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Antioxidative Activity and Safety of the 50% Ethanolic Extract from Red Bean Fermented by *Bacillus subtilis* IMR-NK1

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This study aimed at evaluating the antioxidative activities and the safety of 50% ethanolic extract from red bean fermented by *Bacillus subtillis* IMR-NK1. The antioxidative activities, including α,α-diphenyl-β-picryl-hydrazyl (DPPH) radicals scavenging effects, Fe^{2+}-chelating ability, and reducing power, were studied in vitro. It was found that the antioxidative activity increased with the concentrations of the extract to a certain extent and then leveled off as the concentration further increased. As compared to the commercial antioxidants, the fermented red bean extract showed less scavenging effect on the DPPH radical and reducing power than α-tocopherol and BHT, but better Fe^{2+}-chelating ability. No mutagenicity or toxicity effect toward all tester strains was found in the 50% ethanolic extract of fermented red bean by means of the Ames test. The results suggested that the 50% ethanolic extract was safe in genotoxicity.

KEYWORDS: Fermented red bean; antioxidative activity; *Bacillus subtilis* IMR-NK1; mutagenesis

INTRODUCTION

Free radicals and other reactive oxygen species (ROS) are generated in living organisms through many pathways (*1*). Accumulation of ROS in aerobic organisms is known as an exacerbating factor in cellular injury and the aging process (*2*). In addition, they are considered to induce lipid peroxidation causing the deterioration of foods (*3*). Recently, there is an increasing interest in finding natural antioxidants from plants because they can protect the human body from the attack of free radicals and retard the progress of many chronic diseases (*4*, *5*), as well as retarding the lipid oxidative rancidity in foods. Phenolic compounds, such as flavonoids and phenolic acids, seem to be the most effective chemicals that can be found in many plant raw materials, particularly in fruits, seeds, and herbs (*6*). Their metal-chelating capability, together with the radical scavenging property, has enabled phenolic compounds to be thought of as effective antioxidants derived from natural plants (*7*, *8*).

Beans contain considerable amounts of phenolic compounds (*9*, *10*) that possess varying degrees of antioxidative activity, but the concentration of phenolic compounds in beans may be increased during fermentation (*11*). The traditional Asian fermented soyfoods, such as miso, natto, and tempeh, have been found to exhibit remarkably stronger antioxidative activity than unfermented steamed soybeans (*12*−*14*). Red bean (*Phaseolus radiatus* L. var. *Aurea*) is a leguminous seed, and mainly used as one of the popular ingredients in oriental desserts. However, there were few investigations associated with the antioxidative activities in fermented red beans. In this study, *Bacillus subtilis* IMR-NK1, a superoxide dismutase (SOD) producing bacterium, was applied in the production of Natto-like by means of solid-state fermentation using red bean as media.

To characterize a substance as an antioxidant, it is necessary to assess its interaction against a wide range of species directly responsible for oxidative damage. In particular, a screen of antioxidative ability should include the assessments of the capacity of a putative antioxidant to scavenge radical and ferryl species. Thus, the main objectives of this work were to investigate the antioxidative activity including the radical-scavenging effect, reducing power and Fe^{2+}-chelating ability of the 50% ethanolic extract of red bean fermented by *B. subtilis* INR-NK1. In addition, results were compared with those of commercial antioxidants commonly used in food industry (butylated hydroxytoluene, BHT and α-tocopherol, α-toc). The mutagenicity of the extract of fermented red bean was also investigated.

MATERIALS AND METHODS

Chemicals. α,α-Diphenyl-β-pricryl-hydrazyl (DPPH), α-tocopherol (α-toc), $FeCl_2$, $FeCl_3$, potassium ferricyanide, butylated hydroxytoluene (BHT), trichloroacetic acid and [4,4′-[3-(2-pyridinyl)-1,2,4-triazine-5, 6-diyl] bisbenzenesulfonic acid] (ferrozine) were purchased from Sigma Chemicals Co. (St. Louis, MO). All other chemicals were reagent grade or purer.

Bacteria Strains. The *Salmonella typhimurium* tester strains, including TA97 (hisD6610/rfa/ΔuvrB/pKM101), TA98 (hisD3052/rfa/ΔuvrB/

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pKM101), TA100 (hisG46/rfa/ΔuvrB/pKM101), TA 102 (hisG428/rfa/pKM101, pAQ1) and TA1535 (hisG46/rfa/ΔuvrB), were purchased from Culture Collection and Research Center, Food Industry Research and Development Institution, Taiwan.

