER ASAP

The Literature Cited listing has been corrected August 9, 2004, from the original ASAP posting of August 6, 2004.

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