Isolation and Identification of *B. subtilis*. The soil samples were collected from paddy fields in Taiwan. The dried soils were suspended in tap water, heated to boiling for 20 min in water bath, and then plated in 10 cm-Petri dishes contained DP agar (soluble starch 5%, peptone 1.5%, KH_2PO_4 0.5%, $MgSO_4$·7H2O 0.25%, and agar 1.5%) medium. After two days incubation at 30 °C, the colonies were isolated and tested for free radical scavenging activity using steamed red bean as substrate. The isolated colonies were put in a 250-mL Erlenmyer flask with 10 g of steamed red beans and then incubated at 30 °C for 48 h. After drying at 65 °C, the fermented red bean was pulverized to pass 40-mesh screen, and the oxidative ability was determined. The strain with the highest antioxidative activity was selected and further identified by api 50 CH for this study (BioMerieux sa, France).

Preparation of the 50% Ethanolic Extract from Fermented Red Bean. Red beans (*Phaseolus radiatus* L. var. *Aurea*, Kaoshiung #10) were obtained from Kaoshiung District Agriculture Improvement Station, Taiwan. Red bean sample (1 kg) was soaked in tap water for 8 h, cooked for 1 h, and then cooled to 35 °C. Isolated *B. subtilis* IMR-NK1 was incubated in DP broth at 30 °C for 24 h. Solid-state fermentation was performed by inoculating 50 mL of precultured *B. subtilis* IMR-NK1 into 1 kg of steamed and cooled red bean, and then incubated at 30 °C for 48 h. Fermented red bean powder was obtained by drying the sample with 65 °C hot air for 24 h and pulverizing it to pass a 40-mesh screen. One hundred g of fermented red bean powder was extracted with 500 mL of 50% ethanol at 75 °C for 3 h. The decoction was filtered, and collected, and then dried by a freeze-dryer. The extraction rate of the fermented red bean extract was 9.0%. The extracts were sealed in plastic bottles and stored at −70 °C until used.

Compositions of the 50% Ethanolic Extracts from Fermented Red Bean. The proximate compositions of the fermented red bean extracts were analyzed using the methods of AOAC (method number 934.01 for water, 954.02 for crude fat, 984.13 for crude protein, 962.09 for crude fiber, and 900.02 for ash content determination, respectively) (*15*). The total phenol content (gallic acid equivalent) was analyzed using the Folin−Ciocalteu reagent method (*16*). The anthocyanin content was analyzed using the method of Padmavati et al. (*17*).

Measurement of DPPH Radical-Scavenging Activity. Effect of the 50% ethanolic extract of fermented red bean on DPPH radical was estimated according to the method of Yamaguchi et al. *(18).* An aliquot of the fermented red bean extract (0.2 mL, 0.62∼10 mg/mL), α-toc (0.04∼1.25 mg/mL), or BHT (0.04∼1.25 mg/mL) was mixed with the 100 mM Tris−HCl buffer (0.8 mL, pH 7.4) and then added to 1 mL of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured spectrophometrically at 517 nm. The capability to scavenge DPPH radical was calculated by the following equation:

$$\text{scavenging effect (\%)} = \left[1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right] \times 100 \quad (1)$$

Measurement of Reducing Power. The reducing power of the fermented red bean extract, BHT, and α-toc were determined according to the method of Yen and Chen (*19*). The fermented red bean extract (0.62∼20.0 mg/mL), BHT, or α-toc (0.02∼0.625 mg/mL) was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then the mixture was incubated at 50 °C for 20 min. An equal volume of 1% trichloroacetic acid was added to the mixture, and then it was centrifuged at 6,000 rpm for 10 min to stop the reaction. The upper layer of solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2, and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

Measurement of Fe^{2+}-Chelating Ability. The Fe^{2+}-chelating ability was determined according to the method of Decker and Welch (*20*). The Fe^{2+} was monitored by measuring the formation of ferrous iron−Ferrozine complex. The fermented red bean extract, BHT, or α-toc (0.16∼10 mg/mL) was mixed with 2 mM $FeCl_2$ and 5 mM ferrozine at a ratio of 10:1:2. The mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance of the reaction mixture indicated a higher Fe^{2+}-chelating ability. The capability to chelate the ferrous iron was calculated by the following equation:

$$\text{chelating effect (\%)} = \left[1 - \frac{\text{absorbance of sample at 562 nm}}{\text{absorbance of control at 562 nm}}\right] \times 100 \quad (2)$$

Preparation of Culture Medium. Nutrient broth was prepared by dissolving 25 g of Oxoid nutrient broth in 1 L of water. Glucose minimal agar plate (MA plate) contained 1.5% agar, 0.02% $MgSO_4$·$7H_2O$, 0.2% citric acid, 1% K_2HPO_4, 0.35% $NaHNH_4PO_4$·$4H_2O$, and 2% glucose. Top agar contained 0.75% agar and 0.5% NaCl.

Toxicity Test. Samples were prepared with 0.1 mL of fresh culture of the tester strain (approximately 10^8 cells/mL), 0.1 mL of the 50% ethanol extract of fermented red bean (0.3125−5 mg/mL DMSO), 0.2 mL of phosphate buffer (0.2 M, pH 7.4), and 0.5 mL of S9 mix or instead with phosphate buffer. The series dilutions were made using phosphate buffer and 1 mL of aliquot mixed with 12 mL of nutrient agar. After incubation at 37 °C for 48 h, the number of colonies was counted. A toxicity effect was confirmed if the standard plate count of tested compound was lower than that of control (without adding tested compound).

Ames Test. Mutagenicity was assayed by the standard Ames test (standard plate incorporation assay) (*21*, *22*). A mixture containing 0.1 mL of the 50% ethanol extract of fermented red bean (0.3125−5 mg/plate), 0.5 mL of S9 mix or phosphate buffer, 0.2 mL of 0.5 mM histidine/biotin, and 0.1 mL of fresh culture of the tester strain (approximately 10^8 cells/mL) was added to a tube containing 2 mL of top agar. The tube was then gently vortexed and poured onto the MA plate. The compound was tested with and without S9 mix, and triplicate plates were poured for each dose of mutagen. Diagnostic mutagens including 2-aminofluorene (2-AF) and 4-nitroquinoline-*N*-oxide (4-NQO) served as positive control chemicals. After incubation at 37 °C for 48 h, the number of revertant colonies was scored. A compound was considered a mutagen if there was a 2-fold increase in the number of revertants compared with spontaneous revertants (negative control) or a dose-related increase in the number of revertant in one or more strains.

Statistical Analysis. All data were expressed as mean ± standard deviation. Analysis of variance was performed by using SPSS (SPSS Inc., Chicago, IL). Duncan's new multiple range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The 50% ethanolic extract of the red bean fermented by *B. subtilis* IMR-NK1 used in this study contained 2.76 ± 0.13% crude fat, 26.93 ± 0.09% crude proteins, 14.25 ± 1.01% crude fiber, and 0.44 ± 0.06% ash (dry basis). The total phenol content of the extract was 22.58 ± 0.36 mg (gallic acid equivalent)/g, and the anthocyanin content was 1.00 ± 0.10 μmol/g.

Scavenging of DPPH Radical by the 50% Ethanolic Extract from Fermented Red Bean. The proton-radical scavenging action has been known as an important mechanism of antioxidation. DPPH was used to determine the proton-radical scavenging action of the red bean extract because it possesses a proton free radical and shows a characteristic absorption at 517 nm. The purple color of DPPH solution would fade rapidly when it encountered proton-radical scavengers (*18*). **Figure 1** shows the dose−response curve for the radical-scavenging activity of the fermented red bean extract and commercial antioxidants, α-toc and BHT. At a dosage of 0.62−5 mg/mL, the fermented red bean extract showed 15.3−86.3% scavenging

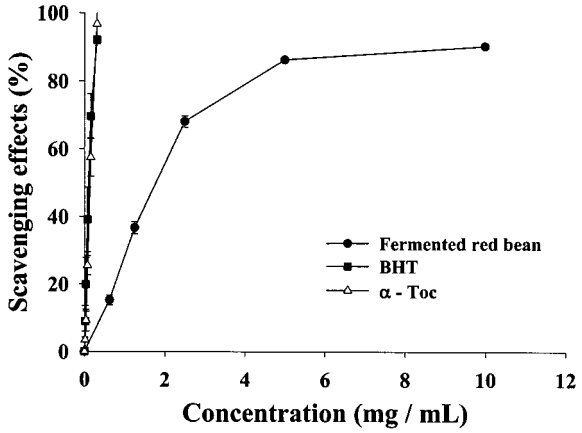

Figure 1. Scavenging effects of the fermented red bean extract, α-toc, and BHT on DPPH radical. Each value represents mean ± SD ($n = 6$).

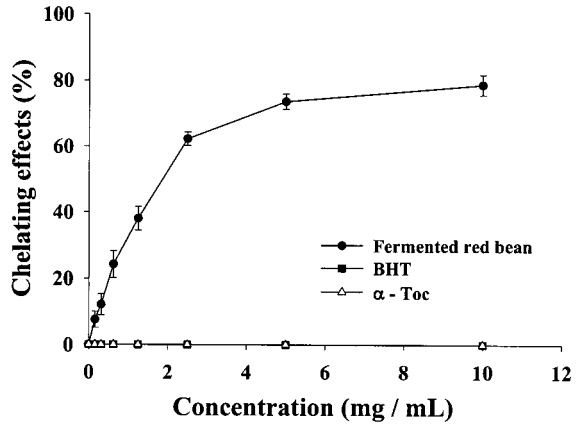

Figure 2. Chelating effects of the fermented red bean extract on Fe^{2+} ion. Each value represents mean ± SD ($n = 6$).

activity of DPPH radical, and the activity increased with the concentrations of the extract. The scavenging effects on DPPH radical leveled off as the concentration further increase. A comparison to the commercial antioxidants showed that the concentrations needed to obtain 80% DPPH radical scavenging activity for the fermented red bean extract, α-toc, and BHT were 3.9, 0.30, and 0.23 mg/mL, respectively. In other words, to reach a similar extent of DPPH scavenging effect the concentration required for the fermented red bean extract was significantly higher than that needed for BHT or α-toc.

Fe^{2+}-Chelating Ability of the 50% Ethanolic Extract from Fermented Red Bean. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (*23*). Because Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. **Figure 2** shows the chelating effect of the fermented red bean extract on ferrous ions. Similarly, the ability of chelating ferrous ions also increased with the concentrations of the extract to a certain extent, and then leveled off as the concentration further increased. At a dose level of 5.0 mg/mL, the chelating effect of the fermented red bean extract could reach to 73.6%. However, α-toc and BHT showed no detectable Fe^{2+}-chelating effect. The results implied that the fermented red bean extract had the Fe^{2+}-chelating effect and could afford protection against oxidative damage.

Reducing Power of the 50% Ethanolic Extract from Fermented Red Bean. In the reducing power assay, the presence of reductants (antioxidants) in tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form. The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. **Figure 3** shows the reducing powers of the fermented red bean extract, α-toc, and BHT. The reducing power (as indicated by the absorbance at 700 nm) also increased with the concentration of the fermented red bean extract to a certain extent, then leveled off with further increase in the concentrations. The reducing powers of BHT and α-toc were more pronounced than that of the fermented red bean extract. For example, the absorbance at 700 nm was found to be 1.08 for the fermented red bean extract at a dose level of 5.0 mg/mL, 1.2 for α-toc at a dose level of 0.312 mg/mL, and 1.35 for BHT at a dose level of 0.312 mg/mL. Although the reducing power of the fermented red bean extract was significantly less than that of BHT or α-toc, it was evident that the fermented red bean extract did have the reducing activity. These results revealed that the fermented red bean extract is an electron donor and can react with free radicals and convert them to more stable products, thus terminating the radical chain reactions (*19*).

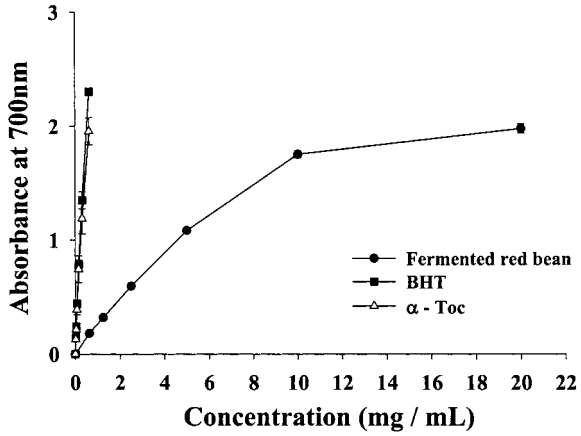

Figure 3. Reducing power of the fermented red bean extract, α-toc, and BHT. Each value represents mean ± SD ($n = 6$). Reducing power: increase in the absorbance at 700 nm indicates the activity of reducing power.

Table 1. Antioxidative Activities of the Fermented Red Bean Extract as Expressed by Antioxidative Activity Constants (k) and Half-Inhibition Concentrations (IC_{50})

antioxidative reaction	k^a (mg/mL)	$IC_{50}{}^a$ (mg/mL)	r^{2b}
DPPH radical	0.74 ± 0.05[a]	1.60 ± 0.07	1.00
Fe^{+2}-chelating ability	0.77 ± 0.15[a]	1.59 ± 0.12	0.98
reducing power	0.37 ± 0.03[b]	NA[c]	0.98

[a] k and IC_{50} values are given as mean ± standard deviation ($n = 6$). [b] r^2, Correlation of determination. [c] NA, not applicable. [a−c]Means with different letters in the same antioxidative reaction differ significantly ($p < 0.05$).

In addition, a high correlation level between the reducing power of the fermented red bean extracts and their anthocyanin contents was observed ($r = 0.926$, $p < 0.05$). Anthocyanins are commonly found in many plants and are responsible for bright colors such as orange, red, and blue. It has been reported that some anthocyanins possess strong antioxidative activity in vitro and in vivo (*24*, *25*). In this study, the anthocyanin content of the fermented red bean extract was found to be 1.00 ± 0.10 mol/g and it may be attributed, in a significant part at least, to the reducing power of the fermented red bean extract.

Concentration-Dependent Kinetics for Antioxidative Activity. As shown in **Figures 1**, **2**, and **3**, the antioxidative effects provided by the fermented red bean extract depended strongly

Table 2. Toxicity of the 50% Ethanolic Extract from Red Bean Fermented by *Bacillus subtilis* IMR-NK1 toward *Salmonella typhimurium* TA97, TA98, TA100, TA102, and TA1535

extract (mg/plate)	no. of colonies (Log CFU/mL)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
control[b]	7.3 ± 1.3	7.3 ± 1.6	6.4 ± 1.1	6.2 ± 1.1	8.7 ± 1.0	9.2 ± 1.4	8.2 ± 1.6	8.1 ± 1.3	8.2 ± 1.5	8.2 ± 1.3
5	7.3 ± 1.2	7.4 ± 1.4	6.2 ± 1.0	6.2 ± 1.4	8.6 ± 0.6	9.3 ± 1.4	8.2 ± 1.1	8.2 ± 1.7	8.3 ± 1.5	8.1 ± 1.0
2.5	7.5 ± 1.6	7.3 ± 1.7	6.3 ± 1.3	6.2 ± 1.2	8.9 ± 1.2	9.2 ± 1.4	8.3 ± 1.2	8.1 ± 1.4	8.2 ± 1.6	8.1 ± 1.6
1.25	7.4 ± 1.6	7.4 ± 1.2	6.4 ± 1.0	6.1 ± 1.0	8.6 ± 0.3	9.2 ± 1.3	8.3 ± 1.8	8.1 ± 1.3	8.3 ± 1.3	8.1 ± 1.3
0.625	8.3 ± 1.3	7.3 ± 1.7	6.2 ± 1.5	6.2 ± 1.4	8.6 ± 0.8	9.1 ± 1.2	8.3 ± 1.5	8.1 ± 1.3	8.3 ± 1.2	8.1 ± 1.4
0.3125	7.3 ± 1.1	7.3 ± 1.7	6.2 ± 0.8	6.2 ± 1.3	8.7 ± 0.8	9.1 ± 1.6	8.2 ± 1.7	8.1 ± 1.5	8.2 ± 1.7	8.1 ± 1.1

[a] Data are mean ± SD of triplicates. [b] The control was without extract.

Table 3. Mutagenicity of the 50% Ethanolic Extract from Red Bean Fermented by *Bacillus subtilis* IMR-NK1 toward *Salmonella typhimurium* TA97, TA98, TA100, TA102, and TA1535

extract	revertant colonies (CFU/plate)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
control[b]	64 ± 10	96 ± 6	19 ± 2	23 ± 2	108 ± 2	135 ± 27	195 ± 4	247 ± 25	9 ± 2	11 ± 1
2-AF (1 μg/plate)	153 ± 21	2190 ± 174	173 ± 15	120 ± 10	793 ± 65	2140 ± 99	1073 ± 327	2277 ± 332	8 ± 0.6	9 ± 2
4-NQO (10 μg/plate)	173 ± 15	980 ± 20	103 ± 6	227 ± 25	250± 50	2533 ± 65	4033 ± 61	1857 ± 233	9 ± 1	9 ± 2
5 (mg/plate)	86 ± 5	100 ± 3	16 ± 4	15 ± 4	77 ± 5	91 ± 3	132 ± 2	203 ± 5	6 ± 1	9 ± 2
2.5 (mg/plate)	79 ± 4	90 ± 8	14 ± 3	16 ± 4	76 ± 6	83 ± 7	209 ± 175	190 ± 18	7 ± 1	8 ± 2
1.25 (mg/plate)	92 ± 4	104 ± 19	10 ± 1	13 ± 3	81 ± 8	90 ± 8	150 ± 20	189 ± 14	6 ± 0.6	6 ± 2
0.625 (mg/plate)	87 ± 4	91 ± 6	12 ± 2	16 ± 5	85 ± 3	81 ± 3	178 ± 13	233 ± 5	9 ± 1	7 ± 1
0.3125 (mg/plate)	87 ± 3	90 ± 1	12 ± 2	12 ± 2	89 ± 9	92 ± 4	149 ± 34	220 ± 14	5 ± 1	5 ± 0.6

[a] Data are mean ± SD of triplicates. [b] The control was without extract.

on its concentration. In general, the antioxidative activity increased with antioxidant concentrations to a certain extent, then leveled off with further increase in the concentrations. The antioxidative activity of the extract of the fermented red bean can be expressed as the following equation (*26*):

$$1 - A/A^* = e^{-kC} \quad (3)$$

,where A is the antioxidative activity at any given antioxidant concentration, A^* is the equilibrium antioxidative activity that remains constant over a large antioxidant concentration, C is the antioxidant concentration, and k is a proportional constant. The proportional constant (k) can be obtained from the slope of a $ln(1 - A/A^*)$ against antioxidant concentration (C) plot. The proportional constant (k) has therefore the unit of reciprocal concentration, and can be viewed as an indication for the antioxidative activity. A higher k value represents a higher antioxidative activity for a specific reaction. In addition, for a specific antioxidative reaction, the half-inhibition concentration (IC_{50}) can be calculated as the antioxidant concentration required for providing 50% of the antioxidative activity.

As shown in **Table 1**, it is evident that the coefficients of determination (r^2) are high in all cases (0.98∼1.00), indicating the proposed concentration-dependent kinetics (eq 3) for the rate of changes in the antioxidative activities of fermented red bean were appropriate. It was found that the fermented red bean had similar antioxidative activity constants (k) for DPPH radical scavenging activity and Fe^{2+} chelating ability indicating that the two antioxidative reactions had similar rates of reaction. A significantly lower k value was found for the reducing power of the fermented red bean indicating a significantly lower rate of reaction for the reducing power compared to the DPPH radical scavenging activity or Fe^{2+} chelating ability.

Toxicity Test. If the chemicals tested for mutagenicity are toxic to the tester strains, it could lead to an erroneous result. It is recommended that a preliminary toxic dose range experiment be performed to determine an appropriate dose range for the mutagenicity assay; results of the toxicity test are shown in **Table 2**. Within the tested dose range, the extract did not show toxicity toward the tester strains with or without the presence of metabolic activator (S9 mix). Becausethere was no toxicity effect on the tester strains, the same dose range of the extract was applied in the mutagenicity assay (Ames test).

Ames Test. Table 3 shows results of the Ames test concerning the mutagenicity of 50% ethanol extract from the fermented red bean. The revertant numbers induced by the extract (0.3125−5 mg/plate) for all tester strains were close to that of the negative control (spontaneous revertant, without extract) and much lower than that of the positive controls (with diagnostic mutagens). The Ames test demonstrated that the fermented red bean extract had no mutagenicity effect under the tested dose range.

This study demonstrated that the fermented red bean extract is safe in genotoxicity and shows antioxidant activity. First, the antioxidative activity of the fermented red bean extract may be attributed to its proton-donating ability as evidenced through

DPPH radicals scavenging results. In addition, the fermented red bean extract had the Fe^{2+}-chelating effect and may afford protection against oxidative damage. Third, fermented red bean extract can also be viewed as an electron donor that could react with free radicals, convert them to more stable products, and terminate radical chain reactions. In light of these antioxidative effects, although fermented red bean extract is not currently used as a food additive, it possesses broad prospects for potential applications and exploitations for the food industry. Several studies have shown that microbial fermented beans possess antioxidative activities (*12*−*14*); for example ,an antioxidant, 2,4,6,3′,4′,-pentahydroxy-chalconol, has been identified from *Aspergillus soyae* fermented black bean (*27*). However, the components responsible for the antioxidative activity of the fermented red bean extract are still unknown. Therefore, it was suggested that further research work was needed on isolating and identifying the antioxidative components in fermented red bean extract.

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